Hindawi Publishing Corporation
Advances in Botany
Volume 2014, Article ID 815769, 11 pages
http://dx.doi.org/10.1155/2014/815769

Review Article

Phytomelatonin: Discovery, Content, and Role in Plants

Marino B. Arnao

Department of Plant Biology (Plant Physiology), Faculty of Biology, University of Murcia, 30100 Murcia, Spain

Correspondence should be addressed to Marino B. Arnao; marino@um.es

Received 16 January 2014; Accepted 19 March 2014; Published 10 April 2014

Academic Editor: Scott Finlayson

Melatonin (N-acetyl-5-methoxytryptamine) is an indolic compound derived from tryptophan. Usually identified as a neurotransmitter or animal hormone, this compound was detected in plants in 1995. Interest in knowing the melatonin content of plants and its possible role therein is growing, as indicated by the increasing number of related publications. Melatonin is present in all plant species studied, with large variations in its level depending on the plant organ or tissue. It seems to be more abundant in aromatic plants and in leaves than in seeds. Regarding its physiological function in plants, melatonin shows auxin activity and is an excellent antioxidant, regulating the growth of roots, shoots, and explants, activating seed germination and rhizogenesis (lateral- and adventitious-roots), and delaying induced leaf senescence. Its ability to strengthen plants subjected to abiotic stress such as drought, cold, heat, salinity, chemical pollutants, herbicides, and UV radiation makes melatonin an interesting candidate for use as a natural biostimulating substance for treating field crops.

1. Introduction

Melatonin is a molecule endowed with a multitude of functions, particularly in mammals. This indoleamine is a hormone that acts in many physiological aspects, influencing mood, sleep, body temperature, the retina, and sexual behavior, among others. Most of these aspects are regulated through or in conjunction with the circadian clock present in animals [1–7]. In addition, melatonin is involved in numerous cellular actions as an antioxidant, acting as an excellent *in vitro* and *in vivo* free radical scavenger [8–15].

Chemically, melatonin (N-acetyl-5-methoxytryptamine) is an indolic compound derived from serotonin (5-hydroxytryptamine) (Figure 1). Both biogenic amines are synthesized from the amino acid tryptophan in a well-characterized biosynthetic pathway in animals and with some particular characteristics in plants. In plants, the auxin, indolyl-3-acetic acid (IAA), bears some resemblance to melatonin since both are indole compounds and have a common biosynthetic pathway through the compound tryptamine in the tryptophan-dependent IAA biosynthetic pathway (Figure 1).

2. Discovery of Melatonin in Plants

The presence of melatonin was described for the first time in the bovine pineal gland by Lerner and coworkers [16]. The isolated substance was able to stimulate melanin aggregation, lightening the skin of frogs and other amphibians but not of mammals. In 1959, melatonin was identified as N-acetyl-5-methoxytryptamine and, soon after, its biosynthetic pathway from tryptophan, with serotonin as intermediary, was discovered [17–19]. In 1959, melatonin was detected in humans [20] and in the 1960s and 70s, its presence was described in many mammals and vertebrates such as birds, amphibians, and fish [21–23]. In the 1980s, melatonin was discovered in invertebrates such as planarians, annelids, molluscs, insects, and crustaceans, among others [24–29].

In higher plants, the first identification of endogenous melatonin was described in 1993 by van Tassel and O'Neill in a congress communication [30]. The authors had detected melatonin by radioimmunoassay (RIA) and gas chromatography with mass spectrometry (GC-MS) in the Convolvulaceae ivy morning glory (*Pharbitis nil* L., *syn. Ipomoea nil* L.) and in tomato fruits (*Solanum lycopersicum* L.), although the results were not published extensively until 1995 [31]. Also in 1995, two papers published simultaneously demonstrated the presence of melatonin in higher plants. Dubbels and coworkers used RIA and HPLC-MS to measure the melatonin levels in extracts of *Nicotiana tabacum* L. and in five edible plants (see Table 1) [32]. Two months later another publication appeared, in which the presence of melatonin in

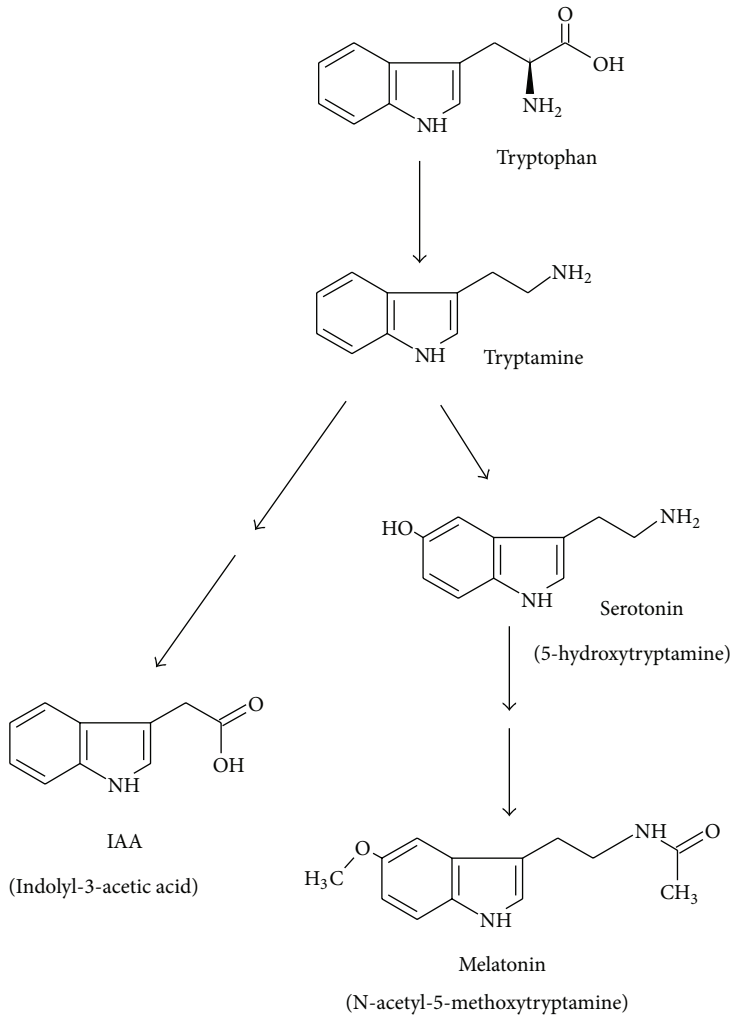

Figure 1: Molecular structure of melatonin and its related compounds in the biosynthesis pathway: tryptophan, serotonin, and tryptamine. This last compound is a precursor of the auxin indolyl-3-acetic acid, IAA.

extracts of a large number of edible plants was quantified by RIA and liquid chromatography (HPLC) with fluorescence detection (Table 1) [33]. Another communication appeared in the same year, in which a Czech research group identified the presence of melatonin in *Chenopodium rubrum* L. using liquid chromatography with mass identification (LC-MS/MS) [34]. Successive studies have quantified the presence of melatonin in many plants and it is now accepted that melatonin is present in animals, plants, and in all the other kingdoms.

3. Extraction, Detection, and Quantification of Phytomelatonin

The first challenge in phytomelatonin quantification was its correct extraction from plant extracts. However, the extraction procedures used for different matrices may pose a problem as regards the measurement of melatonin levels, whatever the subsequent quantification method followed. In higher plants, melatonin levels have been shown to vary from a few picograms up to micrograms per gram of material analyzed. One of the problems concerning the reliable measurement of phytomelatonin that has been mentioned by several authors is the difficulty involved in the extraction and recovery of melatonin in plants [35–39]. At present, several methods of phytomelatonin extraction are used. Generally, it is extracted from liquid nitrogen treated-plant tissue using organic solvents such as methanol, chloroform, or ethyl acetate. However, when aqueous extraction is used, variable or low recovery rates have been reported [37, 40, 41], and due to the amphiphilicity of the melatonin molecule, direct sample extraction procedures (without homogenization of fresh tissues) using only organic solvent are recommended. Hardeland and coworkers mentioned that extraction efficiency is a decisive factor in the global estimation of melatonin content in photoautotrophic organisms [39]. The level of melatonin in different samples was seen to be directly affected by the respective recovery rates of each extraction procedure applied. Thus, the reliability of the melatonin level data will depend on the recovery rate. According to our

Table 1: Data on the presence and content of melatonin in some edible plant organs.

Family	Common name	Specie name	Melatonin content (pg/g FW (DW) tissue)	Reference
Actinidiaceae	Kiwi fruit	*Actinidia deliciosa Liang-Ferg.*	24.4	[33]
Amaranthaceae	Beet root	*Beta vulgaris* L.	2	[32]
Araceae	Taro	*Colocasia esculenta* L.	54.6	[33]
Basellaceae	Indian spinach	*Basella alba* L.	38.7	[33]
Asparagaceae	Asparagus	*Asparagus officinalis* L.	9.5	[33]
Brassicaceae	Cabbage	*Brassica oleracea capitata* L.	107.4	[33]
	white radish	*Raphanus sativus* L.	657.2	[33]
	"	"	485,000 DW	[53]
	Chinese cabbage	*Brassica rapa* L.	112.5	[33]
	mustard seed:			
	black	*Brassica nigra* L.	129,000 DW	[100]
	white	*Brassica hirta* L.	189,000 DW	[100]
Bromeliaceae	Pineapple	*Ananas comosus* L.	36.2	[33]
	"	"	302	[101]
Asteraceae	Shungiku	*Chrysanthemum coronarium* L.	416.8	[33]
	Butterbur (fuki)	*Petasites japonicus Maxim.*	49.5	[33]
	milk thistle seed	*Silybum marianum* L.	2,000 DW	[100]
Cucurbitaceae	Cucumber fruit	*Cucumis sativus* L.	24.6	[33]
	" seed	"	11,000	[89]
Fabaceae	Alfalfa seed	*Medicago sativa* L.	16,000 DW	[100]
	Fenugreek seed	*Trigonella foenum-graecum* L.	43,000 DW	[100]
	Lupin seed	*Lupinus albus* L.	3,830	[44]
Juglandaceae	Walnut	*Juglans regia* L.	3,500 DW	[40]
Poaceae	Rice seed	*Oryza sativa* L.	1,006	[33]
	Barley seed	*Hordeum vulgare* L.	378.1	[33]
	"	"	580	[44]
	Sweet corn	*Zea mays* L.	1,366	[33]
	Oat seed	*Avena sativa* L.	1,796	[33]
	Tall fescue	*Festuca arundinacea Schreb.*	5,288	[33]
Papaveraceae	Poppy seed	*Papaver somniferum* L.	6,000 DW	[100]
Liliaceae	Onion	*Allium cepa* L.	31.5	[33]
	Welsh onion	*Allium fistulosum* L.	85.7	
Lythraceae	Pomegranate	*Punica granatum* L.	540–5,500	[102]
Musacea	Banana	*Musa acuminata* Colla	0.46	[32]
	"	"	8.9	[101]
Oleracea	Olive oil	*Olea europaea* L.	50–119 pg/mL	[103]
Rosaceae	Apple	*Malus domestica Borkh.*	47.6	[33]
	Strawberry	*Fragaria x ananassa Duch.*	12.4	[33]
	"	"	1,400–11,260	[104]
	Almond seed	*Prunus amygdalus Batsch*	39,000 DW	[100]
	Tart cherries	*Prunus cerasus* L.	1,000–19,500	[41]
	Sweet cherries	*Prunus avium* L.	6–224	[105]
	"	"	8,000–120,000	[84]
Rubiaceae	Coffee beans:			
	Robusta	*Coffea canephora Pierr.*	5.8 μg/g DW	[106]
	arabica	*Coffea arabica* L.	6.8 μg/g DW	[106]
Rutaceae	Orange juice	*Citrus x sinensis* L.	150	[101]

Table 1: Continued.

Family	Common name	Specie name	Melatonin content (pg/g FW (DW) tissue)	Reference
Umbelliferae	Carrot	*Daucus carota Hoffm.*	55.3	[33]
	Anise seed	*Pimpinella anisum* L.	7,000 DW	[100]
	Coriander seed	*Coriandrum sativum* L.	7,000 DW	[100]
	Celery seed	*Apium graveolens* L.	7,000 DW	[100]
	Linseed (flax)	*Linum usitatissimum* L.	12,000 DW	[100]
	Fennel seed	*Foeniculum vulgare* L.	28,000 DW	[100]
	Sunflower seed	*Helianthus annuus* L.	29,000 DW	[100]
Solanaceae	Tomato fruit	*Solanum lycopersicum* L.	32.2	[33]
	"	"	0.5	[32]
	"	"	2–16	[52]
	"	"	616–1,068	[37]
	"	"	4,100–114,500	[104]
	"	"	2,500	[107]
	Currant tomato	*Solanum pimpinellifolium* L.	0.1	[32]
	Bell pepper:	*Capsicum annuum* L.		
	green	"	521.4 DW	[108]
	orange	"	581.1 DW	[108]
	red	"	179.5 DW	[108]
	Wolf berry (goji)	*Lycium barbarum* L.	103,000 DW	[100]
	"	"	530,000 DW	[53]
Vitaceae	Grapevine	*Vitis vinifera* L.	5–965	[109]
	"	"	600–1,200	[110]
	"	"	3,000–18,000	[111]
	Redwine	"	140–277 pg/mL	[112]
Zingiberaceae	Cardamom seed	*Elettaria cardamomum* L.	15,000 DW	[100]
	Curcuma	*Curcuma aeruginosa Roxb.*	120,000 DW	[53]
	Ginger	*Zingiber officinale Rose*	583.7	[33]
	"	"	3.2	[37]

data, the inclusion of an ultrasonic treatment in the direct sample extraction procedure resulted in different levels of efficiency (2–20%), depending on the sample, although the inclusion of this treatment in the extraction procedure may lead to a significant increase in phytomelatonin extraction [38, 42]. Other possible problems, such as false-negative and false-positive results, overestimations, losses by destruction, coelution in chromatographic methods, cross-reactivities in immunological procedures, and so forth, have been described in the determination of melatonin in complex matrices [39]. Analysis by liquid chromatography and identification by mass spectrometry (LC-MS/MS) are the most used and recommended techniques for the detection and quantification of phytomelatonin. In this respect, LC-MS/MS with positive electrospray ionization (ESI+) and multiple reaction monitoring (MRM) is widely used. The innovative technique of liquid chromatography with time-of-flight/mass spectrometry (LC-TOF/MS) has also been applied to phytomelatonin detection. However, less specific but very sensitive techniques such as LC with electrochemical or fluorometric detection are also excellent if accompanied by identification by LC-MS/MS [36, 38, 42–49]. Unlike in animals, immunological techniques such as RIA or ELISA present serious problems in plants due to cross-reactivity with coextractives [37, 39, 50].

No exhaustive studies of the melatonin content of edible plants, taking into account variables such as soil, cultivar, growing, post-harvest conditions, and so forth have been made and the lack of contrasted results is one of the main problems in any attempt to explain the meaning of melatonin in plant material and foodstuffs. Interest has focused on measuring its levels because of possible implications for human food consumption, since melatonin from plant foods is absorbed from the gastrointestinal tract and incorporated in the blood stream. Melatonin also crosses the blood-brain barrier and the placenta, being incorporated at subcellular level in the nucleus and mitochondria. Thus, the possibility of modulating blood melatonin levels in mammals and avians through the ingestion of plant-derived foods containing high levels of phytomelatonin could be of potential interest [35, 51]. Table 1 shows the phytomelatonin content of edible plant organs such as fruits, seeds, and roots of the species analyzed to date. In general, seeds present the highest level of phytomelatonin and fruits the lowest. Thus, levels of below 50 $pg \cdot g^{-1}$ of tissue have been seen in the fruits of kiwi, cucumber, banana, apple, and strawberry, while higher levels have been observed in the seeds of mustard, alfalfa, fenugreek, and sunflower, among others (see Table 1). It is interesting to note that the analytical technique used in studies strongly influences the quantitative results. For example, studies made in the 1990's tended to find lower levels of melatonin in plants [32, 33, 52] than is possible with more recent analyses, as can be observed in Table 1 in the case of strawberry and white radish, where

quantitative differences reach up to three orders of magnitude or in the case of tomato fruit, where the values found range from 0.5 to 114,500 $pg \cdot g^{-1}$ of tissue. The explanation is the lack of control in the phytomelatonin extraction process, leading to poor recoveries, and also, in some cases, due to the use of immunological detection techniques, which give rise to large interferences and false negatives. Whatever the case, data for the melatonin content of plant foods are very scarce and no comparisons are possible, especially bearing in mind the large number of variables such as growing conditions (soil, irrigation, photoperiod, irradiance, and harvest), postharvest preservation methods, subspecies, and/or cultivars.

Although more data exist for wild plants, they are still scarce given the great diversity of plants. Table 2 shows the phytomelatonin content of the different wild species studied and in different organs (leaf, fruit, stem, root, flower, and seed). As can be seen, the phytomelatonin content ranges from 6 $pg \cdot g^{-1}$ in the shoots of *Ipomoea nil* L. to 34 $\mu g \cdot g^{-1}$ in the root of *Glycyrrhiza uralensis* Fisch, a difference of 10^6!

These great differences in phytomelatonin content have also been described in Chinese medicinal herbs, as part of a wide-ranging study of 108 common Chinese herbs, where phytomelatonin levels range from 12 $ng \cdot g^{-1}$ DW in *Gardenia jasminoides Ellis* to 2.3 $\mu g \cdot g^{-1}$ DW in *Viola philippica* Cav. [53]. Usually, the phytomelatonin content follows the pattern: leaves > seeds > roots > flowers > fruits, but in most cases data are missing for at least one organ of the plants studied. However, some studies have shown that the melatonin content in different plant organs cannot be considered homogenous. For example, Hernández-Ruiz and Arnao showed that phytomelatonin formed a concentration gradient in *Lupinus albus* L., following the pattern apical > central > basal zone for both roots and hypocotyls [44]. Curiously, this gradient pattern was similar to that observed by IAA, which suggests that both indolic compounds could play similar physiological roles in plants [54].

4. Physiological Roles of Phytomelatonin

Our studies on melatonin were initiated as a natural continuation of our research line on antioxidants in plants and plant foods. The development of new antioxidant estimation methods that can be applied to many types of plant food allowed us to ascertain the characteristics of metabolites possessing interesting antioxidant qualities, such as organic acids, phenolic acids, flavonoids (flavanols, flavanones, and anthocyanins), tocopherols, and carotenoids, among others. Some of these studies on antioxidants can be consulted in our works [55–59]. One of the most interesting groups is the indolic compounds, substances derived mainly from the amino acid tryptophan. These are very diverse and numerous in many plants, which are considered to be of medicinal interest in some cases. They are particularly of high interest because they include the compound indolyl-3-acetic acid (IAA), the most widespread auxin in plants that acts as a growth promoter, among its other physiological functions. So, in a study on the antioxidant properties of various indolic compounds, we found that melatonin had a high antioxidant capacity compared with standard antioxidants such as ascorbic acid or trolox (synthetic analog of vitamin E). Melatonin was seen to have double the antioxidant activity of ascorbic acid or trolox and approximately double that of other indoles such as indolyl-3-acetic acid, indole-3-methanol, indole-3-propionic acid, indole-3-butyric acid, and tryptophan [60].

Parallel to these studies and thanks to a collaboration with Professor J.A. Madrid of the Department of Animal Physiology at the University of Murcia, with whom we studied the influence of various factors on plasma antioxidant levels in rats and their relation with the melatonin content, our group developed a methodology to estimate melatonin in plants and to study their possible physiological role. In our first work on phytomelatonin published in 2004, we hypothesised that melatonin plays a role as plant growth regulator. Generally, the relationship between the molecular structure and biological activity is close. If we observe the molecular structure of melatonin and IAA, both are seen to have a common indole ring but their substituent on the ring is very distinct (see Figure 1). IAA has an acid group in position 3 of the indole ring, while melatonin has an N-acetyl group in position 3 and a methoxy group in position 5. However, the atomic distances between the indole ring (delocalized positive charge) and the acidic group (negative charge) of IAA or between the indole ring and the carbonyl group of melatonin are quite similar (~0.50 nm). This suggested that melatonin might simulate the action of auxin under certain conditions. There is a similar relationship between auxinic activity and synthetic compounds such as 2,4-dichlorophenoxy acetic acid and naphthalene acetic acid. Melatonin stimulates the growth of etiolated hypocotyls of *Lupinus albus* L. in a similar way as in the assay made with IAA [54]. The growth response was melatonin concentration-dependent and exogenous melatonin could replace the auxin stimuli when aerial meristem was excised. This was the first experimental data that clearly demonstrated the auxinic role of melatonin, although, several authors had previously proposed the possible role of melatonin as a plant regulator [61–63]. Our group also reported similar effects of melatonin in the Gramineae *Avena sativa, Triticum aestivum, Hordeum vulgare,* and *Phalaris canariensis* [64]. In these cases, the growth-promoting activity of melatonin reached up to 55% with respect to IAA, while showing a concentration-dependent growth inhibitory effect on the roots, in a similar way to IAA. The growth promoting effect was also observed in lupin cotyledons, which expanded in the presence of exogenous melatonin [65]. Later, the stimulatory growth effect of melatonin on *Brassica* sp. was also demonstrated [66, 67].

Melatonin was also seen to have the capacity to modulate the morphogenesis of plants, in the same way as auxin. For example, melatonin was able to induce rhizogenesis in pericycle cells from *Lupinus albus* L. This physiological role, mainly attributed to IAA, whereby lateral roots can be generated from primary roots or adventitious roots from stems, was also provoked by melatonin at a similar concentration to IAA [68]. Later, this organogenetic effect of melatonin was also demonstrated in *Cucumis sativus, Oryza sativa,* and in *Prunus cerasus* [69–71]. In this sense, recent works demonstrated

Table 2: Data on the presence and content of melatonin in wild higher plants.

Family	Common name	Specie name	Melatonin content (pg/g FW tissue*)	Reference
Adoxaceae	Laurestine	*Viburnum tinus* L.	633 L	[113]
Amaranthaceae	Red goosefoot	*Chenopodium rubrum* L.	240 S	[80]
Anacardiaceae	Pistachio	*Pistacia lentiscus* L.	581 L; 536 F	[113]
	Cyprus turpentine	*Pistacia palaestina Boiss.*	498 L	[113]
Arecaceae	Date palm	*Phoenix dactylifera* L.	469 L	[113]
Asparagaceae	Wild asparagus	*Asparagus horridus* L.	142 L	[113]
	Jew's myrtle	*Ruscus aculeatus* L.	954 L	[113]
Asteraceae	Feverfew	*Tanacetum parthenium* L.	1,3–1,7 (μg/g) L	[114]
	Yarrow	*Achillea millefolium* L.	340,000 L + S	[115]
Brassicaceae	Thale cress	*Arabidopsis thaliana* L.	480,000 L	[49]
	"	"	4,400 Se	
Caprifoliaceae	Honeysuckle	*Lonicera etrusca Santi*	521 L	[113]
Convolvulaceae	Ivy morning glory	*Ipomoea nil* L.	6 S	[52]
Ephedraceae	Joint fir	*Ephedra campylopoda* L.	178 L	[113]
Fabaceae	Chinese liquorice	*Glycyrrhiza uralensis Fisch.*	250 L; 34 (μg/g) R	[94]
	White lupin	*Lupinus albus* L.	8,000–55,000 R	[87]
	"	"	10–75,000 L	[87]
	"	"	3,830 Se	[44]
Hypericaceae	St. John's wort	*Hypericum perforatum* L.	1,8–23 (μg/g) L	[116]
	"	"	1,7 (μg/g) Fl	
Lamiaceae	Sage	*Salvia officinalis* L.	280–400 L	[110]
Lauraceae	Laurel	*Laurus nobilis* L.	8,331 L; 3,710 F	[113]
Meliaceae	White cedar	*Melia azedarach* L.	1,579 L; 585 F	[113]
Moraceae	Common fig	*Ficus carica* L.	12,915 L; 3,959 F	[113]
	Mulberries	*Morus spp.* L.	990 L	[113]
	"	"	1,000–33,000 L	[117]
Myrtaceae	Pineapple guava	*Feijoa sellowiana O.Berg*	1,529 L	[113]
	True myrtle	*Myrtus communis* L.	291 L	[113]
		Myrtus spp.	490 L	[113]
Oleaceae	Olive	*Olea europaea* L.	4,306 L; 532 F	[113]
	Mock privet	*Phillyrea latifolia*	6,337 L; 589 F	[113]
Poaceae	Rice plant	*Oryza sativa* L.	100 L; 500 S;	[70]
	"	"	200 R; 400 Fl	[118]
	Barley plant	*Hordeum vulgare* L.	500–12,000 R; 82,300 S	[64, 86]
	Canary grass	*Phalaris canariensis* L.	26,700 S	[64]
	Wheat plant	*Triticum aestivum* L.	124,700 S	[64]
	Oat plant	*Avena sativa* L.	90,600 S	[64]
Pontederiaceae	Water hyacinth	*Eicchornia crassipes Marth.*	2,900–48,000 L	[82]
Portulacaceae	Purslane	*Portulaca oleracea* L.	19,000 L	[119]
Resedaceae	Arabic desert shrub	*Ochradenus baccatus De* L.	474 L	[113]
Rhamnaceae	Mediterranean buckthorn	*Rhamnus alaternus* L.	584 L; 306 F	[113]
	"	*Rhamnus palaestina Boiss.*	1,167 L; 907 F	[113]
	Christ's thorn jujube	*Ziziphus spina-christi* L.	1,324 L	[113]
Rosaceae	Mediterranean-medlar	*Crataegus azarolus* L.	435 L	[113]
	"	*Crataegus aronia* L.	341 L	[113]
	Shrubby blackberry	*Rubus fruticosus Hegetschw.*	805 L	[113]
Rubiaceae	Narrow-leaved madder	*Rubia tenuifolia D'Urv.*	905 L	[113]
Santalaceae	Osyris	*Osyris alba* L.	844 L	[113]

Table 2: Continued.

Family	Common name	Specie name	Melatonin content (pg/g FW tissue*)	Reference
Smilacaceae	Sarsaparilla	*Smilax aspera* L.	443 L	[113]
Solanaceae	Silverleaf nightshade	*Solanum elaeagnifolium Cav.*	7,895 F	[113]
	Black nightshade	*Solanum nigrum* L.	323 F	[113]
	Tomato plant	*Solanum lycopersicum* L.	15,000–142,000 L	[95]
	"	"	1,400–33,100 S	[95]
	"	"	7,100–10,200 R	[95]
	Tobacco	*Nicotiana tabacum* L.	50 L	[32]
	Devil's trumpet	*Datura metel* L.	1,500 F; 15,000 Fl	[120]
Styracaceae	Drug snowbell	*Styrax officinalis* L.	4,069 L	[113]
Verbenaceae	Common lantana	*Lantana camara* L.	389 L	[113]

*L: leaves; F: fruits; S: shoots; R: roots; Fl: flowers; Se: seeds.

that melatonin does not mimic the auxin signaling response [72, 73].

Our research group was also the first to suggest that melatonin could play a significant role in the foliar senescence processes. The application of exogenous melatonin delays dark-induced senescence in leaves of *Hordeum vulgare* L. [74]. The action of melatonin decreasing the rate of chlorophyll degradation in senescent leaves may be related with their antioxidant capacity, as we and other authors have suggested, but may also be due to the possible regulation of certain markers of senescence, as demonstrated in leaves of apple (*Malus domestica* L.). In melatonin-treated leaves, certain ROS scavenging enzyme activities were enhanced and the upregulation of some senescence-related genes was suppressed, which indicated that melatonin does indeed act as a regulating factor in induced leaf senescence [75, 76]. In addition to its protective effect against senescence, melatonin is also able to promote photosynthetic efficiency, as has been seen in *Malus domestica* [76], *Cucumis sativus* L. seedlings [69], and in *Prunus* sp. [77]. Also, an increase in the efficiency of Photosystem II was observed in melatonin-treated cells of the fresh water Characeae *Chara australis* [78].

The possible role of melatonin as a regulator of light-dark cycles in plants has also been studied. This function has been clearly established in mammals and birds, where its role in photoperiodic regulation has been demonstrated based on the duration and timing of the melatonin signal [8, 79]. In plants, some oscillations in endogenous melatonin levels have been described in *Chenopodium rubrum* L. [34, 80, 81], *Eichhornia crassipes* (hyacinth) [82], *Vitis vinifera cv. Malbec* [83], and *Prunus* [84], pointing to its possible role as light-dark regulator. Also in the green macroalgae *Ulva* sp., a melatonin maximum at night in a long-photoperiod day has been described [85].

However, one of the most interesting aspects was the possible role of melatonin as a protective agent against abiotic stress situations in plants. The excellent properties of melatonin as a natural antioxidant against ROS/RNS and the absence of prooxidant effects have been the subject of a great deal of research. Arnao and Hernández-Ruiz demonstrated that treatment with chemical agents, such as hydrogen peroxide, sodium chloride, or zinc sulphate, stimulates the endogenous level of melatonin in *Hordeum vulgare* roots in a time- and concentration-dependent manner [86]. This increase in melatonin levels probably plays an important role in the antioxidative defense against chemical-induced stress. Also in *Lupinus albus* plants, chemical stressors such as zinc sulphate increased endogenous melatonin levels twelve-fold, possibly through the induction of some enzyme(s) of its biosynthetic pathway [87]. The negative/deleterious effects on plant of other stressors such as copper in soil [67, 88], cold [89–92], herbicides [93], drought [69, 76, 87], light/dark, and UV radiation [44, 82, 87, 94–96] can be reduced or minimized by the presence of melatonin. Generally, these stressor agents provoke an increase in endogenous melatonin levels, suggesting that melatonin may have a significant physiological role in many stress situations, strengthening the cell redox-regulated network [75, 77].

5. Conclusions and Future Perspectives

Melatonin has been seen to be involved in several physiological aspects in plants, where it acts as a circadian regulator, cytoprotector and growth promotor. It also acts in rhizogenesis, cellular expansion and stress-protection. In this respect, several reviews with summarized data can be consulted [39, 43, 45, 46, 97–99]. Currently, two aspects of phytomelatonin arouse the most interest: (i) its application as biostimulator in agriculture and (ii) its use as human natural nutraceutical. As regards the first aspect, trials with melatonin have shown that the application of exogenous melatonin to plants produces an improvement in important aspects of their development, including better adaptation to stress situations such as drought, salinity, pollutants, cold, heat, and radiation, among others. Melatonin also enhances the rate of germination and growth and plant productivity. It acts as a retardant in stress-induced leaf senescence. All this leads us to the idea that exogenous melatonin-treatment of cultivated plants or obtaining melatonin-overproducing plants might help crops resist more easily many adverse environmental conditions from which they normally suffer throughout their development. Another proposal concerns the use of plants rich in melatonin as a tool in phytoremediation techniques for the recovery of contaminated soils such as mining

operations, industrial waste, or soils containing high levels of phytochemicals due to agricultural use.

The second of the above-mentioned aspects refers to the possibility of introducing melatonin-rich plants foods into our diet. Contrary to what frequently occurs with dietary supplements, increasing blood melatonin levels through eating natural foods such as plants could be considered a healthy habit. An oral dose of melatonin of up to 1 gram per day produces no adverse effects in humans. In addition, melatonin is easily absorbed via the gastrointestinal tract. So, its use as a nutraceutical product through the intake of melatonin-rich plants seems to have a promising future as a healthy phytochemical. It would, therefore, seem a worthwhile task to search for plants with high levels of endogenous melatonin that could be used as a natural source of nutraceuticals.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

References

[1] P. M. Fuller, J. J. Gooley, and C. B. Saper, "Neurobiology of the sleep-wake cycle: sleep architecture, circadian regulation, and regulatory feedback," *Journal of Biological Rhythms*, vol. 21, no. 6, pp. 482–493, 2006.

[2] J. E. Jan, R. J. Reiter, M. B. Wasdell, and M. Bax, "The role of the thalamus in sleep, pineal melatonin production, and circadian rhythm sleep disorders," *Journal of Pineal Research*, vol. 46, no. 1, pp. 1–7, 2009.

[3] E. Maronde and J. H. Stehle, "The mammalian pineal gland: known facts, unknown facets," *Trends in Endocrinology & Metabolism*, vol. 18, no. 4, pp. 142–149, 2007.

[4] R. Hardeland, "Melatonin in aging and disease—multiple consequences of reduced secretion, options and limits of treatment," *Aging and Disease*, vol. 3, no. 2, pp. 194–225, 2012.

[5] R. J. Reiter, "Melatonin: clinical relevance," *Best Practice & Research: Clinical Endocrinology & Metabolism*, vol. 17, no. 2, pp. 273–285, 2003.

[6] M. A. Zmijewski, T. W. Sweatman, and A. T. Slominski, "The melatonin-producing system is fully functional in retinal pigment epithelium (ARPE-19)," *Molecular and Cellular Endocrinology*, vol. 307, no. 1-2, pp. 211–216, 2009.

[7] A. Slominski, D. J. Tobin, M. A. Zmijewski, J. Wortsman, and R. Paus, "Melatonin in the skin: synthesis, metabolism and functions," *Trends in Endocrinology & Metabolism*, vol. 19, no. 1, pp. 17–24, 2008.

[8] D.-X. Tan, R. Hardeland, L. C. Manchester et al., "The changing biological roles of melatonin during evolution: from an antioxidant to signals of darkness, sexual selection and fitness," *Biological Reviews*, vol. 85, no. 3, pp. 607–623, 2010.

[9] D.-X. Tan, L.-D. Chen, B. Poeggeler, L. C. Manchester, and R. J. Reiter, "Melatonin: a potent, endogenous hydroxyl radical scavenger," *Journal of Endocrinology*, vol. 1, no. 4, pp. 57–60, 1993.

[10] B. Poeggeler, R. J. Reiter, D.-X. Tan, L.-D. Chen, and L. C. Manchester, "Melatonin, hydroxyl radical-mediated oxidative damage, and aging: a hypothesis," *Journal of Pineal Research*, vol. 14, no. 4, pp. 151–168, 1993.

[11] R. J. Reiter, B. Poeggeler, D.-X. Tan, L.-D. Chen, L. C. Manchester, and J. M. Guerrero, "Antioxidant capacity of melatonin: a novel action not requiring a receptor," *Neuroendocrinology Letters*, vol. 15, no. 1-2, pp. 103–116, 1993.

[12] A. Galano, D.-X. Tan, and R. J. Reiter, "Melatonin as a natural ally against oxidative stress: a physicochemical examination," *Journal of Pineal Research*, vol. 51, no. 1, pp. 1–16, 2011.

[13] E. Gitto, D.-X. Tan, R. J. Reiter et al., "Individual and synergistic antioxidative actions of melatonin: studies with vitamin E, vitamin C, glutathione and desferrrioxamine (desferoxamine) in rat liver homogenates," *Journal of Pharmacy and Pharmacology*, vol. 53, no. 10, pp. 1393–1401, 2001.

[14] J. C. Mayo, D.-X. Tan, R. M. Sainz, M. Natarajan, S. López-Burillo, and R. J. Reiter, "Protection against oxidative protein damage induced by metal-catalyzed reaction or alkylperoxyl radicals: comparative effects of melatonin and other antioxidants," *Biochimica et Biophysica Acta*, vol. 1620, no. 1–3, pp. 139–150, 2003.

[15] R. J. Reiter, D.-X. Tan, L. C. Manchester, and W. Qi, "Biochemical reactivity of melatonin with reactive oxygen and nitrogen species: a review of the evidence," *Cell Biochemistry and Biophysics*, vol. 34, no. 2, pp. 237–256, 2001.

[16] A. B. Lerner, J. D. Case, Y. Takahashi, T. H. Lee, and W. Mori, "Isolation of melatonin, the pineal gland factor that lightens melanocytes," *Journal of the American Chemical Society*, vol. 80, no. 10, pp. 2587–2592, 1958.

[17] A. B. Lerner, J. D. Case, and R. V. Heinzelman, "Structure of melatonin," *Journal of the American Chemical Society*, vol. 81, no. 22, pp. 6084–6085, 1959.

[18] H. Weissbach, B. G. Redfield, and J. Axelrod, "Biosynthesis of melatonin: enzymic conversion of serotonin to N-acetylserotonin," *Biochimica et Biophysica Acta*, vol. 43, pp. 352–353, 1960.

[19] J. Axelrod and H. Weissbach, "Enzymatic O-methylation of N-acetylserotonin to melatonin," *Science*, vol. 131, no. 3409, p. 1312, 1960.

[20] A. B. Lerner, J. D. Case, W. Mori, and M. R. Wright, "Melatonin in peripheral nerve," *Nature*, vol. 183, no. 4678, p. 1821, 1959.

[21] J. K. Lauber, J. E. Boyd, and J. Axelrod, "Enzymatic synthesis of melatonin in avian pineal body: extraretinal response to light," *Science*, vol. 161, no. 840, pp. 489–490, 1968.

[22] P. C. Baker, W. B. Quay, and J. Axelrod, "Development of hydroxyindole-O-methyl transferase activity in eye and brain of the amphibian, Xenopus laevis," *Life Sciences*, vol. 4, no. 20, pp. 1981–1987, 1965.

[23] J. C. Fenwick, "Demonstration and effect of melatonin in fish," *General and Comparative Endocrinology*, vol. 14, no. 1, pp. 86–97, 1970.

[24] B. Vivien-Roels, P. Pévet, O. Beck, and M. Fevre-Montange, "Identification of melatonin in the compound eyes of an insect, the locust (*Locusta migratoria*), by radioimmunoassay and gas chromatography-mass spectrometry," *Neuroscience Letters*, vol. 49, no. 1-2, pp. 153–157, 1984.

[25] B. Vivien-Roels and P. Pévet, "Melatonin: presence and formation in invertebrates," *Experientia*, vol. 49, no. 8, pp. 642–647, 1993.

[26] M. Morita, F. Hall, J. B. Best, and W. Gern, "Photoperiodic modulation of cephalic melatonin in planarians," *Journal of Experimental Zoology*, vol. 241, no. 3, pp. 383–388, 1987.

[27] J. Callebert, J. Jaunay, and J. Jallon, "Control of *Drosophilla* biorhythms," in *Advances in Pineal Research*, vol. 5, pp. 81–84, John Libbey, London, UK, 1991.

[28] I. Balzer, I. R. Espínola, and B. Fuentes-Pardo, "Daily variations of immunoreactive melatonin in the visual system of crayfish," *Biology of the Cell*, vol. 89, no. 8, pp. 539–543, 1997.

[29] D. Abran, M. Anctil, and M. A. Ali, "Melatonin activity rhythms in eyes and cerebral ganglia of *Aplysia californica*," *General and Comparative Endocrinology*, vol. 96, no. 2, pp. 215–222, 1994.

[30] D. van Tassel and S. O'Neill, "Melatonin: identification of a potential dark signal in plants," in *Plant Physiology*, vol. 102, supplement 1, article 659, 1993.

[31] D. van Tassel, N. Roberts, and S. O'Neill, "Melatonin from higher plants: isolation and identification of N-acetyl-5-methoxytryptamine," *Plant Physiology*, vol. 108, supplement 2, article 101, 1995.

[32] R. Dubbels, R. J. Reiter, E. Klenke et al., "Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry," *Journal of Pineal Research*, vol. 18, no. 1, pp. 28–31, 1995.

[33] A. Hattori, H. Migitaka, M. Iigo et al., "Identification of melatonin in plants and its effects on plasma melatonin levels and binding to melatonin receptors in vertebrates," *Biochemistry and Molecular Biology International*, vol. 35, no. 3, pp. 627–634, 1995.

[34] J. Kolar, I. Machackova, H. Illnerova, E. Prinsen, W. van Dongen, and H. van Onckelen, "Melatonin in higher plant determined by radioimmunoassay and liquid chromatography-mass spectrometry," *Biological Rhythm Research*, vol. 26, pp. 406–409, 1995.

[35] R. J. Reiter, D.-X. Tan, S. Burkhardt, and L. C. Manchester, "Melatonin in plants," *Nutrition Reviews*, vol. 59, no. 9, pp. 286–290, 2001.

[36] J. Kolář and I. Macháčková, "Melatonin in higher plants: occurrence and possible functions," *Journal of Pineal Research*, vol. 39, no. 4, pp. 333–341, 2005.

[37] C. Pape and K. Lüning, "Quantification of melatonin in phototrophic organisms," *Journal of Pineal Research*, vol. 41, no. 2, pp. 157–165, 2006.

[38] J. Cao, S. J. Murch, R. O'Brien, and P. K. Saxena, "Rapid method for accurate analysis of melatonin, serotonin and auxin in plant samples using liquid chromatography-tandem mass spectrometry," *Journal of Chromatography A*, vol. 1134, no. 1-2, pp. 333–337, 2006.

[39] R. Hardeland, S. Pandi-Perumal, and B. Poeggeler, "Melatonin in plants: focus on a vertebrate night hormone with cytoprotective properties.," *Functional Plant Science & Biotechnology*, vol. 1, no. 1, pp. 32–45, 2007.

[40] R. J. Reiter, L. C. Manchester, and D.-X. Tan, "Melatonin in walnuts: influence on levels of melatonin and total antioxidant capacity of blood," *Nutrition*, vol. 21, no. 9, pp. 920–924, 2005.

[41] S. Burkhardt, D.-X. Tan, L. C. Manchester, R. Hardeland, and R. J. Reiter, "Detection and quantification of the antioxidant melatonin in Montmorency and Balaton tart cherries (*Prunus cerasus*)," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 10, pp. 4898–4902, 2001.

[42] M. B. Arnao and J. Hernández-Ruiz, "Assessment of different sample processing procedures applied to the determination of melatonin in plants," *Phytochemical Analysis*, vol. 20, no. 1, pp. 14–18, 2009.

[43] M. B. Arnao and J. Hernández-Ruiz, "The physiological function of melatonin in plants," *Plant Signaling & Behavior*, vol. 1, no. 3, pp. 89–95, 2006.

[44] J. Hernández-Ruiz and M. B. Arnao, "Distribution of melatonin in different zones of lupin and barley plants at different ages in the presence and absence of light," *Journal of Agricultural and Food Chemistry*, vol. 56, no. 22, pp. 10567–10573, 2008.

[45] S. D. Paredes, A. Korkmaz, L. C. Manchester, D.-X. Tan, and R. J. Reiter, "Phytomelatonin: a review," *Journal of Experimental Botany*, vol. 60, no. 1, pp. 57–69, 2009.

[46] D.-X. Tan, R. Hardeland, L. C. Manchester et al., "Functional roles of melatonin in plants, and perspectives in nutritional and agricultural science," *Journal of Experimental Botany*, vol. 63, no. 2, pp. 577–597, 2012.

[47] X. Huang and G. Mazza, "Application of LC and LC-MS to the analysis of melatonin and serotonin in edible plants," *Critical Reviews in Food Science and Nutrition*, vol. 51, no. 4, pp. 269–284, 2011.

[48] M. C. Garcia-Parrilla, E. Cantos, and A. M. Troncoso, "Analysis of melatonin in foods," *Journal of Food Composition and Analysis*, vol. 22, no. 3, pp. 177–183, 2009.

[49] F. Gómez, I. G. Hernández, L. D. Martinez, M. F. Silva, and S. Cerutti, "Analytical tools for elucidating the biological role of melatonin in plants by LC-MS/MS," *Electrophoresis*, vol. 34, no. 12, pp. 1749–1756, 2013.

[50] D. L. van Tassel and S. D. O'Neill, "Putative regulatory molecules in plants: evaluating melatonin," *Journal of Pineal Research*, vol. 31, no. 1, pp. 1–7, 2001.

[51] R. Reiter, D.-X. Tan, L. Manchester et al., "Melatonin in edible plants (phytomelatonin): identification, concentrations, bioavailability and proposed functions," *World Review of Nutrition and Dietetics*, vol. 97, pp. 211–230, 2007.

[52] D. L. van Tassel, N. Roberts, A. Lewy, and S. D. O'Neill, "Melatonin in plant organs," *Journal of Pineal Research*, vol. 31, no. 1, pp. 8–15, 2001.

[53] G. Chen, Y. Huo, D.-X. Tan, Z. Liang, W. Zhang, and Y. Zhang, "Melatonin in Chinese medicinal herbs," *Life Sciences*, vol. 73, no. 1, pp. 19–26, 2003.

[54] J. Hernández-Ruiz, A. Cano, and M. B. Arnao, "Melatonin: a growth-stimulating compound present in lupin tissues," *Planta*, vol. 220, no. 1, pp. 140–144, 2004.

[55] M. B. Arnao, A. Cano, and M. Acosta, "Methods to measure the antioxidant activity in plant material. A comparative discussion," *Free Radical Research*, vol. 31, pp. S89–S96, 1999.

[56] M. B. Arnao, "Some methodological problems in the determination of antioxidant activity using chromogen radicals: a practical case," *Trends in Food Science & Technology*, vol. 11, no. 11, pp. 419–421, 2000.

[57] M. B. Arnao, A. Cano, and M. Acosta, "The hydrophilic and lipophilic contribution to total antioxidant activity," *Food Chemistry*, vol. 73, no. 2, pp. 239–244, 2001.

[58] A. Cano, M. Acosta, and M. B. Arnao, "Determination of antioxidant activity: an adaptation for measurement by HPLC," in *Encyclopedia of Chromatography*, J. Cazes, Ed., pp. 1–6, Marcel Dekker Press, New York, NY, USA, 2002.

[59] M. B. Arnao and J. Hernández-Ruiz, "Recommendations for determining antioxidant activity to study redox status and plant stress," *Plant Stress*, vol. 1, pp. 32–36, 2007.

[60] A. Cano, O. Alcaraz, and M. B. Arnao, "Free radical-scavenging activity of indolic compounds in aqueous and ethanolic media," *Analytical and Bioanalytical Chemistry*, vol. 376, no. 1, pp. 33–37, 2003.

[61] S. J. Murch, S. KrishnaRaj, and P. K. Saxena, "Tryptophan is a precursor for melatonin and serotonin biosynthesis in in vitro regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants," *Plant Cell Reports*, vol. 19, no. 7, pp. 698–704, 2000.

[62] S. J. Murch and P. K. Saxena, "Melatonin: a potential regulator of plant growth and development?" *In Vitro Cellular & Developmental Biology—Plant*, vol. 38, no. 6, pp. 531–536, 2002.

[63] I. Balzer and R. Hardeland, "Metatonin in algae and higher plants. Possible new roles as a phytohormone and antioxidant," *Botanica Acta*, vol. 109, no. 3, pp. 180–183, 1996.

[64] J. Hernández-Ruiz, A. Cano, and M. B. Arnao, "Melatonin acts as a growth-stimulating compound in some monocot species," *Journal of Pineal Research*, vol. 39, no. 2, pp. 137–142, 2005.

[65] J. Hernández-Ruiz and M. B. Arnao, "Melatonin stimulates the expansion of etiolated lupin cotyledons," *Plant Growth Regulation*, vol. 55, no. 1, pp. 29–34, 2008.

[66] Q. Chen, W.-B. Qi, R. J. Reiter, W. Wei, and B.-M. Wang, "Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of *Brassica juncea*," *Journal of Plant Physiology*, vol. 166, no. 3, pp. 324–328, 2009.

[67] M. M. Posmyk, H. Kuran, K. Marciniak, and K. M. Janas, "Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations," *Journal of Pineal Research*, vol. 45, no. 1, pp. 24–31, 2008.

[68] M. B. Arnao and J. Hernández-Ruiz, "Melatonin promotes adventitious- and lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L.," *Journal of Pineal Research*, vol. 42, no. 2, pp. 147–152, 2007.

[69] N. Zhang, B. Zhao, H. J. Zhang et al., "Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* L.)," *Journal of Pineal Research*, vol. 54, no. 1, pp. 15–23, 2013.

[70] S. Park and K. Back, "Melatonin promotes seminal root elongation and root growth in transgenic rice after germination," *Journal of Pineal Research*, vol. 53, no. 4, pp. 385–389, 2012.

[71] V. N. Sarropoulou, I. N. Therios, and K. N. Dimassi-Theriou, "Melatonin promotes adventitious root regeneration in in vitro shoot tip explants of the commercial sweet cherry rootstocks CAB-6P (*Prunus cerasus* L.), Gisela 6 (*P. cerasus* × *P. canescens*), and MxM 60 (*P. avium* × *P.. mahaleb*)," *Journal of Pineal Research*, vol. 52, no. 1, pp. 38–46, 2012.

[72] R. Pelagio-Flores, E. Muñoz-Parra, R. Ortiz-Castro, and J. López-Bucio, "Melatonin regulates Arabidopsis root system architecture likely acting independently of auxin signaling," *Journal of Pineal Research*, vol. 53, no. 3, pp. 279–288, 2012.

[73] F. Koyama, T. Carvalho, E. Alves et al., "The structurally related auxin and melatonin tryptophan-derivatives and their roles in *Arabidopsis thaliana* and in the human malaria parasite *Plasmodium falciparum*," *Journal of Eukaryotic Microbiology*, vol. 60, no. 6, pp. 646–651, 2013.

[74] M. B. Arnao and J. Hernández-Ruiz, "Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves," *Journal of Pineal Research*, vol. 46, no. 1, pp. 58–63, 2009.

[75] P. Wang, L. Yin, D. Liang, C. Li, F. Ma, and Z. Yue, "Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate-glutathione cycle," *Journal of Pineal Research*, vol. 53, no. 1, pp. 11–20, 2012.

[76] P. Wang, X. Sun, C. Li, Z. Wei, D. Liang, and F. Ma, "Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple," *Journal of Pineal Research*, vol. 54, no. 3, pp. 292–302, 2013.

[77] V. Sarropoulou, K. Dimassi-Theriou, I. Therios, and M. Koukourikou-Petridou, "Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (*Prunus avium* × *Prunus cerasus*)," *Plant Physiology and Biochemistry*, vol. 61, pp. 162–168, 2012.

[78] D. Lazar, S. J. Murch, M. J. Beilby, and S. Al Khazaaly, "Exogenous melatonin affects photosynthesis in characeae *Chara australis*," *Plant Signaling & Behavior*, vol. 8, no. 3, Article ID e23279, 2013.

[79] R. J. Reiter, "The melatonin rhythm: both a clock and a calendar," *Experientia*, vol. 49, no. 8, pp. 654–664, 1993.

[80] J. Kolář, I. Macháčková, J. Eder et al., "Melatonin: occurrence and daily rhythm in *Chenopodium rubrum*," *Phytochemistry*, vol. 44, no. 8, pp. 1407–1413, 1997.

[81] K. Wolf, J. Kolář, E. Witters, W. van Dongen, H. van Onckelen, and I. Macháčková, "Daily profile of melatonin levels in *Chenopodium rubrum* L. depends on photoperiod," *Journal of Plant Physiology*, vol. 158, no. 11, pp. 1491–1493, 2001.

[82] D.-X. Tan, L. C. Manchester, P. di Mascio, G. R. Martinez, F. M. Prado, and R. J. Reiter, "Novel rhythms of N1-acetyl-N2-formyl-5- methoxykynuramine and its precursor melatonin in water hyacinth: importance for phytoremediation," *The FASEB Journal*, vol. 21, no. 8, pp. 1724–1729, 2007.

[83] H. E. Boccalandro, C. V. González, D. A. Wunderlin, and M. F. Silva, "Melatonin levels, determined by LC-ESI-MS/MS, fluctuate during the day/night cycle in *Vitis vinifera* cv Malbec: evidence of its antioxidant role in fruits," *Journal of Pineal Research*, vol. 51, no. 2, pp. 226–232, 2011.

[84] Y. Zhao, D.-X. Tan, Q. Lei et al., "Melatonin and its potential biological functions in the fruits of sweet cherry," *Journal of Pineal Research*, vol. 55, no. 1, pp. 79–88, 2012.

[85] O. Tal, A. Haim, O. Harel, and Y. Gerchman, "Melatonin as an antioxidant and its semi-lunar rhythm in green macroalga *Ulva* sp," *Journal of Experimental Botany*, vol. 62, no. 6, pp. 1903–1910, 2011.

[86] M. B. Arnao and J. Hernández-Ruiz, "Chemical stress by different agents affects the melatonin content of barley roots," *Journal of Pineal Research*, vol. 46, no. 3, pp. 295–299, 2009.

[87] M. B. Arnao and J. Hernández-Ruiz, "Growth conditions determine different melatonin levels in *Lupinus albus* L," *Journal of Pineal Research*, vol. 55, no. 2, pp. 149–155, 2013.

[88] D.-X. Tan, L. C. Manchester, P. Helton, and R. J. Reiter, "Phytoremediative capacity of plants enriched with melatonin," *Plant Signaling & Behavior*, vol. 2, no. 6, pp. 514–516, 2007.

[89] M. M. Posmyk, M. Bałabusta, M. Wieczorek, E. Sliwinska, and K. M. Janas, "Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress," *Journal of Pineal Research*, vol. 46, no. 2, pp. 214–223, 2009.

[90] X.-Y. Lei, R.-Y. Zhu, G.-Y. Zhang, and Y.-R. Dai, "Attenuation of cold-induced apoptosis by exogenous melatonin in carrot suspension cells: the possible involvement of polyamines," *Journal of Pineal Research*, vol. 36, no. 2, pp. 126–131, 2004.

[91] Y. Zhao, L.-W. Qi, W.-M. Wang, P. K. Saxena, and C.-Z. Liu, "Melatonin improves the survival of cryopreserved callus of *Rhodiola crenulata*," *Journal of Pineal Research*, vol. 50, no. 1, pp. 83–88, 2011.

[92] K. Kang, K. Lee, S. Park, Y. S. Kim, and K. Back, "Enhanced production of melatonin by ectopic overexpression of human

serotonin *N-acetyltransferase* plays a role in cold resistance in transgenic rice seedlings," *Journal of Pineal Research*, vol. 49, no. 2, pp. 176–182, 2010.

[93] S. Park, D.-E. Lee, H. Jang, Y. Byeon, Y.-S. Kim, and K. Back, "Melatonin-rich transgenic rice plants exhibit resistance to herbicide-induced oxidative stress," *Journal of Pineal Research*, vol. 54, no. 3, pp. 258–263, 2013.

[94] F. Afreen, S. M. A. Zobayed, and T. Kozai, "Melatonin in *Glycyrrhiza uralensis*: response of plant roots to spectral quality of light and UV-B radiation," *Journal of Pineal Research*, vol. 41, no. 2, pp. 108–115, 2006.

[95] M. B. Arnao and J. Hernández-Ruiz, "Growth conditions influence the melatonin content of tomato plants," *Food Chemistry*, vol. 138, no. 2-3, pp. 1212–1214, 2013.

[96] A. Conti, C. Tettamanti, M. Singaravel, C. Haldar, S. Pandi-Perumal, and G. Maestroni, "Melatonin: an ubiquitous and evolutionary hormone," in *Treatise on Pineal Gland and Melatonin*, C. Haldar, M. Singaravel, and S. K. Maitra, Eds., pp. 105–143, Science Publishers, Enfield, UK, 2002.

[97] M. M. Posmyk and K. M. Janas, "Melatonin in plants," *Acta Physiologiae Plantarum*, vol. 31, no. 1, pp. 1–11, 2009.

[98] K. M. Janas and M. M. Posmyk, "Melatonin, an underestimated natural substance with great potential for agricultural application," *Acta Physiologiae Plantarum*, vol. 35, no. 12, pp. 3285–3292, 2013.

[99] M. B. Arnao and J. Hernández-Ruiz, "Melatonin: synthesis from tryptophan and its role in higher plants," in *Amino Acids in Higher Plants, CAB Intern.*, J. DeMello, Ed., CAB International, Oxfordshire, UK, 2014.

[100] L. C. Manchester, D.-X. Tan, R. J. Reiter, W. Park, K. Monis, and W. Qi, "High levels of melatonin in the seeds of edible plants: possible function in germ tissue protection," *Life Sciences*, vol. 67, no. 25, pp. 3023–3029, 2000.

[101] M. Sae-Teaw, J. Johns, N. P. Johns, and S. Subongkot, "Serum melatonin levels and antioxidant capacities after consumption of pineapple, orange, or banana by healthy male volunteers," *Journal of Pineal Research*, vol. 55, no. 1, pp. 58–64, 2012.

[102] P. Mena, Á. Gil-Izquierdo, D. A. Moreno, N. Martí, and C. García-Viguera, "Assessment of the melatonin production in pomegranate wines," *LWT—Food Science and Technology*, vol. 47, no. 1, pp. 13–18, 2012.

[103] C. de la Puerta, M. P. Carrascosa-Salmoral, P. P. García-Luna et al., "Melatonin is a phytochemical in olive oil," *Food Chemistry*, vol. 104, no. 2, pp. 609–612, 2007.

[104] M. Stürtz, A. B. Cerezo, E. Cantos-Villar, and M. C. Garcia-Parrilla, "Determination of the melatonin content of different varieties of tomatoes (*Lycopersicon esculentum*) and strawberries (*Fragaria ananassa*)," *Food Chemistry*, vol. 127, no. 3, pp. 1329–1334, 2011.

[105] D. González-Gómez, M. Lozano, M. F. Fernández-León, M. C. Ayuso, M. J. Bernalte, and A. B. Rodríguez, "Detection and quantification of melatonin and serotonin in eight Sweet Cherry cultivars (*Prunus avium* L.)," *European Food Research and Technology*, vol. 229, no. 2, pp. 223–229, 2009.

[106] A. Ramakrishna, P. Giridhar, K. U. Sankar, and G. A. Ravishankar, "Melatonin and serotonin profiles in beans of *Coffea* species," *Journal of Pineal Research*, vol. 52, no. 4, pp. 470–476, 2012.

[107] M. Okazaki and H. Ezura, "Profiling of melatonin in the model tomato (*Solanum lycopersicum* L.) cultivar Micro-Tom," *Journal of Pineal Research*, vol. 46, no. 3, pp. 338–343, 2009.

[108] X. Huang and G. Mazza, "Simultaneous analysis of serotonin, melatonin, piceid and resveratrol in fruits using liquid chromatography tandem mass spectrometry," *Journal of Chromatography A*, vol. 1218, no. 25, pp. 3890–3899, 2011.

[109] M. Iriti, M. Rossoni, and F. Faoro, "Melatonin content in grape: myth or panacea?" *Journal of the Science of Food and Agriculture*, vol. 86, no. 10, pp. 1432–1438, 2006.

[110] P. W. Stege, L. L. Sombra, G. Messina, L. D. Martinez, and M. F. Silva, "Determination of melatonin in wine and plant extracts by capillary electrochromatography with immobilized carboxylic multi-walled carbon nanotubes as stationary phase," *Electrophoresis*, vol. 31, no. 13, pp. 2242–2248, 2010.

[111] S. Vitalini, C. Gardana, A. Zanzotto et al., "The presence of melatonin in grapevine (*Vitis vinifera* L.) berry tissues," *Journal of Pineal Research*, vol. 51, no. 3, pp. 331–337, 2011.

[112] M. I. Rodriguez-Naranjo, A. Gil-Izquierdo, A. M. Troncoso, E. Cantos, and M. C. Garcia-Parrilla, "Melatonin: a new bioactive compound in wine," *Journal of Food Composition and Analysis*, vol. 24, no. 4-5, pp. 603–608, 2011.

[113] R. Zohar, I. Izhaki, A. Koplovich, and R. Ben-Shlomo, "Phytomelatonin in the leaves and fruits of wild perennial plants," *Phytochemistry Letters*, vol. 4, no. 3, pp. 222–226, 2011.

[114] S. J. Murch, C. B. Simmons, and P. K. Saxena, "Melatonin in feverfew and other medicinal plants," *The Lancet*, vol. 350, no. 9091, pp. 1598–1599, 1997.

[115] F. Marioni, A. Bertoli, and L. Pistelli, "A straightforward procedure to biosynthesise melatonin using freshly chopped *Achillea millefolium* L. as reagent," *Phytochemistry Letters*, vol. 1, no. 2, pp. 107–110, 2008.

[116] S. J. Murch and P. K. Saxena, "A melatonin-rich germplasm line of St John's wort (*Hypericum perforatum* L.)," *Journal of Pineal Research*, vol. 41, no. 3, pp. 284–287, 2006.

[117] P. Pothinuch and S. Tongchitpakdee, "Melatonin contents in mulberry (*Morus* spp.) leaves: effects of sample preparation, cultivar, leaf age and tea processing," *Food Chemistry*, vol. 128, no. 2, pp. 415–419, 2011.

[118] S. Park, T.-N. N. Le, Y. Byeon, Y. S. Kim, and K. Back, "Transient induction of melatonin biosynthesis in rice (*Oryza sativa* L.) during the reproductive stage," *Journal of Pineal Research*, vol. 55, no. 1, pp. 40–45, 2013.

[119] A. P. Simopoulos, D.-X. Tan, L. C. Manchester, and R. J. Reiter, "Purslane: a plant source of omega-3 fatty acids and melatonin," *Journal of Pineal Research*, vol. 39, no. 3, pp. 331–332, 2005.

[120] S. J. Murch, A. R. Alan, J. Cao, and P. K. Saxena, "Melatonin and serotonin in flowers and fruits of *Datura metel* L," *Journal of Pineal Research*, vol. 47, no. 3, pp. 277–283, 2009.