

Review

# Melatonin-Induced Water Stress Tolerance in Plants: Recent Advances

Mohamed Moustafa-Farag<sup>1,2\*†</sup>, Ahmed Mahmoud<sup>2,3†</sup>, Marino B. Arnao<sup>4</sup>, Mohamed Sheteiwy<sup>5</sup>, Mohamed Dafea<sup>2</sup>, Mahmoud M. Soltan<sup>6,7</sup>, Amr Elkelish<sup>8</sup>, Mirza Hasanuzzaman<sup>9</sup>, Shaoying Ai<sup>1\*</sup>

<sup>1</sup> Institute of Agricultural Resources and Environment, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, Guangdong, China; m\_m\_kamel2005@gdaas.cn (M.M.-F); aishaoying@gdaas.cn (S.A)

<sup>2</sup> Horticulture Research Institute, Agriculture Research Center, 9 Gmaa St, Giza, 12619, Egypt; mohameddafa@yahoo.com (M.D)

<sup>3</sup> Laboratory of Germplasm Innovation and Molecular Breeding, Institute of Vegetable Science, Zhejiang University, Hangzhou 310058, China.; 11716103@zju.edu.cn (A.M.)

<sup>4</sup> Department of Plant Physiology, Faculty of Biology, University of Murcia, Murcia, Spain; marino@um.es (M.A)

<sup>5</sup> Department of Agronomy, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt; salahco\_2010@mans.edu.eg

<sup>6</sup> Horticulture and Crop Science Department, The Ohio State University, Ohio Agricultural Research and Development Center, Ohio, USA

<sup>7</sup> Vegetable Production under Modified Environment Department, Horticulture Research Institute, Agriculture Research Center, Egypt; dsoltan2012@gmail.com

<sup>8</sup> Botany Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt; amr.elkelish@science.suez.edu.eg

<sup>9</sup> Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh; mhzsauag@yahoo.com

† These authors contributed equally to this work.

\* Correspondence: m\_m\_kamel2005@gdaas.cn (M.M.-F); aishaoying@gdaas.cn (S.A)

**Abstract:** Water stress (drought and waterlogging) is severe abiotic stress to plant growth and development. Melatonin, bioactive plant hormone, has been widely tested in drought situations in diverse plant species, while a few studies on the role of melatonin in waterlogging stress conditions have been published. In the current review, we analyze the bio-stimulatory functions of melatonin on plants under both drought and waterlogging stresses. Melatonin controls the levels of reactive oxygen and nitrogen species and positively changes the molecular defence to improve plant tolerance against drought and waterlogging stress. Moreover, the crosstalk of melatonin and other phytohormones is a key element on plant survival under drought stress, while this relationship needs further investigation under waterlogging stress. In this review, we draw the complete story of water stress on both sides: drought and waterlogging through discussing the previous critical studies under both conditions. Moreover, we suggest several research directions, especially for waterlogging, which remains a big vague piece of melatonin and water stress puzzle.

**Keywords:** Abiotic stress; Melatonin; Water stress; Drought; Waterlogging; Antioxidants; Stress signalling, phytohormones

## 1. Introduction

With the notable increase in global warming, rainfall disparity and poor drainage, water stress (drought and waterlogging) is becoming one of the fiercest environmental challenges in the agriculture sector, mainly in the arid and semi-arid regions for drought stress [1,2]. As is also the case in the areas of heavy rainfall, inadequate draining, and flooding for waterlogging stress [3], which could seriously threaten food security by 2050, as the world's population is predicted to reach ten billion [4]. The key serious impact of water stress is the massive generation of reactive oxygen species

(ROS) and malondialdehyde (MDA) over the cell tolerance ability [5,6]. Thus, directly and/or indirectly damages the cell membrane, nucleic acids, and proteins (Figure 1). This adversely affects gas exchange, photosynthesis, and decrease plant growth, as well as yield quality and quantity [7,8]. Practically, the global scale analysis of published studies over the last four decades on maize and wheat revealed that up to 20-40% yield reductions were due to water scarcity [9]. Meanwhile, the destructive effect of waterlogging on crops yield is estimated by a 40–80% loss in the area more than 1.7 billion hectares [10-12].

Indeed, plants have developed several strategies to cope with water stress. In drought, plants avoid the drastic effects of stress through the induction of stomatal closure, accumulation of compatible solutes, and biosynthesis of wax [4]. Moreover, plants increase their tolerance by the activation of antioxidative abilities and the induction of some molecular chaperones to alleviate oxidative damage [13,14]. In waterlogging, plants avert stress by altering plant metabolism toward anaerobic, glycolytic, and fermentative metabolism. In response to anoxia, the plant activates the antioxidant machinery, expression of heat shock transcript, and accumulation of osmolytes [15]. Previous publications stated that the various plant responses to water stress are mediated by essential regulators such as phytohormones [16]. Among them, melatonin is a unique antioxidant and plant master regulator that protects plants from oxidative stress and regulates various plant responses to environmental disorders, especially water stress [17-19]. Although accumulating reviews about the beneficial effects of melatonin have been published over the last decade, it still needs more discussion to update and discover melatonin functions, especially under biotic and abiotic stresses [20-22]. Herein, we will discuss the most recent and relevant studies of the protective roles of melatonin-induced water stress tolerance, including anatomical changes, physiological and molecular mechanisms as well as its central role in the hormonal system. Grasping of the current situation and considering the future perspectives of melatonin roles in water stress tolerance will also be deeply discussed.

## 2. Melatonin-induced drought stress tolerance

### 2.1. An overview

Among plant growth substances, melatonin (N-acetyl-5-methoxytryptamine) is an amazing and powerful naturally occurring antioxidant that effectively cope with the drastic effects of water deficit in plants [18,23]. Thus, melatonin is strongly recommended to mitigate drought stress in several plant species including model plants [24,25], field crops [26,27], fruit crops [28,29], vegetable crops [30,31] as well as ornamental and medicinal plants [32,33] (Table 1). Melatonin treatment ranged from a very low concentration (50 nM) in grape [34] to high dosage (1 mM) in maize [35] (Table 1). Moreover, melatonin can be applied as different forms to alleviate drought stress including seed priming [36], seed coating [37], direct soil treatment [38], foliar application [33], in nutrient solutions and hydroponic systems [39], supplemented with irrigation [28] and roots pretreatment [40] (Table 1)

**Table 1.** Roles of melatonin in drought stress tolerance.

Common name	Scientific name	Drought treatment	Melatonin Treatment		Effects	Reference
			Concentration *	Application form		
<b>Model plants</b>						
Arabidopsis	<i>Arabidopsis thaliana</i>	Water withholding (21 d)	50 µM	Supplemented with nutrient solution	Stress-responsive genes ▲, soluble sugars ▲	[41]
<b>Field crops</b>						

Common name	Scientific name	Drought treatment	Melatonin Treatment		Effects	Reference
			Concentration *	Application form		
Rice	<i>Oryza sativa</i>	water drainage from vessels (5 d)	100 $\mu$ M	Pretreatment in growing distilled water	Plant growth $\blacktriangle$ , osmoprotectants proline $\blacktriangle$ , Stress-responsive genes $\blacktriangle$ , mitochondrial structure $\blacktriangle$ , ROS $\blacktriangledown$ , electroleakage $\blacktriangledown$	[42]
Maize	<i>Zea mays</i>	Water withholding (8 d), melatonin application during recovery, followed by withholding (8 d).	1 mM	Supplemented with irrigation	Photoprotection (PSII efficiency) $\blacktriangle$	[35]
Maize	<i>Zea mays</i>	30-60 % SWC (8 d)	100 $\mu$ M	Foliar application	Recovering after rehydration $\blacktriangle$ , photosynthesis $\blacktriangle$ , stomatal conductance $\blacktriangle$ , transpiration rates $\blacktriangle$ , cell turgor and water holding capacity $\blacktriangle$ , enzymatic and non-enzymatic antioxidants $\blacktriangle$ , osmotic potential $\blacktriangledown$ , ROS $\blacktriangledown$	[43]
Maize	<i>Zea mays</i>	20% PEG 6000 (3 d)	10–100 $\mu$ M	Foliar application pre-treatment	Photosynthesis $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , carbon fixation $\blacktriangle$ , amino acids and secondary metabolites biosynthesis $\blacktriangle$ , ROS $\blacktriangledown$	[27]
Maize	<i>Zea mays</i>	Water withholding (7 d)	100 $\mu$ M	Two methods (root-irrigation and foliar application)	Photosynthesis $\blacktriangle$ , ROS $\blacktriangledown$	[44]
Maize	<i>Zea mays</i>	40–45% field capacity (50 d)	50 $\mu$ M (foliar spray) and 100 $\mu$ M (soil drench)	Foliar application or soil treatment	Photosynthesis $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , ROS $\blacktriangledown$	[45]
Wheat	<i>Triticum aestivum</i>	40% and 60% field capacity (7 d)	500 $\mu$ M	Soil application	Chloroplast structure $\blacktriangle$ , photosynthesis $\blacktriangle$ , photoprotection $\blacktriangle$ , cell turgor and water holding capacity $\blacktriangle$ , GSH and AsA contents $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , GSH-AsA cycle-related genes $\blacktriangle$ , ROS $\blacktriangledown$ , membrane damage $\blacktriangledown$	[46]
Wheat	<i>Triticum aestivum</i>	30% pot holding capacity (8 d)	100 $\mu$ M	Soil application	Recovering after rehydration $\blacktriangle$ , biomass and root/shoot ratio $\blacktriangle$ , water holding capacity $\blacktriangle$ ,	[47]

Common name	Scientific name	Drought treatment	Melatonin Treatment		Effects	Reference
			Concentration *	Application form		
Wheat	<i>Triticum aestivum</i>	20% PEG 6000 (7 d)	10 and 100 $\mu$ M (variety-dependent)	Seeds treatment	chlorophyll $\blacktriangle$ , photosynthesis $\blacktriangle$ , ROS $\blacktriangledown$ , MDA $\blacktriangledown$ Germination percentage $\blacktriangle$ , germination index $\blacktriangle$ , germination potential $\blacktriangle$ , radicle length and number $\blacktriangle$ , plumule length $\blacktriangle$ , Lysine (germination-related amino acid) $\blacktriangle$	[48]
Tartary Buckwheat	<i>Fagopyrum tataricum</i>	20% field capacity (15 d)	100 $\mu$ M	Foliar application	Water status $\blacktriangle$ , osmoprotection $\blacktriangle$ , secondary metabolites $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , photosynthesis $\blacktriangle$ , ROS $\blacktriangledown$	[49]
Barley	<i>Hordeum vulgare</i>	(Combined drought and cold)	1 mM	Foliar or soil application	Endogenous melatonin $\blacktriangle$ , ABA $\blacktriangle$ , water status $\blacktriangle$ , antioxidants $\blacktriangle$ , photosynthesis $\blacktriangle$ , PSII efficiency $\blacktriangle$	[50]
Soybean	<i>Glycine max</i>	20% field capacity (10 d)	50 $\mu$ M	Seed coating	Seedlings growth $\blacktriangle$ , biomass $\blacktriangle$ , electrolyte leakage $\blacktriangledown$	[37]
Soybean	<i>Glycine max</i>	15 % PEG 6000 (7 d)	100 $\mu$ M	Supplemented with nutrient solution	Seedlings growth $\blacktriangle$ , photosynthesis $\blacktriangle$	[39]
Soybean	<i>Glycine max</i>	45% RSWC (15 d)	100 $\mu$ M	Foliar application	Antioxidant enzymes $\blacktriangle$ , osmolytes $\blacktriangle$ , MDA $\blacktriangledown$	[26]
Soybean	<i>Glycine max</i>	11 d in 20% PEG 6000	100 $\mu$ M	Soil application	Antioxidant enzymes $\blacktriangle$ , ROS $\blacktriangledown$ , oxidative damage $\blacktriangledown$	[38]
Soybean	<i>Glycine max</i>	15 % PEG 6000 (3 days)	100 $\mu$ M	Foliar and root application	Plant growth and flowering $\blacktriangle$ , seed yield $\blacktriangle$ , gas exchange $\blacktriangle$ , PSII efficiency $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , MDA $\blacktriangledown$	[51]
Cotton	<i>Gossypium hirsutum</i>	10% PEG 6000 (7 d)	100 $\mu$ M	Seeds pre-soaking	Number and opening of stomata in cotton testa $\blacktriangle$ , germination parameters $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , osmoprotection $\blacktriangle$ , GA3 $\blacktriangle$ , ABA $\blacktriangledown$ , ROS $\blacktriangledown$ , MDA $\blacktriangledown$	[52]
Alfalfa	<i>Medicago sativa</i>	Water withholding (7 d)	10 $\mu$ M	Soil application	Chlorophyll $\blacktriangle$ , stomatal conductance $\blacktriangle$ , osmoprotection $\blacktriangle$ , Nitro-oxidative homeostasis $\blacktriangle$ , cellular redox disruption $\blacktriangledown$ , MDA $\blacktriangledown$ , ROS $\blacktriangledown$	[53]
Apple	<i>Malus species</i>	Water withholding (6 d)	100 $\mu$ M	Fruits Soil application	Water holding capacity $\blacktriangle$ , chlorophyll $\blacktriangle$ , photosynthesis	[54]

Common name	Scientific name	Drought treatment	Melatonin Treatment		Effects	Reference
			Concentration *	Application form		
Apple	<i>Malus domestica</i>	50 field capacity (3 months with sampling every month)	100 $\mu$ M	Soil application	<p>▲, antioxidants ▲, stomatal opening regulation ▲, melatonin biosynthesis genes ▲, electrolyte leakage ▼, ROS ▼, ABA ▼ through ABA synthesis gene ▼ and catabolic genes ▲</p> <p>Chlorophyll ▲, photosynthesis ▲, photoprotection ▲, antioxidant enzymes ▲, GSH and AsA contents ▲, membrane damage ▼, leaf senescence ▼, senescence-associated genes 12 ▼, pheophorbide a oxygenase-related gene ▼, ROS ▼</p> <p>Photoprotection ▲, leaf thickness ▲, spongy tissue ▲, stoma size ▲, chloroplast structure ▲, enzymatic and non-enzymatic antioxidants ▲, osmoprotectants (free proline) ▲, ultrastructural damage ▼, oxidative injury ▼</p>	[55]
Grape	<i>Vitis vinifer</i>	10% PEG 6000 (12 d)	50, 100 and 200 nM	Roots pretreatment	<p>Photoprotection ▲, leaf thickness ▲, spongy tissue ▲, stoma size ▲, chloroplast structure ▲, enzymatic and non-enzymatic antioxidants ▲, osmoprotectants (free proline) ▲, ultrastructural damage ▼, oxidative injury ▼</p>	[34]
Grapevine	<i>Vitis amurensis</i> , <i>V. vinifera</i> and <i>V. labruscana</i>	10% PEG 6000 (4 d)	Endophyte colonization of secreted-MELATONIN bacteria)	<i>Bacillus amyloliquefaciens</i> SB-9 ( secreted melatonin-endophyte bacteria ) colonization	Melatonin synthesis and its intermediates ▲, plant growth ▲, ROS ▼, MDA ▼	[56]
Grape	<i>Vitis vinifer</i>	Water withholding (18 d)	100 $\mu$ M	Supplemented with irrigation	MDA ▼, relative conductivity ▼	[57]
Grape	<i>Vitis vinifer</i>	Water withholding (18 d)	100 $\mu$ M	Supplemented with irrigation	Chlorophyll ▲, SOD activity ▲	[29]
Kiwifruit	<i>Actinidia chinensis</i> var. <i>deliciosa</i>	Water withholding (9 d) (RWC below 35% field capacity)	100 $\mu$ M	Supplemented with irrigation	<p>Root vigour ▲, osmoprotectants ▲, proteins biosynthesis ▲, chlorophyll ▲, photosynthesis ▲, light energy absorption ▲, photoprotection ▲, CO<sub>2</sub> fixation-associated genes ▲, MDA ▼, cell membranes damage ▼, stomatal closure ▼</p>	[58]

Common name	Scientific name	Drought treatment	Melatonin Treatment		Effects	Reference
			Concentration *	Application form		
Kiwifruit	<i>Actinidia chinensis</i>	water withholding (9 days)	100 $\mu$ M	Irrigation pretreatment	Water holding capacity $\blacktriangle$ , antioxidant enzymes-related genes $\blacktriangle$ , GSH-AsA cycle-related genes $\blacktriangle$ , ROS $\blacktriangledown$ , MDA $\blacktriangledown$	[28]
Chinese hickory	<i>Carya cathayensis</i>	30 % PEG 6000 (10-40 d)	100 $\mu$ M	Foliar application pretreatment	Recovering after rehydration $\blacktriangle$ , photosynthesis $\blacktriangle$ , antioxidants $\blacktriangle$ , osmoprotectants (TSS and proline) $\blacktriangle$ , metabolic pathways-related genes $\blacktriangle$ , antioxidant enzymes-related genes $\blacktriangle$ , ROS $\blacktriangledown$	[59]
Vegetables						
Tomato	<i>Solanum lycopersicum</i>	Water withholding for (5-20 d after moderate drought)	0.1 mM	Supplemented with irrigation	Photosynthesis $\blacktriangle$ , root vigour $\blacktriangle$ , PSII efficiency $\blacktriangle$ , antioxidants $\blacktriangle$ , toxic substances $\blacktriangledown$	[60]
Tomato	<i>Solanum lycopersicum</i>	10% PEG (7 d)	200 $\mu$ M	Foliar application	Chlorophyll $\blacktriangle$ , p-coumaric acid content $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , MDA $\blacktriangledown$	[30]
Pepper	<i>Capsicum annuum</i>	10% PEG (8 d)	50 $\mu$ M	Seeds pretreatment	Water holding capacity $\blacktriangle$ , endogenous melatonin $\blacktriangle$ , GSH content $\blacktriangle$ , chlorophyll $\blacktriangle$ , carotenoids $\blacktriangle$ , proline $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , MDA $\blacktriangledown$	
Watermelon	<i>Citrullus lanatus</i>	Water withholding (4 d)	150 $\mu$ M	Roots pretreatment	Wax accumulation $\blacktriangle$ , melatonin-ABA crosstalk $\blacktriangle$	[40]
Cucumber	<i>Cucumis sativus</i>	18 % PEG 6000 (days)	100 $\mu$ M	Seeds priming and nutrient solution	Seed germination $\blacktriangle$ , root generation and vitality $\blacktriangle$ , root/shoot ratio $\blacktriangle$ , roots vigour $\blacktriangle$ , chlorophyll $\blacktriangle$ , photosynthesis $\blacktriangle$ , chloroplasts ultrastructure $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , ROS $\blacktriangledown$	[61]
Rapeseed	<i>Brassica napus</i>	4% PEG 6000 (7 d)	0.05 mM	In PEG solution	Plant growth $\blacktriangle$ , antioxidants $\blacktriangle$ , osmoprotectants $\blacktriangle$ , ROS $\blacktriangledown$	[62]
Rapeseed	<i>Brassica napus</i>	-0.3 & -0.4 Mpa PEG 6000 (7 d)	500 $\mu$ M	Seed priming	Chlorophyll $\blacktriangle$ , stomatal opening regulation $\blacktriangle$ , chloroplast structure $\blacktriangle$ , cell expansion and cell wall $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , osmoprotectants $\blacktriangle$ , oxidative injury $\blacktriangledown$	[36]

Ornamental and medicinal plants

Common name	Scientific name	Drought treatment	Melatonin Treatment		Effects	Reference
			Concentration *	Application form		
Jinyu Chuju	<i>Dendranthema morifolium</i>	40 % field capacity (6 d)	100 $\mu$ M	Foliar application	Chlorophyll $\blacktriangle$ , photosynthesis $\blacktriangle$ , biomass $\blacktriangle$ , osmoprotectants (TSS and proline) $\blacktriangle$ , cell membrane damage $\blacktriangledown$ , relative conductivity $\blacktriangledown$ , MDA $\blacktriangledown$	[63]
Moldavian balm (Dragon head)	<i>Dracocephalum moldavica</i>	40-60% field capacity	100 $\mu$ M	Foliar application	Plant growth and flowering $\blacktriangle$ , antioxidants $\blacktriangle$ , chlorophyll $\blacktriangle$ , water holding capacity $\blacktriangle$ , ROS $\blacktriangledown$ , MDA $\blacktriangledown$	[64]
Creeping bentgrasses	<i>Agrostis stolonifera</i>	Water withholding (14 d)	20 $\mu$ M	Foliar application	Visual quality $\blacktriangle$ , PSII efficiency $\blacktriangle$ , chlorophyll $\blacktriangle$ , water holding capacity $\blacktriangle$ , melatonin biosynthesis genes $\blacktriangle$ , dehydration responsive genes $\blacktriangle$ , chlorophyll-degradation genes $\blacktriangledown$ , leaf senescence $\blacktriangledown$ , ROS $\blacktriangledown$ , MDA $\blacktriangledown$	[65]
Tall fescue	<i>Festuca arundinacea</i>	Water withholding (10 d)	20 $\mu$ M	Irrigation pretreatment	Plant growth $\blacktriangle$ , chlorophyll $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , ROS $\blacktriangledown$ , MDA $\blacktriangledown$	[66]
Bermudagrass	<i>Cynodon dactylon</i>	Withholding water (21 d)	20 and 100 $\mu$ M	Irrigation pretreatment	Plant growth $\blacktriangle$ , chlorophyll $\blacktriangle$ , survival rate $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , stress-responsive genes $\blacktriangle$ , metabolic regulation $\blacktriangle$ , hormonal signalling-related genes regulation $\blacktriangle$ , ROS $\blacktriangledown$ , electrokage $\blacktriangledown$	[67]
Fenugreek	<i>Trigonella foenum-graecum</i>	19.5 % PEG 6000 (21 d)	100 and 300 $\mu$ M	Foliar application pretreatment	Endogenous melatonin and secondary metabolites $\blacktriangle$ , chlorophyll $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , ROS $\blacktriangledown$	[68]
Coffee	<i>Coffea arabica</i>	40% of max moisture retention capacity (21 d)	300 $\mu$ M	Soil application	Root vigour $\blacktriangle$ , photoprotection $\blacktriangle$ , gas exchange $\blacktriangle$ , carboxylation efficiency $\blacktriangle$ , chlorophyll $\blacktriangle$ , antioxidants $\blacktriangle$ , MDA $\blacktriangledown$	[32]
Tea	<i>Camellia sinensis</i>	20 % PEG 6000 (2 d)	100 $\mu$ M	Foliar application pretreatment	Photosynthesis $\blacktriangle$ , GSH and AsA contents $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , antioxidant enzymes-related genes $\blacktriangle$ , ROS $\blacktriangledown$ , MDA $\blacktriangledown$	[33]
Different crops						
Tobacco, Tomato and	<i>Nicotiana benthamiana, Solanum</i>	Water withholding (6 d)	10 $\mu$ M	Foliar application	MDA $\blacktriangledown$ , drought tolerance $\blacktriangle$	[24]

Common name	Scientific name	Drought treatment	Melatonin Treatment		Effects	Reference
			Concentration *	Application form		
Cucumber	<i>Lycopersicon and Cucumis sativus</i>					

▲, enhanced or ▼ decreased compared to control. ROS, reactive oxygen species; PSII, photosystem II; GSH, glutathione; AsA, ascorbate; MDA, malondialdehyde; ABA, abscisic acid; GA3, gibberellic acid; SOD, superoxide dismutase; TSS, total soluble sugar. \* Only those doses of exogenous melatonin which had the superior positive impacts on plant tolerance against drought stress have been selected.

## 2.2. Melatonin is involved in drought stress tolerance:

Given the wide use of melatonin in drought stress alleviation, it was of interest for the scientific community to investigate the direct evidence of melatonin involvement in drought tolerance. This takes place through melatonin-biosynthesis genes such as tryptophan decarboxylase (TDC), N-acetylserotonin methyltransferase (ASMT), serotonin N-acetyltransferase (SNAT) and caffeic acid O-methyltransferase (COMT). In this respect, the endogenous melatonin levels are changed with the alteration of environmental conditions of plant growth. The melatonin level is increased with a protective role in response to different abiotic stressors such as cold, heat, heavy metals, UV radiation, water deficit, and waterlogging [17,23]. Thus, the expressions of the biosynthesis enzyme transcripts (TDC, SNAT, ASMT, and COMT genes) occur in stressful situations, producing a burst in the levels of endogenous melatonin. The global influence of environmental factors on the melatonin levels of plant organs was demonstrated in barley, tomato, and lupin plants by Arnao and co-workers [69-71]. Some representative examples of melatonin induction by drought can be consulted in studies in Arabidopsis [25], barley [72], bermudagrass [67], apple [54], grapevine [56] and rice [73]. In these cases, an increase in the melatonin level in one or more transcripts of melatonin biosynthesis enzymes between 2-6 fold because stress conditions have been described [74].

## 2.3. Mechanisms of melatonin-induced drought stress tolerance

### 2.3.1. Anatomical changes and physiological mechanisms

In the last few years, the role of melatonin as a multifunctional regulator of the plant status under drought conditions, including (i) anatomical and (ii) physiological aspects have been studied progressively and notably reached more than 42 studies within seven years (Table 1). (i) The anatomical changes are induced by melatonin within the different plant organs including less cell membrane damage [63], more intact grana lamella of chloroplast [46], alleviation of chloroplast ultrastructural damage and preservation of its system [34,61], mitochondrial structure safeguarding [67], cell expansion maintaining [36], better leaf thickness, spongy tissue and stomata size [34,36], cuticle formation [75] and wax accumulation [40]. (ii) By increasing drought severity, melatonin, which is biosynthesized in mitochondria and chloroplasts [76,77], exhibits more defence strategies. It promotes the physiological aspects including antioxidant system [28,59] to alleviate the oxidative damage, leading to less accumulation of H<sub>2</sub>O<sub>2</sub>, O<sup>-2</sup> and NO [26,53], less electrolyte leakage [42], lower lipid peroxidation (MDA reduction) [28,65], lower relative conductivity [57], toxic substances content easing [60] cellular redox disruption limitation [53], better nitro-oxidative homeostasis [53], and enhanced ascorbate (AsA)–glutathione(GSH) cycle capacity (higher GSH and AsA contents) [55]. Such beneficial effects are carried out by regulating the enzymatic activities such as ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), as well as non-enzymatic antioxidants and osmoprotectants (proline and others) [45,64], and also secondary metabolites such as flavonoids, phenolics and phenylalanine ammonialyase [49]. Simultaneously, melatonin improves the plant photosystem indicated by higher chlorophyll content [58], greater photosynthetic rates [44],

and higher transpiration rates [32]. Besides, Melatonin proved to enhance photoprotection via improving the photo-system II efficiency [35]. As a multifunctional substance, melatonin also regulates the osmotic potential of the cell [43] via the accumulation of soluble sugars and proline [62]. Moreover, water status is one of the most important priorities of melatonin to control under drought conditions. In this respect, melatonin enhances plant resistance via higher stomatal conductance [43], higher cell turgor and water holding capacity [65], and stomatal opening regulation [78]. Consequently, the whole plant status is enhanced including seed germination efficiency [48], root generation vitality and strengthen [61], growth and flowering [37,51], visual quality [65], seed yield [39], leaf senescence alleviation [55] and quickly recovering after rehydration [59].

### 2.3.2. Molecular mechanisms

#### Omics of Redox hemostasis and plant build-in-processes

The protective mechanisms of melatonin against drought stress are also studied and focused on the ability of melatonin to protect plant cells against redox homeostasis disruption in response to drought stress. Melatonin regulates ROS/RNS levels and antioxidants-related genes including SOD, POD, CAT, APX, glutathione S-transferase (GSTP), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) [28,31,33,38,44,46,53,59,79,80], as well as osmoprotective elements via regulation of proline biosynthesis genes [53]. Melatonin is not only alleviating oxidative damage, but also regulating plant build-in-associated genes, including carbohydrate/fatty and amino acids metabolism [27,37,38], carbon metabolic pathway [67], nitrogen metabolism and transport [38,55], plant secondary metabolism [59] energy production [38,80], carotenoids metabolism and photosynthesis [28,37,38,59] and cuticle wax biosynthesis [75]. In this regard, the metabolism of carbohydrate/fatty acids is reported to be up-regulated via seed-coating of soybean with melatonin solution as a means to improve its tolerance to drought stress [37]. Melatonin is also a key regulator of nitrogen (N) metabolism and transport as indicated by the higher expression levels of N uptake genes (AMT2-1, AMT1-2, AMT1-6, AMT1-5, NRT1-1, NRT2-5, NRT2, and 7NRT2-4) and metabolic genes (NADH-GOGAT NR, Fd-GOGAT, NiR, and GS) in the leaves of apple trees [55].

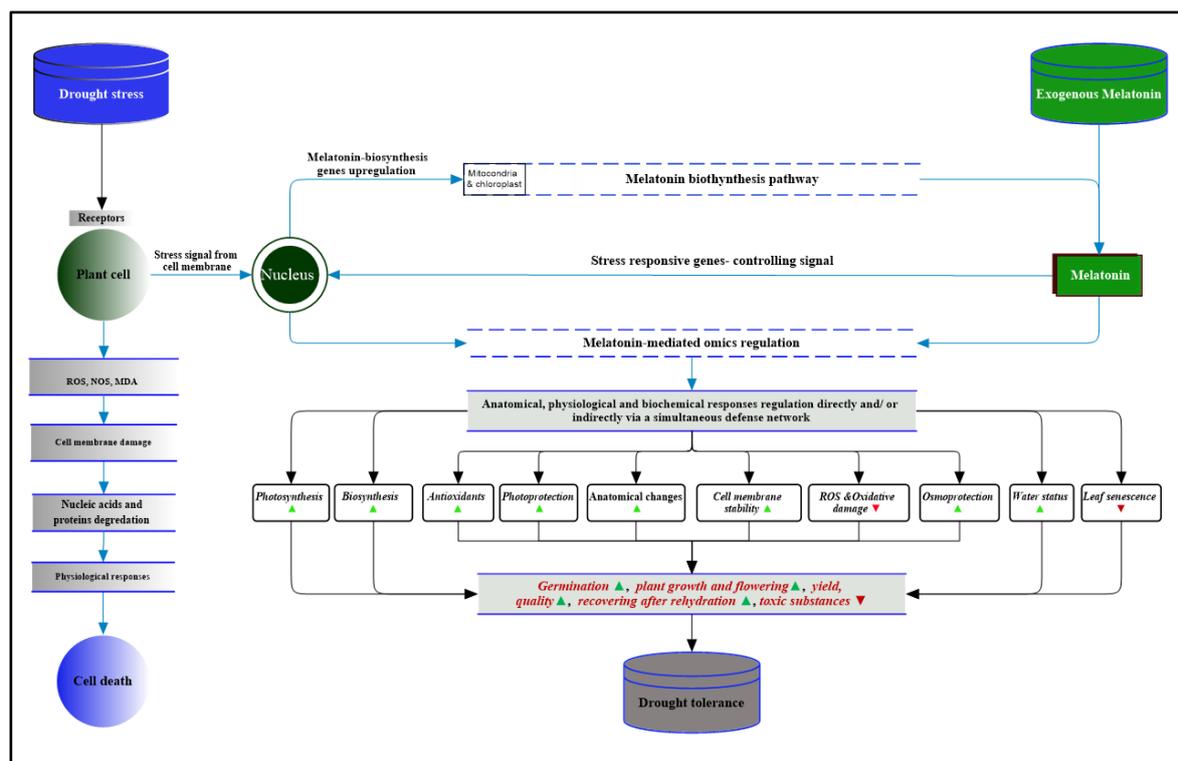
#### Omics of energy production, photosynthesis, and wax biosynthesis

Melatonin promotes energy production under water scarcity through regulating glycolytic protein expression and electron transport in the respiratory chain [80]. Moreover, melatonin governs the photosynthesis process via regulating molecular elements involved in enzymatic activities of CO<sub>2</sub> fixation (PGK, TKT, FBA, RPI, FBP, GAPA, TIM, RPK, Rubisco, SEBP, and RPE) [58], protein expression for carbon fixation [27], light reaction of photosynthesis (cytochrome P450) [38] and tetrapyrrole pigment biosynthesis [38,65,79]. Photosynthesis is also reported to be up-regulated via seed-coating of soybean with melatonin solution as a means to improve its tolerance to drought and salinity stress. Among interesting genes up-regulated by melatonin, there were two subunits of photosystem I (PS I) (PsaG and PsaK), two elements (PsbO and PsbP) related to the oxygen-evolving complex of PS II (oxygen-evolving enhancer proteins) [37]. Moreover, melatonin up-regulates the relative expression of PetF ferredoxin gene (controls the amount of reduced ascorbate and protects chlorophyll from degradation), and the VTC4 gene, encoding the L-galactose 1-P-phosphatase for ascorbate biosynthesis [37]. In another study, Ma, et al. [65] reported that melatonin inhibited the gene expression and enzyme activities of chlorophyll-degradation genes including Chlase, Chl-PRX and PPH in melatonin-treated plants during drought stress, which directly affects photosynthesis performance. On the other hand, Ding, et al. [75] tested the relative expression of four wax biosynthesis-related genes, including KCS1 responsible for fatty acid elongation, CER3 involved in alkane synthesis, TTS1 associated with triterpenoids synthesis and LTP1 accountable for lipids transport. It was remarked that the transcripts of the four genes were triggered by drought stress and were further induced as a result of melatonin treatment, demonstrating the role of melatonin in enhancing wax biosynthesis [75].

## Omics of stomatal movement, autophagy, and others

Melatonin-mediated stomatal closure mechanism is also investigated, suggesting that melatonin is a phytohormone triggering stomatal closure via the signalling pathway of PMTR1, which is controlling H<sub>2</sub>O<sub>2</sub> production. PMTR1 is a phytomelatonin receptor having a receptor-like topology and interacting with the subunit of G-protein A (GPA1) in the plasma membrane [78]. Phytomelatonin–receptor binding regulates the heterotrimeric G-proteins (G $\gamma$  $\beta$  and G $\alpha$ ) dissociation, which enhances NADPH oxidase-dependent H<sub>2</sub>O<sub>2</sub> releasing (RBOH), promoting Ca<sup>2+</sup> influx as well as strengthening K<sup>+</sup> efflux, leading to stomatal closure [78]. Under excessive drought, plant resorts to get rid of dysfunctional or unnecessary cellular components to facilitate the orderly degradation and recycling of cellular components through autophagy mechanism. The regulatory role of melatonin in autophagy is elucidated in wheat seedlings via enhancing metabolic process associated with autophagy represented by up-regulating fused signal recognition particle receptor, Rab-related protein, serine protease, aspartyl protease at the protein or mRNA level [80]. Moreover, melatonin regulates the action of key transcription factors such as Myb4, AP37 and zinc finger [42,67], in parallel with some transporter proteins, including proton transporter (UCP1), potassium transporter (HKT1), and water channel protein (PIP2;1) [42], which are all essential elements in stress tolerance. Besides, melatonin application orchestrates some stress-signalling genes such as calcium and protein kinases-related genes, implying that kinase signalling could prove essential roles in drought tolerance [67].

All in all, as shown in Figure 1, it can be concluded that once the plants feel by water scarcity under drought conditions, the protective and regulatory role of melatonin, in parallel with other anti-stress strategies, is starting to prevent, alleviate or stop the harmful effects of the stress [17,81]. At the cellular level, stress signal from the cell membrane informs the nucleus that "cell life under threat" to cope with the drastic effects of the drought [78,82]. Quickly, the nucleus starts to activate the melatonin biosynthesis pathway from its precursor, tryptophan, in mitochondria, and chloroplasts [76,77,83] through up-regulating the melatonin-biosynthesis genes [54,65]. Remarkably, melatonin starts by sending its feedback to the nucleus about such stress to trigger the appropriate stress response through omics regulation [41,46,55,65]. As a result, the genes involved in the anatomical, physiological and biochemical aspects are regulated directly and/or indirectly via a simultaneous defence network. The omics-mediated responses include photosynthesis, biosynthesis, antioxidants, photoprotection, cell membrane stability, osmoprotection, water status, leaf senescence, oxidative damage alleviation in addition to the anatomical changes. Consequently, the whole plant status is enhanced, including growth and development, flowering, yield, quality and survival rate (recovering after rehydration), while the toxic substances are decreased, which collectively lead to drought tolerance.



**Figure 1.** A schematic model explains the mechanism underlying melatonin-mediated drought stress response. At the cellular level, stress signal from the cell membrane is received by the nucleus, which starts to activate the melatonin biosynthesis pathway from its precursor, tryptophan, in mitochondria and chloroplasts by up-regulating the melatonin-biosynthesis genes. Melatonin sends its feedback to the nucleus about such stress to mediate the omics regulation. Consequently, the genes encoding proteins related to plant anatomical, physiological and biochemical responses are regulated directly and/or indirectly via a simultaneous defence network. The omics-mediated responses include photosynthesis, biosynthesis, enzymatic and non-enzymatic antioxidants, photoprotection, cell membrane stability, ROS and oxidative damage, osmoprotection, water status, leaf senescence in addition to the anatomical changes, which lead to drought tolerance. Consequently, the whole plant status is enhanced, including growth and development, flowering, yield, quality, and survival rate, while the toxic substances are decreased.

### 2.3.3. Melatonin orchestrates other phytohormones in the regulatory-defence network

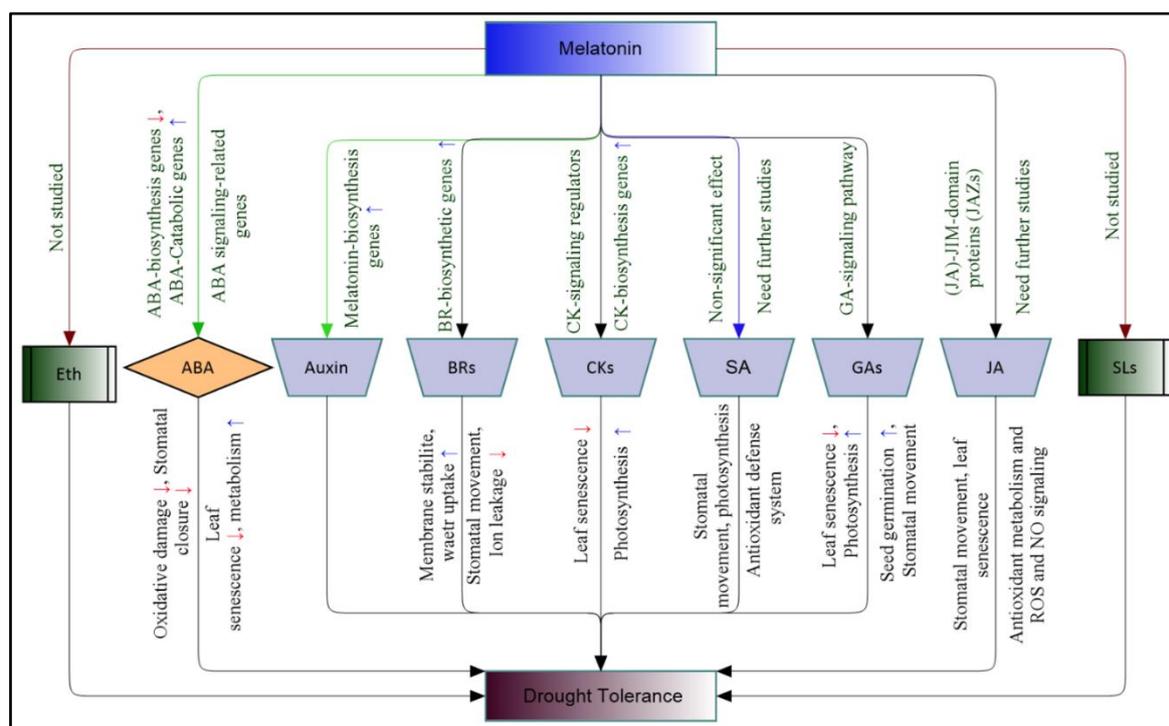
Melatonin is a central molecule in the hormonal system, thus increases the plant tolerance to drought stress through the regulation of phytohormone levels such as abscisic acid (ABA), auxins (Auxs), cytokinins (CKs), gibberellins (GAs), brassinosteroids (BRs), jasmonic acid (JA) and salicylic acid (SA). The key physiological aspects that are much regulated by phytohormones in response to drought stress include antioxidants metabolism, carbohydrate production (carbon metabolism), stomatal movement, and leaf senescence [84]. Drought stress up-regulates ABA, BRs, and JA [59,84] and down-regulates CKs and GAs [52,59], while melatonin enhances the levels of BRs, GAs, JA and CKs [59] and decreases ABA level [59] (Figure 2).

Water scarcity stimulates ABA biosynthesis [14,85], which in turn down-regulates the main metabolic pathways [59], induces stomatal closure [84] and contributes to leaf senescence [84]. Moreover, the over-accumulation of ABA up-regulates the ROS generation pathways and causes oxidative damage [14]. However, melatonin maintains the ABA homeostasis (low to moderate ABA concentrations) by positive regulation of its biosynthetic genes and negative regulation of catabolic genes [52,54,59,72]. Li, et al. [54] clarified that melatonin effectively down-regulates MdNCED3, ABA synthesis genes, and up-regulates its catabolic genes, MdCYP707A2 and MdCYP707A1, causing ABA reduction. Besides, melatonin regulates ABA signalling-related genes such as SnRK2 (SNF1-related protein kinases 2), RCAR/PYR/PYL, and NCED (nine-cis-epoxycarotenoid dioxygenase) [67]. Cytokinins (CKs) are an essential group of phytohormones in the inhibition of leaf senescence and

chlorophyll degradation under water stress, which in turn suppresses CK biosynthesis and transport causing CK-reduction and faster leaf senescence [86-88]. Melatonin treatment up-regulates CK levels and some related-signalling factors leading to better photosynthesis efficiency and drought-induced tolerance [59,65,89]. The first demonstration that melatonin inhibits leaf senescence was made in barley [90]. Melatonin-induced alleviation leaf senescence in creeping bentgrass is associated with the down-regulation of chlorophyll catabolism and the synergistic interaction with CK-biosynthesis genes and signalling pathways in melatonin-treated ipt-transgenic plants [65].

Brassinosteroids (BRs) possess an apparent ability as drought stress-protective molecules in plants [91]. Melatonin regulates the biosynthesis of BRs via the stimulation of various BRs-biosynthetic genes like DWARF4, D11, and RAVL1 [92], which control stomatal movement [93], enhance cell membrane constancy and water uptake and decrease membrane damage-induced ion leakage in case of water limitation [59,94]. Jasmonic acid (JA) is a crucial plant hormone in the regulation of drought responses such as stomatal movement, leaf senescence, antioxidant metabolism, and ROS and NO signalling [95-100]. JA levels are increased in response to drought stress and highly stimulated as a result of melatonin application, which induces drought tolerance [59]. The melatonin-JA crosstalk is stated by regulating molecular transcripts such as JA-JIM-domain proteins (JAZs) in JA signalling [67].

Moreover, melatonin interacts with GAs via GA-signaling, which further controls the biosynthesis of auxins [25,59]. Gibberellins (GAs) are regulators of stomatal movement [101,102], photosynthesis [103], seed germination [104], and leaf senescence [4]. Drought stress inhibits gibberellins (GAs) biosynthesis [52,105], which are much enhanced in response to melatonin treatment causing drought tolerance [59]. Salicylic acid (SA) accumulation plays a vital role in stomatal movement, photosynthesis, and antioxidant defence system [4]. In maize plants, under drought conditions, an increase (but non-significant) in the defence hormone, SA, has been described in melatonin-treated plants [35]. Enhanced drought tolerance was achieved mainly using transgenic plants through overexpressing melatonin-biosynthesis genes under drought conditions [25,106,107], which led to a decrease of indole-3-acetic acid (IAA) may be due to the competition for the same precursor, tryptophan. The plant root is the first plant organ to touch the environment and represents a priority for plant breeders to improve its efficiency under abiotic stresses, including drought. Interestingly, melatonin is targeting plant roots showing auxin-like action [108]. In this regard, Pelagio-Flores, et al. [108] provide direct evidence supporting the mechanism of this action in *Arabidopsis thaliana* via inspiring lateral and adventitious root formation conferring a widespread root system. The auxin-like effect of melatonin in roots was elucidated using the auxin-responsive marker constructs. It is suggesting that melatonin neither nor activates auxin-inducible gene expression nor induces the degradation of HS:AXR3NT-GUS, indicating that root developmental changes elicited by melatonin were independent of auxin signalling [108]. To date, under drought situations, there is no comprehensive study revealing the interaction between melatonin and ethylene or strigolactones, thus need further investigations. All the above details confirmed that melatonin acts as a relevant regulator of many plant hormone elements, so-called as a plant master regulator [109,110], making the plants more tolerant when irrigation water is limited (Figure 2).



**Figure 2.** A schematic model explains the melatonin effect on other phytohormones under drought stress: Under drought, melatonin enhances the levels of brassinosteroids (BRs), cytokinins (CKs), gibberellins (GAs) and jasmonates (JA) and decreases abscisic acid (ABA) level and Auxins. **Eth**, Ethylene; **ABA**, Abscisic acid; **BRs**, Brassinosteroids; **CKs**, Cytokinins; **SA**, Salicylic acid; **GAs**, Gibberellins; **JA**, Jasmonic acid; **SLs**, Strigolactones. Red connectors, not studied; green connectors, reduced; Black connectors, enhanced; blue connectors, non-significant effect. ↑, up-regulated; ↓, down-regulated.

### 3. Melatonin-induced waterlogging stress tolerance

#### 3.1. An overview

Despite the importance of melatonin to mitigate the harmful effects of abiotic stresses, the research on melatonin-induced waterlogging tolerance is recently started to emerge (Table 2). The first report was registered as a patent in 2015 by [Chen, et al. \[111\]](#). In this report, the authors indicated that melatonin has a great ability to eliminate ROS, alleviate oxidative damages, resist waterlogging, and consequently reduce yield and quality loss [111]. After this ground-breaking work, [Zheng, et al. \[112\]](#) elucidated that melatonin is an effective phytohormone to protect apple plants under waterlogging stress. Melatonin application increased endogenous melatonin level, reduced chlorosis and wilting, improved antioxidant enzyme activities, reduced ROS and MDA, enhance Chl content and photosynthesis, suppressed anaerobic respiration and enhanced aerobic respiration, as well as up-regulated melatonin-biosynthesis enzymes (MbT5H1, MbAANAT3, and MbASMT9) [112]. In recent work, [Zhang, et al. \[113\]](#) investigated the impact of melatonin pretreatment on alfalfa under waterlogging stress and indicated that melatonin could alleviate the stress damage and enhances the plant growth, chlorophyll content, and PSII efficiency. Moreover, melatonin treatment increased polyamine (putrescine, spermidine, and spermine) levels and decreased ethylene under stress, which is controlled via changes in gene expression [113].

**Table 2.** Roles of melatonin in waterlogging stress tolerance.

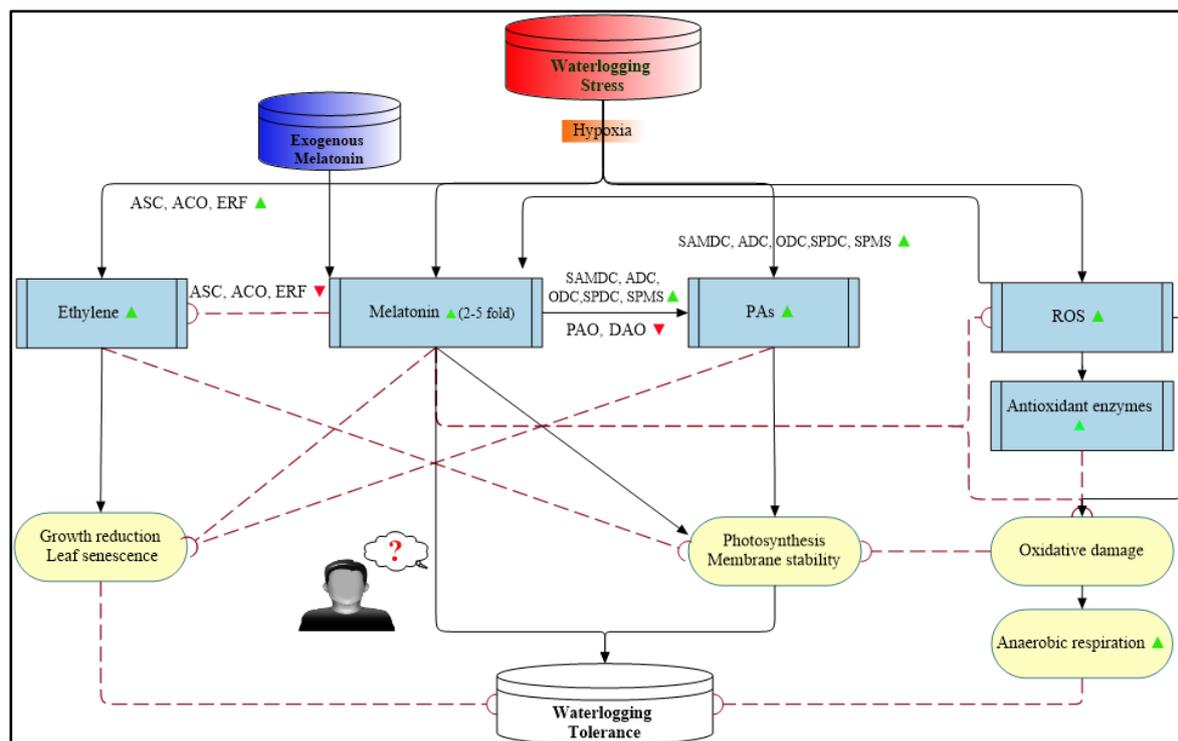
Species	Scientific name	Waterlogging treatment	Melatonin Treatment		Functions	References
			Concentration*	Application form		

Patent on:						
wheat, corn, rice leek Siberian crab apple, soybean, peanut and radish.			100 $\mu$ M	Foliar spraying	ROS $\blacktriangledown$ , oxidative damages $\blacktriangledown$ , yield and quality $\blacktriangle$	[111]
Apple	<i>Malus baccata</i>	Waterloggin g stress (9 d)	200 $\mu$ M (Foliar spraying) 600 $\mu$ M (root irrigation)	Foliar spraying or root irrigation	Endogenous melatonin $\blacktriangle$ , antioxidant enzymes $\blacktriangle$ , chlorophyll $\blacktriangle$ , photosynthesis $\blacktriangle$ , aerobic respiration $\blacktriangle$ , synthetic enzymes $\blacktriangle$ ROS $\blacktriangledown$ , MDA $\blacktriangledown$ , anaerobic respiration $\blacktriangledown$ , chlorosis and wilting $\blacktriangledown$	[112]
Alfalfa	<i>Medicago sativa</i>	Waterloggin g stress (10 d)	100 $\mu$ M	Foliar spraying pretreatment	Endogenous melatonin $\blacktriangle$ , gene expression regulation $\blacktriangle$ , photosynthesis $\blacktriangle$ , electroleakage $\blacktriangledown$ , MDA $\blacktriangledown$ , leaf senescence $\blacktriangledown$ , polyamine and ethylene metabolism reprogramming	[113]

$\blacktriangle$ , enhanced or  $\blacktriangledown$  decreased compared to control. ROS, reactive oxygen species; MDA, malondialdehyde. \* Only those doses of exogenous melatonin which had the superior positive impacts on plant tolerance against waterlogging stress have been selected.

### 3.2. Mechanisms of melatonin-mediated waterlogging stress tolerance

Melatonin application is a practical approach to suppress the drastic effects of waterlogging. To date, there are two published mechanisms induced by melatonin to enforce waterlogging tolerance [112,113]. [Zheng, et al. \[112\]](#) proposed the first mechanism of melatonin-mediated waterlogging tolerance in apple seedlings to keep aerobic respiration and preserve photosynthesis by efficient repression of the ROS burst and consequent mitochondria degradation. [Zhang, et al. \[113\]](#) suggested another model in alfalfa through interacting with/or directly regulating the metabolic pathways of ethylene and polyamines (PAs). Waterlogging stress induced an increase of endogenous melatonin levels of 2-5 fold comparing with un-stressed plants. Melatonin starts by reducing ethylene production via down-regulating the ethylene synthesis-associated genes and alleviating waterlogging-caused growth inhibition, chlorosis, and premature senescence. Then, melatonin enhances PAs levels through promoting the enzymatic activities gene expression involved in PA metabolism [113]. The authors proposed that melatonin increased waterlogging tolerance at least partially by regulating polyamine and ethylene biosynthesis due to ethylene suppression and PAs promoting, leading to the more stable cell membrane, better photosynthesis and less ethylene-responsive senescence [113]. Collectively, Waterlogging induces ethylene, melatonin (2-5 fold), polyamines (PAs), and ROS. Melatonin is also produced in response to ROS generation and exogenous melatonin. Melatonin stimulates PAs biosynthesis, photosynthesis, and membrane stability, while inhibits ethylene biosynthesis, growth reduction, leaf senescence, ROS and oxidative damage. Excessive ROS causes oxidative damage leading to anaerobic respiration, which is scavenged by the antioxidant enzymes. Also, the growth reduction and leaf senescence are increased by ethylene, while decreased by PAs. Moreover, photosynthesis and membrane stability are enhanced by PAs, while reduced by ethylene induction and oxidative damage (**Figure 3**). The role of melatonin in waterlogging tolerance, especially molecular evidences, still needs further studies.



**Figure 3.** A schematic model explains the protective mechanisms of melatonin in waterlogging tolerance. Black-solid arrows indicate the stimulation, while the red-dashed indicate the inhibition. ▲ and ▼ shapes indicate enhanced or decreased, respectively. Waterlogging induces ethylene, melatonin (2-5 fold), polyamines (PAs) and ROS. Melatonin is also induced in response to ROS generation and exogenous melatonin. Melatonin stimulates PAs biosynthesis, photosynthesis, and membrane stability, while inhibits ethylene biosynthesis, growth reduction, leaf senescence, ROS, and oxidative damage. Excessive ROS causes oxidative damage leading to anaerobic respiration, which is scavenged by the antioxidant enzymes. Also, the growth reduction and leaf senescence are increased by ethylene, while decreased by PAs. Moreover, photosynthesis and membrane stability is enhanced by PAs, while reduced by ethylene induction and oxidative damage. The role of melatonin in waterlogging tolerance still needs further studies. This Figure is a combination of the two published mechanisms of [Zheng, et al. \[112\]](#) and [Zhang, et al. \[113\]](#) with some modifications.

#### 4. Conclusion and Future perspectives

Water stress tolerance (drought stress and waterlogging) may be attributed to structural and functional adaptations at the cellular and whole plant levels including root enhancement, growth promotion, oxidative damage alleviation, osmotic potential modulation, leaf water potential and cell wall elasticity controlling, stomatal closure and accumulation of osmolytes, thereby easing the harmful impacts of such destructive stresses [4,15,114]. Melatonin may be considered as a core part of the regulatory network controlling all of these mechanisms and representing a promising material for future studies and practical uses [17,109,110]. Melatonin research has been experiencing hyper-growth in the last two decades; however, its roles in water stress tolerance induction need further investigations. The regulation of melatonin and its metabolism pathway under water stress is still unclear. Understanding the role of melatonin in nutrient uptake will give us an excellent opportunity to take advantage of such a useful molecule for strengthening plant tolerance and adaptability to water stress. Further, in-depth studies to clarify the molecular mechanisms using microarray, transcriptomic and proteomic analyses will help to figure out the genes regulating plant anatomical, physiological and biochemical aspects in response to melatonin exogenous application under water stress. Exploring new receptor-mediated phyto-melatonin signalling plays a role in such physiological processes in the future works. Also, the best known on the relationship of melatonin with other small signalling molecules such as NO and H<sub>2</sub>S can be relevant. The molecular mechanisms unrevealing the crosstalk between melatonin and other phytohormones such as

strigolactones, ethylene in promoting water stress tolerance worth further studies through mutagenesis or genetic modulation; also, the studies on aquatic model plants. The relationship between melatonin and multiple stressors combination is a topic to be taken into account in future research due to the complexity of the interaction of plants with diverse environmental agents. By last, the use of synthetic melatonin, a relatively cheap compound, or of phytomelatonin-rich extracts should be an interesting approach to improve plant tolerance [115,116].

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