J. Pineal Res. 2015; 59:133–150
Doi:10.1111/jpi.12253

Published by John Wiley & Sons Ltd
Journal of Pineal Research

Journal of Pineal Research Molecular, Biological, Physiological and Clinical Aspects of Melatonin

REVIEW ARTICLE

Functions of melatonin in plants: a review

Abstract: The number of studies on melatonin in plants has increased significantly in recent years. This molecule, with a large set of functions in animals, has also shown great potential in plant physiology. This review outlines the main functions of melatonin in the physiology of higher plants. Its role as antistress agent against abiotic stressors, such as drought, salinity, low and high ambient temperatures, UV radiation and toxic chemicals, is analyzed. The latest data on their role in plant–pathogen interactions are also discussed. Both abiotic and biotic stresses produce a significant increase in endogenous melatonin levels, indicating its possible role as effector in these situations. The existence of endogenous circadian rhythms in melatonin levels has been demonstrated in some species, and the data, although limited, suggest a central role of this molecule in the day/night cycles in plants. Finally, another aspect that has led to a large volume of research is the involvement of melatonin in aspects of plant development regulation. Although its role as a plant hormone is still far of from being fully established, its involvement in processes such as growth, rhizogenesis, and photosynthesis seems evident. The multiple changes in gene expression caused by melatonin point to its role as a multiregulatory molecule capable of coordinating many aspects of plant development. This last aspect, together with its role as an alleviating-stressor agent, suggests that melatonin is an excellent prospect for crop improvement.

Marino B. Arnao and Josefa Hernández-Ruiz

Department of Plant Biology (Plant Physiology), Faculty of Biology, University of Murcia, Murcia, Spain

Key words: abiotic stress, antioxidant, biotic stress, circadian rhythm, melatonin, photosynthesis, phytomelatonin, plant growth, plant growth regulator, plant senescence, plant–pathogen interaction, reactive oxygen species (ROS), rhizogenesis, rooting, stress alleviation, stress-responsive factors

Address reprint requests to Marino B. Arnao, Department of Plant Biology (Plant Physiology), Faculty of Biology, University of Murcia, 30100 Murcia, Spain.
E-mail: marino@um.es

Received May 6, 2015;
Accepted June 5, 2015.

Introduction

Melatonin (*N*-acetyl-5-methoxytryptamine) is a pleiotropic molecule with numerous cellular and physiological actions in diverse kingdoms. Discovered in the bovine pineal gland in 1958 [1], it acts as a neurohormone, secreted by the pineal gland into the cerebrospinal fluid and to the bloodstream. This indoleamine makes a relevant contribution to the regulation of many physiological events, such as circadian rhythms, sleep, mood, body temperature, appetite, sexual behavior, retina physiology, and immunological system, among others [2–6]. Additionally, melatonin is involved in numerous cellular actions as an antioxidant, possessing excellent in vitro and in vivo properties as free radical scavenger [7–12].

Since the simultaneous discovery of the presence of melatonin in higher plants in 1995 by Dubbels et al. [13] and Hattori et al. [14], successive studies have led to an accumulation of information about the presence of melatonin in plants and its possible physiological functions, as well as on methods for its extraction and assessment adapted to plants. The evolution in the number of articles (papers and others) published on melatonin in plants is curious. Since the first articles published in 1995, only 37 articles were published on this topic up to 2005 (an average of 3.4 per year) (Fig. 1). Since 2006, the publications on melatonin in plants have shown an exponential increase, reaching a maximum in 2014, with 38 articles. It is expected that in the current year, this number may well be exceeded since only up to April 2015, a total of 28 articles were published. To date, a total of 235 publications (papers and others) have appeared on this topic. Of these, most have been published in *Journal of Pineal Research* (~40%), although in recent years, other journals specializing in plant physiology or food chemistry have shown great interest in sharing the many attractions that plant melatonin offers as a scientific subject of research.

Since the discovery of melatonin in plants, several issues have arisen, mainly in the last 10 yrs. Plant melatonin, also called 'phytomelatonin' by some authors [15], was initially studied as an interesting natural antioxidative molecule. Its presence in foodstuff of plant origin at considerable concentrations (up to mg/100 g FW) and their subsequent incorporation in the human bloodstream after the intake make it an excellent nutraceutical for humans [16–21]. Also, more recently, the possible use of melatonin-rich plants as a recovery-bioagent for chemically contaminated soils has been suggested as an innovative phytoremediation practice [22–25]. However, this review will confine itself to the most recent and relevant studies of melatonin related with plant physiology. Thus, the roles of melatonin related with diverse aspects, such as its protective role against abiotic and biotic stressors, its function as plant regulator in rooting, growth, and other morphogenetic features, changes in melatonin levels that undergo biological rhythms, and its action as a gene expression modulator, are extensively discussed. These lines also should serve to express our appreciation to those

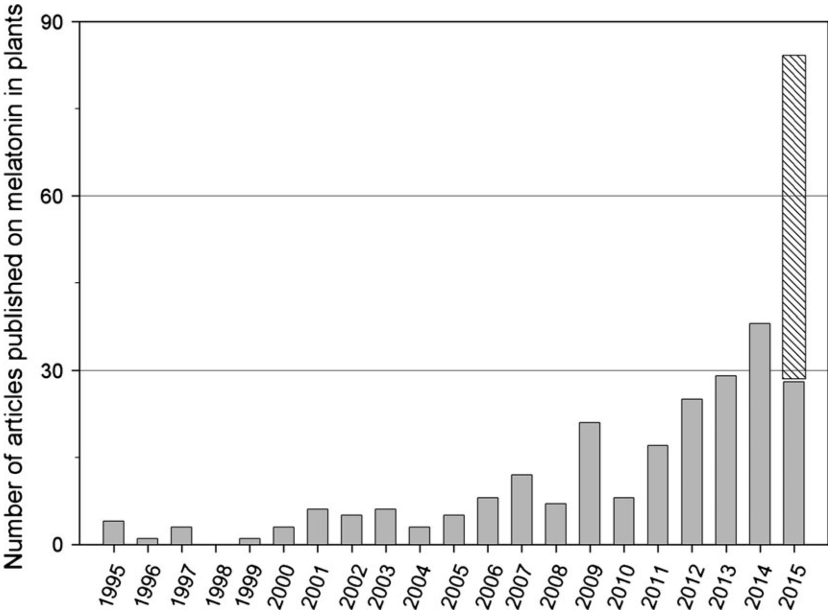


Fig. 1. Evolution of the number of articles related with melatonin in plants since its discovery in 1995. For 2015, the data are based on an extrapolation of the data available to date (first 4 months).

pioneering groups who suggested that melatonin may play an important role in the physiology of plants: the groups of Drs. Kolár and Macháčková in the Czech Republic [26–29], Drs. Saxena and Murch in Canada [30–33], and Drs. Reiter and Tan in USA [34–39], to which we add our first paper on the physiological role of melatonin in lupin published in 2004 [40–42].

Melatonin as an abiotic antistressor

The excellent properties of melatonin as an antioxidative molecule have been widely demonstrated in vitro and in vivo, mainly in animal studies. Melatonin acts as an effective free radical scavenger against hazardous reactive molecules such as hydroxyl radical, superoxide anion, singlet oxygen, hydrogen peroxide, hypochlorous acid, nitric oxide, peroxynitrite anion, peroxynitrous acid, and lipid peroxyl radical, among others. Therefore, melatonin behaves as a generous scavenger of reactive oxygen and reactive nitrogen species (ROS and RNS). Furthermore, melatonin acts as a regulator of many redox enzymes in animal tissues, upregulating antioxidant enzymes such as catalases, peroxidases, and superoxide dismutases, and downregulating pro-oxidant enzymes such as nitric oxide synthases and lipoxygenases, all of which contribute to its action as an anti-inflammatory, anticancerigen, and geroprotecting agent, among others pathological dysfunctions [5, 7–12, 24, 43–47].

Exogenous application of melatonin to plants

In light of its antecedents described in animals and after the detection of melatonin in plants, the possible effects that the treatment of exogenous melatonin might have in different abiotic stress situations were soon studied. The pioneering and most recent and relevant studies in this regard are discussed below.

From the initial studies in carrot culture cells (*Daucus carota*) where the presence of exogenous melatonin attenuated cold-induced apoptosis [48], the possible protective role of melatonin in plants against abiotic stressors was postulated. Thus, many stress situations have been analyzed. Of highlight are the studies in pea plants (*Pisum sativum*), where the application of exogenous melatonin to copper-contaminated soil enhanced the tolerance and survival of plants [23]. Also, the pretreatment of seeds with melatonin reduces copper toxicity in red cabbage seedlings (*Brassica oleracea rubrum*) [49]. Cucumber seeds pretreated with melatonin improve their germination rate during chilling stress with respect to untreated seeds [50]. Also, melatonin improved the survival of cryopreserved callus of the Crassulaceae *Rhodiola crenulata*, a traditional Tibetan herb that grows in extreme conditions of cold, low oxygen, high altitude, and intense UV radiation [51]. In another case, water-stressed cucumber plants treated with melatonin showed a clear increase in the seed germination rate and root growth, which indicates that the application of melatonin minimizes induced water stress [52].

When apple seedlings (*Malus hupehensis*) pretreated with melatonin were subjected to salt stress, shoot height, leaf number, chlorophyll content, and electrolyte leakage, the plants were less affected by the saline stress compared with untreated plants. Interestingly, hydrogen peroxide levels were halved, ROS-metabolizing enzymes (ascorbate peroxidase, catalase, and peroxidase activities) were induced, and Na^+ and K^+ transporters (NHX1 and AKT1) were upregulated, which would help to alleviate saline-induced inhibition [53]. The protective role of exogenous melatonin was also observed in mung bean (*Vigna radiata*) meristem cells after chilling [54]. Similarly, the presence of melatonin in both preculture and regrowth media enhanced the growth of frozen shoot explants of American elm (*Ulmus americana*), demonstrating the usefulness of melatonin for the long-term storage of germplasm for plant cell culture [55]. In a recent study of *Arabidopsis* treated with melatonin and grown at 4°C, melatonin-treated plants had significantly greater fresh weight, primary root length, and shoot height compared with untreated plants, the effect being both time and concentration dependent [56]. Treating *Arabidopsis* with melatonin upregulated the expression of some cold-signaling genes: the C-repeat-binding factors (CBFs), which control the expression of ~100 genes, providing freezing tolerance to the plants; COR15a, a cold-responsive gene regulated by CBFs; CAMTA1, a transcription factor involved in freezing- and drought-stress tolerance, which is related to the Ca^{2+}/calmodulin proteins (well known in melatonin treatment in animal cells); and ZAT10 and ZAT12, two key transcription activators of ROS-related antioxidant genes. Thus, these data point to a role for melatonin in the upregulation of specific cold-responsive genes, supporting the hypothesis that melatonin plays a protective role against abiotic stresses [57].

Similar data were obtained in a recent study with soybean (*Glycine max)* plants. Seeds imbibed with melatonin optimized parameters such as seedling growth, leaf size, plant height, biomass and pod and seed number. Melatonin treatment improved the salt and drought tolerance of plants, demonstrating the significant potential of melatonin for improving field crops, also in this species [58]. Similar results were obtained with the widely used turfgrass, bermudagrass (*Cynodon dactylon*), which was actively protected by exogenous melatonin against salt-, drought-, and cold-stress situations compared with untreated plants. Stressed melatonin-treated plants showed a lower ROS burst, electrolyte leakage, and cell damage and higher plant height/weight, high levels of amino acids, organic acids, sugars, sugar alcohols than untreated plants, clearly affecting the carbohydrate and nitrogen metabolism, mainly solutes involved in the osmotic-stress response [59]. Recently, similar data were obtained in *Citrus aurantium* seedlings [60]; in roots, seedlings, and cotyledons of sunflower [61]; in cucumber seeds [62]; and in *Chara australis* cell cultures [63], all in salt-stressed plants; also in cucumber seedlings under high temperature stress [64], in *Vitis vinifera* cuttings under water-deficient stress [65], and in tomato plants under alkaline stress [66].

The effect of melatonin on the photosynthetic process deserves special consideration. In the pioneering work of Arnao et al. [67], exogenous melatonin retarded induced senescence in barley leaves and delayed the loss of chlorophylls compared with untreated leaves. This effect of melatonin was contrasted with the inductive effect of the hormone abscisic acid (ABA) and the retardant effect of kinetin (a synthetic cytokinin with plant hormone activity) on foliar senescence. Later, this was confirmed in other species such as apple [68–70], *Arabidopsis* [71], cucumber [52], rice [72, 73], and cherry [74]. Interest centered on how melatonin was able to prevent the loss of chlorophylls in stress situations, thus optimizing the photosynthetic process. Exogenous melatonin delayed dark-induced senescence in apple leaves (*Malus domestica*) through the enhancement of some ROS scavenging enzyme activities, which contributed to the elimination of the H_2O_2 excess generated in stressed leaves, while maintaining the ascorbic acid and glutathione content higher than in control leaves [68]. Also, in the long-term application of melatonin to 1-yr-old apple trees under drought conditions, leaf senescence was delayed and accompanied by a significant reduction in chlorophyll degradation. Suppression of the upregulation of the senescence markers, *senescence-associated gene 12* and *senescence 4* transcripts, and of the monooxygenase senescence-related pheophorbide a oxygenase (PaO) clearly indicated a role for melatonin as a regulating factor in induced-foliar senescence [69]. These data were recently confirmed in *Arabidopsis*, where chlorophyllase and PaO were both downregulated by melatonin [71]. More recently, one of the ubiquitin-mediated degradation of auxin/indole-3-acetic acid (AUX/IAA) protein, the *auxin resistant 3/indole-3-acetic acid inducible 17*, was downregulated by exogenous melatonin, providing a direct link between melatonin and natural leaf senescence in *Arabidopsis* [75].

In addition to the protective role of melatonin against leaf senescence, melatonin may also increase photosynthetic efficiency in plants. Melatonin contributed to a better efficiency of photosystem II under dark and light conditions in apple trees, alleviating the inhibition in photosynthesis caused by drought stress and also allowing the leaves to maintain a higher capacity for CO_2 assimilation and stomatal conductance [69]. Similar data were obtained in water-stressed cucumber seedlings. Melatonin treatment reduced chlorophyll degradation, increased the photosynthetic rate and the activities of ROS scavenging enzymes, reversing the adverse effect of water stress [52]. Also, in shoot tip explants of cherry rootstock PHL-C (*Prunus avium* × *Prunus cerasus*), the application of exogenous melatonin at low concentrations slightly enhanced the content of photosynthetic pigments, total biomass, and total carbohydrates, while reducing the proline content of roots, indicating a role for melatonin in the plant stress metabolism [74]. Also, in salt-/drought-stressed soybean, exogenous melatonin promoted plant growth and seed yield, improving tolerance to abiotic stresses. Transcriptome analysis revealed that melatonin upregulated the expression of genes related to photosynthesis, carbohydrate/fatty acid metabolism, and ascorbate biosynthesis. More particularly, some interesting genes upregulated by melatonin include two subunits of photosystem I (PsaK and PsaG), two elements (PsbO and PsbP) related to the oxygen-evolving complex of photosystem II (oxygen-evolving enhancer proteins), the ferredoxin gene PetF, and the VTC4 gene, which encodes the L-galactose 1-P phosphatase involved into ascorbate biosynthesis [58].

Some preservative effects of melatonin on the chlorophyll content have also been described previously in the macroalga *Ulva* sp. [76] and in the freshwater *C. australis,* where a clear increase in the efficiency of the reaction centers of photosystem II was observed [77]. Recently, similar data have been obtained concerning the protective role of melatonin on the photosynthetic pigments in cold-stressed wheat [78]; salt-, cold-, and water-stressed bermudagrass [59]; salt-stressed citrus [60]; salt-stressed sunflower [61]; and tomato plants under alkaline stress [66].

In some cases, a clear relationship between the beneficial effects resulting from exogenous melatonin and changes in morphology and leaf anatomy have been described. Thus, in grape cuttings under water-deficiency stress, melatonin alleviates the oxidative damage by reducing the ROS burst, decreasing malondialdehyde content, and increasing the activity of antioxidative enzymes (catalase, SOD, peroxidase). In addition, the levels of antioxidant metabolites such as glutathione and ascorbic acid increased, as was the proline content [65]. Melatonin-treated plants maintained chlorophyll levels and the efficiency of photosystem II at a similar level as nonstressed plants. Anatomically, water-deficiency stress leads to greater leaf thickness, with thicker cuticles and smaller stomata, especially in mature leaves; this was accompanied by a significant deformation of the palisade and spongy tissues. In melatonin-treated grape plants, greater stomatal pore length and width was correlated with a higher degree of stomata opening. The thickness of leaves, cuticles, palisade, and spongy tissues was also higher than in stressed plants, although not as high as in well-watered plants. Melatonin promotes the high osmotic metabolites levels (as proline), low cell osmotic potential, high cell turgor, and optimal stomata opening, all of which increase CO_2 availability, optimizing the photosynthetic process. Also, in water-stress conditions chloroplast morphology is seriously affected: Chloroplast length decreased and the width increased, giving them a round in shape; chloroplast membrane systems were damaged; starch grains disappeared; and thylakoids were dilated, loosened, and distorted. Chloroplasts of melatonin-treated plants showed a very well-preserved internal lamellar system, and all the damage and destructuration were seriously mitigated [65].

Also under drought conditions, melatonin improves the functioning of the stomata. In two *Malus* species, melatonin pretreatment led to longer and wider stomata and with larger apertures, in both control- and drought-stressed plants. The exogenous application of melatonin through roots optimizes several parameters in leaves such as the relative water content, electrolyte leakage, chlorophyll contents, photosynthetic efficiency, stomatal conductance, hydrogen peroxide levels, and antioxidant enzyme activities. Interestingly, in leaves under drought pretreated with melatonin, ABA level was approximately half those in drought melatonin-untreated leaves. Melatonin downregulates 9-cis-epoxycarotenoid dioxygenase (NCED), a key enzyme in ABA biosynthesis in plants, and upregulates two CYP707 monooxygenases, key catabolic enzymes implicated in the ABA metabolism under drought conditions. According to the authors [79], the lower ABA and H_2O_2 levels in melatonin-treated plants improved stomatal performance, counteracting the stressor conditions.

In a particular interesting paper, the proteomic changes that occur during leaf senescence were studied [80]. Such studies provide a large amount of data and bring to light many of the actions that melatonin can exert in the physiological processes of plants. Melatonin applied to roots for 2 months delayed leaf senescence in apple (*M. hupehensis*) [80]. The proteomic data showed that 622 proteins were altered by the natural senescence process and 309 after melatonin treatment. As a general conclusion, melatonin altered many proteins involved in the senescence process and led to the downregulation of proteins that are normally upregulated during natural senescence. This proteomic study agreed with the same author's data that showed that melatonin treatment provoked higher photosynthetic activity, higher chlorophyll and nitrogen levels, higher total soluble protein and Rubisco contents, in addition to higher levels of total carbohydrates, sorbitol, and starch, compared with control apple leaves [70]. In particular, proteins involved in photosynthesis are strongly regulated during natural senescence. Melatonin mainly upregulated three chloroplastic ATP synthases, two Rubisco small subunits, one Rubisco-interacting protein, and one photosystem I reaction center subunit, which correlates with better photosynthetic activity. Also, melatonin downregulated PaO (related with chlorophyll degradation), one senescence dehydration-associated protein, four dehydrins, and two heat-shock proteins (related to abiotic stress), two α-amylases, one pullulanase (a debranching starch enzyme), two α-glucans, and one α-glucano-transferase. All these are related with starch granule breakdown, which is related to a reduction in photosynthesis and marked starch degradation during leaf senescence, a process that is alleviated by melatonin. Several proteins involved in protein folding and post-translational modifications, such as some MAP kinases, serine/threonine protein kinases/phosphatases, among others (many of which are involved in senescence and stress responses), are downregulated by melatonin [80].

Changes in endogenous melatonin levels by abiotic stressors

As indicated above, many data point to the protective role that the addition of melatonin can have in plants. However, only recently have data clearly demonstrated that endogenous melatonin levels change according to environmental conditions. In the study of *Glycyrrhyza uralensis*, roots multiplied their endogenous melatonin content to about 80 μg/g FW after UV-B radiation treatment, an approximately 7-fold increase over control plants [81]. These striking data suggest that melatonin is accumulated in the plant tissues as a protective molecule in response to different environmental abiotic stressors, such as cold, UV radiation, the light–dark cycle, chemical agents, and water deficit.

Barley and lupin plants treated with different chemical stressors such as zinc salt, hydrogen peroxide, and sodium chloride showed an increase in endogenous melatonin levels [82, 83]. Such induction of melatonin biosynthesis was time and concentration dependent, zinc salt being the best inducer in roots, where it multiplied melatonin levels 6-fold in barley and 12-fold in lupin. In the same study, lupin plants grown at 6°C showed a 2.5-fold increase in their melatonin content compared with plants grown at 24°C [83]. Also in lupin, water restriction during seedling growth provoked a 4-fold increase in endogenous melatonin compared with well-irrigated plants. The global influence of environmental factors on the melatonin levels of plant organs was clearly demonstrated in tomato and lupin plants by Arnao & Hernández-Ruiz [83, 84]. In both cases, plants grown in field conditions contained 10-fold (in tomato) and 3-fold (in lupin) more melatonin than those grown in artificial conditions (culture chambers). The influence of environmental factors on melatonin levels was later demonstrated in water hyacinth plants [36], grape berry skin [85], and cherry fruits [86].

More recently, melatonin levels in sunflower seedlings under salt-stress conditions have been determined [61].

Disregarding the very high melatonin quantification (on the order of μg/g FW), seedlings grown in the presence of NaCl showed up to 2-fold more melatonin in roots than untreated plants and up to 6-fold more in cotyledons. Serotonin levels were also higher in the salt-treated plants. The immunohistochemical localization study showed that the differentiated regions of seedling roots subjected to NaCl treatment exhibit increased accumulation of serotonin and melatonin, in the symplastic zone. Serotonin appeared in the endodermis, pericycle, and vascular bundle of primary roots, while melatonin accumulation was evident in cortical cells, vascular bundles, and exodermis of primary roots. Both indoleamines were detected in the oil body containing cells of cotyledons. So, in salt-stressed seedling cotyledons, the enzyme activity of hydroxyindole-*O*-methyltransferase (HIOMT), the last step in the melatonin biosynthesis pathway (Fig. 2), was induced. This effect correlated with an rise in melatonin level in sunflower cotyledons [61]. In rice, treatment with the herbicide butafenacil provoked an increase in the transcripts of three of the enzymes acting in the melatonin biosynthesis pathway, tryptophan 5-hydroxylase (T5H), tryptophan decarboxylase (TDC), and HIOMT [87]. Also in rice leaves treated with cadmium, the endogenous melatonin level increased 6-fold over the level of control plants. This cadmium-induced synthesis of melatonin was accompanied by the upregulation of TDC, T5H, and HIOMT. However, the expression of serotonin *N*-acetyltransferase (SNAT), the penultimate gene in melatonin synthesis, was downregulated [88]. Similarly, in two *Malus* species, expression of the four melatonin biosynthesis genes, TDC, T5H, SNAT, and HIOMT, was upregulated by drought conditions [79]. All the data available to date showed more evidence as the abiotic stressors induce melatonin biosynthesis and its role as a signal intermediate in the abiotic stress responses. The recent paper on bermudagrass (*C. dactylon*) also points to the effect that several abiotic stressors have on endogenous melatonin levels. Treatment with NaCl, drought, or cold provoked significant melatonin increases (2-, 2.6-, and 3-fold, respectively). These data, together with the effect that exogenous melatonin has on alleviating the cell damage induced by abiotic stress in bermudagrass (mentioned above), led the authors to propose a model involving a possible role for melatonin as a modulator of metabolic homeostasis in the photorespiratory and carbohydrate/nitrogen metabolism [59].

The protective role of melatonin treatment in cold conditions or freezing tissues has been demonstrated [50, 51, 55, 56, 59, 83]. In contrast, a limited number of data have been obtained with heat stress. Thermosensitive *Phacelia tanacetifolia* seeds treated with melatonin reversed the inhibitory effect of high temperature on germination [89]. Also, exogenous melatonin significantly increased the activities of nitrogen metabolism-related enzymes, increasing the nitrate content and restricting the ammonium content at high temperatures, improving the resistance of cucumber seedlings against heat stress [64]. Temperature-dependent melatonin synthesis was closely associated with an increase in both SNAT and HIOMT activities, with high catalytic activities at 55°C. Thus, the daily melatonin levels in field-grown rice plants were unaffected as the positive effect of the relatively high temperature during the day was counteracted by the negative effect of high light [90]. The impact of high temperatures on endogenous melatonin level and thermotolerance factors in *Arabidopsis* was recently studied [91]. Heat stress (37°C) provoked a 2- to 5-fold increase in the endogenous melatonin content of *Arabidopsis* seedlings. Also, exogenous melatonin treatment (20 μM) enhanced the survival rate (~50%) of plants subjected to heat stress (45°C, 120 min) compared with melatonin-untreated plants (survival rate 5%). Heat-shock transcription factors (HSFs) are the major regulators of heat stress-responsive genes. The HSFA1 subtype and some heat-responsive genes (*HSFA2*, *HSA32*, *HSP90*, and *HSP101*) were upregulated by heat stress and exogenous melatonin, conferring relevant thermotolerance to *Arabidopsis* [91].

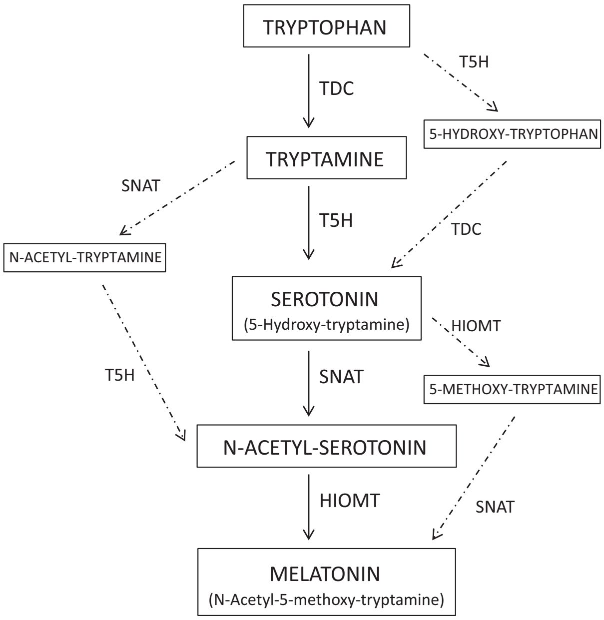


Fig. 2. Biosynthetic pathway of melatonin from tryptophan in plants. The enzymes of the respective steps are as follows: T5H, tryptophan 5-hydroxylase; TDC, tryptophan decarboxylase; SNAT, serotonin *N*-acetyltransferase; and HIOMT, hydroxyindole-*O*-methyltransferase. Dotted lines represent alternative reactions described in particular cases.

With the aim of studying the possible roles of melatonin at physiological level, a variety of transgenic plants expressing ectopic genes, mainly melatonin biosynthesis genes of animal origin, have been used. Table 1 shows the studies on melatonin that have been published with biochemical and physiological objectives using transgenic plants. The most commonly used ectopic genes were those which codify SNAT and HIOMT enzymes (Fig. 2) from different sources, including human and sheep. However, the first to be used was the SNAT gene from *Chlamydomonas reinhardtii* [92]. Some studies have been made with other melatonin biosynthesis genes, such as T5H and TDC. Rice plants have been the most widely used receptor of these ectopic genes, and many rice transgenic plants have been obtained to study the melatonin metabolism (Table 1). Tomato and *Nicotiana sylvestris* plants have also been used. In general, SNAT/HIOMT overexpressing

Table 1. Melatonin-related transgenic plants

Ectopic enzyme gene-modified plant	Melatonin level in transgenic lines	Other levels	Physiological response	Ref.
SNAT from *Chlamydomonas reinhardtii* Overexpressed (OE) in transgenic tomato	↑ up to 8.7-fold	–	No significant morphological changes were observed	[92]
Human SNAT OE in transgenic rice	↑ up to 7-fold	↑ NAT (up to 9-fold) ↑ Chl (up to 2.5-fold)	Increased cold resistance Increased growth of roots and leaves	[94]
Human SNAT/HIOMT OE in *Nicotiana sylvestris*	From not detected in WT to 50 μg/g DW in a transgenic line	–	Increased resistance to UV-B radiation	[95]
Sheep SNAT OE in rice	↑ up to 8-fold	↑Chl ↓MDA, ROS ↑SOD, CAT, APX ↑TDC, T5H, EHIOMT	Increased resistance to herbicide butafenacil Decreased necrotic tissue	[87]
Sheep SNAT OE in rice	↑ up to 4.9-fold	-	Increased root growth Increased biomass	[96]
Sheep SNAT OE in micro-tom tomato	↑ up to 2.3-fold	↓IAA (up to 7.1-fold)	Loss of apical dominance Branching phenotype	[97]
Sheep HIOMT OE in micro-tom tomato	↑ up to 6-fold	↓IAA (up to 1.6-fold)	Increased resistance to drought	
Sheep SNAT OE in transgenic rice	↑ up to 3-fold	–	Increased lustiness Increased biomass Decreased seed yield Retarded flowering	[98]
Rice HIOMT isogenes OE in rice	↑ up to 1.5-fold	–	HIOMT-transcripts upregulated by abscisic acid and jasmonic acid	[99]
Segicuchi Rice Mutant (lack of *T5H*)	↓ up to 280-fold	↑Tryptamine (up to 400-fold) ↑NAc-Tryptamine (up to 245-fold) ↓Serotonin (up to 240-fold)	–	[100]
Rice TDC isogenes OE in rice	↑ up to 31-fold	↑Tryptamine (up to 135-fold) ↑Serotonin (up to 62-fold) ↑NAc-serotonin (up to 4-fold)	–	[101]
Apple HIOMT OE in *Arabidopsis*	↑ up to 4-fold	↓IAA (up to 1.4-fold)	Lower intrinsic ROS Increased lateral roots Increased biomass Greater tolerance to drought	[93]
Rice indoleamine 2,3-dioxygenase OE in tomato	↓ up to 4-fold	–	Decreased lateral leaflets Leaflets more flattened/less serrated	[102]

plants showed a net increase in endogenous melatonin levels as a response. Also, the levels of other related indoles changed in transgenic plants with respect to untransformed plants. Some phenotypic changes are to be expected. For example, the increase in melatonin in overexpressing rice plants was related with greater resistance to butafenacil, a singlet oxygen-generating herbicide [87]. Also, overexpression of apple HIOMT in *Arabidopsis* resulted in a higher endogenous melatonin level, low ROS content, increased biomass and, overall, a greater tolerance to drought treatment compared to wild-type plants [93]. As a rule, a higher resistance to abiotic stress conditions such as drought, cold, and UV-B can be observed in melatonin-rich overexpressing plants compared with wild-type plants. These results demonstrate that the endogenous melatonin, even at very low concentrations (pico- or nanograms per gram of fresh weight), is critical for ROS scavenging and serves as a first line of defense against stressors. Also, a higher root growth rate, robustness, and biomass were observed in the transgenic modified plants (Table 1).

Melatonin as a biotic antistressor

Melatonin can act as a biocide against some fungi and bacteria, although conclusive data on its action mechanism do not exist. Low doses of melatonin show in vitro antimicrobial activity against antibiotic-resistant gram-positive and gram-negative pathogen strains [103], while concentrations much higher showed antibacterial activity against ten different human pathogenic bacteria [104]. Their use as in vivo antibiotic has also been suggested, for example, in human newborns suffering from septicemia [105]. In our preliminary studies, different concentrations of melatonin showed growth inhibition activity against several plant fungal pathogens such as *Alternaria* spp., *Botrytis* spp., and *Fusarium* spp. growing in standard media (Fig. 3, panels A and B). Also, melatonin was capable of decreasing the rate of infection in plant–pathogen attack. In nonsterilized *Lupinus albus* seeds, a low rate of fungal infection (≤40%, compared with a control, unpublished data) was reached when seeds were pretreated with melatonin during the germination process (Fig. 3, panels C–E).

Exogenous melatonin treatments improved resistance against several fungal/bacterial infections, causing plants to develop the diseases at a lower extent. Thus, Yin et al. [106] improved resistance of *Malus prunifolia* against the fungus *Diplocarpon mali* (Marssonina apple blotch), treating trees through their roots by irrigation with melatonin at different concentrations (from 50 to 500 μM). At 20 days, melatonin-treated apple trees showed a lower number of damage leaves, with a higher chlorophyll content, a more efficient photosystem II and a less defoliation that infected-untreated trees. The authors affirmed that exogenous melatonin induces and maintains phenylalanine ammonia-lyase activity and the expression of the protector pathogenesis-related proteins, chitinase, and β-1,3-glucanase, contributing to greater resistance to fungal infection in those treated plants, reducing lesions, inhibiting pathogen expansion, and alleviating disease damage [106]. Moreover, melatonin was capable of reducing 10-fold the multiplication of the virulent bacterial pathogen *Pseudomonas syringae* (*Pst* DC3000) following infection of *Arabidopsis thaliana* leaves. Thus, in *Arabidopsis* and *Nicotiana benthamiana* leaves pretreated with exogenous melatonin (10 μM), the induction of various pathogenesis-related genes and other elements of the plant defense response related with salicylic acid (SA), jasmonic acid (JA), and ethylene have been described. Of note was the rapid induction of the defense genes (only 0.5 hr), which reached a peak 3 hr after melatonin treatment [107]. Recently, the same authors demonstrated that increased pathogen susceptibility in a SNAT knockout *Arabidopsis* mutant was correlated with a reduction in endogenous melatonin level due to SNAT gene inactivation. Also, a decrease in SA level and a strong inhibition of defense genes, including pathogenesis-related proteins PR1, PR5, and defensin 1.2, were observed in mutant lines compared with wild type. Melatonin also induced the nuclear localization of the SA receptor (NPR1), a key factor in the signaling pathway [108]. The data obtained with diverse *Arabidopsis* mutants showed that melatonin acts upstream of the defense genes signaling pathway, inducing the biosynthesis of SA, JA, and ethylene, which, together, elicit disease resistance in a well-known co-action [109]. The emergence of an oxidative burst during the early stage of plant–pathogen interaction seems to increase endogenous melatonin level [107, 108].

The scarce data available in this respect show that melatonin (and perhaps serotonin [110] and *N*-acetylserotonin

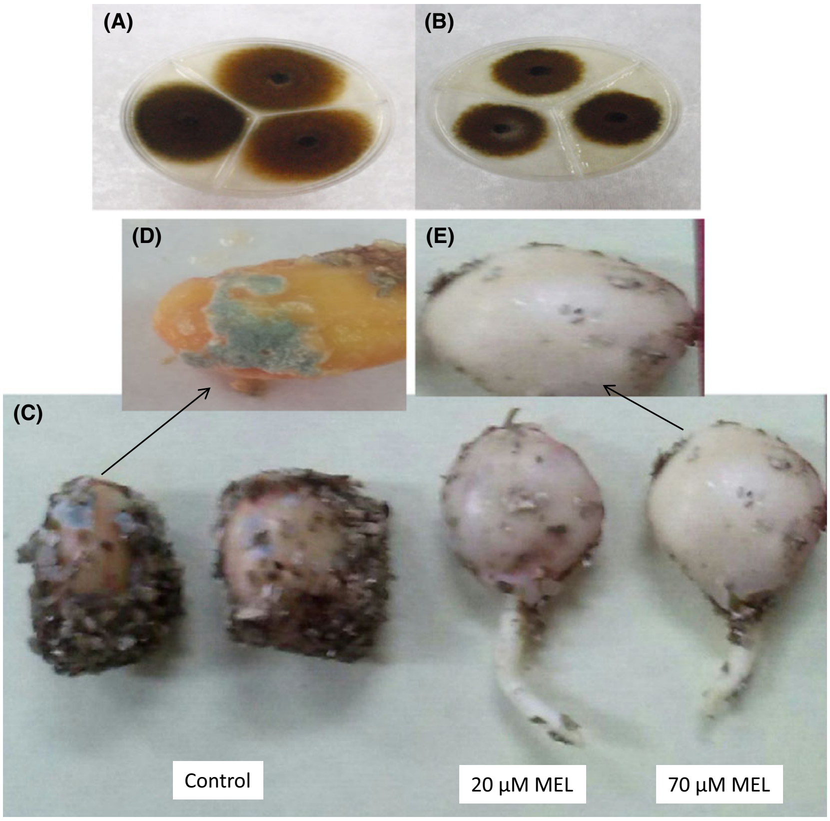


Fig. 3. Effect of melatonin on plant fungal pathogens. Panels A–B: fungitoxicity in in vitro assay of melatonin on *Alternaria* spp. grown in potato dextrose agar (PDA) plates. (A) Control; (B) 4 mM melatonin. Panels C–E: in vivo fungal infection assay on lupin seeds in germination. (C) Nonsterilized lupin seeds germinated on vermiculite substrate previously imbibed with water (Control) or with melatonin at 20 and 70 μM. (D) Detail of fungal infection (*Penicillium* spp.) in control seeds. (E) No fungal infection could be observed in melatonin pretreated seeds.

[107], to a lesser degree) triggers the defense responses, acting as a signal molecule (elicitor) capable of inducing several defense genes related with pathogen resistance. Some plant fungi which establish a mutualistic endophytic relationship with several plant species present higher melatonin levels than are found in plants. In this sense, the possible relationship between plant–pathogen/beneficial fungi and their respective melatonin levels may be an interesting line of study. All the data point to the increase in ROS (oxidative burst) being the common factor between the abiotic and biotic stresses responsible for the increase in melatonin level observed in these situations (Fig. 4).

Melatonin as a biological rhythm regulator

The role of melatonin as a regulator of light–dark cycles has been clearly established in mammals [111–113]. In plants, the circadian oscillator is able to adjust the phase of a variety of biological processes, such as gene and metabolic regulation, protein stability, among others, to coincide with daily and/or seasonal cycles. Thus, circadian regulation increases photosynthesis and growth rates and may affect flowering and seed yield in crops and biotic/abiotic stress responses [114–117]. This role as chronoregulator was the starting point for research into melatonin in plants and, more specifically, its possible involvement as a regulatory molecule in circadian rhythms and in aspects connected with photoperiodicity. In 1997, Kolar et al. [27] demonstrated the presence of melatonin in cultivated 15-day-old *Chenopodium rubrum* plants and observed an oscillating behavior of melatonin levels in 12:12-hr light/dark cycles. Low or undetectable levels of melatonin were found during the light period and a considerable increase (reaching maximum levels of ~250 pg/g FW) in darkness (Fig. 5, panel A). This increase in melatonin during the dark period was similar to that observed in mammals. In plants exposed to different photoperiodic cycles, no relationship between the photoperiod applied and the duration of melatonin increase was observed [118]. The authors concluded that, although the maximum of melatonin always occurred after lights off, melatonin biosynthesis was not directly light-regulated, but showed a circadian rhythm, as in mammals. In *Pharbitis nil* seedlings, van Tassel et al. [119] indicated that no differences in the melatonin content appeared with respect to the light/dark cycle applied.

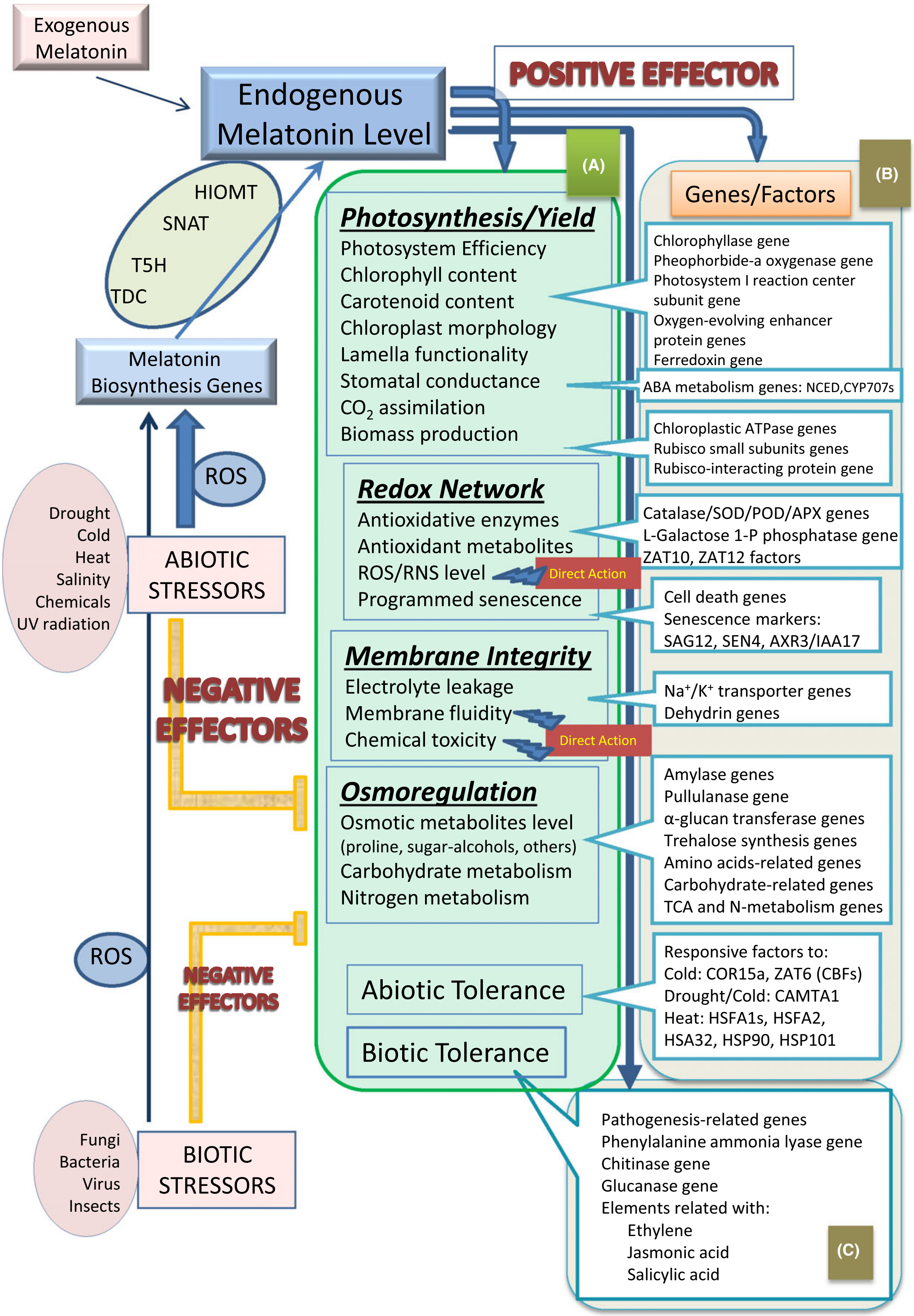
In a significant study by Reiter group's working with water hyacinth (*Eichhornia crassipes*) [36], plants grown in natural conditions presented maximum levels of melatonin, with a peak occurring late in the light phase of the light–dark cycle (Fig. 5, panel B). Also, the catabolite N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), determined for the first time in plants, showed a similar rhythm. Interestingly, when the plants were cultivated in artificial light, melatonin levels were considerably lower, indicating the importance of the quality of light for the melatonin biosynthetic capacity of plants [120]. Similarly, the macroalga *Ulva sp.* presented a melatonin rhythm, with a maximum at night in long-photoperiod day (16 hr light: 8 hr dark) [76].

An oscillating behavior of melatonin levels was observed in the berry skin of *V. vinifera* cv Malbec, during the night/day cycle and under field conditions [85]. The grape skins presented a melatonin peak of 158 ng/g FW at sunrise, decreasing in the next 4 hr to undetectable values both at noon and during the afternoon (Fig. 5, panel C). During the night, melatonin reached their lowest levels ~10 ng/g FW. The author's interpretation was that melatonin levels were probably controlled by the circadian clock and that the decrease in melatonin during the light period would be due to 'melatonin consumption' in grapes as an antioxidant response against solar radiation. More recently, two peaks of melatonin production, at 5:00 and 14:00 hr during a 24-hr period, in two sweet cherry cultivars, have been described (Fig. 5, panel E). This double-peak matched TDC (Fig. 2), suggesting that it acts as a rate-limiting enzyme in melatonin synthesis in plants [86]. Also two peaks were described in apple (*Malus zumi*) leaves, at 14:30 and 5:30 hr (Fig. 5, panel D), slightly after malondialdehyde peaks, suggesting that oxidative stress can induce melatonin biosynthesis [93], as also was proposed in sweet cherry [86]. Our data obtained for lupin and barley are very similar to those for sweet cherry and apple leaves, in which two peaks of melatonin appeared (Fig. 5, panels F and G). In both cases, melatonin peaks were higher in roots than in leaves or cotyledons. The results of the studies cited above (*Chenopodium*, *Eichhornia*, *Vitis*, *Malus, Prunus*, *Lupinus*, and *Hordeum*) indicate that the time of day at which the sample is taken seems to be relevant for melatonin levels in plant tissues, since, depending on the time of day or night, differences of several orders of magnitude are recorded. Whatever the case, a circadian rhythm of melatonin seems to exist in plants. However, the decisive influence of abiotic factors on endogenous melatonin levels and the wide range of melatonin concentrations observed in different plant organs mean that much more rigorous studies need to be performed on this topic.

Melatonin as a plant (hormone) regulator

One of the first roles proposed for melatonin in plants was its possible action as a growth regulator. The structural

Fig. 4. Scheme of melatonin action as positive effector on several physiological processes. Abiotic and biotic stressors provoke an increase into endogenous melatonin level through the upregulation of melatonin biosynthetic genes. Both abiotic and biotic stress effects are mediated by an oxidation burst, ROS induction being the first cellular signal. Stressors act as negative effectors in many cellular and physiological processes such as photosynthesis and membranes integrity (panel A). Endogenous melatonin can change the expression of many genes and regulation factors that attenuate or reverse the negative effects of biotic/abiotic stressors on physiological processes, acting as a positive effector against stress (panels B and C). Also, melatonin can act directly as free radical scavenger (direct antioxidant) on reactive oxygen and reactive nitrogen species, lipid peroxides, and toxic chemicals, controlling relevant aspects such as membrane integrity and the proper functioning of the redox network.


Exogenous Melatonin
Endogenous Melatonin Level
POSITIVE EFFECTOR
HIOMT
SNAT
T5H
TDC
Melatonin Biosynthesis Genes
ROS
Drought
Cold
Heat
Salinity
Chemicals
UV radiation
ABIOTIC STRESSORS
NEGATIVE EFFECTORS
ROS
NEGATIVE EFFECTORS
Fungi
Bacteria
Virus
Insects
BIOTIC STRESSORS
(A)
Photosynthesis/Yield
Photosystem Efficiency
Chlorophyll content
Carotenoid content
Chloroplast morphology
Lamella functionality
Stomatal conductance
CO_2 assimilation
Biomass production
Redox Network
Antioxidative enzymes
Antioxidant metabolites
ROS/RNS level
Direct Action
Programmed senescence
Membrane Integrity
Electrolyte leakage
Membrane fluidity
Chemical toxicity
Direct Action
Osmoregulation
Osmotic metabolites level
(proline, sugar-alcohols, others)
Carbohydrate metabolism
Nitrogen metabolism
Abiotic Tolerance
Biotic Tolerance
(B)
Genes/Factors
Chlorophyllase gene
Pheophorbide-a oxygenase gene
Photosystem I reaction center subunit gene
Oxygen-evolving enhancer protein genes
Ferredoxin gene
ABA metabolism genes: NCED,CYP707s
Chloroplastic ATPase genes
Rubisco small subunits genes
Rubisco-interacting protein gene
Catalase/SOD/POD/APX genes
L-Galactose 1-P phosphatase gene
ZAT10, ZAT12 factors
Cell death genes
Senescence markers:
SAG12, SEN4, AXR3/IAA17
Na^+/K^+ transporter genes
Dehydrin genes
Amylase genes
Pullulanase gene
α-glucan transferase genes
Trehalose synthesis genes
Amino acids-related genes
Carbohydrate-related genes
TCA and N-metabolism genes
Responsive factors to:
Cold: COR15a, ZAT6 (CBFs)
Drought/Cold: CAMTA1
Heat: HSFA1s, HSFA2,
HSA32, HSP90, HSP101
Pathogenesis-related genes
Phenylalanine ammonia lyase gene
Chitinase gene
Glucanase gene
Elements related with:
Ethylene
Jasmonic acid
Salicylic acid
(C)

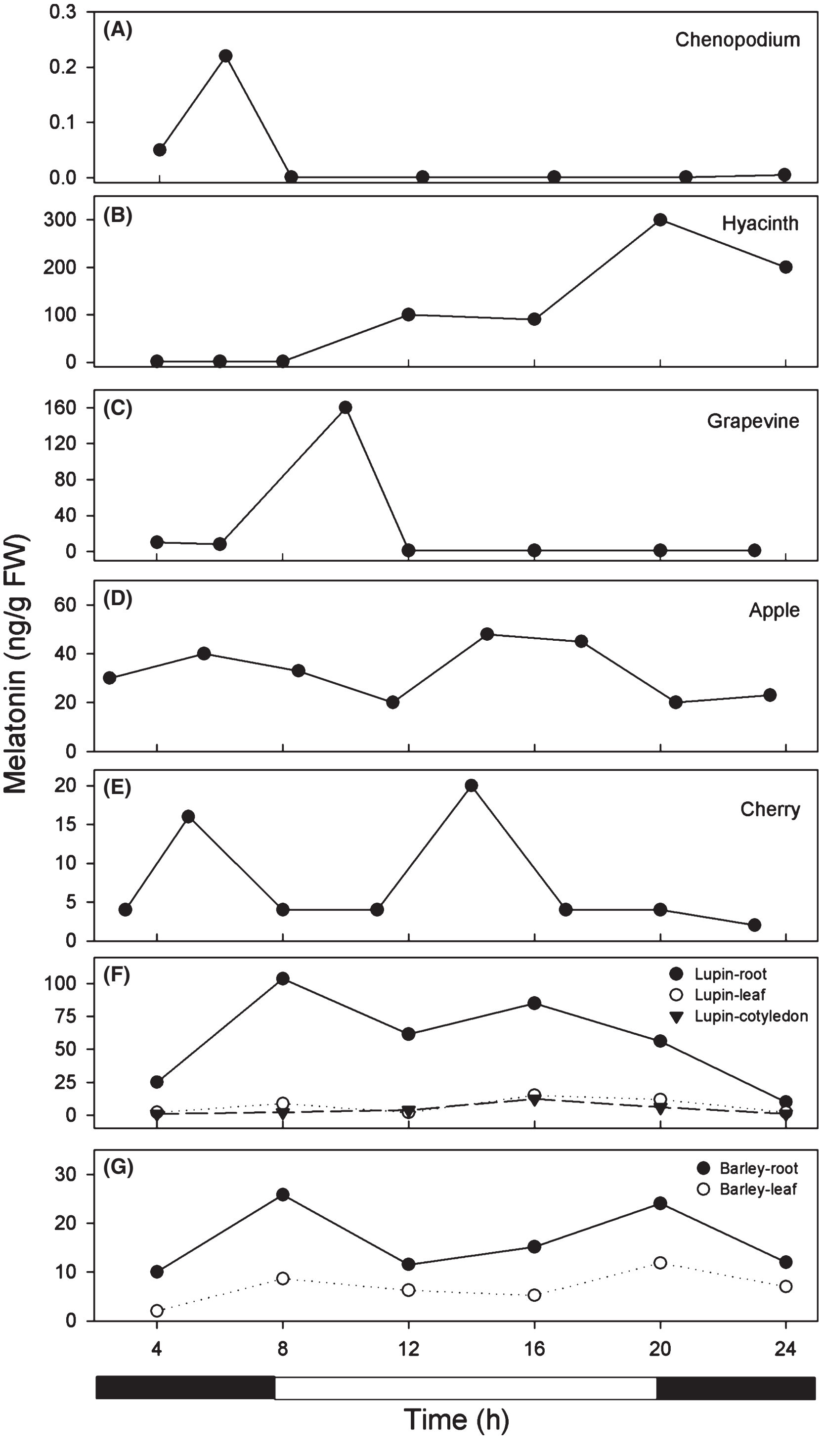


Fig. 5. Changes in melatonin level during a photoperiodic cycle of 12 hr light/12 hr darkness. The approximate data are taken from the respective works cited in the text.

similarity and the common biosynthetic pathway between IAA and melatonin led to suggestions of possible functional similarities between these two indolic molecules [121]. In 2004, the action of melatonin as a growth promoter was demonstrated in etiolated *Lupinus albus* [40]. Similar to IAA, exogenous melatonin induces active growth of hypocotyls at micromolar concentrations, while having an inhibitory effect at higher concentrations. As in the case of IAA, endogenous melatonin showed a concentration gradient distribution in tissues, probably related with the different growth potential of hypocotyls and roots: The most apical zone, being the most actively growing zone, had the highest melatonin content. This relationship has also been described, for the gradient observed between auxin and melatonin [122]. The growth-promoting effect of melatonin was also demonstrated in several monocots. Curiously, the effect of exogenous melatonin was tissue-selective, promoting growth in coleoptiles but inhibiting it in roots, at similar concentrations, which resembles the behavior of IAA [41, 123]. These promoting/

inhibitory, concentration-dependent effects of melatonin have also been described in red cabbage [49] and in mustard [124]. Also in transgenic overexpressing sheep SNAT rice plants (Table 1), the increase in endogenous melatonin level was correlated with the high growth rate of roots [94, 96]. Possibly, melatonin (and also serotonin) restored growth-promoting activity in salt-stressed sunflower seedlings, perhaps making up for the deficiency in IAA caused by disruption of the auxin gradient in primordial roots provoked by salts [61]. More recently, the positive effect of exogenous melatonin on root/shoot growth has been clearly demonstrated in several species such as *Arabidopsis* [56, 57], soybean [58], bermudagrass [59], and *Citrus* [60]. To date, the limited number of studies that exist indicate that actions on the growth process of IAA and melatonin could run in parallel, although any specific relationship between IAA and melatonin is still far from being demonstrated. For example, there are insufficient data on how IAA and melatonin affect mutual endogenous levels, bearing in mind that both indoles share common precursors in the biosynthetic route, tryptophan, and tryptamine [125].

Another action in which auxinic substances are involved is rhizogenesis. Melatonin is able to induce root primordials from pericycle cells in lupin, generating new adventitious or lateral roots [126]. This rhizogenic effect has also been studied in other species such as rice [96], cherry [74, 127], *Arabidopsis* [93, 128, 129], cucumber [52], and pomegranate [130]. Based on the data obtained, melatonin began to be considered as a plant regulator in the formation of adventitious roots. The potentiating effect of melatonin on rooting, when acting together with other auxin such as IAA or indole-3-butyric acid (IBA) is of great interest, especially in recalcitrant species to auxin action [131].

Meanwhile, few advances have been made in understanding the role of melatonin in cultured cells. Since the studies of Saxena group's with *Hypericum perforatum* (St. John's wort) [33] and with *Echinacea purpurea* (purple coneflower) [132], where it was postulated as a potential plant regulator, independent of IAA, in organogenic processes (rhizo- and caulogenesis); and the study of in vitro shoots of *Vaccinium corymbosum* (highbush blueberry), where the morphogenic potential of melatonin was compared with that of IAA and IBA [133], no new studies have appeared. There is great interest that melatonin might have as a dual agent, as cell protector (antioxidant activity), and as plant morphogenic regulator, in in vitro applications. Only recently, an interesting study has been published on the use of melatonin in *Agrobacterium*-mediated plant transformation methods. The presence of exogenous melatonin significantly reduced tissue browning and cell death, without affecting T-DNA integration, increasing the stable transformation frequency of plants [134].

Until now, there is no clear evidence that melatonin may replace IAA in its effect on apical dominance. Okazaki work's with transgenic tomato plants overexpressing the rice indoleamine 2,3-dioxygenase gene, which encodes an enzyme that cleaves the indolic ring, points to a relationship between low endogenous melatonin level and some morphological changes in transgenic tomato leaves, with more flattened and more serrated leaflets than tomato wild-type leaves (Table 1) [102]. More recently, also in transgenic tomato plants but overexpressing sheep SNAT and HIOMT enzymes (see Fig. 2 and Table 1), higher endogenous melatonin levels were correlated with a substantial decrease in endogenous IAA levels in leaves and stems and with a branching phenotype, which suggests that melatonin does not replace IAA in the apical dominance function [97]. Similar findings were obtained in transgenic *Arabidopsis* overexpressing apple HIOMT (Table 1), where an increased melatonin level was matched by an increase in new roots and a strongly decreased levels of endogenous IAA. This last effect indicates that melatonin and IAA probably acts in the same physiological action, but in parallel [93].

Related with plant hormones, the changes induced in gene expression by melatonin are worthy of comment. Thus, with respect to auxin, root primordial induction by melatonin treatment was independent of IAA-signaling activation. This particular effect of melatonin on rhizogenesis was also observed with other auxin, 1-naphthaleneacetic acid (NAA), a synthetic root-promoting plant regulator widely used in agronomic and biotechnological applications. In both cases, IAA and NAA were able to activate the auxin-inducible gene expression marker DR5:GUS in *Arabidopsis*, but not with melatonin, which also activates the generation of lateral roots [128, 129]. In a whole-transcriptome sequencing (RNA-seq) analysis in cucumber roots, the expression of 121 genes were significantly upregulated and 196 downregulated in roots treated with melatonin, which activated rhizogenesis. Several superfamilies of transcription factors such as MYB (plant orthologs of Myb proto-oncogene), MRKY (plant protein which bind at specific DNA sequence through the WRKYGQK peptide), and NAC (no apical meristem/ATAF1-2/cup-shaped cotyledon proteins) are involved in responses related with biotic/abiotic stresses, plant development, hormonal signal transduction, and disease resistance, among others. Some members of these transcription factors and also some ethylene-transcription factors (ERFs) have the ability to negatively regulate root-related genes and therefore suppress root formation. Many of these factors were downregulated by melatonin, allowing new root formation [135]. Curiously, the expression pattern of auxin-related genes exhibited minimal changes in melatonin-treated *Arabidopsis* plants with respect to those that are untreated. Only an IAA-amino synthase was upregulated, with no change in the expression of auxin biosynthetic genes [71].

Auxin-influx carrier protein-1 (AUX1) which belongs to a small gene family comprising four highly conserved genes, AUX1 and LIKE-AUX1 (LAX) (amino acid/auxin permease superfamily), was severely downregulated in response to melatonin treatment in *Arabidopsis*. Of the four AUX/LAX genes, AUX1 regulates lateral root development, root gravitropism, root hair development, and leaf phyllotaxy. These preliminary data show that melatonin can interfere in the action of auxin through changes in auxin carriers that modify local IAA gradients [71, 136]. Thus, except for these last data and the existence of common biosynthetic precursors (tryptophan and tryptamine), a direct connection between IAA and melatonin has not yet been found, attending to their similar physiological actions.

In the case of gibberellins (GA) and ABA, the levels of both plant hormones were altered by melatonin treatment. Thus, melatonin upregulated GA biosynthesis genes such as GA20ox and GA3ox in cucumber seedlings in saline conditions, contributing to a high level of activates GAs as GA_4, promoting salt-inhibited germination process [62, 135]. Also, melatonin treatment provoked the upregulation of ABA catabolism genes (two CYP707 monooxygenases) and downregulated 9-cis-epoxycarotenoid dioxygenase (NCED), a key enzyme in ABA biosynthesis, which resulted in a rapid decrease in ABA level during seed germination under salt stress [62]. Similar data were obtained in apple leaves in drought conditions, where melatonin pretreatment halved the ABA content through regulation of the same ABA biosynthesis and catabolism enzymes, as mentioned above [79].

A novel study on the effect of exogenous melatonin on ethylene metabolism, postharvest ripening, and the quality of tomato fruit showed that tomatoes treated with 50 μM melatonin for 2 hr manifested substantial changes in their fruit ripening parameters, such as lycopene levels, fruit softening, flavor, ethylene signaling, and biosynthesis enzymes with respect to untreated tomatoes [137]. Exogenous melatonin slightly increased ethylene generation and further timing of climacteric peak through the upregulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase expression. Also, the ethylene receptor genes NR and ETR4 and the transducing elements EIL1, EIL3, and ERF2 genes were upregulated. The only known data in this respect were provided in a congress communication in which melatonin produced a strong inhibition (up to 65% in roots, compared with a control) in the rate of ethylene production in etiolated seedlings of lupin [138]. This inhibitory effect on ethylene production manifested itself in vegetative tissues as a regulation by auxin, in which IAA was capable of inducing ACC synthase expression, but also blocked the induction of ACC oxidase expression by ethylene, in accordance with the model of Kang's group in mung bean hypocotyls [139]. Also, in *Arabidopsis* melatonin-treated plants, two ACC synthases were upregulated, one being ACC synthase, which is auxin-inducible according to the above model [71]. Possibly, this opposite effect was due to the differences in the auxin-mediated response between vegetative and reproductive tissues. Also, exogenous melatonin treatment provoked the upregulation of genes related with lycopene biosynthesis-, aroma/flavor-, cell wall structure, and aquaporin in tomatoes, leading to the conclusion that melatonin promotes postharvest tomato fruit ripening through increased ethylene production and signaling [137]. Nevertheless, different effects could appear in vegetative tissues.

With regard to SA and JA, both plant regulators are involved in biotic stress responses, as mentioned above. Also, in the extensive genetic functional analysis of *Arabidopsis* treated with exogenous melatonin, most genes in the ABA, SA, JA, and ethylene pathways were upregulated, confirming that melatonin alters the expression of stress response genes involved in all the steps of the pathway, from receptors through transcription factors. These results confirm the critical roles of melatonin in defense against both biotic and abiotic stresses in plants [71, 108]. In contrast, in a recent work the treatment of rice leaves with different plant hormones (IAA, GA_3, JA, SA, and ethylene, at a fixed concentration) did not provoke the induction of melatonin biosynthesis and only a low level was measured in response to ABA, suggesting that melatonin production is not linked to plant hormone responses [88].

Conclusions and future challenges

Fig. 4 summarizes the most relevant aspects concerning abiotic/biotic stressors and melatonin. Both abiotic and biotic stress effects are mediated by an oxidative burst, ROS induction being the first cellular signal. This ROS burst is probably responsible for the increase in endogenous melatonin levels through upregulation of melatonin biosynthetic genes (at least three of the four enzymes involved). Stressors acts as negative effectors in many cellular and physiological processes, such as photosynthesis and membrane integrity (Fig. 4, panel A). Endogenous melatonin can change the expression of many genes and regulation factors that attenuate or reverse the negative effects of biotic/abiotic stressors on physiological processes, acting in this way as a positive effector against stress (Fig. 4, panels B and C). Also, melatonin may act directly as free radical scavenger (direct antioxidant) on ROS/RNS, lipid peroxides, and toxic chemicals, controlling relevant aspects such as membrane integrity and the proper functioning of redox network. Thus, melatonin acts as a first barrier against the ROS burst and, in a second line of defense, changes the expression of many responsive stress genes. Clearly, the action of melatonin becomes more evident and concise in challenging physiological situations, when plants are subjected to stressful environments or aggressive conditions such as severe stress and/or pathogen infections. Although there are many aspects that remain to be investigated, a role for melatonin as common effector in biotic and abiotic stresses has been proposed, suggesting that melatonin may play a relevant role in the growth/defense balance as modulator [140–143].

As regards to future challenges, all aspects of melatonin metabolism must be considered to be of great interest. Although many considerations on biosynthetic enzymes and their regulation are beyond the scope of this review, much information on the kinetic properties and cellular localization of the enzymes involved in plant melatonin biosynthesis (T5H, TDC, SNAT, and HIOMT) has recently become available (Fig. 2), almost all in rice plants [90, 99, 101, 144–148], but some in *Arabidopsis* [93, 149, 150]. It is necessary to extend the studies to other species. With respect to melatonin degradation, melatonin is catabolized into various metabolites, including 4-hydroxymelatonin, 2-hydroxymelatonin, cyclic 3-hydroxymelatonin, melatonin dioxetane, AFMK, and N^1-acetyl-5-methoxykynuramine (AMK) in the kynuric pathway [24, 151, 152] and 6-hydroxymelatonin, 6-sulfatoxymelatonin, 5-methoxytryptamine, 5-methoxytryptophol, and 5-methoxyindolil-3-acetic in the indolic pathway [24, 151, 153, 154]. Only AFMK has been reported in the water hyacinth [36]. Practically everything in this respect remains to be studied in plants, although some recent studies detected 2-hydroxymelatonin as the main product of degradation of melatonin in rice [88, 148, 155]. Related to this, the detection of some melatonin isomers, mainly in plant-fermented beverages (wine, fruit juices) [156, 157], generated some confusion until the unequivocal identification of the main isomer was identified as tryptophan ethyl ester [158–160]. Nevertheless, the presence of melatonin conjugated with carbohydrates and/or amino acids can be expected in plant extracts, in a similar way to the metabolism of other plant hormones (e.g. auxin). As regards the origin of melatonin, the most recent data suggest that it is generated in the mitochondria and chloroplast [147–149, 161]. Nevertheless, data exist that show that roots have the highest melatonin content of the whole plant, presumably being synthesized in the leucoplastid of roots, this remains to be confirmed. Also, the detection of melatonin in xylem and/or phloem elements/vessels should be investigated.

The physiological actions of melatonin through gene regulation will probably involve their interaction with a receptor. Although the hormone/receptor interaction in plants is much more complex than in animals, the detection and characterization of potential melatonin receptor (s) in plants is also a primary objective. Undoubtedly, the extensive knowledge we have on its receptors and antagonists in vertebrates may help in this respect [162]. Also, in the interplay between ROS and plant hormones, melatonin should be taken into account [140]. In fact, recently melatonin has began to be considered by experts in plant hormones [131].

Despite the absence of conclusive data, much information is already available about the role of melatonin in relevant aspects of the physiology of plants, such as rooting, photosynthesis, and resistance to stressful environments. This should be corroborated, verifying its role as a biostimulator and stress-protective molecule in field trials. Possibly, for an overview of the physiological processes in which melatonin is involved, it will be necessary to understand its relation with circadian rhythms. Available data on circadian changes in melatonin levels should be contrasted with the influence of melatonin on the central regulatory elements of the biological clock in *Arabidopsis* as CCA1, LHY, and TOC1 [163]. In animals, melatonin plays an important role as a chronobiological agent, acting as a darkness signal providing information to the brain and peripheral organs, regulating circadian phases, and maintaining rhythm stability. Recently, the role of melatonin as a proteasome inhibitor in a feedback clock gene regulation mechanism has been proposed in animals [164]. The similarity in pacemaker architecture (feedback regulation) and the influence of environmental factors (synchronization) in the circadian clock (in animal and plant) mean that much of what is known about melatonin in animals might be studied in plants [165].

To conclude, the potential of melatonin to strengthen plants subjected to multiple abiotic/biotic stressors has opened up an interesting area of study for this natural substance, especially for crop improvement and pathogen protection.

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