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Review

Phytomelatonin: An Emerging Regulator of Plant Biotic Stress Resistance

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Melatonin has diverse functions in plant development and stress tolerance, with recent evidence showing a beneficial role in plant biotic stress tolerance. It has been hypothesized that pathogenic invasion causes the immediate generation of melatonin, reactive oxygen species (ROS), and reactive nitrogen species (RNS), with these being mutually dependent, forming the integrative melatonin–ROS–RNS feedforward loop. Here we discuss how the loop, possibly located in the mitochondria and chloroplasts, maximizes disease resistance in the early pathogen ingress stage, providing on-site protection. We also review how melatonin interacts with phytohormone signaling pathways to mediate defense responses and discuss the evolutionary context from the beginnings of the melatonin receptor–mitogen-activated protein kinase (MAPK) cascade in unicellular green algae, followed by the occurrence of phytohormone pathways in land plants.

Highlights

Melatonin enhances the biotic stress tolerance against pathogen attack.

Melatonin modulates melatonin receptor–mitogen-activated protein kinase cascades, typical phytohormone signaling pathways to contribute to the direct defense response.

Melatonin in Plants: A Multiregulatory Molecule

Melatonin (*N*-acetyl-5-methoxytryptamine) is a ubiquitously distributed molecule that exists in all kingdoms including plant species [1]. The term 'phytomelatonin' was first introduced in 2004. The compound is related to diverse physiological actions, including seed germination, growth, rooting, photosynthesis, and protection against stresses [2] and is recognized as a multiregulatory molecule with a possible role as a plant master regulator [2]. That phytomelatonin is a protective agent against various stresses is widely accepted and the means of conferring abiotic tolerance by melatonin are well documented [2–7]; however, its effects on biotic tolerance have been much less frequently studied and have not been summarized comprehensively [8]. Therefore, we here review the role of melatonin in plant biotic stress tolerance, explore the mechanisms, and consider the role of melatonin in the context of evolution and the potential that this powerful molecule has in controlling plant diseases.

Melatonin Enhances Biotic Stress Tolerance

Melatonin application substantially enhances biotic tolerance in plants (Table 1). Exogenous melatonin treatment plays a role against fungal pathogen infection. Supplemental melatonin at 0.05–0.5 mM improved resistance against Marssonina apple blotch caused by *Diplocarpon mali* [9]. Melatonin also attenuates fungal infection in potato [10], cotton [11], and *Lupinus albus* [6]. A range of melatonin concentrations inhibits the growth of fungal pathogens including *Botrytis*, *Alternaria*, and *Fusarium* spp. [6]. In general, melatonin helps plants to resist fungal infection, reduce lesions, and inhibit pathogen expansion and alleviates disease damage.

Arabidopsis (*Arabidopsis thaliana*)/*Pseudomonas syringae* pv. tomato DC3000 (*Pst* DC3000) is the most widely used model in plant–bacterial pathogen interaction studies [12]. Pretreatment with melatonin enhanced disease defense against *Pst* DC3000 in arabidopsis and tobacco (*Nicotiana tabacum*) [13,14]. A marked reduction in endogenous melatonin levels due to the inactivation of serotonin *N*-acetyltransferase, the enzyme that controls melatonin production, led to increased susceptibility to *Pst* DC3000 [15]. Phytomelatonin, therefore, confers plant bacterial tolerance.

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Table 1. Examples of the Effects of Melatonin on Plant Biotic Tolerance

Plant species	Pathogen	Melatonin level	Main effect	Refs
Rice (*Oryza sativa* L.)	–	Melatonin-rich transgenic rice	Increase disease resistance protein	[20]
Arabidopsis (*Arabidopsis thaliana* L.)	–	Exogenous treatments (1.0 mM)	Activate disease resistance protein (TIR-NBS class)	[21]
Switchgrass (*Panicum virgatum* L.)	–	Melatonin-rich transgenic plants	Activate MAPK signaling pathway and a resistance protein guards the plant against pathogens	[19]
Apple [*Malus prunifolia* (Willd.) Borkh. cv. Donghongguo]	Marssonina apple blotch	Exogenous treatments (0.05, 0.1, or 0.5 mM)	Increase bacterial tolerance; activate plant defense-related enzymes	[9]
Lupin (*Lupinus albus* L.)	*Penicillium* spp.	Exogenous treatments (20.0 μM, 70.0 μM)	Improve resistance against pathogens	[6]
Potato (*Solanum tuberosum* L.)	*Phytophthora infestans*	Exogenous treatments (6.0 mM, 10.0 mM)	Attenuate potato late blight; various metabolic processes in *P. infestans* are dramatically suppressed	[10]
Cassava (*Manihot esculenta* Crantz)	Cassava bacterial blight	Downregulation of melatonin biosynthesis	Decrease disease resistance	[54]
Cotton (*Gossypium* spp.)	*Verticillium dahliae*	Exogenous treatments (10.0 μM)	Enhance cotton resistance to *V. dahlia*; promote lignin and gossypol accumulation	[11]
Arabidopsis and tobacco (*Nicotiana tabacum* L.)	*Pseudomonas syringae* DC3000	Exogenous treatments (10.0 μM)	Suppress bacterial propagation; induce various PR genes and defense genes	[13]
Arabidopsis	*P. syringae* DC3000	Exogenous treatments (20.0 μM)	Increase biotic tolerance; upregulate PR genes and *NPR1*	[14]
Arabidopsis	*P. syringae* DC3000	Melatonin biosynthesis mutant	Decrease resistance to infection; reduce SA levels; decrease induction of defense genes	[13]
Arabidopsis and tobacco	*P. syringae* DC3000	Exogenous treatments (1.0 μM)	Increase bacterial tolerance; activate MAPK signaling cascades	[41]
Tomato (*Solanum lycopersicum* L.) and tobacco	Tobacco mosaic virus	Exogenous treatments (100.0 μM)	Improve resistance; increase expression levels of PR1 and PR5	[17]
Rice	RSV	Exogenous treatments (10.0 μM)	Lead to lower disease incidences increase expression of *OsPR1b* and *OsWRKY45*	[18]

Glossary

Horizontal gene transfer (HGT): the direct movement of genetic information between different species without reproduction, a process between prokaryotes and eukaryotes, and between the DNA-containing organelles including the nucleus, mitochondrion, and chloroplast of eukaryotes.
Hypersensitive response (HR): rapid and localized cell death evoked via pathogen attack in higher plants. It is not a disease syndrome but an efficient, host-regulated defense response conferring the neutralization of intruding pathogens.
Melatonin–ROS–RNS loop: under biotic stress conditions, melatonin, ROS, and RNS promote the production of each other, forming the melatonin–ROS–RNS feedforward loop.
Mitogen-activated protein kinase (MAPK): a broad range of highly conserved serine-threonine kinases regulating various cellular roles in the regulation of gene expression, cellular growth, and stress responses.
Programmed cell death (PCD): a type of normal cell suicide via specialized and genetically regulated cellular machinery to kill itself.
Reactive nitrogen species (RNS): several NO-derived compounds from NO· and $O_2^{\cdot-}$.
Reactive oxygen species (ROS): the derivatives of molecular oxygen produced as an attribute of aerobic life.
Redox homeostasis: the dynamic equilibrium between ROS and RNS formation and removal to maintain the optimum physiological redox steady state. It is essential for the response to stressful challenges.
Systemic acquired resistance (SAR): a distinct signal transduction pathway that plays an important role in long-lasting protection against a broad spectrum of microorganisms. SAR is closely related to the accumulation of pathogenesis-related proteins, contributing to plant resistance.

In contrast to diseases caused by bacteria and fungi, viral diseases are more difficult to control once an infection develops [16]. The first investigation related to melatonin conferring viral tolerance showed that exogenous application of melatonin significantly reduced virus infection in *Nicotiana glutinosa* [17]. Moreover, melatonin pretreatment also led to lower disease incidences in rice (*Oryza sativa*) [18]. Melatonin-mediated plant resistance to viruses provides an important new means to potentially control plant viral diseases.

Recently, the analysis of gene expression has provided further evidence for the involvement of melatonin in modulating biotic stress resistance. The application of melatonin to plants or over-expression of melatonin biosynthesis genes contributes to plant-pathogen tolerance by inducing pathogenesis-resistance (PR) genes (*PR1*, *PR5*, *NPR1*, and *PDF1.2*) and activating disease

resistance proteins [TIR-NBS class, **MAPKs** (see Glossary)]; these findings show that melatonin may be a defensive agent in plants against pathogens [9,13,19–21].

Besides pathogens, insects also cause huge losses and substantially reduce crop yields [22]. Plant secondary metabolites serve as juvenile hormone antagonists against insects and are used to kill the insects [23]. Dopamine (a catecholamine), with a skeleton similar to that of melatonin, functions as an antiherbivore defense in *Ulvaria obscura* [24]. Melatonin (an indoleamine) thus may also work in defense against insect attack [25].

Melatonin–ROS–RNS Feedforward Loop Conferring Plant-Pathogen Tolerance at the Early Infection Stage

Under stressful conditions, plant cellular responses are primarily initiated by the interaction of the extracellular signal with plasma membrane receptors, altering **redox homeostasis** and resulting in excessive production of **ROS** and **RNS**, with the major reactants including hydrogen peroxide (H_2O_2), the superoxide anion ($O_2\cdot^-$), nitric oxide (NO·), and the alkoxyl radical (RO·), etc. [2,4,26,27]. ROS and RNS display dual functions in plant cells. At lower levels, they primarily work as secondary messengers to maintain redox homeostasis and are a requisite for growth and function as secondary messengers [4,28]. At higher endogenous levels, ROS and RNS are toxic and harmful to the cells due to their radical and/or highly oxidative nature [27]. If the additional oxidative stress is not counteracted, it can lead to irreversible and seriously oxidative effects and, finally, to cell death [27,29].

Stress conditions are usually accompanied by an oxidative burst. However, there are significant differences in how an excess of ROS and RNS influences plant stress tolerance under various abiotic and biotic stress conditions. During abiotic stress, the oxidative burst results in severe damage to proteins, lipids, and even nucleic acids at the whole-plant level [4,27]. These severe consequences can be avoided in part due to the existence of a set of antioxidant agents (metabolites and enzymes) that protect cells during the antistress response; they neutralize and eliminate ROS and RNS overproduction [27]. By contrast, when recognizing pathogen ingress, plants initiate a resistance response aimed at interrupting pathogen growth and disease development, including activation of local and systemic defenses and the induction of a localized plant **programmed cell death (PCD)** at the site of infection; this is referred to as the **hypersensitive response (HR)** [30,31]. The ROS burst, which mainly occurs in chloroplasts and mitochondria, is necessary for the induction of resistance responses [27,32–36]; that is, the activation of local and systemic defenses and PCD [4]. Compared with abiotic stress, ROS scavenging enzymes are initially downregulated when a pathogen attacks, in agreement with what is observed for alternative oxidases, which correlate with increased ROS levels initiating PCD to inhibit pathogen spread [34,37]. Thus, the oxidative burst has been proposed as a main, ubiquitous plant response to pathogen attack following successful recognition [14].

RNS and ROS signaling pathways in plant biotic interactions are closely connected [36]. Both modulate the upregulation of downstream genes related to plant stress responses in primary metabolism or phytohormonal signaling [38]. Generally, these signals act together and maintain their respective networks in a coordinated manner in response to disease intrusion (Figure 1).

Biotic stresses usually increase endogenous melatonin, ROS, and RNS simultaneously (Figure 1) [15,39]. In biotic adversities, the effective co-interaction of a complex redox network among ROS, RNS, and melatonin becomes obvious [1]. *Pst* DC3000 invasion caused a significant elevation of endogenous melatonin (two- to fourfold after 24 h) and NO· (two- to threefold from 3 to 24 h) in arabidopsis [14]. Rice stripe virus (RSV) ingress enhanced the content of melatonin and RNS in

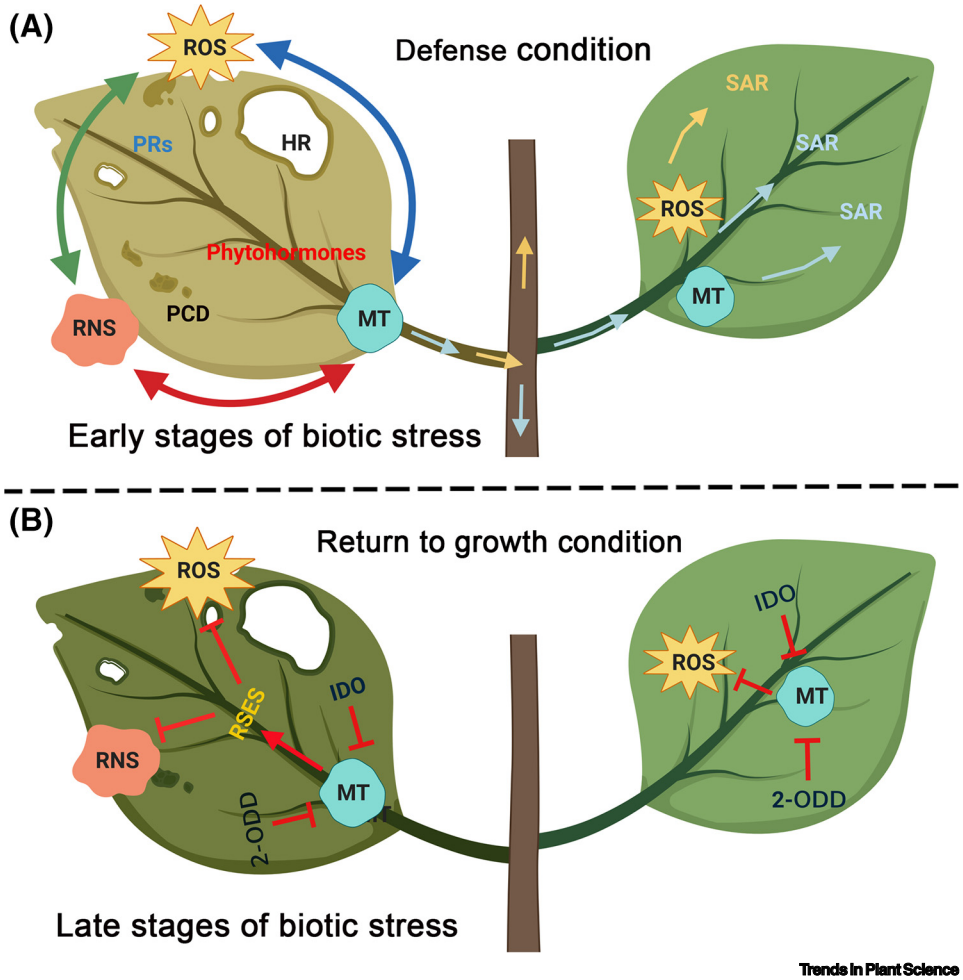

Figure 1. Interaction between Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), and Melatonin Modulating the Balance between Defense against Biotic Stresses and Growth. (A) In the early stages of biotic stress, melatonin, ROS, and RNS promote the production of each other, forming the melatonin–ROS–RNS feedforward loop, conferring on the plant biotic stress resistance. (B) Over the course of biotic stresses, the accumulated melatonin suppresses ROS and RNS overproduction and an excessive resistance response. See the main text for additional details. Abbreviations: PR, pathogenesis-resistance gene; HR, hypersensitive response; PCD, programmed cell death; MT, melatonin; SAR, systemic acquired resistance; RSES, radical scavenger enzyme system; 2-ODD, 2-oxoglutarate-dependent dioxygenase; IDO, indoleamine-2,3-dioxygenase. This figure was created using BioRender (https://biorender.com/).

rice as well [18]. Thus, the increase in endogenous melatonin, ROS, and RNS is considered a self-regulating network, with the components directly interacting with each other and via regulation of their biosynthesis and catabolic genes.

Melatonin initially increases the production of NO· and ROS under biotic stress (Figure 1). Relatively higher concentrations of phytomelatonin result in the production of RNS and ROS, which in turn confer plant tolerance against biotic stressful environments [40]. The application of melatonin enhances the generation of NO· or H_2O_2 in plants infected with the bacterial pathogen *Pst* DC3000 [13,41,42]. This is further evident from the fact that melatonin enhanced ROS production in apple tissues infected with the fungus *D. mali* [9] and in stored fresh strawberry fruits with fungal infection [43]. By inhibiting *S*-nitrosylation activity, phytomelatonin efficiently

stimulates NADPH oxidase to induce H_2O_2 levels in tomato (*Solanum lycopersicum*) [44]. Melatonin also induces gene expression in the NO· biosynthesis pathway [e.g., nitric oxide synthase (NOS), nitrate reductase (NR)] [14,17,45,46] and increases the levels of a radical species of nitrogen [27,47]. Melatonin also interacts with nitrogen-containing radicals to form *N*-nitrosomelatonin [47], which further enhances the NO· generation [1]. The melatonin receptor (i.e., CAND2/PMTR1) interacts with G protein α subunits, which stimulates AtNOS1/AtNOA1 and thus mediates the generation of NO·, as well as promoting NOS-like molecules by upregulating RbOH in arabidopsis [48].

By contrast, melatonin treatment reduces ROS production under abiotic stresses and normal conditions [49–51]. Clearly, under abiotic stress conditions, melatonin may play a role in clearing the excessive ROS and RNS, whereas it seems to stimulate the production of ROS and RNS to promote cell death and halt pathogen intrusion under biotic stress conditions. This response mechanism of melatonin may be related to the differences in plant sensing of and response to biotic and abiotic stresses. Studies have shown that different stresses induce different chemical signals in individual plant cells [52]. Therefore, melatonin may interact with different chemical signals to regulate ROS and RNS production positively or negatively under different stressful conditions. Compared with abiotic stress, intruding microorganisms release specific effectors [53]; these proteins are sensed by melatonin, possibly mediating the burst of ROS and RNS. Regardless, the exact mechanisms of how melatonin exerts different responses under different conditions remain unclear.

Second, ROS and RNS promote the production of phytomelatonin (Figure 1). Melatonin induction in arabidopsis after infection with *Pst* DC3000 was independent of H_2O_2 and NO individually, but dependent on the combination of H_2O_2 and NO [41]. Also, increased ROS and RNS levels lead to the upregulation of melatonin biosynthesis genes resulting in elevated melatonin levels in stressed plants [2,6,12,25,27,54,55].

Third, the reaction of NO· with $O_2\cdot^-$ is apparent under pathological conditions [2]. NO·, by interacting with respiratory complex III, inhibits electron transfer, and increases $O_2\cdot^-$ formation [56] or positively regulates the ROS network through NO·- dependent post-translational modification [12]. Likewise, this occurs in the relationship between ROS and RNS.

Thus, during biotic stress melatonin increases endogenous ROS and RNS levels, and, curiously, ROS and RNS also enhanced melatonin levels, and ROS and RNS interact with each other [12,27]. Given the complexity of the mechanisms that may be operative in the redox network, it is difficult to place each element in a series of linear events [27]. Here, we propose that when plants are infected with pathogens, melatonin, ROS, and RNS each promotes the production of the others, forming the melatonin–ROS–RNS feedforward loop.

The burst of melatonin, ROS, and RNS contributes to rapid plant biotic tolerance. NO· is an excellent candidate to serve as a signaling messenger acting over short distances (organelle and cell level) in response to local pathogen invasion, while melatonin can be transported easily via the xylem from roots to leaves and other organs [57]. Due to its stability and amphiphilic nature, melatonin is a suitable candidate to act as a long-distance message transmitter, inducing systemic disease resistance in the entire plant [27]. Furthermore, ROS is also considered a suitable candidate for traveling long distances [58]. The combination of RNS, ROS, and melatonin spreads the biotic stressful signal from the site of initiation (root, stem, or leaf) to the entire plant and confers on the plant biotic tolerance at the early stage of infection.

Melatonin–ROS–RNS Feedforward Loop Recovers to a Normal Level at the Late Infection Stage

After stimulation of plant immunity, an excess of ROS and RNS must be effectively cleared, contributing to redox homeostasis, avoiding potential damage to cellular components, and maintaining follow-up growth [27,59,60]. The balance between ROS and RNS, and the balance between these chemical species and melatonin, determine the equilibrium or homeostasis of the redox network [27]. The equilibrium is generally sustained through enzymatic and nonenzymatic antioxidants [60,61]. A major function of melatonin is to serve as an antioxidant to act against the extra production of radical species, with two major chemical mechanisms comprising single-electron transfer and hydrogen transfer in organisms [27,62–65]. Due to its indirect actions, melatonin activates the gene expression of antioxidant enzymes such as SOD, catalases, and others (Figure 1) [2,27,65]. The melatonin derivatives also have been reported as positive ROS and RNS scavengers, including *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK), cyclic-3-hydroxymelatonin (c3OHM), and *N*1-acetyl-5-methoxykynuramine (AMK), all of which have high efficiency as scavengers of ·OH, and particularly ·OOH in the case of c3OHM [5,63]. Via these multiple means, melatonin alleviates oxidative damage in plants.

For melatonin *per se*, the increased melatonin at the early infection attains normal concentrations via metabolic genes such as indoleamine 2,3-dioxygenase (IDO) or 2-oxoglutarate-dependent dioxygenase (2-ODD) [5,66,67]. At the end, plant cells recover from the condition of the burst of ROS, RNS, and melatonin.

Melatonin Regulates Biotic Stress by Crosstalk with Plant Disease-Resistance Signaling Pathways

The regulatory roles of H_2O_2, NO·, and melatonin have been reported as upstream signaling molecules in plant innate immunity (Figure 2) [1,68]. Pathogen invasion leads to the activation of the **melatonin–ROS–RNS loop**, conferring the plant disease resistance. The role of ROS and RNS in pathogen tolerance has been fully illustrated; however, how melatonin responds to intrusion at the molecular level has not been summarized.

MAPK cascades are the most well-studied signal transduction modules that play pivotal roles against pathogen attack in the plant [69]. On sensing a pathogen infection, MAPKKK is phosphorylated and phosphorylates MAPKK, which in turn phosphorylates and activates MAPKs [69,70]. Active MAPKs subsequently phosphorylate downstream components and trigger defense responses [69,70]. MAPK cascades are documented to be responsible for triggering the melatonin-induced defense mechanisms [41,42]. Further biochemical and genetic analysis showed that melatonin-mediated resistance is triggered by MAPK signaling via OXI1/MAPKKK3–MAKK4/5/7/9–MAPK3/6 cascades [41,42]. Although many MAPK components are activated by melatonin, the direct link between melatonin and the MAPK cascades remains unknown. The first identification of a plant melatonin receptor, CAND2/PMTR1, suggests that the MAPK cascade activation may be mediated by the CAND2/PMTR1-melatonin complex (Figure 2) [48], forming the CAND2/PMTR1–MAPK cascade pathway conferring biotic stress resistance.

Phytohormones and their metabolites are core regulators of plant defense [71,72]. The intricate network of phytohormone signaling pathways ensures that plants activate the necessary defense responses against pathogens, and balance plant defense and growth [71,73,74]. The salicylic acid (SA) pathway is another important signaling pathway that mediates host responses against microbial pathogens [75]. Genetic interactions between melatonin and SA have been widely documented during pathogen infection (Figure 2) [13,15,41,42,47,76,77]. For example, the

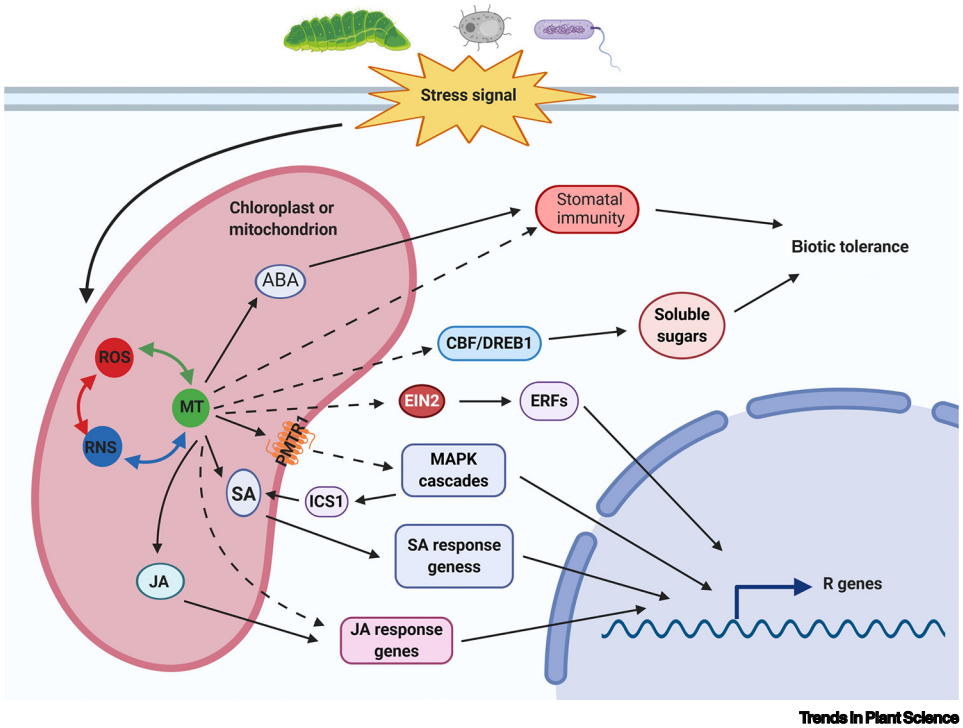

Figure 2. Current Linkages between Melatonin and Plant Disease-Resistance Signaling Pathways. After sensing the biotic stress signal, plant cells rapidly produce large amounts of melatonin in mitochondria and chloroplasts, inducing the melatonin–reactive oxygen species (ROS)–reactive nitrogen species (RNS) feedforward loop. Then, melatonin interacts with other disease-resistance signaling pathways including phytohormones and CAND2/PMTR1–mitogen-activated protein kinase (MAPK) cascade pathways to modulate the biotic stress responses. Unbroken lines represent direct interactions and broken lines indicate indirect interactions. See main text for additional details. Abbreviations: MT, melatonin; JA, jasmonic acid; SA, salicylic acid; ABA, abscisic acid; ET, ethylene; EIN2, ethylene insensitive 2; R genes, resistance genes; PMTR1, phytomelatonin receptor 1; ICS1, isochorismate synthase 1; CBF/DREB1, C-repeat-binding factor/dehydration responsive element-binding factor 1; ERF, ethylene response factor. This figure was created using BioRender (https://biorender.com/).

application of melatonin enhances the synthesis of SA in plants infected with pathogens [13,17,41]. Several pathogen-responsive genes induced by SA, such as *PR1*, *PR5*, *ICS1*, and *PDF1.2*, are upregulated in response to melatonin treatment [14,15,17]. Moreover, the *snat* mutant, a melatonin biosynthesis mutant, exhibits reduced SA levels compared with the wild type [15]. Furthermore, melatonin is reported to promote the synthesis of SA through the MAPK cascades [41].

Ethylene (ET) also plays an essential role in regulating plant disease resistance [78]. Although both melatonin and ET are correlated with plant defense responses, the crosstalk between melatonin and ET signaling has not been well studied. Weeda *et al.* showed that most of the identified genes in the ET pathway were upregulated in response to melatonin treatment in arabidopsis [21]. Similar responses were also observed in melatonin-treated watermelon [79]. Furthermore, melatonin-induced pathogen-responsive genes were partially suppressed in a knockout arabidopsis mutant of ethylene insensitive 2 (*ein2*) [13], the key regulatory component of the ET signaling pathway [13,42]. The *RAV* genes which encode ET response factors (ERFs), were shown to be essential for resistance against cassava bacterial blight through the induced expression of genes associated with melatonin biosynthesis [54]. Thus, ET signaling may function synergistically with melatonin to modulate the defense-related genes and disease resistance (Figure 2).

In addition to these classic plant disease-resistance pathways, several other plant hormones or signals are involved in melatonin-mediated plant basal defense responses as well. For example, melatonin application induced the accumulation of jasmonic acid (JA) and glycerol and, ultimately, a rise in pathogen-related gene expression [21,51,77,80]. In plants, the increased production of melatonin enhanced transcript levels of *CBF/DREB1*s and soluble sugar accumulation, such as sucrose, resulting in improved resistance to the bacterial pathogen [14]. Li *et al.* reported that melatonin-mediated resistance to *Verticillium dahliae* in cotton involves the promoted antifungal activity of gossypol and lignin biosynthesis [11].

Stomata constitute a major port for pathogen entry [81]. On pathogen infection, plants synthesize abscisic acid (ABA) and initiate defense mechanisms, including the expression of defense-related genes and regulation of the stomatal aperture conferring resistance to the pathogen [82,83]. Recently, melatonin was found to mediate ABA metabolism and stomatal behavior in *Malus prunifolia* and drought-sensitive *Malus hupehensis* [81]. Thus, melatonin promotes stomatal closure and actively blocks pathogen invasion by inducing ABA signaling. Furthermore, Wei *et al.* showed that melatonin governs receptor-dependent stomatal closure [48], indicating that melatonin also directly regulates stomatal immunity through an ABA-independent mechanism (Figure 2).

Similar to phytomelatonin, auxin is an indole derivative, and they share the same biosynthetic precursor, tryptophan [84]. Like melatonin, auxin is also involved in biotic stress signaling pathways. For example, several auxin-responsive and auxin-related genes respond to biotic stress [85]. In biotic stress, auxin acts as the positive regulator of JA/ET-mediated resistance and antagonists of SA-mediated resistance [86]. Together, the indole derivatives auxin and melatonin potentially modulate biotic stress by crosstalk with other phytohormones.

Melatonin–ROS–RNS Feedforward Loop and Melatonin-Related Biotic Stress Pathways Located at Chloroplasts and Mitochondria

ROS and RNS are mainly produced in mitochondria and chloroplasts under biotic conditions [32–34]. In agreement with the distribution of ROS and RNS, both of these organelles are believed to generate melatonin [87–90]. Chloroplasts are the sources of signaling molecules in plant biotic interactions, including the phytohormones SA, JA, and ABA [32,91,92]. Systemic signal crosstalk with melatonin further induces defense reactions at sites away from the primary infection, resulting in the following **systemic acquired resistance (SAR)** [32]. Chloroplasts are, therefore, critical for defense, with the revelation that microbe-derived effectors target chloroplasts or chloroplast-localized proteins, and may mediate plant biotic interactions in various distances [32,92,93].

Acetyl-CoA, a key substrate for melatonin generation, is synthesized in the mitochondria [94–96], suggesting the complete biosynthesis of melatonin in the organelle. Plant mitochondria are also involved in the perception of biotic stress and initiation of responses in plant PCD and HR, with complex synergistic or possibly antagonistic interactions among SA, NO·, and ROS [34,47,97]. Compartmentalization of signaling proteins allows them to represent particular functions in metabolically diverse microenvironments. Currently, some melatonin activities are mediated by G protein-coupled receptors [87,98,99]. CAND2/PMTR1, the phytomelatonin receptor, interacts with the G protein subunit (GPA1) [48]. The MT1 receptor, generally confined to the limiting cell membrane, has also been identified in the mitochondrial outer membrane [88], indicating that plant melatonin receptors may be localized at the outer membrane of mitochondria as well. While MAPK, one key factor in the CAND2/PMTR1–MAPK cascade, usually operates in the cytosol, there is also evidence that MAPK components may appear in the chloroplast [100,101].

In summary, mechanistic studies have revealed that melatonin may interact with other plant disease-resistance signaling pathways, such as the MAPK cascades, SA pathway, ET pathway, JA pathway, and stomatal immunity, to mediate plant defensive posture (Figure 2). The production of phytomelatonin in both mitochondria and chloroplasts provides maximal on-site protection for these two critical organelles. The overlap of the production sites for both ROS and RNS and melatonin in the two organelles further indicates that the melatonin–ROS–RNS feedforward loop is located at the two organelles and cooperates in response to biotic stresses. As expected, both of these organelles play key roles in biotic tolerance [32,34,92,93,97]. They provide an opportunity for the interaction of melatonin, ROS, and RNS and downstream phytohormones under biotic stress. It is hypothesized that the integrative melatonin–ROS–RNS loop is possibly located in the mitochondria and chloroplasts [102,103], where the biotic tolerance conferred by the entire loop could be realized. However, the molecular mechanisms underlying the crosstalk remain unclear. The identification and investigation of novel components that mediate crosstalk between melatonin and other regulatory signals will provide new insights into the understanding of plant disease resistance.

The Evolution of Biotic Tolerance of Melatonin Is Secondary after Its Role in Scavenging Radicals

Melatonin can be traced back to the origin of life [67,104,105]. It is speculated that it was first produced in bacteria before endosymbiosis (Figure 3). Mitochondria and chloroplasts, originating from α-proteobacteria and cyanobacteria, respectively, via endosymbiosis, retained the ability to produce melatonin [87]. Consistent with this, all plants potentially synthesize this critical molecule in the mitochondria and chloroplasts (Figure 3) [87–90]. Melatonin's chemical structure has remained the same for billions of years [87,106]. Based on the evolutionary history of melatonin, however, it seems that melatonin not only retained its primary function as an antioxidant but acquired other important biological actions during plant evolution [107].

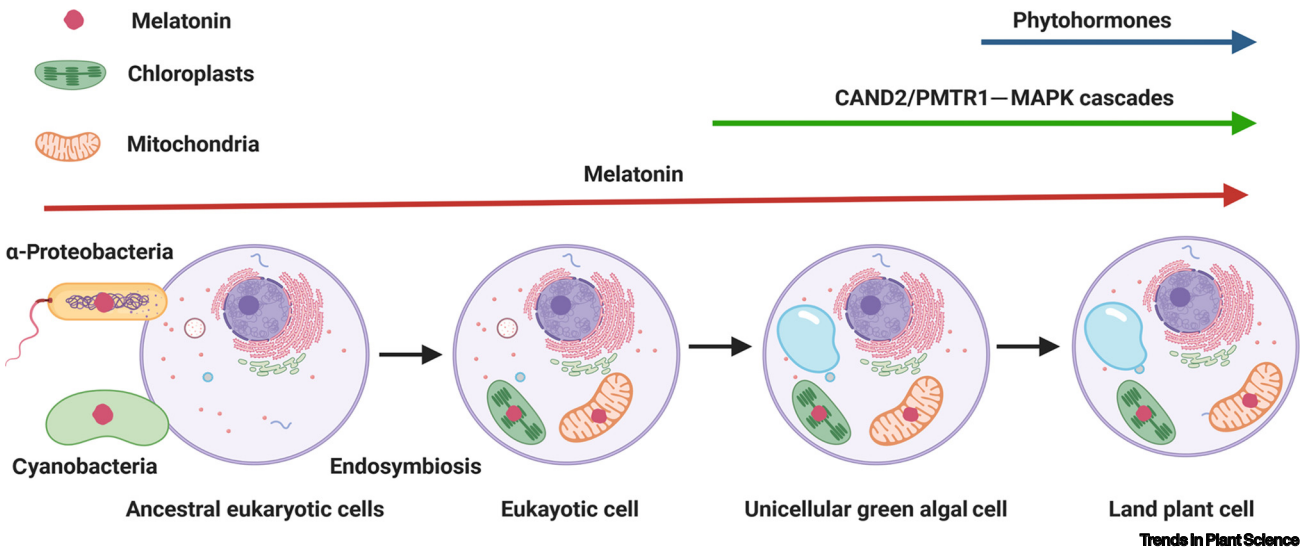

Figure 3. The Evolution of Melatonin–Phytohormone and CAND2/PMTR1–Mitogen-Activated Protein Kinase (MAPK) Cascade Pathways. It is believed that melatonin first evolved in prokaryotes at the beginning of life on Earth. Via endosymbiosis, ancestral eukaryotic cells digested α-proteobacteria and cyanobacteria and evolved into eukaryotic plant cells with mitochondria and chloroplasts, thus having the ability to synthesize melatonin. Melatonin receptors first occur in two tested unicellular green algae, *Coccomyxa subellipsoidea* and *Botryococcus braunii*. MAPK cascades appear in another unicellular green alga, *Chlamydomonas reinhardtii*. Later, after plant terrestrialization, phytohormone pathways appear to increase plant biotic stress tolerance with an interaction with melatonin. This figure was created using BioRender (https://biorender.com/).

Land plants began to emerge on earth around 200 million years ago. Immunity in plants may have evolved after plants became land dwellers and had to respond to the associated pathogens or insects [108,109]. Phytohormones are key factors for plant defense [71,72], protecting plants from pathogen attack and balancing plant growth and defense [71,73,74]. SA, JA, ABA, and ET are core members of the response to biotrophic and necrotrophic pathogens [71,110]. The timing of the evolution of most phytohormone signaling pathways coincides with land colonization, which is a likely need for plant adaptations to the presence of pathogens (Figure 3) [71]. During plant terrestrialization, these related phytohormones and other biotic stress genes that enhance plant resistance to various stresses were obtained by **horizontal gene transfer (HGT)** from soil bacteria [111,112]. Melatonin evolved at the birth of life while hormones began to emerge after plant terrestrialization. Melatonin functions upstream of the various hormones in response to the pathogen invasion.

Together with phytohormones, another plant biotic stress resistance pathway mediated by melatonin is the CAND2/PMTR1 (melatonin receptor)–MAPK cascade pathway [41,42,48,69,70]. Melatonin receptors subsequently developed after the appearance of melatonin. Generally, melatonin receptors are hypothesized to originate from multicellular organisms, including both plants and animals [87]. The BlastP tool was used to evaluate the taxa that may be the first plant for the birth of CAND2/PMTR1 [searching the NCBI database and the plant genomic database of Phytozome v12.1 (https://phytozome.jgi.doe.gov/pz/portal.html) with the conserved domain Tmemb40 (Pfam ID: PF10160) of CAND2/PMGR1 from arabidopsis as a query]. The results indicate that the plant melatonin receptor is absent in *Cyanophora paradoxa*, a cyanobacterium, but first appears in two tested unicellular green algae, *Coccomyxa subellipsoidea* and *Botryococcus braunii* (Table S1 in the supplemental information online); later, the receptor spread to various land plants, like the moss *Physcomitrella patens*, the lycopod *Selaginella moellendorffii*, and seed plants (Figure 3).

With regard to MAPK cascades in the CAND2/PMTR1–MAPK cascade pathway, it appears in the unicellular green alga *Chlamydomonas reinhardtii* and diverged in land plants (Figure 3) [113]. Particularly, the *Chlamydomonas* genome encodes a single MAKK belonging to the MAKK3 structural class, indicating that this chimeric arrangement has emerged in the lineage of early photosynthetic eukaryotes [114]; furthermore, the MAPK cascades are shown to be highly conserved throughout evolution [114,115], possibly due to their indispensable roles in land plant survival. The similar evolutionary models for the plant melatonin receptor and MAPK cascades, especially their first emergence in a unicellular green alga, suggests that CAND2/PMTR1–MAPK pathways may have evolved almost simultaneously. Obviously, the CAND2/PMTR1 receptor probably evolved after melatonin, followed by the evolution of the MAPK cascades [41,42].

Concluding Remarks and Future Perspectives

Accumulating evidence indicates that melatonin interacts with disease-resistance signaling pathways to modulate biotic stress responses. Compared with the spontaneous lowering of excessive oxidation under abiotic stress conditions, plants initiate an oxidative burst aimed at the activation of local and systemic defenses and induction of the localized HR after infection. When infected by pathogens, endogenous ROS, RNS, and melatonin rapidly upregulate, producing a positive regulatory loop where the three factors are mutually dependent. The loop is located upstream in plant innate immunity, leading to both the HR and downstream biotic tolerance signals, including the melatonin receptor–MAPK cascade and phytohormone pathways. The melatonin–ROS–RNS loop and the downstream biotic tolerance pathways are situated at mitochondria and chloroplasts, where they cooperate in response to biotic stresses.

Outstanding Questions

Based on the difference between biotic and abiotic responses in reference to the underlying role of melatonin, is there a specific melatonin receptor associated with the biotic stress response?

How does melatonin increase ROS and RNS under biotic stress, but eliminate ROS and RNS under abiotic stress and normal conditions?

Further molecular crosstalk networks related to melatonin must be identified under biotic stress.

Additional investigations on the origin and evolution of melatonin and its associated pathways for disease tolerance are needed.

How melatonin functions differently in response to various pathogens or insects is in need of clarification.

Genetic transformation with key candidate genes responsible for the biosynthesis of melatonin to improve endogenous melatonin levels should be conducted, aimed at increasing the plant biotic stress tolerance and crop production.

Melatonin *per se* acts as a natural biostimulatory treatment for crop production [7]. For example, melatonin-treated soybean produced more pods and seeds and a greater yield than the control plants both in a greenhouse and in the field [116]. Melatonin treatment postponed apple tree flowering, potentially prolonging the harvest time and increasing the yield [117]. These findings suggest a new approach for improvement of crop yields as well as to protect them against biotic stress. Particularly, as melatonin is safe and shows benefits in humans, exogenous pretreatment of plants with this molecule represents a promising strategy to protect them against pathogenic infection [8,9]. Melatonin may reduce dose levels and enhance the efficacy of fungicides against potato late blight [10]. This would be beneficial for human health and the environment. The application of melatonin in crop production is a frontier that should be aggressively explored [27], which may, as a result, improve crop safety and yield.

Looking ahead, further efforts are needed to arrive at the following frontiers: (i) optimize the proper use of melatonin on crop production; (ii) explore the molecular crosstalk networks related to melatonin; and (iii) increase endogenous melatonin levels by overexpressing key candidate genes responsible for the biosynthesis of melatonin (see Outstanding Questions).

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Supplemental Information

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