

SEMINAR REPORT

Functional analyses of phyto melatonins by omics approach

By

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2018-11-007

Presented on 3/01/2020

Submitted in partial fulfilment of requirement of the course

MBB 591 Masters' Seminar (0+1)



CENTRE FOR PLANT BIOTECHNOLOGY AND MOLECULAR BIOLOGY

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CERTIFICATE

This is to certify that the seminar report entitled “**Functional analyses of phyto melatoninins by omics approach**” has been solely prepared by **Nivethitha B (2017-11-001)**, under my guidance and has not been copied from seminar reports of any seniors, juniors or fellow students.

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1. Introduction

In the growth and development process of plants, numerous factors, especially environmental stress are involved in moulding the status of the plant. Biotic and abiotic stress will induce yield reduction, growth retardation, senescence and even death. Plants develop various strategies to alleviate the damage induced by different stress, to overcome the limitations of their sessile nature. Plenty of molecules such as ethylene, nitric oxide, hydrogen sulfide, calcium and phytohormones, such as jasmonic acid gibberellin acid and abscisic acid, are involved in plant stress responses. Nowadays, another amazing molecule named melatonin draws the attention of researchers. Plenty of investigations found that melatonin plays an essential role in plant development and stress responses. Melatonin was so-named, because, it could reverse the darkening effect of melanocyte stimulating hormone (MSH) in animals. It was portrayed exclusively as an animal hormone for many decades (Arnao and Hernandez-Ruiz, 2019).

Melatonin (N-acetyl-5-methoxy-tryptamine) is the ubiquitous molecule that is found in almost all the organism starting from, unicellular bacteria, dinoflagellates, higher plants and animals. It is also an evolutionarily conserved and a pleiotropic molecule. It is an amphiphilic / amphipathic molecule having both hydrophilic and hydrophobic parts, thereby showing affinity to water and lipid layers. The word ‘MEL’ can be used as the short form of melatonin.

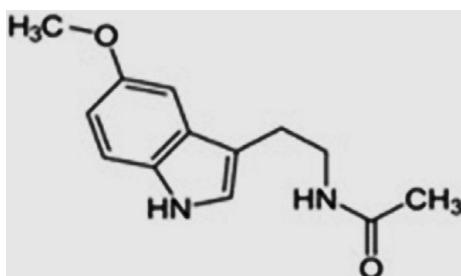


Fig. 1 Chemical structure of melatonin

2. Discovery of melatonins

The presence of melatonin was described for the first time in the bovine pineal gland by Lerner and co-workers in 1958. In 1959, melatonin was detected in humans and in the 1960s. In 1959, melatonin was detected in humans and in the 1960s and 70s, its presence was described in many mammals and vertebrates such as birds, amphibians, and fish. Later, in the year 1980s, melatonin was discovered in invertebrates such as planarians, annelids, molluscs, insects, and crustaceans, among others its presence was described in many mammals and vertebrates such as birds, amphibians, and fish. In higher plants, the first identification of endogenous melatonin

was described in 1993 by van Tassel and O'Neill in a congress communication. The authors had detected melatonin by radioimmunoassay (RIA) and gas chromatography with mass spectrometry (GC-MS) in the Convolvulaceae ivy morning glory (*Pharbitis nil* L., syn. *Ipomoea nil* L.) and in tomato fruits (*Solanum lycopersicum* L.), although the results were not published extensively until 1995. Two papers published simultaneously in 1995 demonstrated the presence of melatonin in higher plants. Dubbels and co-workers used RIA and HPLC-MS to measure the melatonin levels in extracts of *Nicotiana tabacum* L. and in five edible plants. Two months later another publication appeared, in which the presence of melatonin in extracts of a large number of edible plants was quantified by RIA and liquid chromatography (HPLC) with fluorescence detection (Arnao, 2014).

3. Role of melatonins in animals

Melatonin is well-known as a sleeping hormone since it controls the sleep wake cycle in human. Other than that, it has so many functions in animals, especially human beings. It is also known as darkness hormone since it is secreted by pineal gland in response to dark. Thus during daytime the MEL level will be less and as the night proceeds its level gradually rises and gives signals to our eyes to sleep. It is also a potent antioxidant, scavenging free radicals and thus it prevents membrane damage. This will be further responsible for anti-cancerous, anti-ageing and anti-inflammatory properties of MEL. Finally it is the biological modulator of the most important physiological activities in body including sleep, mood, circadian rhythm, body temperature, food intake, retinal physiology, sexual behaviour, immune system (Debnath *et al.*, 2019). Thus it is now gaining importance in the treatment of jet lag to improve the quality of sleep and diseases like Ebola (Masters *et al.*, 2014). Many tablet formulations of MEL are commercially available in the market.

4. Phytomelatonin

The term 'phytomelatonin' denotes melatonin of plant origin. It is also used to denote the endogenous MEL in plants. It was first proposed in a paper about the treatment of liver cancer in rats (Black *et al.*, 2004). Its presence is confirmed experimentally in most of the plants. It is present all the parts of plants. The concentration of phytomelatonin across the plants parts varies drastically with leaves showing the least and seeds showing the maximum. Among the plants certain medicinal herbs shows the highest concentration (1000ng/g) in several herbs, which is 15 - 100 fold higher than the average level in other plants. Coffee beans and cereals also has high content of MEL. The concentration will vary among species or cultivars and also dependent on growth and development stages and tissue categories. The

concentration is higher in plants compared to animals to cope with the environment stress as they are immobile (Arnao and Hernandez-Ruiz, 2018).

5. Biosynthetic pathways of phytomelatonin

Melatonin and phytomelatonin are synthesized from the amino acid tryptophan in both animals and plants. In a wide range of plant species, melatonin biosynthesis begins from tryptophan. It is catalysed by tryptophan decarboxylase (TDC) and converted to tryptamine, and then tryptamine 5-hydroxylase (T5H) catalyses tryptamine to serotonin, which will be converted to melatonin via two steps. In some other plants, such as *Hypericum perforatum*, tryptophan is catalysed into 5-hydroxytryptophan by tryptophan 5-hydroxylase (TPH), and then TDC/AADC (aromatic-L-amino-acid decarboxylase) converts 5-hydroxytryptophan to serotonin. This pathway is the same as that in animals. In the next two steps, serotonin is converted to N-acetyl-serotonin by serotonin N-acetyl transferase (SNAT)/arylalkylamine N-acetyl transferase (AANAT), and then N-acetyl-serotonin methyl transferase (ASMT)/hydroxyindole O-methyltransferase (HIOMT) catalyzes N-acetyl-serotonin into melatonin. Additionally, tryptamine can be catalysed by SNAT into N-acetyl-tryptamine, which is not further converted into N-acetyl-serotonin by T5H. It is difficult to determine whether there is a pathway to convert N-acetyl-tryptamine into N-acetyl-serotonin. The other pathway is to convert serotonin into 5-methoxy-tryptamine by HIOMT and, finally, to catalyse 5-methoxy-tryptamine into melatonin by SNAT. Recently, a reverse melatonin pathway was reported, in which N-acetyl-serotonin is converted into serotonin by N-acetyl-serotonin deacetylase. Furthermore, tryptophan is not only the resource of melatonin, but also the precursor of indole-3-acetic acid (IAA), maybe implying the multifunctional role of melatonin in plants (Fan *et al.*, 2018). Study on rice indicated that the conversion of serotonin occurred markedly through tryptophan –tryptamine–serotonin, known as the tryptamine pathway (Debnath *et al.*, 2019).

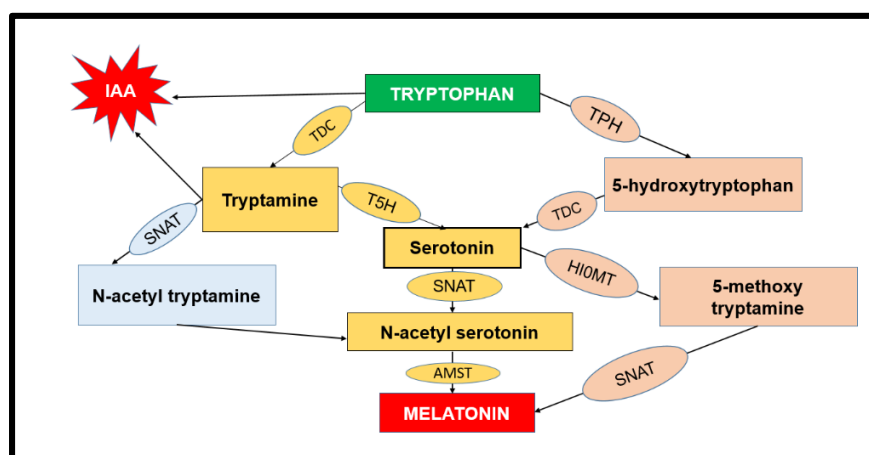


Fig. 2 Proposed biosynthetic pathway of phytomelatonin

6. Physiological roles of melatonin in plants

Plant melatonin is involved in multiple physiological actions include promoting seed germination, growth and development, rooting, photosynthesis, flowering, fruit ripening, delayed leaf senescence and protection against biotic and abiotic stressors. All these functions are inter-related involving complex mechanism. This is why it called as a multifunctional signalling molecule (Arnao and Hernandez-Ruiz, 2019).

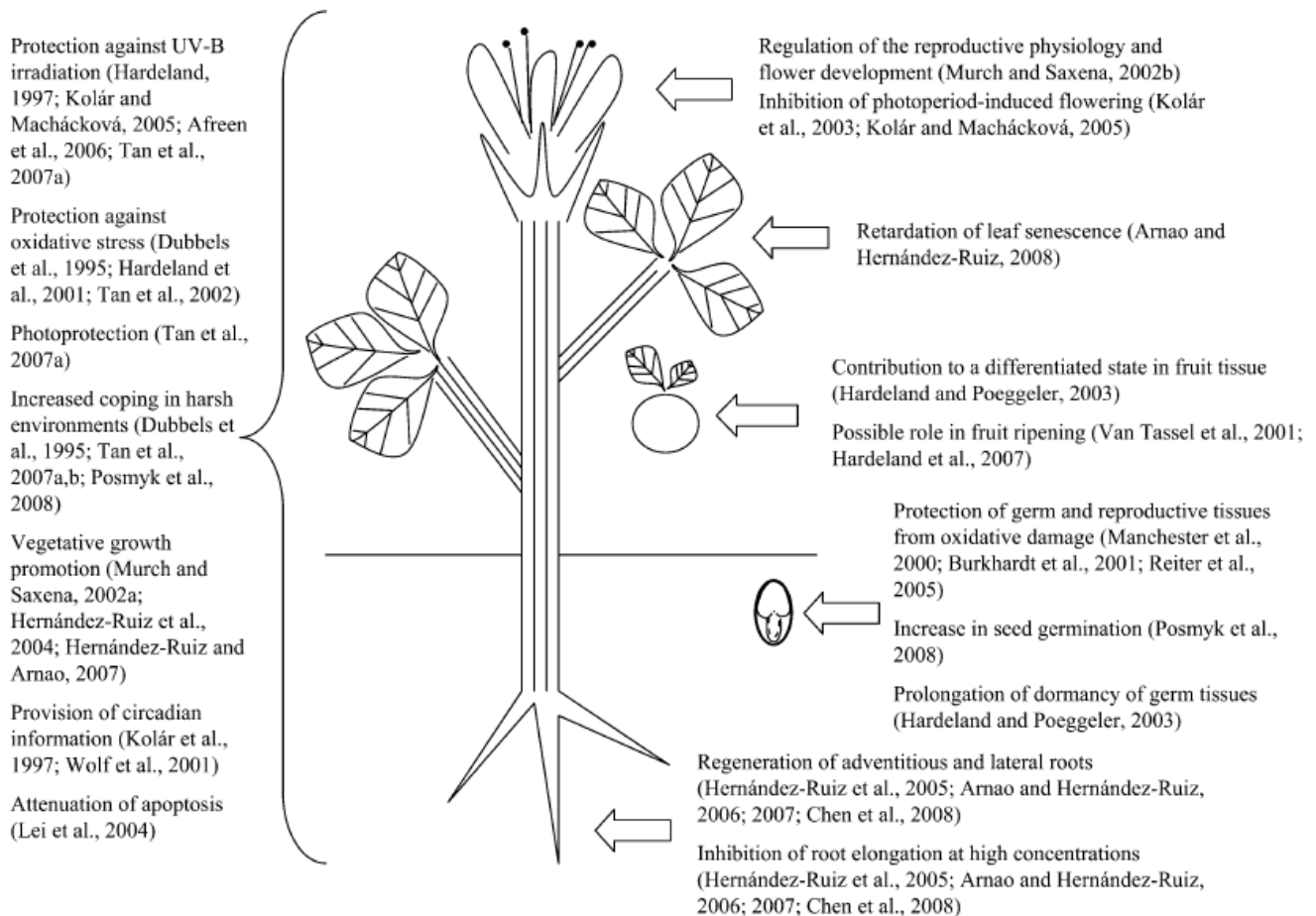


Fig. 3 Proposed functions attributed to melatonin in higher plants

(Paredes *et al.*, 2009)

Similar to plant hormones, melatonin has a range of possible cellular and physiological effects, such as changes in intracellular Ca^{2+} and in the permeability of membranes mediated by ion transporters, optimizing efficiency and leaf water/ CO_2 exchange changes in the opening and/or closing of stomata, in carbohydrate, lipid, and nitrogen metabolisms, and also in osmoprotector metabolites .

7. Molecular influence of exogenous MEL

Endogenous melatonin level increases in response to stress. Application of exogenous melatonin also increases the endogenous melatonin level and has the same molecular influence. Genome-wide transcriptomic profiling genes reveals that the genes were differentially regulated in MEL treated plants when compared to control. Transcription factors like DREB WRKY MYB AND HSF are highly induced by MEL (Zhan *et al.*, 2019). Gene ontology report reveals that melatonin regulates many of the genes are involved in primary and secondary metabolism. Biosynthesis genes of phytohormones and various stress response genes are also regulated by MEL. Different genes were regulated by different concentrations of MEL. This shows the concentration dependent activity of MEL (Wang *et al.*, 2017).

8. Functional analyses of MEL

Phytomelatonin is having multiple inter-related functions. So, the functions of phytomelatonin can be grouped as an antioxidant and as a plant growth regulator. Omics approaches like transcriptomics, proteomics and metabolomics are used to investigate the functions, by providing the global view of changes at varying levels of gene expression. The functions of phytomelatonin along with the appropriate omics tool used for its analysis will be detailed below.

8.1. Phytomelatonin as an antioxidant

MEL is an effective antioxidant by acting both directly and indirectly. It is five times more effective than GSH in neutralizing hydroxide (OH⁻). It is also 15 fold more effective than exogenous scavenger mannitol. This also contributes to the stress tolerance in plants provided by the phytomelatonin (Debnath *et al.*, 2019).

8.1.1. Direct antioxidant

In plant cells, free radicals are produced continuously which damages the cell integrity. An antioxidant is a molecule that scavenges the free radical produced, thus preventing the damages caused to the cell membranes. ROS (Reactive Oxygen Species) like O₂⁻ (superoxide anion) and RNS (Reactive Nitrogen Species) like ·NO (nitric oxide) are the common free radicals. Since it is an amphiphilic molecule, it can easily through cell membrane to cytoplasm and subcellular partitions and effectively scavenges free radicals directly (Debnath *et al.*, 2019)

Shi *et al.* (2015) found that exogenous melatonin significantly alleviated hydrogen peroxide (H₂O₂)-modulated plant growth, cell damage, and ROS accumulation in Bermuda

grass. 76 proteins significantly influenced by melatonin during mock or H₂O₂ treatment were identified by gel-free proteomics using iTRAQ (isobaric tags for relative and absolute quantitation). Metabolic pathway analysis showed that several pathways were markedly enhanced by melatonin and H₂O₂ treatments, including polyamine metabolism, ribosome pathway, major carbohydrate metabolism, photosynthesis, redox, and amino acid metabolism. The study provided more comprehensive insights into the physiological and molecular mechanisms of melatonin in Bermuda grass responses to direct oxidative stress. This may relate to the activation of antioxidants, modulation of metabolic pathways, and extensive proteome reprogramming.

8.1.2. Indirect antioxidant

MEL acts as indirect antioxidant by triggering plant's natural antioxidant system.

Plants usually have two type of antioxidant system (Zhan *et al.*, 2019)

- Enzymatic antioxidant system
- Non – enzymatic antioxidant system

Enzymatic antioxidants include –

- ✓ SOD (Superoxide dismutase)
- ✓ CAT (Catalase)
- ✓ POD (Peroxidase)
- ✓ GR (Glutathione reductase)
- ✓ DHAR (Dehydroascorbate Reductase)

Non – enzymatic antioxidants include -

- ✓ GSH (Glutathione)
- ✓ AsA (Ascorbic acid)
- ✓ Carotenoids
- ✓ Tocopherols
- ✓ Flavonoids

MEL acts a signalling molecule at cellular level up-regulating the genes coding for these enzymes. Metabolites of MEL also have anti-oxidative capacity, thus increasing the efficiency as an antioxidant. These all provides the raised mechanism to stress response.

8.1.3. MEL mediated stress tolerance

8.1.3.1. Abiotic stress tolerance

MEL provides tolerance to plants against abiotic stressors like drought, cold, heat, salinity, heavy metals and UV radiations. by regulating genes involved in the DREB/CBF, HSF, SOS and ABA pathways.

8.1.3.1.1. Drought tolerance

ABA will be produced as a stress response in plants. MEL provides drought tolerance in plants by up-regulation of ABA catabolism genes like *CYP707A1* and *CYP707A2* and down-regulation of ABA biosynthesis genes like *NCED3* and chlorophyll degradation enzymes - *PAO* and *SAG*. It also causes the up-regulation of CBFs / DREBs (Arnao and Hernandez-Ruiz, 2019).

Wang *et al.* in 2013 reported that exogenous application of 100 μM concentration of melatonin provides drought tolerance in Apple (*Malus prunifolia*) by modulation of photosynthesis genes *SAG12* and *PAO*. The same concentration of MEL also found to improve tolerance to drought in tomato by modulation of photosynthesis & ROS genes (Liu *et al.*, 2015).

Concentration of melatonin (μM)	Plant species	Changes and mechanism	References
100	Apple	Photosynthesis, Modulation of <i>SAG12</i> and <i>PAO</i>	Wang <i>et al.</i> , 2013
100	Apple	ABA & ROS	Li <i>et al.</i> , 2015
100	Tomato	Photosynthesis & ROS	Liu <i>et al.</i> , 2015

Table 1 Genes responsible for MEL-mediated drought tolerance

8.1.3.1.2. Cold tolerance

MEL alleviates cold stress by up-regulation of *Cold responsive genes (COR)15*, transcription factors of chilling stress tolerant genes - *C₂H₂-type zinc finger transcription factor (ZAT) 10*, *ZAT6*, *ZAT10* & *ZAT12*, CBFs and polyamine (PA) synthesis genes like SAMDC-1.

A study conducted by Bajwa and co-workers in 2014 using transcriptomics revealed that modulation of CBF, *COR*, *ZAT10* and ROS genes will be responsible for MEL-mediated cold tolerance in *Arabidopsis thaliana*. In cucumber, up-regulation of *CsZat12* and modulation of PA and ABA metabolism genes provides tolerance to cold stress (Zhao *et al.*, 2017).

Transcriptional regulation of cold-responsive genes is crucial for exogenous melatonin-mediated cold tolerance in plants. Nonetheless, how melatonin regulates cold responsive genes was largely unknown. In a study by Li *et al.* in 2016, cucumber seedlings at four-leaf stage was MEL pretreated 150 μ M for 3 days and exposed to cold stress (4°C) for 36 h. The four treatments considered for the study are CK (control), Cold, MT (MEL treated) and MT + Cold (Fig. 4).

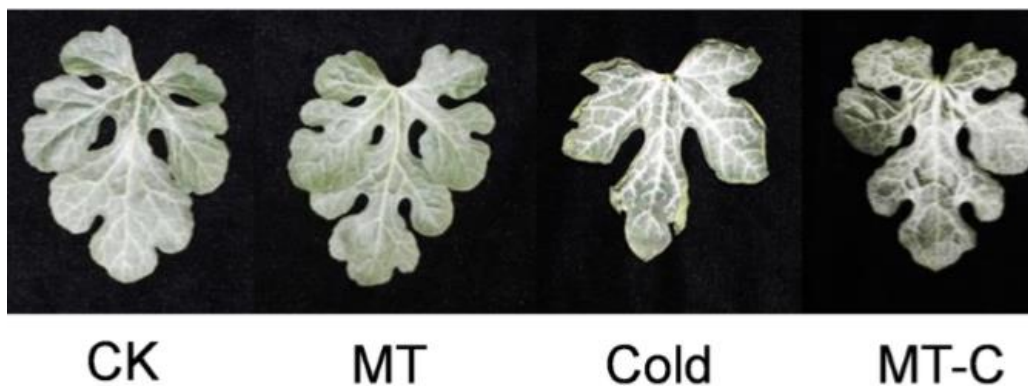


Fig. 4 Treatments of cucumber leaves

High-throughput sequencing of miRNA & mRNA was done to reveal the miRNAs & mRNAs responsive to MEL was found. Prediction of target mRNAs cleaved by miRNA was done using functional annotation by Gene Ontology (GO) classification of potential target genes cleaved by miRNAs based on their involvement in various biological processes (Fig.5)

They found that exogenous melatonin improved cold tolerance in watermelon by regulating expression of microRNAs (miRNAs). They also identified a set of miRNAs that were regulated by melatonin under unstressed or cold conditions. Importantly, mRNA-seq analysis revealed that melatonin induced down-regulation of some miRNAs, such as miR159-5p, miR858, miR8029-3p, and novel-m0048-3p correlated with the up regulation of target

genes involved in signal transduction (CDPK, BHLH, WRKY, MYB, and DREB) and protection/detoxification (LEA and MDAR) under cold stress (Fig. 6). These results suggest that miRNAs may be involved in melatonin-mediated cold tolerance in watermelon by negatively regulating the expression of target mRNAs.

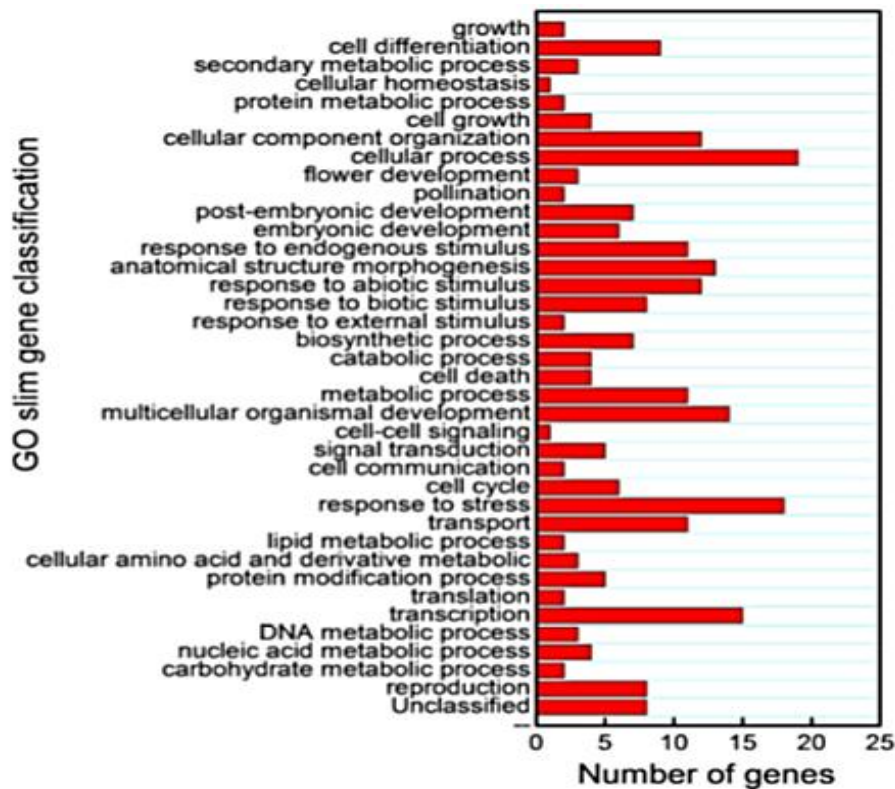


Fig. 5 Potential target Genes cleaved by miRNAs

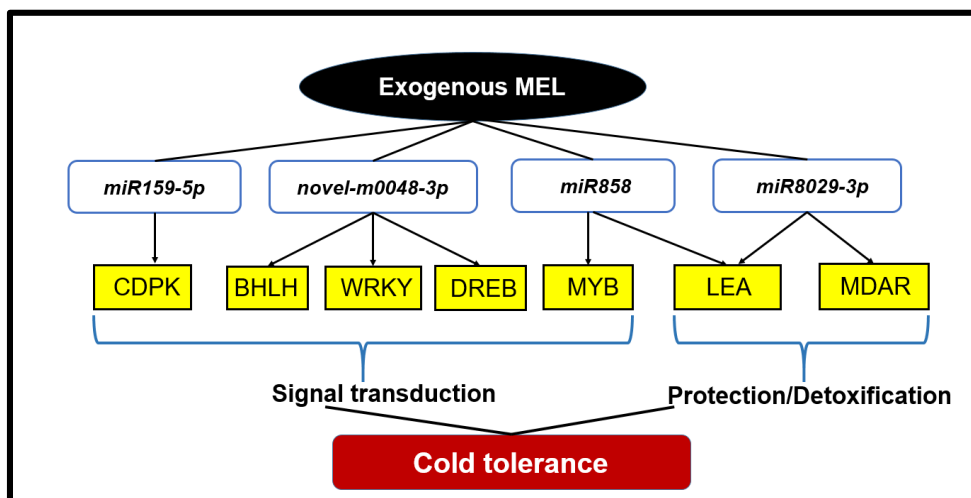


Fig. 6 Proposed regulatory pathway of miRNAs in MEL mediated cold tolerance

8.1.3.1.3. Salinity tolerance

In salt stressed plants when high amount of sodium ion enters into plant cell, it is harmful to cytosolic enzymes. Normally, the three mechanisms to prevent Na⁺ accumulation of cytoplasm are restriction of Na⁺ influx, active Na⁺ efflux and compartmentalization of Na⁺ into vacuoles. These will be executed by MEL through the up-regulation of the following ion homeostasis genes and some ROS genes (Zhan *et al.*, 2019).

- *NHX1* encoding vacuolar Na⁺/H⁺ exchanger which aids in the transfer of Na⁺ into vacuoles
- *SOS1* encodes plasma membrane Na⁺/H⁺ anti-transporter and transports Na⁺ out of cells
- *AKT1* encoding K⁺ channel protein which helps in absorbing K⁺ from soil and transporting it to roots

Zhang *et al.* in 2017 using proteomics revealed that the regulation of energy metabolizing proteins by MEL will be responsible for MEL mediated cold tolerance in cucumber. Redox homeostasis and up-regulated *MdNHX1* and *MdAKT1* by MEL is responsible for cold tolerance in *Malus hupehensis* which is revealed using transcriptomics.

Plant species	Omics	Changes and mechanism	Reference
<i>Malus hupehensis</i>	Transcriptomics	Redox homeostasis Up-regulated <i>MdNHX1</i> and <i>MdAKT1</i>	Li <i>et al.</i> , 2012
Rice	Transcriptomics	Redox homeostasis Down regulation of <i>SAG</i>	Liang <i>et al.</i> , 2015
Cucumber	Proteomics	Regulation of energy metabolizing proteins	Zhang <i>et al.</i> , 2017
Wheat	Proteomics	Regulation of energy metabolism and autophagy proteins	Cui <i>et al.</i> , 2018

Table 2 Genes responsible for MEL mediated salt tolerance

8.1.3.2. Biotic stress tolerance

MEL provides tolerance to biotic stress especially to wide range of plant pathogens. Application of MEL up-regulates defensive genes encoding PR proteins – PR- 1, PR -2, PR -5 which holds anti-microbial properties. MAPK cascades is involved in diverse signalling pathways triggered in response to stress especially, MPK3 / MPK6 plant defense signalling cascades is triggered by pathogens. MEL also triggers MPK3 / MPK6 and provides immunity (Kanwar *et al.*, 2018).

In a study by Chen *et al.* in 2019, exogenous melatonin ($200 \mu\text{g mL}^{-1}$) significantly inhibited the growth of Rice bacterial leaf streak (BLS), caused by *Xanthomonas oryzae* pv. *oryzicola* (Xoc) and reduced the mRNA expression level of seven cell division-related genes. Melatonin reduced the pathogenicity of Xoc towards the susceptible rice IR24. $200 \mu\text{g mL}^{-1}$ MEL added in nutrient broth medium for growing *Xanthomonas oryzae* pv. *oryzicola*. Rice cultivar IR24 was grown in a greenhouse, and 3-week-old leaves were used for testing

The lesion length in IR24 infected by Xoc pre-treated with melatonin ($200 \mu\text{g mL}^{-1}$) was more than 23% smaller than the lesion length of the control group. Similarly, infection of IR24 leaves by Xoc pre-treated with melatonin ($200 \mu\text{g mL}^{-1}$) reduced the bacterial population by 45%. The biofilm formation, swimming motility and extracellular protease activities of Xoc also decreased under melatonin treatment. Melatonin only slightly changed the morphology of Xoc but significantly reduced the mRNA expression of toxin genes. Furthermore, treating leaves with exogenous melatonin reduce the incidence of BLS by 17%

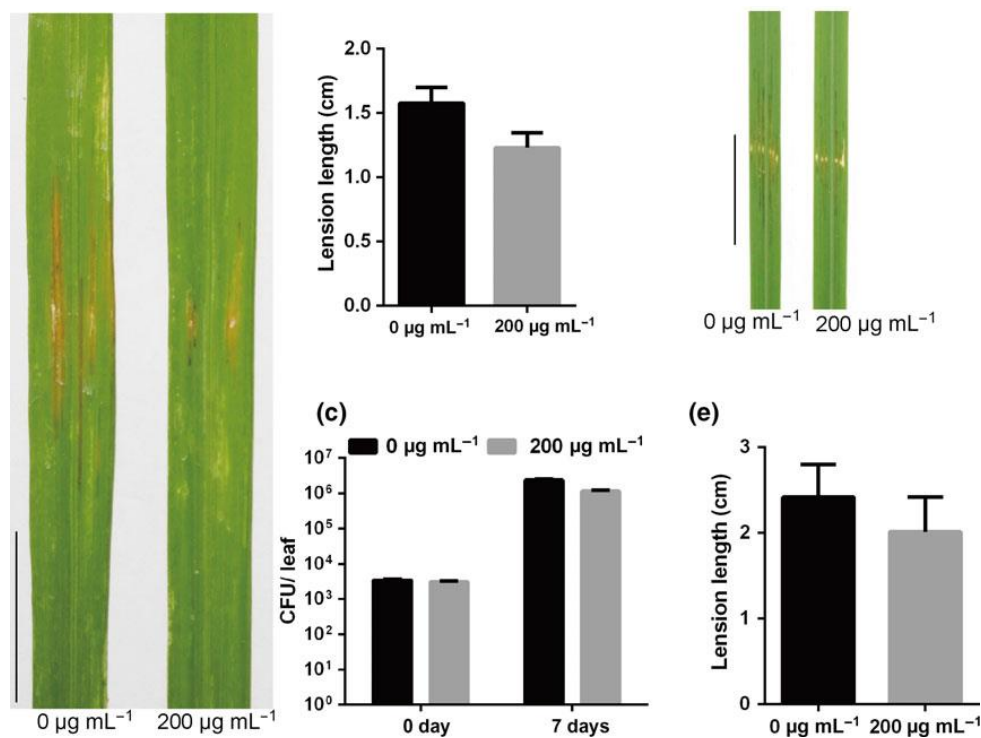


Fig. 7 Reduction of the pathogenicity of Xoc by melatonin

Melatonin was found to reduce the pathogenicity of Xoc. Therefore, the effect of 24 h treatment with melatonin on the expression of toxin genes and other crucial virulence genes was investigated using RT-PCR analysis and mRNA expression levels were obtained (Fig. 8). All the three toxin genes were down-regulated. Furthermore, the mRNA level of *Toxin A* was 3-fold lower in the treatment group than in the control group. One virulence transcription

regulator (LysR-Tf), two antibiotic-resistance operons (MarR Tf-A and MarR Tf-A) and two phosphate transporters (PstB, PstS) were also down-regulated in the treated RS105 group relative to the control group.

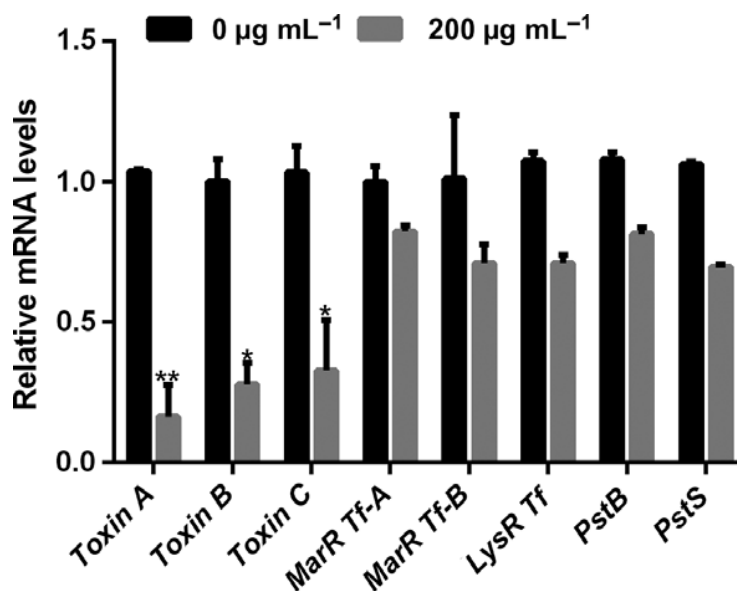


Fig. 8 Suppressed mRNA levels of toxin genes of Xoc in MEL treated plants

Plant Species	Pathogen / Disease	Changes and Mechanism	Omics	Reference
Arabidopsis	<i>Pseudomonas syringe pv. tomato</i> (Pst) DC3000	Sugar and glycerol accumulation	Metabolomics	Quian <i>et al.</i> , 2015
Banana	Fusarium wilt	Regulation of <i>MaHSP90s</i>	Transcriptomics	Wei <i>et al.</i> , 2017
Tomato	Tobacco Mosaic Virus	Upregulation of PR-1 and PR - 5	Transcriptomics	Zhao <i>et al.</i> , 2019
Banana	Post-harvest anthracnose	Enhanced MAPK signaling pathways	Transcriptomics and proteomics	Li <i>et al.</i> , 2019

Table 3. MEL mediated immunity against various pathogens

8.2. Phytomelatonin as a plant growth regulator

8.2.1. Regulation of primary metabolism

MEL is a regulator primary metabolism in response to stress. It up-regulates genes involved in carbohydrate metabolism and nitrogen metabolism. It also modulates proteins involved in lipid metabolism and ribosome synthesis (Shi *et al.*, 2015). Wei *et al.* in 2014 reported the increase in leaf size, height, pods and seed number of soybean by the application of MEL.

Wan *et al* in 2018 investigated the roles of melatonin and serotonin in modulating plant growth and defense by integrating physiological and transcriptome analyses in *Arabidopsis*. Moderate concentrations of melatonin and serotonin did not affect primary root (PR) growth but markedly induced lateral root (LR) formation. Both melatonin and serotonin locally induced the expression of the cell-wallremodeling-related genes LBD16 and XTR6, thereby inducing LR development. Treatment with 50 μ M serotonin significantly improved PSII activity, and the transcriptome data supported this result. Melatonin and serotonin slightly affected glycolysis and the TCA cycle; however, they markedly regulated the catabolism of several key amino acids, thereby affecting carbon metabolism and energy metabolism. Melatonin and serotonin improved iron (Fe) deficiency tolerance by inducing Fe-responsive gene expression

In a study by Quian *et al.* in 2015, exogenous melatonin treatments of 28-day-old of soil-grown WT (Col-0) *Arabidopsis* plants were watered with nutrient solution containing 20 μ M melatonin from below in pots with plants. Comparative metabolomic analysis by GC-TOF-MS metabolite profiling analysis was performed for identification of metabolites. Heat map was generated which shows the effect of exogenous melatonin on endogenous levels of 18 sugars and 5 sugar alcohols (Fig. 9)

Thus they found that the endogenous levels of both soluble sugars (fructose, glucose, melibiose, sucrose, maltose, galatose, tagatofuranose and turanose) and glycerol were commonly increased after both melatonin treatment and Pst DC3000 infection in *Arabidopsis*.

Further it was shown that exogenous pre-treatment with fructose, glucose, sucrose, or glycerol increased innate immunity against Pst DC3000 infection in wild type (Col-0) *Arabidopsis* plants, but largely alleviated their effects on the innate immunity in SA-deficient NahG plants and NO-deficient mutants. This indicated that SA and NO are also essential for sugars and glycerol-mediated disease resistance. Moreover, exogenous fructose, glucose,

sucrose and glycerol pre-treatments remarkably increased endogenous NO level, but had no significant effect on the endogenous melatonin level.

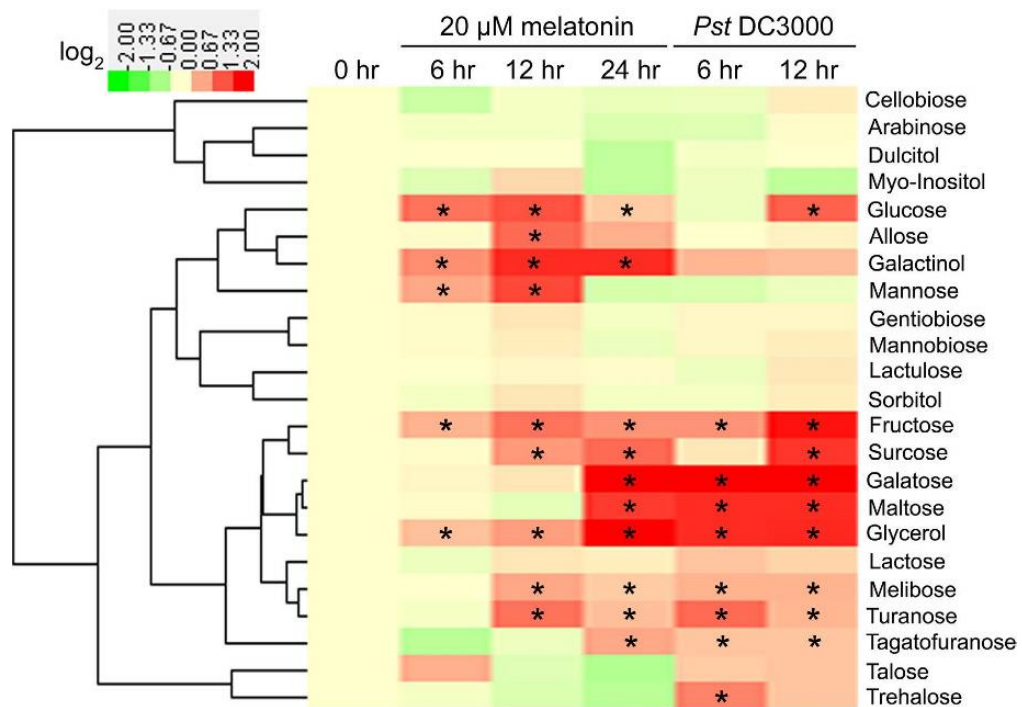


Fig. 9 Heat map showing the effect of exogenous melatonin on endogenous sugar levels

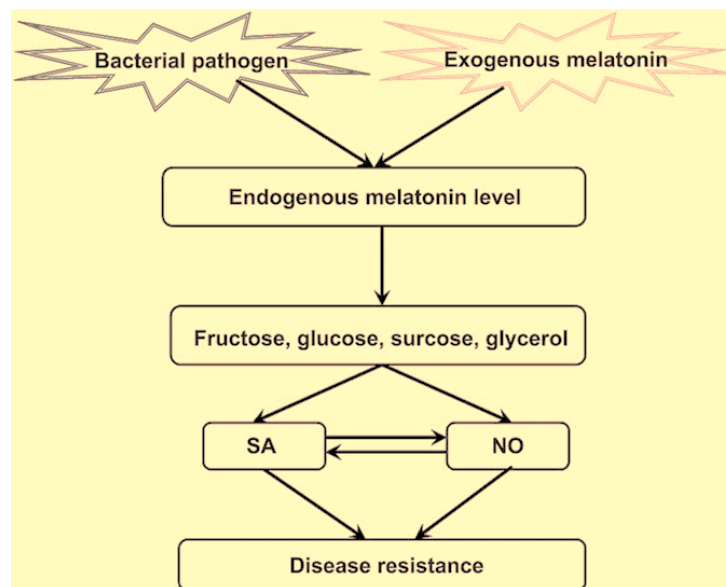


Fig. 10 Pathway of sugar mediated innate immunity

8.2.2. Improved photosynthesis and delayed senescence

Photosynthesis is a key biological process that determines plant survival and reproductivity. MEL relieves chloroplast from stress - induced damage by regulating

senescence related protein – *SAG* and suppresses chlorophyll degrading enzyme – *PAO* (Liang *et al.*, 2015). MEL alleviates stomatal limitation in cucumber & watermelon (Li *et al.*, 2017) and improves chlorophyll content in rice (Wang *et al.*, 2013).

8.2.3. Effect on seed germination

Seed germination is a critical and complex process in the plant life cycle. Dynamic balance of endogenous ABA and GA is crucial for seed germination. ABA synthesis genes is up-regulated during abiotic stress. GA acts a antagonist for ABA. MEL causes the up-regulation of GA synthesis genes – *GA20ox* and *GA3ox* and ABA catabolic genes – *CYP707A* and *CYP707A2* and down-regulation of ABA synthesis genes – *NCED1* and *ZEP* (Zhan *et al.*, 2019).

Although previous studies have found that melatonin can promote seed germination under salt stress, the involvement of melatonin in the regulation of proteomic changes remains poorly understood. In a study by Zhang *et al.* in 2017, priming seeds of cucumber with MEL (1 μ M) for 24hrs to promote seed germination under NaCl stress and proteomic analysis was done. (Fig. 11) A total of 157 proteins were significantly influenced (ratio ≥ 2 or ≤ -2) by melatonin during seed germination under salt stress using a label-free quantitative technique. GO analysis revealed that several pathways were obviously regulated by melatonin, including ribosome biosynthesis, lipid metabolism, carbohydrate metabolism, and storage protein degradation. Not only stress-tolerant proteins but also proteins that produce ATP as part of glycolysis, the citric acid cycle, and the glyoxylate cycle were up-regulated by melatonin. Thus the study provides the new evidence that melatonin alleviates the inhibitory effects of NaCl stress on seed germination by promoting energy production.

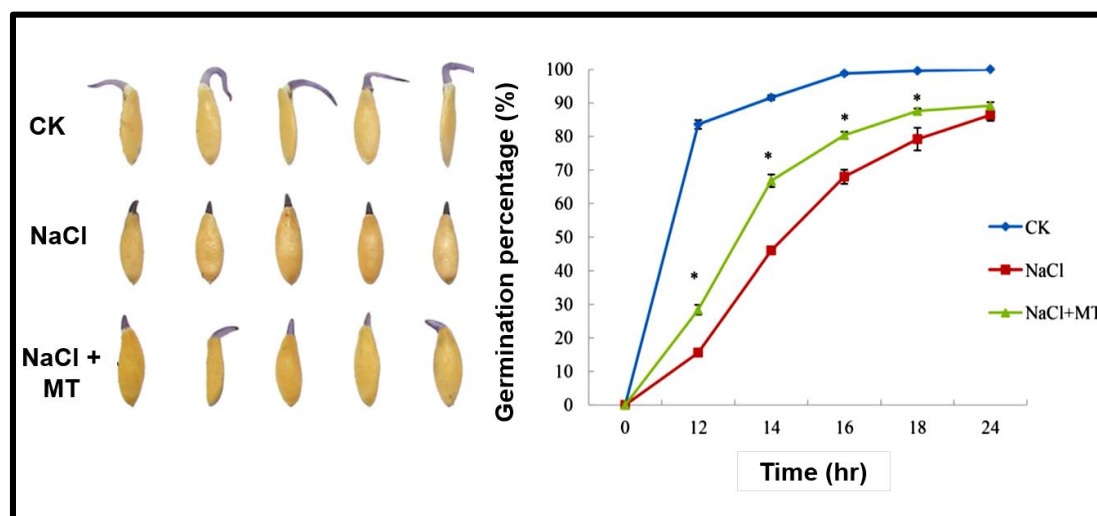


Fig. 11 Effect of MEL in seed germination of cucumber

8.2.4. Effect on fruit ripening

MEL regulates genes of secondary metabolism and steps in carotenoid biosynthesis. It also up-regulates transcripts of ethylene signal transduction and related genes. Thus it promotes fruit ripening and softening by improved content of ripening and anthocyanin related proteins.

The study on the effect of melatonin on the postharvest ripening and quality improvement of tomato fruit was carried out by Sun *et al.* in 2015. The tomatoes were immersed in exogenous melatonin for 2 h, and then the related physiological indicators and the expression of genes during post-harvest life were evaluated. Compared with control check (CK), the 50 μ M 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.

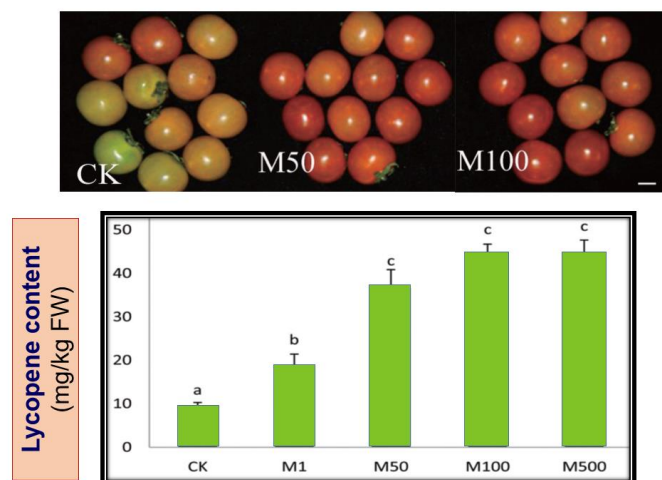


Fig. 12 Lycopene content of tomato at 17 d after MEL treatment

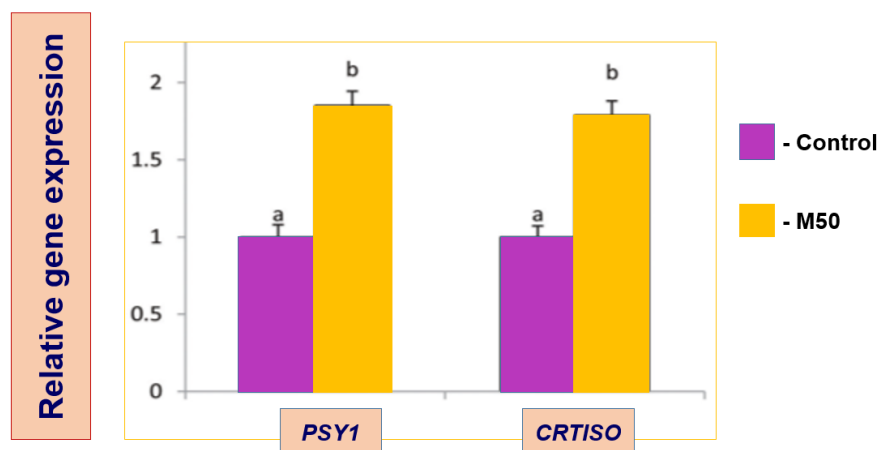


Fig. 13 Real-time PCR analysis of *PSY1* and *CRTISO* expression

8.2.5. Crosstalk with phytohormones

Phytomelatonin interacts with other phytohormones and also regulates their biosynthesis genes. Usually, it up-regulates biosynthesis genes of GA, PA, cytokinin and ethylene and down-regulates ABA biosynthesis genes (Zhang et al., 2015). Since tryptophan acts as a common ancestor for both IAA and MEL, both share similar properties. The increased endogenous MEL concentration decreases the endogenous levels of the auxin, IAA. Both of them encourages the vigorous growth of hypocotyl of *Lipus albus* at μM concentration and inhibitory consequences at higher accumulation. At lower concentration it mimics IAA functions. Root promotion effects of MEL was confirmed on rice, cucumber and pomegranate.

9. Summary

- ✓ MEL is an ubiquitous molecule with multitude of functions
- ✓ MEL in plants has great physiological significance
- ✓ Regulation of other phytohormones by MEL
- ✓ Exogenous MEL triggers biosynthesis of endogenous MEL
- ✓ Exogenous MEL have same molecular influence as endogenous
- ✓ Functions - interrelated involving complex mechanisms
- ✓ Omics provides the global view of changes at molecular level

10. Future thrust

Appropriate application of omics for analysing the functions of melatonins will give us much deeper understanding on its functions, so that phytomelatonin could be effectively applied as a bio-stimulator in agriculture and ultimately as a nutraceutical for human consumption.

11. Discussion

1. Among the edible plants, which is having the highest amount of MEL?

Coffee bean is having the highest amount of MEL (5800ng/g of DW) and among direct edibles Radish is having the highest amount (485 ng/ g of DW). Cereals especially germinated seeds and tomato are also having the more phytomelatonin content.

2. Through which means the exogenous MEL are applied to the plants?

Application means vary according to the plant parts and purpose. For seeds, priming with solution of MEL can be done. Spraying is the widely adopted method since it can be applied for whole plant, or leaves, fruits etc. For roots, it is applied through irrigation water.

3. Do higher concentration of phyto melatonin have any negative effect on plants and animals?

So far no reports have shown that MEL is having negative effect on both plants and animals.

4. Why human needs to consume MEL through foods, though it is already present in humans?

Mostly all the organisms will be having MEL at definite amounts. But plants being a sessile organism have higher amounts than humans to overcome various stressors. So, in cases like sleeping disorders or jetlag where, one needs to consume MEL externally, phyto melatonin from edibles will be a better option.

5. Are these phyto melatonin available commercially in India or other countries?

MEL are available commercially as tablet and also liquid formulations. But phyto melatonin are not commercialized yet.

6. Do cooking spoils the phyto melatonin present in the edible vegetables?

Cooking may not spoil the phyto melatonin content in edibles like tomato since, the roasted coffee bean acquires higher content of MEL than the fresh ones.

7. Is the application of phyto melatonin is practised in the field anywhere?

No, it is not practised anywhere. It is used only for the research purposes.

8. Can the phyto melatonin be used for delaying ripening, since it the desired property at present?

Till now the reports have shown that MEL can only used in improving ripening and flavour. In contrast its use for delaying ripening may be done by acquiring deeper knowledge in molecular mechanism of MEL governed fruit ripening and carotenoid biosynthesis, which still requires more intensive research using appropriate omics tool.

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13. Abstract

Plants have developed various mechanisms to cope with the environmental stress for overcoming the limitations of sessile nature. Plenty of molecules such as phytohormones and polyamines are involved in the plant stress response. To this list, adds a new molecule, melatonin (N-acetyl-5-methoxy-tryptamine), which acts as a master regulator in plants. It was portrayed exclusively as an animal hormone for many decades (Arnao and Hernandez-Ruiz, 2019).

Melatonin is a universal molecule that is present in plants and animals. The first evidence confirming its existence was in edible plants like Tomato, Cucumber and Banana (Dubbels *et al.* & Hattori *et al.*, 1995). Melatonin of plant origin is known as phytomelatonin (Arnao and Hernandez-Ruiz, 2018). It has been detected in many plants with content variation across different parts. The concentration is usually higher in seeds and medicinal herbs. It shares many similarities with indole-3-acetic acid (IAA) since they both are synthesized from a common precursor, tryptophan. Tryptamine pathway is the common biosynthesis pathway for melatonin in most of the plants.

Multiple physiological actions of phytomelatonins include promotion of seed germination, plant growth and development, rooting, photosynthesis, flowering, fruit ripening, delayed leaf senescence and protection against biotic and abiotic stressors (Arnao and Hernandez-Ruiz, 2019). It has a predominant role as an antioxidant, acting directly by scavenging the free radicals and indirectly by triggering the activities of other antioxidants.

Phytomelatonin regulates many genes involved in metabolic pathways and stress response. It improves tolerance to cold, heat, salt, and drought stress by regulating genes involved in the DREB/CBF, HSF, SOS, and ABA pathways, respectively. The regulation of different genes at varying concentrations prove the concentration dependent activity of melatonin in plants (Wang *et al.*, 2018). Endogenous melatonin level increases in response to stress. Application of exogenous melatonin also increases the endogenous melatonin level and has the same molecular influence.

Omics approaches like transcriptomics, proteomics and metabolomics are used to investigate the functions, by providing the global view of changes at varying levels of gene expression. High-throughput miRNA and mRNA sequencing revealed that, negative regulation of miRNAs upon melatonin treatment caused the up-regulation of the target genes involved in

signal transduction and protection, which will be responsible for cold tolerance in watermelon (Li *et al.*, 2016).

Thus, functional analyses of melatonins in plants could pave the way for effective application of phytomelatonin as a biostimulator in agriculture and ultimately as a natural nutraceutical for human consumption.