Melatonin in business with abiotic stresses in plants

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Abstract Melatonin (MEL) is the potential biostimulator molecule, governing multiple range of growth and developmental processes in plants, particularly under different environmental constraints. Mainly, its role is considered as an antioxidant molecule that copes with oxidative stress through scavenging of reactive oxygen species and modulation of stress related genes. It also enhances the antioxidant enzyme activities and thus helps in regulating the redox homeostasis in plants. Apart from its broad range of antioxidant functions, it is involved in the regulation of various physiological processes such as germination, lateral root growth and senescence in plants. Moreover this multifunctional molecule takes much interest due to its recent identification and characterization of receptorCandidate G-protein-Coupled Receptor 2/Phytomelatonin receptor(CAND2/PMTR1) in *Arabidopsis thaliana*. In this compiled work, different aspects of melatonin in plants such as melatonin biosynthesis and detection in plants, signaling pathway, modulation of stress related genes and physiological role of melatonin under different environmental stresses have been dissected in detail.

Keywords Antioxidant · Environmental stresses · Melatonin · (CAND2/PMTR1) · Reactive oxygen species

Introduction

Melatonin (MEL) (N-acetyl-5-methoxytryptamine) is naturally occurring bioactive indolamine molecule, present in phylogenetically distinct organisms. It is a small molecule and has a skeleton of benzene and indol ring with methoxy side chain and an alkylamide side chain respectively, having a molecular weight of 232.28 g/mol. It was first discovered and extracted from the bovine pineal gland of cow and also reported in humans (Lerner et al. 1958, 1959). Due to its ability to change (lighten) the skin color in amphibians and certain fishes by assembling the melanin (pigment granules), it is named as melatonin. This pleiotropic molecule governs a wide range of biological functions. In animals, it modulates various physiological actions and hence acts as biological modulator of circadian rhythms, sleep, mood, retina physiology, food intake, locomotory activity, sexual behaviour, immunology and seasonal reproductive physiology (Reiter et al. 1993; Hardeland 2013; Carrillo-Vico et al. 2013; Majidinia et al. 2018). The rhythmic secretion of MEL serves as a signaling molecule of darkness hence controls various physiological actions like (sleep—/wake-up cycle; endocrine secretion cycles) and studies showed that abnormal change in rhythmic production of MEL is responsible for many disorders (Maronde and Stehle 2007; Hardeland 2012) e.g., Alzheimer's disease and Parkinson's syndrome (Srinivasan et al. 2005).

MEL was not discovered in plants before 1995 and a research report appeared in science changed the view of researchers that MEL was confined not only in animals but

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also present in the plants. In 1991, MEL was discovered in photosynthetic dinoflagellate *Lingulodinium polyedrum* previously known as (*Gonyaulax polyedra*). This study infused curiosity among the scientists for detection of MEL in other plants and then it was detected in *Pharbitis nil* in 1993, however this finding could not published before 1995. Now the MEL detection is reported in different plants such as tomato, banana, cucumber, beet-root with the varying concentrations of 50.6, 46.6, 8.6, 0.2 ng/100 g of plant tissue (peel of plant material was not included) respectively, detected by high performance liquid chromatography/mass spectrometry (HPLC) and radioimmunoassay (RIA) (Dubbels et al. 1995). However, they also reported that potato was devoid of MEL.

MEL is widely distributed in phylogenetically distinct lineages ranging from microscopic organism such as bacteria, unicellular and multicellular algae, and animals to higher plants (Kolář and Macháčeková 2005; Posmyk and Janas 2009; Stehle et al. 2011). During the last 20 years, researchers have taken much interest in exploring the phytomelatonin and it is reported extensively in edible and medicinal plants (Posmyk and Janas 2009; Tan et al. 2012; Arnao 2014). It has been extracted in different plant tissues of around 150 plant species (Hardeland et al. 2007; Gomez et al. 2013; Arnao 2014; Hardeland 2016). Researchers initially believed that it plays similar role in plants like in animals and they investigated its role in circadian rhythm, however studies have indicated that in some plants MEL level oscillates like in *Vitis vinifera*, grape plant (Boccalandro et al. 2011), *Chenopodium rubrum* (Wolf et al. 2001) and *Prunus savium* (Zhao et al. 2013). This change in the levels of MEL could be due to endogenous regulatory mechanisms of photoperiodism like flowering. But conclusive evidences are lacking to prove that it is involved in circadian rhythms. It is reported that MEL levels are changing due to environmental constrains (Tan et al. 2012). MEL is detected in variety of plant tissues such as in seeds, leaves, fruits, flowers and roots (Afreen et al. 2006) and its concentrations varies in different plant species and also varies within the same plant (Hattori et al. 1995; Kolář and Macháčeková 2005). Thus, MEL emerged as a potential anti-stressor molecule because of its protective functions under different environmental stress conditions such as drought, salt, heavy metal, heat, chilling and UV- light stress in plants. Therefore, this review will address the dynamic aspects of melatonin including its biosynthesis and detection in plants, as a stress responses regulator and modulation of stress related genes. Furthermore, an effort has been made to summarize its involvement in growth and development, seed priming and its signaling.

Biosynthesis and detection of phytomelatonin

Endogenous level of MEL in plants is comparably higher than animals (Tan et al. 2012). This sets the basis for looking an alternative mechanism for biosynthesis of MEL in plants. DNA sequences of serotonin N-acetyltransferase (SNAT) enzyme which catalyzes the final step of MEL synthesis in plants, shows more similarity to cyanobacteria rather than animal sequences. Cyanobacteria are able to synthesize this indolamine (MEL) which further corroborates the fact that the modern chloroplast has been evolved from cyanobacteria as per the endosymbiotic theory (Tan et al. 2012; Zhao et al. 2019). Later on, it was hypothesized that the main site of phytomelatonin biosynthesis could be chloroplast (Zheng et al. 2017). They cloned *MzASMT9* gene N-acetylserotonin-O-methyltransferase 9 (ASMT), last enzyme for the biosynthesis of MEL obtained from *Malus zumi*. Through in vivo techniques like fluorescence and Western blot it was confirmed that *MzASMT9* was present in chloroplast and this study results that the site of biosynthesis of MEL is chloroplast. Moreover chloroplast also synthesizes MEL in vitro (Zheng et al. 2017).

In vertebrates biosynthetic pathway of MEL is extensively studied (Reiter 1991; Falcón et al. 2009). Tryptophan serves as a precursor for biosynthesis of MEL in plants: tryptophan, an amino acid is converted into 5-hydroxytryptophan by an enzyme tryptophan 5-hydroxylase (T5H, E.C.1.14.16.4). 5-hydroxytryptophan is then converted into serotonin (Falcón et al. 2009). In *Hypericum perforatum* L. plant, commonly known as St. John's Wort, formation of serotonin has been reported from 5-hydroxytryptophan in presence of enzyme tryptophan decarboxylase (TDC) (Murch et al. 2000; Murch and Saxena 2006). In most plants, such as rice (*Oryza sativa*), (Kang et al. 2007), pepper (*Capsicum annuum*) (Park et al. 2009), Madagascan periwinkle (*Catharanthus roseus*) (DeLuca et al. 1989) and tobacco (*Nicotiana tabacum*) (DiFiore et al. 2002), tryptophan is first converted into tryptamine by the enzyme tryptophan decarboxylase (TDC, E.C. 4.1.1.28), this pathway is most commonly found in plants. At C-5 position tryptamine is then hydroxylated by the enzyme tryptamine 5-hydroxylase (T5H) and is converted into serotonin (Fujiwara et al. 2010). In animals as well as in plants, serotonin N-acetyltransferase (SNAT, E.C. 2.3.1.87) enzyme catalyzes the conversion of serotonin to N-acetylserotonin. Finally methylation of N-acetylserotonin by the hydroxyindole-O-methyltransferase (HIOMT, E.C.2.1.1.4) also called as acetyl serotonin methyl transferase (ASMT) leads to the formation of MEL. In biosynthesis of MEL, HIOMT enzyme acts as rate limiting enzyme. The other route of MEL synthesis is also evaluated in which serotonin is converted into 5-methoxytryptamine by the enzyme HIOMT and 5-methoxy-

tryptamine is finally catalysed by SNAT into MEL (Tan et al. 2016; Choi et al. 2017). Moreover, from tryptophan through indole-3-acetylaldehyde, Auxin (IAA) is also biosynthesized from tryptamine, thus tryptophan serves as precursor for both MEL and IAA (Debnath et al. 2019) (Fig. 1).

Techniques by which phytomelatonin is analyzed are very important for apprehending its biosynthesis, physiological role and regulatory mechanism. For the quantification of MEL, techniques like radioimmunoassay (RIA) and Enzyme-linked Immunosorbent Assay (ELISA) have been used from long time (Dubbels et al. 1995; Pape and Luning 2006). ELISA is very convenient as its kits are available in market for its precise detection, but major problem of this technique is antibody cross reactivity which lowers its accuracy and specificity (Gomez et al. 2013). Analysis of MEL by gas chromatography followed by mass spectrometry technique has been applied which leads to improved specificity and sensitivity, although it requires complex sample preparation for its analysis (Ragab et al. 2006). Before the analysis of phytomelatonin, samples (plant extract) are prepared with utmost care as endogenous MEL concentration is very low and complicated to determine. Various techniques are employed for sample purification e.g., solid phase extraction (SPE) (Gomez et al. 2015), micro-extraction packed sorbent

(MEPS) (Huang and Mazza 2011a, b; Byeon and Back 2014; Gomez et al. 2015), liquid/liquid extraction (LLE) (Arnao and Hernández-Ruiz 2009a; Byeon and Back 2014). Solid phase extraction and liquid/liquid extraction are most common approaches in practice for sample purification (Gomez et al. 2015). Also HPLC technique is mostly used for the determination of MEL and for this various detectors are required like electro-chemical detector (ECD) (Zettersten et al. 2009), UV-detector (Ly et al. 2008) and fluorescence detector (FD) (Padumanonda et al. 2014). Among the above mentioned detectors, MS detector has comparatively high specificity and sensitivity. HPLC followed by MS technique has been shown most powerful approach for determination of MEL (Huang and Mazza 2011b; Gomez et al. 2013). Another simple and quick method was developed for the analysis of MEL in plant tissue by UHPLC (UltiMate 3000 High Performance Liquid Chromatography) followed with High Resolution Orbitrap Mass Spectrometry (Ye et al. 2017). In this method, isotope-marked MEL, MLT-d4 is used as internal standard. The use of the (MLT-d4) minimizes the interference of sample as it contains a number of metabolites (Ye et al. 2017). The analytical outcome of plant extract could be maximized by liquid liquid extraction (LLE) purification method.

Fig. 1 Biosynthesis of Melatonin: Dark red arrow shows melatonin biosynthesis in animals, while green arrow shows the pathways of melatonin biosynthesis in plants and yellow arrow shows the regulating steps. TDC, T5H, SNAT, HIOMT/ASMT are abbreviative

forms of enzymes (tryptophan decarboxylase, tryptophan 5-hydroxylase, serotonin N-acetyltransferase, hydroxyindole-O-methyltransferase/acetyl serotonin methyl transferase respectively (Modified from Zhang et al. 2015)

Physiological roles of melatonin under abiotic stresses

Effect of melatonin on growth and development

MEL, a low molecular weight amphiphilic indoleamine signalling molecule found in all examined plants at distinct levels from pico to nanograms per gram of plant tissue (Fan et al. 2018). Indole-3-acetic acid (IAA) and other indoleamine compounds are very important for growth and development in the plants such as Auxins. MEL is also an indoleamine molecule and shares a common precursor (tryptophan) for biosynthesis with IAA. Due to similarity in the chemical structure of MEL and IAA, it was suggested in earlier studies that MEL has the growth promoting activity probably by increasing the auxin concentration or having auxin like activities (Chen et al. 2009). MEL and IAA were applied in a wide range of concentration to etiolated hypocotyls of *Lupinus albus* L., both the indoleamine compounds enhanced the growth of plant at lower concentrations (micromolar) and decrease the growth at higher concentration. Similar investigation were carried in various plants such as in *Phalaris canariensis* L. (canary grass), *Triticum aestivum* L. (wheat), *Hordeum vulgare* L. (barley) and *Avena sativa* L. (oat) (Hernandez-Ruiz et al. 2005; Arno and Hernandez Ruiz 2007). MEL is involved in multiple growth and developmental processes in plants and its roles in plants particularly under stress conditions have been explored in recent years (Table No. 1).

MEL is involved in a number of physiological actions in plants such as regulation of growth and metabolism (Wan et al. 2018), improves the growth and photosynthetic pigments in broccoli (Gul et al. 2018), lateral or adventitious root induction (Arnao and Hernández-Ruiz 2017a; Wen et al. 2016), delays leaf senescence (Liang et al. 2015), heavy metal tolerance (Farouk and Al-Amri, 2019), mitigated the negative effect of salinity stress (Chen et al. 2018), alleviates the negative effects of cold stress in melon (Zhang et al. 2017b), enhanced drought stress tolerance (Yang et al. 2019; Campos et al. 2019), protects chlorophyll pigments under stress conditions (Szafrńska et al. 2016), improving seed germination under salt stress (Castanares et al. 2019), alleviates the oxidative stress and promotes the growth under salt stress (Zhang et al. 2017a), maintains the ion homeostasis by up regulation of ion channel genes MdNHX1 and MdAKT1 (Li et al. 2012), scavenges the ROS and thus inhibits electrolyte leakage results in improving electron transport chain (Posmyk et al. 2008). MEL also modulates the gene expression and interacts with other phytohormones, IAA, cytokinin, gibberellic acid, ethylene and abscisic acid (Arnao and Hernández-Ruiz 2017b). However, its receptor was not

identified and it was a question mark how plants sense the MEL and now recently its receptor (CAND2/PMPTR1) has been identified in Arabidopsis and MEL is considered as a new phytohormone.

Effect of melatonin on seed priming

Various techniques are employed with the aim to increase the tolerance for stresses, priming is one of the most reliable technique by which raised plants could be tolerant for future abiotic and biotic stresses. Priming in plants can be induced naturally in response to external environment constrains and enhances the ability of facing a particular stress thereafter (Filippou et al. 2013). High salinity results in ionic toxicity and also osmotic stresses (Munns and Tester 2008) due to accumulation of Na⁺ in the cytosol or abnormal distribution of K⁺ with the disruption of normal Na⁺/K⁺ ratio. It has been reported that pre-treatment with MEL to *Malus hupehensis* provided tolerance to salt stress (Li et al. 2012). Another study revealed that either coating or pre-treatment with MEL increased tolerance to salt stress (Wei et al. 2014). Moreover, it has also been revealed in a recent report that MEL mitigates the salt stress in wheat seedlings through the regulation of polyamine metabolism (Ke et al. 2018).

Effect of melatonin on senescence

Senescence is a regulatory physiological process in plants and is determined by internal and external factors. Foliar senescence rate varies in different species and is dependent on the external environment. Detachment of leaf involves degradation of chlorophyll, deprivation of molecules, nutrient relocation, alterations in plant growth regulators, deterioration of cell walls and at last cell death (Lim et al. 2007). The role of MEL was determined in leaves of *Hordeum vulgare* L. which were placed in medium with or without MEL in petri plates and it was observed that MEL treated leaves had delayed senescence. However, the mechanism of it was not revealed but it might be due to its antioxidant actions or regulation of senescence related genes (Arnao and Hernández-Ruiz 2009b). In another study, apple leaves were kept in dark in order to evoke senescence rate and it was observed that treated leaves with 10 mM MEL delayed chlorophyll degradation and has high efficiency of PS II than control leaves (Wang et al. 2012). A similar result was also noted in apple plant with delayed senescence by treatment of MEL (Wang et al. 2013a).

Role of melatonin under abiotic stresses

MEL has amphiphilic property and could enter into the sub cellular sections which make it more reasonable and

Table 1 Effect of melatonin on growth, photosynthetic and biochemical attributes in different plants under various abiotic stresses

Plant species	Effects of melatonin	References
<i>Hypericum perforatum</i> L.	Indolamine level was elevated at uninucleate microsporogenesis stage indicating its role in reproduction.	Murch and saxena (2006)
Trangenic tobacco	Checks the reproduction of white fly.	Thomas et al. (1995)
<i>Malus domestica</i>	Alleviated the oxidative stress, delays leaf senescence and enhanced photosystem II (PSII) efficiency under drought stress.	Wang et al. (2013a, b)
<i>Triticum aestivum</i> L.	Mitigates salinity induced stress by regulation of polyamine metabolism.	Ke et al. (2018)
<i>Cicer arietinum</i> L.	Increase in vegetative growth parameters photosynthetic pigments and enhanced nutritional values of seeds.	Dawood (2018)
<i>Solanum lycopersicum</i> L.	Conferred tolerance to acid rain by increasing ROS scavenging and photosynthesis.	Debnath et al. (2018)
<i>Camellia sinensis</i>	Enhances the tolerance to the different abiotic stresses	Li et al. (2019)
<i>Solanum lycopersicum</i> L.	Prevents damage to proteins and membranes under combination of heat and salinity stress and limits the ROS production.	Martinez et al. (2018)
<i>Cucumis sativus</i> L.	Promotes germination of seeds under salt stress and also enhanced the levels of proteins for energy production	Zhang et al. (2017a, b)
<i>Lycopersicon esculentum</i> L.	Alleviates the inhibitory effect of cold stress and protects the photosynthetic apparatus.	Yang et al. (2018)
<i>Chara australis</i>	Increases quantum yield of photosystem.	Lazar et al. (2013)
<i>Brassica oleracea</i>	Accumulation of anthocynin by up regulation of biosynthetic gene of anthocynin.	Zhang et al. (2016)
<i>Cucumis sativus</i> L.	Increases germination rate, stimulates lateral root and protects chlorophyll damage under drought stress	Zhang et al. (2013)
<i>Oryza sativa</i>	Alleviates the inhibitory effects on growth of plants under cold-stress and protects photosynthetic apparatus against oxidative stress.	Han et al. (2017)
<i>Glycine max</i>	Improved plant height, size of leaf and also seed number.	Wei et al. (2014)
<i>Hordeum vulgare</i> L.	Delays senescence by protecting chlorophyll degradation	Arnao and Hernández-Ruiz (2009b)
Transgenic Arabidopsis	Enhances drought tolerance by over expression of the wheat TaCOMT gene.	Yang et al. (2019)
<i>Coffea arabica</i> L.	Improves drought tolerance and has positive effect on carboxylation process.	Campos et al. (2019)
<i>Pyrus communis</i> L.	Delays post-harvest senescence.	Liu et al. (2019)

powerful antioxidant (Tan et al. 2012). Apart from this, metabolites associated with MEL also acts as antioxidants. Thus, MEL together with its coupled intermediates, functions as scavenger of various free radicals (Tan et al. 2015). Despite in vivo synthesis of MEL, plants are able to absorb and accumulate MEL from the surrounding environment such as soil or when applied exogenously (Tan et al. 2007). Thus plants build up high concentration of MEL, which indicates that it serves as important molecule and regulates functions such as growth and reproduction, delay in senescence, improves fruit ripening and increases fruit yield (Dubbels et al. 1995; Arnao and Hernández-Ruiz 2014). The most promising function of this pleiotropic molecule is the alleviation and protection against stress conditions (Zhang et al. 2015; Wei et al. 2014). It has a complex association with stress signaling cascades by regulating the ROS levels and redox state. A number of

studies have confirmed that MEL acts as powerful antioxidant with a prominent ability for ROS scavenging (Tan et al. 2000; Allegra et al. 2003). It is also evaluated that the derivatives of MEL such as 3-hydroxymelatonin (3OH-Mel), N¹-acetyl-N²-formyl-5-methoxyknuramine (AFMK), N-acetyl-5-methoxyknuramine (AMK) have the antioxidant property, which implies that MEL has higher antioxidant capabilities than vitamins like C, E and K (Tan et al. 2015). Additionally it has amphiphilic property and hence penetrates into cell compartments. MEL along with its related intermediate molecules could scavenge ROS molecules more effectively, thus have a powerful antioxidant action which makes it highly efficient molecule for protection against oxidative stress (Tan et al. 2015). Due to lipophilic property and antioxidant actions, MEL protects lipid peroxidation, hence increases viability and rate of germination of seeds (Van-Tassel and O'Neil 2001).

Oxidation of lipids is decreased by elevating endogenous level of MEL, which suggests its antioxidant action (Zhao et al. 2013). Thus, MEL has potent role in protecting plants from oxidative stress even at low concentration. However, exogenously supplied MEL is also absorbed and accumulated in plant organs (Zhang et al. 2015). There are number of studies which indicated that MEL protects important biomolecules such as lipids, proteins, DNA, RNA from oxidative damage (Sliwinski et al. 2007; Galano et al. 2018). Researchers reported that the antioxidant action of MEL works in number of ways, acts as a direct scavenger of free radicals, up regulation of antioxidant enzymes, stimulate glutathione synthesis, facilitates other antioxidants, protection of free radical scavenging enzymes, scavenging of free radicals in mitochondrial electron transport chain, alleviates the photo inhibition of photo system II (Cui et al. 2017; Galano et al. 2018).

It has been studied that ozone tolerant species have higher MEL levels compared to plants which are sensitive to ozone (Dubbels et al. 1995). Simopoulos et al. (2005) observed that Mediterranean and Alpine plant species and their counterparts have different melatonin levels. Mediterranean and Alpine plant exposed to high UV had high MEL concentration than their counterparts exposed to low UV radiations. MEL biosynthesis genes in transgenic *Nicotiana sylvestris* alleviated the DNA damage induced by UV-B radiation (Zhang et al. 2012), which suggests defensive function of MEL against UV stress. MEL also provides the line of defence against the heavy metals toxicity (Romero et al. 2014). Exogenous application of MEL alleviates the cadmium induced phytotoxicity in tomato plants by activating different mechanism such as, enhancements in antioxidant potential, subsequent sequestration of Cd and phytochelatin biosynthesis (Hasan et al. 2015a, b) and toxic effects of Cu^{2+} ions were also reduced by pre-treatment of MEL to the seeds of *Brassica oleracea* (Posmyk et al. 2008). Cao et al. (2019) also revealed the beneficial role of MEL in mitigation of Cu^{2+} toxicity in cucumber and provide tolerance by improving Cu^{2+} sequestration, carbon metabolism and inhibition of overproduction of ROS.

Exogenously sourced MEL on cucumber plants provided tolerance to salt stress and also improved growth and photosynthesis by mitigating the oxidative stress through increases the activity of antioxidant and relative enzymes (Wang et al. 2016). Finding of Sadak (2016) revealed that wheat grains primed with 500 mM concentration of MEL effectively alleviated the salinity stress in the plant. Moreover it has also been revealed that MEL acts as an effective biostimulator (Kołodziejczyk et al. 2016) and pre-treatment of the seeds with MEL under cold stress provided tolerance by detoxifying the ROS and up regulation of stress related proteins. Low concentration of

exogenous MEL alleviated the impact of salt stress in *Actinidia* seedlings by increasing the antioxidant substances and endogenous levels of MEL was increased by the up regulation of *SNAT*, which is the one of the MEL biosynthesis pathway enzyme (Xia et al. 2017). It was observed that MEL prevents proteins and membranes from oxidation, resulted by salt and heat stress. Gene expression of many antioxidant genes and related antioxidant enzymes particularly ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GPX) and phospholipid hydroperoxide glutathione peroxidase (Ph-GPX) was up-regulated in MEL treated plants under stress conditions. These antioxidants and related antioxidant enzymes nullified the effects of ROS and maintained the redox state of cells (Wang et al. 2016). It was also revealed that plants treated with MEL have high photosynthetic rate and efficiency of PSII under salt and heat stress. Liu et al. (2018) reported that exogenous application of MEL alleviates salt stress in rapeseed seedlings by improving H_2O_2 -scavenging capacity through increased activity of antioxidant enzymes and accumulation of osmoregulatory substances.

It has been studied that MEL alleviates drought stress by over expression of biosynthetic genes of MEL in transgenic tomato plant which conferred tolerance to water stress (Wang et al. 2014). Cui et al. (2017) studied the response of wheat seedling to MEL in alleviation of drought stress. Seedlings of wheat treated with MEL remarkably enhanced the tolerance by reduction of ROS level, reduction in membrane damage, increased photosynthetic rate and improved capability of photosystem II (Fv/Fm). Ye et al. (2016) also studied the role of MEL under water stress in *Zea mays* L. and the treated plants had improved the activities of antioxidant defence system and also in DPPH activity which in turn could have checked the oxidative damage and favoured photosynthesis. A recent report also confirmed the involvement of MEL in drought stress tolerance in *Dracocephalum moldavica*, and effectively reduced the adverse effect of imposed stress. Foliar application of MEL has significant impact and reduced the adverse effect of stress condition through up-regulation of various antioxidant enzymes and also reduced the H_2O_2 content and lipid peroxidation (Kabiri et al. 2018). Similarly, another recent investigation indicated that pretreatment of oat seedlings under polyethylene glycol (PEG) imposed drought stress with 100 μM MEL had reduced contents of H_2O_2 and $\text{O}_2^{\bullet-}$ and enhanced the antioxidant enzymes activities (Gao et al. 2018). Qiao et al. (2020) also reported that exogenous MEL increased the tolerance to PEG-induced drought stress by improved activity of aquaporin and increasing the hydraulic conductance in maize plants. Moreover, in another recent study Bidabade et al. (2020) showed that MEL treatment provides drought

stress tolerance and also increases production of essential oil in two *Salvia* cultivars.

It has been observed that rice plants grown in adverse condition like high temperature had elevated level of MEL which suggests its protective role against high temperature stress (Byeon and Back 2014). High temperature disturbs the fluidity of membrane and enzyme efficiency which negatively affects the growth of the plants. The seeds of *Phacelia tanacetifolia* when treated with MEL exhibited tolerance to heat and high light stress (Tiryaki and Keles 2012). Xu et al. (2016) also revealed that MEL increases thermotolerance in tomato plants by promoting cellular protein protection and degradation of heat induced denatured proteins. Moreover, it has been reported that freezing stress leads to an increase in MEL level (Shi and Chan 2014). They assessed the endogenous MEL concentration in leaves of the *Arabidopsis* before and after cold stress and observed that exogenous MEL enhanced the tolerance to cold stress. It was evaluated that ZAT6, cysteine2/histidine2-type zinc finger transcription factor were involved in the regulation of MEL-mediated cold stress tolerance. Recently, it is shown that MEL application has protective role to PSII under cold stress in rice plants. Seed soaking and dipping of root in MEL were found efficient to cope with cold stress (Han et al. 2017).

Role of MEL was also analysed under the Paraquat (PQ) stress, which is an inhibitor of photochemical reaction and causes overproduction of ROS like O_2^- and other ROS species. Treatment of MEL significantly improved the oxidative stress in seeds of *Pisum sativum* L. (Szafrńska et al. 2016). MEL synthesis enzymes namely MeTDC2, MeASMT2 interact with ascorbate peroxidase MeAPX2 and reduce the oxidative stress by regulating redox homeostasis in cassava (Bai et al. 2020). Besides exogenous supplementation of MEL, engineered MEL enriched plants developed by transgenic approach shows promising results. Melatonin-rich transgenic (MRT) rice plants treated with butafenacil (singlet oxygen generation herbicide) showed resistance by scavenging (ROS) in vivo in transgenic plants (Park et al. 2013). Over expression of MEL synthesis genes (ovine AANAT and HIOMT) in switchgrass elevates MEL production which exhibits improvement in growth attributes and provides resistance to salt stress (Haung et al. 2017). Furthermore, Kang et al. (2010) studied that transgenic rice seedling with high levels of MEL by over expression of human serotonin N-acetyltransferase showed increase in chlorophyll synthesis and promotes cold-stress resistance. Similarly Lee and Back (2017) reported that overexpression of one of the MEL biosynthesis enzyme rice serotonin N-acetyltransferase1 in transgenic rice plants developed tolerance to cadmium stress. Moreover, ectopically expressing of MzASMT, or TaCOMT in *Arabidopsis* boosted the MEL level and

enhanced the tolerance to drought stress (Zuo et al. 2014; Yang et al. 2019). Therefore, based on the number of findings compiled in this review, it was revealed that MEL plays diverse role in plant growth and development particularly under different environmental stresses and help to develop adaptive activities in plants against different environmental stresses as depicted in Fig. 2.

Signaling and mechanism of action of melatonin

Various physiological processes are governed by the phyto-melatonin such as enhancement in seed germination and growth, heavy metal tolerance and enhancement in net photosynthetic rate. It has also regulatory role in important processes like fruit ripening, senescence and lateral root development (Chen et al. 2009; Hasan et al. 2015a, b; Arnao and Hernández-Ruiz 2017a). Moreover, MEL also serves as promising biostimulator against abiotic stresses (Wei et al. 2014). Like other phytohormones, it is involved in cellular processes such as proper dynamics of intracellular Ca^{2+} , opening and or closing of stomata and regulates ion transport (Li et al. 2012, 2017a, b). Since receptor and phytohormone interaction is very important for triggering downstream signalling. Its receptor was not identified to consider it as plant hormone. Now recently phyto-melatonin receptor (CAND2/PMTR1) has been identified in model plant *Arabidopsis thaliana* and it is considered as plant hormone (Wei et al. 2018). CAND2/PMTR1 has a receptor like structure and interacts with α subunit of G protein (GPA1), thereby induces its expression. Interaction of CAND2/PMTR1 and phyto-melatonin results in dissociation of $G\alpha$ and $G\gamma b$ subunits which increases influx of Ca^{2+} by activating NADPH oxidase-dependent H_2O_2 production. The influx of Ca^{2+} promotes efflux of K^+ which ultimately causes the closure of stomata. Therefore, closure of stomata is regulated by application of MEL through its interaction with receptor mediated H_2O_2 Ca^{2+} signaling cascade (Fig. 3). However, closure of stomata was concentration dependent (Wie et al. 2018). Hence, based on several finding it can be summarized that MEL regulates receptor mediated down-stream signaling pathways in plants, which results in adaptive strategies and modulates plant metabolism (Fig. 3).

MEL regulates gene expression particularly under stressed conditions of plants as reported in number of plants such as cold relative genes (ZAT6, ZAT10, ZAT12, COR15a and CAMTA1) were transcriptionally up-regulated in *Arabidopsis thaliana*. These genes improve the tolerance in *Arabidopsis thaliana* to low temperature stress (Bajwa et al. 2014; Shi and Chen 2014). MdNHX1, MdAKT1, and Na^+/H^+ vacuolar antiporter genes in *Malus domestica* were considerably up-regulated by MEL and hence mitigated the negative effects of the salt stress

Fig. 2 Depicts the diverse role of MEL in plants under different abiotic stress conditions and its involvement in different growth and developmental processes. Colored boxes depict the responses of MEL in plants under different stressors. ↓-denotes inhibition; ↑-denotes promotion

(Li et al. 2012). *SENESCENCE-ASSOCIATED GENE 12* (*SAG12*) and *PHEOPHORBIDE A OXYGENASE* (*PAO*) genes were down regulated considerably by the application of MEL. It also helps in quenching, improves the capability of Photosystem II (PSII) and thus enhances the net photosynthetic rate (Wang et al. 2013b). Impact of exogenous MEL was analysed at transcriptome level in *Arabidopsis* (Weeda et al. 2014). A total of 1308 genes were modulated, out of which 742 were down regulated and 566 genes were up regulated. It was also revealed that *AtIAA17* was directly associated with MEL-mediated aging of the leaf (senescence). Shi et al. (2015a) found that at different development stages, the concentration of endogenous MEL varied significantly which was dependent on the stage of development. They observed that *AUXIN RESISTANT 3* (*AXR3*)/*INDOLE-3-ACETIC ACID INDUCIBLE 17* (*IAA17*) expression profile was suppressed significantly with the treatment of MEL. Suppression of *IAA17* by

exogenous application of MEL could have inhibitory effect on *SENESCENCE 4* (*SEN4*) and *SENESCENCE-ASSOCIATED GENE 12* (*SAG12*). Thus, *IAA17* gene regulates the senescence in *Arabidopsis*. Moreover, heat tolerance was also enhanced in *Arabidopsis* by up-regulation of heat-shock factors (*HSFA1s*) significantly by the supplementation of MEL (Shi et al. 2015b). Ozgur et al. (2017) studied the role of MEL under stress on Unfolded Protein Response Genes (*UPR*) at the transcriptional level in endoplasmic reticulum of *Arabidopsis thaliana*. The findings indicated that the MEL concentration enhanced and *UPR* components decreased in Tunicamycin (Tm) treated endoplasmic reticulum (ER) in stressed plants. They concluded that stress in ER induced by Tm was related to sensor/transducer *bZIP28*, *bZIP17*, *RE1A*, *IRE1B*, apoptosis genes *AGB1* and ER-associated degradation (*ERAD*) genes, (these ER genes were up-regulated with Tm treatment), which were down regulated reverse to control level with

Fig. 3 Model of likely MEL signaling in plants. Interaction of MEL and CAND2/PMTR1 (receptor) causes dissociation of G α and G γ subunits which increases influx of Ca²⁺ by activating NADPH

oxidase-dependent H₂O₂ production and finally regulates the genetic expression (Arnao and Hernández 2019)

25 μ M concentration of MEL in roots. Hence MEL copes with deleterious impact of Tm induced ER stress. In oat seedling, pre-treated with MEL shows up-regulation drought associated genes: *DREB2*, *WRKY1*, and *MYB* and *Asmap1* and *Aspk11* which are mitogen activated protein kinases MAPKs. These findings also suggested that MEL alleviates drought stress tolerance by involving MAPKs cascades (Gao et al. 2018).

MEL attains pin interest of researchers due to its photocatalytic action as ROS scavenger (Hardeland 1996; Fan et al. 2018). MEL interacts with ROS by electron (e⁻) donation to produce melatoninylation and donation of H atom from N atom or through nitrosation and substitution at C2, C4 and C7 positions. Moreover, MEL has capability to repair damaged biomolecules such as it has ability to form guanosine from guanosine radical by electron transfer (Tan et al. 2002). Tan (1993) determined the interaction of MEL with hydroxyl radical ($\cdot$ OH). However, the mechanism of interaction of MEL with free radicals is not fully elucidated. Among the ROS, $\cdot$ OH is the most reactive free radical having a positive reduction potential ($E^{\circ} = 2.31$ V) (Reiter et al. 2000a; Reiter et al. 2000b; Halliwell and Gutteridge 2015). Once the hydroxyl radical is formed, it immediately reacts with almost any molecules with high rate constants. MEL scavenges the $\cdot$ OH with the rate constant $0.6 \times 10^{11} \text{ M}^{-1} \text{ s}^{-1}$ (Poeggeler et al. 1996). Photolysis of H₂O₂ leads to generation of $\cdot$ OH radicals which are captured by 5,5-dimethylpyrroline-N-oxide (DMPO) (a

spin trapping agent) which is determined by the electron spin resonance spectroscopy (ESR). These studies further ascertained that MEL is a potent scavenger of $\cdot$ OH (Stasica et al. 1998; Mahal et al. 1999). It is capable of donating electrons to $\cdot$ OH and could acts as a reductive agent. H₂O₂ is produced in vivo by many enzymes such as xanthine, D-amino acid oxidase. Generally it is less reactive and has no electric charge, but with the transition metal ions like Fe²⁺, it becomes highly reactive. MEL scavenges H₂O₂ in dose-dependent manner (Tan et al. 2000). Singlet oxygen ($^1\text{O}_2$) is formed during photosensitization process of substrates like biological pigments (Halliwell and Gutteridge 2015). It is a type of ROS produced in vivo and has high potential of damaging macromolecules. MEL is capable of reducing $^1\text{O}_2$ -dependent 2,2,6,6-tetramethylpiperidine oxide radical formation during photo-excitation reaction. Moreover, it has been observed that the $^1\text{O}_2$ formed by UV light is scavenged by MEL with a rate constant of $2.20 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ (Roberts et al. 2000). MEL has a high capacity of scavenging free radicals and helps in maintaining micro-environment of biological membrane, hence acts as a powerful antioxidant and important molecule for the defense of cells. 3-hydroxymelatonin (3OH-Mel), N¹-acetyl-N²-formyl-5-methoxykynuramine (AFMK) are oxidation products of MEL and acts as a reductive force, donates two electrons (Tan et al. 2001) at a time and supposed to be unique compared to other cellular antioxidants as they can donate only one electron during

scavenging of free radicals (Reiter 2000b; Tan et al. 2007; Galano et al. 2018).

Conclusion and future remarks

Melatonin acts as the potential biostimulator molecule in plants. Different approaches have been adopted by the plants in response to ROS which serves as biological signals and triggers stress responsive genes. It is concluded that plants require adequate level of MEL for various physiological processes, such as germination, lateral root induction, photosynthetic performance, antioxidant activity and senescence, and particularly for mitigation of various environmental stresses. Therefore, MEL inflicted signaling pathways have been studied during abiotic stresses and still more to be explored the signaling pathways against broad range of stressors. Considerable success has been attained in recent years, which helped a subtle understanding of MEL inflicted physiology, site of synthesis and biosynthetic pathway, but many components in its signaling network are yet to be divulged in detail.

Moreover, there are various issues concerned with MEL which needs to be further elucidated. Like its receptor (CAND2/PMTR1) has been identified, other receptors or binding sites for MEL could be identified. Role of MEL under biotic stress is limited and it would be new approach to study the role of exogenous supplementation of MEL against nematodes, fungi and viruses. As MEL acts as powerful antioxidant but its reaction mechanism with ROS and its downstream signaling pathway needs further dissection. In addition to this, transcription factors and regulatory proteins involved in MEL mediated response could also be explored.

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