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REVIEW PAPER

Phytomelatonin: a review

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Abstract

Melatonin (*N*-acetyl-5-methoxytryptamine) has been detected in a number of plant species. Indeed, there exists evidence that this classically-considered animal indole is actually both synthesized in and taken up by plants. Among the actions that melatonin may carry out in plant tissues, its role as an antioxidant or growth promoter is most strongly supported by the experimental evidence. Other suggested functional implications include the co-ordination of photoperiodic responses and regulation of plant reproductive physiology, defence of plant cells against apoptosis induced by harsh environmental conditions, its participation as a free radical scavenging agent and/or up-regulator of certain protective enzymes in the senescent process. This review presents a detailed summary of the investigations that have been performed to date in the plant melatonin (phytomelatonin) field. The purpose of this summary is to bring the reader up to date on what is known about melatonin in plants and to encourage plant scientists to investigate this novel research topic; this would certainly assist in solving the numerous questions that still remain regarding the role of melatonin in plants.

Key words: Alga, angiosperm, antioxidant, growth promoter, melatonin, plant, phytomelatonin.

Introduction

Although there were some preliminary indications (Van Tassel *et al.*, 1993, 1995; Kolár and Machácková, 1994; Kolár *et al.*, 1995), the first complete publications showing that melatonin indeed existed in plants were independently provided by Dubbels *et al.* (1995) and Hattori *et al.* (1995). Since then, there has been an ever-growing number of studies reporting the detection of the indole in a variety of vegetables, cereals, fruits, seeds, and even medicinal herbs (Reiter *et al.*, 2007). In fact, numerous publications have confirmed melatonin's presence in the plant kingdom (Murch *et al.*, 1997; Manchester *et al.*, 2000; Chen *et al.*, 2003; Reiter *et al.*, 2007). Although the presence of melatonin in plants seems to be a universal phenomenon, there is still an absence of information on its occurrence in very important plant species outside the angiosperms, with the exception of the micro- and macroalgae (Balzer and Hardeland, 1996; Fuhrberg *et al.*, 1996; Balzer *et al.*, 1998; Lorenz and Lüning, 1998; Hardeland, 1999; Pape and Lüning, 2006) and other photoautotrophic microorganisms (Hardeland and Poeggeler, 2003).

The reason for this may be attributed to inefficient detection methods and the lack of experimental systems to explore the biochemical and molecular aspects of melatonin in plants. Nevertheless, in subsequent years, some methodological protocols were designed in order to obtain quick, reliable results on plant melatonin content (Cao *et al.*, 2006). In addition, extraction methods have been adjusted and optimized to the particular complexities that plant samples normally involve (Mercolini *et al.*, 2008). Although the biochemical pathways and enzymatic mechanisms of plant melatonin formation have yet to be explored, studies using radioisotope tracer techniques indicate that, in higher plants, tryptophan is the common precursor for both serotonin and melatonin as well as for indole-3-acetic acid (IAA) (Murch *et al.*, 2000). In addition, some investigations reported that plants may be able to absorb melatonin from the soil in which they are grown (Tan *et al.*, 2007*a*, *b*).

The differential synthesis of melatonin, depending on the degree of light to which the short-day plant *Chenopodium*

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rubrum L. (red goosefoot) was exposed, with the highest levels during the scotophase of the light–dark cycle, suggested that melatonin in plants may have functions analogous to those in animals. Thus, the melatonin cycle may provide information about circadian time, i.e. it may be implicated in photoperiodism in plants as well as in animals (Kolár *et al.*, 1999; Kolár and Machácková, 2001; Machácková and Krekule, 2002). Further studies, however, showed that the production of the indole increased under high UV intensities (Tettamanti *et al.*, 2000; Afreen *et al.*, 2006), with peaks occurring late in the light phase of the light–dark cycle (Tan *et al.*, 2007*a*). This prompted the conclusion that one important function of melatonin may be the scavenging of free radicals, thereby protecting plants against oxidative stress and reducing the damage of macromolecules in a manner similar to that in animals (Tan *et al.*, 2007*a*). Certainly, there are data indicating that elevated melatonin may confer higher antioxidant protection in plants (Manchester *et al.*, 2000; Reiter and Tan, 2002; Reiter *et al.*, 2005), especially in environments where free radicals cannot be detoxified enzymatically, as in the case of dry seeds (Hardeland *et al.*, 2007). Also, the morphogenetic and photoprotective actions of the indole in plants may be of importance (Hardeland, 2008). However, other functions besides the normal scope of this molecule have been proposed in plants (Fig. 1). Melatonin is seen to be an agent with auxinic activity and to promote vegetative growth similar to IAA (Hernández-Ruiz *et al.*, 2004, 2005; Afreen *et al.*, 2006; Arnao and Hernández-Ruiz, 2006, 2007; Chen *et al.*, 2008; Posmyk *et al.*, 2008).

This review is constructed to provide botanists, plant physiologists, and plant researchers in general, with a state-of-the-art view of the current knowledge of the characterization and possible functions of melatonin (phytomelatonin) in plants, in the hope of stimulating studies that will clarify the exact physiological roles that this intriguing molecule may play in plants.

Evidence of melatonin synthesis

There is little information on whether melatonin in plant products is synthesized *in situ*. However, there is a modicum of evidence suggesting that plants are equipped with the molecular machinery for melatonin biosynthesis. The first study of melatonin synthesis in any plant, *Hypericum perforatum* L. (St John's wort), was reported by Murch *et al.* (2000). The rather high quantities of the indole that the authors had previously detected in this so-called medicinal plant (Murch *et al.*, 1997) led them to investigate the potential occurrence of the melatonin biosynthetic pathway in St John's wort and to quantify the potential incorporation of radiolabel from tryptophan into auxin and indoleamine metabolites under low and supplemented light conditions.

The isotope tracer method showed that the carbon skeleton of tryptophan was incorporated into serotonin and melatonin in higher plants (Murch *et al.*, 2000). Also, under low light conditions, more radiolabelled serotonin was recovered than was melatonin; as the light intensity increased, this ratio was reversed suggesting that the metabolism of tryptophan to melatonin is influenced by light intensity.

Afreen *et al.* (2006) examined melatonin levels in *Glycyrrhyza uralensis* Fischer under lights of different spectral quality; they observed the promotion of the synthesis of melatonin in the plant roots when they were exposed to UV-B irradiation. The authors speculated that the elevated melatonin production under UV-B was an adaptive reaction of plants to tolerate these harmful rays and they surmised that the changing melatonin concentrations were a consequence of its synthesis by the plant (Afreen *et al.*, 2006).

Using a liquid chromatography electrospray ionization/tandem mass spectrometry method and deuterated-melatonin as a reference standard, Tan *et al.* (2007*a*) identified unexpectedly high melatonin levels in *Eichhornia crassipes* (Mart.) Solms (common water hyacinth), especially when they were grown under sunlight. The levels of melatonin in leaves of hyacinth under sunlight (10 000–15 000 $\mu W\ cm^{-2}$) were 48 ± 14.3 ng g^{-1} (08.00–12.00 h) and under the artificial light (400–450 $\mu W\ cm^{-2}$) they were 2.9 ± 0.64 ng g^{-1} (08.00–12.00 h), respectively. These authors also tested whether the water hyacinth had the capacity to synthesize melatonin. For this purpose, some plants were provided tryptophan, a precursor of melatonin, in the media in which the plants were grown. Melatonin levels in the plants treated with tryptophan were significantly higher than those in control plants, providing additional evidence for the conversion of tryptophan to the indoleamine in plants (Tan *et al.*, 2007*a*). Recall, however, that in contrast to the observations of Tan *et al.* (2007*a*), diurnal fluctuations of melatonin with the highest levels during the dark phase of the light–dark cycle were previously reported in the short-day plant *Chenopodium rubrum* L. (Kolár *et al.*, 1997). Therefore, further investigations are required to elucidate the actual diurnal pattern of the synthesis of melatonin in plants. Also, the particular biosynthetic and enzymatic pathways involved in melatonin formation require clarification. In this regard, the selected melatonin-rich germplasm line of St John's wort recently identified by Murch and Saxena (2006) may facilitate fundamental studies on melatonin biosynthesis and metabolism in plants.

Evidence of melatonin uptake

Whereas plants that contain melatonin may produce the indole, it is also possible that they are able to absorb melatonin from the soil or the medium in which they are grown. This supposition was mentioned prior to the first studies showing the incorporation of melatonin from the culture medium into the plant (Reiter *et al.*, 2001), taking into account that in the photoautotroph, dinoflagellate *Lingulodinium polyedrum* Stein (syn. *Gonyaulax polyedra*

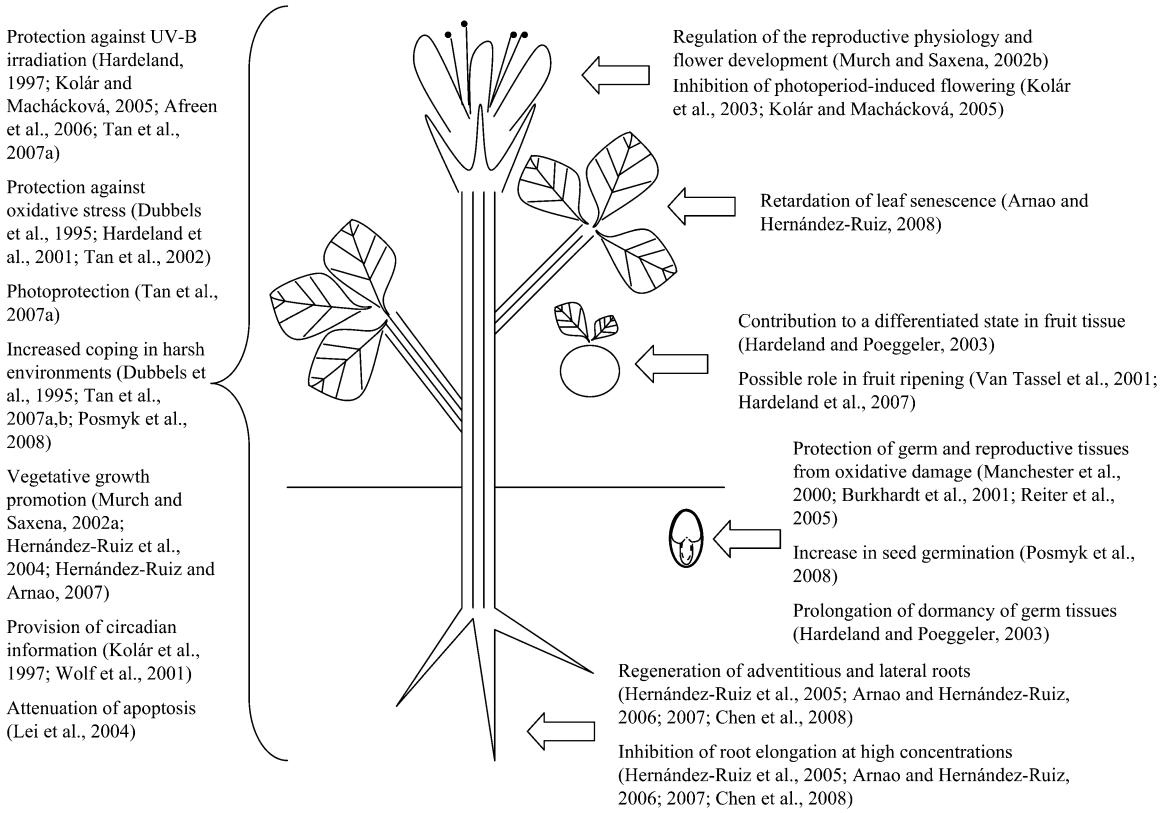

Fig. 1. Reported or proposed functions attributed to melatonin in higher plants.

Stein) exogenously added melatonin was, reportedly, rapidly taken up (Mueller and Hardeland, 1999). In fact, Burkhardt *et al.* (2001), who reported the presence of melatonin in two varieties of *Prunus cerasus* L. (tart cherry), mentioned the possibility that, besides its potential synthesis in the cherry fruit, melatonin may be absorbed through the roots and transported to the fruit. Since many microorganisms, including bacteria and fungi, contain melatonin (Manchester *et al.*, 1995; Hardeland and Poeggeler, 2003), their decomposition in the soil may release melatonin into the surrounding material and, as a result, the rootlets of the plants may absorb this product and recycle it.

Recent observations by Tan *et al.* (2007*a*, *b*) provide a certain basis for this speculation. These authors tested whether exogenous melatonin could be extracted from the growth medium by the water hyacinth and observed that, when melatonin at a concentration of 5 μM was provided in the growth medium, the melatonin levels in the leaves of hyacinth were dramatically elevated when compared to the plants which grew in media lacking additional melatonin. This documented that at least the water hyacinth has the capacity to absorb exogenous melatonin via the roots (Tan *et al.*, 2007*a*). Moreover, the application of the same dose of melatonin of the copper-polluted soil of growing *Pisum sativum* L. (pea) resulted in an increase in the survival of the plants when compared to those that had not received the melatonin-enriched solution. These findings indirectly suggested that melatonin in the water was taken up by the pea plants and protected the plants from copper toxicity (Tan *et al.*, 2007*b*). Not only are the roots able to absorb melatonin, but recent research from Arnao and Hernández-Ruiz (2008*a*) has shown that the leaves also have this capability. Thus, they observed that incubating *Hordeum vulgare* L. (barley) leaves with increasing concentrations of exogenous melatonin in the growing medium resulted in a dose-dependent accumulation of the indole in the leaves. Similar results have been reported in the cotyledons of *Lupinus albus* L. (Hernández-Ruiz and Arnao, 2008). Due to the fact that melatonin possesses both lipophilic and hydrophilic properties, it may be easy for the molecule to cross morpho- and physiological barriers with minimal difficulty, resulting in the rapid transport of the molecule into plant cells.

Melatonin in plant material

Melatonin has been detected in the roots, leaves, fruits, and seeds of a considerable variety of plant species. In the flowering plants or angiosperms, its presence has been described in a number of families belonging to both the Magnoliopsida and Liliopsida classes (see Supplementary Table S1 at *JXB* online; Table 1). Most of the genera investigated to date for their melatonin content appertain to families important in terms of their abundance in the plant kingdom or due to their high economic value (Reiter, 1999; Tettamanti *et al.*, 2000; Reiter *et al.*, 2007). These include

the Apiaceae (or Umbelliferae), Asteraceae (or Compositae), Brassicaceae (or Cruciferae), Fabaceae (or Leguminosae), Lamiaceae, Rosaceae, and Solanaceae in terms of dicotyledons; and Alliaceae, Poaceae (or Gramineae), and Zingiberaceae for the monocotyledons. Thus, melatonin may be viewed as a molecule with a widespread occurrence in the Magnoliphyta division, with the potato tuber being, to date, the only plant tissue that has been reported to be devoid of detectable levels of the indoleamine (Dubbels *et al.*, 1995; Badria, 2002).

Excluding flowering plants, the available information on the presence of melatonin is rather scarce. The other divisions comprising the superdivision Spermatophyta (Pinophyta or conifers, Cicadophyta, Ginkgophyta, and Gnetophyta) have not been examined for their melatonin content. This is also the case for the Marchantiophyta (liverworts), Anthocerotophyta (hornworts), Bryophyta (mosses), Lycopodiophyta (clubmosses), and Pteridophyta (ferns and horsetails). Algae are an exception, as melatonin has been found in members of the Chlorophyta or green algae (including genera of the unicellular organisms *Chlamydomonas* Ehrenberg, *Dunaliella* Teodoresco, or *Acetabularia* J.V.F. Lamouroux, and recently in the macroalga *Ulva lactuca* L.), as well as in the Rhodophyta [red algae; *Palmaria palmata* (L.) Kuntze, *Porphyra umbilicalis* (L.) Kützing, *Chondrus crispus* Stackhouse] and Phaeophyta [brown algae; *Pterygophora californica* Ruprecht, and *Petalonia fascia* (O.F. Müller) Kuntze] (Balzer and Hardeland, 1996; Fuhrberg *et al.*, 1996; Balzer *et al.*, 1998; Lorenz and Lüning, 1998; Hardeland, 1999; Pape and Lüning, 2006). Melatonin has also been detected in unicellular photosynthesizing organisms from the phylum Dinoflagellata, including *Lingulodinium polyedrum* Stein or *Pyrocystis acuta* Kofoid (Poeggeler *et al.*, 1991; Balzer *et al.*, 1993; Hardeland, 1993; Poeggeler and Hardeland, 1994; Hardeland and Fuhrberg, 1996; Fuhrberg *et al.*, 1997; Hardeland and Poeggeler, 2003).

Examination of reports on melatonin in plant materials show that the quantity of the indole varies widely according to the plant species studied. Levels of melatonin in the

Table 1. Scientific names, common names, and tissues of plants belonging to the class Liliopsida (monocots) where melatonin has been identified

The data are arranged by family.

Liliopsida (monocots)				
Family	**Scientific name**	**Common name**	**Tissues**	**Reference**
Alliaceae	*Allium cepa* L.	Onion	Homogenate of edible tissue	Hattori *et al.*, 1995; Badria, 2002
	Allium fistulosum L.	Welsh onion	Homogenate of edible tissue	Hattori *et al.*, 1995
	Allium sativum L.	Garlic	Homogenate of edible tissue	Badria, 2002
Araceae	*Colocasia esculenta* (L.) Schott	Taro	Homogenate of edible tissue	Hattori *et al.*, 1995
Asparagaceae	*Asparagus officinalis* L.	Asparagus	Homogenate of edible tissue	Hattori *et al.*, 1995
Bromeliaceae	*Ananas comosus* (L.) Merr.	Pineapple	Homogenate of edible tissue	Hattori *et al.*, 1995; Badria, 2002
Musaceae	*Musa ensete* J.F.Gmel.	Banana	Homogenate of edible tissue	Badria, 2002
	Musa sapientum L.	Banana	Homogenate of (edible) tissue	Dubbels *et al.*, 1995
Poaceae (Gramineae)	*Avena sativa* L.	Oat	Etiolated coleoptile, homogenate of edible tissue	Hattori *et al.*, 1995; Hernández-Ruiz *et al.*, 2005
	Festuca arundinacea Schreb.	Tall fescue	Homogenate of edible tissue	Hattori *et al.*, 1995
	Hordeum vulgare L.	Barley	Etiolated coleoptile, homogenate of edible tissue	Hattori *et al.*, 1995; Badria, 2002; Hernández-Ruiz *et al.*, 2005; Arnao and Hernández-Ruiz, 2008
	Lophatherum gracile Brongn.	Damzjuye	Dried powder[a]	Chen *et al.*, 2003
	Oryza sativa L.	Rice	Homogenate of edible tissue	Hattori *et al.*, 1995; Badria, 2002
	Phalaris canariensis L.	Canary grass	Etiolated coleoptile	Hernández-Ruiz *et al.*, 2005
	Triticum aestivum L.	Wheat	Etiolated coleoptile	Hernández-Ruiz *et al.*, 2005
	Zea mays L.	Corn	Homogenate of edible tissue	Hattori *et al.*, 1995; Badria, 2002
Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms	Common water hyacinth	Flower, leaf	Tan *et al.*, 2007
Ruscaceae	*Ophiopogon japonicus* (L. f.) Ker Gawler	Mondo grass, fountain plant	Dried powder[a]	Chen *et al.*, 2003
Zingiberaceae	*Curcuma aeruginosa* Roxb.	Erzhu	Dried powder[a]	Chen *et al.*, 2003
	Elettaria cardamomum (L.) Maton	Green cardamom	Seed	Manchester *et al.*, 2000
	Zingiber officinale Roscoe	Ginger	Homogenate of edible tissue, tuber	Hattori *et al.*, 1995; Badria, 2002; Pape and Lüning, 2006

[a] Dried powder derived from flowers, seeds, leaves, roots, and stems.

range of ng g^{-1} to μg g^{-1} tissue have normally been reported in medicinal plants, many of them indigenous to China or the Mediterranean or alpine areas (Murch *et al.*, 1997, 2004; Tettamanti *et al.*, 2000; Chen *et al.*, 2003) Seeds have also been reported to possess high levels of melatonin (typically in the ng g^{-1} tissue range), although with considerable interspecific disparity, from 189 ng g^{-1} dry seed found in *Brassica hirta* Moench (white mustard) to only 2 ng g^{-1} dry seed described for *Silybum marianum* (L.) Gaertn. (milk thistle) (Manchester *et al.*, 2000; Reiter *et al.*, 2005). The lack of central vacuoles in seed cells and, therefore, a much higher proportion of cytoplasm may be the reason for this variation. Other reasons may relate to differences in the water content of the seeds, the abiotic environment in which the plants were grown, and intrinsic genetic variability of the plants (Manchester *et al.*, 2000). Perhaps the rationale for the rather high levels of melatonin in seeds is functional, i.e. to protect the delicate and lipid-rich tissues of the embryo from oxidative stress (Manchester *et al.*, 2000; Iriti and Faoro, 2006).

Common fruits, however, seem to have a lower amount of the indoleamine (Dubbels *et al.*, 1995; Hattori *et al.*, 1995; Badria, 2002), with the exception of tart cherries (*Prunus cerasus* L.), where higher concentrations of melatonin than those measured in other fruits has been reported (13.46 ng g^{-1} and 2.06 ng g^{-1}, for the Montmorency and Balaton varieties, respectively) (Burkhardt *et al.*, 2001).

Many authors have focused on the differences in melatonin content, suggesting various hypotheses to explain the widely varying levels. The reasons proposed can be divided into those related to the extraction methods used to recover melatonin from plant material and those inherent to the particular molecular constituents of plants. Thus, whereas taking samples of animal blood or urine is simple, non-destructive, and results in relatively clean samples that can often be assayed directly for the presence of melatonin [e.g. direct radioimmunoassay (RIA) or direct injection onto a high-performance liquid chromatography (HPLC) column], plants must normally be destructively sampled by extraction followed by extensive purification before the indole products can be assayed (Van Tassel and O'Neill, 2001). These procedures usually involve a high level of oxidants being formed, such as H_2O_2 and radicals deriving from it, that can easily lead to melatonin destruction (Poeggeler and Hardeland, 1994), a phenomenon aggravated by the lack of efficient techniques to mix larger pieces of plant tissues with preserving solutions before shock-freezing, a step normally required in these determinations (Hardeland and Poeggeler, 2003). As an example, when comparing the melatonin content of fresh leaves from *Tanacetum parthenium* (L.) Schultz-Bip. (feverfew) with freeze-dried and oven-dried leaves, Murch *et al.* (1997) observed losses during the drying process of 15% and 30%, respectively. In addition, the complex chemistry of plant tissues which often contain large amounts of carbohydrates, lipids, and pigments, may induce false positives or false negatives (Van Tassel and O'Neill, 2001). This diverse range of primary and secondary metabolites, with pharmacological and antioxidant properties in many cases, may interfere with RIA or enzyme-linked immunosorbent assay determinations or co-elute with melatonin in the HPLC or other separation methods, due to the capacity of these molecules either to mimic melatonin or to cross-react within immunoassays, making chromatographic resolution difficult (Van Tassel and O'Neill, 2001; Caniato *et al.*, 2003; Hardeland and Poeggeler, 2003; Kolár and Machácková, 2005).

The physicochemical properties of melatonin have also been mentioned as a possible explanation for the above-mentioned variations in the amounts of melatonin in plant extracts. Since the indole may co-extract and partition with chlorophyll and phenolic compounds, as well as sublimate during vacuum drying and be susceptible to destruction by the impurities commonly found in organic solvents, its levels may vary artificially (Van Tassel and O'Neill, 2001). Melatonin can easily be lost by binding to glass, polyvinyl-polypyrrolidone, or nylon-membrane filters (Van Tassel, 1997).

Some authors have highlighted another difficulty related to the partitioning of melatonin in plant tissues. Since it is not clear how much of the indole resides in the large central vacuole, as compared to the cytoplasm, determinations of melatonin using fresh or dry weight (or protein) as reference values may grossly underestimate the cytoplasmic concentrations. Moreover, the possible presence of melatonin in cell walls has never been studied (Hardeland, 1997; Hardeland and Poeggeler, 2003).

Interestingly, the melatonin levels also vary within different tissues of a given plant. For example, in *Glycyrrhyza uralensis* Fischer, a medicinal plant traditionally used for its anti-viral, anti-tumour properties and also as a natural sweetener, melatonin has been detected in the root tissues of 3- and 6-month-old plants, but not in the root tissues of 1-month-old seedlings or in the stem (Afreen *et al.*, 2006). In addition, exposure to UV-B stimulated melatonin synthesis in the root tissues. Tan *et al.* (2007*a*) also found a difference in the tissues of *Eichhornia crassipes* (Mart.) Solms; the levels of melatonin and of its related metabolite, *N*1-acetyl-*N*2-formyl-5-methoxykynuramine (AFMK), were much higher in flowers than they were in leaves. An opposite pattern was reported in *Datura metel* L. (devil's trumpet), where the average concentration detected in leaves was roughly 5-fold higher than that of unopened flowers (Cao *et al.*, 2006). In the leaves of *Portulaca oleracea* L. (purslane), a vegetable consumed mainly in the eastern Mediterranean region, very high levels of melatonin in leaves have been reported (Simopoulos *et al.*, 2005).

As a further complication, studies performed in the same tissue and the same plant species have generated widely divergent data. An interesting example is represented by the species *Lycopersicon esculentum* Mill. (domesticated tomato). Thus, Hattori *et al.* (1995) reported 32.2 pg g^{-1} tissue, while Dubbels *et al.* (1995) obtained 50.6 ng 100 g^{-1} and 16.6 ng 100 g^{-1} for the cultivars 'Sweet 100' and 'Rutgers California Supreme', respectively. This shows that there exists intraspecific differences among members of this plant species. More recently, Pape and Lüning (2006)

detected a concentration of melatonin of about 1200 pg g^{-1} (fresh weight). The grade of ripeness in *Lycopersicon esculentum* Mill. seems to play a role in the final melatonin content of the plant. Van Tassel *et al.* (2001) harvested tomatoes at the mature green stage and allowed them to ripen under controlled conditions. Tissue samples from four fruits at each stage of ripeness (mature green, breaker, turning, pink, light red, and mature red) were frozen, extracted, and HPLC fractionated. They reported that mature green tissue had the lowest amounts of melatonin, whereas mature red tissue had the highest. They also obtained consistently lower values when the method of measurement was gas chromatography-mass spectrometry compared with those estimated using RIA. Similar intraspecific variations have been reported for the grape (*Vitis vinifera* L.), with Nebbiolo and Croatina cultivars having the highest melatonin concentration (0.9 ng g^{-1} and 0.8 ng g^{-1}, respectively), and the Cabernet Franc cultivar having the lowest detected values (0.005 ng g^{-1}) (Iriti *et al.*, 2006). Since the experiments with grape were performed using skins, the authors assumed that the melatonin concentration in grape seeds may be still higher, and even enhanced by the use of certain agrochemical treatments of grapevines, such as the plant defence activator benzothiadiazole (Iriti and Faoro, 2006). In the case of wine, Guerrero *et al.* (2008) showed that the concentration of melatonin in the Spanish red wines ranged around 50–80 pg ml^{-1}. Moreover, Spanish white wines exhibited slightly lower levels of melatonin than Spanish red wines. The exceptions were the Reserva type of Ribera del Duero (red wine) aged for 5 years, with an indole content of around 200 pg ml^{-1}, and sherry wine, whose concentration of melatonin was below 10 pg ml^{-1} (Guerrero *et al.*, 2008). Cultivar, agro-meteorological conditions, vintage, and wine-making procedures are among the possible reasons why the amount of melatonin present in these particular wines differ from those reported by other authors (Iriti, 2008; Mercolini *et al.*, 2008).

Studies using *Echinacea purpurea* (L.) Moench explants revealed an accumulation of significantly higher amounts of melatonin in plants exposed to thidiazuron, a synthetic cytokinin-like plant growth regulator (Jones *et al.*, 2007). Moreover, de la Puerta *et al.* (2007) also found differences in olive oil samples when they compared refined olive oil and extra-virgin olive oil registered designations of origin (D.O.), and also between the latter (for example, 71 pg ml^{-1} in D.O. Bajo Aragón and 119 pg ml^{-1} in D.O. Baena). They proposed as a possible explanation for the diversity of concentration differences in the heat-treatment or chemical processing of the product. In fact, the hydrothermal treatment of *Fagopyrum esculentum* Moench (buckwheat) seeds, followed by dehulling and milling processes, decreased the amount of melatonin by 3-fold on average (Zieliński *et al.*, 2006). Similarly in the tart cherry, as mentioned above, a strain difference was encountered; the Montmorency cherries contain approximately 6 times more melatonin than do Balaton cherries (Burkhardt *et al.*, 2001). Nevertheless, the amount of melatonin in the cherries, either Balaton or Montmorency, did not vary according to when they were harvested (July and August) or the orchards where they were grown, although the mean melatonin levels differed significantly regarding the tree where the fruits were harvested (Burkhardt *et al.*, 2001).

Another characteristic responsible for the seemingly divergent findings may be the presence, in the plant, of a circadian rhythm of melatonin synthesis. For example, in *Eichhornia crassipes* (Mart.) Solms it was observed that melatonin levels exhibited an obvious novel diurnal rhythm under natural environmental conditions, with a peak occurring late in the light phase of the light–dark cycle (Tan *et al.*, 2007*a*). Also, the melatonin-derived metabolite, AFMK, displayed a similar rhythm. These investigators proposed that the highest level of melatonin near sunset may represent the accumulated melatonin synthesized during daily sunlight exposure. When the levels of melatonin were measured in plants grown under artificial night, the concentration of the indoleamine was dramatically lower (Tan *et al.*, 2007*a*), showing that the quality of light also determines the melatonin content. In the short-flowering plant, *Chenopodium rubrum* Fischer, a rhythm of melatonin has also been reported, but contrary to the case of the common hyacinth, high values are reached during darkness, while low levels were attained during the day (Kolár *et al.*, 1997; Wolf *et al.*, 2001).

To obtain reliable results, factors such as the characteristics of the place where plants were grown, the ripening stage, the light regime, the type of tissue analysed, if phytochemicals or chemical processes were employed, the inherent molecular and circadian features of the plant, etc, seemingly have to be taken into account. In addition, and apart from the application of appropriate internal and external calibration measures, researchers are urged strictly to apply preservative conditions of extraction, as well as controlling the yield by the determination of recovery, and very importantly to obtain similar values by two methodologically different procedures (Hardeland and Poeggeler, 2003; Hardeland and Pandi-Perumal, 2005).

In this regard, and knowing the difficulty that melatonin extraction and quantification from plants involves, some analytical methodologies have recently been created in order to obtain rapid and accurate results. For example, Cao *et al.* (2006) designed a quick method for the reproducible concurrent detection and quantification of melatonin by means of chromatography-tandem mass spectrometry with electrospray ionization, atmospheric pressure chemical ionization, and atmospheric pressure photoionization. Electrospray ionization proved more reliable in routine tests to resist the effect of the sample matrix, especially for low melatonin contents. The limit of detection of melatonin in the plant extract was 5 pg ml^{-1} and the limit of quantification was 0.02 ng ml^{-1}. Other techniques have been developed to measure the melatonin in a given plant sample, adjusting the methodological steps to its characteristics. This is the case of the recently-released work by Mercolini *et al.* (2008), who have optimized an analytical method based on HPLC coupled to fluorescence detection for the determination of melatonin in red and white wine.

Also, the extraction procedures of melatonin in plant material have recently gained attention. Thus, Arnao and Hernández-Ruiz (2008*b*) evaluated the recovery rate of the indole using direct sample extraction and a homogenized sample extraction procedure. They found higher recovery rates with the direct sample extraction procedure (more than 90%) than with the homogenized sample procedure, where the percentage of recovery was around 50%.

These studies offer an optimistic view in terms of the determination of melatonin in plant materials in the future. As in the case of animals, where there is a wide range of commercial products specific for a certain species, hopefully there will soon be methods specifically designed for plants, which will take into account the characteristics of these complex matrices and, consequently, will avoid the interfering difficulties encountered by researchers in the past.

Melatonin as an antioxidant in plants

The fact that melatonin is a proven potent free-radical scavenger and a broad spectrum antioxidant in animals (Tan *et al.*, 2002) led researchers to deduce that the indole presumably acted in a similar manner in plants. This action was proposed for the first time by Dubbels *et al.* (1995), as a possible explanation for the divergent results in the melatonin content obtained in *Lycopersicon esculentum* Mill. and *Lycopersicon pimpinellifolium* (L.) Mill. (wild tomato); the latter species contains about 5-fold less melatonin than the former. The authors associated this result with the fact that the domesticated tomato is known to be more tolerant of high ozone levels than the ozone-susceptible wild strain, probably indicating a more active melatonin generating system. Also, leaves of different varieties of *Nicotiana tabacum* L. (tobacco) were differentially sensitive to ozone damage, with the sensitivity being reduced in leaves with the highest melatonin concentrations (Dubbels *et al.*, 1995). Therefore, sufficiently high melatonin levels seem to protect plants against damage from ozone, a free radical generator, which in high concentrations decreases plant height, results in visible foliar injury at all growth stages, and progressively reduces the fresh weight of fruits when applied at flowering (Dubbels *et al.*, 1995; Badria, 2002).

Another potential physiological function of melatonin related to antioxidation is speculated to be photoprotection. During photosynthesis, large quantities of free radicals or reactive oxygen species, including high levels of H_2O_2 and singlet oxygen, are generated. In addition, with increasing exposure to light during the photophase, plastidial photoprotection is diminished with impairments in the violaxanthin cycle, and a progressive destabilization of light-harvesting complexes and photosystems occurs when excessive superoxide is formed. Tan *et al.* (2007*a*) put forward the idea that the accumulated melatonin and AFMK observed during the late night phase in *Eichhornia crassipes* (Mart.) Solms which is synthesized during the day, may serve to protect against the pending free radical damage from toxic reactive oxygen and reactive nitrogen species. In addition, melatonin has also been proposed to exert photoprotective effects against UV exposure in algae and higher plants (Hardeland, 1997; Tan *et al.*, 2007*a*). This possibility is supported by an observation of Tettamanti *et al.* (2000), who showed that alpine and Mediterranean plants exposed to high UV in their natural habitat contain more melatonin that the same species living under lower UV exposure. Afreen *et al.* (2006) documented that high-intensity UV-B radiation was twice as effective as low intensity UV-B radiation in stimulating an increase in melatonin concentrations in the roots of *Glycyrrhyza uralensis* Fischer, suggesting to the authors that this radiation promotes melatonin production as a means of protecting the plant from free radicals and other oxidizing molecules that result from UV irradiation (Reiter and Tan, 2002; Kolár and Machácková, 2005). These findings support the concept that one important role of melatonin in plants may be to protect the plant from any stressful condition and to prevent injuries induced by oxidative stress at the cellular level (Hardeland *et al.*, 2001; Tan *et al.*, 2002).

The high amounts of melatonin detected in seeds have been hypothesized to be essential for protecting germ and reproductive tissues from oxidative damage due to UV light, drought, extremes in temperature, and environmental toxins (Manchester *et al.*, 2000; Burkhardt *et al.*, 2001). As an example, its presence in *Juglans regia* L. (walnut) may be to protect the highly oxidizable lipids from oxidation, thereby preserving the viability of the nut (seed) so it will subsequently germinate successfully (Reiter *et al.*, 2005). Given that nuts/seeds contain an abundance of readily oxidizable fats and since melatonin functions as an antioxidant, it was to be expected that seeds would contain the indole since reducing oxidative damage in these organs is important considering they represent the next generation (Reiter *et al.*, 2007). It has also been suggested that the amphiphility of melatonin may favour its accumulation especially in oily seeds, presumably providing antioxidative protection within a dormant and more or less dry system, in which enzymes are poorly effective and cannot be up-regulated (Hardeland *et al.*, 2007). A high content of melatonin may also aid the seed in the process of sprouting. In this respect, Cho *et al.* (2008) suggested an increased melatonin content as an explanation for the improvement of 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity in sprouting *Helianthus annuus* L. (sunflower) seeds.

Plant oxidative stress can also be initiated by contaminants in the environment (Kolár and Machácková, 2005). Since it has been shown that in adverse environments, such as cold temperature, plant melatonin production is elevated (Tan *et al.*, 2000), melatonin may assist plants in coping with harsh environmental stresses, including extremely hot or cold weather or soil and water pollutants (Tan *et al.*, 2007*a*, *b*). These characteristics may even provide these plants with utility for phytoremediation (Tan *et al.*, 2002). There are some recent studies that point in that direction. For example, Tan *et al.* (2007*b*) showed that copper

contamination of soil was lethal to *Pisum sativum* L. (pea) seeds; however, melatonin added to the soil significantly enhanced their tolerance to that metal contamination and, therefore, increased their survival. Similarly, Posmyk *et al.* (2008) found a protective effect of melatonin during hydropriming in germinating *Brassica oleracea rubrum* L. (red cabbage) seeds and young seedlings against toxic concentrations of copper. In fact, they observed in the seedlings from non-melatonin-treated seeds grown in the presence of copper, an increase in lipid peroxidation as well as an inhibition of growth while also affecting DNA endoreplication and cell division. Conversely, in seedlings from plants treated with solutions of melatonin (1 μM or 10 μM), copper-induced cell injury was inhibited (Posmyk *et al.*, 2008). In the case of *Eichhornia crassipes* (Mart.) Solms studies have shown that this plant species tolerates contamination by the phosphorus pesticide ethion (Xia and Ma, 2006), the heavy metal mercury (Riddle *et al.*, 2002), and the carcinogen arsenic (Misbahuddin and Fariduddin, 2002). This plant has been used to remove these pollutants from wastewater generated from industrial and agricultural sources (Trivedy and Pattanshetty, 2002; Jayaweera and Kasturiarachchi, 2004). Tan *et al.* (2007*a*) proposed that the rather high levels of melatonin and AFMK in the water hyacinth indicate that this plant has an elevated antioxidant capacity that may explain their high tolerance to pollutants, which are initiators of oxidative stress. Therefore, both melatonin and AFMK probably assist the water hyacinth in defending against environmental stresses caused by heavy metals or toxic chemicals.

Melatonin as a growth promoter in plants

Melatonin has been suggested to function as an auxin to promote vegetative growth in a number of plant species, although the first experiments aimed at testing this novel capacity were not successful (for a review see Kolár and Machácková, 2005). However, the observations that auxin-induced root and cytokinin-induced shoot organogenesis were inhibited by alterations in the endogenous concentration of melatonin and inhibitors of the transport of serotonin and melatonin (Murch *et al.*, 2001), led to the suggestion that melatonin may act as a potential regulator of plant growth and development (Murch and Saxena, 2002*a*). Since then, a variety of studies have confirmed this supposition. Thus, Hernández-Ruiz *et al.* (2004) incubated etiolated hypocotyls from *Lupinus albus* L. (lupin) in the presence of a range of concentrations of melatonin and IAA. Both compounds were seen to be distributed in lupin tissues in a similar concentration gradient and produced an active promotion of growth at concentrations in the micromolar range, but a growth inhibitory effect at high concentrations in intact and de-rooted lupins. When the zone of hormone synthesis was eliminated, the plants showed minimal growth, an effect that was reversed when IAA or melatonin were applied. Because the meristematic zone had been excised, the authors attributed the observed hypocotyl growth to cell expansion in the tissues, where melatonin together with IAA may play a role. Furthermore, both indoles induced the appearance of root primordia from pericycle cells, modifying the pattern of distribution of adventitious or lateral roots, the time-course, the number and length of adventitious roots, and the number of lateral roots. In this study, melatonin produced the maximum number of roots/hypocotyls with similar values to IAA for root length in practically the entire range of concentrations tested (Arnao and Hernández-Ruiz, 2006). The same group reported similar effects of melatonin in the monocotyledons *Triticum aestivum* L. (wheat), *Avena sativa* L. (oat), *Hordeum vulgare* L., and *Phalaris canariensis* L. (canary grass), i.e. an active promotion of growth and a growth inhibitory effect at high concentrations due, probably, to auxin-induced ethylene biosynthesis (Hernández-Ruiz *et al.*, 2005). Particularly, and taking the optimal degree of growth promotion obtained in coleoptiles with IAA as 100%, the optimal growth-promoting effect of melatonin was 10% for oat, 20% for wheat, 32% for the canary grass, and 55% for barley coleoptiles, which the authors regarded as a considerable auxinic effect (Hernández-Ruiz *et al.*, 2005). Similarly, the root growth ranged between 56% for the canary grass to 86% for wheat, with the growth-promoting effect in lupin tissues was up to 63% in different bioassays (Hernández-Ruiz *et al.*, 2004, 2005). Melatonin also stimulates the expansion of etiolated cotyledons of *Lupinus albus* L., to a similar extent as that observed for IAA (Hernández-Ruiz and Arnao, 2008).

Melatonin in a dose-dependent manner also promoted the vegetative growth and development of *Glycyrrhiza uralensis* Fischer (Afreen *et al.*, 2006). In fact, the results obtained by this group revealed evidence that the concentration of melatonin in their plant model increased as the plant grew, with the values in 6-month-old plants being 4-fold or even higher that those from 3-month-old plants. They also found that the concentration of melatonin recorded and the plant growth were highest in the plants grown under red light, suggesting that there was a relationship between growth and development of the plant and the concentration of the indole (Afreen *et al.*, 2006). Chen *et al.* (2008) described similar effects in *Brassica juncea* (L.) Czern. (wild leaf mustard). Here 0.1 μM melatonin showed a stimulatory effect on root growth while 100 μM was inhibitory, with the stimulatory effect only being detectable in young seedlings. Also, endogenous free levels of IAA increased at low melatonin concentrations, while at high melatonin concentrations IAA was not significantly elevated and root elongation was strongly inhibited. This led the authors to suggest that the suppressive effect exerted by melatonin on root growth seemed to involve mechanisms not related to IAA (Chen *et al.*, 2008). Interestingly, in thidiazuron-treated leaf explants of *Echinacea purpurea* (L.) Moench, concentrations of melatonin and serotonin have been shown to increase after the use of auxin transport and action inhibitors (Jones *et al.*, 2007). Moreover, the supplementation of the thidiazuron medium with lidocaine, a sodium channel blocker, resulted in elevated levels of

serotonin and melatonin but not auxin and significantly decreased the rate of thidiazuron-induced regeneration in the explants (Jones *et al.*, 2007). Taking into account that the levels of both serotonin and melatonin and also of auxin raised by exposure to thidiazuron associated with the induction of regeneration, the authors proposed that melatonin may act as a hormone independently or in concert with auxin and its own precursors and metabolites. In turn, Posmyk *et al.* (2008) found that melatonin pretreatment increased the germination of seeds from *Brassica oleracea rubrum* L. by about 17% in water and by about 12–14% in the presence of copper. The growth rates of 1 μM or 10 μM melatonin treated seedlings were also higher than those from the controls or from seeds hydroprimed with distilled water only. Since both melatonin and IAA are structurally related and indole derivatives, the authors suggested the possibility that the applied melatonin could be metabolized to IAA or an IAA agonist in plant tissue, due to the fact that, at least in animals, melatonin can be converted to 5-methoxyindolacetic acid, a compound that exhibits low auxin activity (Hardeland *et al.*, 1993; Katekar, 1997).

Melatonin's action in plants via Ca^{2+}-calmodulin may be a potential mechanism of signalling in plants in view of the Ca^{2+}-dependent activity of auxin in several physiological responses. Melatonin is known to have a high affinity for calmodulin and, moreover, it has remarkable effects on the cytoskeleton in plants (Hardeland, 1997). Melatonin causes protein-kinase Ca^{2+}-dependent inhibition, with the interaction of calmodulin-kinase being relevant to rearrangements of the cytoskeleton, which represents some of the earliest effects described for melatonin, including plants (Hardeland, 2008). In fact, melatonin was reported to stimulate the microtubule assembly in *Haemanthus katharinae* Baker while, in contrast, it caused microtubule depolymerization, disrupting the mitotic apparatus in the onion root tip (Kolár and Machácková, 2005). On the other hand, the activation of calcium channels that change cell polarity resulted in an increase in melatonin (and the related indoles auxin and serotonin) in *Echinacea purpurea* (L.) Moench explants, together with an inhibition in thidiazuron-induced callus induction (Jones *et al.*, 2007). Since, at physiological concentrations, melatonin has been proposed to antagonize the inhibition of tubulin polymerization caused by calmodulin, thereby stimulating microtubule growth, and at pharmacological levels the indole binds to tubulin preventing its polymerization (Benítez-King and Antón-Tay, 1993), these observations may possibly be related to the growth-promoting effects with low concentrations of melatonin and the inhibition provoked by high concentrations of melatonin reviewed in this section.

Other functions of melatonin in plants

Since, in animals, melatonin is generally considered to be the chemical expression of darkness (Reiter, 1991), the first studies performed in plants to elucidate the physiological role of this molecule were aimed at determining whether it would function similarly in plants. It was reasonable to assume that the melatonin rhythm in plants could be a night signal, co-ordinating responses to diurnal and photoperiodic environmental cues, such as flowering, a short-day response. This possibility was indirectly supported by finding in the short-day plant *Chenopodium rubrum* L., that melatonin exhibits a robust circadian rhythm with the peak occurring at night (Kolár *et al.*, 1997; Wolf *et al.*, 2001) and also because exogenously applied melatonin substituted for darkness in the bioluminescence response of *Lingulodinium polyedrum* Stein (Balzer and Hardeland, 1991) and the growth response of *Pterygophora californica* Ruprecht (Fuhrberg *et al.*, 1996). Thus, melatonin was shown to exert a suppressive effect on the flowering of both *Chenopodium rubrum* L. and in the long-day plant *Arabidopsis thaliana* (L.) Heynh. (Kolár *et al.*, 2003; Kolár and Machácková, 2005). In contrast to some vertebrates, where melatonin administration causes changes that mimic short-day responses, the indole application neither induced flowering after a short non-inductive night nor had effects on the period or phase of the circadian rhythm in photoperiodic sensitivity to a single night of various durations. This led the researchers to conclude that exogenous melatonin may control other early processes, for example, in *Chenopodium rubrum* L. the transition to flowering (Kolár and Machácková, 2005). Similarly, no promotion of flowering after melatonin application was found in the short-day plant *Pharbitis nil* (L.) Choisy, in the water plants *Spirodela polyrhiza* (L.) Schleid., *Lemna minor* L., and *Lemna trisulca* L. or in the long-day species *Chenopodium murale* L. (Kolár and Machácková, 2005). These authors concluded that, considering the data obtained, melatonin did not regulate daily rhythms in higher plants, although they added that some effects might be perhaps discovered in the future.

Melatonin has also been proposed to play a significant role in the regulation of the reproductive physiology and flower development of *Hypericum perforatum* L. (St John's wort) because the highest concentrations of the indole are detected during uninucleate microsporogenesis and the enhanced regenerative potential of isolated anthers at the stage of elevated melatonin contents (Murch and Saxena, 2002*b*). The authors speculated that the reproduction-related actions of melatonin described in this plant model may occur in other higher plants as well.

On the other hand, it has been reported that melatonin supplementation attenuates cold-induced apoptosis in *Daucus carota* L. (carrot) root cell suspensions in a process that does not relate to reactive oxygen species generation (Lei *et al.*, 2004). Thus, pretreatment with melatonin for 5 d significantly attenuated cold-induced apoptosis as indicated by changes in the number of TUNEL-positive cells, a reduced level of DNA fragmentation and fewer morphological changes associated with programmed cell death; these changes were accompanied by a significant elevation in the levels of the polyamines, putrescine and spermidine (Lei *et al.*, 2004). The authors suggested that the effect of

melatonin on polyamine levels may be related to some photoperiodic events. However, a possible interrelation between auxin, melatonin, and polyamines has been suggested due to the fact that classical hormones which stimulate plant development (auxins, cytokinins, and gibberellins) increase the content of polyamines. Given that melatonin has been reported to cause auxinic-like actions (see previous section), this relationship cannot be ruled out (Hernández-Ruiz *et al.*, 2005).

Melatonin might also contribute to the maintenance of dormancy germs or to a differentiated state in fruit tissue (Hardeland and Poeggeler, 2003). Elevated levels in some juicy fruits may indicate a role in fruit ripening and/or a function in the maintenance of developmental stages that may extend to the persistence of dormancy (Van Tassel *et al*, 2001; Hardeland *et al.*, 2007).

Recently, it has been reported that *Hordeum vulgare* L. leaves treated with melatonin solutions clearly slowed the senescence process, as estimated from the chrorophyll lost in leaves; this protective effect against senescence was melatonin concentration-dependent (Arnao and Hernández-Ruiz, 2008*a*). These authors suggest that melatonin may have a specific action on the chrorophyll-degrading enzymeschlorophyllase, pheophorbide α oxygenase or red-chlorophyll catabolite reductase. They also did not preclude the possibility that the indole may prevent the generation of free radicals and thereby delay the senescence process (Arnao and Hernández-Ruiz, 2008*a*).

Concluding remarks

Although melatonin has been identified in a remarkably large number of species, the percentage in terms of the entire plant kingdom is still low. The results of the investigations on phytomelatonin have uncovered several facts: melatonin is present in a wide number of plant products; the concentration of melatonin varies extremely widely in different plants; and melatonin is unequally distributed in plant parts. While many unanswered questions remain, the high levels of this indolic compound in some plant tissues (Reiter *et al.*, 2007), the identification of its biosynthetic pathway from tryptophan (Murch *et al.*, 2000), the evidence that melatonin can be taken up from soil or growing medium and incorporated into the plant tissues (Tan *et al.*, 2007*a*, *b*; Arnao and Hernández-Ruiz, 2008*a*), and the functions attributed to this molecule in plants, including its role as an antioxidant (Hardeland, 2008) or as a growth promoter (Arnao and Hernández-Ruiz, 2006) among others, strongly suggest that melatonin should be considered as a bioactive functional plant substance.

The recent development in methodological approaches that allow quick and reliable results of the presence of melatonin in plants (Cao *et al.*, 2006; Mercolini *et al.*, 2008) will undoubtedly prompt investigations into its role in plants, including confirming functions that have already been proposed and also perhaps revealing new actions. In terms of the application of beneficial plant properties to human and animal health, it will be essential to identify the importance of melatonin in edible foodstuffs. Interestingly, it has been shown that an extract of *Humulus lupulus* L. (hops) has considerable affinity for the serotonin (5-HT4e, 5-HT6, and 5-HT7) and melatonin (ML1 and ML2) receptors (Abourashed *et al.*, 2004), as well as decreasing body temperature after oral administration (Butterweck *et al.*, 2007), a typical observation reported after melatonin administration to humans. This temperature lowering response was also shown to be antagonized by the competitive melatonin antagonist lunzindole (Butterweck *et al.*, 2007). The authors suggested that the hypothermic, and therefore the sleep-inducing effects of hop extract may be mediated through the activation of melatonin receptors. These data suggest that hops may contain melatonin and that the indole present in hop tissues may be responsible of the actions described.

It is important to determine whether some of the positive health effects of plant extracts observed in humans may be exerted by melatonin and to document whether melatonin may be working synergistically with other molecules consumed in plant products. Also, tests should be performed to determine if the actions attributed to other plant compounds are, in fact, carried out by melatonin. If melatonin has health benefits when consumed in the diet, it is likely that plants could be genetically engineered to produce increased amounts of the important indole. Hopefully, in the future, the word phytomelatonin will be a common term in the plant physiology and botanical research reports.

Supplementary data

Supplementary data can be found at *JXB* online.

Table S1. Families, scientific names, common names, and plant tissues from the Magnoliopsida class (dicots) where the presence of melatonin has been described.

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