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Distribution of Melatonin in Different Zones of Lupin and Barley Plants at Different Ages in the Presence and Absence of Light

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In animals, melatonin (*N*-acetyl-5-methoxytryptamine) has several physiological roles, mostly related with circadian and seasonal rhythms. In 1995, it was detected in a variety of edible plants, and it is known that melatonin from plant foods is absorbed from the gastrointestinal tract and incorporated in the blood stream. This indoleamine also crosses the blood−brain barrier and the placenta, being incorporated at the subcellular level. The possibility of modulating blood melatonin levels in mammals and avians through the ingestion of plant foodstuffs seems to be an interesting prospect. However, data concerning the melatonin content of edible plants are scarce and have not been contrasted. Obtained with very different analytical tecnhiques, in some cases inappropiate, the quantitative data show a high degree of variation. Possibly for the first time in plants, we have used liquid chromatography with time-of-flight/mass spectrometry to identify melatonin. This sophisticated technique, combined with the more commonly used liquid chromatography with fluorescence detection for melatonin quantification, has permitted us to describe the distribution of this compound in different organs and zones in plants. Also, changes in melatonin levels with age and the possible influence of a light/dark photoperiod or constant darkness on its levels are studied. The proposal, applied here to lupin (*Lupinus albus* L.) and barley (*Hordeum vulgare* L.), may also serve as a model for application to other plant foodstuffs.

KEYWORDS: Barley; growth; *Hordeum vulgare;* melatonin; LC−-fluorescence; LC−TOF/MS; lupin; *Lupinus albus*; phytomelatonin

INTRODUCTION

Melatonin (*N*-acetyl-5-methoxytryptamine), first described as a vertebrate hormone produced by the pineal gland, is a substance with a broad array of functions. It mediates the regulation of circadian and seasonal rhythms and can act in animals as an anti-inflammatory, immunomodulator and geroprotector, while showing antitumoral and anticancerigenic functions (*1−3*). Another very interesting aspect is its role as antioxidant molecule, which has been clearly demonstrated in animal cells, both in cell cultures and in in vivo systems. Melatonin acts by scavenging many types of reactive oxygen/nitrogen species (ROS/RNS) and also organic radicals, protecting cells from possible damage by a variety of radical species. In animals, the antioxidant actions of melatonin include the upregulation of antioxidative enzymes such as glutathione peroxidase, glutathione reductase, catalases, CuZnSODs, etc. Such cellular protection could be the central mechanism operating in the effects of melatonin observed in a variety of dysfunctions and diseases: cardiovascular and neurodegenerative disorders, or cancer initiation and growth, etc. (*4−8*). Many of these dysfunctions seem to be related to alterations in the regulated oscillations of the endogenous levels of melatonin (chronodisruption) and subsequently in the normal circadian rhythms.

Melatonin has also been detected in such taxa as bacteria, fungi and plants. In higher plants, it was discovered in 1995 in studies made using edible plants (*9, 10*). In these studies, its detection and quantitation was limited to edible parts and medicinal plants. However, whole plants were not systematically analyzed, and cultivation conditions were not taken into consideration. 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 the 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 could be of potential interest (*11, 12*).

In plants, melatonin has been seen to be involved in several physiological aspects, acting as circadian regulator, cytoprotector, cytoskeletal modulator and growth promotor, and in rhizogenesis, cellular expansion and stress-protection (*13−18*). In this respect, several reviews with summarized data can be consulted (*12, 19−21*).

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Since the pioneering studies of Dubbels et al. (*9*) and Hattori et al. (*10*), both in 1995, which measured melatonin level by radioimmunoassay in edible plants such as tomato, potato, onion, rice, cucumber, banana, apple, strawberry, kiwi, etc., very few data have been amassed. However, it is known that the levels of melatonin in plant foods vary considerably, from picograms to micrograms per gram of plant material. Generally, seeds and leaves present the highest level and fruits the lowest (*11, 21*). Particularly interesting studies are those looking at melatonin in seeds such as almond, sunflower, alfalfa, black and white mustard, coriander, etc. (*22*), virgin Spanish olive oil (*23*), several grape cultivars (*24*), walnuts (*25*), cherries (*26*), medicinal plants such as St. John's wort (*27*), feverfew (*28*) and medicinal herbs of Chinese origin (*29*), where the high melatonin content of some of these herbs (1−3 μg/g dry weight) is of note. However, in no case has the level of melatonin estimated been contrasted, and there has been no systematic evaluation of melatonin levels in plant foods. Also, in many cases, no clear identification of the molecule has been offered.

Reliable melatonin quantitation in plants is accompanied by methodological problems involving its extraction, recovery and measurement (*20, 21, 30, 31*). This indoleamine has been measured using liquid chromatography with electrochemical or fluorescence detection, while liquid chromatography−tandem mass spectrometry is strongly recommended for the unequivocal identification of the molecule, although even in this case matrix problems may seriously affect the quantification limits (*10, 12−16, 26, 30*). Furthermore, immunological techniques, such as RIA or ELISA, have presented serious problems for use in plants because of cross-reactivity with other metabolites of plant extracts, resulting in false-positive and overestimations of endogenous melatonin (*11, 12, 17, 20, 31*).

In this work, we study the endogenous melatonin level of two edible plants, a monocot (*Hordeum vulgare* L.) and a dicotyledon (*Lupinus albus* L.), to ascertain its distribution in different plant organs and zones. The innovative technique of liquid chromatography with time-of-flight/mass spectrometry (LC−TOF/MS) was used, possibly for first time in plants to identity melatonin, while the more commonly used technique of liquid chromatography with fluorescence detection was used to quantify melatonin in small amounts of plant tissues. In addition, changes in melatonin levels with age and the possible influence of light on its levels were studied, comparing light-grown and dark-grown plants.

MATERIALS AND METHODS

Reagents. Melatonin (N-acetyl-5-methoxytryptamine) was purchased from Acros Organics Co. (Geel, Belgium). The solvents chloroform, water and acetonitrile (HPLC grade) were obtained from Scharlau Chemie (Barcelona, Spain). The different reagents and salts (analytical grade) used were obtained from Merck (Darmstadt, Germany).

Plant material. Lupin (*Lupinus albus* L.) and barley (*Hordeum vulgare* L.) seeds were sterilized in 10% hypochlorous acid solution for 5 min, washed three-times with distilled water and then soaked in distilled water for 24 h at 24 °C in darkness. Lupin and barley plants were grown in pots (60 × 50 mm ø) with vermiculite (an inert substrate) in a controlled chamber at 24 °C and relative humidity of 50−65%. The light-grown plants were grown in a photoperiodic cycle (16 h light: 8 h dark) of 80−90 μmol photon m^{-2} s^{-1} photosynthetic photon flux density (PPFD) supplied by 36 W Sylvania-Grolux fluorescent tubes. The PPFD was measured with a Delta OHM-HD9021 quantum sensor (Padova, Italy). The dark-grown plants were grown in the same way at 24 °C but in darkness.

Melatonin measurements in different zones. To measure the melatonin content in plant samples a direct-sample extraction procedure was used. For this, 0.2−0.5 g fresh weight of tissue was cut into sections (3−5 mm) and, without homogenization, placed in vials containing 3 mL of chloroform, overnight (15 h) at 4 °C in darkness with shaking. The sections were discarded after being washed with 0.5 mL of solvent and then evaporated to dryness under vacuum using a SpeedVac ThermoSavant mod. SPD111V coupled to a refrigerated RVT400 vapor trap (USA). The dry residue was redissolved in 0.5 mL of acetonitrile, filtered (0.2 μm) and analyzed by LC with fluorescence detection. The procedures were carried out in dim artificial light. For LC−TOF/MS analysis, at least 5 g of fresh plant material was used.

These same procedures were applied to standard melatonin solutions at different concentrations (0.01 and 0.1 μM) to calculate the recovery percentage with the sample extraction procedure. A recovery rate of 95% in spiked samples was obtained and used for the final calculation of melatonin in plant tissues (*16*).

Melatonin Analysis by Liquid Chromatography with Fluorescence Detection. A Jasco liquid chromatograph (Tokyo, Japan), a Waters Spherisorb-S5 ODS2 column (250 × 4.6 mm) and a fluorescence detector Jasco FP-2020-Plus were used to measure melatonin levels. An excitation wavelength of 280 nm and an emission wavelength of 350 nm were used. The isocratic mobile phase consisted of water: acetonitrile (60:40) at a flow rate of 0.2 mL/min. The data were analyzed using the Jasco ChromPass 1.8.6 Data System Software. An in-line fluorescence spectral analysis (using the Jasco Spectra Manager Software) compared the excitation and emission spectra of standard melatonin with the corresponding peak in the samples.

Liquid Chromatography with Time-of-Flight/Mass Spectrometry (LC−TOF/MS). Identification of melatonin in plant extracts was confirmed using an Agilent 6220 Accurate-Mass TOF LC/MS equipped with an electrospray interface operating in positive ion mode. The LC system was an Agilent 1200 series consisting of two pumps and a Zorbax Rapid Resolution C_{18} (2.1 mm × 50 mm, d.f. = 1.8 μm) column at a flow rate of 0.4 mL/min. A linear gradient of 5 to 95% of acetonitrile in water with 0.1% formic acid for 15 min, was used. The injection volume was 40 μL. The following operation parameters were used for the LC−TOF/MS measurements. The capillary voltage was 2200 V, the gas temperature was 350 °C, nitrogen drying gas was set at 11 L min^{-1} and the nebulizer pressure was 45 psi. The dielectric capillary exit was 120 V (fragmentor setting) and the skimmer 60 V. The octapole DC1 was set to 35.5 V and octapole RF to 250 V. The instrument was tuned and calibrated with the standard calibration solution provided by the manufacturer with reference *m/z* of 149.02332 and 322.048121. LC−TOF/MS accurate mass spectra were recorded across the range from 140 to 350 *m/z* at a scan rate of one second per spectrum. The data recorded were processed with the Agilent MSD TOF Software.

Statistical Analysis. For the melatonin level data, differences were determined using the SPSS 10 program (SPSS Inc., Chicago), applying the LSD multiple range test to establish significant differences at $P < 0.05$.

RESULTS AND DISCUSSION

Melatonin in plant samples was determined using a fluorescence detector coupled to the LC. In the conditions described (see Materials and Methods section), melatonin eluted at 11.5 min. The melatonin peak was confirmed by (i) its retention time, (ii) detection and quantification by spiking the standard into the plant samples with nanograms and (iii) obtaining the respective excitation and emission spectra. The comparative spectral analysis showed the same peak/valley relation as observed in standard melatonin spectra. The calculation of first- and second-order derivatives for each spectrum confirmed the identity of melatonin in the corresponding eluting-peak (data not shown). A linear regression curve of standard melatonin with the equation $y = 2.06x + 3.0$ ($R^2 = 0.9990$), where y = peak area (mV·min) and x = pg of melatonin injected, was used to determine the amount of melatonin in each injection.

In previous studies, we confirmed the presence of melatonin in lupin and several poaceans (barley, oat, wheat, canary grass)

using liquid chromatography with mass−mass spectrometry (LC−MS/MS) with positive electrospray ionization (ESI+) and the multiple reaction monitoring (MRM) mode (*13−16*). In the present study, we used the innovative technique of liquid chromatography with time-of-flight/mass spectrometry (LC−TOF/MS), possibly for the first time for detecting melatonin in plant samples. **Figure 1A** shows a representative extracted ion chromatogram (EIC) at m/z 233.13000 ± 0.1 of a standard solution of melatonin. In the chromatographic conditions assayed, melatonin was detected at 6.6 min. The ESI+ accurate mass spectra showed the detection of $[M + H]^+$, $[M + Na]^+$ and $[M + K]^+$, which correspond to protonated, sodium and potassium base peak ion, respectively (**Figure 1B**). The EIC of the LC−TOF/MS analysis of plant samples point to the presence of melatonin through the detection of $[M + H]^+$ (**Figure 1C** and **1D**). The mass data analysis of lupin root samples showed an accurate mass of 232.12157 (experimental mass), which matches the calculated melatonin mass (m/z 232.12118) with an error of 1.68 ppm (**Figure 1D**). This is below the widely accepted accuracy threshold for confirming elemental compositions, established as 5 ppm (*32*). In the same way, the presence of melatonin in different zones of barley and lupin plants was confirmed (data not shown).

Figure 2 shows the levels of melatonin in roots, hypocotyls, primary leaves, and cotyledons in 6, 12 and 18 day old lupin seedlings grown in a light/dark photoperiod (**Figure 2A**) or in constant darkness (**Figure 2B**). In light-grown plants of all ages analyzed, roots presented the highest melatonin content, followed by leaves, hypocotyls and cotyledons. The levels of melatonin increased with age only in roots and leaves, with a tendency for the content (12−14 ng/g FW of melatonin) to divide evenly between both organs at 18 days. Endogenous melatonin levels in hypocotyls and cotyledons showed no statistically significant differences with age, the content remaining at 1−2 ng/g FW.

Figure 2B shows the distribution at 6 and 12 days of melatonin in etiolated lupin plants grown in darkness. In this case, the plants were not kept any longer in pots because they were no longer viable after 13−14 days in constant darkness. In these dark-grown plants, too, the roots contained most of the melatonin, with levels similar to those seen in the roots of light-grown plants. According to the data of **Figure 2A** and **2B**, light does not seem to play a significant role in the biosynthesis and accumulation of melatonin in lupin plants, at least in the growing conditions assayed.

In the case of barley plants, the melatonin content of roots and leaves grown in a light/dark photoperiod reached similar levels to lupin, with a rate of melatonin accumulation in 6 day old plants that was higher than in light-grown lupin plants, as can be seen in **Figures 3A** and **2A**. Unlike in light-grown lupin, both roots and leaves of 6 day old light-grown barley plants, presented similar level of melatonin (~8 ng/g FW), perhaps due to the lower development rate of light-grown lupin leaves with respect to light-grown barley leaves. Thus, a high degree of development could be related with a high melatonin content. As time progressed (6, 12 and 18 days old), melatonin increased in the organs, reaching a similar melatonin level as seen in light-grown lupin plants (**Figures 2A** and **3A**).

In 6 dayold dark-grown barley plants, melatonin was mainly present in roots, after which levels decreased. In 12 day old plants, leaves and roots presented statistically similar levels (**Figure 3B**). Significant differences were observed between light-grown barley and etiolated-barley plants: 12 day old light-grown plants showed a high melatonin level. The lower melatonin content of older dark-grown plants could be related with the cessation of growth due to darkness.

We also analyzed the initial melatonin content of lupin and barley seeds (**Table 1**). In lupin, hydrated seeds (after 24 h of imbibition in water) showed differing amounts of melatonin, the seed coat containing a high amount of melatonin (~37 ng/g FW), close to 90% of the total, while only 10% was contained in the cotyledons. Lupin-seed flour (from nonimbibed seeds) showed a value of ~0.5 ng/g dry weight. It seems, then, that the imbibition process itself is a relevant step in the appearance of free melatonin in lupin seeds or, at least, that it is necessary for the activation of the seed metabolism, thereby increasing considerably the melatonin content in this organ. Comparing these data with **Figure 2**, melatonin in cotyledons seems to be consumed during plant growth, falling from 3.8 in hydrated seeds to ~1.0 ng/g FW in 18 day old plants, which is the opposite of what occurrs in roots and leaves, where melatonin accumulated with age. In the case of barley, hydrated seeds and seed-flour showed the lowest melatonin content measured (**Table 1**).

To obtain a more exhaustive idea of how melatonin is distributed in plant tissues, a detailed zone-by-zone study in roots and leaves was made. **Table 2** shows the distribution of melatonin in 12 day old lupin and barley plants. In lupin, roots and hypocotyls were divided into three equal zones: apical, central and basal. In the case of the hypocotyl, the apical zone was the upper section nearest the aerial-meristematic region while the basal zone was the nearest to the hypocotyl-root division. In the case of primary roots, the apical zone was the region containing the root meristem, while the basal zone was the next to the basal-hypocotyl zone. In dark-grown and light-grown lupin plants, both the roots and the hypocotyls presented a similar distribution of melatonin, approximately half being observed in the apical zones and a quarter in each of the other zones (central and basal). Such a distribution was probably related to the different growing potential of these zones in lupin: the most apical zone, being the most actively growing zone, had the highest melatonin content. This relationship has been described previously, when a gradient between auxin and melatonin was considered (*13*). In the case of barley, the roots and leaves were divided into two zones: basal and apical. In this case, except in dark-grown roots, a roughly equal distribution (~50:50) of melatonin was observed (**Table 2**). As **Table 2** shows, levels of up to ~20 ng/g FW for lupin roots and ~13 ng/g FW for barley leaves were measured, demonstrating that substantial amounts of melatonin are accumulated in specific zones or tissues. Thus, in the determination of melatonin in plant foodstuffs, special attention should be paid to the particular distribution of melatonin in different zones or tissues.

No exhaustive studies of the melatonin content in edible plants, taking into account variables such as soil, cultivar, growing and postharvest conditions, etc., have been made, and the lack of contrasted results is one of the main problems in the attempt to explain the meaning of melatonin in plant material and foodstuffs (*12, 19−21*). However, the melatonin levels determined in this work can be compared with those provided by other authors. Using a radioimmunoassay kit (RIA) for melatonin and screening a large number of edible plants, Hattori et al. (*10*) demonstrated that chickens fed with plant products rich in melatonin show high blood melatonin levels and that the melatonin is capable of binding to melatonin receptors. Barley, rice, corn and oat showed melatonin contents of 0.37, 1.0, 1.3 and 1.8 ng/g of seed, respectively. The data for barley seed are close to our estimates (see **Table 1**), although the exact

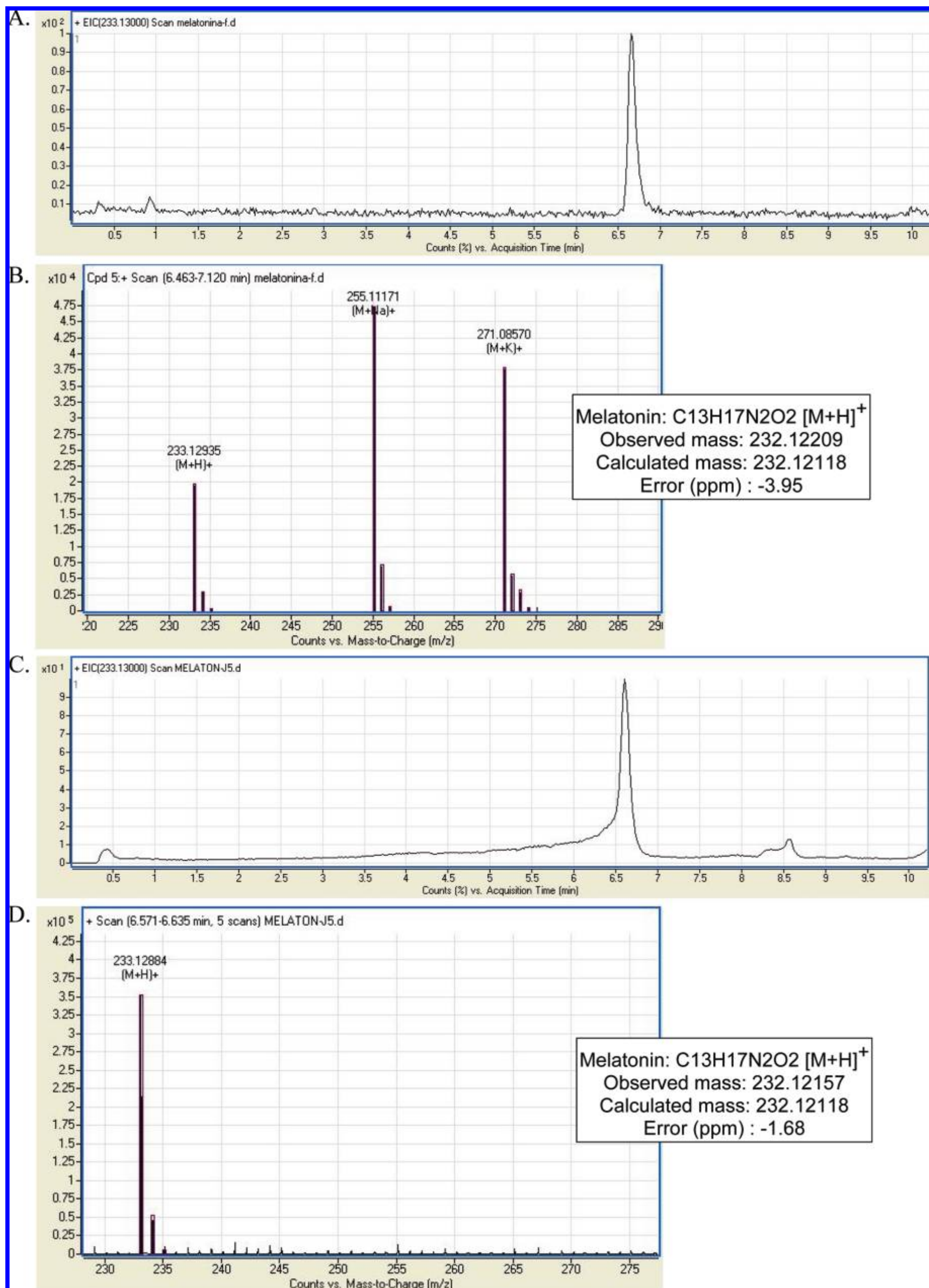

Figure 1. Representative chromatograms and mass spectra of standard melatonin and lupin root sample using liquid chromatography with time-of-flight/mass spectrometry (LC−TOF/MS). Panel **A**: Extracted ion chromatogram (EIC) at *m/z* 233.13000 of a standard solution of melatonin. Panel **B**: Accurate mass spectra of standard melatonin solution showing different derivates of the base peak ion with the mass data analysis of protonated $(M + H)^{+}$. Panel **C**: EIC as in (**A**) of a lupin root sample. Panel **D**: Protonated base peak ion of melatonin in lupin root sample and their corresponding accurate mass analysis.

sample type (fresh, dry, entire seed, flour) used by the above authors is not clear. Another specific study on seeds, using RIA and LC with coulometric-array-electrochemical detection, was developed by Manchester et al. (*22*), who analyzed 15 types of edible seeds such as anise, coriander, celery, flax, alfalfa, fennel, sunflower and mustard, among others. Black and white mustard

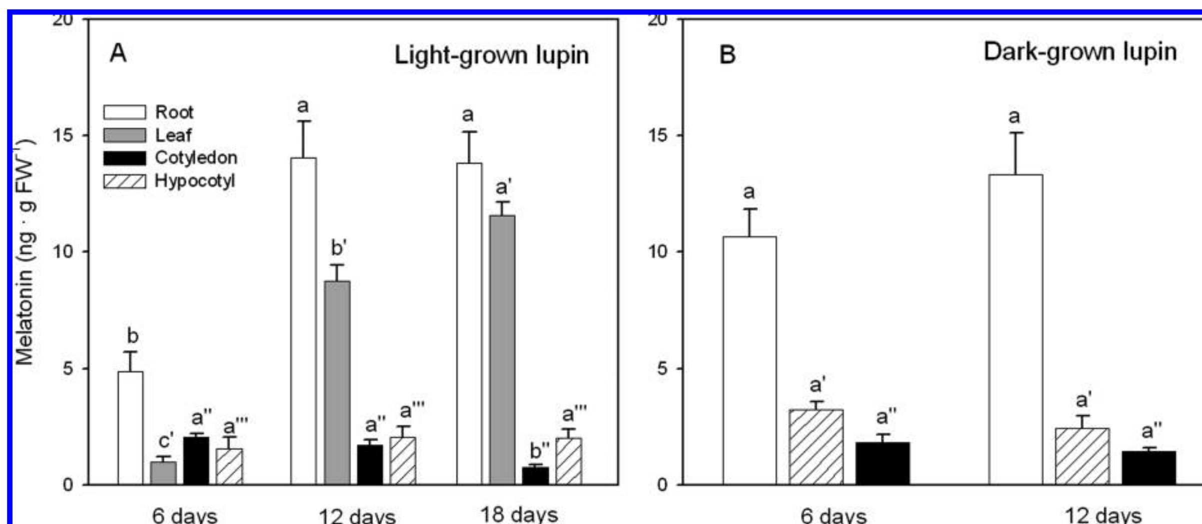

Figure 2. Distribution of endogenous melatonin in different organs (root, leaf, cotyledon and hypocotyl) of 6, 12 and 18 day old lupin plants. Panel **A**: Lupin plants grown in a photoperiodic cycle of 16 h light/8 h dark. Panel **B**: Lupin plants grown in constant darkness. Error bars represent standard errors of the mean ($n = 12$). Different superscript letters, for each organ, indicate statistically significant differences at $P < 0.05$.

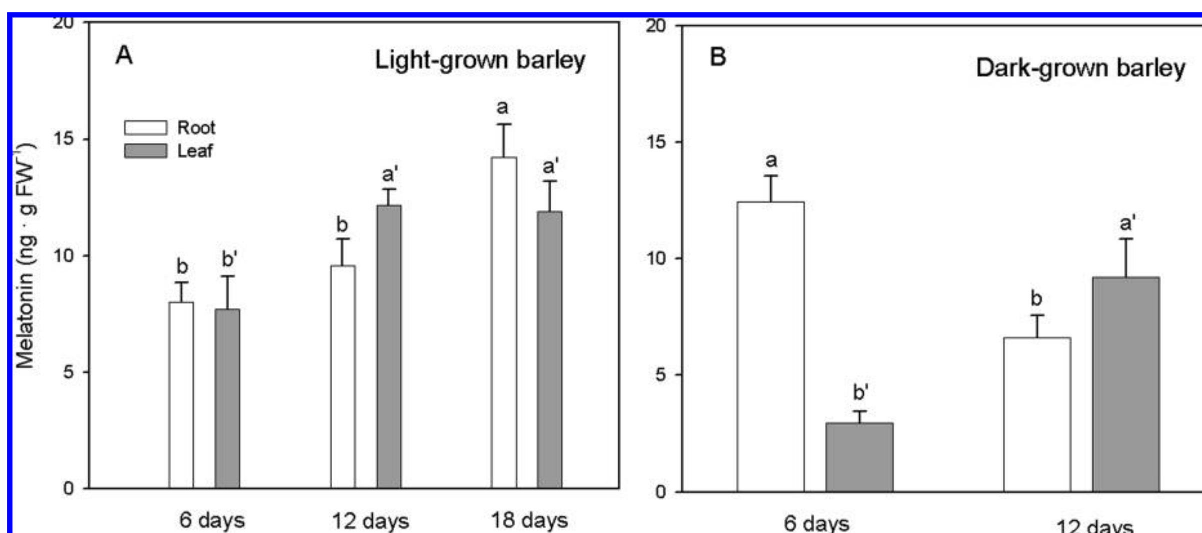

Figure 3. Distribution of endogenous melatonin in different organs (root and leaf) of 6, 12 and 18 day old barley plants. Panel **A**: Barley plants grown in a photoperiodic cycle of 16 h light/8 h dark. Panel **B**: Barley plants grown in constant darkness. Error bars represent standard errors of the mean ($n = 12$). Different superscript letters, for each organ, indicate statistically significant differences at $P < 0.05$.

Table 1. Melatonin Content in Hydrated Seeds and Seed Flour of Lupin and Barley

species	melatonin[a] (ng/g FW or DW)
Lupinus albus	
seed-cotyledons	3.83 ± 0.21 b (FW)
seed-coat	37.50 ± 2.30 a (FW)
seed-flour	0.53 ± 0.04 (DW)
Hordeum vulgare	
seed	0.58 ± 0.05 (FW)
seed-flour	0.09 ± 0.01 (DW)

[a] Values are mean ± SE ($n = 5$). Values followed by different letters are significantly different at $P < 0.05$.

presented the higher melatonin levels (130−190 ng/g DW), while most presented values below to 15 ng/g DW. The high melatonin levels measured in seeds led the authors to propose a protective function for melatonin in plant germ tissues. Another interesting study by Reiter et al. (*25*) demonstrated that the melatonin contained in walnuts (∼3.5 ng/g DW) can increase blood melatonin concentrations, as reflected in the increased antioxidant capacity of serum in rats.

For vegetables and fruits, data are scarce. Hattori et al. (*10*) and Dubbels et al. (*9*), using RIA kits, analyzed the edible part of tomatoes, cucumber, spinach, cabbage, radish, onion, ginger, carrot, among others, finding melatonin contents of between 0.02−0.62 ng/g FW. In the fruits analyzed (banana, strawberry, kiwi, apple, pineapple), no more than 0.05 ng/g of fruit was found. Cherry, with between 1 and 20 ng/g FW, depending on the variety and orchard, is possibly the fruit with highest melatonin content (*26*). Several varieties of grapes and wines presented melatonin contents below 1 ng/g FW (*24*). Also, in a study made in Spanish virgin olive oils, values between 0.05−0.12 ng/mL of melatonin were detected (*23*).

These low melatonin contents in vegetables and fruits contrast with the high melatonin levels of some medicinal plants. One of most studied medicinal plant is St. John's wort (*Hypericum perforatum* L.), in which a melatonin content of 1,750 and 4,400 ng/g FW in leaves and flower, respectively, was detected using LC with electrochemical detection (*27, 28*). Other interesting medicinal plants such as feverfew (*Tanacetum parthenium* L.) and *Scutellaria biacalensis* presented melatonin contents of

Table 2. Distribution of melatonin in different organs and zones of 12 day-old light-grown and dark-grown barley and lupin plants.

species/organ/zone	dark-grown melatonin[a] (ng/g FW)	dark-grown % in organ[b]	light-grown melatonin (ng/g FW)	light-grown % in organ
Lupinus albus				
hypocotyl				
apical	3.54 ± 0.30 a	48.8	3.10 ± 0.24 c	50.5
central	1.83 ± 0.12 b	25.3	1.89 ± 0.20 b	30.9
basal	1.89 ± 0.18 b	25.9	1.14 ± 0.10 d	18.5
root				
basal	5.94 ± 0.61 d	14.9	8.46 ± 0.80 c	20.0
central	14.04 ± 0.78 b	35.1	15.15 ± 1.47 b	35.8
apical	19.98 ± 1.70 a	50.0	18.69 ± 1.62 a	44.2
Hordeum vulgare				
leaf				
apical	9.61 ± 0.79 c	52.1	10.92 ± 0.83 b	45.0
basal	8.82 ± 0.71 d	47.9	13.34 ± 1.10 a	55.0
root				
basal	5.46 ± 0.50 c	27.4	8.55 ± 0.82 b	44.5
apical	9.61 ± 0.89 b	72.7	10.62 ± 0.99 a	55.4

[a] Values are mean ± SE ($n = 9$). Values followed by different letters, for each species and organ, are significantly different at $P < 0.05$. [b] Distribution in percentage was calculated taking into account that the different organs were divided in equal sizes.

between 2,000 and 7,000 ng/g (*28*). Chen et al. published an extensive screening of 108 common Chinese medicinal herbs (*29*) using LC−fluorescence and LC−MS/MS. Ten of the plants had a melatonin content of between 1,000 and 3,800 ng/g DW, and 64 herbs had more than 10 ng/g DW. In other cases, such as in *Glycyrrhiza uralensis*, a surprising 80,000 ng/g FW of melatonin has been described (*33*).

As regards specific organs, the melatonin contained in different parts of entire plants (leaf, root, fruit, seed) has been analyzed in very few species, among then *Hypericum perforatum (27), Eichhornia crassipes (18) and Pharbitis nill (17)*. However, in no case was a detailed analysis made, although, in general, higher melatonin levels were observed in leaves and roots, as they were in the present study.

To summarize, the data presented in this study represent an attempt to minimize the more widespread factor that occurs observing whole data on melatonin content in plants, its high variability. The data obtained by screening melatonin content in lupin and barley plants grown in different conditions (light/dark photoperiod and constant darkness) are based on a solid methodological support involving (i) clear identification of melatonin using LC−TOF/MS, (ii) accurate melatonin quantification in plant samples using LC−fluorescence, and (iii) high melatonin recovery rates (95%). Melatonin quantification in different organs and zones showed a pronounced distribution gradient, especially in roots. This experimental approximation (or similar ones) can be used to obtain more accurate data on melatonin levels in edible plants, and to provide a basis for contrasting data, an indispensable step. Only if this is done will we be able to establish the real importance of plant melatonin, so-called phytomelatonin (*12*), in the diet and in chronodisruption.

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