Scientia Horticulturae 172 (2014) 242–247

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Profiling the melatonin content in organs of the pepper plant during different growth stages

Ahmet Korkmaz [a,b,*], Özlem Değer [a], Yakup Cuci [c]

[a] Kahramanmaraş Sütçü Imam University, Department of Horticulture, Kahramanmaraş, Turkey
[b] Kahramanmaraş Sütçü İmam University, Research and Development Center For University – Industry and Public Relations, Kahramanmaraş, Turkey
[c] Kahramanmaraş Sütçü Imam University, Department of Environmental Engineering, Kahramanmaraş, Turkey

ARTICLE INFO

Article history:
Received 26 December 2013
Received in revised form 29 March 2014
Accepted 17 April 2014
Available online 4 May 2014

Keywords:
Fruit
HPLC–FD
Melatonin
Pepper
Seed
Seedling

ABSTRACT

Melatonin (N-acetyl-5-methoxytryptamine), a well-known animal hormone, was first isolated from bovine pineal gland and, since then, it was discovered in evolutionary distant organisms including bacteria, algae, invertebrates, and finally in higher plants. Although the presence of melatonin in plants seems to be a universal phenomenon, there is still an absence of information on its occurrence in important plant species, one of which is pepper (*Capsicum annuum* L.). Our objective was to determine the presence of melatonin in different organs (leaves, roots, fruits and seeds) of two pepper cultivars and its variation during various growth stages (germination, seedling, flowering, and harvest) using HPLC–FD. We found very high concentration of melatonin in seedlings at the cotyledon stage (108.6–111.6 ng g^{-1} FW) with a progressive decline as the plants matured. Melatonin was also found to accumulate highly in mature seeds, and to varying concentrations in fruits, leaves, and roots depending on the developmental stage, strongly suggesting that melatonin controls these developmental processes.

1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is an indoleamine initially isolated for the first time from the bovine pineal gland more than half a century ago (Lerner et al., 1958). Since then, it was discovered in evolutionary distant organisms including bacteria, algae, invertebrates, vertebrates and in finally higher plants (Gomez et al., 2012; Hardeland and Poeggeler, 2003; Migliori et al., 2012; Tan et al., 2012). The first evidence that melatonin exists in plants came from two independent groups in 1995 (Dubbels et al., 1995; Hattori et al., 1995), and in subsequent years, melatonin has also been detected in the roots, leaves, fruits, flowers, and seeds of a variety of vegetables, cereals, fruits, and in medicinal herbs (Paredes et al., 2009; Posmyk and Janas, 2009; Reiter et al., 2007). Recently, some plants have been genetically engineered to produce elevated amounts of melatonin (Okazaki et al., 2009a; Park et al., 2013a,b).

It has been postulated that plant melatonin may serve as a photoperiodic and circadian rhythm regulator (Kolár et al., 1999; Kolár and Macháčková, 2005) and as a universal antioxidant because of its wide distribution in fungi, algae, bacteria, animals and plants (Galano et al., 2011, 2013; Tan et al., 2012). The fact that melatonin is a proven potent free-radical scavenger and a broad spectrum antioxidant in animals led researchers to deduce that this molecule presumably acts in a similar manner in plants (Arnao and Hernández-Ruiz, 2009a; Van Tassel and O'Neill, 2001). Studies also suggest that melatonin plays a major role in many functions in plants, including regulation of mitosis (Banerjee and Margulis, 1973), delaying flower induction (Kolár et al., 2003), adventitious and lateral root regeneration (Arnao and Hernández-Ruiz, 2007), the stimulation of hypocotyl, coleoptile, and root growth (Chen et al., 2009; Hernández-Ruiz et al., 2005), and protection against cold stress (Posmyk et al., 2009), salinity stress (Li et al., 2012), and toxic copper ions (Posmyk et al., 2008). While numerous studies have suggested that melatonin possesses important functions in the plants, extensive research is still necessary to clarify the role and mechanism of action of melatonin on whether it acts as an independent plant growth regulator, as a factor mediating the activity of other substances affecting plant growth or as a molecule involved in regulation of growth but whose activity is generally ascribed to other compounds such as auxin (Posmyk and Janas, 2009).

Melatonin concentrations in the plants differ not only from species to species but also among varieties within the same species (Hattori et al., 1995; Posmyk and Janas, 2009; Reiter et al., 2007).

* Corresponding author at: Kahramanmaraş Sütçü Imam University, Department of Horticulture, Kahramanmaraş, Turkey.
Tel.: +90 344 280 2035; fax: +90 344 280 2002.
E-mail addresses: akorkmaz@ksu.edu.tr, akorkmaz@hotmail.com (A. Korkmaz).

http://dx.doi.org/10.1016/j.scienta.2014.04.018
0304-4238/

While there was no melatonin detected in potato tuber (Badria, 2002), the highest melatonin content (>3700 ng g^{-1}) is reported to be in medicinal plants and herbs which are commonly used to possibly defer aging and to treat diseases associated with free radicals (e.g. neurological disorders) (Chen et al., 2003). Moreover, the melatonin levels also vary within different organs or tissues of a given plant and the highest melatonin contents have generally been reported in reproductive organs such as flowers, fruits and the seeds (Paredes et al., 2009).

Pepper (*Capsicum annuum* L.), a member of *Solanaceae* (Nightshade) family along with other important vegetable species such as tomato, eggplant and potato, is produced in large areas throughout the world with production exceeding 29 million tons in 2010 (FAO, 2010). Although the presence of melatonin in plants seems to be a universal phenomenon, there is still an absence of information on its occurrence in very important plant species, one of which is pepper. To date, the only evidence that melatonin exists in pepper came from Huang and Mazza (2011) who reported that melatonin content ranged between 24.3 pg g^{-1} and 581.1 pg g^{-1} depending on the extraction solvents used and the color of the fruit. In the current study, our objective was to determine the presence of melatonin in different organs (roots, leaves, fruits and seeds) of two pepper cultivars and the distribution and accumulation of melatonin in these organs during various growth stages (germination, seedling, flowering and harvest) using HPLC–FD, which would reveal its physiological roles during these developmental stages of plant and fruit growth.

2. Materials and methods

2.1. Plant material

Seeds of 'Sena' red pepper (*C. annuum* L.), an open pollinated cultivar, were obtained from Agricultural Research Institute (Kahramanmaras, Turkey). Seeds of 'Mert' sweet pepper, an F_1 hybrid, were purchased from Yüksel Seed Company (Antalya, Turkey). Fruits of 'Sena' are mostly suitable for crushed hot red pepper or paprika production while 'Mert' fruits are used for green or red fresh consumption.

2.2. Chemicals and reagents

Melatonin and all other chemicals all of which were analytical grade or better were purchased from Sigma–Aldrich Chemicals (St. Louis, MO). To prepare stock solution, 1 mg of melatonin was dissolved in 1 mL ethanol and the total volume was brought to 10 mL by adding the mobile phase (see below). Standard solutions in 10 different concentrations (0.5–100 ng mL^{-1} range) were obtained by diluting stock solution with the mobile phase.

2.3. Seed germination

Seeds were disinfested in 1% (active ingredient) sodium hypochlorite solution for 10 min to eliminate possible seed-borne microorganisms, rinsed for 1 min under running water then were dried for 30 min at room temperature. Single layers of pepper seeds from each cultivar were placed in 10 cm × 10 cm × 4 cm (l × w × h) covered transparent plastic boxes on double layers of filter paper moistened with 15 mL of double distilled water and kept in a growth room at 25 ± 1 °C (day/night) under cool fluorescent lamps (225 μmol m^{-2} s^{-1}) for 14 h day^{-1}. When the cotyledons were fully opened (cotyledon stage, 15–17 days after planting, DAP), the seedlings were sampled for melatonin analysis 2 h after the dark period had started. A total of 16 samples in 4 replicates (4 samples per replication) from each cultivar was collected and immediately frozen at −80 °C until melatonin extraction.

2.4. Seedling growth

The seeds were planted at a depth of 1.0 cm into 5.5 cm-deep flat cells (75 cm^3) filled with growth medium consisting of peat and perlite in the ratio of 3:1. The flats were watered regularly with tap water and kept in a growth room at 25 ± 1 °C (day/night) under cool fluorescent lamps (225 μmol m^{-2} s^{-1}) for 14 h day^{-1}. The seedlings were watered as needed and fertilized with liquid fertilizer (18 N, 18 P, 18 K + trace elements) at the rate of 150 ppm N starting from the first true leaf stage. When the seedlings had fully developed 4 true leaves (35 DAP), they were sampled for MEL analysis 2 h after the dark period had started. Leaf samples were taken from the latest fully developed leaf while root samples were taken after the roots had been washed under running water and dried with paper towel to remove the surface water. A total of 16 samples in 4 replicates (4 samples per replication) from each cultivar was collected and immediately frozen at −80 °C until melatonin extraction.

2.5. Plant growth under greenhouse conditions

The seedlings were raised under same conditions as explained above and when they had 4 true leaves, they were transplanted into 10 L pots filled with peat and perlite in the ratio of 3:1. The plants were kept in an unheated greenhouse under natural photoperiod and fertilized with liquid fertilizer (20 N, 20 P, 20 K + trace elements) at the rate of 150 ppm N via drip irrigation twice a week. When the plants reached the flowering stage (86 DAP), leaf, root and flower samples were taken in the manner as described above. Developing fruits were collected at 6, 10, 14, 18, 22, 26, 30, and 48 days after flowering (DAF). Fruits at 30 DAF were defined as mature green. After the green mature stage, some fruits were allowed to stay on the plants until their color turned to red 48 DAF (mature red). Leaf, root, and seed samples from plants were also collected 30 and 48 DAF (or 116 and 134 DAP) for melatonin analysis.

2.6. Melatonin extraction

To measure melatonin content in pepper plant samples, a slightly modified version of direct sample extraction procedure reported by Arnao and Hernández-Ruiz (2009b) was employed. One g frozen plant tissue was placed in test tubes containing ethyl acetate (3 mL). After leaving overnight (17 h) at 4 °C in darkness with shaking, the tubes with plant tissues were centrifuged at 6000 × *g* and 4 °C for 20 min. The supernatant was transferred to another tube and the remaining plant residue was washed with 0.5 mL ethyl acetate. The extract and washing from each sample were evaporated to dryness under vacuum using a CentriVap vacuum concentrator (Labconco, Kansas City, MO) coupled to a refrigerated CentriVap −50 °C vapor trap. The residue was redissolved in methanol (0.5 mL), filtered (0.45 μm) and analyzed using HPLC with fluorescence detection. Extraction of melatonin from plant tissues and HPLC analysis were always carried out under dim light.

2.7. Melatonin analysis

Shimadzu, Prominance UFLC equipment utilizing an Intersil ODS-2 column (GL Sciences, 5 μm, 150 mm × 4.6 mm) with fluorescence detection was used to measure melatonin levels. An excitation wavelength of 280 nm and an emission wavelength of 350 nm were used. The mobile phase was constituted of methanol: 0.1 mM Na_2HPO_4/H_3PO_4 buffer (40:60, v/v) pH 4.5 at a flow rate of 0.4 mL min^{-1}. Twenty microliter extracted samples were injected