

Determining diurnal and seasonal changes in melatonin and tryptophan contents of eggplant (*Solanum melongena* L.)

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Abstract: The objective of this study was to simultaneously determine changes in tryptophan and melatonin contents of eggplant seedlings at two different growth stages during a 24-h period. Variations in tryptophan and melatonin levels in different organs (roots, leaves, fruits, and seeds) and the distribution and accumulation of these molecules in these organs during various growth stages were also investigated. In eggplant seedlings, melatonin showed a strong peak at the beginning of the dark period, while tryptophan levels were at the lowest. Melatonin was also high in the middle of the light period and an inverse relationship was observed between melatonin and tryptophan levels. These results indicated that melatonin fluctuations in eggplant seedlings are likely the result of the combined effects of endogenous and environmental factors. Melatonin was found in high quantities in seedlings and mature seeds, and its concentration in leaves and roots decreased dramatically as the growth stages progressed. On the other hand, tryptophan content in leaves and roots increased markedly as the growth season progressed, and the highest tryptophan levels were detected in roots of older plants, flowers, and fruits at mature seed stage. Significant seasonal variations in melatonin levels suggest that melatonin may play a key role in eggplant developmental processes.

Key words: Circadian rhythm, eggplant, HPLC-FD, growth stage, melatonin, tryptophan

1. Introduction

Melatonin, also known as N-acetyl-5-methoxytryptamine, was first isolated from the bovine pineal gland in the 1950s as an important animal hormone (Lerner et al., 1958). Since then, it was found in varying amounts in almost all life forms including eukaryotic unicellular organisms, prokaryotes, fungi, algae, animals, and plants (Posmyk and Janas, 2009; Tan et al., 2012). Although some preliminary findings had been reported previously (Van Tassel et al., 1993; Kolár and Macháčkova, 1994), the first evidence that melatonin indeed existed in plants came independently from two different groups of researchers (Dubbels et al., 1995; Hattori et al., 1995). In the following years, the existence of melatonin in various quantities has also been proven in different organs of a variety of plants, including cereals, vegetables, fruits, and medicinal herbs (Chen et al., 2003; Paredes et al., 2009; Posmyk and Janas, 2009; Arnao, 2014). Recently, genetically engineered rice and tomato plants were reported to have enhanced melatonin contents (Okazaki et al., 2009; Byeon et al., 2014).

It has been suggested that melatonin may serve as a circadian and photoperiodic rhythm regulator (Kolár et al., 1999), and as a powerful free-radical scavenger and universal antioxidant due to its ubiquitous presence in various life forms (Tan et al., 2012). In animals, the primary role of melatonin is to regulate the circadian rhythm; therefore, it is named as the hormone of darkness, because its levels peak at night and decline to a minimum during the day (Hardeland, 2008). The presence of melatonin in several plant species from different families initiated a growing interest in whether similar patterns may exist in plants. Kolár et al. (1999) reported significantly higher melatonin levels during the night than the daytime in the leaves of a short-day plant, *Chenopodium rubrum*. In contrast to these findings, Tan et al. (2007) documented that melatonin levels in water hyacinth (*Eichhornia crassipes*) exhibited a diurnal rhythm but peak levels occurred close to the end of the light period rather than the dark period. The authors also found that melatonin levels in plants grown under strong sunlight were significantly higher than in plants grown under much

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dimmer indoor light, further confirming melatonin's role as a free radical scavenger. Another rhythmic pattern of melatonin variation was found in green macroalgae, in which melatonin levels exhibited a semilunar rhythm with higher melatonin levels occurring at the time of low tides when the chance of oxidative stress was higher (Tal et al., 2011). Additionally, several studies demonstrated dual melatonin peaks in the circadian cycle of some plants. For example, two peaks of melatonin production, just before sunrise and late in the afternoon when peak temperatures occurred, have been reported in sweet cherry fruits (Zhao et al., 2013) and in apple leaves (Zuo et al., 2014) during a 24-h period. Similar results were also found in the leaves and roots of lupin and barley plants (Arnao and Hernández-Ruiz, 2015a). All of these studies indicated that changes in melatonin content during a 24-h period seem to be influenced by the time of day at which the sample was taken and the conditions of the surrounding environment under which the plants were being grown. However, additional research is obviously necessary to clearly demonstrate the changes in melatonin content in plants raised under varying conditions.

Although the biosynthetic pathway of melatonin synthesis in animals has been thoroughly studied and characterized in detail in plants (Zhang et al., 2015), there is still some ambiguity in the biosynthetic pathway that needs to be resolved. Starting with the amino acid precursor tryptophan, melatonin is synthesized in four successive steps, the intermediate molecules being tryptamine (5-hydroxytryptophan in animals), serotonin, and N-acetylserotonin (Reiter et al., 2015). In plants, the conversion of tryptophan to tryptamine catalyzed by the enzyme tryptophan decarboxylase is known to be the rate-limiting step (Zhao et al., 2013). On the other hand, Kang et al. (2013) presented new evidence that the last step in the pathway (conversion of N-acetylserotonin to melatonin), regulated by the enzyme N-acetyl serotonin methyltransferase, determines melatonin production in plants. Phytomelatonin levels vary not only among plant species but also within varieties of the same species (Paredes et al., 2009; Posmyk and Janas, 2009; Arnao and Hernández-Ruiz, 2015b). While no detectable amount of melatonin was reported in potato (Badria, 2002), the highest melatonin contents ($> 3700 \text{ ng g}^{-1}$) were detected in Chinese herbs and medicinal plants that are frequently used to delay aging and to cure diseases resulting from the action of free radicals (e.g., neurological disorders) (Chen et al., 2003). Additionally, melatonin levels also differ within tissues or organs of a given plant depending on physiological and environmental conditions. Reproductive organs such as flowers, fruits, and seeds generally have higher melatonin content than leaves and roots (Paredes et al., 2009).

Eggplant (*Solanum melongena* L.), a member of the family Solanaceae (nightshades), is a very important vegetable species cultivated in large areas with its production surpassing $49 \times 10^6 \text{ t}$ in 2013 (<http://faostat3.fao.org/browse/Q/QC/E>). Even though melatonin was proven to be present in numerous plant species, there is very little information about its presence in some major crop species, one of which is eggplant. In this study, the first goal was to simultaneously determine the changes in melatonin and its precursor tryptophan levels in eggplant seedlings at two different growth stages during a 24-h period using high-performance liquid chromatography with fluorescence detection (HPLC-FD). Our second objective was to determine the variation in tryptophan and melatonin levels in various organs (leaves, roots, fruits, and seeds) and the changes in the accumulation of these molecules in these organs throughout the entire growth stage of eggplant, from germination to harvest. This will reveal more information to better understand the physiological roles of these compounds in plants.

2. Materials and methods

2.1. Plant material

Hadrian F₁ eggplant seeds were purchased from Antalya Tarım Seed Company (Antalya, Turkey). This cultivar is mostly grown for single-season cultivation in greenhouses and has dark purple fruits of 22–25 cm long weighing around 200 g.

2.2. Chemicals and reagents

Melatonin, tryptophan, and the rest of the chemicals used in this study were products of Sigma-Aldrich Chemicals (St. Louis, MO, USA). Main stock solutions were prepared by dissolving 1 mg of melatonin and 10 mg of tryptophan in 1 mL of ethanol. The total volume of the main stock solutions was brought to 10 mL by adding the mobile phase (see below). Ten different concentrations (0.5–100 ng mL⁻¹ range for melatonin and 1–1000 ng mL⁻¹ range for tryptophan) of working stock solutions were obtained by diluting the main stock solution with the mobile phase.

2.3. Seed germination

Eggplant seeds treated with 5% sodium hypochlorite solution for 10 min to eliminate surface infestations were rinsed under tap water for 1 min, and then left to surface-dry for 30 min at 25 °C. The seeds were placed in 10 × 10 × 4 cm (l × w × h) covered transparent plastic boxes lined with moistened double-layer filter paper. The boxes were kept in a conditioned room at 25 ± 1 °C (day/night) under cool fluorescent lamps providing approximately 250 μmol m⁻² s⁻¹ light intensity for 16 h day⁻¹. When germinated seedlings reached the cotyledon stage (15–18 days after planting, DAP), they were sampled for melatonin and tryptophan analysis at 4-h intervals for a total of 6 times during a 24-h period. The actual time of sampling was 30 min after each indicated sampling time (Figure 1). A total

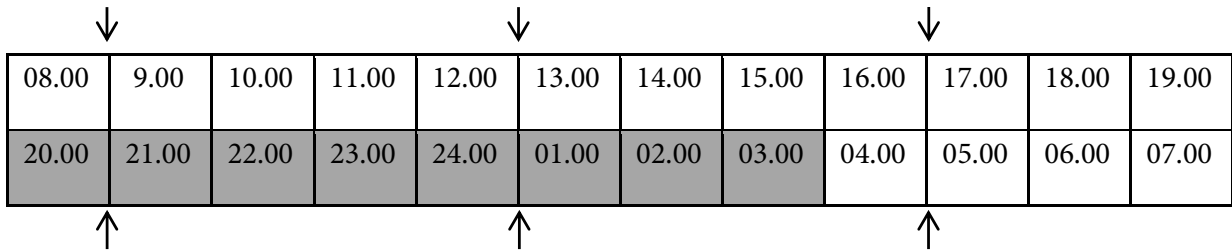


Figure 1. Time of sampling during 24-h period. Dark area indicates the daily dark period while arrows indicate the actual timing of sampling.

of 8 samples in 4 replicates (2 samples per replication) from each sampling time were taken and immediately placed in a freezer at -80°C until extraction.

2.4. Seedling growth

Seeds were planted into 5.5-cm-deep flat cells (75 cm^3) containing perlite and peat in the ratio of 1:3. The flats were placed in a walk-in growth chamber at $25 \pm 1^{\circ}\text{C}$ (day/night) under cool fluorescent lamps providing approximately $250\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ light for 16 h day^{-1} . The seedlings were watered as needed and fertilized (20 N, 20 P, 20 K + trace elements) once a week at the rate of 100 ppm N, starting from the appearance of the first true leaf. Seedlings with 4 fully developed true leaves (transplant stage, 40 DAP) were sampled for melatonin and tryptophan analysis as explained above. Samples from the latest fully developed leaves and washed and surface-dried roots were taken for tryptophan and melatonin analysis and the number of samples for each sampling time was the same as mentioned above.

2.5. Plant growth under greenhouse conditions

Eggplant seedlings were grown under similar conditions as described previously until reaching 4 true-leaf stage. At this stage, the seedlings were transplanted into 10-L pots filled with a mix of peat, perlite, and garden soil in the ratio of 3:1:3 by volume. The plants were placed in an unheated glass greenhouse under a natural photoperiod and fertilized with water-soluble fertilizer at the rate of 150 ppm N twice a week through drip irrigation. During the entire growing period, temperatures in the greenhouse varied between $30\text{--}35^{\circ}\text{C}$ and $20\text{--}24^{\circ}\text{C}$ during day and night, respectively. When all plants reached the flowering stage (about 83 DAP), flower, leaf, and root samples were collected for melatonin and tryptophan analysis 30 min after the onset of dark period. Samples from developing fruits were taken at 7, 10, 15, 20, 25, and 50 days after flowering (DAF). Fruits obtained at 25 DAF were defined as marketable fruits at harvest stage. Some fruits were left to grow on plants until their seeds matured (50 DAF, mature seed stage). Root, leaf, and seed samples were also taken at

25 and 50 DAF (or 108 and 133 DAP) for tryptophan and melatonin analysis.

2.6. Sample extraction

A slightly modified version of the method developed by Arnao and Hernández-Ruiz (2009) was used to simultaneously determine tryptophan and melatonin contents in samples. Briefly, the test tubes containing 3 mL of chloroform and 0.5 g of frozen plant tissue were placed on a shaker in the dark for 17 h at 4°C . The tubes were centrifuged for 20 min at $6000 \times g$ and 4°C , and after transferring the supernatant to another tube, plant residue was washed with 0.5 mL of chloroform. The liquid part of each sample was evaporated using a CentriVap vacuum concentrator (Labconco, Kansas City, MO, USA) attached to a refrigerated CentriVap vapor trap. The residue was redissolved in 0.5 mL of methanol, filtered with a $0.45\text{-}\mu\text{m}$ filter, and used for analysis. Due to the sensitivity of melatonin to light, extractions and analysis were always performed under very low light conditions.

2.7. Chromatographic conditions

A HPLC system that consisted of an Intersil ODS-2 column (GL Sciences, $5\ \mu\text{m}$, $150 \times 4.6\text{ mm}$) equipped with Prominence UFLC equipment (Shimadzu, Kyoto, Japan) and an RF-20A fluorescence detector was used for simultaneous measurement of melatonin and tryptophan. Melatonin and tryptophan were detected using excitation and emission wavelengths at 280 and 350 nm, respectively. A buffer mixture of $0.1\text{ mM Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ and methanol (60:40, v/v, pH 4.5) was used as the mobile phase, which was delivered using a quaternary pump at a flow rate of 0.4 mL min^{-1} . The injection volume of the extract was $20\ \mu\text{L}$ and column oven temperature was 35°C . Between each sample reading, a blank sample was run through the system to clean the autosampler needle. Melatonin and tryptophan contents in each sample were calculated by comparing the sample peak area with the standard curves for melatonin and tryptophan and the values were expressed as ng g^{-1} fresh weight ($\text{g}^{-1}\text{ FW}$) of tissues.

2.8. Statistical analysis

Analysis of variance (one-way ANOVA) was conducted using the SAS statistical software program and Fisher's least significant difference (LSD) test was performed to identify significant differences among the treatment means.

3. Results

The results showed that melatonin and tryptophan contents of seedlings at the cotyledon stage fluctuated significantly during the 24-h period (Figure 2). The highest melatonin ($33.0 \text{ ng g}^{-1} \text{ FW}$) and the lowest tryptophan ($32.6 \text{ ng g}^{-1} \text{ FW}$) levels were determined in the beginning of the dark period. Melatonin content was also quite high ($28.7 \text{ ng g}^{-1} \text{ FW}$) 4 h after the light period started, but dropped to its lowest level of $6.1 \text{ ng g}^{-1} \text{ FW}$ 4 h later (middle of the light period) and remained quite low until the end of the light period. On the contrary, tryptophan was at its highest level ($84 \text{ ng g}^{-1} \text{ FW}$) in the middle of the light period and started to decline as the light period progressed, reaching its lowest level ($32.6 \text{ ng g}^{-1} \text{ FW}$) by the beginning of the dark period.

Significant diurnal changes in both melatonin and tryptophan contents were also observed in the leaves of eggplant seedlings at transplant stage (Figure 3A). Melatonin levels were quite similar to those determined in the seedlings at cotyledon stage. The highest melatonin ($44.5 \text{ ng g}^{-1} \text{ FW}$) and the lowest tryptophan ($3.5 \text{ ng g}^{-1} \text{ FW}$) levels were determined in the beginning of the dark period (Figure 3A). Moreover, in the middle of the dark period and during the entire light period, melatonin levels remained quite low with the only exception of a slight increase to its second peak ($17.0 \text{ ng g}^{-1} \text{ FW}$) occurring 8 h

after the light period started. In contrast, tryptophan levels started from quite a low value of $16.0 \text{ ng g}^{-1} \text{ FW}$ in the beginning of the light period and increased significantly to around $55\text{--}59 \text{ ng g}^{-1} \text{ FW}$ as the light period progressed. However, tryptophan levels fell quite sharply to the lowest of about $4.0 \text{ ng g}^{-1} \text{ FW}$ by the end of the light period and remained low towards the middle of the dark period.

The trend of change in melatonin content of eggplant roots during the 24-h period was similar to the changes observed in the leaves (Figure 3B). Melatonin content in roots was quite high at the beginning of the dark period, but fell drastically, reaching the lowest level by 4 h after the light period started. On the other hand, even though tryptophan levels of eggplant roots doubled during the light period and in the middle of the dark period, these changes were not statistically significant.

Leaf melatonin and tryptophan contents were also quantified at various growth stages (Figure 4A). Melatonin content at the seedling stage (40 DAP) was relatively high with a value of $44.5 \text{ ng g}^{-1} \text{ FW}$, which decreased very sharply to $3.4 \text{ ng g}^{-1} \text{ FW}$ by the flowering stage (83 DAP) and remained very low for the remainder of the growing season. In contrast, the changes in tryptophan content of eggplant leaves were quite opposite. Tryptophan levels at the seedling and the flowering stages were very low, both being around $4 \text{ ng g}^{-1} \text{ FW}$, but they increased significantly as the growth season progressed, finally reaching $64 \text{ ng g}^{-1} \text{ FW}$ by the time the seeds matured in the fruits (133 DAP).

A similar trend was also observed in eggplant roots, where melatonin and tryptophan contents were at the highest and lowest levels, respectively, at the seedling stage (Figure 4B). As the plants reached the flowering

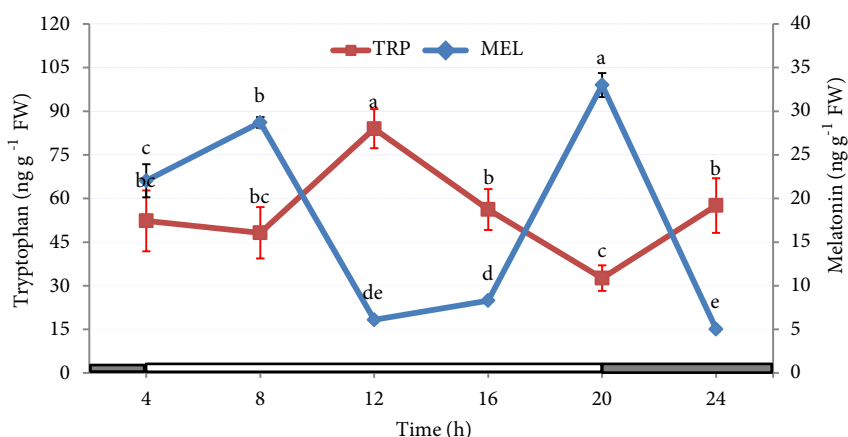


Figure 2. Changes in tryptophan (TRP) and melatonin (MEL) levels in eggplant seedlings at cotyledon stage during a photoperiodic cycle of 16 h of light/8 h of darkness. The seedlings were 15–18 days old at this stage. Results are the average value of two sample extractions taken in 4 replications ($n = 8$). Vertical bars represent mean \pm SE. The open bar at the bottom indicates light, while the closed bar indicates darkness during the 24-h period. Values with different letters are significantly different at $P \leq 0.05$.

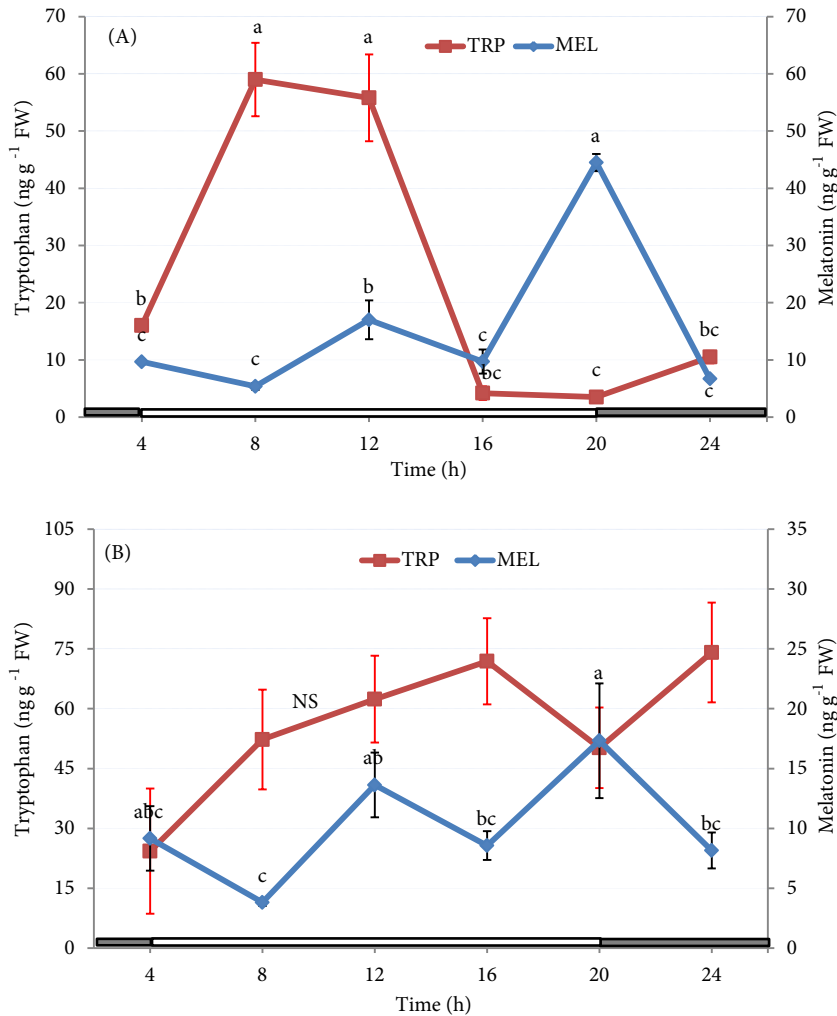


Figure 3. Changes in tryptophan (TRP) and melatonin (MEL) levels of (A) leaves and (B) roots of eggplant seedlings at transplant stage during a photoperiodic cycle of 16 of h light/8 h of darkness. Results are the average value of two sample extractions taken in 4 replications ($n = 8$). Vertical bars represent mean \pm SE. The open bar at the bottom indicates light, while the closed bar indicates darkness during the 24-h period. Values with different letters are significantly different at $P \leq 0.05$. NS = not significant.

stage, melatonin decreased to the lowest level, about 3 ng g⁻¹ FW, and remained so for the rest of the season until the mature seed stage. However, tryptophan exhibited a steady increase from the lowest level at the seedling stage to flowering stage and harvest stage, finally attaining its highest level of 222.8 ng g⁻¹ FW at mature seed stage. It should also be noted that at seedling stage the melatonin level in the leaves was 2.5 times higher than the level detected in the roots, whereas a reverse trend was found for tryptophan (Figure 4A). Tryptophan levels were several orders of magnitude higher in the roots than the leaves in every growing phase measured (Figures 4A and 4B). At the end of the growing season (mature seed stage), however, as melatonin contents of the leaves and roots were almost the

same, tryptophan levels were more than three times higher in the roots than in the leaves.

Melatonin and tryptophan contents in developing eggplant fruits (Figures 5A and 5B) and in seeds (Figure 6) were also determined. Melatonin content was high, 8.1 ng g⁻¹ FW, at the flowering stage (0 DAF). It declined progressively to the lowest level by 15 and 20 DAF (1.8 ng g⁻¹ FW), and increased slightly to about 2.5 ng g⁻¹ FW as the fruits reached harvest and mature seed stages at 25 and 50 DAF, respectively (Figure 5B). Tryptophan content, on the other hand, was quite high in the range of 150 to 180 ng g⁻¹ FW in flowers and very young fruits (7 DAF), and fell drastically as the fruit growth progressed, reaching the lowest level of 3.9 ng g⁻¹ FW at 15 DAF. However, it

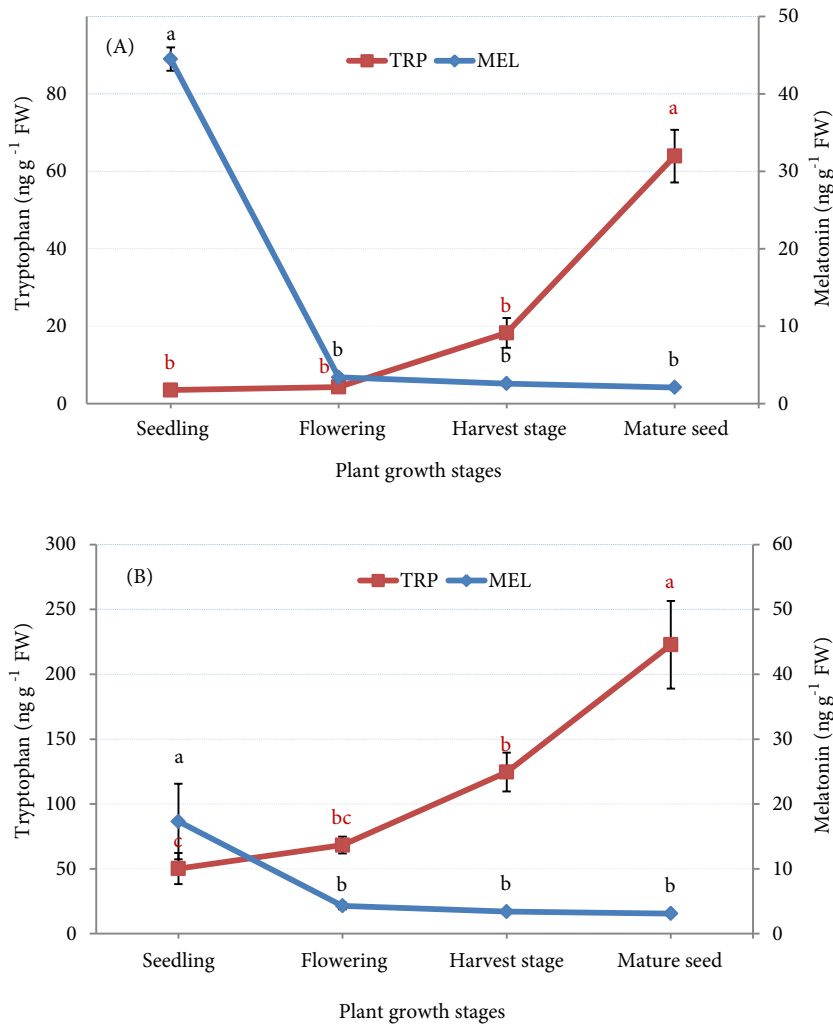


Figure 4. The changes in tryptophan (TRP) and melatonin (MEL) levels of (A) leaves and (B) roots of eggplant at various plant growth stages. Results are the average value of two sample extractions taken in 4 replications ($n = 8$). Vertical bars represent mean \pm SE. Values with different letters are significantly different at $P \leq 0.05$.

increased sharply to the highest level of $269.6 \text{ ng g}^{-1} \text{ FW}$ in fruits harvested at mature seed stage (50 DAF). Melatonin content was $40.2 \text{ ng g}^{-1} \text{ FW}$ in seeds at harvest stage (25 DAF) and substantially increased to $68.8 \text{ ng g}^{-1} \text{ FW}$ at the mature seed stage (50 DAF) (Figure 6). Tryptophan content of seeds, however, was considerably lower than melatonin content, and although the level of tryptophan increased from harvest stage to mature seed stage, the difference was not significant (Figure 6).

4. Discussion

As elaborated previously in the introduction, melatonin levels usually exhibit a rhythm of lower values during daytime and higher levels at night. In addition, light exposures uniformly suppress melatonin production in

insects and mammals; thus, melatonin is known as the chemical expression of darkness in animals (Hardeland and Poeggeler, 2003; Paul et al., 2015). However, studies of daily fluctuations of melatonin levels in plants have demonstrated contrasting results. To date, there are no reports directly linking variations in melatonin content to circadian fluctuations controlled by a plant's biological clock. It is suggested that variations in melatonin content are likely the result of combined effects of endogenous and environmental factors triggered by natural conditions (Boccalandro et al., 2011). For example, no solid evidence was reported for melatonin increasing significantly during the night in any parts of morning glory and tomato grown under 12:12-h light:dark photoperiods (Van Tassel et al., 2001), as it occurs in animals. On the other hand, a

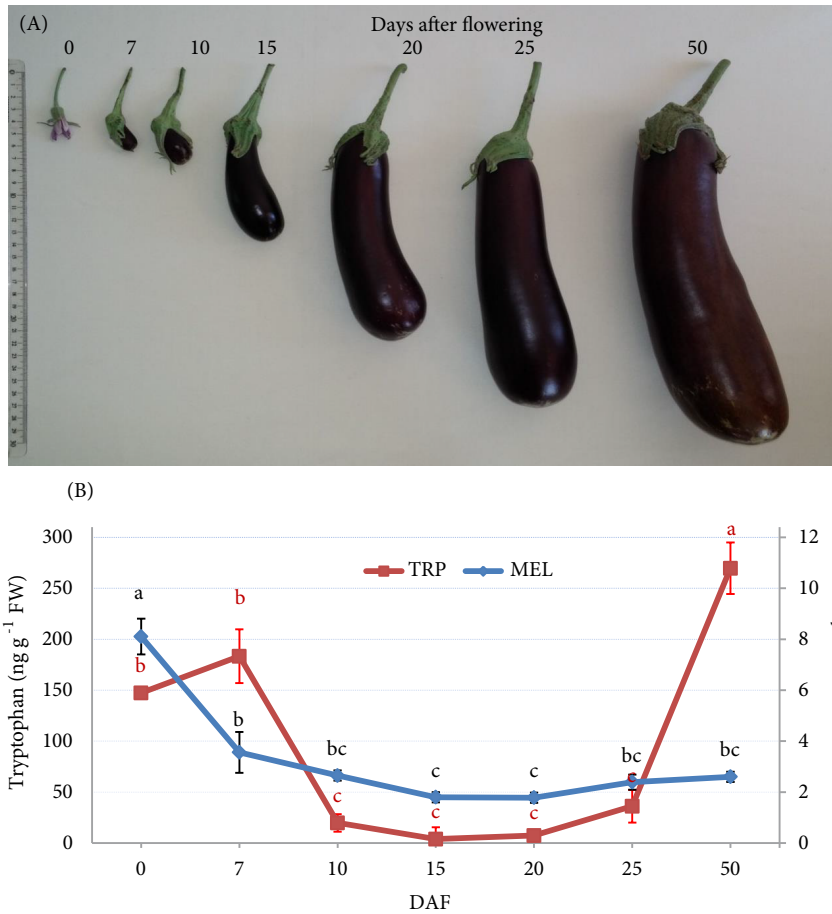


Figure 5. The appearance of eggplant fruits (A) and tryptophan (TRP) and melatonin (MEL) levels of eggplant fruits (B) at different stages of development. Results are the average value of two sample extractions taken in 4 replications (n = 8). Vertical bars represent mean ± SE and DAF denotes days after flowering. Values with different letters are significantly different at P ≤ 0.05.

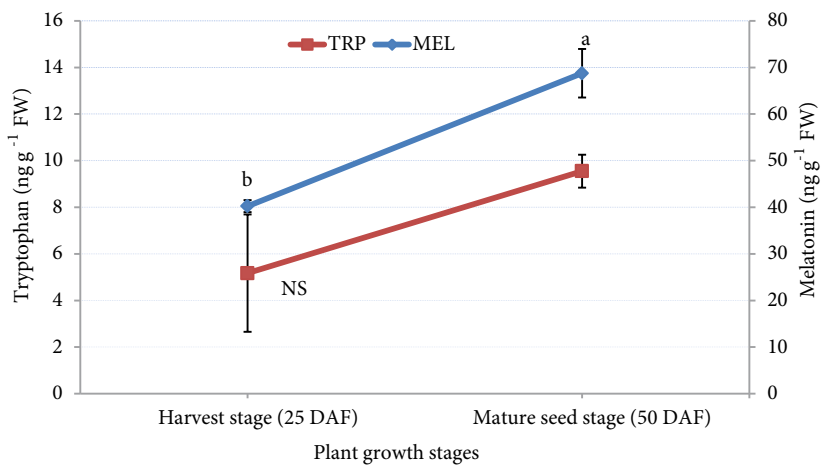


Figure 6. Tryptophan (TRP) and melatonin (MEL) levels of eggplant seeds at two different fruit maturation stages. Results are the average value of two sample extractions taken in 4 replications (n = 8). Vertical bars represent mean ± SE and DAF denotes days after flowering. Values carrying different letters are significantly different at P ≤ 0.05. NS = not significant.

daily rhythm of a strong nocturnal maximum followed by very low levels during the light period was found in the shoots of *Chenopodium rubrum* (Wolf et al., 2001). Another significant result identified in this work was that the duration of maximum melatonin content was not photoperiod-dependent, but the highest levels always occurred 2 h after the dark period initiated in plants growing under 16:8-h light:dark photoperiodic conditions (Wolf et al., 2001).

To the best of our knowledge, there is no information available in the literature about daily changes in tryptophan content in plants and variations in melatonin content in eggplant. In this report, diurnal trends of change in tryptophan and melatonin levels in eggplant seedlings were established for the first time. In our experiment, where the plants were grown under constant temperature and 16:8 light/dark photoperiodic conditions, melatonin levels showed a strong peak at the beginning of the nocturnal period, during which time tryptophan levels plunged (Figures 2, 3A, and 3B). Additionally, melatonin levels exhibited smaller peaks in the middle of the light period; however, the time of maxima differed with seedling age. In younger seedlings (cotyledon stage), for example, melatonin displayed a second peak 4 h after light exposure, while in the leaves of older seedlings (transplant stage) the appearance of the second peak shifted towards the middle of the light period. Moreover, perhaps the most interesting finding was the inverse relationship observed between melatonin and tryptophan levels, i.e. the highest melatonin contents usually corresponded to the lowest tryptophan levels. This could be attributed to the fact that tryptophan is the precursor of melatonin and it increases while melatonin decreases and vice versa. Two peaks of melatonin production in plants are not rare and have previously been documented. For example, Zhao et al. (2013) reported dual peaks of melatonin production during a 24-h period in cherry fruits grown under natural environment (i.e. plants exposed to daily changing light and temperature conditions). The authors concluded that one peak was induced by darkness, and the second peak by high light and temperatures that occurred later in the day corresponding to higher free radical accumulation. Even though the light levels to which the plants were exposed were lower in our study, the second melatonin peak could possibly be caused still by light exposure considering the high sensitivity of young seedlings to the conditions of the surrounding environment at this growing stage. Additionally, Boccalandro et al. (2011) found melatonin levels in fruits of *Vitis vinifera* grown under field conditions to fluctuate during a 24-h period, reaching a strong peak in the morning. They also reported that melatonin levels in grape berries dropped significantly with increasing

sunlight and continued to be low throughout the day, finally recovering around late night. In contrast, melatonin levels of *Arabidopsis* seedlings, grown under constant temperature and 12:12 light/dark photoperiod with a light intensity of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$, were not diurnally regulated (Hernández et al., 2015). Taken together, our data suggest that melatonin levels in eggplant seedlings are probably controlled by the plant's own circadian clock and conditions of the surrounding environment. However, it is important to note that sampling time during the day seems to be highly relevant for melatonin levels in plant tissues, since differences of several orders of magnitude have been reported depending on the time of day and night. It is also clear that additional studies should be carried out to understand the changes in melatonin content in plants and the decisive influence of environmental factors on endogenous melatonin levels.

Similar to melatonin, plant hormone indole-3-acetic acid (IAA) is also synthesized from tryptophan and both compounds exhibit similar growth-promoting or growth-inhibiting properties in plants. Melatonin is reported to be a molecule having an auxinic activity, where it promotes and inhibits vegetative growth in a similar fashion to IAA (Hernández-Ruiz et al., 2005). Moreover, synchronous circadian concentration changes (double peaks in a 24-h duration) in melatonin, serotonin, and IAA, all of which are produced from the same precursor, tryptophan, are reported in the family Characeae, indicating a possible crosstalk between IAA and melatonin biosynthesis pathways (Beilby et al., 2015).

In this report, tryptophan and melatonin were also quantified in various organs and at different growth stages of eggplant; to date, this is the first study to examine the presence of these molecules in eggplant throughout its life cycle. Our study revealed that melatonin levels were quite high and tryptophan contents were low in earlier stages of plant growth. This is expected since the germination and early seedling growth stages are highly critical in the life of any plant species, significantly affecting the performance of plants in their later stages of growth. However, field conditions are rarely optimum for seed germination, and stressful conditions in the field are known to induce plants to make structural and biochemical adjustments, allowing them to tolerate adverse conditions, especially in early life stages (Pratap and Sharma, 2010). Therefore, higher melatonin content determined in early stages of plant growth following germination could be associated with the antioxidant nature of melatonin, leading to elevated levels of stress tolerance.

High melatonin levels observed in leaves and roots of eggplant seedlings decreased drastically by the flowering stage and remained low, while tryptophan levels increased

significantly as the growth season advanced (Figures 4A and 4B). Melatonin is synthesized in chloroplasts and mitochondria in plants (Tan et al., 2013) and may be transported to any plant tissue, including meristems, flowers, seed, fruits, and so on, where it is in higher demand (Arnao and Hernández-Ruiz, 2013). This would explain why significantly lower melatonin contents in leaves and roots were detected at the flowering stage. It is possible that the need for higher melatonin levels in other parts of the plant such as flowers, fruits, or seeds may have triggered the import of some melatonin produced by these organs. Another possibility could be that the rate of melatonin production may have been reduced in these organs since the precursor molecule (tryptophan) levels increased dramatically starting from the flowering stage. Confirmatory findings were also reported in pepper, a close relative of eggplant, in which there was the least amount of melatonin in the leaves at flowering stage and melatonin levels in the roots declined gradually with progressing growth season (Korkmaz et al., 2014).

In general, such reproductive parts of plants as flowers, fruits, and especially the seeds contain higher levels of melatonin compared to other parts of plants (Arnao, 2014; Arnao and Hernández-Ruiz, 2015). Since the seed, especially its germ tissue, is highly susceptible to damage caused by oxidative stress, melatonin might be present in higher amounts as an important part of its antioxidant defense system (Manchester et al., 2000). The reproductive stage is the most critical developmental stage in a plant's life cycle, and plants at this stage are highly vulnerable to hostile environmental conditions (Thakur et al., 2010). Park et al. (2013) reported that melatonin is synthesized transiently in the panicle of rice, whereas no upsurge in melatonin content was observed in the flag leaf during flowering, and that the panicle had six times higher melatonin content than the flag leaf. Higher levels of melatonin during flowering may imply that melatonin possesses a vital role in protecting the flowers from stresses as pollination accelerates the initiation of senescence, leading finally to flower death (O'Neil, 1997).

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We found relatively high quantities of melatonin and tryptophan in flowers and their levels declined substantially as the fruits reached harvest stage (Figures 5A and 5B). Additionally, melatonin content of fruits remained unchanged at mature seed stage, at which time tryptophan content was at its highest level. Furthermore, significant increases in melatonin content in seeds were observed as the seeds matured, whereas tryptophan content remained unchanged (Figure 6). Similar findings were also reported in *Datura metel*, where serotonin and melatonin contents were quite high in the least developed flower buds with their levels progressively decreasing as the flower buds and fruits matured (Murch et al., 2009). Similarly, the highest melatonin content in tomato (Okazaki and Ezura, 2009) and pepper (Korkmaz et al., 2014) was detected in seedlings and seeds, supporting the notion that higher melatonin content is essential for guarding these tissues from various stresses.

In conclusion, diurnal and developmental differences in melatonin and tryptophan contents in eggplant were established in this research. Eggplant seedlings exhibited dual peaks of melatonin production, with a strong peak in the beginning of the nocturnal period and a smaller peak in the middle of the light period. Tryptophan levels, on the other hand, displayed quite the opposite trend with a strong peak in the middle of the light period and very low levels in the beginning of the dark period. High-level melatonin production in the beginning of the dark period seems to be associated with the plant's own biological clock, while the other peak is likely to be related to the surrounding environment. Melatonin accumulated in large amounts in young seedlings and mature seeds, confirming previous findings that it may be vital in protecting the germ tissues and newly germinated seedlings from the damaging effects of varying environmental conditions.

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