Bionatura Journal
Ibero-American Journal of Biotechnology and Life Sciences

Review

Melatonin, a controversial compound in plants, is related to the transport of sodium and potassium ions and proline content.

Dayron Otero-Rodriguez [1†], *Camilo Escalante-Magaña* [1†], *Maria de Fátima Medina-Lara* [1], *Ileana Echevarria-Machado* [1], *and Manuel Martínez Estevez* [1*]

[1]Integrative Biology Unit. Yucatan Scientific Research Center. Mérida, 97205, Yucatán, Mexico
† Both authors contribute equally * Correspondence: luismanh@cicy.mx

ABSTRACT

Melatonin, known primarily for its role in animals, also performs essential functions in plants, where it acts as a growth regulator, antioxidant, and modulator of stress responses. However, their role remains controversial because of the differences observed between species and experimental conditions. In the context of sodium–potassium ion transport (Na^+ / K^+), melatonin contributes to maintaining ionic balance, especially under salt stress. It does this by stimulating the activity of ionic transporters, such as the anti-carrier Na^+ / H^+, which expels sodium from cells or stores it in vacuoles, and by promoting the retention of potassium, which is crucial for metabolic and photosynthetic functions. In addition, melatonin influences the levels of proline, a key amino acid involved in the response to abiotic stress. It promotes its accumulation by activating biosynthetic genes, such as P5CS, and by improving antioxidant metabolism, protecting cells against oxidative damage. Although its effects are promising, the variability of results in different plants and conditions has generated debates about its effectiveness and precise mechanisms. More research is needed to clarify its role in plant physiology.

Keywords: Melatonin; Salinity tolerance; Oxidative stress; Ion transport; Proline accumulation

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a low-molecular-weight organic compound that acts as a pleiotropic signaling molecule, performing vital functions in a variety of physiological processes in both animals and plants[1]. It was first identified in animals in 1958 in the bovine pineal gland[2]. It is considered a hormone that regulates various biological processes, such as antioxidant activity, enhancement of the immune system, circadian rhythms, sleep physiology, seasonal reproductive physiology, and thermal homeostasis[3]. In the plant environment, the presence of melatonin was observed for the first time in vascular plants in 1995[4,5]. Since then, melatonin has been reported to play a crucial role as an antioxidant, participating in the regulation of reactive oxygen species (ROS) and lipid peroxidation in animal tissues[6]. This antioxidant activity is not limited only to animals but also extends to plants and other photosynthetic organisms, such as algae[7], which reinforces the hypothesis that melatonin acts as a universal antioxidant, as proposed by8 in 1993. Research in plants has shown that melatonin can interact with a wide range of ROS and other reactive nitrogen-containing molecules (RNS), which makes it an essential component for the functionality of biological membranes, especially mitochondrial ones [8,9]. Melatonin has been confirmed to play a protective role in plants against various types of biotic and abiotic stress[10].

The structural similarity of melatonin with indole-3-acetic acid (IAA, auxin) has led many researchers to explore its function in plants[11]. In addition to its antioxidant properties, melatonin has been shown to induce substantial changes in gene expression and affect various physiological processes in plants. An important advance in the research of plant melatonin was the discovery of its first specific receptor, which has led some to consider it a new plant hormone[12].

Currently, there is detailed knowledge about biosynthesis, degradation, conjugation, transport, and signal transduction mediated by melatonin in plants. These advances suggest that melatonin could have hormonal effects in plants, similar to those observed in animals[13]. Concerning its biosynthesis, tryptophan is known to be its precursor, and the enzymes involved in its synthesis in plants differ from those that act in animals[13]. Light and temperature are crucial factors in the regulation of melatonin biosynthesis in plants, which highlights the importance of the environment in the function of this molecule[14].

Melatonin in Plants: Functions, Applications, and Controversies

Melatonin plays a multifaceted role in plants, intervening in diverse processes, such as growth promotion, rooting induction, tropism[18], germination of seeds and photosynthesis, and optimizing the efficiency of water/CO2 exchange in leaves. In addition, it regulates vital physiological processes such as maturation, senescence, the internal biological clock, and parthenocarpy[19].

Although initially considered an indolamine neurotransmitter in animals, melatonin has emerged as a multifunctional signaling molecule in plants. Its presence in plants was initially investigated because of its antioxidant properties, and its identification in plant-based foods has led to its recognition as a nutraceutical for humans[20]. Recently, its potential use in phytoremediation has also been suggested as a bioagent for the recovery of chemically contaminated soils[21].

Phytomelatonin, as has been called in some studies[22], has been the subject of great interest for its ability to regulate a wide range of physiological functions in plants, such as protection against biotic and abiotic stressors, and its role in the regulation of growth, rooting, and other morphogenetic aspects. It has also been identified as a modulator of biological rhythms and gene expression (Figure 1).

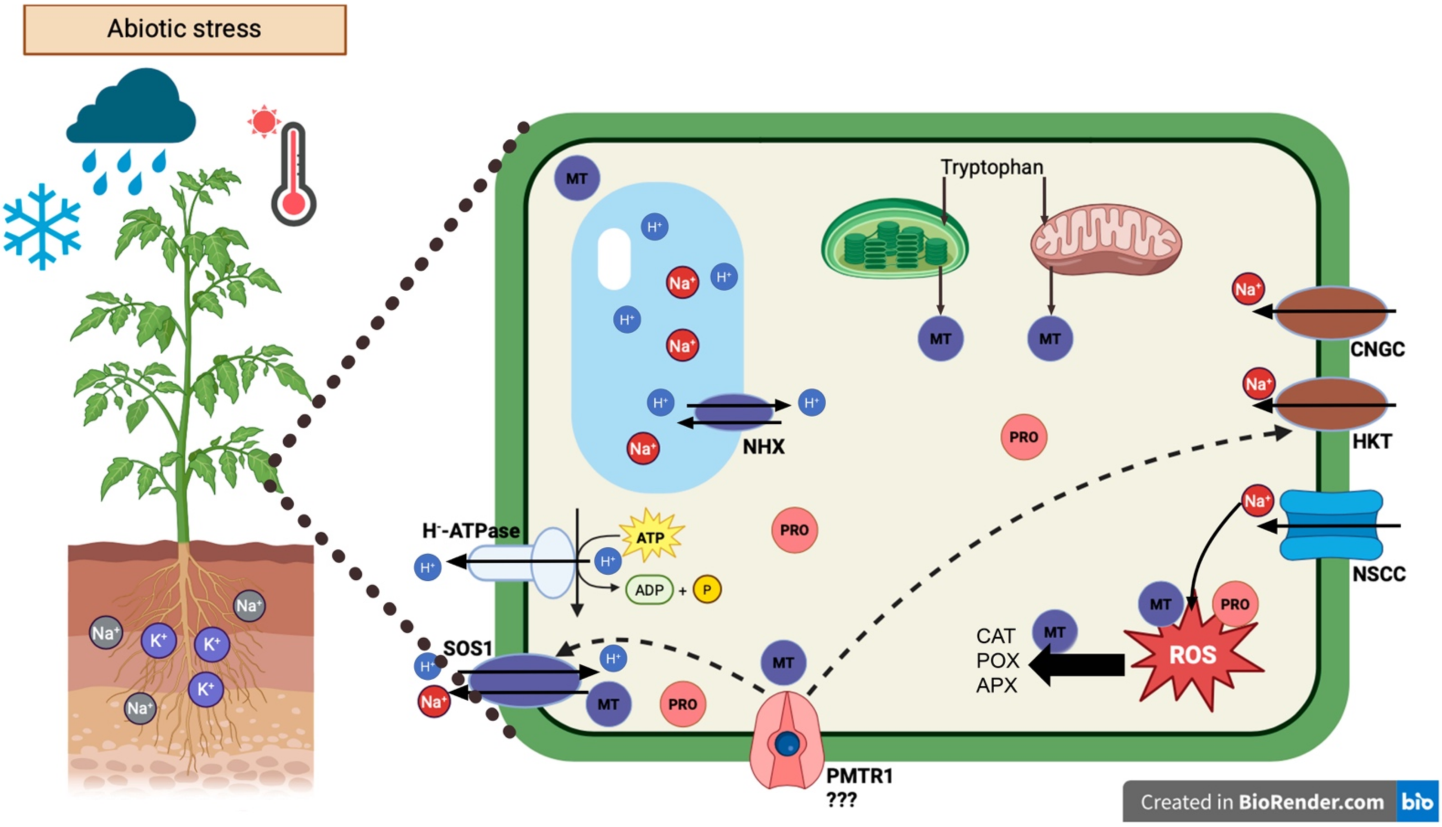

Figure 1. Representation of the role of melatonin in plants, highlighting its potential influence on potassium-sodium (K^+/Na^+) ion transport and the activation of ROS-scavenging enzymes under salt stress conditions. Melatonin acts as a multifunctional regulator, modulating responses that promote ionic homeostasis and salt stress tolerance in plants. MT, Melatonin; Pro, Proline; NCSS, Non-Selective Cation Channels; HKT, High-Affinity Potassium Transporter; CNGC, Cyclic Nucleotide-Gated Channels; NHX, Na^+/H^+ Exchanger; PMTR, Plant Melatonin Receptor; SOS1, Salt Overly Sensitive 1 (Na^+/H^+ Anti-porter); H^+-ATPase, Plasma Membrane Proton Pump.

Hypothesis and Initial Studies on the Effects of Melatonin on Plants

In his early research, melatonin in plants was thought to play a role similar to that in mammals, particularly in regulating circadian rhythms and photoperiodicity, processes associated with flowering. These studies were complemented by research on photoreceptors, vernalization, and the hormones that regulate biological rhythms in plants.

In 1997 Kolar et al. [23] reported the presence of melatonin in 15-day-old *Chenopodium rubrum* plants. These studies revealed an oscillating behavior in melatonin levels, with low or undetectable concentrations during the day and a significant increase during the dark, reaching a maximum of 240–550 pg/g fresh weight[23]. This oscillation pattern, similar to that observed in animals, raised great expectations about the possible role of melatonin in plant circadian rhythms.

However, in subsequent studies, which were carried out in plants exposed to different photoperiods (6, 12, and 18 hours of darkness), no significant modifications were observed in the duration of the increase in melatonin as a function of the photoperiod, although the maximum melatonin peak was always observed. Produced in the dark[24]. In a more recent work, the same group investigated the effects of the exogenous application of melatonin on the flowering of *Chenopodium rubrum* and reported that although melatonin did not induce toxic effects or alter the shape, color or number of leaves, the exogenous application of very high concentrations of melatonin (100 and 500 μM) had a slight inhibitory effect on flowering without modifying the rhythm or phase of the process[25].

Melatonin and Its Protective Function in Plant Cells

In recent research, an experimental approach analogous to that used in animals has been applied to explore the role of melatonin in plants. This compound, known for its ability to protect against oxidative damage caused mainly by free radicals, has shown promising effects in recent studies focused on plant organisms. Although most of the research on melatonin has focused on animals and cell systems, some studies have suggested that melatonin may also play a crucial role in protecting plants against oxidative stress and other types of cell damage [26,27].

Despite the lack of conclusive evidence to support the protective effect of melatonin in plants, several hypotheses have emerged from the presence of this molecule in various plant organs. Hardeland proposed that melatonin could be involved in the maintenance of seed dormancy based on the high concentrations observed in these seeds[27]. These findings suggest that melatonin can regulate physiological processes that allow seeds to remain dormant until environmental conditions are favorable for germination.

A related study was carried out by Manchester *et al*. (2000)[26]. They indicated that variations in melatonin levels in different cultivars of *Nicotiana tabacum* could be correlated with their susceptibility to ozone, suggesting that melatonin could be significant in the response of plants to environmental stress. This compound could contribute to the protection of plants against the toxicity of free radicals generated by pollution, helping them manage the oxidative damage induced by pollutants such as ozone.

On the other hand, Murch and Saxena (2002) [29] investigated the indole content in *Hypericum perforatum* during flower development and reported that both serotonin and melatonin reached high levels during the critical stages of this process. This coincides with the greater regeneration potential of the isolated anthers, suggesting that melatonin could function as a protective agent against stress, providing an adaptive mechanism that favors the reproduction of the plant under unfavorable conditions.

Melatonin receptors in plants

Among the basic principles that must be taken into account to determine whether there are hormonal functions in the animal or plant kingdom is the presence of an agent responsible for the interaction with the hormone, which triggers the elements of the signaling chain; this agent is known as the receiver. One of the most important limitations of studies on melatonin in plants has been the lack of an identified receptor.

This has also been an obstacle to considering melatonin as a plant hormone. However, as with other plant hormones, receptor identification only occurred after many relevant biochemical and physiological data for melatonin were obtained. The turning point came with the recent identification of CAND2/PMTR1, a phytomelatonin receptor discovered in *Arabidopsis (A. thaliana)*[30].

Located in the plasma membrane with a receptor topology, it interacts with the G protein subunit (GPA1), and melatonin induces its expression in different tissues. Phytomelatonin-receptor binding triggers the dissociation of Ggb and Ga, which activates the production of H_2O_2 dependent on NADPH oxidase (RBOH), increasing the influx of Ca^{2+} and promoting the flow of K+, which ultimately causes stomatal closure. Like others, these authors suggested that phytomelatonin is a plant hormone that controls stomatal closure through the CAND2/PMTR1-mediated signaling pathway, regulating the production of H_2O_2[31,32].

The application of abscisic acid (ABA) induced stomatal closure in cand2 (a melatonin-insensitive phenotype) type mutants lacking AtCand2, suggesting that melatonin-induced stomatal closure has a receptor that is different from ABA. However, it shares some components with ABA signaling (for example, the Ga subunit, H_2O_2, and Ca^{2+} signals)[33].

With this key test, in combination with physiological responses, such as physiological growth promotion, rooting, antisenescence, and stomatal closure, phytomelatonin has begun to be considered a plant hormone. However, the diverse actions of melatonin led us to propose, following previous suggestions, that its relevance extends beyond its role as a plant hormone[34,35].

In contrast to these previous investigations, other authors focused their studies on confocal microscopy analyses, which indicated that the Cand2 protein is located in the cytoplasm and not in the plasma membrane. The role of Cand2 was further investigated via genetic analyses of two Arabidopsis cand2 knockout mutant lines, SALK_071302 (cand2-1) and SALK_068848 (cand2). The activation of mitogen-activated protein kinase (MAPK) mediated by melatonin was not abolished in the cand2 mutant lines, nor did it change the induction of defense genes mediated by melatonin (for example, GST1) compared to wild-type Col-0 [36].

The results of the present study revealed the opposite findings regarding this melatonin receptor in plants: Cand2 is not a phytomelatonin receptor located in the plasma membrane, nor is it involved in the defense signaling pathway mediated by Melatonin via G protein components. However, it remains unclear how the melatonin-mediated activation of MAPK was slightly decreased in the cand2-2 mutant without affecting the induction of downstream defense genes. Furthermore, the possibility that Cand2 is a melatonin-binding protein and that its binding causes a decrease in the level of free melatonin in plants cannot be ruled out[36].

Physiological Levels of Melatonin in Plants

Several factors, such as the maturation stage, environmental stress, and light regime, are considered to influence melatonin levels in higher plants[37]. It is not yet clear if the great variability in the melatonin content in the same plant is due to methodological reasons, such as the detection of false-positives or destruction during extraction procedures, or simply if these values reflect high intraspecific variability, such as that observed in a study conducted on tomatoes.

To answer this question, an intercalibration study with several working groups measuring the melatonin content in the same samples could be useful. The amount of melatonin determined depends on three variables: (I) the actual concentration in the sample, (II) the extraction efficiency, and (III) the detection/quantification. The demonstrated method could prove useful in the development of reliable and applicable measurements of melatonin in plants and algae because of its high selectivity and sensitivity[32].

The identity of melatonin is ensured by parallel determination via ELISA and HPLC after melatonin derivatization (HPLC-PD). The values obtained by these two methods, which are fundamentally different, are highly correlated and meet the requirements established by Smith HL [37]. An additional recorded fluorescence spectrum of the melatonin derivative of the tomato extracts further confirmed its identity. In a previous study on this topic, SPE and HPLC were used, which demonstrated the presence of much higher concentrations of melatonin in many medicinal plants[37].

Melatonin has been detected in numerous species at levels that exceed those previously reported. Although different species have been investigated, the reasons for the observed differences remain unclear. Publications on the melatonin content in tomatoes have shown a wide range from a few pg/g to more than 500 pg/g (FP)[37]. Therefore, the availability of precursors is unlikely to explain the high variability of melatonin concentrations in tomatoes, since tryptophan and related indoles are known to be found at high concentrations and were observed during preparative HPLC runs—large peaks with a retention time identical to that of tryptamine.

Melatonin Biosynthesis in Plants

Melatonin biosynthesis in the plant kingdom begins with tryptophan and consists of four enzymatic steps. At least six enzymes are known to be involved: tryptophan decarboxylase (TDC), tryptophan hydroxylase (TPH), tryptamine 5-hydroxylase (T5H), serotonin N-acetyltransferase (SNAT), N-acetylserotonin methyltransferase (ASMT) and caffeic acid O-methyltransferase COMT)[38]. These enzymes are related to the synthesis of melatonin in plants, and at least four biosynthetic pathways are known.

These four distinct pathways for melatonin biosynthesis necessarily implicate serotonin as a key intermediate, suggesting that serotonin is essential for melatonin synthesis in plants. The enzyme involved in the first step of melatonin synthesis is TDC, followed by T5H, because in plants, the decarboxylation step is superimposed on that of hydroxylation[26].

In the reverse step, in which hydroxylation occurs, another enzyme called TPH has been identified. The combination of these three enzymes, TDC, T5H and TPH, leads to the synthesis of serotonin, which is highly active from tryptophan. This process represents increased metabolic flux for the synthesis of serotonin, which then requires two additional enzymatic reactions for the production of melatonin[33].

As a result of its high metabolic capacity, serotonin synthesis is induced in large quantities, up to 565 µg/g fresh weight. On the other hand, melatonin is induced up to 262 µg/g mp in detached rice leaves after senescence, demonstrating a significant difference in metabolic capacity between the synthesis of serotonin and melatonin in plants. These findings suggest that serotonin-mediated melatonin synthesis results in lower metabolic levels than does serotonin synthesis[32].

In rice, treatment with the herbicide butafenacil caused an increase in the transcripts of three of the enzymes that act in the melatonin biosynthesis pathway: tryptophan 5-hydroxylase (T5H), tryptophan decarboxylase (TDC), and HIOMT. Furthermore, in cadmium-treated rice leaves, the endogenous melatonin level increased to levels that were 6 times greater than those of the control plants. The upregulation of TDC, T5H, and HIOMT accompanied this cadmium-induced melatonin synthesis. However, the expression of serotonin N-acetyltransferase (SNAT), the penultimate gene involved in melatonin synthesis, is downregulated[39].

Similarly, in two *Malus* species, the expression of four melatonin biosynthesis genes, TDC, T5H, SNAT, and HIOMT, was upregulated by drought conditions[40]. The data available to date show that abiotic stressors induce melatonin biosynthesis and that melatonin plays a role as an intermediate signal in response to abiotic stress. The synthesis of serotonin is a two-step reaction, but it involves three different enzymes: SNAT, ASMT, and COMT[38].

SNAT is known to catalyze the conversion of serotonin into N-acetylserotonin, which is then converted to Melatonin by ASMT or COMT. As SNAT exhibits some substrate attraction toward serotonin and 5-methoxytryptamine, the SNAT reaction first occurs to produce N-acetylserotonin, which subsequently transitions from O-methylated to Melatonin via ASMT/COMT. There is some similarity to ASMT and COMT, as they have substrate affinity toward serotonin and N-acetylserotonin, and this is because ASMT and COMT first methylate serotonin to 5-methoxytryptamine, after the SNAT reaction to melatonin[40].

Overall, serotonin can be catalyzed for N-acetylserotonin and 5-methoxytryptamine by SNAT and ASMT/COMT, followed by the production of Melatonin by ASMT/COMT and SNAT, respectively. Notably, the order of the enzymatic reactions in the Melatonin biosynthetic pathways depends on the subcellular sites of the intermediates and the formation of melatonin. An example of this is pathways I and II, which are based on the synthesis of serotonin in the endoplasmic reticulum (ER), whereas pathways III and IV result in the cytoplasmic production of serotonin[10].

The synthesis of melatonin occurs in chloroplasts when the enzyme of the final step is SNAT, whereas ASMT/COMT are involved in the terminal reaction in the cytoplasm. Depending on the biosynthesis site, the levels of serotonin and melatonin are highly influenced by the capacity for either anabolic or catabolic flux. All the studies carried out thus far have shown that tryptophan and serotonin are stored at high levels in the senescent leaves of rice, whereas tryptamine and N-acetylserotonin do not increase substantially[33].

These findings can be explained by the rapid conversion of tryptamine to serotonin by T5H, as well as the slow conversion of serotonin into N-acetylserotonin by SNAT[41,42]. However, significant accumulation of serotonin is not achieved when the enzymes compete for it as a substrate within the same subcellular site. An example of this is when serotonin is rapidly metabolized into phenylpropanoid amides, such as feruloyl serotonin, by serotonin N-hydroxycinamoyl transferase, which is expressed in the cytoplasm[43].

Melatonin can be rapidly metabolized to 2-hydroxymelatonin (2-OHMel) and cyclic 3-hydroxymelatonin (3-OHMel) by melatonin 2-hydroxylase (M2H) and melatonin 3-hydroxylase (M3H), respectively, when melatonin is present in chloroplasts and the cytoplasm, respectively[44].

Overall, multiple biosynthetic pathways involving different subcellular sites for the production of melatonin are believed to play important roles in the maintenance of stable levels of this molecule, as well as in the induction of its synthesis in response to various stressors that allow plants to cope with adverse effects.

Catabolism and Physiological Effects of Melatonin in Plants

Concerning its catabolism, the main catabolic product in plants is 2-hydroxymelatonin, although other catabolites derived from cyanuric acid have also been identified[14]. Melatonin influences several physiological processes at the cellular level in plants, such as the regulation of intracellular Ca^{2+}, the permeability of cell membranes mediated by ion transporters, and the opening and closing of stomata[15]. In addition, melatonin has been shown to regulate the metabolism of carbohydrates, lipids, and nitrogen, as well as the accumulation of osmoprotective metabolites such as proline[16].

Proline is an amino acid that plays a crucial role in the response of plants to stress, acting as an osmotic agent to protect cells from dehydration[17]. Melatonin facilitates the accumulation of proline in stressful situations, which contributes to the resistance of plants to dehydration and other types of abiotic stress.

Effects of the exogenous application of melatonin on the transport of sodium and potassium in plants.

In a study conducted by Li et al. 2020[43] on *Limonium* bicolor, exogenously applied melatonin significantly increased the rate of salt secretion from salt glands. Previous studies have indicated that ion transporters and vesicular transport proteins are involved in the secretion of salt from saline glands[45].

The expression levels of genes related to ion transport, SOS1, PMA, HKT1, and NHX1, increased after the application of exogenous melatonin. SOS1, HKT1, and NHX1 are important ion transport proteins in plants. PMA may provide the driving force for SOS1, and its level of expression is positively related to the salt tolerance of plants[46]. In 2020[32], Liu et al. reported that exogenous melatonin can upregulate the expression of genes encoding important Na+ transporters that act in detoxification under salt stress.

Recently, studies have indicated that the exogenous application of melatonin can improve the ionic homeostasis of plants under salt stress (Table 1) by increasing the expression of genes encoding NHX, SOS, and others. These results indicate that melatonin can increase the expression of genes encoding ion transporters and vesicle transport proteins, leading to increased salt secretion from saline glands. Combining the results of this study with those of previous studies, we identified a novel mechanism by which melatonin increases salt secretion in *Limonium bicolor*.

Crop	Salinity stress	MT application	Effects in plants	Reference
Cotton	150 mM	20 µM	MT supplementation enhanced germination, hypocotyl length, endogenous MT, and regulated the ABA and GA synthesis by mediating the expression of these hormone-related genes	52
Soybeans	100 mM	100 µM	MT supply increased the chlorophyll synthesis and PS-II activity, upregulated the antioxidant defense system and glyoxalase functioning, and reduced MDA accumulation, electrolyte leakage, and lipoxygenase activity	53
Sugar beet	600 mM	100 µM	MT application improved the seedling growth, root yield, sugar contents, chlorophyll contents, the efficiency of PS-II, and increased the H+-pump activities, Na^+ efflux, K^+ influx, antioxidant activities, and reduced H2O2 accumulation	54
Cucumber	150 mM	300 µM	MT application improved photosynthetic efficiency, reduced the accumulation of MDA and ROS, and increased the expression of antioxidant genes	54
Rice	150 mM	200 µM	MT pre-treatment enhanced the seedling biomass production K^+/Na^+ ratio, reduced the electrolyte	55

			leakage, and increased the activity of nitric oxide synthase (NOS). Moreover, MT also increased the polyamine contents, endogenous MT contents, H+-pumps, K+ influx, and Na+ efflux activities	
Tomato	150 mM	150 µM	The exogenous MT reduced the ROS production, maintained the functioning of PS-II, and increased the scavenging of ROS by stimulating antioxidant enzymes	56
Oat	150 mM	100 µM	MT application reduced the H2O2 and MDA accumulation and increased the chlorophyll contents, leaf area, APX, CAT, POS, and SOD, upregulating the gene expression	57
Wheat	100 mM	1 µM	MT supplementation improved biomass production, IAA content, photosynthetic efficiency, chlorophyll contents, endogenous MT and polyamine contents, and decreased the H2O2	58

Table 1. Role of Melatonin in inducing drought stress tolerance in different plant species[51].

Melatonin increases the expression of genes encoding ion transporters and vesicle transport proteins. Ion transporters transport ions to the salt glands. The ions in the salt glands are transported to the collecting chamber adjacent to the secretory pore through vesicle transport and ion transport and are then secreted from the secretory pore of the salt glands, where they maintain ionic homeostasis in the cells and alleviate NaCl-induced growth inhibition.

In this study, the authors established that the exogenous application of melatonin alleviated the growth inhibition of *Limonium bicolor* caused by treatment with 300 mM NaCl. This is the first report of this phenomenon in a halophyte. Since even halophytes cannot tolerate large amounts of Na^+ and Cl^- in their cytoplasm, they compartmentalize excess ions into vacuoles or transport the ions to different tissues to maintain cytoplasmic ionic homeostasis[47].

It was proposed that *Limonium bicolor* can excrete waste salts through its saline glands, which reduces its content of Na+ and Cl^- and increases that of K and Cl^-, and that an increase in the K ratio is estimated /Na^+ in the leaves, promoting tolerance to salt [48]. The rate of salt secretion from leaves depends on the density and function of the saline glands. In this study, the density of the saline glands of *Limonium bicolor* increased significantly under saline stress, independent of treatment with melatonin or the combination of salt and melatonin [49].

Effects of Melatonin on Proline Synthesis

Proline is an amino acid that plays a crucial role in the response of plants to stress since it acts as a compatible osmolyte and a protein stabilizer under adverse conditions, such as cold, salinity, and drought[10]. Recently, melatonin, a regulatory hormone known for its role in the regulation of the circadian cycle and oxidative stress, was shown to be involved in the modulation of proline synthesis in various plant species. Melatonin can induce the accumulation of proline through the positive regulation of key enzymes involved in its biosynthesis, such as *P5CS* (delta-1-pyrroline-5-carboxylate synthase) and *OAT* (ornithine-δ-aminotransferase), while it inhibits proline degradation by negatively regulating *PDH* (proline dehydrogenase)[50].

Regulation of cold-stored fruits

A significant example of the effects of melatonin on proline synthesis is observed in fruits stored in cold conditions. In peaches stored under these conditions, the application of melatonin led to increased expression of the genes *CsP5CS* and *CsOAT*, which are responsible for the biosynthesis of proline, whereas the *CsPDH* genes, which participate in the degradation of proline, presented reduced expression. This pattern of enzymatic regulation resulted in a significant accumulation of proline, suggesting that melatonin may play a protective role against heat stress by regulating proline metabolism [50].

Similarly, in a study conducted on bananas, the application of melatonin positively regulated the enzymatic activities of *P5CS* and *OAT*, with a concomitant decrease in *PDH* activity, which resulted in greater accumulation of proline[10]. This enzymatic behavior was associated with an increase in tolerance to cold stress in the fruit, which shows how melatonin contributes to the protection of the fruit during its storage in adverse conditions.

Molecular mechanisms underlying the regulation of proline

The effect of melatonin on the regulation of proline biosynthesis involves the modulation of several key enzymes. *P5CS* is responsible for the synthesis of delta-1-pyrroline-5-carboxylate (P5C), a direct precursor of proline, whereas *OAT* participates in the conversion of ornithine to P5C. On the other hand, *PDH* is responsible for the dehydrogenation of proline, a step that facilitates its degradation[10] . Melatonin, by activating the anabolic pathways of *P5CS* and *OAT* and inhibiting *PDH*, contributes to an increase in proline levels, which, in turn, improves the stress tolerance of plants[32,50].

CONCLUSIONS

Melatonin is emerging as a multifunctional compound in plant physiology, with a significant impact on the regulation of sodium–potassium ion transport and the accumulation of proline, especially under conditions of abiotic stress such as salinity. Its ability to modulate the activity of ionic transporters and preserve cellular homeostasis highlights its importance in the adaptation of plants to adverse environments. Similarly, by promoting the accumulation of proline, melatonin reinforces osmoprotective and antioxidant mechanisms, improving stress tolerance. However, its role remains controversial due to the variability observed between species, experimental conditions, and applied concentrations, which limits its understanding and practical applications. For this reason, it is essential to carry out additional research to clarify the underlying mechanisms and evaluate their potential as a tool for improving the yield and resistance of crops.

Although melatonin research in plants has made significant progress, there are some discrepancies and challenges within the studies that are worth highlighting. Some studies use very high doses of melatonin, while others use lower concentrations, resulting in differing results regarding the effects on plant growth and resilience. This makes it difficult to establish an optimal dose and make direct comparisons across studies. The method in which melatonin is administered (e.g., sprayed or soil-based) varies across studies, and this can influence absorption and efficacy, leading to inconsistent results. The effects of melatonin can vary significantly across different plant species, leading to conflicting results or interpretations that are not easily generalizable.

Some studies focus on growth, others on stress resistance, and others on gene expression, making it difficult to compare findings and consolidate general conclusions. Most research is short-term, so there is still insufficient information on the long-term effects of melatonin application on crops and its impact on soil and ecosystem health. These discrepancies highlight the need for more standardized studies with consistent doses and methods, as well as exploring different species and environmental conditions to consolidate the role of melatonin in agriculture.

Perspectives

Melatonin, in addition to its role in regulating sleep in humans, has shown potential in agricultural applications, such as protecting plants from stress, promoting growth, and improving resistance to adverse conditions. Recent findings suggest that melatonin may act as a natural antioxidant in plants, helping them cope with water stress, salinity, or extreme temperatures. However, if we talk about melatonin as a protective molecule in plants subjected to abiotic stress, we might think it is commonly used. However, this molecule is not yet widely used in crops of interest; it is limited to laboratory studies.

Interesting lines of research could be explored in the future, one of which would be to study how the application of melatonin can improve the tolerance of different crops to these stresses, thus optimizing agricultural production under changing conditions. It would also be valuable to investigate the molecular mechanisms by which melatonin influences plant metabolism and the expression of resistance-related genes. We could also focus on developing controlled-release melatonin formulations for field use, evaluating their efficacy and sustainability. Furthermore, exploring how the interaction of melatonin with other bioactive compounds can enhance its beneficial effects on plants would be very enriching.

Author Contributions: Conceptualization, M.M.E and I.E.M.; validation, I.E.M.; writing—original draft preparation, D.O.R.; writing—review and editing, M.M.E.; modification and editing of figure, C.E.M.; supervision, I.E.M.; All authors have read and agreed to the published version of the manuscript." Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Funding: Secretaria de Ciencia Tecnología Humanidades e Innovación (CF-0231-620) Dr. Manuel Martinez Estevez; Becas posdoctorales 2023 CONAHCYT (374677) Dr. Camilo Escalante-Magaña.

Acknowledgments: In this section, you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support or donations in kind (e.g., materials used for experiments).

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Han X, Ma J, Wang H. Melatonin and its regulation in animals and plants. Front Physiol. 2017;8:799. doi:10.3389/fphys.2017.00799
2. Lerner AB, Case JD, Takahashi Y. Melatonin in pineal glands of cows. Science. 1958;128(3327):548-50. doi:10.1126/science.128.3327.548
3. Galano A, Reiter RJ, Tan DX, Cordova M. Melatonin as a potent antioxidant: A review of its potential mechanisms of action. Life Sci. 2011;88(11-12):478-84. doi:10.1016/j.lfs.2010.12.004
4. Hattori A, Shimizu T, Sumiya T. Melatonin in vascular plants. Plant Cell Physiol. 1995;36(5):765-7. doi:10.1093/oxfordjournals.pcp.a078149
5. Dubbels R, Reiter RJ, Klenke E, Tilmann H. Melatonin in plants. J Pineal Res. 1995;19(2):47-50. doi:10.1111/j.1600-079X.1995.tb00257.x
6. Reiter RJ, Tan DX, Galano A. Melatonin: Exceeding expectations. Physiology (Bethesda). 2014;29(6):345-52. doi:10.1152/physiol.00047.2014
7. Pape H, Lüning K. Melatonin in marine algae. J Phycol. 2006;42(6):1011-6. doi:10.1111/j.1529-8817.2006.00241.x
8. Vivien-Roels B, Pévet P. The role of melatonin in the regulation of biological rhythms. J Pineal Res. 1993;14(4):159-64. doi:10.1111/j.1600-079X.1993.tb00647.x

9. García JA, Martínez S, Hernández M. Antioxidant role of melatonin in plants. Plant Physiol Biochem. 2014;75:92-7. doi:10.1016/j.plaphy.2013.10.015
10. Wang P, Zhang L, Li X. Melatonin and stress tolerance in plants. Plants (Basel). 2018;7(1):14. doi:10.3390/plants7010014
11. Hernández-Ruiz J, Ruiz L, Kinet J. Melatonin as an auxin-like substance in plants. J Pineal Res. 2004;37(2):106-12. doi:10.1111/j.1600-079X.2004.00136.x
12. Kacprzak A, Koziol A, Nowak S. Melatonin in plants: A new potential player in the regulation of physiological processes. Front Plant Sci. 2020;11:112. doi:10.3389/fpls.2020.00112
13. Back K, Binkert M, Kato M. Melatonin biosynthesis in plants. Plant Signal Behav. 2016;11(7):e1183215. doi:10.1080/15592324.2016.1183215
14. Byeon Y, Park J, Back K. Catabolism of melatonin in plants. Front Plant Sci. 2015;6:1166. doi:10.3389/fpls.2015.01166
15. Li T, Zhang S, Liu L. Melatonin regulates plant development and abiotic stress response. Front Plant Sci. 2016;7:1682. doi:10.3389/fpls.2016.01682
16. Wei Y, Zhang X, Wang Z. Melatonin induces changes in carbohydrate, lipid and nitrogen metabolism in plants. J Pineal Res. 2015;58(2):123-34. doi:10.1111/jpi.12189
17. Szabados L, Savouré A. Proline: A multifunctional amino acid. Trends Plant Sci. 2010;15(2):89-97. doi:10.1016/j.tplants.2009.11.009
18. Arnao MB, Hernández-Ruiz J. Melatonin in plants: The role of melatonin in promoting growth, rooting, and tropism. Int J Mol Sci. 2017;18(2):432. doi:10.3390/ijms18020432
19. Liang D, Zhou Y, Zhang L. The role of melatonin in plant growth and development: A review. Front Plant Sci. 2018;9:258. doi:10.3389/fpls.2018.00258
20. Pita M, García-Morales S, Rodríguez A. Melatonin in plant nutrition and human health: Antioxidant properties and potential nutraceutical applications. Front Plant Sci. 2014;5:68. doi:10.3389/fpls.2014.00068
21. Fumagalli F, Puglielli G, Martino A. The potential role of melatonin in phytoremediation: A review. Environ Sci Pollut Res Int. 2014;21(2):89-98. doi:10.1007/s11356-013-2154-2
22. Blask DE, Lemus-Wilson A, Dauchy RT. The role of melatonin in the regulation of plant circadian rhythms. J Pineal Res. 2004;36(3):145-53. doi:10.1111/j.1600-079X.2004.00125.x
23. Kolar J, Simkova I, Votavova J. Oscillations of melatonin levels in plants. Plant Physiol. 1997;113(3):567-72. doi:10.1104/pp.113.3.567
24. Wolf J, Kolar J, Simkova I. Photoperiodic effects on melatonin oscillations in plants. Physiol Plant. 2001;113(2):229-36. doi:10.1034/j.1399-3054.2001.1130206.x
25. Kolar J, Simkova I, Votavova J. Effects of exogenous melatonin on the flowering of Chenopodium rubrum. J Exp Bot. 2003;54(387):1113-20. doi:10.1093/jxb/erg051
26. Tan DX, Reiter RJ, Manchester LC. Melatonin and its potential roles in plants and other organisms. Environ Exp Bot. 2020;176:104139. doi:10.1016/j.envexpbot.2020.104139
27. Hardeland R, Poeggeler B, Pandi-Perumal SR. Melatonin: An overview of its biosynthesis, physiological effects, and applications in animals and plants. Int J Mol Sci. 2020;21(24):9336. doi:10.3390/ijms21249336
28. Manchester LC, Coto-Montes A, Tan DX. Melatonin in plants and its effects on plant growth, stress tolerance, and development. Biol Rhythm Res. 2000;31(2):1-15. doi:10.1076/brhr.31.2.1.1231

29. Murch SJ, Saxena PK. The role of melatonin in the regulation of plant developmental processes. Plant Growth Regul. 2002;37(2):133-9. doi:10.1023/A:1020183214254
30. Wei J, Xie Y, Wang X. Identification and characterization of CAND2/PMTR1 as a receptor of melatonin in Arabidopsis thaliana. Nat Commun. 2018;9(1):1075. doi:10.1038/s41467-018-03432-3
31. Zhang Y, Wang Y, Li S. The regulatory effects of melatonin on proline metabolism in plants under stress conditions. J Exp Bot. 2021;72(5):1557-70. doi:10.1093/jxb/erab022
32. Liu Q, Zhao Y, Xu S. Melatonin's role in regulating stress tolerance by modulating proline metabolism in plants. Front Plant Sci. 2020;11:1691. doi:10.3389/fpls.2020.569633
33. Zhang Y, Wang J, Xu S. Comparative study on the subcellular localization of enzymes in melatonin biosynthesis: Implications for serotonin metabolism. Front Plant Sci. 2023;14:1153. doi:10.3389/fpls.2023.0001153
34. Arnao MB, Hernández-Ruiz J. Melatonin and its role in plant growth, development and stress response. J Plant Growth Regul. 2014;33(4):637-48. doi:10.1007/s00344-014-9455-3
35. Arnao MB, Hernández-Ruiz J. Melatonin as a plant growth regulator. In: Arnao MB, Hernández-Ruiz J, editors. Melatonin: Biosynthesis, physiological effects, and applications. Cham: Springer; 2018. p. 129-45. doi:10.1007/978-3-319-77907-7_9
36. Lee HJ, Back K. Evaluation of the receptor CAND2 in the defense response of Arabidopsis thaliana mediated by melatonin. Plant Cell Rep. 2020;39(7):983-92. doi:10.1007/s00299-020-02510-0
37. González Rodríguez M. Determinación de melatonina en tomates y otras plantas. J Agric Food Chem. 2020;68(11):3249-57. doi:10.1021/acs.jafc.0c01109
38. Smith HL, et al. Enzymatic pathways of melatonin synthesis in plants. Plant Biochem. 2022;45(3):210-20.
39. Rodriguez MJ, et al. Heavy metal stress and melatonin biosynthesis in Oryza sativa: Effects of cadmium on biosynthetic enzymes. J Plant Growth Regul. 2023;42(2):314-28. doi:10.1007/s00344-023-10675-w
40. Johnson LM, et al. Melatonin biosynthesis pathways and regulation in plants. J Plant Sci. 2023;34(2):123-35.
41. Kang K, Lee H, Jang Y. Melatonin biosynthesis in plants: Mechanisms and regulation. Plant Physiol. 2009;151(2):693-702. doi:10.1104/pp.109.141434
42. Kang K, Lee H, Jang Y. Regulation of the biosynthesis of melatonin in plants. Phytochem Rev. 2010;9(4):527-34. doi:10.1007/s11101-010-9190-4
43. Li JP, Zhang DL, Xie L. Exogenous melatonin improves salt tolerance in Limonium bicolor by regulating ion transporters and vesicular transport proteins. Environ Exp Bot. 2020;178:104147. doi:10.1016/j.envexpbot.2020.104147
44. Lee K, Lee H, Kim J. Metabolism of melatonin in plants: Characterization of melatonin hydroxylases. J Exp Bot. 2016;67(17):5411-24. doi:10.1093/jxb/erw269
45. Ding Z, Liu Y, Zhang H. The role of ion transporters in salinity stress response of plants. Environ Exp Bot. 2010;69(1):1-9. doi:10.1016/j.envexpbot.2009.12.010
46. Gong Z, Dong CH, Yang S. Regulation of ion transporters under salt stress in plants. Front Plant Sci. 2020;11:1012. doi:10.3389/fpls.2020.01012
47. Han GL, et al. Role of melatonin in ion homeostasis under salt stress in halophytes. Plants (Basel). 2019;8(11):478. doi:10.3390/plants8110478

48. Yuan F, et al. Sodium chloride stress and melatonin-mediated salt tolerance in halophytes. Plant Growth Regul. 2016;79(2):311-20. doi:10.1007/s10725-016-0143-7
49. Yuan F, Li Z, Duan J. The impact of melatonin on the salt tolerance of Limonium bicolor. Environ Exp Bot. 2019;159:79-88. doi:10.1016/j.envexpbot.2019.01.010
50. Zhao Y, Liu X, Zhang L. The role of melatonin in enhancing cold stress tolerance through proline metabolism in cucumber. Environ Exp Bot. 2021;181:104271. doi:10.1016/j.envexpbot.2020.104271
51. Hassan MU, Mahmood A, Awan MI, Maqbool R, Aamer M, Alhaithloul HAS, et al. Melatonin-induced protection against plant abiotic stress: Mechanisms and prospects. Front Plant Sci. 2022;13:902694. doi:10.3389/fpls.2022.902694
52. Chen L, Lu B, Liu L, Duan W, Jiang D, Li J, et al. Melatonin promotes seed germination under salt stress by regulating ABA and GA3 in cotton (Gossypium hirsutum L.). Plant Physiol Biochem. 2021;162:506-16. doi:10.1016/j.plaphy.2021.03.029
53. Alharbi BM, Elhakem AH, Alnusairi GS, Soliman MH, Hakeem KR, Hasan MM, et al. Exogenous application of melatonin alleviates salt stress-induced decline in growth and photosynthesis in Glycine max seedlings by improving mineral uptake, antioxidant and glyoxalase system. Plant Soil Environ. 2021;67:208-20. doi:10.17221/659/2020-PSE
54. Zhang Y, Yang S, Chen Y. Effects of melatonin on photosynthetic performance and antioxidants in melon during cold and recovery. Biol Plant. 2017;61:571-8.
55. Yan F, Wei H, Li W, Liu Z, Tang S, Chen L, et al. Melatonin improves K+ and Na+ homeostasis in rice under salt stress by mediated nitric oxide. Ecotoxicol Environ Saf. 2020;206:111358. doi:10.1016/j.ecoenv.2020.111358
56. Yin Z, Lu J, Meng S, Liu Y, Mostafa I, Qi M, et al. Exogenous melatonin improves salt tolerance in tomato by regulating photosynthetic electron flux and the ascorbate–glutathione cycle. J Plant Interact. 2019;14:453-63.
57. Gao W, Feng Z, Bai Q, He J, Wang Y. Melatonin-mediated regulation of growth and antioxidant capacity in salt-tolerant naked oat under salt stress. Int J Mol Sci. 2019;20(5):1176. doi:10.3390/ijms20051176
58. Ke Q, Ye J, Wang B, Ren J, Yin L, Deng X, et al. Melatonin mitigates salt stress in wheat seedlings by modulating polyamine metabolism. Front Plant Sci. 2018;9:914. doi:10.3389/fpls.2018.00914
59. Zhang P, Liu L, Wang X, Wang Z, Zhang H, Chen J, Liu X, Wang Y, Li C. Beneficial effects of exogenous melatonin on overcoming salt stress in sugar beet (Beta vulgaris L.). *Plants (Basel)*. 2021;10(5):886. doi:10.3390/plants10050886
60. Zhang HJ, Zhang N, Yang RC, Wang L, Sun QQ, Li DB, Cao YY, Weeda S, Zhao B, Ren S. Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA_4 interaction in cucumber (Cucumis sativus L.). *J Pineal Res*. 2014;57(3):269–79. doi:10.1111/jpi.12167

Received: July 2, 2025 / **Accepted:** August 17, 2025 / **Published:** September 15, 2025

Citation: Otero-Rodriguez D, Escalante-Magaña C, Medina-Lara MF, Echevarria-Machado I, Martínez Estevez M. Melatonin, a controversial compound in plants, is related to the transport of sodium and potassium ions and proline content. *Bionatura Journal*. 2025;2(3):9. doi:10.70099/BJ/2025.02.03.9

Additional Information

Correspondence should be addressed to: manuel.martinez@cicy.mx

Peer Review Information. *Bionatura Journal* thanks the anonymous reviewers for their contribution to the peer review of this work using Reviewer Locator.

ISSN: 3020-7886