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REVIEW

Melatonin in flowering, fruit set and fruit ripening

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Abstract

Key message **Melatonin induces a delay in flowering stabilizing DELLA proteins and also promotes the transcription of FLC. In fruit set, melatonin is able to induce parthenocarpy. Melatonin promotes ripening and retards senescence of fruits.**

Abstract Melatonin is an animal hormone involved in many regulatory processes such as those related to sleep. Melatonin was discovered in plants in 1995 and is called phytomelatonin. Also in plants, a great variety of physiological processes have been described in which melatonin plays a role. In plants, melatonin is mainly involved in stress situations but also in germination, plant growth, rhizogenesis, senescence and as a protector agent improving important processes such as photosynthesis, CO_2 uptake, cell water economy and primary and secondary metabolism. Melatonin has been related to changes in the majority of plant hormones. Many revisions of stress situations have been published. However, melatonin and plant reproductive development have been poorly studied. The aim of this review is to provide an overview of works related to flowering, fruit set and development, including parthenocarpy and fruit ripening/senescence, and the role played by melatonin in the same.

Keywords Flowering · Fruit set · Melatonin · Parthenocarpy · Quality fruits · Reproductive development · Ripening · Shelf-life fruit · Senescence

Introduction

The compound *N*-acetyl-5-methoxytryptamine, commonly known as melatonin, was initially discovered as an animal hormone, but in 1995 was discovered in plants. In animals, melatonin influences many physiological aspects such as sleep, mood, body temperature, endocrine hormone levels, the immunological system and sexual behavior (Arnao and Hernández-Ruiz 2015a). Melatonin has also been widely studied in many plant species, and several important roles have been attributed to it. In general, melatonin plays an important role in stress situations against both abiotic (UV radiation, salinity, drought, extreme temperatures, chemical contaminants, etc.) and biotic stressors (bacteria, fungi and viruses) (Arnao and Hernández-Ruiz 2014, 2015b). Thus, melatonin seems to have some protective role in physiological aspects such as germination, photosynthesis, water economy and primary and secondary metabolisms and in growth and vegetative development. In all these process, melatonin has a direct regulatory effect, acting as scavenger of reactive oxygen species (ROS) and reactive nitrogen species (RNS) or an indirect effect by enhancing changes in gene expression of stress-specific transcription factors among other elements, including plant hormones such as IAA, gibberellins, cytokinins, abscisic acid and ethylene, and also other plant regulators such as salicylic acid, jasmonates, brassinosteroids, strigolactones and polyamines (Arnao and Hernández-Ruiz 2018a, b, 2019c, 2020; Sharif et al. 2018). However, the number of studies focusing on reproductive development is considerably lower. From the initial studies on *Chenopodium rubrum* by Kolar and co-workers in 1995 to the present day, many advances have been made and interesting articles published. The present contribution represents a global revision of the studies related to melatonin and plant reproductive development. In addition, the possible beneficial postharvest application of melatonin is discussed.

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Melatonin in flowering

Early studies on the possible role of melatonin in flowering were involved the short-day plant *Chenopodium rubrum* (Machackova and Krekule 2002; Kolar et al. 2003). In these studies, high concentrations of melatonin inhibited flower induction by an average of 40–50% when applied in the period between 2 h before and 2 h after the beginning of the inductive dark period. An agonist of melatonin used in animal studies, the compound CGP-52608 (1-[3-allyl-4-oxothiazolidine-2-ylidene]-4-methyl-thio semicarbazone), was effective in inhibiting flower induction. However, melatonin treatment had no effect on the phase of the flowering, suggesting that it controlled some process before floral transition (Kolar and Machackova 2005). These studies formed part of research into the possible role of melatonin in plants as a chronoregulator of circadian rhythms, in a similar way as it functions in animals (Kolar et al. 1995, 1997; Wolf et al. 2001). In a similar study in the long-day plant *Arabidopsis thaliana*, flowering was slightly delayed in plants treated with 100 or 500 µM melatonin, which affected leaf and rosette numbers and anthesis. These data formed part of a PhD thesis and, unfortunately, do not seem to have been published in any journal (Kolar 2003). Also, in initial experiments, on the floral development of St John's wort (*Hypericum perforatum*), the appearance of a melatonin peak in the intermediate stages of flower development was described. According to the authors, this melatonin peak matched the increased regenerative potential of isolated anthers, so melatonin accumulation in specific tissues and stages was interpreted as a signal in gametophytic development, which resulted in viable microspores (Murch and Saxena 2002). The same authors, working with flowers of devil's trumpet (*Datura metel*), a narcotic plant used in natural medicine around the world, the highest concentration melatonin was found at as the flower buds matured, suggesting melatonin played a protective role in the reproductive tissues (Murch et al. 2009).

In the herbaceous peony (*Paeonia lactiflora*), the melatonin content has been studied during its different flower developmental stages. During the development of flowers, the melatonin content increased in the flower-bud stage (S1), decreased (S2: initiating bloom stage) and then peaked in the bloom stage (S3). Finally, the melatonin content in the wither stage (S4) was a little higher than the value in S2. These variations were directly related to TDC transcript expression (Fig. 1). The authors also studied the effect of different parts of the light spectrum on the melatonin content during flowering, showing that sun exposure and blue light induce melatonin production, while in shade conditions and white and green lights lower values were registered. Also, "dual peaks" of melatonin occurred during a 24-h light/dark cycle, overlapping with temperature and light intensity patterns and with a decrease in the malondialdehyde (MDA) content, an indicator of membrane lipid peroxidation. The authors pointed out the difficulty in drawing any conclusions from these data on the role of melatonin in the floral development of peony. However, the data suggest a protective role of melatonin against situations of light stress and other agents in peony flowers (Zhao et al. 2018). In cut anthurium flowers (*Anthurium andraeanum*), this protective role of melatonin was also demonstrated during low-temperature storage (Aghdam et al. 2019).

In a study of field-grown rice plant, the melatonin contents at three different reproductive stages (pre-flowering, flowering and postflowering) were determined. It was seen that melatonin biosynthesis in the panicle (flower) was sixfold higher than that found in the flag leaves. This induction was parallel to the induction of melatonin biosynthetic enzymes (TDC, T5H and COMT) (Fig. 1), suggesting that melatonin may participate in flower development (Park et al. 2013). According to the authors, melatonin may contribute to the regulation of flowering time in a manner similar to that of ascorbic acid in *Arabidopsis*, where an ascorbic acid-deficient *Arabidopsis* mutant showed an early-flowering phenotype, whereas flowering was delayed in a mutant rich in ascorbic acid. However, although the data do not confirm a direct relationship between flowering and the antioxidant status of tissues, a relationship between ascorbic acid levels and possible hormonal and metabolic changes was suggested (Kotchoni et al. 2009). Also in rice plants growing in field conditions, phenotypes of melatonin-rich transgenic plants overexpressing sheep SNAT had an endogenous melatonin content threefold seen in wild-type plants and a twofold increase in biomass. Flowering was delayed by 1 week in the transgenic lines. The grain yields of the melatonin-rich transgenic lines were reduced by 33% on average (Byeon and Back 2014).

In *Arabidopsis thaliana*, at least six distinct signaling flowering pathways have been identified including photoperiod, vernalization, ambient temperature, autonomous, gibberellic acid (GA) and age pathways. The key autonomous pathway gene flowering locus C (FLC) encodes a MADS box-containing transcription factor which negatively regulates floral transition by binding to the promoters of Suppressor of overexpression of ConstanS1 (SOC1) and flowering locus T (FT). Both FLC and Squamosa promoter-binding protein-like (SPL) encode products related to the DELLA proteins, which act as negative regulators of GA signaling (Wigge et al. 2005; Li et al. 2016; Yu et al. 2012; Searle et al. 2006).

The first study that provided a direct link between melatonin and floral transition was that of Shi and co-workers

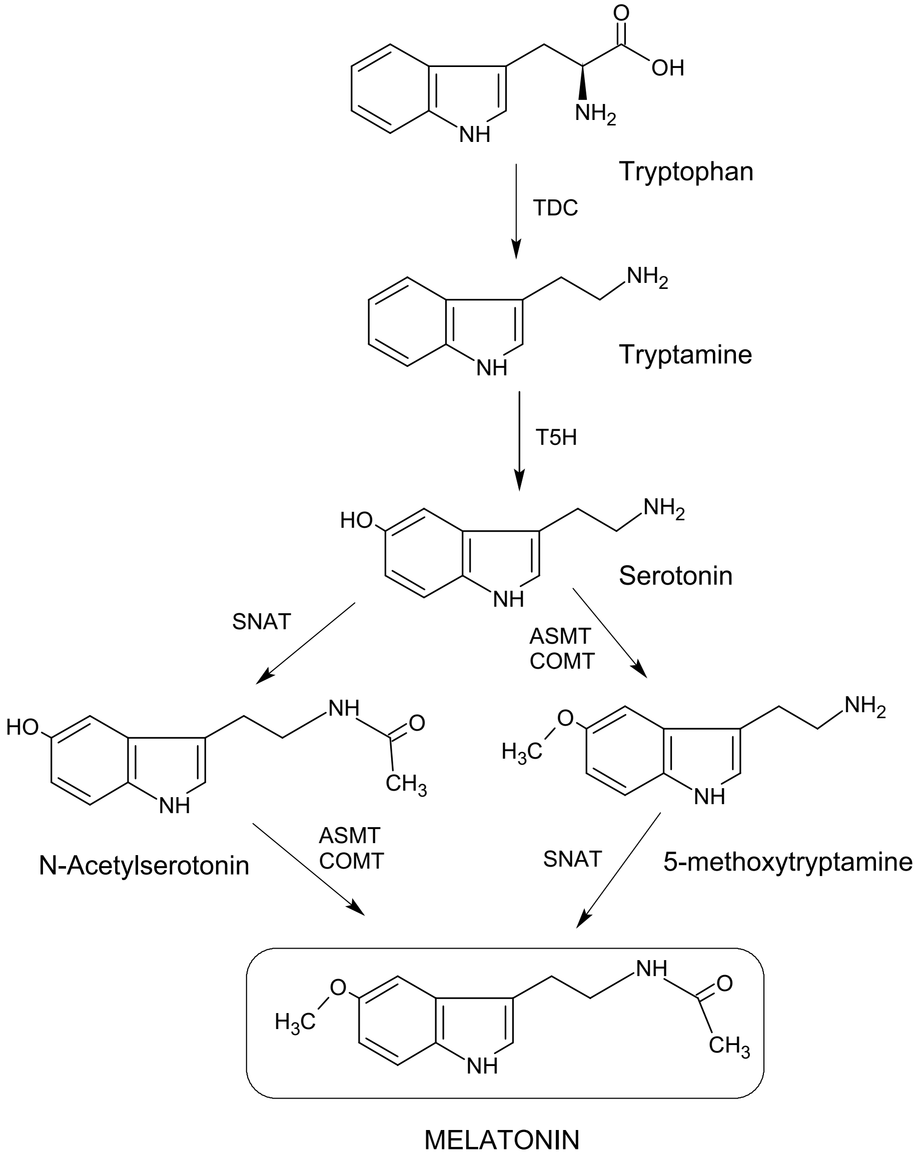

Fig. 1 Biosynthesis pathway of melatonin in plants. The enzymes of the steps are: *TDC* tryptophan decarboxylase, *T5H* tryptamine 5-hydroxylase, *SNAT* serotonin *N*-acetyltransferase, *ASMT* acetylserotonin methyltransferase, *COMT* caffeic *O*-methyltransferase

(Shi et al. 2016). In this study, the novel involvement of DELLAs and *FLC* in melatonin-mediated flowering in *Arabidopsis* was demonstrated. Thus, 500 µM melatonin-treated plants flowered 5 days later and had more rosette leaves than untreated plants. The results suggested that exogenous melatonin retards flowering. Melatonin increased the protein stabilizations of two DELLA proteins, without regulating the transcripts of *DELLAs*. Moreover, exogenous melatonin had no significant effect on endogenous GA levels, indicating that melatonin affects DELLA proteins independently of GA accumulation. Melatonin induces the endogenous nitric oxide (NO) levels and may be involved in melatonin-mediated signaling pathways (Shi et al. 2015; Mukherjee 2019). Moreover, the contents of DELLA proteins are induced by NO, inducing NO-mediated photomorphogenesis and root growth (Lozano-Juste and León 2011; Bai et al. 2014). Thus, it was concluded that NO may be involved in melatonin-mediated DELLA stabilization, with a clear late-flowering effect (Shi et al. 2016). Also, melatonin regulates flowering time by promoting the transcription of *FLC*, which in turn inhibits the transcription of *FT*. The suggestion is that melatonin-mediated flowering is dependent on FLC, but not on FT. Also, some inhibitory effect on SOC1 is produced by exogenous melatonin through SPL inhibition of DELLA-via (Fig. 1). Recent evidence of the role of melatonin in flowering was observed in a *snat* mutant of *Arabidopsis* (Fig. 1). *SNAT2* was abundantly expressed in flowers. The *snat2* mutant produced less melatonin than the wild type, but only in flowers, not affecting to the melatonin levels in leaves, pointing to a tissue-specific expression together with SNAT1 (which is preferentially expressed in mature leaves). The *snat2* plants showed a slight delay flowering but with significant differences compared to exogenous melatonin treatments in GA-related genes such as *ent*-kaurene synthase, MYB33 (a GA-responsive inhibitor factor) and FT expression. According to the authors, these contradictory results (between *snat2* plants and exogenous melatonin application in wild-type plants) are explained by the fact that high-dose melatonin (0.5 mM) using in Shi et al. (2016) retards the growth of *Arabidopsis* seedlings and that exogenous melatonin did not mimic perfectly the effects of endogenous melatonin on flowering (Lee et al. 2019). Clearly, this aspect requires more research.

Strigolactones (SLs) are a class of carotenoid-derived compound with plant regulator activities, which participate in shoot branching, lateral root formation, germination, leaf senescence, stem secondary growth and flowering (Waters et al. 2017). In an interesting study on flowering in *Arabidopsis thaliana*, mutant plants with deficient in SL content or signaling flowering earlier and exogenous melatonin delayed flowering in both WT and SL mutants, but with a threshold level of endogenous melatonin of 7–8 ng/g FW, while when the tissue melatonin content fell below a threshold of 0.9–1.0 ng/g, flowering was accelerated. These data imply that, in the absence of a sufficient amount of melatonin, SL acts to downregulate *SPL* genes, thereby inducing earlier flowering; however, when the tissue was exogenously boosted with melatonin, a delay in flowering was induced as a result of the upregulation of *FLC*. Thus, the presence of SL acts to suppress melatonin signaling and/or synthesis, thereby linking SL with melatonin in the flowering response. The flowering delay by melatonin operates via the expression of *FLC*, which is triggered when the endogenous melatonin level exceeds a threshold concentration. Exactly how SL regulates the biosynthesis of melatonin is still unknown (Zhang et al. 2019).

Figure 2 compiles the data of the studies made in *Arabidopsis* on flowering related to melatonin (Shi et al. 2016; Zhang et al. 2019), including the relevant data about the possible regulation of GA content by melatonin (Shi et al. 2019). Melatonin upregulated GA biosynthesis genes such as GA20ox and GA3ox in cucumber seedlings in saline conditions, contributing to a high level of GAs as GA_4, promoting a salt-inhibited germination process (Zhang et al. 2014). In cotton seedlings, exogenous melatonin produced an increase in the GA content (Xiao et al. 2019), while in pear, melatonin induced parthenocarpy (Liu et al. 2018b) (see below). The contradictory effect of melatonin in some reproductive aspects respect to vegetative responses (i.e., stress situations) is an interesting behavior which will require more effort and research (Fig. 2).

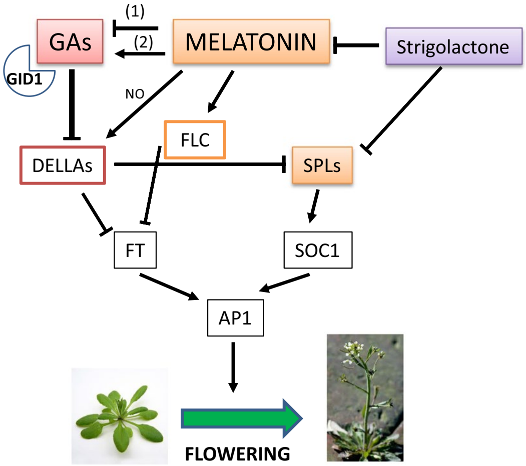

Fig. 2 Proposed role of melatonin in flowering. Melatonin acts by modulating the expression of GAs, DELLAs and FLC, delaying flowering in *Arabidopsis*. Strigolactone acts by inhibiting the biosynthesis/signaling of melatonin and also inhibits SPLs, resulting in earlier flowering. *GAs* gibberellins, *GID1* GA receptor, *DELLAs* transcriptional repressors of GA responses, *FLC* flowering locus C (core repressor of vernalization), *SPL* Squamosa promoter-binding protein-like, *FT* flowering locus T, *SOC1* suppressor of overexpression of Constans 1, *API1* Apetala-1. (1) in *snat2* plants, with low endogenous melatonin levels, decreased GAs levels have been suggested by Shi et al. (2019); (2) in some stress situations, exogenous melatonin treatments induce GA biosynthesis and an increase in GA levels; also parthenocarpy induction (see text)

Melatonin in fruit set and development: parthenocarpy

Several experiments have been carried out to analyze the role of melatonin in fruit set and fruit development. In initial studies, melatonin was seen to be present at high concentrations for the first 10 days after anthesis in devil's trumpet fruits (*Datura metel*), with the highest concentration measured in the ovule and the lowest in the fleshy fruit (Murch et al. 2009). During the development of cherry fruits (*Prunus avium*), the highest levels of melatonin were reached in stage II, embryo development and endocarp lignification, which has also been related to ROS accumulation in "watery" seeds, indicating that oxidative stress induced rapid melatonin biosynthesis (Zhao et al. 2013). In Micro-Tom tomato fruits, melatonin was also accumulated during fruit development, with a maximum in the last stages, when the fruits turn to a pink-red color (Okazaki and Ezura 2009). It is significant that tomato seeds had the highest melatonin concentration, which suggests a possible protective role for melatonin in seeds, as was earlier proposed (Manchester et al. 2000). A similar conclusion was reached in a study of fruit development (véraison) in Merlot wine grapes, in which the maximum melatonin level was reached in green fruits (Murch et al. 2010). In *Hypericum perforatum*, the highest levels were observed during uninucleate formation. High melatonin levels were also associated with a higher regeneration potential of isolated anthers (Murch and Saxena 2002). In two cultivars of pepper (*Capsicum annuum*), high amounts of melatonin were detected in the flowers, young and mature red fruits and in the seeds. The melatonin content in the fruit and seeds increased substantially as the fruit and thereby seed maturation progressed. According to the authors, the fact that higher levels of melatonin were detected in seedlings at the cotyledon stage as well as in reproductive organs and mature fruits of both cultivars supports the conclusion that higher levels of melatonin are geared toward protecting these organs from environmental assaults (Korkmaz et al. 2014).

In a more exhaustive work in *Red Fuji* apple fruits (*Malus domestica*), the expression of melatonin biosynthesis genes (Fig. 1) fully coordinated with melatonin production in apple fruit development. Melatonin levels in fruits exhibited an inverse relationship with the content of malondialdehyde, a product of lipid peroxidation. During fruit development, two major melatonin peaks appeared coinciding with the rapid growth of fruits and increased respiration and ROS (Lei et al. 2013). In a study in tomato fruits (*Solanum lycopersicum*), an increase in the serotonin (a precursor of melatonin) content was observed during fruit development in all fruit tissues (exocarp, mesocarp, placenta, columella, seed and jelly), reaching a maximum in the fully ripe stage (Hano et al. 2017). In different varieties of hemp (*Cannabis sativa*) plants, the melatonin content of mature seeds was 4–10 times higher than in leaves, stem or flower heads (Allegrone et al. 2019). Whatever the case, at present it is unknown whether melatonin peaks act as a signal of the fruit development process or as a response to ensure the proper redox status of fruits and seeds during their development.

Undoubtedly, the works related to the melatonin and development of fruits of which have had the greatest impact are those carried out by Xu et al. (Zhai et al. 2018; Liu et al. 2018b, 2019a, b). Parthenocarpy, the production of seedless fruit without ovule fertilization, can be natural or artificial. Plant hormones such as auxin and gibberellins can induce parthenocarpy in various horticultural species (Ozga and Reinecke 2003; Srivastava and Handa 2005). In *Starkrimson* pear (*Pyrus communis*), melatonin induces parthenocarpy by increasing GA_3 and GA_4 biosynthesis, producing fruits similar to those originated by hand pollination, through cell division and mesocarp expansion in the fruits. The seeds of exogenous melatonin-treated fruit were undeveloped and aborted later in the fruit-setting stage. In the study, the GA_3 and GA_4 contents of ovaries increased, the fruit shape index increased significantly, fruit transverse diameter decreased and the longitudinal diameter clearly increased, all effects similar to those of GA treatment. Melatonin induced significant upregulation of GA20ox (it produces active GAs) and downregulation of GA2ox (it produces inactive GAs), which established an increased level of active GAs. In contrast, the IAA content as well as the number of seed cell layers in the ovary did not increase after melatonin treatment and the IAA content declined after pollination compared with the control. These results contradict other results in which IAA induced parthenocarpy in eggplant (Du et al. 2016). Auxin-signaling-related genes did not change after melatonin treatment, showing that IAA is not a major factor in pear parthenocarpy. Photosystems I and II-related, chlorophyll-related and carbohydrate-related genes were upregulated after melatonin treatment. Transcriptome data showed that the numbers of cell cycle- and cell expansion-related genes were modified by melatonin treatment. Plant cyclins and expansins play important roles in cell division and plant development (Choi et al. 2006). In pear parthenocarpy, cyclins and 9–12 differentially regulated expansins were upregulated by melatonin. Thus, melatonin promotes cell division and cell expansion through the GA pathway, a conclusion confirmed by cell histology observations (Liu et al. 2018b).

Melatonin in fruit ripening and senescence

Many data on the role of melatonin in fruit ripening and senescence have been obtained. Table 1 shows the studies on fruit ripening using melatonin as a physiological modulator.

The effect of exogenous melatonin on fruit ripening has focused mainly on the relationship between melatonin and the plant hormone ethylene. In the first study, tomatoes treated with 50 µM melatonin showed substantial changes in their fruit ripening parameters, such as lycopene levels, fruit softening, flavor and ethylene-signaling and biosynthesis enzymes with respect to untreated tomatoes. Exogenous melatonin slightly increased ethylene generation and the subsequent timing of the climacteric peak through the upregulation of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase expression. Also, the ethylene receptor genes, NR and ETR4, and the transducing elements, EIL1, EIL3 and ERF2, were upregulated by melatonin (Sun et al. 2015).

In a differential proteomic analysis of tomato fruits, many proteins involved in ripening, cell wall, carbohydrate, flavonoid and fatty acid biosynthesis were influenced by melatonin. Also, some proteins related to anthocyanin biosynthesis during fruit ripening increased, suggesting that the exogenous application of melatonin positively regulates fruit ripening, while negatively regulating fruit senescence (Sun et al. 2016). Moreover, melatonin-treated tomato plants through seed-priming had much higher yields, as well as higher ascorbic acid, lycopene and Ca levels, while the content of N, Mg, Cu, Zn, Fe and Mn decreased. By contrast, plants irrigated weekly with nutrient solutions supplemented with melatonin showed significant improvements in their

Table 1 Plant species used in fruit ripening studies with melatonin treatments

Specie	Best melatonin treatment	Mode	References
Tomato	50 µM, 2 h	Dipping of fruits	Sun et al. (2015)
	"	"	Sun et al. (2016)
	0.1 mM, spray	In seeds, roots and fruits	Liu et al. (2016)
Peach	0.1 mM, 10 min	Dipping of fruits	Gao et al. (2016)
Strawberry	0.1 mM, 5 min	Dipping of fruits	Aghdam and Fard (2017)
	0.1–1 mM, 5 min	"	Liu et al. (2018a)
	0.1 mM, 5 min	"	El-Mogy et al. (2019)
Grape berry	0.1 mM, 10 s	Dipping of fruits	Xu et al. (2017)
Pear	0.1 mM, 12 h	Dipping of fruits	Zhai et al. (2018)
	50 µM, 2 h	"	Liu et al. (2019a)
	0.1 mM, spray	In 5-year-old trees	Liu et al. (2019b)
Plum	0.1–1 mM, 40 days	Dipping of fruits	Bal (2019)
Litchi	0.4 mM, 5 min	Dipping of fruits	Zhang et al. (2018)
Banana	0.2–0.5 mM, 2 h	Dipping of fruits	Hu et al. (2017)
	10 mM, 3 min	Dipping of fruits	Li et al. (2019)

contents of soluble solids, ascorbic acid, lycopene, citric acid and P compared with control plants that received only a standard nutrient solution. Sucrose and glucose contents increased following melatonin treatment. As tomato fruits develop, levels of soluble galactose increase, thereby stimulating ethylene production and, subsequently, promoting ripening. Also, melatonin-treated plants have significantly higher levels of citric acid. The flavor of fruits is optimal when there are high concentrations of both sugars and organic acids. In general, melatonin increased both tomato fruit yield (by up to 13%) and quality (Liu et al. 2016).

In peach fruits, melatonin treatment effectively slowed senescence in the two peach cultivars assayed, as indicated by reduced weight loss, the incidence of decay and the respiration rate, while firmness and the total soluble solids and ascorbic acid contents were maintained. Similarly, the postharvest application of melatonin delayed senescence and maintained the quality of peach fruit, which may be attributed to its capacity to mediate antioxidative actions, suggesting that melatonin treatment could be considered as a promising method for delaying senescence and maintaining fruit quality of postharvest peach fruit (Gao et al. 2016).

In melatonin-treated strawberry, improvements in postharvest decay indicators and high ATP content, antioxidant activity and polyphenol levels have been described (Aghdam and Fard 2017). Similar data were provided by Liu et al. (2018a). In strawberry fruits treated with 0.1–1.0 mM melatonin for 5 min and then stored at 4 °C and 90% humidity, reduced decay and weight loss were observed. Also, senescence parameters (color, firmness, the total soluble solids content, titratable acidity, hydrogen peroxide and MDA) were clearly delayed by melatonin treatments. By contrast, total phenolics and flavonoid contents increased, resulting in a higher antioxidant capacity. Moreover, melatonin treatment enhanced the expression of melatonin biosynthetic genes including TDC, T5H, SNAT, COMT and ASMT and consequently increased the content of endogenous melatonin (Fig. 1). More recently, the effect of melatonin on aroma profile of strawberries has been studied. Melatonin treatment delayed fruit deterioration and also significantly reduced CO_2 production compared to control fruits. The relative abundance of the majority of volatile organic compounds (VOCs) was not affected, but some relevant VOCs were altered, which may affect consumer perception of quality (El-Mogy et al. 2019).

In grape berries, melatonin treatment enhanced ethylene production, which partially contributed to polyphenol accumulation (anthocyanins) and an increase in antioxidant activities (Xu et al. 2017). In *Moldova* grape berries, melatonin accumulated rapidly from the onset of véraison, reached a maximum 94 days after bloom and then exhibited low levels in the late stages of berry ripening. Treatment with between 10 and 100 µM melatonin increased the levels of ABA, hydrogen peroxide and ethylene production and promoted berry ripening compared with a control treatment. The suppression of ethylene biosynthesis had the greatest inhibitory effects on melatonin-induced berry ripening compared with the effects of ABA and hydrogen peroxide. Melatonin also promoted ethylene production via ABA. The authors pointed out that the use of melatonin during berry ripening probably accelerates berry ripening (Xu et al. 2018).

During the cold storage of pears, melatonin has the potential to retain the commercial value of postharvest pear fruits and delay senescence by limiting ethylene production and the reactive oxygen burst (Zhai et al. 2018). In pear fruits, too, melatonin and also nitric oxide decreased the rate of polygalacturonase and cellulase upregulation, inhibited the expression of ethylene biosynthesis genes (ACS and ACO) and reduced respiration and ethylene production rates, delaying fruit senescence (Liu et al. 2019a). In another interesting work by the same authors, melatonin increased fruit weight by 47.85% compared to the control. During the period of fruit maturation, melatonin increased the content of soluble sugars, particularly sucrose and sorbitol, probably as a result of improved starch accumulation. Expression levels of the invertase gene were lower in melatonin-treated fruit, which resulted in lower enzyme activity, whereas the activity of sucrose phosphate synthase increased due to enhanced levels of expression. Thus, the application of melatonin improved the sucrose content of pear fruits. In short, this study shows that the application of melatonin to pear trees may be used for the production of larger and sweeter fruits of higher economic value (Liu et al. 2019b).

Immediately after harvest, plum fruits (*Prunus domestica*) were immersed in several melatonin solutions at 1 °C for 40 days. It was found that the 0.1 and 1 mM melatonin solutions were the most effective treatments for reducing weight loss and maintaining greater firmness. The ascorbic acid content, total phenolic content and antioxidant activity were greater in melatonin-treated plums than the control. The authors suggested that melatonin treatment may be a useful technique to extend the postharvest life of plum while maintaining an acceptable fruit quality (Bal 2019).

In litchi fruits (*Litchi chinensis*), fruits harvested at commercial maturity and immersed in 0.4 mM melatonin at 25 °C for 5 min strongly suppressed pericarp browning and delayed discoloration during storage. Melatonin treatment reduced relative membrane leakage rate and inhibited the generation of ROS and MDA. Melatonin treatment markedly promoted the accumulation of endogenous melatonin, delayed the loss of total phenolics, flavonoids and anthocyanins and enhanced the activities of antioxidant enzymes such as SOD, CAT, APX and GR and the expression of four genes encoding enzymes for the repair of oxidized proteins. By contrast, the activities of browning-related enzymes including polyphenol oxidase and peroxidase were reduced. These findings suggested that the delay of pericarp browning and senescence by melatonin in litchi fruit can be attributed to the maintenance of redox homeostasis through improvement in the antioxidant capacity and the modulation of the repair of oxidatively damaged proteins (Zhang et al. 2018).

In a study of several banana (*Musa acuminata*) varieties, the exogenous application of melatonin resulted in slower postharvest banana ripening. The effect was concentration dependent, with the 200 and 500 µM treatments being the most effective. Exogenous melatonin also led to high endogenous melatonin content, reduced ethylene production through the regulation of *ACO1* and *ACS1* expression and delayed sharp changes in quality indices. The authors proposed that melatonin may be used as an indicator for banana fruit ripening in various varieties and that the repression of ethylene biosynthesis and postharvest ripening by melatonin can be used for the biological control of postharvest fruit ripening and quality (Hu et al. 2017). In another study in banana, exogenous melatonin treatment significantly reduced the incidence of anthracnose in ripe yellow banana fruit and delayed fruit senescence. However, melatonin treatment did not affect the growth of *Colletotrichum musae* in vitro. Also, after melatonin treatment, auxin, ethylene and mitogen-activated protein kinase (MAPK) signaling pathways were enhanced, which might enhance fruit resistance by regulating their physiological characteristics, disease-resistant proteins and metabolites (Li et al. 2019). With respect to melatonin action against plant pathogens in fruits, several models have been studied in both viral and fungal diseases. Thus, melatonin improved resistance and reduced lesions in different plant diseases such as tobacco mosaic virus and apple stem grooving virus (Chen et al. 2019; Zhao et al. 2019) and was also effective against the fungi *Fusarium, Diplocarpon, Botrytis, Alternaria, Penicillium, Rhizopus, Podosphaera and Phythophthora*, generally inducing SA and JA immunoresponses and leading to an extension of the shelf life and quality of fruits (Arnao and Hernández-Ruiz 2015b, 2018a, b; Hernández-Ruiz and Arnao 2018; Sharif et al. 2018; Moustafa-Farag et al. 2020).

In résumé, melatonin treatments can have different effects on ethylene biosynthesis enzymes, inducing ACO and ACS in tomato fruit and in *Moldova* grape berry, promoting its ripening; and, in contrast, melatonin downregulates the same enzymes in pear, strawberry, plum, litchi and banana, decreasing ethylene generation and improving extended shelf life through delay senescence. The physiological state of the fruit and the concentration of melatonin used, together with the treatment times, seem decisive to understand the effects produced. At this point, it is important to note that while the ripening is a genetically regulated process and clearly marked by the action of ethylene, senescence is an oxidative process that involves deterioration and disorders. As both processes are not clearly separated at a specific point or time, they are regulated in different ways as expressed in Fig. 3. These findings in general suggest that melatonin treatment may be a useful technique to extend the postharvest life and improve the quality of fruits. Figure 3 depicts a scheme whereby the role of melatonin and the redox network are integrated with respect to ripening and senescence (Kumar et al. 2014; Mukherjee 2019; Xu et al. 2019; Arnao and Hernández-Ruiz 2019a, c).

Conclusions and perspectives

In flowering, the appearance of a melatonin peak during the intermediate stages of flower development has been described, and a protective role of melatonin during

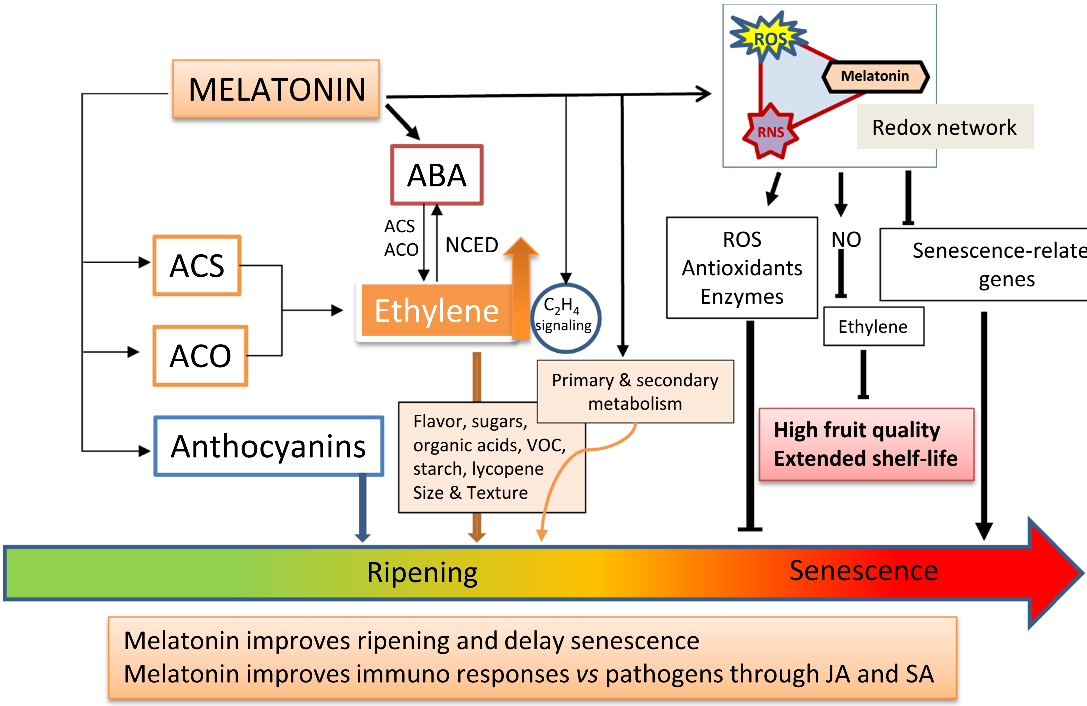

Fig. 3 Proposed role(s) of melatonin in fruit ripening and senescence. Melatonin acts by upregulating both ethylene biosynthesis and anthocyanin biosynthesis enzymes, accelerating fruit ripening and also promotes ABA biosynthesis on diverse elements of ethylene signaling and the enhancement of diverse steps of primary and secondary metabolism. The effect of melatonin in senescence is the contrary, delaying it. Melatonin, through the redox network involving ROS and RNS (mainly NO), decreases the ROS level, upregulating diverse antioxidative enzymes and metabolites, which delay senescence. Also, several senescence-related genes are downregulated by melatonin. All this improves fruit quality and extends fruit shelf life

flowering against situations of stress such as light and temperature has been proposed. However, the direct correlation between the melatonin peak(s) and the induction of melatonin biosynthetic enzymes suggests that melatonin may participate in flower development. In recent studies in *Arabidopsis,* exogenous melatonin induced a delay in flowering. Melatonin increased the protein stabilizations of DELLA proteins, but also induced endogenous NO levels with a clear late-flowering effect. Melatonin regulates flowering time by promoting the transcription of *FLC*, which, in turn, inhibits the transcription of *FT.* Also, the role of melatonin in flowering was demonstrated in a *snat* mutant of *Arabidopsis,* which produced less endogenous melatonin than the wild type, possibly affecting GA biosynthesis and *FT* expression and slightly retarding flowering. The presence of exogenous SL acts to suppress melatonin signaling and/or biosynthesis, thereby inducing earlier flowering.

In fruit set, the most significant role of melatonin is its capacity to induce parthenocarpy. This effect in pear is the consequence of the induction of GA biosynthesis, producing fruits similar to those originated by hand pollination, promoting cell division and mesocarp expansion. Also, plant cyclins and expansins were upregulated by melatonin, in addition to other gene elements related to carbohydrate metabolism and photosynthesis.

Melatonin promotes the ripening and retards the senescence of fruits. Melatonin acts through the up- and downregulation of many gene elements related to ethylene, anthocyanins, flavonoids and cell wall enzymes, among others, such as senescence-related genes, carbohydrate metabolism and the ASC-GSH cycle. The redox network involving melatonin, ROS and RNS has a central role controlling redox homeostasis during ripening and senescence, in a similar way to that which occurs in stress situations (Arnao and Hernández-Ruiz 2019a). This different action of melatonin in ripening and senescence can be explained by different action on several transcripts, being senescence-related genes downregulated, whereas ripening-related genes were upregulated. All this adds up to an optimal ripening process and a delay in the senescence of melatonin-treated fruits, resulting in an extended shelf life and higher-quality fruits. The stimulation by melatonin of plant immune responses against fruit diseases contributes greatly to maintaining the fruit in healthy conditions.

With respect to future perspectives, the possible use of melatonin to retard flowering needs more experimentation. Until now, only some data exist in *Arabidopsis*, and it will be necessary to apply this knowledge to horticultural species. Also, the precise role of strigolactones as inhibitors of the action of melatonin in flowering should be studied in depth. The effect of melatonin in flowering has not been studied by comparing short, long and neutral day plants, which seems essential before any firm conclusions can be reached about flowering. As regards fruit set, more data on the cross talk between melatonin and plant hormones such as auxin, GA and cytokinins are essential. Also, the induction of parthenocarpy in pear by melatonin should be confirmed in other species, in both horticultural (tomato, egg, pepper, cucumber) and tree (apple, plum, peach) fruits to really establish the possibilities of melatonin as a fruit set improver.

However, the effects of melatonin in postharvest are much better known. There are quite a few studies that show the field of action of melatonin as a ripening inducer, but especially as a retarding agent for the senescence of fruit. In this sense, the concentration of melatonin and the time of application seem to be key aspects to obtain good results concerning fruit preservation. In some cases, melatonin treatments can relax the often narrow range of conservation conditions such as temperature, making fruit more able to withstand wider variations in this important parameter. Another aspect to consider is the legal possibility of using synthetic melatonin for this purpose. In a recent publication, we analyze—from the physiological and legal point of view—the possibilities of applying melatonin within the framework of agricultural production in the European Community (Arnao and Hernández-Ruiz 2019b). Recently, nitric oxide and hydrogen sulfide have attracted great interest. These physiological gases are usually involved in many of the responses mediated by melatonin or vice versa and in the epigenetic control of fruit ripening. It is necessary to investigate the gene regulation of this trio (NO, H_2S and melatonin), which are clearly related to the redox network and its synergistic/antagonistic effects on the flowering, fruit development and ripening of fruits.

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