Melatonin Application And Breeding Approach For A Postharvest Quality And Delayed Senescence In Horticultural Crops

[1]Sahana, [2]Airadevi P Angadi, [3]R M Hosamani
[1]M. Sc. (Agri.) student,[2]Scientist (Horticulture),[3]Professor of Horticulture
[1]Department of Horticulture,
[1]College of Agriculture, University of Agricultural Sciences, Dharwad, India.

Abstract - The marketability of horticultural produce is directly affected by their shelf life, which determines acceptability for commercial purposes. There are many approaches to manage postharvest quality but melatonin treatment and breeding was found to be effective. Melatonin treated fruits (Apple, Banana, Grapes, Litchi, Mango, Peach), vegetables (Tomato, Mushroom) and flowers (Carnation, Cut Rose, Cut Tuberose) showed maximum shelf life as it decreases ethylene production and retained quality parameters. The treatment also significantly enhanced the levels of endogenous melatonin, antioxidant components (total phenolics, flavonoids and anthocyanin) and antioxidant enzyme activities of the fruit. It also inhibited the other oxidative stress markers such as O_2, H_2O_2, MDA, protein carbonyl content and upregulated the expressions of antioxidant genes.

Index Terms – Melatonin, Postharvest treatment, Fruits and vegetables, Postharvest technology, Shelf life, Vase life, Breeding of horticulture crops, Genome editing, CRISPR/Cas9, Mutation breeding.

1. Introduction

Following harvest, fresh produce undergoes respiration, a process involving the consumption of stored carbohydrates, intake of oxygen, release of carbon dioxide and heat. Managing postharvest ripening or senescence is essential for preserving the quality of fruits. Application of exogenous melatonin is one of the chemical treatments to maintain postharvest quality and to delay senescence. Melatonin is an indoleamine neurohormone found in both plants and animals. It mainly acts as antioxidant, growth regulator, bio-stimulator, anti-stress promoter. Melatonin which is already exists inside the plant parts is called as endogenous melatonin. The amount of endogenous melatonin is generally low in fruits and vegetables, its low content may not be enough to improve the post-harvest preservation, so exogenous melatonin is utilized for improving the post-harvest preservation. Breeding also helps to extend the shelf-life of the produce and improve the postharvest quality of fruits and vegetables. Several plant breeding techniques have been attempted to understand ethylene regulation pathways and ethylene-dependent biochemical and physiological processes.

2. Melatonin biosynthesis

The melatonin biosynthesis in plants commences with tryptophan. Under normal plant growth, l-tryptophan is first carboxylated by l-tryptophan decarboxylase (TDC) into tryptamine in cytoplasm. The second committed enzyme tryptamine-5-hydroxylase (T5H), which catalyzes tryptamine into serotonin and requires acetyl-coenzyme A (Ac-CoA) on the endoplasmic reticulum. Usually in fresh plants, the third step is the acetylation of serotonin into *N*-acetylserotonin by serotonin *N*-acetyltransferase (SNAT) in the chloroplast. *N*-Acetylserotonin methyltransferase (ASMT) is believed to be the last enzyme, it catalyzes *N*-acetyl-serotonin into melatonin by methylation reaction in the cytoplasm (Xie *et al*., 2022).

3. Endogenous melatonin

Melatonin which is already exist inside the plant parts is called as endogenous melatonin. The content of MEL was found to vary in different plants in morning glory and tomato melatonin content generally increased during ripening. MEL content in tomato plants cultured in open fields was higher than that of plants cultured in chambers, the MEL level of *Vitis vinifera* decreased dramatically in day time and was highest in the darkness. Content of endogenous MEL increased under stress condition (Fan *et al*., 2018).

4. Functions of melatonin in horticultural crops

It helps in improving seed germination, regulation of circadian rhythms, modification of root system architecture, vegetative development, regulation of photosynthetic machinery, role as potential growth regulator, enhanced seedling health index, protection against environmental stresses, fruit ripening, maintenance of ROS homeostasis, cell protection, regulation of antioxidant enzymes pool, retardation of leaf senescence, modulation of flowering development, inhibition of root elongation, balanced of mineral nutrient homeostasis and reproductive development.

5. Mechanism of exogenous melatonin when it is subjected to fruits and vegetables

The aging process of fruits and vegetables involves a breakdown in cell membrane integrity and function, leading to increased membrane leakage. This deterioration is attributed to the over production of reactive oxygen species (ROS), including O_2^-, H_2O_2, hydroxyl radical (OH^-) and singlet oxygen ($1O_2$), which are potent compounds capable of damaging biological macromolecules and influencing the metabolism of post-harvest fruits and vegetables. Melatonin primarily functions as a potent scavenger of free radicals by enhancing the levels of antioxidant enzymes, non-enzymatic antioxidants and enzymes involved in oxidative protein repair. This action helps to eliminate excess reactive oxygen species from post-harvest fruits and vegetables while also promoting the GABA shunt pathway. Consequently, levels of hydroxyl radicals and hydrogen peroxide decrease, reducing the extent of membrane lipid peroxidation and thereby shielding cells from oxidative harm, ultimately prolonging shelf life. Additionally, the application of exogenous melatonin elevates levels of JA and SA, triggering plant defense responses against pathogens, bolstering pathogen resistance and extending shelf life. Diseases or senescence in post-harvest fruits and vegetables generate substantial amounts of ROS, leading to lipid peroxidation and subsequent decay (Xu *et al*., 2019).

6. Role of melatonin

Melatonin is responsible for maintainence of quality parameters like pigment, firmness and so on. It acts on enzymes which are involved in ethylene synthesis pathway and also helps in expression of genes which are responsible for pigment development. It also helps in decay control by lowering the activities of PME (Pectin methylestarase), PG (Polygalacturonase), PL (Pectate lyase) and RG (Rhamnogalacturonase), these enzymes involved in fruit tissue softening. Melatonin delays the ripening through repressing ethylene biosynthesis. It lowers the activities of enzymes which are responsible for ethylene synthesis. Genes which are responsible for ethylene biosynthesis are - *MaACO1*, *MaACS1*. Melatonin maintains the turgidity, visual appearance and thereby it delays the senescence.

6.1. Quality improvement

6.1.1. Melatonin treatment in tomato (Var. Bmei)

The effect of melatonin on the postharvest ripening and quality improvement of tomato fruit was carried out. The tomatoes were immersed in exogenous melatonin for 2hr and then the expression of genes involved in fruit color development were evaluated. Compared with control check (CK), the 50 µM melatonin treatment significantly increased lycopene levels by 5.8-fold. Meanwhile, the key genes involved in fruit color development, including phytoene synthase1 (PSY1) and carotenoid isomerase (CRTISO), showed a 2-fold increase in expression levels (Sun *et al*., 2015).

6.1.2. Melatonin treatment in white mushroom (*Agaricus bisporus*)

White mushrooms were subjected to different concentrations (0.05, 0.1 and 0.2 mM) of melatonin for evaluation. Results indicated that treatment with 0.1 mM melatonin significantly maintained desirable characteristics such as high hardiness, fracturability and chewiness in the mushrooms while suppressing adhesiveness, preserving white color, minimizing cap opening and enhancing flavor, leading to higher acceptability scores and reduced electron leakage rates. Additionally, melatonin treatment regulated respiratory states in the mushrooms, improving oxidative phosphorylation and enhancing mitochondrial efficiency, ultimately delaying senescence in the white mushrooms (Li *et al*., 2021).

6.2. Decay control

6.2.1. Improvement of shelf life and postharvest quality of grapes

Fengzao grapes underwent immersion in melatonin solutions at different concentrations (0, 0.05, 0.1, 0.5 and 1.0 mM) for 2 hours followed by storage at 24 ± 1 ˚C for 15 days. The application of melatonin significantly prolonged grape senescence across all treatment groups compared to the control, with the most substantial delay observed in the 0.5 mM treatment. Additionally, there was a notable decrease in browning and mildew occurrence in berries treated with melatonin compared to the control group (Sun *et al*., 2020).

6.2.2. Melatonin treatment in litchi

The Feizxiao litchi was treated with melatonin solution concentrations of 0.2 and 0.6 mmol/L and stored at 4˚C for 12 days. The results confirmed that the browning index of the 0.6 mmol/L melatonin treated fruits was significantly lower than that of the 0.2 mmol/L melatonin treatment and the weight loss rates of the litchi treated with 0.2 and 0.6 mmol/L melatonin were 7.67 % and 22.46 % lower than the control, respectively. Compared with the control group, the appearance of melatonin treatment exhibited a positive effect on intrinsic red-green color retention (Xie *et al*., 2022).

6.3. Controlled ripening

6.3.1. Melatonin treatment in banana

The investigation into the role of melatonin in postharvest ripening and quality across different banana varieties with varying ripening periods revealed that treatments of 200 and 500 µM were notably more effective compared to the 50 µM treatment. Furthermore, the application of exogenous melatonin resulted in increased levels of endogenous melatonin, decreased ethylene production via the regulation of *MaACO1*, *MaACS1* expression and delayed rapid shifts in quality indices (Hu *et al*., 2017).

6.3.2. Melatonin treatment in 'Guifei' mango

The study examined how melatonin influences ripening and softening in stored mango fruit by investigating its relationship with ethylene and abscisic acid (ABA) biosynthesis. Results indicated that treating 'Guifei' mangoes with melatonin (0.5 mM, immersed for 1 hour) effectively slowed down changes in ripening parameters such as firmness, pulp colour, β-carotene levels, soluble solids content (SSC), titratable acidity (TA) and respiration rate. Moreover, melatonin significantly postponed climacteric ethylene production and 1-aminocyclopropane-1-carboxylic acid (ACC) levels in mango fruit during storage. This delay in ethylene production and ACC levels could be attributed to decreased activities of ACC synthase (ACS) and ACC oxidase (ACO) enzymes (Liu *et al*., 2020).

6.3.3. Melatonin treatment in apple (var. Fuji)

The study examined the impact of melatonin treatment on the ripening of 'Fuji' apples stored at 1 °C for 56 days. Apples, harvested at the commercial ripening stage, were subjected to 1 mmol L^{-1} melatonin treatment. Results revealed that compared to the control group, melatonin-treated apples exhibited notable reductions in ethylene production (from day 28 to day 56) and weight loss (from day 14 to day 56) during storage. Additionally, the melatonin treatment maintained superior skin structure in the apples throughout the storage period. The decrease in ethylene production was associated with down regulation of gene expressions including *MdACO1*, *MdACS1*, *MdAP2.4* and *MdERF109* (Onik *et al*., 2021).

6.4. Increased shelf life

6.4.1. Melatonin treatment in carnation

The effect of different melatonin concentrations (0.01, 0.1 and 1 mM) on the vase life of cut carnations flowers cv. Baltico was evaluated. The greatest delay in senescence was observed with 0.1 mM MT concentration, increasing vase life up to 10 days more as compared to control carnations. The highest dose evaluated (1 mM) maintained all the parameters evaluated but showed the wilting symptoms earlier. For this reason, 0.1 MT concentration could be a tool capable of improving carnation vase life for longer time, increasing the commercial potential of this cut flower (Lezoul *et al*., 2022).

6.4.2. Melatonin treatment of cut roses (First Red)

The flowering stems of *Rosa hybrida* cv. 'First Red' were immersed in melatonin solutions at concentrations of 0, 0.1, 0.2 and 0.3 mM for 30 minutes before being placed in distilled water for assessment. Melatonin application significantly extended vase life and maintained water content compared to the control, particularly with the 0.2 mM concentration, which nearly doubled vase life, being 1.9 times higher than the control. Melatonin treatment notably boosted phenol and glutathione (GSH) levels, as well as the activities of CAT, APX and GR enzymes in comparison to untreated flowers. Furthermore, melatonin treated flowers exhibited significantly greater radical scavenging capacity than the control, resulting in reduced H_2O_2 production and lipid peroxidation, thus contributing to membrane stability maintenance (Mazrou *et al*., 2022).

6.4.3. Melatonin treatment in cut tuberose (cv. Single)

Effects of pre harvest treatment of different concentrations of exogenous melatonin [0 mM (MT0), 0.5 mM (MT1), 0.7 mM (MT2), 1 mM (MT3)] of tuberose are evaluated in cv. Single. The 0.07 mM and 1 mM preharvest melatonin treatments were the most effective in delaying the senescence of tuberose cut flowers by improving leaf gas exchange, TSP, soluble sugar contents, decreasing proline, H_2O_2 and MDA contents (Zulfiqar *et al*., 2023).

6.5. Chilling injury allivation

6.5.1. Melatonin treatment in peach (var. Chuanzhongdao)

Effects of 0.1 mM melatonin on chilling injury, membrane fatty acid content and phenolic metabolism in peach fruit were studied during storage at 1°C for 28 days. Melatonin treatment delayed the development of chilling injury in peach fruit, as was illustrated by melatonin treated fruit showing lower chilling injury incidence, chilling injury index and firmness loss than the control. Melatonin treatment prevented membrane lipid peroxidation and contributed to maintaining a higher ratio of unsaturated to saturated fatty acids in peach fruit (Gao *et al*., 2018).

7. Breeding for improving postharvest quality and delaying senescence

7.1. Genome editing in petunia

The transcript levels of PhACO genes (*PhACO1*, *PhACO3* and *PhACO4*) in Petunia cv. Mirage Rose are associated with ethylene production at different flowering stages were investigated. *PhACO1* was subsequently edited using the CRISPR/Cas9 system and its role in ethylene production was investigated. *PhACO1*-edited T_0 mutant lines, regardless of mutant type, exhibited significantly reduced ethylene production and enhanced flower longevity compared with wild-type (Xu *et al*., 2020).

7.2.Genome editing in *Solanum melanogena* L.

Polyphenol oxidases (PPOs) facilitate the oxidation of polyphenols, leading to the browning of eggplant flesh upon cutting. Employing a CRISPR/Cas9-based mutagenesis strategy, three specific PPO genes (*SmelPPO4*, *SmelPPO5* and *SmelPPO6*) exhibiting elevated transcript levels in the fruit post-cutting were targeted for knock-out. The induced mutations were reliably passed down to the offspring and correlated with diminished PPO activity, consequently resulting in reduced browning of eggplant flesh post-cutting (Maioli *et al*., 2020).

8. Conclusion

Melatonin regulates many physiological functions but it plays a very important role in managing postharvest quality. Though it is very effective chemical but it is not commercially used in India because of high cost and undetermined toxicity. There are many breeding techniques to improve postharvest quality, delaying senescence and increase shelf life in which genome editing tools are used the most. Nowadays CRISPR/Cas9 is getting hyped as it helps in increasing shelf life of many horticultural crops.

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