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MINI REVIEW

Melatonin in higher plants: occurrence and possible functions

Abstract: Melatonin may be ubiquitous in the plant kingdom. This review considers the evaluation of methods of melatonin determination in plant material and possible melatonin functions in plants. Concerning the determination methods, the only reliable techniques are liquid chromatography – mass spectrometry or gas chromatography – mass spectrometry after some purification steps of the extract. Melatonin was shown to delay flower induction in some photoperiodic plants and in the dinoflagellate *Lingulodinium* it replaces, in part, the requirement of darkness for cyst formation. Melatonin may also have a function as an antioxidant and it may possess some auxin-like effects. Finally, it may act as a signal for interaction of plants with herbivores and pests. Further research is needed to clarify these potential functions.

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Introduction

After its original discovery in the bovine pineal gland [1], melatonin was found in many species of animals. Melatonin is widespread and most extensively studied in vertebrates – mammals (e.g. [2, 3]), birds, reptiles, amphibians, bony fishes, cartilagenous fishes (reviewed in Ref. [4]), and cyclostomes [5]. However, its occurrence was also confirmed in several invertebrate phyla (reviewed in Refs [6–8]). The first report of melatonin presence outside the animal kingdom, in the dinoflagellate alga *Lingulodinium polyedrum* [9], suggested that it is a very ubiquitous compound, and that at least some of its functions might be evolutionarily conserved. Since then, melatonin was indeed found in many algae and in several species of fungi, protists, and even prokaryotes (reviewed in Refs [8, 10]). The work in *Lingulodinium* also prompted a search for melatonin in higher plants. The first reports, published in 1995 [11–13], clearly demonstrated its presence in various angiosperm species. After 10 yr of research, it is obvious that melatonin is rather common in higher plants but there still remain many unanswered questions. In this mini-review, we focus on two issues: (i) evaluation of methods employed for sample preparation and melatonin determination in plants, (ii) hypotheses and evidence concerning melatonin's functions in plants.

Methods of melatonin determination in higher plants

Various methods of melatonin extraction, purification, and determination were employed for samples from algae to higher plants. Some authors used very simple extraction solvents – ethanol [14], 10% Na_2CO_3 [11], phosphate-buffered saline [12], or potassium phosphate buffer [15]. However, Poeggeler and Hardeland [16] pointed out that material from unicellular eukaryotes and plants often contains compounds (e.g. chelated iron or redox-active biomolecules) that promote melatonin degradation via photooxidation or free radical-mediated oxidation. They recommend 0.4 M $HClO_4$ for protein precipitation (which eliminates possible enzymatic melatonin degradation), and potent hydroxyl radical scavengers (acetone, Tris/HCl, or Tricine/NaOH buffer) with added antioxidants for extraction. The procedure should also be carried out in dim red light or darkness to avoid photooxidation [9, 16]. Several researchers subsequently used acetone-, Tris-, or Tricine-based extraction mixtures, sometimes including antioxidants, $HClO_4$, or the chelator EDTA [9, 17–21]. Hernández-Ruiz et al. [22] also added an antioxidant (butylated hydroxytoluene) to their extraction solvent, ethyl acetate.

Melatonin was sometimes measured directly in the extracts [9, 12, 14, 20]. Nevertheless, Van Tassel and O'Neill [23] and Kolář [24] found further sample purification highly desirable because it removes compounds that would otherwise interfere with melatonin determination. Several studies used solid-phase extraction on C18 cartridges, sometimes followed by high-performance liquid chromatography (HPLC) fractionation [17, 18, 21]. Other authors extracted melatonin from an aqueous extraction solution into an organic solvent – chloroform or diethyl-ether [11, 15]. Immunoaffinity chromatography was also employed for sample purification [25, 26]. In this case, immunoaffinity columns were chosen because they were successfully used before liquid chromatography – mass spectrometry (LC-MS) analysis of melatonin in human

serum [27]. Moreover, immunoaffinity purification is also part of some clean-up protocols for determination of plant hormones, e.g. cytokinins [28] and indole-3-acetic acid [29].

Melatonin was determined in plants by methods previously employed in animals. The methods differ in their sensitivity and specificity. HPLC with fluorescence detection was not sensitive enough to prove melatonin presence in *C. rubrum* shoots [24] but was successfully used to quantify melatonin in Chinese medicinal herbs [30]. HPLC with eletrochemical detection is more sensitive in comparison [16] and was often used in algae and higher plants (e.g. [9, 14, 15, 20]) in spite of its relatively low specificity – many compounds with comparable oxidation potentials may have retention times very similar to melatonin. Radioimmunoassay (RIA) is more specific and is frequently employed in animals (e.g. [31–33]). However, detailed investigations suggest that plant extracts contain many substances cross-reacting with anti-melatonin antibodies [23]. Melatonin levels in plants determined by RIA alone and not validated by other methods [11, 12] may thus be overestimated, as was demonstrated, e.g. for *Pharbitis nil* and tomato [18]. In contrast to animals, melatonin RIA is therefore not a reliable method in plants. Gas chromatography – mass spectrometry (GC-MS) or LC-MS, which offer high sensitivity and excellent detection specificity, can overcome the problems discussed above. Melatonin can be measured directly by LC-MS or it can be converted to a volatile (e.g. pentafluoropropionyl – [34]) derivative and analyzed by GC-MS. Both methods have been used for melatonin quantification in animals (e.g. [34, 35]). In plants, CG-MS was applied to confirm melatonin identity [11] and to measure melatonin content in *P. nil* and tomato [18]. Liquid chromatography – tandem mass spectrometry (LC-MS/MS) was also used in several studies to prove melatonin's identity (e.g. [19, 22, 30]) or to determine its levels [21, 25].

Liquid chromatography – tandem mass spectrometry offers distinct advantages: first, it does not require melatonin derivatization in contrast to GC-MS, and secondly, the double ion selection method provides the greatest specificity for melatonin currently achievable. However, Kolář [26] and J. Kolář and K. Wolf (unpublished data) observed that the area of melatonin peak detected by LC-MS/MS was severely reduced in some series of samples. This was likely due to a so-called 'matrix effect'. This phrase refers to changes (usually reduction) in ionization efficiency – and consequently in the peak area – of an analyte, which are caused by coeluting compounds from biological material and/or from chemicals or labware used for sample preparation. Matrix effects are often observed in LC-MS/MS analyses [36–38]. In the case discussed here, it would be expected that the signal of the ^{2}H-melatonin internal standard in tissue extracts spiked with ^{2}H-melatonin would be diminished in comparison with a solution containing only the pure substance. This was indeed demonstrated for the affected plant samples (E. Witters, J. Malbeck and A. Trávníčková, unpublished results). The matrix effect does not pose a serious problem for melatonin quantification because the ionization of both the endogenous compound and the deuterated internal standard added to each sample should be influenced to the same extent [39]. However, it is obvious that the detection limit of the method is greatly deteriorated. The possibility of matrix effects should therefore be kept in mind while developing methods for LC-MS measurement of melatonin in plants. Matrix effects, when encountered, can be minimized or eliminated by a more selective extraction, a more efficient chromatographic separation, or a change of the ionization method [37, 38].

Melatonin occurrence in higher plants

Melatonin was found in many species of angiosperms, in both dicotyledons and monocotyledons. Its occurrence was not yet investigated in other groups of higher plants – mosses, ferns, gymnosperms, etc. The first preliminary report [13] was in *P. nil* (Japanese morning glory) where melatonin was determined by RIA and GC-MS. Melatonin was then identified in *Nicotiana tabacum* and in edible organs of tomato, banana, cucumber, and beetroot; it was not detected in potato. The levels were 2–510 pg/g fresh weight as determined by RIA, and melatonin identity in tomato extracts was confirmed by GC-MS spectra [11]. Hattori et al. [12] reported melatonin in 24 species of edible plants from 12 families of both dicotyledons and monocotyledons. Its content ranged from 10 to 5300 pg/g fresh weight (RIA) and was generally the highest in seeds of *Poaceae*, e.g. of rice and oat. Melatonin concentrations up to 250 pg/g fresh weight were later detected in *Chenopodium rubrum* shoots using LC-MS/MS [21]. Since then, the presence of melatonin was reported in many other plants, with concentrations usually in the range of picograms to nanograms per gram of tissue, as summarized in Table 1.

There is obviously convincing evidence that melatonin occurs in higher plants. However, not all the data are equally reliable. Most importantly, some researchers did not prove melatonin identity beyond a reasonable doubt, e.g. by mass spectrometric methods. In addition, some of the reported levels may be inaccurate. On one hand, recovery of extraction procedures was not properly determined in some cases. Because melatonin is rather unstable in plant extracts, the published concentrations might be underestimated [6]. On the other hand, it was already mentioned above that plant material contains large amounts of compounds that cross-react with antibodies used in RIA, as was pointed out by Van Tassel and O'Neill [23]. When RIA was employed as the only method of melatonin quantification (e.g. [11, 12]), the values may have been overestimated. Indeed, several plants that were reported to have high melatonin levels, e.g. oat and ginger [12], were later found to contain only trace amounts [17]. Also, RIA measurements provided much higher melatonin concentrations than GC-MS in *P. nil* and tomato [18]. As regards the frequently used HPLC methods with electrochemical or fluorescent detection, it would be desirable to confirm the quantitative results (and not only melatonin identity) by substantially more specific mass spectrometric detection.

Melatonin daily profiles have only been studied in two species so far; both plants are important models for research on photoperiodic flower induction. Preliminary results suggested a diurnal rhythm with a night maximum in *P. nil* shoots [13]. However, repeated experiments did not

Table 1. A summary of important publications that reported melatonin in higher plants

Reference	Plant species and organs analyzed	Melatonin concentration	Method of melatonin determination
[15]	tart cherries (*Prunus cerasus*), fruit	2–13 ng/g FW	HPLC-ECD
[11]	5 edible species, edible parts; tobacco (*Nicotiana tabacum*), leaves	edible plants: 2–510 pg/g FW; tobacco: 40–100 pg/g FW	RIA; GC-MS to confirm identity
[12]	24 edible plants (monocot and dicot species), edible parts	10–5300 pg/g FW	RIA; HPLC-FD to confirm identity
[22]	lupin (*Lupinus albus*), hypocotyl	9–28 ng/g FW	HPLC-ECD; LC-MS/MS to confirm identity
[30]	Chinese medicinal herbs (108 species screened), various organs	up to 3800 ng/g DW (more than 10 ng/g DW in 64 species)	HPLC-FD; LC-MS/MS to confirm identity
[21]	*Chenopodium rubrum*, shoots	up to 250 pg/g FW (LC-MS/MS)	LC-MS/MS, RIA
[14]	15 species of edible plants, seeds	2–190 ng/g dry seed	RIA; HPLC-ECD to confirm identity
[104]	St. John's wort (*Hypericum perforatum*), developing flowers	up to 4000 nmol/g FW	HPLC-ECD; validated by LC-MS/MS and RIA
[105]	feverfew (*Tanacetum parthenium*), *Hypericum perforatum*, and two *Scutellaria* species, leaves or flowers	0.1–2.5 μg/g DW	not reported
[106]	huang-qin (*Scutellaria baicalensis*), in vitro propagated shoots from 26 distinct germplasm lines	9–44000 nmol/g DW	HPLC-ECD; validated by LC-MS/MS and RIA
[18]	morning glory (*Pharbitis nil*), shoots; tomato, fruit	morning glory: up to 12 pg/g FW (GC-MS); tomato: up to 17 pg/g FW (GC-MS)	GC-MS, RIA
[25]	*Chenopodium rubrum*, shoots	up to 100 pg/g FW	LC-MS/MS

FW, fresh weight; DW, dry weight; HPLC, high-performance liquid chromatography; ECD, electrochemical detection; FD, fluorescence detection; LC-MS/MS, liquid chromatography – tandem mass spectrometry; GC-MS, gas chromatography – mass spectrometry; RIA, radioimmunoassay.

show any clear pattern of changes in a 12 hr light/12 hr dark regime [18]. In contrast, a clear daily rhythm with a sharp maximum at night and very low levels during the day was found in shoots of *C. rubrum* [21]. In this plant, the highest melatonin concentrations always occur 6 h before lights on in different photoperiodic regimes [25].

Very little is known about melatonin biosynthesis in higher plants. In one study, ^{14}C-tryptophan was fed to in vitro grown *Hypericum perforatum* plantlets. Tryptophan was metabolized to indoleacetic acid, tryptamine, 5-hydroxytryptophan, serotonin, and melatonin [19]. 5-Hydroxytryptophan and serotonin are melatonin precursors in vertebrates [40], suggesting the melatonin biosynthetic pathway in higher plants might be similar to that in animals.

Possible melatonin functions in higher plants

Regulation of circadian rhythms and photoperiodic reactions

Most vertebrates studied so far exhibit daily rhythms in blood levels of melatonin, with low concentrations during the day and high at night. These rhythms persist under constant darkness and are therefore circadian [4, 41]. It was thus proposed that melatonin mediates time-of-day information to target cells throughout the body (e.g. [41]). Several lines of evidence indeed support the involvement of melatonin in vertebrate circadian rhythmicity. Importantly, circadian rhythms are affected by exogenous melatonin treatment (reviewed in Refs [42, 43]): depending on the experimental setting, melatonin can cause arrhythmicity, change the free-running period or phase, or entrain free-running rhythms. Daily melatonin profiles may also encode photoperiodic information as lengthening of the night prolongs the duration of elevated melatonin levels [4, 41]. Melatonin clearly affects mammalian photoperiodism (reviewed in Refs [41, 44, 45]). Treatments that disrupt the daily oscillations in melatonin levels (removal of the pineal gland or continuous-release melatonin implants) influence photoperiodic reactions. Photoperiodic responses are mainly determined by the daily duration of high melatonin levels; for example, melatonin application at appropriate times of day can elicit short-day responses in animals kept in long days. The situation is more complex in nonmammalian vertebrates where pinealectomy and/or melatonin administration influence seasonal reproduction in some species but not in others [4]. In vertebrates, melatonin thus regulates circadian rhythms and photoperiodism. Daily and/or circadian rhythms in melatonin levels were also found in some invertebrate species, suggesting similar roles in rhythmicity and/or photoperiodism (reviewed in Ref. [6, 7]).

Melatonin was also found in many species of algae (reviewed in Ref. [10]). Five species of green algae *Chlamydomonas*, *Dunaliella*, and *Acetabularia* show daily melatonin rhythms with night maxima which continue in constant conditions [46]. There is also some evidence for similar diurnal oscillations of melatonin levels in the red alga *Porphyra umbilicalis* [47]. In the dinoflagellate

L. polyedrum, melatonin concentrations also exhibit a daily rhythm with a night maximum [9]. In addition, melatonin and its metabolite 5-methoxytryptamine promote photoperiodically controlled encystment [48] and affect the circadian bioluminescence rhythm [6] in this organism. It was also reported that melatonin application causes phase shifts of a bioluminescence rhythm in the dinoflagellate alga *Pyrocystis acuta* [49] and possibly in several other dinoflagellates [10]. These findings resemble melatonin's roles in animal rhythmicity and photoperiodism, suggesting that the involvement of melatonin in time measurement might be evolutionarily conserved and shared by most eukaryotes.

Several authors therefore tested whether melatonin affects circadian rhythms and/or photoperiodic reactions also in higher plants. This possibility is indirectly supported by the finding that melatonin levels in the dicot plant *C. rubrum* exhibit a diurnal rhythm with a night maximum [21, 25]. Because melatonin application in long days causes short-day responses in some vertebrates, effects of melatonin administration were investigated in several species of plants with photoperiodically controlled flowering. However, no promotion of flowering after melatonin application was found in the short-day plant *P. nil* [17]. Similarly, melatonin treatment 1, 2, or 4 hr before lights off does not induce flowering of the water plants *Spirodela polyrhiza*, *Lemna minor*, and *Lemna trisulca* in noninductive photoperiods [50]. 10^{-4} or 5×10^{-4} M melatonin also does not significantly influence flowering of the long-day plant *Chenopodium murale* [51].

The most convincing effect of melatonin on a photoperiodic response in a higher plant was found in the short-day plant *C. rubrum* [52]. In contrast to some vertebrates, melatonin application does not induce a short-day response (i.e. flowering) after a short (6–8 hr) noninductive night. Instead, 100 or 500 μM melatonin applied 1 hr before the beginning of an inductive 12-hr darkness significantly lowers the percentage of flowering plants. Uptake experiments revealed that high melatonin levels in the plant (50–90% of the applied amount) are reached during the first half of darkness and are maintained until at least 24 hr after the end of this dark period. Exogenous melatonin could therefore influence both photoperiodic flower induction and/or subsequent developmental processes in the shoot apical meristem. Because 500 μM melatonin inhibits flowering only when applied 3 hr before or during the first half of a 12-hr dark period, it probably affects some early steps of the transition to flowering. The first step is the measurement of night length by a circadian oscillator. *Chenopodium rubrum* has a circadian rhythm in photoperiodic sensitivity to a single night of various durations [53]. However, melatonin treatment has no effect on the period or phase of this rhythm. Exogenous melatonin must thus control some other early process(es) of *C. rubrum* transition to flowering.

Melatonin application also delays flowering of the long-day plant *Arabidopsis thaliana* [26]. *Arabidopsis thaliana* plants treated with 100 or 500 μM melatonin flower slightly later than control plants, especially in terms of their ontogenetic age, i.e. leaf number; numbers of rosette and total leaves at anthesis are significantly increased by 8–10%. Because melatonin suppresses flowering in both the short-day *C. rubrum* and the long-day *A. thaliana*, this effect could be a more general phenomenon among higher plants. The lack of melatonin's influence on flowering in some species (see above) might have been caused merely by an inappropriate experimental setup or by an inappropriate method or timing of treatment. Importantly, only the ability of melatonin to promote flowering in noninductive photoperiods was tested in most cases. Nevertheless, it should be stressed that melatonin concentrations required for the inhibition of flowering exceed the naturally occurring levels by several orders of magnitude, at least in *C. rubrum* where the endogenous concentrations were found to be 10^{-12} mol/g fresh weight or less [21]. The results are thus encouraging but do not prove that endogenous melatonin affects plant photoperiodic responses. It is of course possible that only a small proportion of the applied melatonin is transported to target cells. But it cannot be ruled out that high levels of exogenous melatonin may in fact suppress flowering by a mechanism not related to the mode of action of the endogenous compound. Further studies are needed to identify the exact mechanism(s) of melatonin's action on flowering.

Very little is known about melatonin effects on circadian rhythms of higher plants. Kolář [26] tested melatonin, several related indole derivatives, and compounds that are melatonin agonists or inhibitors of its biosynthesis in animals. Neither melatonin nor any of the other chemicals specifically affected two circadian rhythms: cotyledon movement in *C. rubrum* (after pulse application at two different circadian times), and bioluminescence in *N. tabacum* seedlings carrying a luciferase reporter gene controlled by a circadian clock-regulated cab2 promoter (during continuous application to nutrient medium). Melatonin also does not influence the circadian rhythm of photoperiodic time measurement in *C. rubrum* (see above). These data indicate that melatonin probably does not regulate daily rhythms in higher plants; but some effects might be perhaps discovered in the future if other treatment schedules, species, or rhythms are investigated.

Other functions related to those in animals

In animals, melatonin not only regulates photoperiodism and circadian rhythmicity but also has many other functions. Melatonin may play some of these roles also in higher plants.

Melatonin binds to Ca^{2+}-calmodulin in animal cells [54] and antagonizes some of its effects [55]. In MDCK cells treated with 10^{-9} M melatonin, melatonin co-localizes with calmodulin and changes its subcellular distribution [56]. Because Ca^{2+}-calmodulin regulates many calcium-dependent cellular functions, these findings suggest that melatonin may affect a broad range of processes via this interaction. For example, melatonin inhibits the activity of Ca^{2+}-calmodulin-dependent nitric oxide synthase in rat cerebellum, possibly by acting as a calmodulin antagonist [57]. On the contrary, melatonin showed no calmodulin antagonism in the case of T-lymphocyte activation [58]. Calmodulin is a calcium signal transducer also in plants [59], so melatonin effects on plant processes controlled by calmodulin are worth investigating.

Melatonin affects the cytoskeleton, causing either microtubule enlargement or depolymerization. It was proposed that at physiological concentrations (10^{-9} M) melatonin antagonizes the inhibition of tubulin polymerization caused by calmodulin and thus stimulates microtubule growth. At pharmacological levels (10^{-7}–10^{-5} M), melatonin binds to tubulin and prevents its polymerization (reviewed in Ref. [60]). These effects were observed in both animals and plants. In higher plants, 10^{-4} M melatonin increases birefringence of the mitotic spindle (i.e. stimulates microtubule assembly) in *Haemanthus katherinae* [61]. In contrast, 8×10^{-4} M melatonin causes microtubule depolymerization and therefore disrupts the mitotic apparatus in onion root tips [62].

Finally, melatonin is a radical scavenger and an antioxidant in animals. Melatonin was shown to protect organisms against reactive oxygen and nitrogen species (ROS and RNS) [63–66]. Melatonin is in many animals produced also in other organs than pineal gland and a hypothesis was suggested that this melatonin has other than hormonal functions, e.g. radical scavenging [67]. Actions of melatonin as an antioxidant can be classified into four categories: (a) direct free radical scavenging; (b) stimulation of activities of antioxidant enzymes; (c) increasing the efficiency of mitochondrial oxidative phosphorylation and reducing electron leakage; (d) augmenting the efficiency of other antioxidants [64].

Melatonin has the ability to neutralize the highly toxic hydroxyl radical ($OH^{\bullet}$) [68]. The proposed product of this reaction is cyclic 3-hydroxymelatonin, which also has antioxidant properties. Reaction of melatonin with hydrogen peroxide was shown in vitro and its product again has antioxidant properties [69, 70]. These cascades of scavenging events may be the reason for the unexpectedly high efficiency of melatonin in reducing radical damage in vivo. Melatonin was shown also to scavenge superoxide ($O_2^{\bullet -}$) and $NO^{\bullet}$ radicals [71–73] and also haloperoxyradicals, e.g. $CCl_3O_2^{\bullet}$ [74].

Melatonin increases activities of several antioxidant enzymes: superoxide dismutase (SOD, both MnSOD and CuSOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GRd) and glucose-6-phosphate dehydrogenase [63, 75]. Melatonin stimulates the rate-limiting enzyme of glutathione (GSH) synthesis, γ-glutamylcysteine synthase, and thus increases intracellular concentration of GSH [76]. Moreover, amplification of GPx activity in rat brain by melatonin was observed and melatonin deficiency after pinealectomy reduces the activity of this enzyme [77]. Melatonin also increases the expression of genes coding for antioxidative enzymes, especially SOD [78]. Additionally, melatonin has antiapoptotic effects [79] and is able to prevent neurons in patients with Alzheimer disease from mutilation and death [80, 81]. Similar beneficial effects of melatonin have been observed in prematurely born children with septic shock [82] and in respiratory distress syndrome [83].

The specific functions of melatonin at the mitochondrial level are under intensive investigation. A relationship is suggested on the basis of high ROS and RNS production in mitochondria, occurrence of GPx and GRd in mitochondria, anti-apoptotic effects of melatonin (apoptotic signals originating in mitochondria) and putative existence of mitochondrial binding site for melatonin [84, 85]. In in vitro experiments, melatonin prevented oxidative damage in mitochondria induced by t-butyl hydroperoxide [86]. Melatonin also elevated ATP production in mitochondria [87]. The proposed actions of melatonin at the mitochondrial level have recently been reviewed by Leon et al. [88].

Melatonin shows a synergism with 'classical' antioxidants like GSH and vitamins E and C [89]. Under high oxidative stress melatonin was shown to be superior to vitamins E and C in reducing damage [65]. Melatonin acts as an ABTS cation radical scavenger with much higher efficiency than other antioxidants with which melatonin acts synergistically [67].

In plants, antioxidant properties of melatonin were described as well, but the functioning of melatonin as an antioxidant in vivo is much less clear than in animals. In the alga *L. polyedrum*, high levels of melatonin rescued cells from lethal oxidative stress caused by H_2O_2 and this effect was not because of induction of antioxidative enzymes [90]. Sublethal oxidative stress induced by different agents was also counteracted by melatonin [8, 91]. In tomato, a correlation between susceptibility to ozone and melatonin levels was described [11]. Melatonin was also reported to delay senescence of leaves, both in monocots and dicots [92]. High levels of melatonin found in seeds led to the suggestion that melatonin functions as a protective antioxidant [14].

Functions specific to higher plants

Not all melatonin's functions in higher plants have to duplicate those in animals. For example, melatonin (N-acetyl-5-methoxytryptamine) is structurally related to the plant hormone auxin (IAA, indole-3-acetic acid); both are indole derivatives. It is therefore possible that melatonin (either endogenously occurring or exogenously applied) may exhibit some auxin-like effects in plants. It could hypothetically bind to auxin receptors and act directly as an auxin agonist. However, it does not meet the requirements for an agonist very well. It was concluded from a comparison of auxin activities of various compounds that the minimal structural requirements for auxin activity are the presence of an aromatic group and a carboxyl group or groups that can mimic them in terms of electron distribution and polarity [93]. The melatonin molecule does have an aromatic (indole) part but lacks the acidic carboxyl or similar group.

In agreement with these theoretical considerations, it was demonstrated that melatonin has no auxin activity in two model systems: the wheat coleoptile segment straight growth biotest [26] and the decapitated *Coleus* shoot phototropic test [94]. But a recent paper by Hernández-Ruiz et al. [22] shows an auxin-like activity of melatonin in *Lupinus albus*. Applied melatonin stimulated elongation growth of *L. albus* hypocotyls in a concentration range similar to the promotive concentrations of IAA. In three different biotests, maximal growth activation by melatonin was 22–63% of that caused by IAA treatment. Moreover, the highest levels of endogenous IAA and melatonin were detected in the hypocotyl zone with the maximal growth

rate, supporting the notion that melatonin may indeed have a hormonal function in this case.

Although these results are very interesting, future studies should also consider the possibility that the applied melatonin could be metabolized to IAA or an IAA agonist in plant tissues. For instance, melatonin can be converted to 5-methoxyindoleacetic acid, at least in animals [95], and this compound exhibits low auxin activity in the split pea stem bioassay [93].

Murch et al. [96] studied melatonin's effects on organogenesis in in vitro cultured stem sections of *H. perforatum*. They tested melatonin, serotonin, the auxin IAA, and several inhibitors of auxin or indoleamine action or transport. In most variants supplemented with the inhibitors (alone or combined with IAA), reduced root regeneration correlated with decreases in the levels of both IAA and melatonin. While IAA alone strongly stimulated root formation, melatonin addition to the culture medium had only minimal influence on root regeneration. Further experiments are therefore necessary before we can assess the role of melatonin in plant organogenesis, either in vitro or in vivo.

Melatonin may also have ecological functions in higher plants. Reiter et al. [97] suggested that melatonin in plants could increase the levels of this indoleamine in animals that consume such plants. Indeed, this group recently found melatonin in walnuts and showed that daytime blood levels of melatonin are markedly increased in rats after they consume walnuts [98]. Because melatonin influences many aspects of animal physiology, particularly reproduction, it might adversely affect herbivores or pests, e.g. insects. Melatonin would thus play a similar role as many secondary metabolites, especially alkaloids. The involvement of melatonin in plant–animal interactions has not been investigated so far, and remains purely speculative. However, melatonin and several related indoleamines affect insect reproduction. Pharmacological doses of melatonin suppress fertility in *Drosophila* [99]. *Drosophila melanogaster* reproduction is also severely reduced on a medium containing tryptamine or serotonin, and preference tests suggest that tryptamine might be an antiattractant for *D. melanogaster* [100]. Reproduction of sweet potato whiteflies is markedly lower on tobacco plants transformed with the gene for tryptophan decarboxylase from *Catharanthus roseus*. These plants accumulate tryptamine and either this compound or some of its metabolites are active against the insect [101].

Conclusions and perspectives

We have summarized the occurrence, possible effects and potential roles of melatonin in higher plants. First of all, we stressed how difficult it is to perform reliable determinations of melatonin levels in plant material. Further research should address the methodical problems of melatonin quantification in higher plants and should focus more on those plants in which higher levels of melatonin were found (i.e. reliably higher than detection limits of current analytical methods). Melatonin is definitely present in plants, but its functions remain unclear. It is obvious that the data now available are insufficient to make any definitive conclusions. Melatonin may influence photoperiodic reactions, such as flowering in *C. rubrum* [52], or it may act as an antioxidant [8]. We should also stay open to the possibility that in plants, melatonin can have function(s) different from those in animals. For example, it may possess some auxin-like effects [22], or act as a signal in communication with herbivores or pests. Only further research will define the roles of melatonin in plants.

In the last several years, attention was also paid to possible use of plants with high melatonin levels as a dietary supplement to increase its blood plasma levels in humans (e.g. [97]). Plant melatonin may indeed improve human health. As described above, melatonin is a potent radical scavenger and antioxidant. Melatonin seems to act protectively in some diseases, e.g. in neurodegenerative diseases [102]. Dietary intake of melatonin has been shown to increase melatonin uptake by tumor tissue and retard tumor growth [103]. Some medicinal plants, e.g. *H. perforatum*, contain high melatonin levels (see Table 1 for references). Given the current strong demand for natural healthcare products, this line of research can bring promising and perhaps commercially interesting results.

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