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# Review of Melatonin in Horticultural Crops

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## Abstract

Melatonin is an indoleamine, abundant in animals and plants, which has the functions of regulating circadian rhythm, improving immunity and anti-aging in animals, and is a good health care product beneficial to human health. Recent studies have shown that melatonin has physiological functions including regulating plant growth, promoting seed germination, controlling root development and delaying leaf senescence. The antioxidant properties of melatonin give it the ability to strengthen plants' resistance to stress. The comprehensive researches in recent years, involving five aspects of "the biosynthetic pathway of melatonin in plants, the melatonin in horticultural crops and its influencing factors, the roles of melatonin in the growth and development of horticultural crops, in the response to stress of horticultural crops, the signal transduction network of melatonin in regulating plant growth and the development and stress resistance," are reviewed in the present paper. The application of melatonin in horticulture production is also discussed, which can provide a theoretical reference for the application of melatonin in horticultural production.

**Keywords:** development, growth, horticultural crops, melatonin, stress tolerance

## 1. Introduction

Melatonin, *N*-acetyl-5-methoxytryptamine, is an indole-like tryptamine. In 1958, Lerner et al. [1] extracted melatonin from the pineal gland of cattle and found that it was a kind hormone-like substance, which was widely involved in the growth and development regulation, as well as signal transduction, in animals. Before the 1990s, melatonin was recognized as an animal hormone. However, Balzer and Hardeland [2] successfully isolated and identified melatonin from single-celled algae, indicating the existence of natural melatonin in the plant kingdom. Since then, melatonin has become a research hotspot and received extensive attention from researchers in the field of plant and agricultural science. With the rapid development of material separation and identification technology, plant physiology and cell biology, molecular biology, sequencing technology and other research methods, scientists have conducted more comprehensive and in-depth studies on the synthesis pathway, concentration, distribution and biological function of melatonin in plants. Moreover, melatonin has a variety of regulatory effects in plants and has many benefits for human health. Therefore, the research on melatonin in horticultural crops is fast increasing, which is no less than that in model plants of *Arabidopsis thaliana*. Therefore, this review comprehensively and systematically introduced the research progress on melatonin and its function in horticultural

crops, and looked forward to the future research to provide some theoretical basis for the application of melatonin in horticultural crop production.

## 2. The biosynthetic pathway of melatonin in plants

Melatonin is a small molecule, which can shuttle freely in and between cells due to its hydrophilic and lipophilic molecular structure [3]. Using St. John's wort (*Hypericum perforatum* L. cv. Anthos) seedlings as the plant material, Murch et al. [4] exogenously supplied ^{14}C labeled tryptophan and found the presence of radioactive tryptamine, 5-hydroxytryptophan, serotonin, indoleacetic acid and melatonin via adopted isotope tracer approach. Among them, tryptophan and 5-hydroxytryptophan are the synthetic precursors of melatonin in animals. The study indicated that the biosynthetic pathway of melatonin was similar and conserved from animals to plants. Nowadays, the biosynthesis of melatonin using L-tryptophan as substrate has been evidenced in plants with the following steps [5]: L-tryptophan is decarboxylated under the catalysis of L-tryptophan decarboxylase (TDC) to form tryptamine; tryptophan reacts further with tryptophan 5-hydroxylase (T5H) to produce serotonin; serotonin-*N*-acetyltransferase (SNAT) catalyzes the production of *N*-acetylserotonin; and *N*-acetylserotonin can be further catalyzed by *N*-acetylserotonin *O*-methyltransferase (ASMT) or caffeic acid *O*-methyltransferase (CAMT) to produce melatonin (**Figure 1**).

At present, key genes of melatonin synthesis, *TrpDC*, *T5H*, *AcSNMT* and *HIOMT*, have been successfully cloned from plants and their expression patterns have also been carried out [6]. In recent years, plant chloroplasts and mitochondria are considered as the key organelles of the melatonin synthesis [7]. The hypothesis to establish the basis for melatonin is an important biological antioxidant, and chloroplasts and mitochondria are main reactive oxygen species (ROS) sources; these two organelles generate large amounts of melatonin, which is used to remove
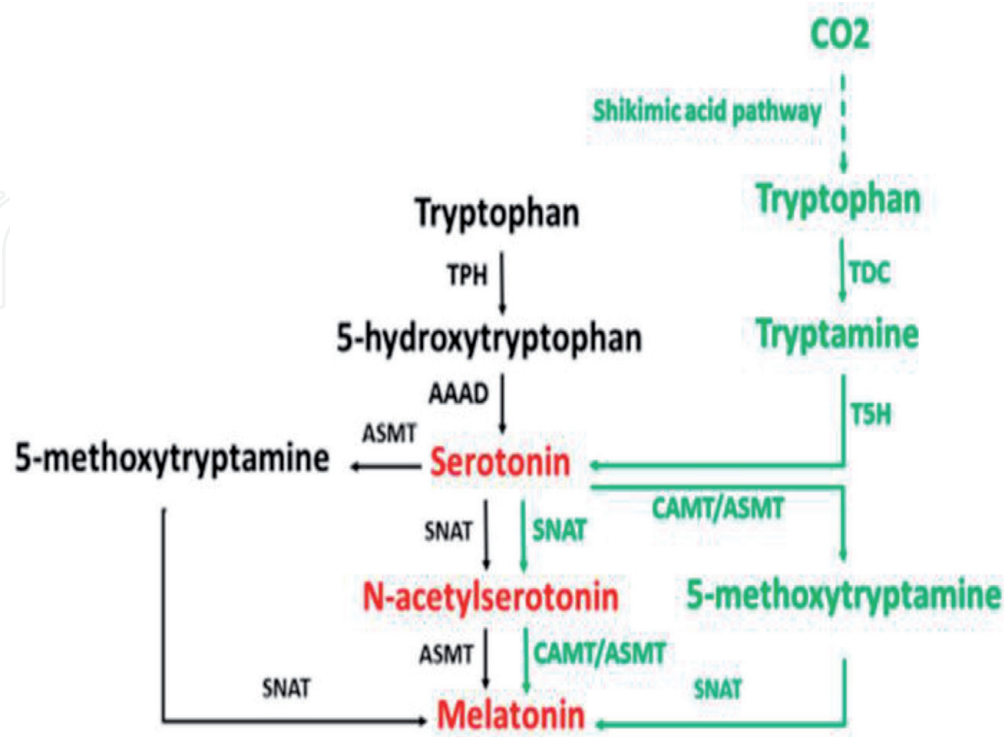


**Figure 1.**
*A comparison of the biosynthetic pathways of melatonin in animals and in plants. Black occurs only in animals; green occurs only in plants; and red occurs in both animals and plants.*

excess ROS to reduce the oxidation of cells. However, a recent study in rice (*Oryza sativa* L.) has shown that the key melatonin synthesis gene *COMT* is located in plant chloroplasts and has demonstrated that melatonin synthesis is increased through the 5-MT pathway [8]. CAND2/PMTR1, a hypothetic plant melatonin receptor, recently has been identified in *Arabidopsis thaliana* [9]. It is located in the plasma membrane with a receptor-like topology and interacts with the G-protein subunit (GPA1), while expression in different tissues is induced by melatonin. The binding of plant melatonin receptors triggers Gγb and Gα activation of NADPH oxidase-dependent $H_2O_2$ production (RBOH), increasing $Ca^{2+}$ inflow, promoting $K^+$ outflow, and ultimately leading to stomatal closure [10]. The cloning of these melatonin-metabolizing genes and the discovery of the receptors can lay a certain foundation for the study of melatonin-related functions in the future.

## 3. Concentrations of melatonin in horticultural crops and its influential factors

Although policies regarding the free sale, universal use and food supplement of melatonin are still controversial, most developed countries have classified melatonin as an over-the-counter drug and allowed it to be sold freely in pharmacy stores. Currently, melatonin is regarded as an over-the-counter medicine and health care product to relieve sub-health and improve sleep [11]. Some studies indicated that regular consumption of melatonin-rich foods can significantly improve human health [12–14]. In view of the health benefits of melatonin, more and more nutritionists begin to pay attention to the amount of melatonin in food, hoping that people can get more natural melatonin from daily food.

Two research groups identified melatonin in some edible plants in 1995. Vantassel et al. [15] have identified the presence of plant-derived melatonin in higher plants of morning glory (*Ipomoea nil* L.) and tomato (*Solanum lycopersicum* L.) by radio immunoprecipitation and gas chromatography-mass spectrometry analysis; this was also the first report of plant-derived melatonin in horticultural crops. Since then, melatonin has been isolated and identified in numbers of horticultural crops (**Table 1**). Melatonin levels are usually high in seeds and low in fruits in these edible organs of horticultural crops. According to the statistics, melatonin concentrations in various tissues of plants generally conform to the following rules from high to low: seeds, leaves, roots, flowers and fruits [16]. Moreover, the melatonin concentrations are not evenly distributed in the same tissue. Using white lupine (*Lupinus micranthus* Guss.) leaves as an example, the highest concentrations of melatonin are in the leaf tip, then in the middle leaf, and the least in the leaf base [17]. Through comparative analysis, the distributed gradient of melatonin is similar to the distribution of auxin, which indicates that these two indoleamine compounds may play similar or synergistic roles in plants [18].

The melatonin concentrations in horticultural crops are closely influenced by species, varieties, growing environment, cultivated methods, harvesting time and processing methods. As shown in **Table 1**, melatonin concentration in sweet cherry (13.46 ng $g^{-1}$) is threefold higher than that in tomato. (4.1 ng $g^{-1}$) [19]. In different varieties of tomato, the melatonin concentrations fluctuated greatly from 0.5 pg $g^{-1}$ to 114.5 ng $g^{-1}$. This difference is largely influenced by the genotype of the variety itself. Climate and environmental factors in different years have significant effects on the melatonin concentrations of horticultural crops. For example, the melatonin concentrations of 'Marbone' tomato harvested in 2010 were six times higher than those in 2009, while the melatonin concentrations of 'Festival' strawberries harvested in 2010 were three times lower than those

| Common name | Specie name | Melatonin content (pg g$^{-1}$) |
|---|---|---|
| Sweet cherries | *Prunus avium* L. | 8000–120,000 (FW) |
| Tart cherries | *Prunus cerasus* L. | 1000–19,500 (FW) |
| White radish | *Raphanus sativus* L. | 657.2 (FW) |
| Ginger | *Zingiber officinale* Rose | 583.7 (FW) |
| Pomegranate | *Punica granatum* L. | 540–5500 (FW) |
| Shungiku | *Chrysanthemum coronarium* L. | 416.8 (FW) |
| Pineapple | *Ananas comosus* L. | 302 (FW) |
| Chinese cabbage | *Brassica rapa* L. | 112.5 (FW) |
| Cabbage | *Brassica oleracea capitata* L. | 107.4 (FW) |
| Carrot | *Daucus carota* L. var. *sativa* Hoffm. | 55.3 (FW) |
| Taro | *Colocasia esculenta* L. | 54.6 (FW) |
| Apple | *Malus domestica* Borkh. | 47.6 (FW) |
| Spinach | *Basella alba* L. | 38.7 (FW) |
| Onion | *Allium cepa* L. | 31.5 (FW) |
| Cucumber | *Cucumis sativus* L. | 24.6 (FW) |
| Kiwi fruit | *Actinidia Chinensis* | 24.4 (FW) |
| Strawberry | *Fragaria* x *ananassa* Duch. | 12.4 (FW) |
| Asparagus | *Asparagus officinalis* L. | 9.5 (FW) |
| Banana | *Musa acuminata* Colla | 8.9 (FW) |
| Beet root | *Beta vulgaris* L. | 2 (FW) |
| Tomato | *Solanum lycopersicum* L. | 0.5–114,500 (FW) |
| Thyme | *Thymus vulgaris* L. | 38,000 (DW) |
| Chinese liquorice | *Glycyrrhiza uralensis* Fisch. | 34,000 (DW) |
| Coffee beans | *Coffea* sp. | 5800–6800 (DW) |
| Feverfew | *Tanacetum parthenium* L. | 1700 (DW) |
| Mulberry Morus | *Morus alba* L. | 1510 (DW) |
| Black pepper | *Piper nigrum* L. | 1092 (DW) |
| Kidney bean sprouts | *Phaseolus vulgaris* L. | 529 (DW) |
| Aloe | *Aloe vera* L. | 516 (DW) |
| White radish Raphanus | *sativus* L. | 485 (DW) |
| Jujube | *Ziziphus jujube* Lam. | 256 (DW) |
| White mustard seed | Brassica hirta L. | 189 (DW) |
| Qin Jiao | *Gentiana macrophylla* Pall. | 180 (DW) |
| Mustard seed | *Brassica nigra* L. | 129 (DW) |
| Goji berry | *Lycium barbarum* L. | 103–530 (DW) |
| Almond seed | *Prunus amygdalus* Batsch | 39 (DW) |
| Sunflower seed | *Helianthus annuus* L. | 29 (DW) |
| Anise seed | *Pimpinella anisum* L. | 7 (DW) |
| Coriander seed | *Coriandrum sativum* L. | 7 (DW) |
| Celery seed | *Apium graveolens* L. | 7 (DW) |

| Common name | Specie name | Melatonin content ($pg\,g^{-1}$) |
|---|---|---|
| Walnut | *Juglans regia* L. | 3.5 (DW) |
| Bell pepper | *Capsicum annuum* L. | 0.179–0.581 (DW) |

**Table 1.**
*The content of melatonin in horticultural crops [16].*

in 2009 [20]. The concentrations of melatonin in field-cultivated tomato were significantly higher than those in the phytotron-cultivated tomato, and higher than those in the vitro-cultivated tomato [21]. Riga et al. [22] found that fruit bagging can significantly increase the melatonin concentrations in most tomato varieties but reduce the concentrations of melatonin in pepper (*Capsicum annuum* L.). The dynamic change of melatonin concentrations in mammals is regulated by photoperiod and circadian rhythm; the change of melatonin concentrations in plants is also following an analogous pattern. Thus, the harvest time in one day also has an impact on the concentrations of melatonin in the product organs. Zhao et al. [23] showed that the melatonin concentrations of cherries picked at night were significantly higher than those when picked at day times. However, the peak value of melatonin concentrations in cherry fruits usually occurred at 14:00, when the temperature was the highest and the light was intense. This result suggests that melatonin is not only responding to the photoperiod but also involved in a photoprotective mechanism and free radicals scavenging against light damage during photosynthesis. Other studies have shown that the harvest time can also affect the melatonin concentrations in the organs of products. The concentrations of melatonin in cherry usually increase with fruit ripening [24]. The melatonin concentrations of mulberry leaves decreased with the maturity process [25]. The concentrations of melatonin initially increased and then decreased with the chili (*Capsicum annuum* L.) maturation [26]. Similarly with mammals, the highest melatonin concentrations usually exist in young tissues, followed by mature tissues, and then in aging tissues, which indicates that melatonin offers juvenile protection or acts as an aging antagonistic substance. Post-harvest processing has a great influence on the concentrations of melatonin in horticultural products. For example, Kirakosyan et al. [27] compared the melatonin concentrations of frozen, freeze-dried powder, juice and dried fruits of cherry. They found that the cherry juice and cherry dried fruits did not contain melatonin. However, melatonin was detected in frozen cherries and freeze-dried powder, and the melatonin concentrations in freeze cherry were significantly higher than those in freeze-dried fruits. These results indicate that melatonin is not stable and easily degrades upon destruction of the cellular structure during form processing. Yeast is beneficial to produce melatonin during grape wine making [28]. The melatonin concentrations of mulberry tea are only 15% of those in fresh leaves when deep processing of mulberry leaves for green tea and black tea takes place [25]. In addition, melatonin can be detected in most Chinese teas, such as Longjing tea and oolong tea. Therefore, the selection of varieties with high melatonin level, appropriate climatic conditions, reasonable use of bagging cultivation technology and determination of appropriate harvesting time is important to maintain the high melatonin concentrations in horticultural crops. For the fruit and vegetable deep processing products, appropriate processing technology should be considered to maximally maintain the natural melatonin in the products. However, there is still a lack of systematic research on the influence of these factors on melatonin concentrations.

## 4. Roles of melatonin in regulating growth and development of horticultural crops

### 4.1 Effects of melatonin on growth and yield formation of horticultural crops

Hernández-Ruiz et al. [18] were the first to propose that melatonin was a hormone-like growth regulator in plants. Because melatonin promotes the hypocotyl growth of albino lupine (*Lupinus micranthus* Guss.) in vitro, its action is related to the concentration gradient in plant tissues. The conclusion was consistent within barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.) and sunflower (*Helianthus annuus* L.) [29]. In terms of chemical structure, the side chain of melatonin has no carboxyl group of IAA, and its growth-promoting activity is about 10–50% of the effect of IAA. Moreover, melatonin does not bind to the IAA receptor, indicating that although melatonin and IAA are co-indoleamine compounds, their signal regulation mechanism is quite different. Since no specific binding receptor of melatonin has been found in plants, melatonin cannot be defined as a plant hormone. Nevertheless, more and more studies are focusing on exploring and identifying the functional and signaling networks that melatonin performs in plants.

Byeon and Back [30] obtained transgenic rice with excessive melatonin accumulation via overexpression of sheep *SNAcT* gene in rice. The growth potential of transgenic rice was significantly higher than that of wild rice and showed the characteristics of delayed flowering and reduced yield. This is consistent with the viewpoint mentioned in Section 2 of this report that "melatonin is a kind of anti-aging substance," indicating that melatonin can inhibit reproductive growth and promote nutritional growth. According to this characteristic, we predicted that through the modification of melatonin-related genes or the exogenous spraying or watering melatonin, the horticultural crops with vegetative organs, as products, could effectively obtain higher economic yield, but the specific effect needs further test to verify.

Other studies have shown that 50 μmol $L^{-1}$ of melatonin solution can significantly promote the growth of soybean seedlings and increase the yield of soybean [31]. Liu et al. [32] found that in the late stage of pear fruit development, spraying of 100 μmol $L^{-1}$ of melatonin solution into the pear tree could promote the accumulation of endogenous melatonin in pear fruit. Melatonin increased the size of pear fruit by increasing the net photosynthetic rate and maximum quantum efficiency of photosystem II. During the ripening period, melatonin increased the concentrations of soluble sugar, especially sucrose and sorbitol, which may be the result of improving the accumulation of starch and promoting the expansion of pear fruit, which had a significant effect on increasing yield. In a study on grapes, Meng et al. [33] found that spraying of 100 mol $L^{-1}$ of melatonin solution on young grape fruits could promote the accumulation of endogenous melatonin in grape fruits and promote the expansion of grape fruits, which had a significant effect on increasing production.

From these effects of melatonin on crop growth and yield, it becomes apparent that the promotion effect of melatonin on crop growth is a common feature. The difference was that the increase of endogenous melatonin concentrations reduced the rice yield, while the exogenous melatonin treatment increased the soybean and grape yield. The effect of melatonin on crop yield seems contradictory; however, we think that first, the soybean experiment adopted melatonin in treatment at seedling stage, but no melatonin was added at the yield formation stage. However, the melatonin level of transgenic rice was high in the whole growth period, so soybean showed an increase in yield, and rice showed a decreased yield. Second, according

to the phenotypic difference, melatonin promoted plant growth in soybean more than it did in rice. The full growth of vegetative body provides more abundant photosynthetic products for reproductive growth. Third, the essence of melatonin inhibiting yield formation lies in inhibiting reproductive growth, that is, seed formation. The berry of grape is developed from ovary, which is similar to tomato and other berry crops. The expansion of ovary in the early stage of fruit development needs the stimulation of IAA to form a strong storage for nutrition. The effects of melatonin and IAA are similar, which may be the specific mechanism of melatonin in promoting the expansion of grape fruit. Fourth, Zhao et al. [34] showed that low concentrations of melatonin (10 μmol L$^{-1}$) could promote the metabolism of sugars, photosynthesis, loading and transportation of sucrose in the maize phloem, thus promoting the growth of maize plants. However, high concentrations of melatonin (1 mmol L$^{-1}$) could inhibit sucrose loading in phloem, resulting in the accumulation of excessive sucrose, hexose and starch in leaves. As a result, leaf photosynthesis and the growth of maize plants were inhibited. Zhang et al. [35] also obtained similar results on the effects of melatonin concentrations on flowering in apple tree. Overexpression of *SNAcT* gene in rice is similar to the continuous supplementation of melatonin, while in the soybean and grape trials, the short-term supplementation of melatonin resulted in the difference in melatonin supply. Combined with the results of Zhao et al. [34], we analyzed that the concentrations of melatonin in rice was too high, inhibiting the loading of sugars in the phloem, which leads to reduced transport of sugars to the endosperm to form a low-yielding phenotype. The concentration of melatonin in soybean and grape is suitable, which is conducive to the transport of sugar to endosperm and ovary, thus forming a high-yield phenotype. In conclusion, these four factors may be the main reasons behind the differences in the characterization of melatonin on yield. Based on previous studies, we predicted that the right amount of melatonin could increase the yield of root vegetables, leaves, stems, fruits and fruit vegetables as well as the pomology with flesh as edible organs, while the effect on the yield of cauliflower vegetables (such as cauliflower) and pomology with seeds as edible organs (such as walnuts) remains to be verified. However, different crops have different demands on melatonin in different periods. In view of the importance of yield characters in agricultural production, the influence of melatonin on the yield of horticultural crops should become a scientific research hotspot in the future.

## 4.2 Melatonin regulates the ripening, aging and preservation of horticultural crops

Ripening, aging and preservation are contradictory processes in the production of horticultural products. Interestingly, more and more studies have shown that melatonin can be used to flexibly regulate the ripening, aging and storage of horticultural crops under different concentrations and conditions. Sun et al. [36] found that pretreatment of tomato with 50 μmol L$^{-1}$ and 100 μmol L$^{-1}$ melatonin could promote tomato ripening. Its regulatory mechanism can be summarized as follows: melatonin activates the expression of *ACS4* gene and enhances the synthesis of ethylene. Ethylene signal promoted the synthesis of lycopene by expression of *PSY1* gene through the signal transduction pathways such as *NR*, *ETR4*, *EILs* and *ERF2*, which made the tomato turn color, regulated the expression of *TBG4*, *PG2A*, *Exp1*, *XTH5* and *PE1* genes to degrade the cell wall, softened the fruit, regulated the expression of *PIPs* gene to control fruit water loss, and regulated the expression of *ADH2* and *AAT* genes to promote sugar conversion. Thus, phenotypic characteristics promoting tomato ripening were formed. Similar results have been found in grapes [37]. Lei et al. [38] showed that the synthesis of melatonin

in the ripening process of apple fruits was mainly catalyzed by L-tryptophan decarboxylase (TrpDC), tryptophan hydroxylase (T5H), 5-hydroxytryptophan *N*-acetyltransferase (SNAcT) and *N*-acetyl-5-hydroxytryptophan methyltransferase (AcSNMT). Moreover, the research group proposed that during the ripening process of apple fruits, melatonin and malondialdehyde (MDA) concentrations were always negatively correlated. So they speculated that the main role of melatonin in the ripening process of apple fruits was ROS scavenging. When different concentrations of melatonin were used to treat fruits of different species and ripeness, the results were often opposite. Liu et al. [39] found that treatment with melatonin after harvest effectively delayed the senescence of strawberry fruits and reduced the rate of decay, severity of decay and weight loss of strawberry fruits. The main mechanism is to reduce the total soluble solid, $H_2O_2$ and MDA concentrations of the fruit, promote the accumulation of total phenolic substances and flavonoids, improve the antioxidant capacity of the fruit, delay its color development, and maintain the hardness and titratable acidity.

Shi et al. [40] showed that the concentrations of endogenous melatonin *Arabidopsis thaliana* increased continuously during the process of seedling, maturation and aging. Exogenous spray of melatonin could significantly inhibit the aging process of *Arabidopsis thaliana*. In view of the close relationship between melatonin and IAA, they found that melatonin reduced the expression of *AXR3* and *IAA17* genes, antagonized by IAA signal, which induced the expression of *SEN4* and *SAG12* genes and led to aging. Thus, high concentration of melatonin inhibits the aging process of plants by reducing the expression of *IAA17* and related genes. Arnao and Hernández-Ruiz [41] found that melatonin treatment (1 mmol $L^{-1}$) significantly inhibited the aging and chlorophyll degradation of barley leaves. Through hormone simulation experiment, the research group believed that the main mechanism of melatonin in inhibiting leaf senescence was synergistic kinetin (KT) and antagonistic abscisic acid (ABA). Wang et al. [42] showed that long-term irrigation of melatonin significantly inhibited the aging of apple leaves. Through proteomics analysis, it was found that melatonin inhibited the activities of most hydrolytic enzymes in plasmids of apple leaves, which were involved in the hydrolysis, redox and stress response, transcriptional regulation, photosynthesis and other senescence-related processes of macromolecular proteins.

## 4.3 Effects of melatonin on root development of horticultural crops

Endogenous melatonin has similar physiological functions with IAA, which promotes root development, elongation and lateral and adventitious root development. Chen et al. [43] found that 0.1 μmol $L^{-1}$ melatonin promoted the elongation of mustard (*Brassica juncea* L.) root, while 100 μmol $L^{-1}$ melatonin could inhibit its elongation, which was consistent with the physiological concentrations of IAA. The results of IAA concentrations' determination showed that melatonin could induce the accumulation of IAA in mustard roots, which indicated that there was an interaction between melatonin and IAA. In *Arabidopsis thaliana*, melatonin increases the appearance of adventurous roots twofold and the appearance of lateral roots by up to threefold, but has no effect on root hair density [44]. In addition, a large number of lateral roots were induced in three *Arabidopsis thaliana* transgenic lines that produced excessive melatonin compared with the WT lines [45]. Zhang et al. [46] showed that exogenous application of 500 μmol $L^{-1}$ melatonin significantly promoted the occurrence of lateral roots of cucumber. Moreover, transcriptome analysis showed that melatonin caused the upregulation of 121 genes and downregulation of 196 genes. Through GO and pathway enrichment analysis, Zhang et al. believed that melatonin could promote lateral root development of cucumber by activating

root-related hormone and transcription factor pathways and reducing oxidative damage caused by respiration during root genesis. Exogenous melatonin could promote the accumulation of nitric oxide (NO) in the periderm of young stem and the tip of new adventitious root, indicating that melatonin-induced NO may be involved in the regulation of adventitious root regeneration. In addition, exogenous application of 50 μmol $L^{-1}$ melatonin significantly promotes the regeneration of tomato adventitious roots [47]. NO acts as the common downstream signal of melatonin and IAA in the process of melatonin-induced root generation by promoting the synthesis, polar transport and hormone signal perception of IAA.

Although melatonin is closely related to IAA in the process of affecting plant root development, melatonin-induced root morphogenesis is independent of auxin signaling [44]. Melatonin promotes the elongation of the principal root and lateral root in *Arabidopsis thaliana*. However, melatonin and IAA have no signal cross-linking and independent signal transduction pathways during root development via auxin signal response label DR5: *uidA* approach. It can be seen that the interaction between melatonin and IAA, two indolamines with similar chemical structure, is extremely complex. Therefore, the following conclusions were made: melatonin is similar to IAA in the process of affecting plant root construction depending on obvious concentration effect. The melatonin-induced root development is relatively independent of IAA in the signal transduction pathway. However, melatonin can promote root development through IAA synthesis, polar transport and hormone perception. In addition, melatonin plays key role in ROS scavenging for the vigorous development of root system, which ensures a good redox balance and the smooth progress of metabolism in cell. The effects of melatonin on root growth make it a broad application prospect in horticulture industry. For example, cucumber and watermelon root usually develop weakly. Thus, pumpkin or gourd is often used as grafting rootstock in these vegetable productions. Although rootstock application enhances root activity, it tends to decrease the sensory quality of the fruit. If proper amount of melatonin is applied to cucumber and watermelon, it may be a good substitute technology for grafting. The application of melatonin to promote adventitious root production is more extensive, such as the grafting technology of "double broken root" for melon and some other fruit vegetables, the induction of root buds for asexual propagation materials, such as potato (*Solanum tuberosum* L.), the cuttage propagation for fruit trees, vegetables and flowers, and the root induction for tissue culture of horticultural crops.

## 5. Role of melatonin in stress response of horticultural crops

Large numbers of studies have shown that the endogenous melatonin of plants often changes greatly under the stimulation of stress factors, including light intensity, light quality, temperature, water and oxygen, as well as the stimulation of salinity, ultraviolet (UV-B), paraquat, diseases and insect pests, etc. At the same time, exogenous addition of melatonin or enhancement of plant endogenous melatonin synthesis, through gene editing technology, can improve the plant's resistance to adversity.

### 5.1 Regulation of melatonin resistance to abiotic stress

The efficient utilization of light energy for plant growth, development, yield and quality by light has always been the core of scientific research in horticulture production. Melatonin, on the other hand, has been shown to have a circadian rhythm in mammals, so it is inferred that there should also be a myriad of links

between endogenous melatonin of plant and light environmental factors. Melatonin improved the growth performance of yeast under UV radiation and reduced the mortality [48]. The mechanism proposed suggests that melatonin increased the expression of antioxidant genes and DNA-repairing genes.

Kolar et al. [49] used 100 mmol L$^{-1}$ and 500 mmol L$^{-1}$ of melatonin solutions to treat the cotyledon and germ of the short-day plant *Chenopodium rubrum* (*Chenopodium rubrum* L.), which could significantly inhibit the flowering rate under the short-term conditions, and only applied melatonin before the end of the light treatment or the first half of the dark treatment was effective. This suggests that melatonin levels are influenced by circadian rhythms and regulate the photoperiod of plants, which in turn affects some early steps in flowering. In addition, the application of shading technology in the cultivation of capsicum can significantly reduce the melatonin level of capsicum fruits, which indicates that solar radiation can cause the increase of melatonin concentrations [50]. Studies in wheat also show that the concentrations of melatonin in leaves under light condition are significantly higher than they are under dark condition. At the same time, the light treatment significantly increases the concentrations of melatonin synthesis precursors (tryptamine, 5-hydroxytryptamine and *N*-acetyl-5-hydroxytryptamine) in leaves [36]. This suggests that light promotes plants to convert L-tryptophan into melatonin. Light quality also affects the endogenous melatonin concentrations of licorice (*Glycyrrhiza uralensis* L.), following the rule that red light > blue light > white light [51]. The induced effect of light on melatonin is consistent with the hypothesis that chloroplast is the key organelle for melatonin synthesis [52], which is based on the following: pepper peel and wheat leaves are rich in chloroplasts, while light will inevitably produce excess light energy while promoting plant photosynthesis, resulting in the combination of free electrons and $O_2$ to form ROS. In addition, single light masses, such as red light and blue light, may specifically increase the conversion efficiency of light energy to electric energy at photosystem I (PSI) or photosystem II (PSII), thus increasing the imbalance of electron transfer between PSII and PSI, creating conditions for free electrons to combine with $O_2$ to generate ROS. Since melatonin is an important antioxidant in organisms, it is of certain biological significance to induce chloroplasts to produce a large amount of melatonin to remove excess ROS so as to reduce the oxidative pressure faced by cells. Unfortunately, at present, there is no direct evidence for the systematic studies about the light quality and light intensity that affect melatonin concentrations for alleviating the strong light damage in plants. Therefore, the mechanism of melatonin metabolism on light in plants and melatonin response to light needs further exploration and verification.

Temperature is a key environmental factor that affects horticultural crops, especially vegetable crops that are planted off season. Inappropriate temperature leads to substantial loss of yield and poor quality of horticultural crops. For this reason, horticultural researchers have been working on temperature adaptation mechanisms and efficient and safe plant growth regulators to cope with sudden temperature changes. Shi et al. [53] confirmed that melatonin was induced by high temperature in *Arabidopsis*, and the application of 20 µmol L$^{-1}$ melatonin significantly improved the expression of heat shock factor (*HSFA1s* and *HSFA2s*) and heat shock protein (*HSP90* and *HSP101*), which led to increased survival rate of *Arabidopsis* under high temperature stress. Jia et al. found that application of 29.0 mg L$^{-1}$ melatonin can enhance the high temperature stress tolerance of cherry radish (*Raphanus sativus* L. var. *radculus pers*), which increased the biomass to 12.9% and the soluble protein to 18.7%. At the same time, the activity of antioxidant enzyme, especially for POD, was enhanced and the lipid peroxidation was reduced under adverse conditions [54]. For melatonin-regulated plant cold tolerance, Lei et al. [55] found that very low concentration of exogenous melatonin (21.5 nmol L$^{-1}$) could significantly

improve the cell viability of carrots, under low-temperature stress, enhance the stability of cell membrane structure, and inhibit orderly degradation of DNA caused by programmed cell death. When tomato was treated with low temperature, the melatonin concentrations and the expression of synthetic control genes were significantly increased [56]. With the extension of stress time, the melatonin concentrations showed an increasing trend, indicating that melatonin played an important role in plant resistance to low-temperature stress. Appropriate osmotic stress can improve the germination rate of cucumber seeds under low-temperature stress [57]. At the same time, osmotic stress intensity was positively correlated with endogenous melatonin concentrations in cucumber seed germination. Further studies showed that the endogenous melatonin, induced by osmotic stress, was beneficial to remove peroxidation damage and stabilize membrane structure under low-temperature stress. However, the excessive endogenous melatonin induced by hyperosmotic stress destroyed the oxidation equilibrium state of protein, but reduced the resistance of cucumber shoots to low temperature. In tomato, the maximum quantum yield (Fv/Fm) of PSII was significantly reduced during chilling, which was effectively alleviated by exogenous melatonin. This is because melatonin induces the expression of violaxanthin de-epoxidase gene, enhances the enzyme activity of violaxanthin de-epoxidase and the enhancement of de-epoxidation state of xanthophyll pigments, promotes the non-photochemical quenching and alleviates the photoinhibition during chilling [58]. In addition, studies on the improvement of plant cold tolerance by exogenous melatonin have also been reported in Kiwifruit (*Actinidia Chinensis*) and *Arabidopsis thaliana*. Wang et al. [59] found that the treatment of Kiwifruit seedlings with 100 µmol L$^{-1}$ melatonin could significantly relieve the growth inhibition and chlorophyll degradation, improve antioxidant enzyme activity and eliminate ROS under low-temperature stress. Exogenous melatonin can induce the expression of a series of low-temperature responsive transcription factors (*CBFs*, *DREBs*, *COR15a*, *CAMTA1* and *ZATs*) [60], which indicates that melatonin has a physiological function in responding to low temperature and transcriptional activation of related metabolic processes.

Horticultural crops require lots of water in their cultivation. Water stress or physiological drought will affect the growth and development of crops and make a significant impact on the yield. Exogenous MT promoted the accumulation of soluble sugar and protein under stress, thereby alleviating the damage of rapeseed seedlings under drought stress [61]. Many orchards in arid/semi-arid areas (especially in mountainous areas) are in a state of long-term water shortage. Although fruit trees can grow, their productions are affected. Melatonin treatment significantly improved the drought resistance of wheat seedlings, including reduced membrane damage, more complete chloroplast grana lamella, higher photosynthetic rate, maximum efficiency of photosystem II, and higher cellular turgor and water-holding capacity [62]. Zuo et al. [45] cloned *AcSNMT*, a key gene for drought-induced melatonin synthesis, from drought-tolerant apple rootstock (*Malus zumi* Mats) and heterologous expressed it in *Arabidopsis thaliana*. The subcellular localization analysis showed that *AcSNMT* gene was mainly located in the nucleus and cell membrane, and the synthesized melatonin could effectively remove drought-induced ROS and improve the growth potential and survival rate of transgenic plants under drought stress. Gong et al. [63] also verified the regulation of melatonin on ROS metabolism under drought stress and the drought-resistant mechanism in tomato. In addition, Meng et al. [64] also found that melatonin could protect the chloroplast membrane structure and grana lamella structure of grape under drought stress, increase the thickness of leaves and the tightness of palisade tissue, and regulate stomatal closure to reduce water loss.

There are about 831 million hectares of saline-alkali land in the world, including 397 million hectares of neutral saline soil and 434 million hectares of alkaline saline soil, accounting for 10% of the world's arable land [65]. Salinity stress can lead to the reduction of water availability and nutrient imbalance, seriously restricting agricultural production. In addition, facility horticulture is also faced with the problem of soil secondary salinization due to the closed environment, lack of rain water leaching in the soil, excessive fertilization and other factors. Therefore, how to improve the salinity tolerance of horticultural crops becomes a key link in the development of characteristic horticulture industry in saline and alkaline areas. Ke et al. [66] demonstrated that melatonin pretreatment regulated polyamine metabolism in wheat and reduced the damage of salt stress. They also believed that melatonin induces enzyme activity that stimulates ROS to clear antioxidant defenses in response to salinity. In addition, exogenous melatonin can also prevent the accumulation of triacylglycerol and promote fatty acid β-oxidation and energy conversion under salt stress conditions. So it is helpful for improving PM $H^+$-ATPase activity, activating gene expression of $Na^+$-$K^+$ reverse transporter, and maintaining $K^+/Na^+$ homeostasis of sweet potato (*Solanum tuberosum* L.) [67]. Application of 0.5 µmol $L^{-1}$ exogenous melatonin can alleviate symptoms of green leaf loss, reduce the accumulation of $Na^+$, improve phenols, ascorbic acid and glutathione etc., and promote the related activity of antioxidant enzymes, to alleviate the oxidative damage in salt-stressed tomato [68]. Similarly, root treatment with melatonin alleviated the damage of photosynthetic capacity and oxidative stress and improved antioxidant enzyme activity in salt-stressed watermelon [69]. Zhang et al. [70] also demonstrated in cucumber that exogenous melatonin can upregulate the gibberellin (GA) signaling pathway through the upregulated GA biosynthesis genes (*GA20ox* and *GA3ox*) and inhibit abscisic acid (ABA) signaling pathway through the upregulation of ABA catabolism genes (*CYP707A1* and *CYP707A2*) and downregulation of an ABA biosynthesis gene (*NECD2*), thus promoting the germination rate of cucumber seeds under salt stress. The accumulation of endogenous melatonin in sunflower can be induced by salt stress for 48 h, and the distribution of melatonin accumulation induced by salt stress in the root vascular bundles and cortex is also regionalized. For example, the concentrations of melatonin in sunflower cotyledons and oil-rich tissues are significantly higher than those in other tissues [71]. Moreover, exogenous melatonin can promote the elongation of hypocotyl and root growth of sunflower seedlings under salt stress, and alleviate the inhibition of salt stress on root development of sunflower seedlings to some extent. In addition, our study showed that exogenous addition of 0.5 µmol $L^{-1}$ melatonin could significantly improve the biomass of tomato seedlings, protect photosynthetic organs, promote the activity of antioxidant system, and balance the $Na^+$-$K^+$ metabolism of tomato plants under the stress of alkaline salt ($NaHCO_3$) [68]. It has been confirmed that melatonin regulates the physiological processes, such as $Na^+$ detoxification, dehydration resistance, high pH buffering and ROS scavenging through DREB1 a and IAA3 pathways [72]. These basic studies on exogenous melatonin improving the saline-alkali tolerance of horticultural crops provide theoretical support for the innovation of horticultural crop cultivation technology in saline-alkali areas.

**5.2 The regulation of melatonin resistance to biological stress**

Plants are often attacked by fungi, bacteria, viruses and pests during their growth and development. Under biological stress, plants produce endogenous hormone-regulated responses, such as salicylic acid (SA), jasmonic acid (JA), ethylene (Eth) and abscisic acid (ABA). Studies in recent years have shown that

melatonin can interact with the signaling pathways of biological stress regulated by SA and JA, and negatively regulate plant resistance to biological stress.

The bacterial disease model strains *Pseudomonas syringe pv.* Tomato DC3000 (DC3000) can induce the accumulation of endogenous melatonin, which may play an important role in plant disease resistance response [73, 74]. When 10 μmol $L^{-1}$ solution of melatonin was sprayed on *Arabidopsis* or tobacco leaves, gene expression of disease-course related proteins and defense-related genes activated by SA and Eth signals were induced to reduce the incidence of DC3000. However, exogenous melatonin has no disease-resistant induction effect on *Arabidopsis thaliana* SA and Eth signal mutants of *npr1*, *ein2* and *mpk6*, which indicates that melatonin has close communication with SA and Eth signals. In addition, exogenous melatonin can promote the translocation of the inhibitor *NPR1* of pathophysiology-related protein (*PR1*) from the cytoplasm to the nucleus [75]. All these evidences indicate that melatonin acts as the upstream signal of SA to activate the disease-resistant response chain in plants.

Application of exogenous melatonin to cotton could induce the expression of phenylpropanoid mevalonate (MVA), gossypol and other pathway-related genes, thus leading to increased lignin and gossypol concentrations in metabolites of this pathway and thus enhancing the resistance of cotton to Verticillium wilt [76]. Liu et al. [77] found that spraying of 50 mol $L^{-1}$ of melatonin solution on tomato fruits can induce and enhance the activity of disease-resistant proteases CHI, GLU, PAL and PPO, and significantly enhance disease resistance to *Botrytis cinerea* by regulating ROS accumulation and JA defense signaling pathways. Shi et al. [78] showed that DC3000 could promote the accumulation of melatonin and NO in *Arabidopsis thaliana*, while exogenous melatonin could induce the production of NO. Moreover, both exogenous melatonin and NO treatment of *Arabidopsis thaliana* can enhance its disease resistance and activate the expression of defense genes related to SA signal. This indicates that NO, as the downstream signal of melatonin, acts as the second messenger, communicates the signal network between melatonin and SA, and activates the disease-resistant regulatory network of plants.

In addition to the disease resistance mechanism mediated by SA signal, there is mechanical disease resistance that consists of epidermal tissue, cell wall, phenylalanine pathway-mediated disease resistance mechanism, etc. in plants. Zhao et al. [79] indicated that exogenous melatonin could downregulate the expression of invertase inhibitors of *Arabidopsis thaliana*, activate cell wall invertase activity, promote sucrose metabolism, and accumulate hexose. However, melatonin-induced cell wall invertase activity can increase the strength of cell wall through synthesis of cellulose, xylose and galactose and promote the deposition of callose in cell wall. Melatonin can improve the disease resistance of plants by improving cell wall composition and structure. In the same year, the group of Yin et al. [80] found that exogenous melatonin can reduce marssonina apple blotch and its main regulation mechanism for melatonin can maintain intracellular redox state after infection, improving the phenylalanine ammonia enzyme, polyphenol oxidase, chitinase and glucanase course related to the activity of defense enzymes.

## 6. Melatonin regulates the signal transduction network of plant growth and stress resistance

As mentioned above, melatonin is widely involved in the regulation of plant growth, development and resistance. Based on this, we sorted out the signal pathways involved in melatonin and summarized the schematic diagram of the signal

transduction network regulated by melatonin (**Figure 2**). The main function of melatonin is to promote the biosynthesis of IAA and cooperate with IAA to promote the elongation and expansion of cells, which is manifested in the induction of root growth, lateral root occurrence, adventitious root occurrence and fruit expansion. Although both of them are indoleamine compounds, melatonin and IAA do not share a set of signal transduction networks [44]. Interestingly, melatonin inhibits the expression of auxin antagonistic transcription factor *IAA17*, which blocks auxin signaling [40]. This is equivalent to melatonin indirectly amplifying the IAA signal. In this regard, melatonin and IAA are not absolutely isolated, but have a signal dialogue. In addition, our study also showed that melatonin can promote the polar transport and perception of IAA in tomato root development [47]. In addition, melatonin can induce GA and inhibit ABA synthesis during seed germination [70]. The physiological effects of GA are mainly growth stimulation and aging delay, while ABA is an aging induction hormone. Unfortunately, the relationship of melatonin-GA-ABA is currently limited to germination experiments, and no more conclusions have been drawn to support its regulatory mechanism. Researchers have recently revealed the antioxidant function of melatonin and the inhibitory function of *IAA17* in plant anti-aging process [40, 41]. We believe that melatonin can delay the degradation of chlorophyll, maintain a good redox balance in leaf tissue, and synergistically promote the functions of aging antagonistic hormones, which are the main regulatory mechanisms to inhibit the aging of plant leaves. Healthy leaves can carry out photosynthesis better, which explains that melatonin promotes plant growth from the perspective of carbon nutrition and improves the physiological mechanism of yield. Another interesting issue is that melatonin can induce the release of Eth during the ripening process of tomato fruits, which promotes the ripening of fruits and improves the quality of commodities [36]. However, Eth is also a plant senescence-inducing hormone, which seems to contradict the above-mentioned idea. We speculate that melatonin may have spatio-temporal specificity of developmental period and tissues due to its multipathway properties in synthesis, which needs systematic analysis and experimental verification from the composition of promoter elements. Unfortunately, this area of research is still blank.
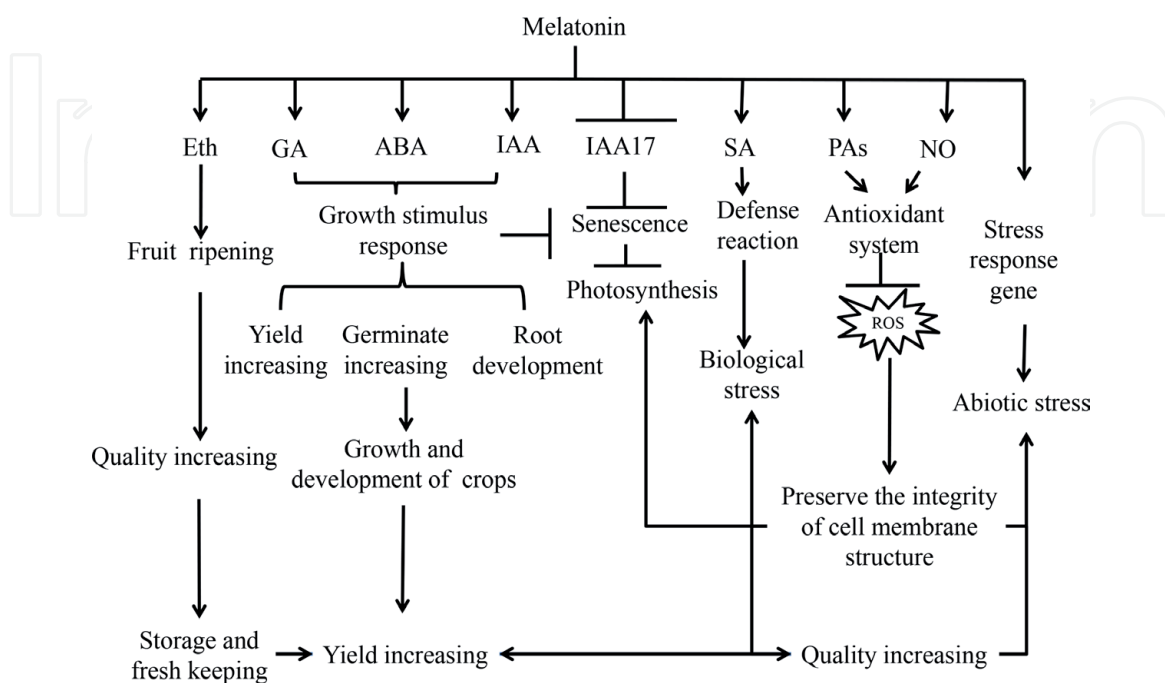


**Figure 2.**
*The signal transduction network of melatonin in plant growth, development and stress tolerance.*

Environmental stress and hormones can induce plant cells to produce polyamine (PAs) and NO. However, both PAs and NO are free radicals with strong reactivity. As two active small molecule signaling substances, they are easy to gain and lose electrons. Lei et al. [55] found that melatonin can induce the synthesis of polyamine, under low-temperature stress, and enhance cold resistance in carrot. However, NO is produced by melatonin during the induction of disease resistance [78], alkali resistance [71] and rooting [47], and NO is needed as the downstream signal. In recent years, we have found that NO is the downstream signal of PAs in tomato stress response, and it can activate several plant stress tolerance signaling pathways including antioxidant system [81]. Of course, the mechanical strengthening of melatonin on cell wall tissues [79] and the contact reaction between melatonin and ROS [82] also contributed to the acquisition of plant resilience traits. In addition, melatonin can directly regulate the expression of stress-related functional genes through transcription factor activation, such as senescence-associated genes (SAGs) [41], C-repeat binding factor (*CBFs*) and low-temperature response gene (*COR15a*) [60], heat shock factor (*HSFAs*) and heat shock proteins (*HSPs*) [53], drought response binding factor (*DREBs*) and drought stress resistance genes (*CAMTA1*) [60], plasma membrane $Na^+/H^+$ reverse transporters (*SOS*), vacuole membrane $Na^+/H^+$ reverse transporters (*NHX*) and also $Na^+$ transporters (*HKT*), etc. [59]. In conclusion, these signal transduction network pathways of melatonin systematically enhance the plant's resistance to adversity and improve the survival rate and growth potential of plants under adverse conditions.

## 7. Conclusion and prospect

Melatonin is widely found in plant tissues, but its concentration in plants is still very low and has obvious tissue specificity. The pan-frying, deep-frying, stir-frying, steaming and stewing techniques commonly used in Chinese food culture are not conducive to the preservation of melatonin in food. Therefore, the food sources mainly focusing on the acquisition of melatonin nutrition are mainly focused on gardening crops like fruits and vegetables suitable for fresh eating. However, melatonin is a substance with similar hormone activity in plants. The use of gene editing technology to comprehensively increase the melatonin concentrations in plants may destroy the balance of melatonin metabolism in plants and bring some adverse effects on the growth and development of plants. Based on this, we propose two suggestions for improving the concentrations of melatonin in horticultural crops: (1) inducing the expression of edible organs or specific developmental periods in horticultural crops by using tissue-specific or inducible promoters combined with melatonin synthesis of key genes. For example, tomato E8 promoter could be used to specifically express the key gene of melatonin synthesis in the fruit, and the effect of excessive melatonin accumulation on the plant was reduced on the basis of increasing the concentrations of melatonin in tomato fruit. Another example is that the chemical-induced expression system TetR combined with melatonin synthesis of key genes can be used to induce the expression of horticultural products in a time period prior to harvesting, which can improve the melatonin concentrations of the harvested products. (2) The *Arabidopsis thaliana* mutant library was used to dig out the mutant with excessive melatonin accumulation, locate the key gene and use CRISPR-Cas9 to conduct targeted gene editing on horticultural crops to create a new horticultural germ plasm rich in melatonin.

The study of melatonin can promote the improvement of global ecological environment. In the horticultural production system under great pressure in the natural environment and frequent outbreak of biological stress, the study on melatonin

promoting growth and anti-stress regulation mechanism and the successful cases of exogenous melatonin promoting crop growth and enhancing resistance are expected to make great contributions to the high yield and high-quality production of horticultural crops. Based on the current research status, we make a prediction on the application of melatonin in horticulture production, hoping to attract more and more attention. (1) For cucumbers and others prone to premature aging, horticultural crops can be applied by exogenous melatonin to alleviate the aging process. (2) Leek, asparagus and most fruit trees show aging trend with the extension of cultivation year. The application of exogenous melatonin also plays a role in the rejuvenation of this kind of aging horticultural crops. (3) Taking advantage of the anti-aging and drought resistance properties of melatonin, it can be used for fresh cut flowers, fruits and leafy vegetables. (4) Melatonin can promote root growth and withstand low temperature in plants, alleviating the reduction in root growth and absorption of fertilizer and water that are caused by the excessive low ground temperature, thereby reducing heating costs, improving the utilization rate of $CO_2$, and promoting the efficacy of fertilizer. (5) At present, shade means are often used in the over-summer cultivation of ginger and other light-tolerant and shade-tolerant horticultural crops to reduce leaf senescence and root loss caused by the damage of strong light to leaf photosynthetic organs. Melatonin is introduced into the cultivation technology of this type of crops by utilizing the mechanism of accumulation of melatonin in chloroplast and protection of photosynthetic organs and inhibition of leaf senescence. (6) In cucumber and pumpkin grafting, the technology of double broken root grafting is often adopted. Melatonin can promote the occurrence of adventitious root and prevent the infection of wound. At the same time, melatonin also has the function of promoting graft wound healing, which has great application potential in some fruit trees and flowers with low graft survival rate. (7) Taking advantage of the characteristics of melatonin in delaying senescence, MT is used as a flower-/fruit-preserving agent for vegetable crops in winter and summer to improve pollen vitality, increase fruit setting rate and reduce malformation fruit rate. (8) As a plant growth regulator, melatonin can be used in plant growth promotion and disease prevention to reduce the use of pesticides. Moreover, as a new type of growth regulation substance, it has the characteristics of high efficiency and environmental protection. Its development and utilization can greatly promote the healthy growth of horticultural crops, improve the fertilizer utilization ratio and reduce the occurrence of plant diseases and insect pests and pesticide dosage. It can play an important role in the process of using fewer chemical fertilizers and pesticides in China, and has a great development potential in promoting the healthy development of horticulture industry.

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