Melatonin Application in Forage Grass Abiotic Stresses Tolerance

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Climate change related abiotic stress has been potentially impacting the quantity and quality of forage grass. Melatonin, a multifunctional molecule that has been found to be present in all plants examined to date, plays a crucial role in improving forage grass tolerance to both biotic and abiotic stresses. However, research on melatonin's role in forage grass is still developing.

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1. Introduction

Melatonin (N-acetyl-5-methoxytryptamine), a versatile small molecule, is widely present in humans, animals, and plants [1]. Studies have shown that melatonin can influence the entire life cycle of plants, from seed germination to growth, maturation, aging, and recovery from stress [2]. The biosynthesis of melatonin begins with the production of tryptophan through the oxalate pathway in chloroplasts, which is then converted into serotonin via the catalysis from biosynthetic enzymes, and finally is synthesized into melatonin [3]. The biosynthetic enzymes of melatonin mainly include tryptophan decarboxylase (TDC), tryptophan hydroxylase, tryptamine 5-hydroxylase (T5H), serotonin N-acetyltransferase (SNAT), N-Acetyl serotonin methyltransferase (ASMT), arylalkylamine N-acetyltransferase, and hydroxyindole-O-methyltransferase (HIOMT) [4]. The TDC enzyme plays an important role in melatonin biosynthesis by catalyzing the conversion of tryptophan into tryptamine. The T5H enzyme then initiates the conversion of tryptamine into serotonin. At the same time, T5H can convert tryptophan into 5-hydroxytryptophan, which is then converted into serotonin under the catalysis of aromatic-L-amino acid decarboxylase (AADC). SNAT and HIOMT enzymes convert serotonin into N-acetyl serotonin or N-acetyl tryptamine in chloroplasts or mitochondria. The final step in melatonin biosynthesis is the synthesis of melatonin through the O-methylation of N-acetyl serotonin by the action of ASMT [5].

Moreover, melatonin, as an antioxidant, can alleviate damage caused by abiotic stress, regulate photosynthesis, ion balance, and stress signal transduction, and directly enhance the activity of various antioxidant enzymes and enzymes involved in photosynthesis in plant cells, thus protecting plants from cell damage caused by oxidative stress [6]. Therefore, it is predicted that melatonin will play an important role in helping plants adapt to adverse environmental conditions, particularly in the current context of climate change.

2. Melatonin Regulates the Growth and Development of Forage Grass

The characteristics of forage grass that is growing well include lush overall growth, large and dense leaves, thick and strong stems, and a stable and lush underground root system [7]. The quality of the growth conditions directly impacts the quality of forage grass, which, in turn, affects the availability of high-quality feed for the livestock industry [8]. It also indirectly affects humans' healthy intake of meat and milk protein through the adequate supply of forage grass. However, under the influence of drought, alkali salt, and heavy metal stresses, the growth time for forage grass is short, the leaves and stems are short and thin, the overall growth shape is small, and the resistance is weak [9].

2.1. Seed Germination and Seedling Growth

Seed germination and seedling growth (including root and stem growth) are two important stages of forage grass growth and development [10]. The regulating effect of melatonin is manifested in the germination rate (GR), germination potential (GP), germination index (GI), plant height (PH) stem thickness (ST), and root length (RL) in the stage of seed germination and seedlings. They all show good results compared to untreated ones after melatonin treatment. For example, after pretreatment with 300 μmol/L melatonin, the GR and GP of hybridization pennisetum seeds under 150 mM NaCl salt stress increased by 20% and 10%, respectively [11]. Under the condition of PEG-23% drought treatment and 75 μmol/L exogenous melatonin treatment for 24 h, the GR, GP, GI, and vitality index of 'WuSu No.1' awnless brome seeds increased by 34.24%, 68.71%, 69.61%, and 78.14%, respectively [12]. Under the condition of 0.15 mM NaCl salt stress treatment, the GR and GP of alfalfa seed of the 'Chifeng' variety increased by 53.8% and 53.5%, respectively, and the GI increased by 55.8% after the supplementation of 300 μmol/L melatonin pretreatment [13]. Under drought stress, the growth of 'Sanditi' alfalfa seedlings showed improvement in PH, ST, and RL after 100 μmol/L melatonin treatment [14]. Similarly, oat was improved by melatonin treatment under salt stress; their PH, ST, RL, fresh weight (FW), dry weight (DW), and relative water content significantly improved [15].

2.2. The Mechanism of Melatonin Regulation in the Growth and Development Stage

Melatonin exerts noticeable effects on the germination and growth stages of forage grass plant seeds. Its primary mechanism of action involves the regulation of stress-related gene expression, thereby enhancing germination and promoting resistance to stress, as well as facilitating overall growth and development. However, further research is still needed on which pathways are affected by the expression of these partial genes. Exogenous melatonin treatment promoted the up-regulation of alfalfa stress-related genes Dehydration-Responsive Element (DRE), Auxin Response Factor (ARF), Homeodomain-Leucine Zipper (HD-ZF), Myeloblastosis (MYB), and Refinder of the Embryo Morphogenesis (REM) [16]. In alfalfa grown under cadmium stress conditions, the application of exogenous melatonin stimulated the upregulation of ABC transporter and PCR2 transcripts and reduced the accumulation of cadmium content [17].

3. Melatonin Regulates the Photosynthesis of Forage Grass

3.1. The Effects of Melatonin Application on Photosynthesis

Photosynthesis is a very important pathway in the growth and development of any plant. Plants convert light energy into chemical energy [18]. Whether this process works normally is one of the criteria for normal plant growth [19]. Photosynthetic pigments play an important role in the photosynthesis process. Under abiotic conditions, the photosynthesis of forage grass will be affected. The reason is mainly because abiotic stress destroys the homeostasis of normally growing forage grass. The most obvious manifestation is the release and over accumulation of reactive oxygen species in the body. When reactive oxygen species (ROS) are excessively accumulated, the biosynthesis of chlorophyll will be disrupted, and the chlorophyll content and the light absorption and photochemical efficiency will be reduced, further leading to a series of photosynthetic imbalances and deterioration [20]. Therefore, being able to effectively maintain the chlorophyll content and the stability of the photosynthetic electron transport chain to remove reactive oxygen species is the main starting point for regulating abiotic stress damage and ensuring the normal operation of photosynthesis [21]. After melatonin treatment of alfalfa under salt stress, it was found that melatonin has obvious effects on increasing the chlorophyll content and maintaining photosynthesis.

3.2. The Mechanism of Melatonin Regulation on Photosynthesis

The mechanisms whereby melatonin regulates photosynthesis in forage grass include (1) melatonin regulating the expression of genes related to chlorophyll synthesis and degradation, and (2) melatonin regulating the operation of the photosynthetic transfer chain and related components. For example, under drought stress, the expression of the chlorophyll degradation genes *Chlorophyllase* (*Chlase*)*, Pheophytinase* (*PPH*)*,* and *Chlorophyll peroxidase* (*Chl-PRX*) in *Agrostis stolonifera* after melatonin treatment was found to be significantly reduced, and at the same time, the activity of chlorophyll degradation gene enzymes was significantly reduced [22]. Under high-temperature stress, the expression of the senescence-related genes *LpSAG12.1* and *Lph36* in perennial ryegrass treated with melatonin was significantly inhibited, resulting in a decrease in chlorophyll content [23]. Therefore, melatonin affects chlorophyll content directly by regulating the expression of chlorophyll synthesis and degradation genes and indirectly affects chlorophyll content by regulating the expression of aging-related genes.

Photosynthetic electron transport is an important process of photosynthesis and is easily damaged. The photosystem II (PSII) is an important thylakoid membrane protein in the photosynthetic electron transport chain. It is a large multi-subunit pigment-protein complex. It is also an important part of the photosynthetic electron transfer process. When drought causes the electron transfer of PSII to be inhibited, ROS accumulate excessively in the thylakoid membrane, reducing the electron transfer efficiency of PSII. However, when researchers used melatonin to treat wheat grown under drought conditions, they found that the degradation of proteins on the thylakoid membrane caused by drought was inhibited, and *D1, Lhcb5, Lhcb6, PsbQ,* and *PsbS* protein levels increased; it also promoted the dephosphorylation of *LCHII, CP43,* and *D1* to protect the photophosphorylation system [24]. Carbon assimilation is driven by CO_2 and limited by low levels or the activation of photosystem I (PS I). The study found that melatonin can enhance α-amylase activity under cold stress. β-amylase activity promotes carbon assimilation in the CHL b-deficient mutant ANK32B of wheat, enhances ATPase activity and sucrose synthesis, and maintains a high-chlorophyll content.

4. Melatonin Regulates the Antioxidant Mechanism of Forage Grass

The Effects of Melatonin on Antioxidant Mechanism

Abiotic stress causes an increase in the content of reactive oxygen in plants, which leads to the disruption of cell integrity and metabolic pathways. Previous studies have demonstrated that melatonin can improve the ROS clearance efficiency under abiotic stress conditions [25]. Melatonin may help to reduce ROS in two ways: (1) melatonin regulates the activity of different enzymatic and non-enzymatic antioxidants; and (2) melatonin itself can directly scavenge ROS [26]. The antioxidant nature of melatonin is due to the redox active properties of the molecule, as well as metabolites originating during its metabolism [27], including cyclic 3-hydrox-ymelatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, N1-acetyl-5-methoxykynuramine, 6-hydroxymelatonin, and 2-hydroxymelatonin [28]. More interestingly, one molecule of melatonin can simultaneously clear multiple ROS, while the ratio of other antioxidants is only 1:1 or lower. In the ROS, the hydroxyl radical (·OH) is one of the most toxic species. But melatonin’s role as a ·OH radical scavenger is quite amazing [29]. The other is the antioxidant properties of the two side chains of the melatonin molecule: the carbonyl part of the C3 amide functional group and the nitrogen in the carbonyl group play a key role in quenching. Thus, the reaction of melatonin with ROS involves electron supply to form melatonin cationic radicals, nitrogen atom supply to hydrogen, nitrosation, addition reaction, substitution, and ROS reduction [27]. Physiological and mechanistic explanations of melatonin-mediated scavenging of ·OH radicals include the addition of ·OH radicals to the C3 site of the indole ring, tautomerism of enolimide to ketoamine, cyclization between the C2 and N sites of the indole ring, and the reaction of the side chain of the ring with a second ·OH radical to form cyclic 3-hydroxymelatonin and water [29]. Those findings showed that melatonin has the capacity to save and help forage grass to adapt to the environment.

When forage grass is attacked by stresses that cause active oxygen accumulation, research shows that it is able to clear the active oxygen by enhancing its antioxidant and non-enzymatic activities [25]. Melatonin can remove reactive oxygen species. For example, Under drought stress, Studies on alfalfa [30], tall fescue [31], oat [32], and perennial ryegrass [33] all show that exogenous melatonin treatment could enhance the activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), and reduce malondialdehyde (MDA), H_2O_2, O_{21}, and other active oxygen species. Further studies found that melatonin induced an increase in SOD, APX, CAT, and POD coding gene transcriptional levels and their activities [34]. Similar results were found for salt stress or heavy metal toxicity [35].

5. Melatonin Regulates Ion Homeostasis of Forage Grass

5.1. The Effects of Melatonin on Ion Homeostasis

Absorption of ions by plants and maintenance of ion homeostasis in the body are two of the important factors for survival under stress. Under stress, melatonin can help to regulate the ion absorption and ion transporters of forage grass, including Na^+, K^+, and $Cl^−$ transporters, as well as phosphate and sulfur ions to help forage grass establish ion homeostasis [36]. For example, under salt stress, exogenous melatonin treatment promotes plasma absorption of nitrogen, phosphorus, potassium, calcium, and magnesium in alfalfa [37]. Under salt stress, melatonin treatment reduces Na^+ accumulation and promotes K^+ absorption.

5.2. The Mechanism of Melatonin Regulation on Ion Homeostasis

Ions are transported into the plant by H+ pumps, ion channels, and ion channel proteins. So, what role does melatonin play in the activity of ion channels and ion channel-related proteins? Studies have shown that melatonin can improve the absorption and content of sulfur by upregulating genes involved in sulfur transport and metabolism, including sulfur transporters such as ATP thioacylase, 5′-adenylate sulfate reductase, sulfite reductase and O-acetylserine-mercaptan hydrolase [38]. Melatonin also can mediate the regulation of H^+-ATPase activity, which directly affects nutrient and ion absorption and transport, including up-regulation in the gene expression of enzymes, such as *HA2*, *HA3*, *HA4*, *HA8*, and *HA9* [39]. Under the stimulation of ROS, melatonin regulates the level of Ca^{2+} signaling by activating NADPH oxidase activity, thereby promoting Ca^{2+} inflow and maintaining ion homeostasis in vivo. For example, melatonin regulates gene transcription of Ca^{2+} signaling in alfalfa (cyclic nucleotide-gated channel CGs; CAM/calmodulin-like proteins, CAM/CMLs, and calc-dependent protein kinases (CDPKs)) [40].

6. Melatonin Interacts with Other Plant Hormones

6.1. The Effects of Melatonin on Other Plant Hormones

Hormones are produced in the plant, and act on a certain tissue at a certain time, playing a role in growth regulation; so, each hormone exists in the plant body at the same time and there is a certain connection and interaction. Melatonin is one of the endogenous plant hormones of crops, which has been proven to be inseparable from other plant hormones. Melatonin is involved in the synthesis and degradation of other plant endogenous hormones and plays a regulatory role. A large number of studies in other crop fields have found that there are interaction relationships between melatonin and other hormones, such as indole-3-acetic acid (IAA), gibberellin (GA), abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), and cytokinin (CK). (1) Melatonin treatment enhances the accumulation of distal root auxin signals and regulates auxin biosynthesis to control root structure and promote lateral root development [41]. (2) Melatonin concentration affects the IAA biosynthesis; high concentrations inhibit the promotion effect of low concentrations [42]. (3) Stress can promote an increase in ABA levels, and melatonin can mediate ABA metabolism and inhibit ABA synthesis [43]. (4) GA and ABA are antagonists. Melatonin can regulate ABA catabolic genes, GA biosynthesis genes, and ABA biosynthesis genes [44]. The interaction between melatonin and JA or SA has also been well documented for the upstream signaling pathways of defense genes involved in JA and SA biosynthesis [45]. (5) Melatonin regulates the expression of JA biosynthesis genes (*AOC and LoxD*) and genes in JA signaling pathways. (6) Melatonin triggers plant immunity, thereby promoting melatonin-mediated SA synthesis [46].

6.2. The Mechanism of Melatonin Regulation on Other Plant Hormones

There are two relationships between melatonin and other hormones in the pasture field: (1) the relationship between the change in melatonin levels and the content of other hormones; and (2) the relationship between the change in melatonin levels and the biosynthetic expression of other hormones. Exogenous melatonin treatment had a significant effect on IAA content in alfalfa leaves. At 150 mM NaCl, 10 µM melatonin treatment induced a salinity increase, increased auxin content, and significantly increased endogenous melatonin accumulation in alfalfa leaves. High-salt concentration increased IAA content in roots, and exogenous melatonin further increased IAA levels. Under 200 mM NaCl, 15 µM melatonin increased the content of IAA in roots. Salt stress causes a significant increase in IAA/MT ratios in roots and leaves, whereas melatonin treatment reduces IAA/MT ratios in alfalfa leaves [47]. Under drought stress, exogenous melatonin treatment also promoted an increase in IAA and GA3 contents in oat leaves and inhibited the increase of ABA content [15]. Under normal conditions, exogenous melatonin treatment may inhibit seed germination and increase abscisic acid content by up-regulating the expression of abscisic acid related synthesis genes. Under normal growth and development conditions, melatonin treatment synergistically inhibited the germination of Arabidopsis seeds with ABA and up-regulated the expression of NCED3 and ABA2 genes related to ABA synthesis. Melatonin and GA3 produce antagonistic effects and jointly regulate the expression of *ABO5* and *GASA6* genes related to seed germination [48]. In wild ryegrass (*Elymus nutans*), the expression of cold-response genes (*CBF9*, *CBF14*, and *COR14a*) in the ABA independent pathway was upregulated after exogenous melatonin treatment, while additional treatment with the ABA biosynthesis inhibitor fluridone significantly inhibited melatonin induced ABA accumulation, suggesting that ABA-dependent pathways also contribute to melatonin induced cold tolerance [49]. Under high-temperature conditions, exogenous melatonin treatment regulated the up-regulation of cytokinin (CK) biosynthesis genes *LpIPT2* and *LpOG1* and their signaling response transcription factors (B-type ARRs), the transcription of key transcription factors in the ABA signaling pathway encoded by *LpABI3* and *LpABI5*, and the ABA biosynthesis genes *LpZEP* and *LpNCE*. The expression of D1 decreased, while the endogenous melatonin content increased [24].

7. Conclusions

dogenous melatonin content of various varieties and materials and its correlation with life and stress resistance must be an important direction for future research. (2) Under abiotic stress, for the growth and development of forage grass or for the physiological and biochemical response, melatonin treatment can significantly alleviate stress damage and improve stress resistance, and its effect is significantly or even stronger than that in other plants. (3) The regulating mechanisms of melatonin treatment include the up-regulation and expression of stress-related genes to alleviate stress and regulate the expression of photosynthesis, antioxidants, ion homeostasis, and other hormone-related synthesis genes to adapt to abiotic stress. (4) It is certain that melatonin will become an effective weapon that can be applied for the adaptation from abiotic stresses one day; maybe it is a long way away, but it still can be achieved.

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