(Review Article)

Phytomelatonin: A new class master plant hormone with multiple roles

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Abstract

Phytomelatonin, a plant hormone derived from tryptophan, has emerged as a fascinating molecule with diverse physiological functions in plants. In recent years, extensive research has shed light on the multiple roles of phytomelatonin in various aspects of plant growth, development, and stress responses. The accumulating evidence emphasizes the importance of phytomelatonin as a key regulator in plant physiology and highlights its potential applications in crop improvement and stress tolerance. This review article aims to provide a comprehensive overview of the current knowledge regarding phytomelatonin and its multifaceted roles in plants. We discuss the biosynthesis of phytomelatonin and its involvement in regulating plant growth processes such as circadian rhythm, root development, and flowering. Furthermore, we highlight the crucial roles of phytomelatonin in modulating plant responses to various abiotic and biotic stress factors. Even though the effectiveness of melatonin through exogenous application has been well established in plant' tolerance to stressful conditions, studies have just concentrated on the significance of high endogenous melatonin levels in enhancing the plant's ability to withstand stressors. Therefore, this review will also underline the importance of endogenous melatonin and shed light on how plants with high levels of melatonin respond to various stress conditions. Overall, this review will provide a foundation for further research on phytomelatonin and its potential implications in plant science and agriculture.

Keywords: Antioxidant Capacity; Endogenous Melatonin Content; Growth Regulator; Phytomelatonin; Stress Tolerance

1. Introduction

Melatonin, at first glance known for its role in the regulation of circadian rhythms and sleep-wake cycles in animals, has recently emerged as a fascinating and versatile signalling molecule in the plant kingdom [1,2]. Ever since melatonin's presence in various plant species was determined in 1995 [3,4,5], researchers have striven to unravel its diverse functions and regulatory roles within the plant systems. Termed "phytomelatonin," this plant-specific form of melatonin has sparked significant interest in the scientific community due to its multiple roles and potential implications for plant growth, development, and stress responses [6]. This review aims to emphasize the significance of phytomelatonin in optimizing plants' performance under both normal and stress conditions, starting from the discovery of melatonin in plants and delving into the regulatory mechanisms of phytomelatonin biosynthesis and its multifaceted roles in plant physiology as evidenced by current research. Understanding the intricate functions of phytomelatonin and harnessing the benefits of exogenous melatonin could unlock new horizons in plant biology and agricultural practices. As we confront the challenges of climate change and increasing demands on agricultural resources, the insights gained from melatonin research will offer a promising avenue to cultivate resilient and thriving plant systems in the face of adversity. This will not only offer hope for sustainable agricultural practices but also pave the way for innovative approaches in addressing global agricultural challenges.

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2. Melatonin and its discovery

Melatonin was initially discovered within the bovine pineal gland through the efforts of Lerner and his associates in 1958 [7]. This compound elicited melanin accumulation in the skin of frogs and other members of the Caeciliidae family, although its effects on mammals were found to be negligible. Subsequent investigations led to the recognition of melatonin as N-acetyl-5-methoxytryptamine, along with the elucidation of its biosynthetic pathway originating from serotonin and tryptophan [8,9,10]. The presence of melatonin in the human body was first detected in 1959, subsequently establishing itself as a prominent focus of research within the endocrinology area [11]. During the 1960s and 1970s, melatonin was found in various organisms such as birds, amphibians, fish, mammals, and other vertebrates [12,13,14] and investigations revealed the presence of melatonin in various invertebrate organisms including planarians, annelids, mollusks, insects, and crustaceans [15-19]. The first study on the relationship between the day-night rhythm and photoperiod in plants was conducted on *Gonyaulax polyhedra*, a photosynthetic protozoan with brown chromatophores that undergo color changes when present in large numbers in water [20]. The existence of endogenous melatonin in higher plants was first documented in a conference abstract presented by van Tassel and O'Neill in 1993 [21]. These researchers conducted research on melatonin detection in *Pharbitis nil L.*, a plant belonging to the Convolvulaceae (morning glory) family, and in *Solanum lycopersicum* L. (tomato) fruits, belonging to the Solanaceae (nightshade) family. However, they waited until 1995 to publish their results comprehensively [3]. Meanwhile, two separate papers were published, providing the evidence for the occurrence of melatonin in higher plants. Dubbels et. al [4] conducted an investigation wherein they quantified melatonin levels in *Nicotiana tabacum L.* plants, establishing the widespread occurrence of melatonin in plants. Their findings revealed that melatonin is distributed across various plant organs, including leaves, roots, stems, fruits, and seeds. A subsequent publication, merely two months later, quantitatively determined the existence of melatonin in 24 different edible plant samples, and detectable amounts of melatonin were found in all investigated plants using immunological methods [5]. In this study, it was determined that the highest melatonin content was found in rice, which belongs to the Poaceae (grass) family. Furthermore, in 1995, a Czech research group, under the leadership of Kolar and his colloquies [22], made a notable discovery by confirming the presence of melatonin *in Chenopodium rubrum L.*, a distinct plant species. These successive and autonomous investigations have unequivocally verified the existence of melatonin in higher plants, thereby firmly establishing its presence across various biological life forms, including animals, plants, and other organismal classes [23,24,25]. Through numerous studies conducted on plants over the years, the accumulated evidence regarding melatonin has led to its consideration as a potential regulator of plant growth, ultimately culminating in its recognition as a plant hormone [26].

2.1. The biosynthesis of melatonin in plants

In plants, melatonin biosynthesis pathway has been extensively investigated. A series of studies utilizing molecular biology and enzymatic kinetic methodologies have determined that the melatonin synthesis pathway in plants exhibits a notable level of complexity, distinct from that observed in animals, with significant differences between the two pathways [27].

The process of melatonin synthesis in plants involves the participation of unique enzymes that have not been previously identified in invertebrates, highlighting the distinctive biochemical pathways utilized by plants for melatonin production. In plants, the biosynthesis of melatonin is initiated with the enzymatic conversion of the amino acid L-tryptophan into L-tryptamine through the action of tryptophan decarboxylase. Subsequently, a hydroxylation reaction is facilitated by tryptamine 5-hydroxylase, leading to form 5-hydroxytryptamine, an intermediate in the melatonin synthesis pathway. The soluble enzyme responsible for the conversion of L-tryptamine to 5-hydroxytryptamine displays the highest activity in plant roots and thus it has been suggested to function as a growth-promoting regulatory factor, as suggested by Lee and Back [28]. However, the precise mechanism of serotonin-to-melatonin conversion in plants is not completely understood yet, as the simultaneous presence of two intermediate products, N-acetyl-5-hydroxytryptamine, and 5-methoxytryptamine, has been detected, indicating the complexity of this biosynthetic pathway. In the realm of plant biology, it is postulated by researchers that the processes of N-acetylation of 5-hydroxytryptamine followed by methylation to yield melatonin (A→M) and methylation of 5-methoxy tryptamine followed by acetylation to form melatonin (M→A) can occur either concurrently or independently. A study conducted in 2019 revealed that under typical environmental conditions, melatonin biosynthesis predominantly follows the A→M pathway. However, when plants are subjected to abiotic and biotic stressors like drought, salt, heat, cold, heavy metals, and pathogen infection, there is a notable shift toward melatonin production via the M→A pathway, as documented by Ye and his colloquies [29]. These enzymatic conversions are facilitated by various enzymes, including serotonin N-acetyltransferases, N-acetylserotonin-O-methyltransferases, and caffeic acid-O-methyltransferases. Moreover, it should be noted that plants can synthesize tryptophan de novo via the shikimate pathway, meaning that the initial substrate

for melatonin production in plants is not limited to tryptophan alone, as is the case in animals [30,31]. The graphical representation of the melatonin synthesis pathways in plants is depicted in Figure 1.
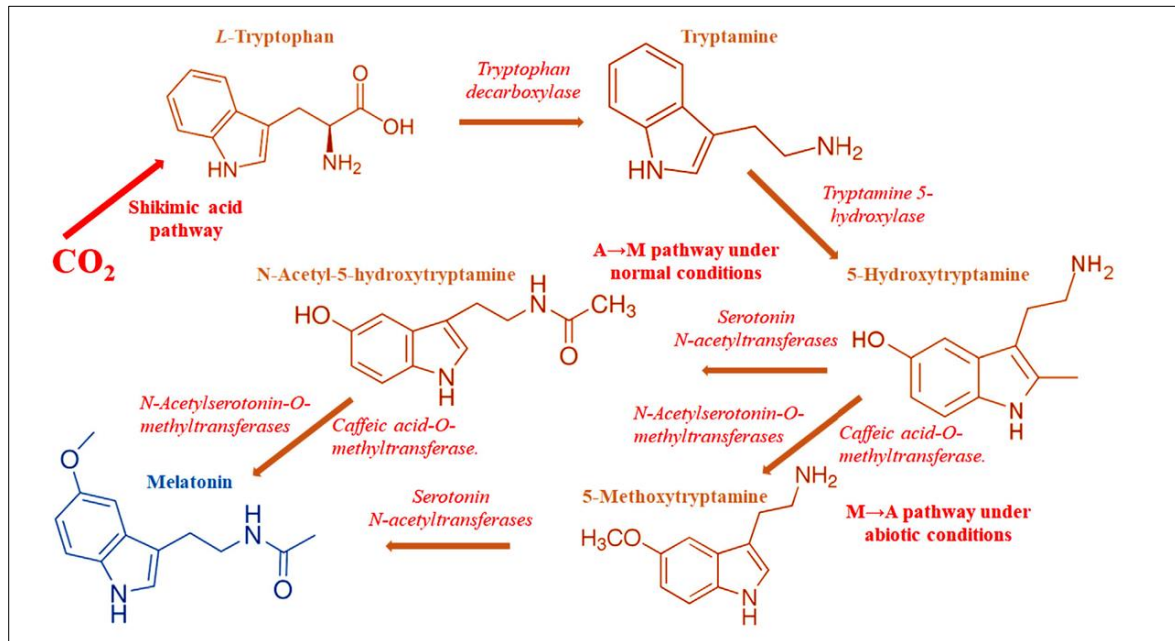
Figure 1 Schematic representation of the melatonin synthesis pathway in plants (adopted from Juhnevica-Radenkova and her colloquies [32]

2.2. The roles of melatonin in plant growth and development

Among the studies aiming to determine the specific roles of melatonin in plants, its potential role as a growth regulator has been extensively investigated. It has been found that melatonin acts as a growth promoter in etiolated bitter lupin (*Lupinus albus*) by exerting effects similar to indole-3-acetic acid (IAA), an auxin, and stimulates the active growth of hypocotyls at micromolar concentrations. However, at higher concentrations, it exhibits inhibitory effects [33,34]. Melatonin has been shown to induce the form of root primordia and promote the adventitious or lateral roots in bitter lupin [35]. This rhizogenic effect has been observed in various plant species, confirming its widespread occurrence. Studies have reported this effect in red cabbage [36], cucumber [37], rice [38], pomegranate [39], as well as in four cherry species [40,41]. These findings highlight the consistency of the rhizogenic effect of melatonin across different plant species. Melatonin plays a regulatory role in various aspects of plant development, including root, leaf, flower, and fruit development, as well as nutrient and water uptake, photosynthesis, and metabolic pathways. Notably, the external application of melatonin has been shown to significantly enhance the growth of seminal roots in wild rice plants cultivated under continuous light conditions [42]. Furthermore, when melatonin is applied before seeding, it improved nutrient balance by increasing leaf K+ and Ca+ contents and K^+/Na^+ and Ca^{2+}/Na^+ ratios in corn plants exposed to salt stress conditions [43]. These findings emphasize the wide-ranging influence of melatonin on plant growth and productivity. A similar effect has been observed in soybean subjected to salt and drought stress, where pre-treatment with melatonin has resulted in a significant increase in the seedling growth, leaf size, biomass, and number of flowers, pods, and seeds compared to control plants [44].

Various studies have demonstrated that the growth enhancement and improvement in yield and yield components induced by melatonin are associated with changes in gene expression related to cell division control, photosynthesis regulation, carbohydrate metabolism, and biosynthesis of fatty acids or ascorbate. While low concentrations of melatonin seem to promote hypocotyl elongation, it has been determined that high concentrations of melatonin harm hypocotyl growth in Arabidopsis plants [45]. Exogenous melatonin at a concentration of 50 μM has been shown to regulate fruit ripening and softening, water loss, and flavor formation [34,46]. Moreover, proteomic analyses have revealed that melatonin-induced fruit ripening may involve changes in proteins associated with ripening-related pathways and anthocyanin accumulation pathways [47].

Photosynthesis, a fundamental physiological process crucial for plant survival and productivity, is regulated by various factors [48]. Abiotic stresses typically disrupt photosynthesis by inducing stomatal closure, leading to a significant reduction in transpiration rate and subsequently improving water use efficiency. However, the exogenous application

of melatonin to tomato plants under low temperature stress has been found to increase important photosynthetic parameters including net photosynthetic rate, transpiration rate, stomatal conductance, electron transport rate, and maximum quantum efficiency of photosystem II (Fv/Fm) [49], moreover melatonin treatment has been shown to prevent photosystem II photoinhibition under cold stress. Similarly, it enhances the photosynthetic rate and chlorophyll content in maize plants under drought stress [50]. Additionally, supplementation of 100 μM melatonin to the soil has been observed to enhance photosynthetic capacity, chlorophyll, and total soluble sugar contents in various plants including apple, rice, grape, cherry, and germinating plants [51,52,53]. Treatment with 1 mM melatonin has significantly delayed leaf senescence in barley, preserving chlorophyll pigments to a maximum extent [54,]. Furthermore, the application of melatonin to the hydroponic growth medium of *Chara australis* plants has shown a tendency to increase the quantum efficiency of photosystem II by 34% [55].

Leaf senescence is a genetically programmed process characterized by the degradation of macromolecules, including chlorophyll, and the sequential transfer of nutrients to other parts of the plant [56,57]. Pretreatment with melatonin has been shown to effectively suppress the upregulation of senescence-associated genes SAG 12 and pheophorbide A oxygenase, which are involved in leaf senescence and the regulation of photosystem II function, particularly under drought stress conditions in apple seedlings [58]. Hong and his colloquies [59] reported that melatonin significantly inhibits chlorophyll degradation, delays leaf senescence, and suppresses the expression of genes associated with aging in rice. High-throughput RNA sequencing in rice has demonstrated that melatonin regulates several leaf senescence-associated transcription factors, including SGR and NAC, highlighting the crucial role of melatonin in modulating the process of plant aging [60]. Moreover, melatonin has been found to downregulate the expression of aging-related genes and regulate leaf senescence in ryegrass under heat stress. Proteomic-based approaches have further demonstrated the impact of melatonin pretreatment on the expression of proteins associated with aging in tomatoes [47].

2.3. The circadian rhythm and annual variation of melatonin

Melatonin plays a significant role in the perception of daily and seasonal time in both animals and humans. In mammals, melatonin is typically produced during the night, and its levels in the blood decrease under light conditions [61]. Therefore, fluctuations in melatonin levels in the bloodstream assist tissues and cells in perceiving daily or seasonal time. In addition, melatonin applications can act as photoperiodic regulators or circadian rhythm regulators in animals and humans, as they imitate dark conditions [62]. The circadian rhythm, also known as the biological clock, regulates the physiological, behavioral, and metabolic functions of various organisms, ranging from bacteria and fungi to plants and animals, in approximately 24-hour cycles corresponding to the Earth's rotation [63]. In plants, melatonin functions as a 24-hour rhythm regulator, and its synthesis increases in the dark. One of the earliest studies to demonstrate the variation of melatonin within 24 hours in plants was conducted on 15-day-old *Chenopodium rubrum L.* plants grown under a 12-hour dark/12-hour light photoperiod. It was found that melatonin levels were low during the light period, but reached their highest level towards the end of the dark period in this study [64].

In general, it is reported that melatonin functions as a 24-hour rhythm regulator in plants, and its synthesis levels increase during the dark period [63]. However, some researchers have reported that melatonin synthesis reaches its highest level just before sunset [65]. For instance, in a study conducted under field conditions, the variation in melatonin levels within a 24-hour time frame was determined in the skins of Malbec grape berries. It was found that the highest level of melatonin was detected at sunrise, and the melatonin level decreased in the subsequent hours [66]. The researchers have reported that the reason for this is that melatonin levels are controlled by the circadian rhythm, and that the decrease in melatonin levels during the light period may be attributed to the excessive consumption of melatonin associated with the activation of antioxidant systems in response to stress caused by solar radiation [66]. Some researchers argue that melatonin is continuously present in plants but its levels can vary throughout the day [67]. In a study involving green macroalgae, it was observed that the highest melatonin levels were detected at 02:00 during the nighttime under a photoperiod of 16 hours of light followed by 8 hours of darkness. Conversely, the lowest melatonin levels were observed at 14:00 [68]. The variation in melatonin content is believed to be associated with enzymatic activities regulated by the circadian rhythm and influenced by exposure to light, leading to the deformation of melatonin. Furthermore, in natural conditions, it has been observed that the melatonin levels in water hyacinth plants increased towards the end of the light period, and this phenomenon may be associated with photosynthesis and daylight protection processes [65]. It has been reported that in the Hadrian F_1 eggplant variety, the melatonin content reached its peak twice during the day, one peak occurring at the beginning of the dark period, while the other peak in the middle of the light period [69]. In eggplant, the melatonin content decreased as the developmental stages progress in leaves and roots. However, in flowers, higher melatonin contents were observed, which decreased along with fruit growth but increased again during harvest and seed maturation [70]. It is believed that the high melatonin content in both seedlings and mature seeds plays a protective role against potential environmental stress factors during these growth stages. Similarly, in a study conducted on different pepper varieties at different growth stages and organs, it was observed that

the highest melatonin content was determined in seedlings at cotyledon-stage (108.6-111.6 ng g^{-1}). As the plants grow progressed, the melatonin content decreased in leaves but significantly increased in fruits and seeds [71].

Melatonin regulates cellular and organismal processes such as cell renewal, hormone secretion, photosynthesis, and flowering by coordinating the response mechanisms according to their internal clocks. Some researchers propose that melatonin in plants can regulate circadian rhythm, gene and metabolite regulation, protein stability, and many biological processes involving daily and seasonal cycles. It can influentially enhance photosynthesis and growth rates, influence flowering to increase seed yield, and impact biotic and abiotic responses [72,73]. When examining studies demonstrating the seasonal and annual rhythms of melatonin, it is observed that the first investigation on this topic was conducted by Kołodziejczyk and her colloquies [74]. In this study, the changes in melatonin content within maize and cucumber seeds were monitored during one year of storage, and it was determined that the melatonin levels in both maize and cucumber seeds varied throughout the storage period. In both plant species, the melatonin levels in the seeds, which initially ranged between 10-20 ng g^{-1} at the beginning of storage, rapidly increased during the 4th and 5th months of storage, reaching to the levels of 60-80 ng g^{-1}. This increase is believed to participate in a defense mechanism to protect the seeds against adverse stress factors. Similarly, studies conducted on stored pepper seeds for two years have revealed significant rise in seed melatonin content during the winter months and a decrease during the summer months [75]. After storing lettuce seeds for a period of 24 months, it was determined that their endogenous melatonin content remained at minimum levels in August but reached maximum levels during the winter months [76]. In a study conducted on the roots and leaves of eggplant plants, a similar relationship between melatonin and tryptophan content was observed. Specifically, there was an inverse relationship between melatonin and tryptophan contents, whereby an increase in one corresponded a decrease in the other [70].

2.4. The role of melatonin in plants as an antioxidant

Plant cells produce significant amounts of reactive oxygen species (ROS) in response to various environmental stimuli, including hydroxyl radicals (·OH), superoxide anions (O2·), singlet oxygen ($_1$O^2), and hydrogen peroxide (H$_2$O$_2$). This situation leads to membrane damage and disruption of cellular organelle integrity. To counteract this issue and safeguard against oxidative harm, plants have evolved efficient enzymatic and non-enzymatic antioxidant defense systems [77]. However, under prolonged and severe stress conditions, the natural defense mechanisms of plants may prove inadequate to cope with oxidative stress and its harmful effects. Melatonin actively participates in tolerance mechanisms against various stressors such as drought, alkalinity, salinity, heat, metal stress, acid rain, and pathogens and it accomplishes this by enhancing enzyme activity and scavenging ROS [78-81]. Numerous scientific investigations have provided compelling evidence that melatonin plays a regulatory role in the biosynthesis and functioning of various biomolecules, including chlorophyll, nitrogen-related compounds, proteins, and RuBisCo. Through its involvement in the antioxidant defense system, melatonin effectively regulates oxidative stress [82,83,84]. Plants utilize both enzymatic and non-enzymatic antioxidants as part of their internal defense systems to withstand unfavorable environmental and growth conditions [85,86]. The enzymatic antioxidant defense system in plants consists of essential enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), glutathione S-transferase (GST), and peroxiredoxin (PRX) [50]. Additionally, non-enzymatic antioxidants including glutathione (GSH), ascorbic acid (AsA), carotenoids, tocopherols, and flavonoids also play a role in maintaining ROS homeostasis in plants. Melatonin serves a significant function in plants as both a direct ROS scavenger and an indirectly as an antioxidant. It effectively regulates the levels and toxicity of H$_2$O$_2$ during harsh environmental conditions by utilizing the ROS-scavenging activity of antioxidant enzymes and the capacity of the ascorbate (ASA)/glutathione (GSH) cycle. Studies have demonstrated that melatonin can scavenge up to 10 free radicals, surpassing the capability of antioxidant enzymes, which typically scavenge only one radical per molecule [50]. Melatonin is acknowledged as an initial antioxidant molecule that assists plants in alleviating oxidative stress by reducing the levels of ROS and reactive nitrogen species (RNS) [87]. In a study conducted by Korkmaz and his colloquies [88], it was observed that *Capsicum annuum* plants exposed to cold stress exhibited a significant increase in the activities of key antioxidant enzymes, namely SOD, CAT, and POX. Previous studies have demonstrated that pre-treatment with melatonin in tomato seedlings can confer beneficial effects under heat-stress conditions. These effects include increased pigment content and gas exchange parameters, enhanced activities of key enzymes such as RuBisCo and FBPase, and upregulated expression of photosynthetic genes [89]. Additionally, Ali and his colloquies [90] reported that the exogenous application of melatonin in tomato plants led to increased activities of antioxidant enzymes such as APX, SOD, POX, and CAT, improving salt tolerance.

Siddiqui and his colloquies [91] observed that the exogenous application of melatonin had beneficial effects on tomato seedlings under salt stress conditions. Melatonin supplementation improved the balance of Na$^+$/K$^+$ ions, enhanced relative water content (RWC), and increased the activities of antioxidant enzymes including SOD, CAT, and POX. Furthermore, it significantly improved the activity of L-cysteine desulfhydrase, while reducing the content of MDA and

H_2O_2 in the seedlings. Additionally, Karaca and Cekic [92] reported that exogenous melatonin promoted GR, SOD and APX activities, chlorophyll content, and increased p-coumaric acid content, while reducing the MDA content in tomatoes under drought stress. Zhang and his colloquies [93] conducted a study and documented that melatonin played a protective role in tomato seedlings by activating antioxidant enzymes like APX and CAT. This led to a reduction in ROS production and effectively mitigated the harmful effects of Cu^{2+} toxicity. Debnath and her colloquies [94] showed in their study that melatonin application enhanced photosynthesis and antioxidant defense mechanisms in plants subjected to simulated acid rain (SAR) stress. This treatment effectively repaired the grana lamellae of chloroplasts. Umapathi et al. [95] discovered that melatonin increased the levels of natural antioxidant enzymes and metabolites in tomatoes (*Solanum lycopersicum* L.) by directly scavenging ROS. The research conducted by Jahan and colloquies [96] revealed that melatonin played a crucial role in maintaining the pools of AsA and GSH, which contributed to the defense of cellular integrity by reducing electrolyte leakage. Additionally, melatonin upregulated the expression of respiratory burst oxidase homolog, effectively limiting the production of ROS.

2.5. Effects of endogenous melatonin on stress tolerance

Melatonin biosynthesis genes derived from animal sources, such as sheep, have frequently been utilized in studies involving transgenic plants. These plants are genetically modified to express ectopic genes, particularly those encoding SNAT and HIOMT enzymes. Using these ectopic genes allows researchers to explore the potential physiological roles of melatonin. Apart from the SNAT and HIOMT genes, other melatonin biosynthesis genes such as T5H and TDC have also been investigated in various studies. Rice plants have been the primary targets for introducing these ectopic genes, resulting in the generation of numerous transgenic rice plants to explore melatonin metabolism. Tomato and *Nicotiana sylvestris* plants have also been employed in some investigations. In general, plants overexpressing SNAT/HIOMT genes have shown a significant increase in endogenous melatonin levels and demonstrated enhanced tolerance to various stress conditions. In the case of rice plants, the elevation of melatonin levels through overexpression has been associated with improved resistance against the herbicide butafenacil, which generates $_1O^2$ [97]. Furthermore, the introduction of the hydroxy indole-O-methyltransferase (HIOMT) gene from apples into Arabidopsis plants has led to an increase in endogenous melatonin levels, a reduction in ROS content, an enhancement in biomass, and an improved tolerance to drought stress [98]. Generally, plants with high melatonin levels achieved through overexpression demonstrate increased resilience against abiotic stressors including drought, cold, and UV-B radiation. These findings highlight the critical role of endogenous melatonin in ROS scavenging, even at lower concentrations (in picograms or nanograms per fresh weight), acting as a primary defense mechanism against stressors. Additionally, transgenic plants with modified melatonin levels have exhibited improved root growth rates, resilience, and overall biomass. In a study involving transgenic rice (*Oryza sativa*) seedlings that were engineered to overexpress the sheep serotonin N-acetyltransferase gene, it was observed that shoot melatonin levels in transgenic seedlings were ten times higher compared to non-transgenic counterparts, leading to a twofold increase in root growth [38].

Under optimal growth conditions, the levels of melatonin in plant tissues generally remain relatively stable. However, environmental factors including high light intensity, low temperatures, elevated temperatures, drought, and exposure to heavy metals strongly influence melatonin levels [99,100,101]. Under salt stress, it has been determined that the endogenous melatonin content in sunflower seedlings is 2-fold higher in the roots and 6-fold higher in the cotyledons compared to non-stressed control plants. Furthermore, a 72% increase in the enzyme activity of HIOMT was observed in the stressed seedlings compared to non-stressed ones [102]. In rice seedlings, it has been found that under oxidative stress caused by the herbicide butafenacil and cadmium, there was a significant increase in the activities of intermediate enzymes involved in melatonin biosynthesis, such as T5H, TDC, and HIOMT, resulting in up to a 6-fold increase in the detected endogenous melatonin levels [97, 103]. Furthermore, an investigation focusing on the role of endogenous melatonin in *Arabidopsis thaliana*'s resistance to gray mold (*Botrytis cinerea*) infection revealed an upregulation in the expression of transcription factor genes associated with resistance in the jasmonic acid signaling pathway. This upregulation subsequently resulted in an increased production of ASMT or SNAT enzymes. Moreover, higher melatonin levels were found to enhance the antioxidant enzymes activities of SOD and POX, elevate jasmonic acid levels, and alleviate plant disease symptoms. Additionally, a reduction in lesion size on the leaves was observed [104]. Under the influence of cadmium stress in tomato seedlings, it has been observed that the transcription of the caffeic acid O-methyltransferase 1 (COMT1) gene, which is involved in melatonin biosynthesis, stimulates the accumulation of endogenous melatonin and its role in enhancing cadmium tolerance [105]. Similarly, in tomato seedlings treated with selenium, the transcription of genes involved in melatonin biosynthesis, such as TDC, T5H, SNAT, and ASMT, is triggered, leading to the turnout of melatonin. This increase in melatonin biosynthesis plays a role in the detoxification of cadmium [106].

The effect of environmental factors on the levels of melatonin in plant organs has been demonstrated through numerous studies. In a study conducted with wild tomato (*L. pimpinellifolium*) and cultivated tomato (*L. lycopersicum*) grown at

high altitudes under ozone stress, it was found that the endogenous melatonin level in the leaves of cultivated tomatoes was 5 times higher compared to the wild tomato variety. These results were attributed to the greater tolerance of the cultivated tomato variety to ozone stress [4]. The same researchers, when investigating the response of different tobacco (*Nicotiana tabacum*) varieties to high ozone stress, found that varieties with high ozone tolerance had higher levels of endogenous melatonin. They stated that an increase in melatonin concentration played an active role in scavenging the free radicals generated as a result of stress. A study conducted with Chinese licorice (*Glycyrrhiza uralensis*) investigated the endogenous melatonin synthesis in plant organs and the effects of externally applied red, blue, and white light (control), as well as UV-B radiation (280-315 nm), on plant growth. The study revealed that the highest endogenous melatonin content was found in the root tissues, and the melatonin content in all tissues was found to increase with plant development [107]. The endogenous melatonin content in plants exposed to stress was found to vary depending on the applied stress and its duration, with the highest melatonin content obtained from treatments with 10 mM H_2O_2 and 1 mM zinc sulfate [108]. In another study focusing on white lupin plant tissues (*Lupinus albus* L.), the endogenous melatonin concentration was investigated under various stress conditions. It was discovered that chemical stress induced by $ZnSO_4$ or NaCl caused significant alterations in the endogenous melatonin concentration, with the highest impact observed with $ZnSO_4$, followed by NaCl, cold stress, and drought stress ($ZnSO_4$ > NaCl > cold stress > drought stress). Furthermore, the application of $ZnSO_4$ was found to significantly increase the endogenous melatonin concentration in the roots of white lupin plants by at least 12-fold compared to non-stressed plants [109]. Additionally, the melatonin content in the roots of plants grown at 6°C was approximately 2.5 times higher than those grown at 24°C [110]. These findings provide clear evidence that melatonin serves as a signaling molecule or a direct stress-preventive compound in various stress conditions, leading to an upregulation of endogenous melatonin production in response to stress [109]. In another study conducted by the same researchers, it was determined that the endogenous melatonin content in the leaves of field-grown tomatoes was 10 times higher compared to in vitro-grown plants, and 7 times higher compared to plants grown in a climate chamber. This increase observed in tomatoes grown in open-field conditions was attributed to their exposure to a higher number of biotic and abiotic stress factors. It was suggested that open-field cultivation exposes tomato plants to a greater variety of biotic and abiotic stresses, leading to the observed higher melatonin content [111]. Furthermore, it has been determined that herbicide treatments applied to transgenic rice seedlings with high endogenous melatonin content reduced stress-induced chlorosis, as well as decreases the H_2O_2 and MDA levels. These treatments also lead to an increase in chlorophyll content and the activities of SOD and CAT enzymes [97]. In Arabidopsis seedlings exposed to high-temperature stress (37 °C) for different durations (0, 15, 30, 60, 120, and 180 min), it has been reported that the endogenous melatonin content was 2 to 5 times higher compared to non-stressed seedlings [112]. In another study conducted to investigate the endogenous melatonin content in pistachio seeds, four different varieties of pistachio trees grown under field conditions with a wide cultivation area were examined. It was stated that the endogenous melatonin content could be influenced by the variety's location or weather conditions, resulting in different melatonin contents among them [113]. In a study conducted to elucidate the role of endogenous melatonin in plant thermotolerance, transgenic tomato plants that were unable to produce melatonin due to the suppression of the COMT1 gene involved in melatonin biosynthesis were subjected to high-temperature stress. It was observed that under high-temperature stress, the EC percentage and MDA concentration in tomato leaves increased. Additionally, significant decreases in the activities of two key antioxidant enzymes, APX and CAT, as well as in the GSH/GSSG and AsA/DHA ratios, which play significant roles in plant thermotolerance, were observed [114].

Recent studies have highlighted the potential use of endogenous melatonin levels as a physiological marker for screening genotypes with stress tolerance. For instance, in a study investigating the relationship between endogenous melatonin levels and germination and emergence performance under chilling stress (15 °C) in 28 pepper (*Capsicum annuum* L.) genotypes, it was observed that genotypes with higher seed melatonin content exhibited greater tolerance to stress. Furthermore, it was noted that seed genotypes with endogenous melatonin concentrations below 2 ng g^{-1} displayed elevated levels of MDA and H_2O_2, along with reduced antioxidant enzyme activities. The positive relationship observed between the seed melatonin content and chilling stress tolerance indicated the potential use of endogenous melatonin levels as a criterion for selecting varieties that are tolerant to chilling stress [115]. Additionally, Köklü Ardıç et al. [116] assessed the endogenous melatonin content in 60 pepper genotypes and established a significant relationship between endogenous melatonin levels and drought stress tolerance. They also proposed that endogenous melatonin levels could serve as an initial criterion for selecting drought-tolerant varieties.

3. Conclusion

The studies conducted so far have clearly demonstrated the critical role of increased endogenous melatonin levels in effectively coping with stress. However, further research is warranted to explore the relationship between endogenous melatonin levels and stress tolerance under various stress conditions, in order to gain a deeper understanding of this association. Additionally, there is a need to delve into the post-translational modulation of melatonin biosynthesis genes

and the regulation of associated proteins, as these areas still hold much knowledge to be uncovered. These investigations will be instrumental in the selection and development of new plant genotypes with desirable traits and ensure the foundation for future research endeavors, emphasizing the significance of endogenous melatonin levels in enhancing plant stress tolerance.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to disclosed.

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