Roles of melatonin in abiotic stress resistance in plants

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Abstract

In recent years melatonin has emerged as a research highlight in plant studies. Melatonin has different functions in many aspects of plant growth and development. The most frequently mentioned functions of melatonin are related to abiotic stresses such as drought, radiation, extreme temperature, and chemical stresses. This review mainly focuses on the regulatory effects of melatonin when plants face harsh environmental conditions. Evidence indicates that environmental stress can increase the level of endogenous melatonin in plants. Overexpression of the melatonin biosynthetic genes elevates melatonin levels in transgenic plants. The transgenic plants show enhanced tolerance to abiotic stresses. Exogenously applied melatonin can also improve the ability of plants to tolerate abiotic stresses. The mechanisms by which melatonin alleviates abiotic stresses are discussed.

Key words: Abiotic stress, genetic modification, mechanism, melatonin, plants, tolerance.

Introduction

Melatonin is an indoleamine synthesized throughout the plant kingdom. It is amphiphilic, which enables it to cross cell membranes easily and enter subcellular compartments (Shida et al., 1994). Melatonin was named as such due to its ability to lighten skin in certain fish, reptiles, and amphibians (Lerner et al., 1958). In vertebrates, melatonin is mostly known as a biological modulator of mood, sleep, sexual behaviour, seasonal reproductive physiology, circadian rhythms, and immunology (Arnao and Hernández-Ruiz, 2006; Reiter et al., 2010; Cipolla-Neto et al., 2014). In plants, research efforts over the past decade have focused on determining its many roles in plant physiology.

Solid evidence implicates melatonin as a growth promoter and rooting agent (Hernández-Ruiz et al., 2004, 2005; Arnão and Hernández-Ruiz, 2007; Hernández-Ruiz and Arnão, 2008 Chen et al., 2009; Sarrou et al., 2014; Zhang et al., 2014). In addition to its roles in plant development, melatonin plays an important role in plant stress defence. Plants may frequently encounter stressful environmental conditions. Various plant species rich in melatonin have shown a higher capacity for stress tolerance. Exogenous treatment or ectopic overexpression of melatonin biosynthetic genes can also improve the resistance against a series of stressors, including extreme temperature, drought, salinity, radiation, and chemical stresses, all of which lead to the reactive oxygen species (ROS) generation. Melatonin is also involved in stress-affected developmental transitions, including flowering, fruiting, and senescence. To understand these phenomena and in order to take full advantage of this molecule in agriculture, we must determine the reason for the existence of melatonin in plants and the biochemical mechanisms in response to external stimuli.

We begin this review by emphasizing the roles of melatonin and its contribution to alleviate abiotic stress in plants. Various abiotic stresses inhibit plant growth via different mechanisms, but all cause a rise in ROS levels, and disrupt the ROS homeostasis. The mechanisms of the involvement of melatonin in modulating stress resistance are summarized herein.
Melatonin biosynthesis pathway in plants

Isotope tracer experiments have confirmed that melatonin is synthesized in plants (Murch et al., 2000). It has been suggested that plant melatonin is synthesized via similar biosynthetic pathways to those in animals (Fig. 1). Additionally, the enzymes which take part in melatonin biosynthesis have been cloned. Melatonin biosynthesis in plants occurs via four consecutive enzymatic steps (Kang et al., 2011). The first committed enzyme is tryptophan decarboxylase (TDC), which catalyses the conversion of tryptophan into tryptamine. TDC has been cloned in several plant species including rice (Kang et al., 2008), pepper (Park et al., 2009), Catharanthus roseus (De luca et al., 1989), and tobacco (Di Fiore et al., 2002). TDC serves as a bottleneck in regulating serotonin (precursor of melatonin) biosynthesis since TDC expression is very low or negligible.

The second step in melatonin biosynthesis is catalysed by the cytochrome P450 enzyme tryptamine 5-hydroxylase (T5H), which hydroxylates the C-5 position of tryptamine to form serotonin (Fujiwara et al., 2010). T5H has been isolated in rice sekiguchi lesion (sl) mutants by map-based cloning (Fujiwara et al., 2010). T5H is constitutively expressed in healthy rice (Oryza sativa) plants (Kang et al., 2007).

The final two enzymes on the melatonin biosynthetic pathway are arylalkylamine N-acetyltransferase (AANAT) and 5-acetylserotonin methyltransferase (ASMT). Currently, there are no known AANAT homologous genes in higher plants; however, AANAT has been cloned in the unicellular green alga Chlamydomonas reinhardtii (Okazaki et al., 2009). Kang et al. have cloned rice serotonin N-acetyltransferase (SNAT) genes, which encode an enzyme catalysing conversion of serotonin into 5-acetylserotonin. The last enzyme, ASMT, which was known as HIOMT (hydroxyindole-O-methyltransferase), has been purified in rice via heterologous Escherichia coli overexpression (Kang et al., 2013). ASMT is the rate-limiting enzyme of melatonin biosynthesis. Subcellular localization shows that SNAT protein is localized in chloroplasts, whereas ASMT is found in the cytoplasm (Byeon et al., 2014a).

Genetic modification changes stress tolerance in plants

The first melatonin biosynthesis gene, TDC, has been overexpressed in rice (Byeon et al., 2014b). Melatonin accumulation is seed specific in TDC3 transgenic lines; transgenic seeds exhibited melatonin concentrations 31-fold higher than those in wild-type seeds. The level of melatonin intermediates also increased in both homologous and ectopic TDC overexpression lines (Kang et al., 2008; Kanjanaphachoat et al., 2012). Transgenic rice plants that overexpress TDC exhibit delayed senescence of leaves (Kang et al., 2009), while suppression of TDC by RNA interference (RNAi) produced lower serotonin levels and promoted the senescence process. Fungal infection of peach fruit led to an increase of TDC gene expression (Park et al., 2009), indicating that melatonin is responsive to pathogen attack. Rice roots maintain high TSH activity, and TSH expression can be induced by Magnaporthe grisea infection, the causal pathogen of rice blast disease. Exogenously applied serotonin, the penultimate substrate for melatonin biosynthesis, increased resistance to rice blast infection in plants (Fujiwara et al., 2010). Exogenously applied melatonin improved resistance to Marssonina apple blotch (Diplocarpon mali) which is one of the most serious diseases leading to defoliation during the growth season (Yin et al., 2013). This also implies that melatonin plays an important role in plant innate immunity.

An AANAT gene homologue is absent in the plant genome. Okazaki et al. (2009) have isolated an AANAT from the unicellular green alga Chlamydomonas reinhardtii and introduced it into the Micro-Tom tomato genome. Its ectopic overexpression in tomato successfully resulted in higher melatonin content.
rice plants, SNAT is substituted for AANAT to encode the gene for the penultimate enzyme in plant melatonin biosynthesis (Kang et al., 2013). Transgenic rice expressing the human serotonin N-acetyltransferase gene (SNAILANAT) exhibited high levels of melatonin and elevated chlorophyll synthesis during cold stress, suggesting that melatonin plays a role in cold stress tolerance (Kang et al., 2010). Transgenic rice seedlings expressing ovine AANAT display enhanced seminal root elongation (Park and Back, 2012), seedling growth (Byeon and Back, 2014), and resistance to herbicide-induced oxidative stress (Park et al., 2013b). Microarray analysis of this transgenic line clearly demonstrated that the senescence-associated proteins are significantly down-regulated (Byeon et al., 2013). High temperatures and dark conditions increased melatonin levels by increasing the activities of the final two enzymes in melatonin synthesis (Byeon and Back, 2013). This implies a role for melatonin in defence against high temperature. Transgenic Micro-Tom tomato plants overexpressing the homologous ovine AANAT and HIOMT genes display loss of apical dominance and enhanced drought tolerance (Wang et al., 2014). Furthermore, rice ASMT mRNA can be induced upon abscisic acid (ABA) and methyl jasmonic acid treatments, suggesting the potential involvement of melatonin in response to various stresses (Park et al., 2013a). Transgenic plants of Nicotiana sylvestris expressing an AANAT gene and a HIOMT gene have enhanced production of melatonin. When exposed to ultraviolet (UV)-B radiation, melatonin exhibits a protective effect against UV-B-induced DNA damage (Zhang et al., 2012).

**Melatonin levels in plants under stress conditions**

It is widely reported that the melatonin concentration inside the cell is responsive to external conditions. A rapid change in light, temperature, and various environmental stress agents may increase melatonin levels. The level of melatonin in plants is also variable under different environmental conditions, especially under stress. Arnao and Hernández-Ruiz determined the melatonin level in barley roots (Arnao and Hernández-Ruiz, 2009b) and lupin (Arnao and Hernández-Ruiz, 2013) under natural or artificially induced adverse conditions. The former experiment treated plants with the chemical stress agents sodium chloride, zinc sulphate, or hydrogen peroxide. Drought, anaerobic, pH, and cold stress were included in the latter experiment. However, all chemical stresses produced similar changes in melatonin levels. Low temperature and drought cause pronounced changes in the endogenous level of melatonin following the chemical stress ZnSO₄ or NaCl. Restricting the oxygen supply to roots also caused a slight increase in root melatonin levels. Under pH stress, alkalinization of the medium lowered the root melatonin content, whereas acidification resulted in a slight increase. Additionally, the responses in both lupin and barley were clearly dose and time dependent. The authors concluded that nearly all stress factors can cause an up-regulation in melatonin biosynthesis in the investigated plants.

Environmental conditions decisively affect the melatonin content of tissues. Plants grown indoors, in moderate conditions, have lower melatonin content than those cultivated in the field with more variable conditions. Plants grown in sunlight possess three times more melatonin in roots and 2.5 times more in leaves compared with those grown under artificial light (Tan et al., 2007a). In pepper fruits, the melatonin content of shaded fruit is lower than that of non-shaded fruit, which indicates that solar radiation causes a rise in melatonin levels (Riga et al., 2014). The melatonin levels in Glycyrrhiza uralensis under lights of different spectral quality have also been examined. Melatonin levels in the roots can be enhanced when plants are exposed to UV-B irradiation. The authors speculate that the elevated melatonin production under stress may be an adaptive reaction of plants to tolerate adverse environmental conditions (Afreen et al., 2006). Furthermore, plants exhibiting different susceptibilities to ozone injury vary in their melatonin content. Plant species which are more resistant to ozone damage maintain higher melatonin levels than sensitive species (Dubbels et al., 1995). Alpine and Mediterranean plants exposed to high UV in their natural habitat contain much higher melatonin levels than the same species living under lower UV exposure (Simopoulos et al., 2005). Therefore, a key role for melatonin may relate to the processes of photosynthesis or photoprotection.

Melatonin and its metabolite N¹-acetyl-L¹-formyl-5-methoxykynuramine (AFMK) are present in the pollutant-tolerant plant, water hyacinth. Studies have shown that the hyacinth can tolerate contamination of wastewater generated from industrial and agricultural sources (Trivedy and Pattanshetty, 2002; Singhal and Rai, 2003; Munavalli and Saler, 2009), such as nitrogen and phosphorus (Jayaweera and Kasturiarachchi, 2004), the pesticide ethion (Xia and Ma, 2006), the heavy metal mercury (Riddle et al., 2002), and the carcinogenic arsenic (Misbahuddin and Fariduddin, 2002). Artificially supplemented melatonin can make this plant even more useful for phytoremediation, because it is a powerful antioxidant capable of elevating their tolerance to pollutants and removing additional contaminants. Since water hyacinth has high levels of melatonin and AFMK, we hypothesize that the elevated concentration of these natural antioxidants may be associated with the high tolerance of this plant to pollutants and to its utility in phytoremediation.

Rice seedlings at high temperatures and under dark conditions show enhanced melatonin synthesis due to increased serotonin N-acetyltransferase and N-acetylsertotonin methyltransferase activities (Byeon and Back, 2013). In green microalgae Ulva sp., temperature and the heavy metals cadmium, lead, and zinc induced a rise in melatonin levels (Tal et al., 2011). Exposure to cadmium induces a significant rise in melatonin levels in the algae, while lead and zinc exposure also induced an increase in melatonin levels, but to a lesser extent. This suggests that melatonin participates in adaptation to environmental stress.

A high content of melatonin may also play a protective role in seeds during sprouting. In plants, the melatonin levels vary substantially among organs, from picograms to micrograms per gram of tissue (Chen et al., 2003; Arnao and Ruiz, 2013).
Exogenously applied melatonin affects stress tolerance in plants

The cloned genes of melatonin synthesis enzymes prove that the plants have the necessary enzymatic machinery for melatonin biosynthesis. In addition to in vivo synthesis, plants can also absorb exogenously provided melatonin from the environment and accumulate it in their organs (Tan et al., 2007a). Tritium-labelled melatonin was readily taken up by Chenopodium rubrum seedlings and remained stable 37h after application (Kolar et al., 2003).

The effect of exogenously applied melatonin ranges from a significant amelioration to being ineffective or even toxic. The concentration is the cause of the difference. Melatonin may play significantly different roles in regulating plant growth and development under low and high concentrations in the same species. In the case of wild leaf mustard (Brassica juncea), a low level of melatonin (0.1mM) stimulated root growth, while a high level (100mM) inhibited growth (Chen et al., 2009). Additionally, melatonin promotes rooting at a low concentration but inhibits growth at high concentrations in cherry tissue culture (Sarropoulou et al., 2012a). Since melatonin levels in plants vary from picograms to micrograms per gram of tissue, the high concentrations used, namely 100mM, would never be achievable in any plant. Indeed, this is many orders of magnitude above physiological concentrations. Concentrations which are too high can cause toxic effects. In the analysis of gene expression modulated by melatonin, it was found that low and high levels of melatonin had a differential effect. Not all genes regulated by low melatonin were regulated by high melatonin (Weeda et al., 2014). This means that melatonin may work in different ways at low and high concentrations. High concentrations of melatonin may severely reduce ROS in cells, thereby affecting ROS-dependent signal transduction and inhibiting cell growth (Afreen et al., 2006). Plant species vary in their sensitivity to melatonin. Melatonin has an effect on lupin and barley root physiology. However, lupin roots are more sensitive to melatonin than barley, since the responses in lupin roots need 24h, while barley roots need 72h to show a similar response.

Roots directly influence the stress resistance of plants. Roots are very sensitive to changes in pH, salinity, oxygen availability, toxic elements, and water potential in their rhizosphere. A strong root system helps resist the adverse effects of stress. The function of melatonin as a rooting agent has been demonstrated many times. For example, melatonin has an effect on the regeneration of lateral and adventitious roots in etiolated hypocotyls of Lupin albus and B. juncea (Arnao and Hernández-Ruiz, 2007; Chen et al., 2009). Melatonin induced the appearance of root primordia from pericycle cells, modified the distribution pattern of adventitious or lateral roots, and affected the timing of adventitious root formation, the number and length of adventitious roots, and the number of lateral roots. Melatonin also modulates Arabidopsis root system architecture by stimulating lateral and adventitious root formation (Pelagio-Flores et al., 2012; Koyama et al., 2013). Transgenic rice seedlings expressing ovine SNAT, which is believed to be the rate-limiting enzyme in melatonin biosynthesis, exhibits enhanced seminal root growth (Park and Back, 2012). In tissue culture systems, melatonin also has a function in root regeneration. The explants used in these experiments were mostly from woody plant species (Murch et al., 2001; Sarropoulou et al., 2012b; Sarrou et al., 2014). An increase in the endogenous concentration of melatonin correlated with an increase in de novo root formation in St. John’s wort (Hypericum perforatum cv. Anthos) (Murch et al., 2001). It is well documented that the control of cellular redox homeostasis is highly related to root development (Passaia et al., 2014). The root is often implicated as the potential site for melatonin biosynthesis due to the high melatonin levels found therein. Furthermore, there is a clear concentration gradient of melatonin in lupin roots, between apical and basal zones and between the main (primary) and lateral (secondary) roots (Arnao and Hernandez–Ruiz, 2007; Hernandez–Ruiz and Arnao, 2008). Generally, the highest melatonin concentration is found in the most actively growing zones.

Under high salinity and drought conditions, plants have reduced growth and a marked decline in their net photosynthetic rates and chlorophyll content. Water stress also causes an inhibition of seed germination and root vitality. However, pre-treatment with melatonin significantly reverses this inhibition (Li et al., 2012; Zhang et al., 2013, 2014). This is demonstrated by comparing reductions in shoot height, leaf numbers, root systems, photosynthetic capacity, and biomass production. Pre-treatment with melatonin significantly alleviates this growth inhibition, thus enabling plants to maintain a robust root system and improve photosynthetic capacity.

Exposure to low temperature triggers biochemical and physiological changes in plants and causes a loss of vigour and reduced growth rate. Cell membrane systems are the primary sites of chilling injuries. Low temperature leads to changes in cell structure, cell membranes, and cell wall composition (Kratsch and Wise, 2000). The effect of chilling stress depends on the degree of severity and the time of exposure. The protective effect of exogenous melatonin during chilling stress has been described. Mung bean (Vigna radiata L.), a plant originating from the tropics, is highly vulnerable to chilling (Hung et al., 2007). Three-day-old seedlings were

Hernández-Ruiz, 2006, 2009a; Hernández-Ruiz and Arnao, 2008; Paredes et al., 2009; Posmyk and Janas, 2009), with the highest levels detected in reproductive organs, particularly in seeds. In sprouting sunflower seeds, an increase in melatonin content was detected (Cho et al., 2008). Since the germ tissue is highly vulnerable to oxidative damage, we surmise that melatonin might be present as an important component of its antioxidant defence system as a free radical scavenger. Thus, melatonin in seeds may be essential for protecting germ tissue and reproductive tissue of plants from oxidative damage (Manchester et al., 2000). Melatonin levels in plants increase considerably under various stress conditions. Such an increase is believed to be beneficial for stress resistance. Exogenous application of melatonin is often implicated as a means of ameliorating the detrimental effects of stress.
exposed to 5 °C for 2 d then returned to optimal temperature (25 °C). The seedlings from seeds primed with melatonin showed a 20% increase in root length and had less disorganized cell ultrastructure (Szafranska et al., 2013). Similar effects of melatonin have also been found in cucumber under osmotic stress (Zhang et al., 2013). Melatonin-treated mung beans also had an increased accumulation of total phenolic compounds and proline (Szafranska et al., 2012). Cucumber seeds treated with melatonin exhibit an improved germination rate during chilling stress (Posmyk et al., 2009). Plant growth data indicate that Arabidopsis plants treated with low concentrations of melatonin (10 μM and 20 μM) show enhanced primary root growth and seedling fresh weight. Pre-treatment with melatonin attenuated apoptosis induced by cold temperature in cultured carrot suspension cells (Lei et al., 2004). Cold stress-induced shrinkage and disruption of carrot cell plasma membranes were almost completely alleviated by melatonin treatment. This suggests that melatonin is helpful in coping with harsh environments by maintaining membrane integrity. Lupin plants grown in a cold environment (6 °C) show a 2.5-fold increase in melatonin levels compared with control plants grown at 24 °C. Cryopreservation is a process during which cells or tissues are cooled to sub-zero temperatures. During cryopreservation, environmental changes including osmotic injury, desiccation, and low temperature can impose a series of stresses on plants (Zhao et al., 2011). The survival rate of the cryopreserved Rhodiola crenulata callus is ~60%. When the callus was pre-treated with 0.1 μM melatonin prior to freezing in liquid nitrogen, the survival rate significantly increased. Melatonin significantly enhanced the recovery of cryopreserved shoot tips of American elm (Ulmus americana L.) (Uchendu et al., 2013). Shoot explants grown in melatonin-enriched media showed increased regrowth. Melatonin can significantly reverse the inhibitory effects of light and high temperature on germination of photosensitive and thermosensitive Phacelia tanacetifolia Benth seeds (Tiryaki and Keles, 2012).

Melatonin delays drought- and dark-induced leaf senescence in apple (Wang et al., 2012, 2013a, b). Melatonin helped maintain photosystem II (PSII) function under stress and delayed the typical reduction in chlorophyll content. Detached leaves treated with 10 mmol 1-1 melatonin showed noticeably slower dark-induced senescence. Melatonin treatment can suppress the up-regulation of senescence-associated gene 12 (SAG12), a marker gene for senescence during dark-induced senescence, implicating that melatonin can inhibit senescence. In barley leaves treated with melatonin solutions, there is a clear slowing of chlorophyll loss in leaves, which is one of the main processes that occurs during leaf senescence (Arnao and Hernández-Ruiz, 2009c). Melatonin can also alleviate paraquat-induced photobleaching. Leaves treated with paraquat in the absence of melatonin became completely photobleached, while leaves treated with 1 mM melatonin remained green, similar to leaves in the absence of paraquat (Weeda et al., 2014). Rice leaves subjected to senescence treatment exhibit enhanced endogenous melatonin levels (Byeon et al., 2012). Also, in melatonin-rich transgenic rice lines, senescence-associated proteins were significantly down-regulated (Byeon et al., 2013). To sum up, melatonin is closely related to the delay of senescence.

In animals, the antioxidant effects of melatonin on various oxidative stresses, such as cadmium and nickel, have been extensively investigated (Wang et al., 2011). Previous studies showed that melatonin treatment might be effective in modulating some neurotoxic effects induced by metals. In the field of medicine, melatonin has been recognized in the prevention of and clinical recovery from heavy metal intoxication (Flora et al., 2013). In green algae, exogenous melatonin can relieve cadmium-induced stress (Tal et al., 2011). High levels of copper contamination are toxic to pea plants; however, melatonin added to the soil significantly enhanced their tolerance to copper contamination and increased their survival rate (Tan et al., 2007b). Melatonin treatment also improved seed germination and seedling growth of cabbage in the presence of CuSO4 (Posmyk et al., 2008).

**Mechanisms of melatonin-mediated stress tolerance and detoxification**

As plants are sessile, they can only adjust their own physical conditions to resist adverse environments. So when faced with a harsh environment, a rapid and tremendous change must occur inside the plant cells in order to survive. A cell is separated from its surrounding environment by a physical barrier, namely the plasma membrane. This membrane is selectively permeable to small molecules and ions. Melatonin is an amphipathic molecule which can easily diffuse through cell membranes into the cytoplasm and enter subcellular compartments.

Drought limits plant growth due to photosynthetic decline. Salinity interferes with plant growth as it leads to physiological drought and ion toxicity. Extreme temperatures impair plant growth or even cause death by affecting membrane fluidity and enzyme activity. Radiation stress can damage macromolecules, such as DNA and proteins, generate ROS, and impair cellular processes. Heavy metals such as copper and zinc are essential for normal plant growth, but excess heavy metals are toxic. The metal ions bind to sulphhydril groups in enzymes and other proteins, thus inhibiting their activity or disrupting their structure (Hall, 2002). In addition, heavy metals cause oxidative deterioration of biomolecules by initiating free radical-mediated chain reactions resulting in lipid peroxidation, protein oxidation, and oxidation of nucleic acids. All the abiotic stresses cause an enhanced generation of ROS (Prasad et al., 1994; Foyer et al., 1997). While ROS are clearly required for growth and function as secondary messengers in signal transduction (Baxter et al., 2014), high concentrations of ROS can trigger genetically programmed cell suicide events (Foyer and Noctor, 2005). Furthermore, excess levels of ROS can have detrimental effects as a result of their ability to cause lipid peroxidation in cellular membranes, DNA damage, protein denaturation, carbohydrate oxidation, pigment breakdown, and impaired enzyme activity (Bose et al., 2014). Chloroplasts and mitochondria are a major source of ROS production in plant cells since photosynthetic and
respiratory processes generate ROS (Mittler, 2002). There is a delicate balance between ROS generation and scavenging. Major ROS-scavenging enzymes of plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione peroxidase (GPX). In addition to the enzymes that detoxify ROS, antioxidants such as ascorbate, glutathione (GSH), and tocopherol play an important role in the regulation of cellular ROS homeostasis.

Studies on how melatonin interacts with stress signalling mechanisms have identified a complex relationship with ROS (Fig. 2). Evidence confirms that melatonin is a broad-spectrum direct antioxidant which can scavenge ROS with high efficiency. A detailed knowledge of melatonin chemistry and molecular interactions with ROS and with strong oxidants has been documented (Tan et al., 2000; Allegra et al., 2003). In addition, melatonin treatments modulate the antioxidant enzymes by both up-regulating the transcript level and increasing the activity levels. Melatonin also has the capacity to enhance cellular antioxidant defence mechanisms by regenerating endogenous antioxidants, such as glutathione and vitamins C and E. They also influence cellular signalling and trigger redox-sensitive regulatory pathways. Under salinity and drought stress, the exogenous application of melatonin significantly suppressed the production of H$_2$O$_2$ and hydroxyl radicals (Li et al., 2012; Zhang et al., 2013). The transcript levels of the genes encoding SOD, APX, CAT, and peroxidase are also up-regulated by melatonin (Rodriguez et al., 2004). Melatonin treatment leads to the maintenance of higher contents of ascorbic acid (AsA) and GSH (Wang et al., 2012). In higher plants, the AsA–GSH cycle is an important antioxidant system protecting against oxidative stress. The theory that the addition of melatonin lessens oxidative damage caused by stress by directly scavenging ROS, and enhancing antioxidants and the activities of antioxidative enzymes has been confirmed in many experiments. Cellular responses to stress are initiated primarily by the interaction of the extracellular material with a plasma membrane protein. This means that stresses are first perceived by receptors present on the membrane of the plant cells. The signal is then transduced downstream, resulting in the generation of secondary messengers including calcium and ROS. Melatonin alters the expression of genes involved in the signal transduction steps along the way. RNA-seq analysis revealed that six stress receptors and 14 genes involved in calcium-dependent signalling are up-regulated in response to melatonin (Weeda et al., 2014). Melatonin interactions with Ca$^{2+}$–calmodulin have been shown to regulate many calcium-dependent cellular functions in animal cells (Posmyk and Janas, 2009).
interactions may also occur in plants. Many transcription factors have been identified as being up-regulated by melatonin treatment, and the majority of these are stress-related transcription factors including WRKY, NAC domain-containing proteins, and zinc finger-related transcription factors. This is consistent with the study on cucumber under salinity stress (Zhang et al., 2014). Many genes related to redox homeostasis were identified among the transcripts that were altered by melatonin. Most genes identified were related to biotic and/or abiotic stress responses. Gene expression of oxidative stress-alleviating enzymes including peroxidases, glutathione peroxidase, haloperoxidase, and thioredoxin reductase was induced in melatonin-rich rice and melatonin-treated plants (Byeon et al., 2014). This implicates that melatonin may have a role in protecting important molecules such as nucleic acids, proteins, and lipids against oxidative damage.

Melatonin alters the expression of a large number of plant stress defence-related genes. In addition to the stress receptors, stress-associated calcium signals, and plant stress defence-related transcription factor genes, melatonin also regulates several specific genes related to specific stresses. For example, melatonin may preserve chlorophyll content. Melatonin significantly down-regulated the expression of chlorophyllase (CLH1), which is a light-regulated enzyme involved in chlorophyll degradation (Weeda et al., 2014). This is consistent with the study conducted on melatonin affecting senescence in apple leaves. In the experiment, exogenous melatonin inhibited transcript levels of pheide a oxygenase (PAO), which is another key enzyme involved in chlorophyll degradation (Wang et al., 2013b). This may provide another mechanism by which melatonin preserves chlorophyll content during senescence, in addition to attenuating ROS. These findings can explain the means by which melatonin preserves the chlorophyll content in leaves, delays senescence, and enhances photosynthetic rates. The detrimental effects of high salt result from both a water deficit caused by osmotic stress and the interference of excess sodium ions with key biochemical processes (Zhang and Blumwald, 2001). NHX1 and AKT1 are ion channel genes which possibly contribute to the maintenance of ion homeostasis. Expression of NHX1 and AKT1 is greatly up-regulated in leaves, thus improving salinity resistance in plants exposed to exogenous melatonin (Li et al., 2012). Furthermore, cell death-associated genes identified were mostly down-regulated by melatonin treatment (Weeda et al., 2014). This result provides another explanation for the melatonin-related antiapoptotic process. It is well established that the antiapoptotic action of melatonin in animal cells is mainly based on its antioxidant property (Lei et al., 2004), whereas in the inhibition of cold-induced apoptosis by melatonin in plants, melatonin may exert its antiapoptotic action through polyamines rather than ROS attenuation. It is documented that high polyamine levels in plant cells are correlated with tolerance of a number of biotic and abiotic stresses (Bouchereau et al., 1999), especially chilling injury (Kim et al., 2002). Polyamines are also implicated, directly or indirectly, in pathways regulating programmed cell death (Moschou and Roubelakis-Angelakis, 2014). Melatonin up-regulates the expression of C-repeat-binding factors (CBFs)/drought response element-binding factors (DREBs), a cold stress-responsive gene, COR15a, a gene encoding the expression of a transcription factor involved in freezing and drought stress tolerance, CAMTA1, and transcription activators of ROS-related antioxidant genes, ZAT10 and ZAT12, following cold stress (Bajwa et al., 2014). The up-regulation of cold signalling genes by melatonin may stimulate the biosynthesis of compounds which protect the membrane structure against peroxidation during chilling. Melatonin has an important role in modulating the expression of a wide range of genes, reflecting its pleiotropic physiological roles in plants (Byeon et al., 2013; Weeda et al., 2014; Zhang et al., 2014).

Metal exposure is associated with several toxic effects. Excess metals can interact with biological macromolecules causing oxidative damage. An adsorptive voltammetric study showed that melatonin and its precursors can bind to several toxic metals: aluminium with melatonin, tryptophan, and serotonin; cadmium with melatonin and tryptophan; copper with melatonin and serotonin; Fe$^{3+}$ with melatonin and serotonin; Fe$^{2+}$ with tryptophan only; lead with melatonin, tryptophan, and serotonin; and zinc with melatonin and tryptophan (Limson et al., 1998). Electrochemical studies show that melatonin can bind to both Cu$^{2+}$ and Cu$^{1+}$ which cause free radical damage (Parmar et al., 2002). It is documented that melatonin affects biological systems not only through direct quenching of free radicals but also via chelation of toxic metals (Flora et al., 2013; Romero et al., 2014).

**Conclusions and perspectives**

Hundreds of articles relating to melatonin in plants have been published in the last 20 years. Research shows that melatonin plays critical roles in plant defence systems. Almost all the abiotic stresses which cause oxidative stress can be alleviated by melatonin treatment if the concentration of melatonin is appropriate. Researchers have demonstrated possible mechanisms of this protective effect.

However, there is still much that remains unknown and needs to be elucidated in the future. The precise location of melatonin biosynthesis in plants requires further investigation. Since melatonin is extremely labile, it is difficult to monitor the transport of melatonin in plant organelles and organs (van Tassel and O’Neill, 2001). Tan et al. (2013) mentioned that in most cells, mitochondria and chloroplasts may be the primary sites of melatonin synthesis. The root is perhaps the organ mentioned most frequently as the potential site of melatonin biosynthesis; however, evidence supporting this theory is limited and the unequivocal site of melatonin biosynthesis remains unknown. Melatonin has pleiotropic physiological roles in plants. Unfortunately, no specific phenotype associated with melatonin has been found in higher plants. Currently, no melatonin receptor(s) or binding site(s) have been identified in plants. In animals, various subtypes of receptors have been characterized, and their genes have been sequenced (Reppert, 1997; von Gall et al., 2002). The possible presence of specific melatonin receptors in plant cells cannot be ruled out and is an important aspect in the study...
of signal transduction of melatonin in plants. Analysis of the differential expression data generated by microarray and RNA-seq can offer new information for identifying putative melatonin receptor(s) in plants. Alternatively, since melatonin has a similar structure to auxin, it could possibly interact with the auxin receptor. In addition to melatonin, several isomers were identified in plants and plant products such as wine and bread (Vicente Gomez et al., 2012; Vitalini et al., 2013; Yilmaz et al., 2014). The study of melatonin isomers is an emerging area of research as they probably share many of the biological functions of melatonin (Tan et al., 2012). It is another promising area of research to explore. All of this warrants further investigation in the near future.

The goal of this review is to update the reader on the roles of melatonin in plant abiotic stress resistance and to encourage plant scientists to investigate further the mechanism of melatonin-related resistance. We have summarized the biosynthesis, response to harsh conditions, roles in regulating stress resistance, and possible mechanisms. First of all, melatonin can be synthesized and taken up by plants. Genetic modification can enhance melatonin synthesis in transgenic plants and improve resistance to adverse conditions. Plants accumulate high levels of melatonin when faced with harsh environments. Exogenously applied melatonin helps improve tolerance to stresses. We also summarized the mechanisms of melatonin-induced abiotic stress resistance. In order to better understand the roles of this molecule and make full use of such roles, many more investigations must be conducted.

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