Serotonin and Melatonin: Role in Rhizogenesis, Root Development and Signaling

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Abstract The indoleamines serotonin (5-hydroxyl-tryptamine) and melatonin (*N* acetyl 5 methoxy-tryptamine) are naturally occurring signaling molecules first discovered in animals but now known to be present in plants from diverse angiosperm families. They perform multiple functions and impact diverse aspects of a plants life. Their role in rhizogenesis is well documented. Melatonin and serotonin induce root elongation, formation and growth of lateral and adventitious roots and root hair development thereby altering the root architecture. Their mode of action in rooting appears to be diverse—they may act in concert with auxins or through independent signaling pathways. As mediators of biotic and abiotic stress, there is considerable interaction of these molecules with ROS and NO species and crosstalk with other plant growth regulators. Analysis of transcript and gene expression profiles has provided insights into the mechanism and pathway of melatonin/serotonin induced promotion of root induction and growth. As of today no receptor has been identified for serotonin. A receptor for melatonin was reported in *Arabidopsis thaliana,* but a subsequent report identified the protein as being located in the cytoplasm.

Abbreviations

AR	Adventitious root
ASMT	*N* Acetyl serotonin *O*-methyl transferase
Col1	Essential gene in JA signaling
CAMT	Caffeic acid *O*-methyl transferase
DEG	Differentially expressed genes
Et	Ethylene
GSNOR	*S* Nitrosoglutathione reductase
HSP	Heat shock protein
JA	Jasmonic acid
JAR1	Essential gene in JA signaling

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LR	Lateral root
LRP	Lateral root primordia
MAPK	Mitogen activated protein kinase cascade
NO	Nitric acid
NR	Nitrate reductase
PAT	Polar auxin transport
RAM	Root apical meristem
RCD 1	Radical-induced cell death 1
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SNAT	Serotonin *N* acetyl transferase
TF	Transcription Factor
T5H	Tryptophan 5 hydroxylase
TDC	Tryptophan decarboxylse
TIBA	Tri-iodo benzoic acid
WT	Wild type

1 Introduction

The indole amines melatonin (*N* acetyl 5 methoxy tryptamine) and its precursor serotonin (5-hydroxy tryptamine) are naturally occurring signaling molecules that mediate a range of physiological activities in humans, animals and plants (Arnao 2014; Erland et al. 2015).

In plants serotonin was discovered in the medicinal herb *Mucuna pruriens* L. (Bowden et al. 1954) in 1954. Melatonin was isolated from bovine pineal gland in 1958 (Lerner 1958) and was considered to be a molecule unique to the animal kingdom until it was identified in the unicellular dinoflagellate *Lingulodinium polyedrum* (syn. *Gonyaulux polyedra*) (Balzer and Hardeland 1991). In higher plants melatonin was detected in the ivy morning glory (*Pharbitis nil* L. syn. *Ipomoea nil* L.) and fruits of *Solanum lycopersicum* (van Tassel and O‘Nielle 1993; van Tassel et al. 1995) in *Chenopodium rubrum* (Kolar et al. 2003) and in *Nicotiana tabacum* and many edible plants (Dubbels et al.1995; Hattori et al.1995).

Melatonin is present in more than 300 plant species representing almost all angiosperm families (Paredes et al. 2009; Simlat et al. 2018; Yan et al. 2020), while serotonin is reported from a fewer but significant number of plants (Gonzalez-Gomez et al. 2009; Huang and Mazza 2011), including many edible, medicinal and horticultural plants (Chen et al. 2003; Reiter et al. 2007; Jemima et al. 2017; Arnao and Hernadez-Ruiz 2018; Yan et al. 2020) in endogenous concentrations ranging from pico to micrograms per g of dry tissue (Jemima et al. 2017; Mir et al. 2020).

Melatonin occurs in almost all plant tissues—root, shoots, leaves, flowers, fruits, seeds and bulbs (Nawaz et al. 2016). Melatonin levels are usually high in seeds and low in fruits and show a gradient from high to low in seeds, leaves, roots, flowers and fruits in many plants (Arnao 2014). Endogenous concentration of melatonin can vary depending on the genotype, external factors like temperature, photoperiod and developmental stage (Zhao et al. 2012). Endogenous levels of melatonin are enhanced by biotic and abiotic stress (Reiter et al. 2015). A concentration gradient of melatonin, from apical to basal parts of the hypocotyl and root, similar to the gradient exhibited by auxins, was seen in *Lupinus albus* L (Arnao and Hernandez–Ruiz 2006).

Serotonin has been found in roots, leaves, fruits and seeds of several plant species (Erland et al. 2018). The endogenous content of serotonin is also known to vary in response to developmental and seasonal changes, under light and dark conditions, and is upregulated in response to biotic stress (Ramakrishna et al. 2011; Ishihara et al. 2008). In Rice, two TDC-like enzymes involved in the biosynthesis of melatonin and serotonin from tryptophan, encoded by LOC_Os08g04540-*TDC1* and LOC_Os08g04560-*TDC3*, are highly induced by both abiotic and biotic stresses. Both *TDC1* and *TDC* 3 are induced by a broad spectrum of pathogens (Dharmawardhana et al. 2013). Melatonin is a good bio-stimulator, improving not only seed germination, seedling/plant growth but also crop production especially under stress conditions.

Endogenous concentrations of melatonin and serotonin are reported to increase at specific stages of flower development in *Datura metel* L. (Murch et al. 2010) and synchronously with circadian rhythms in *Chara australis* (Beilby et al. 2015).

Melatonin and serotonin influence almost all aspects of a plants growth and survival by regulating germination and seedling development (Simlat et al. 2018), morphogenesis (Ramakrishna et al. 2009), parthenocarpy (Liu et al. 2018) root induction, growth and architecture and tropic responses (Pelagio-Flores et al. 2011, 2012; Arnao and Hernandez-Ruiz 2014), delaying senescence (Kang et al. 2009) flowering (Kolar et al. 2003), tolerance to biotic (Ishihara et al. 2008) and abiotic stress (Posmyk et al. 2008; Lei et al. 2004; Bajwa et al. 2014) improving iron deficiency tolerance by inducing Fe-responsive gene expression (Wan et al. 2018) Serotonin is present in the xylem and phloem parenchyma cells suggesting its involvement in maintaining the cellular integrity for facilitating efficient nutrient recycling (Ramakrishna et al. 2009).

Melatonin is an evolutionarily ancient, conserved molecule. As a strong antioxidant (Tan et al. 2015) and a scavenger of reactive oxygen and nitrogen species, melatonin protects plants from oxidative stress and stress due to salinity, heavy metals, drought, extreme temperature, herbicides, pathogens and senescence (Mukherjee et al 2014; Bajwa et al. 2014); Lei et al. 2004; Hardeland 2009; Arnao 2014; Shi et al. 2015b; Pelagio-Flores et al. 2016). Melatonin accumulation in response to these factors acts as a signal to activate transcription factors and antioxidant genes that mitigate these diverse biotic and abiotic stresses. The growth promoting role of melatonin was demonstrated in *Lupinus* albus (Hernandez-Ruiz et al. 2004). In the rooting processes of primary, secondary and adventitious roots, melatonin regulates the expression of many factors, such as PIN auxin transporters and AUX1, and others (Arnao and Hernadez-Ruiz 2018; Wen et al. 2016; Wang et al. 2016).

2 Biosynthetic Pathway

In plants, the indoleamines melatonin and serotonin are synthesized primarily in the root through a common biosynthetic pathway operative in chloroplasts and mitochondria (Tan et al. 2013; Tan and Reiter 2019; Wei et al. 2021). Tryptophan, biosynthesized through the shikimic acid pathway, is the common precursor for the synthesis of serotonin, melatonin, auxin as well as indole alkaloids. TDC is a rate limiting step in melatonin biosynthesis (Zhao et al. 2018).

The biosynthetic pathway for serotonin and melatonin was first described by Murch et al. (2000) in *Hypericum perforatum* L. through the use of C14 labeled tryptophan. The pathway (classical pathway) is almost identical in animals and plants. An alternate pathway for melatonin biosynthesis was proposed (Tan et al. 2016; Geun-Hee et al. 2017) Melatonin is synthesized from tryptophan through four reaction steps involving several enzymes (Fig. 1).

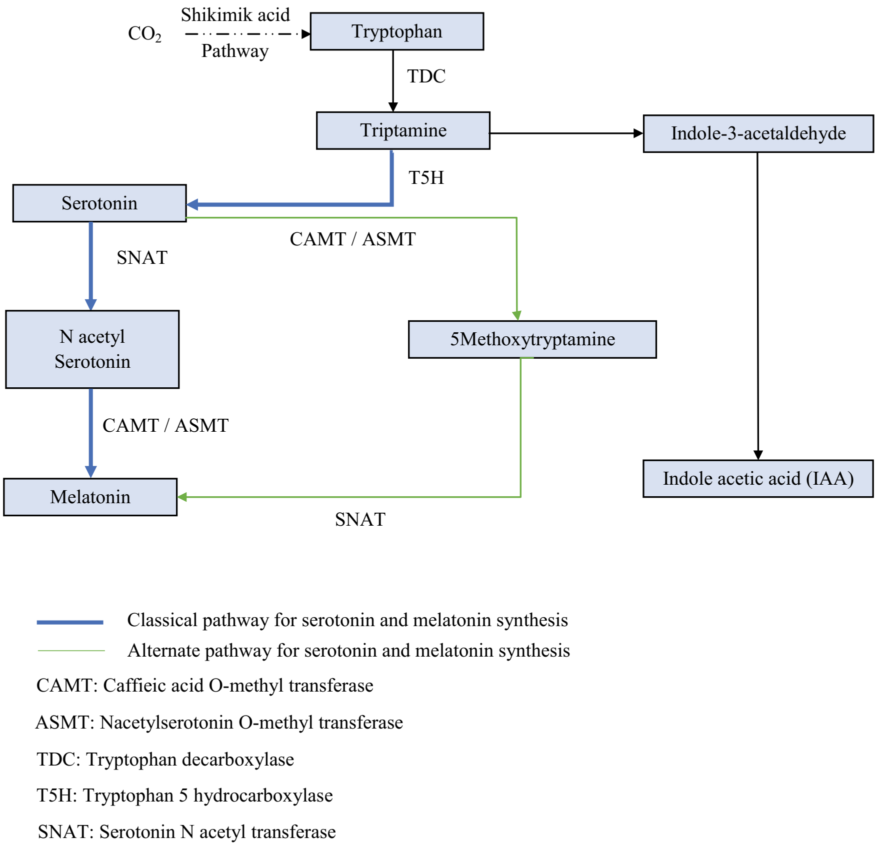


Fig. 1 Biosynthetic pathways of serotonin, melatonin and IAA in plants

Genes of the biosynthetic pathway have been identified and cloned in rice, apple, cassava (Kang et al. 2012; Lee et al. 2014; Lei et al. 2013; Wei et al. 2021). Melatonin can regulate its own biosynthesis by upregulating the gene expression SNAT, ASMT, and CAMT. Over-expression of TDC increases the content of 5-hydroxytryptamine and also confers stress resistance (Kang et al. 2007; Yu et al. 2019; Moustafa-Farag et al. 2020).

Upstream TF s of some melatonin biosynthesis enzymes have been identified. These include heat shock factor 20 (Me Hsf20), Me WrKY79 which bind to the promotor region of MeASMT 1. Transcripotome analysis has revealed the network between melatonin biosynthetic enzymes and signaling pathways for stress resistance (Wei et al. 2021). Melatonin biosynthetic enzymes also interact with ascorbate peroxidase resulting in improved oxidative stress resistance (Bai et al. 2020). Abiotic stress induces an increase in endogenous melatonin through the upregulation of melatonin biosynthetic genes. This is a useful strategy, given the strong antioxidant property of melatonin.

Melatonin can be metabolized to hydroxyl melatonin, β hydroxyl melatonin or converted to a cyclic form. Several studies have reported that the melatonin metabolite, cyclic-3-hydroxymelatonin, is more potent than melatonin to scavenge hydroxyl radical and other ROS (Tan et al. 2015).

3 Role of Melatonin and Serotonin in Rooting

Root initiation, development and architecture are critical for the growth and survival of plants. Besides anchorage, roots source water and nutrients from the soil and are exposed to a multitude of biotic and abiotic stress. Roots are the primary interface between the plant and the soil, and they sense and respond to unfavorable soil environments, enabling plants to overcome these stress related challenges. The primary root (PR) is embryogenic in origin, being formed from the radical and developed by the activity of the root apical meristem. Lateral roots (LR) are initiated from primordia formed on the pericycle of the primary root (Pelagio-Flores et al. 2011). The lateral roots (LR) in turn produce many more LRs which together form a robust root system. Adventitous roots (AR) are formed by redifferentiation of meristematic tissue at the base of the stem, after removal of the primary root system. Auxins establish a new meristem at the base of the stem (Pagnussat et al. 2004). Root hairs arise from specialized epidermal cells called trichoblasts, and vastly increase the root surface area (Slovak et al. 2016).

The redirection of plant growth is initiated by changes in the relative ratio of plant growth regulators, auxin and cytokinin (Skoog and Miller 1957). Cytokinin signaling leads to the formation of embryonic root but cytokinin induced disruption of the auxin gradient leads to the formation of lateral roots (Werner et al. 2009). The control of post-embryonic root growth and LR formation is tightly regulated by auxin (IAA). IAA moves throughout the plant in the phloem or by a more controlled polar transport system (polar auxin transport (PAT) (Baluska et al. 2010). PAT is regulated by AUXIN RESISTANT 1/LIKE AUX1 (AUX1/LAX) uptake proteins, PIN-FORMED (PIN) efflux carriers and P-GLYCOPROTEIN (MDR/PGP/ABCB) efflux/conditional transporters (Swarup et al. 2008; Mravec et al. 2009; Slovac et al. 2016).

Differentiation is now recognized as a complex process regulated by multiple endogenous factors like hormonal interactions and signals, nutrient status and external factors like biotic and abiotic stress and light and temperature regimes (Casimiro et al. 2003; Lopez-Bucio et al. 2003; Peret et al. 2009).

The roles of melatonin and serotonin in plant growth and differentiation is well documented (Hardeland 2015; Erland et al. 2015, 2018; Arnao and Hernandez-Ruiz 2018). Exogenous application of melatonin and serotonin promotes/inhibits elongation of the primary root and initiation and growth of lateral and adventitious roots in many plant species often resulting in an altered root architecture.

Murch et al. (2001) published one of the earliest reports on the effect of melatonin and serotonin on root organogenesis in the medicinal plant St. John's wort (*Hypericum perforatuum* L.). Low concentrations of IAA and melatonin decreased de novo root formation, while increased levels of melatonin led to a corresponding increase in root initiation. Increased serotonin levels increased shoot formation, indicating that a balance between endogenous levels of melatonin and serotonin regulates morphogenesis in plants.

In *Lupinus alba* (Hernandez–Ruiz et al. 2004) melatonin induced LR and AR formation from pericycle and stem cuttings at a similar concentration as IAA. The growth response was concentration dependent and exogenous melatonin could replace the auxin stimuli when the apical meristem was excised. This was the first experimental data that clearly demonstrated the auxinic role of melatonin.

IAA and melatonin induced rhizogenesis in hypocotyl cultures of *Lupinus albus* through the initiation of root primordia on the pericycle., producing an increased the number of newly formed lateral roots. The number and length of adventitious roots were also enhanced which impacted the root structure (Arnao and Hernández-Ruiz 2007). Since then a number of reports have confirmed the role of MT as well as 5HT in the induction of lateral and adventitious roots in a number of diverse species including *Arabidopsis thaliana*,*Brassica juncea*,*Vigna radiata*,*Oryza sativa*, *Prunus* and *Malus* species (Table 1).

4 Melatonin and Serotonin—Auxin like Function in Root Induction?

IAA and MT are structurally related, both are biosynthesized from tryptophan and elicit similar responses (Murch et al. 2000; Tan et al. 2016). In many plants exogenous MT up regulates endogenous IAA levels (Chen et al. 2009; Wang et al. 2016). However, the IAA content decreased in transgenics over expressing MT biosynthesis genes (Zuo et al. 2014). IAA improves root growth in a dose dependant manner, with

Table 1 Effect of melatonin and serotonin on root induction and growth

	Plant	Treatment	Observation	Reference
1	*Arabidopsis thaliana* transgenic line	Ten-day-old seedlings treated with melatonin/IAA/NAA	Enhanced number of lateral roots	Koyama et al. (2013)
2	*Arabidopsis* Ecotype Col-o	In vitro grown seedlings cultured on 10–300 µM serotonin	LR development at low concentrations (10–160 mM) higher concentrations inhibited PR growth and LR development	Pelagio-Flores et al. (2011)
3	*Arabidopsis thaliana* Col0, Ws and Ler ecotypes)	Etiolated hypocotyl from in vitro grown seedlings	melatonin did not inhibit primary root growth even at 200 µm, increased lateral root number and density in all three ecotypes	Pelagio-Flores et al. (2012)
4	*Arabidopsis* ecotype Columbia (Col-0), mutant and wildtype lines	In vitro raised seedlings	serotonin-induced root growth inhibition due to a ROS imbalance at the root tip in a process mediated by RCD1 and Et–JA crosstalk	Pelagio-Flores et al. (2016)
5	*Arabidopsis* Ecotype Col-o	3-day-old in vitro grown seedlings	Melatonin suppressed Pr growth in a dose dependent manner. Inhibited polar auxin transport	Wang et al. (2016)
6	Ecotype Col-o *Arabidopsis*	Melatonin and serotonin applied exogenously	PR growth unaffected LR formation increased at higher dose	Wan et al. (2018)
7	*Arabidopsis thaliana*	In vitro seedlings treated with ten pM to 500 micro-M Melatonin	Primary growth inhibited, lateral root growth enhanced in a dose dependent manner. Melatonin altered expression of 16 auxin related genes	Ren et al. (2019)

(continued)

Table 1 (continued)

	Plant	Treatment	Observation	Reference
8	*Brassica juncea* L	2 and 4 days old seedlings	0.1 microM melatonin stimulated root growth, 100 microM inhibitory for 2d old seedlings	Chen et al. (2009)
9	*Cucumis sativus* L	Seedlings treated with PEG and melatonin	Increase in number of lateral roots compared to control	Zhang et al. (2012)
10	*Cucumis sativus* L	Seeds primed in melatonin germinated in DW/PEG/Melatonin	n Melatonin improved root volume, diameter, improved water stress tolerance	Zhang et al. (2013)
11	Cucumis sativus L. cv. Jingyu-1	Seeds subjected to Nacl stress, treated with melatonin	Radical emergence 0.1–100 microM Melatonin reduced the inhibitory effects of high salinity on germination	Zhang et al. (2014)
12	St. John's wort (*Hypericum* perforatum L.)	In vitro raised shoot explants treated with IAA, melatonin, serotonin	Rooting increase in the endogenous concentration of melatonin increased root formation increased serotonin levels increased shoot formation	Murch et al. (2001)
13	*Hypericum perforatum L.* germplasm lines created by mutation and haploid breeding	medium supplemented with melatonin, serotonin, trptophan, intermediates of the biosynthetic pathway	Partially recovered growth and regenerative recalcitrance of the germplasm	Erland et al. (2018)
14	*Hypericum perforatum* L	Root explants from WT/anther culture (line112/mutant line 4 on modified MS medium with 10 μM tryptophan, or IAA	Shoot formation increased in line 112 but inhibited in line 4. Differential effect of short pulse and prolonged exposure	Erland and Saxena (2019)
15	*Helianthus annuus*	2.4.6.day old seedlings treated with melatonin, serotonin and Na Cl	Elongation of primary root	Mukherjee et al. (2014)

(continued)

Table 1 (continued)

	Plant	Treatment	Observation	Reference
16	*Hordeum vulgare*	Seeds germinated for 72 h on filter paper soaked with serotonin	Serotonin increased root length but had no effect on root weight and mitotic index	Csaba and Pal (1982)
17	*Lupinus albus L.* KBSH 44	6-day-old de-rooted lupin hypocotyls treated with melatonin and IAA	Formation of root primordia from pericycle cells, development of lateral and adventitious roots	Arnao and Hernández-Ruiz (2007)
18	*Malus prunifolia*	Nodal explants treated with melatonin. IBA	AR formation through increased function of *MdWOX11*	Mao et al. (2020a)
19	*Oryza sativa* cv. Dongjin (WT and transgenics expressing sheep SNAT)	3, 10 day old seedlings of WT and T3 transgenic lines	In transgenic lines, seminal root length was 75% longer, and root biomass increased 44% compared to WT In WT melatonin stimulated root growth at lower concentrations, inhibitory at higher concentrations	Park and Back (2012)
20	*Oryza sativa* ssp. Japonica	Seedlings in hydroponic cultures with melatonin (0, 10, 20, and 50 µmol/L)	embryonic and crown root elongation inhibited, number and length of LR increased in 10 and 20 µmol/L melatonin	Liang et al. (2017)
21	cherry rootstock PHL-C (*Prunus avium* L. x *Prunus cerasus*	Shoot explants (from in vitro regenerated plants) treated with melatonin	Melatonin increased root induction and root length Inhibitory at higher concentrations	Sarropoulou et al. (2012a)

(continued)

Table 1 (continued)

	Plant	Treatment	Observation	Reference
22	in vitro cultures of (*Prunus cerasus* L.), Gisela 6 (*P. cerasus, P. canescens*), and M, M 60 (*P. avium, P. mahaleb*	Shoot explants from in vitro regenerated plantlets	Melatonin increased the number roots number, length and percentage of rooting in three commercial cherry rootstocks at low concentrations. inhibited root growth, at higher concentrations	Sarropoulou et al. (2012b)
23	*Punica granatun* cv Wonderful	Shoot cuttings from four year old plant	Melatonin increased number and length of roots, improved rooting percentage	Sarrou et al. (2014)
24	*Solanum lycopersicum*	10 day old de- rooted seedlings treated with melatonin, NO donours and scavenger	Exogenous melatonin induced ARF, NO accumulation	Wen et al. (2016)
25	*Juglans nigra* x *Juglans regia* clone A35	Shoot explants from in vitro raised plants	Endogenous serotonin increased in the shoot top of shoots that rooted in an auxin containing medium. The level of serotonin was unchanged at the bases of these shoots	Gatineau et al. (1997)
26	Leaves of Aspen (*Populus tremuloides* x P. *trémula)*	Aspen leaves treated with crude extract of serotonin from embryos of *Juglans ailanthifolia* var. ailanthifoliaCarr	Serotonin influenced rhizogenesis in Aspen leaves cultivated in *vitro* to the same extent as indole-3-acetic acid	Regula et al. (1989)
27	*Vigna radiata*	Melatonin pretreated 3d old seedlings, chilled at 5 °C for 2 days and transferred to 25 °C	Root growth after transfer to 25 °C. 20% increase in root length in MT treated seeds over control	Szafrańska et al. (2012)
28	*Vitis vinifera* L Rootstock 5BB and cv. Cabernet Sauvignon	2-bud cuttings with basal ends dipped indifferent concentration of melatonin	Root induction genotype dependant. MT decreased rooting compared to IAA	Gokbayrak et al. (2020)

an inhibitory effect at higher concentrations. The root promoting effect of melatonin is also expressed in the formation of lateral and adventitious roots as a dose dependant response, but the stimulatory and inhibitory concentration may vary from plant to plant-eg. melatonin is inhibitory to cherry root stock at5mM whereas it is inhibitory at 100 mM in *Brassica juncea* roots (Sarropoulou et al. 2012a, b; Chen et al. 2009).

There are fewer reports of a stimulatory effect of melatonin and serotonin in the growth of primary roots. The growth-promoting effect of melatonin is high when a stress condition affects plant development, as in the case of salinity induced stress in *Helianthus* (Mukherjee et al. 2014) *Zea mays* (Ren et al. 2020; Su et al. 2021) and *Cynodon* (Oxidative stress) (Shi et al. 2015a, b, c), *Arabidopsis* (cold stress) (Bajwa et al. 2014). Arnao and Hernandez-Ruiz (2006) proposed that melatonin may have auxin like functions in the regulation of plant growth and development.

In *Arabidopsis thaliana* (Pelagio-Flores et al. 2012) primary root growth was unaffected at high concentrations (600-lm) of melatonin, but the number of lateral roots increased three -fold over the control at lower concentrations (150–600-lm) of exogenously supplied melatonin. The CycB1:uidA marker which is active only in mitotic cells, was not expressed in primary root tips. The marker was expressed in lateral root primordia (LRP) at the development stage 1 (as defined by Malamy and Benfey 1997). The lateral roots were produced by the maturation of preformed LRPs. Melatonin also failed to activate the auxin mediated degradation of the Aux/IAA protein indicating that melatonin action was through an auxin independent signaling pathway (Pelagio-Flores et al. 2012). In this study melatonin modulated root system architecture by stimulating lateral and adventitious root formation but minimally affected primary root growth and root hair development.

Pelagio-Flores et al. (2011) also investigated the role of serotonin in rhizogenesis and root growth in WT and transgenic lines *of Arabidopsis thaliana.* Serotonin treatment stimulated LRP formation by decreasing auxin responses during LRP development. Exogenous application of serotonin inhibited root developmental processes which are under auxin control, such as primary root growth, LR formation and root hair development. Serotonin blocked auxin-responsive DR5:uidA and BA3:uidA gene expression and auxin-regulated LR formation. Mutant analyses indicated that serotonin inhibited primary root growth and promoted adventitious root formation independently of the auxin-related loci axr2-1, axr4-1 and aux1-7 but required AXR1 and AXR2. This indicated that serotonin regulates root development probably by acting as a natural auxin inhibitor (Pelagio-Flores et al. 2011).

NaCl stress blocked IAA biosynthesis/transport resulting in accumulation of serotonin and melatonin in the roots and cotyledons of *Helianthus annuus* indicating that NaCl-induced endogenous serotonin accumulation possibly regulates root growth, independent of auxin action (Mukherjee et al.2014).

In *Arabidopis* moderate concentrations of melatonin and serotonin did not affect primary root (PR) growth but induced lateral root (LR) formation through the expression of cell-wall-remodeling genes *LBD16* and *XTR6* (Wan et al. 2018). The authors concluded that melatonin and serotonin do not have auxin-like activity.

5 Insights from Gene Expression Patterns

Techniques like RT PCR and in silico analysis pinned the role of melatonin and serotonin in mediating morphogenesis through altered transcription and gene expression patterns. Wang et al. (2016) found high concentration (600 μM) of melatonin inhibited root growth in *Arabidopsis thaliana* by reducing root meristem and down regulating auxin biosynthesis, the expression of PINFORMED (PIN-PIN1/3/7) proteins as well as the auxin response. The PIN formed proteins are secondary transporters involved in the efflux of auxin from cells. Transcription and protein expression levels of Pin 1,3 and7 were down regulated. Melatonin mediated decrease of the root apical meristem (RAM) was not altered by the auxin transport inhibitor TIBA suggesting that melatonin altered RAM by influencing polar auxin transport (PAT). Expression of key auxin biosynthesis genes (YUC1, YUC2, YUC5, YUC6 and *TAR2*) was also down regulated. This combined effect of melatonin- decreased auxin biosynthesis and altered PAT, resulted in a reduced root apical meristem and inhibited root growth. The authors proposed that melatonin regulates root growth in *Arabidopsis*, through auxin biosynthesis and polar transport, which cause optimal auxin accumulation and distribution in the root apex during the developmental process (Wang et al. 2016) In another study in *Arabidopsis* (Wan et al. 2018) abundance of the auxin carrier AUX1 and PIN 1,2,4,7 was unaltered in response to moderate concentrations of melatonin and serotonin. In *Malus domestica* increased IAA levels and overexpression of MdWOX11 led to increased AR formation in transgenic lines (Mao et al. 2020a, b).

Transcriptome analysis showed that melatonin regulates root development in a partially auxin-dependent manner in rice (Liang et al. 2017). Genome wide expression profiling by RNA-sequencing revealed that a total of 120transcription factors (TF), were up- or down-regulated in the melatonin treated samples compared with the control. The expression of roughly 25 auxin-induced TFs were upregulated whereas the expression of several auxin-inhibited TFs were down–regulated. Among 44 co-up- or co-down- regulated TFs, 21 genes, were specifically or primarily expressed in roots identifying these TFs as key regulators of melatonin signaling pathway (Liang et al. 2015, 2017).

Melatonin induced lateral roots had improved osmic tolerance (Zhang et al. 2013) Analysis of RNA seq profiles in melatonin treated seedlings of *Cucumis sativus* (Zhang et al.2014) revealed differential expression of transcription factors. Ethylene-responsive transcription factors and NAC domain containing proteins were down-regulated by melatonin. In WRKY (a stress related TF) over-expressed lines, lateral root formation was upregulated. Genes that participate in cell wall biogenesis were up-regulated. Melatonin also up regulated peroxidase which controls cell elongation in roots through its auxin oxidase activity. The authors concluded that melatonin affected LR formation in an auxin independent manner (Zhang et al. 2014).

Genome wide expression profiling by RNA sequencing in rice showed that root architecture is modulated by melatonin through auxin signaling pathways (Liang et al. 2017). Melatonin activated the expression of many auxin induced TFs amongst which 21 genes showed a root specific expression. Many of the DEGs were involved in auxin stimulus response and the auxin mediated signaling pathway. Melatonin induced modification of root architecture through increased length of root hairs and an increase in the number of roots, is a well characterized auxin response (Overvoorde et al. 2010; Liang et al. 2017).

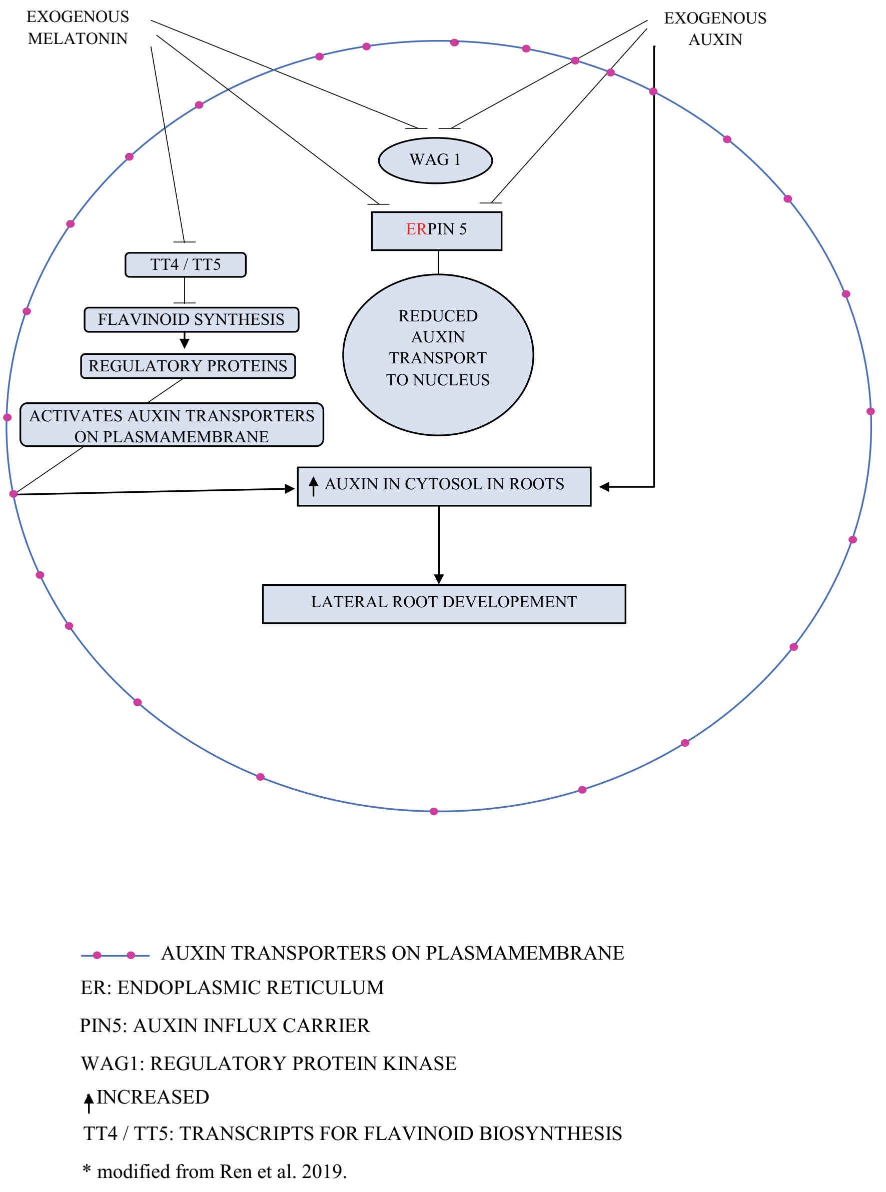
However, according to Pelagio-Flores et al. (2012), melatonin does not regulate AXR3/IAA17 nor activate auxin-inducible gene expression in root development in *Arabidopsis*. The differences in the observation of the two groups may be due to the differential expression profiles of auxin related genes or due to species specific responses (Liang et al. 2017).

According to Yan et al. (2020) melatonin-induced root development is independent of IAA in the signal transduction pathway but melatonin can promote root development through IAA synthesis, polar transport and hormone perception.

Transcriptome analysis revealed that auxin metabolism-related genes exhibited minimal changes in melatonin-treated *Arabidopsis* plants with respect to untreated plants. Only one IAA-amino synthase was upregulated, with no change in the expression of auxin biosynthesis genes (Weeda et al. 2014). Several auxin influx carrier proteins (AUX1/LAX) were down regulated in response to melatonin. AUX 1 regulates lateral root development, root hair development and the gravitropic response (Swarup and Péret 2012) Several efflux genes (PIN 1,2 and3) and auxin signaling transduction genes (IAA19 andIAA24) were upregulated. (Weeda et al. 2014; Arnao and Hernández-Ruiz 2015a, b, c). The same auxin PIN proteins (Pin 1/2/3) were down-regulated in *Lycopersicon* (Wang et al. 2016). In the roots of lupin and some monocots a melatonin gradient similar to IAA gradient is operative (Hernandez-Ruiz and Arnao 2008).

Melatonin and auxin share structural similarity and a common precursor, but there is lack of consensus on the signaling pathway of the two molecules (Wang et al. 2016; Wen et al. 2016; Pelagio-Flores et al. 2012; Ren et al. 2019).

In *Arabidopsis thaliana* (Ren et al. 2019) identified 16 auxin related genes whose expression was altered on exogenous application of melatonin. Many genes coding for auxin transport (PIN5, TT4, TT5)and LAX2 and the auxin /IAA proteins (IAA3 and IAA 17) were down regulated indicating that melatonin modulates lateral root development by regulating the intracellular distribution of auxins. In the *Arabidopsis* ecotype Col-0, auxin and melatonin acted synergistically to promote lateral root envelopment but in the ecotype Ler-O, the two indoleamines had an additive effect which was not expressed in the knock out mutants. The authors proposed a model (Fig. 2) to explain auxin, melatonin reactions in lateral root development. Auxin transporters present in the plasma membrane allow inflow of exogenous auxin into the cell. Exogenous auxin and melatonin inactivates the PIN 5 influx carrier located in the endoplasmic reticulum directly or indirectly through the protein kinase WAG1, thereby inhibiting auxin transport from the endoplasmic reticulum to the nucleus. Exogenous melatonin also down-regulates flavonoid biosynthesis by reducing TT4and TT5 transcripts. Down regulation of flavonoids activates auxin transporters in the plasma membrane. This dual action of melatonin—inactivating PIN 5 and activating auxin transporters in the plasma membrane through the TT4



—●—●— AUXIN TRANSPORTERS ON PLASMAMEMBRANE

ER: ENDOPLASMIC RETICULUM

PIN5: AUXIN INFLUX CARRIER

WAG1: REGULATORY PROTEIN KINASE

↑INCREASED

TT4 / TT5: TRANSCRIPTS FOR FLAVINOID BIOSYNTHESIS

* modified from Ren et al. 2019.

Fig. 2 Proposed mechanism of auxin/melatonin interaction in lateral root development (Ren et al. 2019) and TT5 transcripts, results in a high level of auxin in the cytosol, leading to calcium signaling and increased lateral roots. Cross talk between melatonin and the flavanoid pathway regulates lateral root development (Ren et al. 2019). (with permission provided under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.)

6 Gravitropic Response—An Auxin Like Response to Melatonin

Application of either IAA or melatonin enriched agar blocks elicited a gravitropic response in roots of *Lupinus*. Disruption of the endogenous level of auxin or melatonin through the agar blocs resulted in a gradient that induced the gravitropic response (Arnao and Hernandez-Ruiz 2017). Roots of rice seedlings also showed a gravitropic curvature when treated with exogenous melatonin. The difference in tip angles between the treatments and the control, showed that melatonin modulates root growth through effects on the auxin signaling pathway (Liang et al. 2017).

7 Nitric Oxide, Auxin and Melatonin Signaling Pathways in Root Induction

NO (nitric oxide) and auxin signaling pathways are interconnected in regulating several plant responses. NO and auxins interact to regulate growth, development, and morphology of plant roots (Sanz et al. 2015). In the auxin -regulated formation of adventitious roots in cucumber hypocotyl cuttings, NO interaction with auxins involve the regulation of Ca^{2+}—dependent protein kinase (CDPK) activity. Ca^{2+} and CDPK act as downstream messengers in the signaling pathway triggered by auxins and NO to promote AR development (Pagnussat et al. 2002). NO activates at least two different pathways during the induction of AR (cGMP-dependent and cGMP independent pathways) that involves a MAPK signaling cascade. The MAPK signaling cascade involved in ARF in cucumber explants is cGMP-independent (Pagnussat et al. 2004).

NO donours can mimic the effect of auxin suggesting an important role of NO in auxin induced processes (Chen and Kao 2012). Melatonin increases the nitric oxide (NO) level through the upregulation of nitrate reductase (Arnao and Harnedez-Ruiz 2018). NO as a down stream signal is involved in melatonin induced AR formation in *Solanum lycopersicum* (Wen et al. 2016). In S*olanum lycopersicum* melatonin triggered NO production by upregulating nitrate reductase and down regulating the expression of GSNOR. NO induced auxin accumulation led to ARF induction. Enhanced endogenous concentrations of IAA and IBA, expression of several genes of the auxin signaling pathway–auxin carriers (PIN1,3,7) and auxin signaling transduction genes (IAA19, IAA24) in response to melatonin, indicated that melatonin influenced auxin transport, accumulation and signal transduction through the NO signaling pathway (Wen et al. 2016; Arnao and Harnedez-Ruiz 2018). NO is required for growth and development of roots; Sanz et al. 2015). NO can modify hormonal action by chemical modification of transcription factors/chemical modification of proteins (enzymes) or by directly interacting with plant hormones, leading to changes in hormone levels and signaling patterns (Asgher et al. 2017).

8 Tryptophan as an Inductive Signal

Tryptophan is the precursor of three classes of growth modulators—auxins as well as serotonin and melatonin. Erland and Saxena (2019) proposed that tryptophan also functions as an inductive signal and triggers diverse morphogenetic pathways. Exogenous application of tryptophan resulted in root formation while IAA treatment resulted in de novo shoot formation. Trptophan appeared to have altered the melatonin and serotonin balance and serotonin acted as a transient signal modulating diverse morphogenetic pathways (Erland and Saxena 2019).

9 Stress and Rhizobiology: Role of Melatonin and Serotonin

Roots being the interface between the soil and the plant is subject to diverse biotic and abiotic stress. Roots perceive the stress and respond, often through enhanced endogenous melatonin production, in an effort to alleviate the stress induced harmful effects. Melatonin is a powerful antioxidant and has a cascade effect i.e. melatonin through its secondary and tertiary derivatives is able to neutralize many toxic oxygen derivatives (Tan et al. 2015). One melatonin molecule can scavenge up to ten reactive oxygen species (ROS).Melatonin produced as a response to external stress improves the survival of plants under such conditions. The growth-promoting effect of melatonin is high when a stress condition affects plant development, as in the case of root growth in *Helianthus annuus* (Mukherjee et al. 2014).

Growth inhibition of roots can occur due to deficiency in auxin concentrations caused by disruption of the acropetal gradient of PIN proteins (Sun et al. 2008) In *Arabidopsis thaliana* high salt stress enhanced the proliferation of LR due to accumulation of auxin in the developing primordia in response to salt stress (Zolla et al. 2010). ABA synthesis and ethylene signaling network was also involved in the response.

Exogenous stress disturbs the ion and redox homoeostasis in plants, resulting in the accumulation of ROS (reactive oxygen species) and RNS (reactive nitrogen species). ROS and RNS play a dual role—they are harmful at higher concentrations, damaging membranes, organelles and even nucleic acids but at lower concentrations they act as signaling molecules that re-establish homoeostasis (Arnao and Hernandez-Ruiz 2019a, b). ROS production is needed by plants as it functions as a secondary messenger in signal transduction (Baxter et al. 2014). Melatonin biosynthesis is upregulated by external stress and increased levels of endogenous melatonin mitigate the effect of the stress by directly scavenging ROS/RNS or indirectly by upregulating the expression of genes coding for antioxidant enzymes—catalase/peroxidase/superoxide dismutase etc. (Arnao and Hernandez-Ruiz 2020a, b). Weeda et al. (2014) showed that plants vary in their sensitivity to melatonin and some genes are regulated by low concentrations of melatonin while others are regulated by higher concentrations.

Melatonin production, induced by abiotic stress also increases the level of NO by upregulation of nitrate reductase. Melatonin and NO induce changes in hormonal levels and also alter the expression of Tfs and hormone signaling elements that lead to easing the stress (Arnao and Hernandez-Ruiz 2018).

In *Zea mays* seedlings exogenous melatonin enhanced salt tolerance through osmotic adjustment, ion balance, and alleviation of salt-induced oxidative stress (Ren et al. 2020). Melatonin alleviated high salinity and proline induced water stress and promoted seed germination in *Cucumis sativus* by upregulating the activity of ROS scavenging enzymes (Zhang et al. 2012, 2013). Melatonin promoted the expression of stress tolerant proteins as well as proteins involved in ATP production and promoted the degradation of storage proteins to produce energy for germination of *Cucumis sativus* seeds. Melatonin also regulated heat shock proteins to protect seed germination under salt stress (Zhang et al. 2017).

In mutant and transgenic lines of *Arabidopsis thaliana*, stress due to incubation with Hydrogen—rich water stimulated the expression of the hydrogenase gene CrHYD1. A hydrogen signaling cascade upregulated melatonin biosynthesis. Melatonin re-established ion and redox homoeostasis in part through Na^+/H^+ antiport across the plasma membrane leading to improved tolerance to salinity stress (Su et al. 2021). The authors concluded that melatonin acts downstream to the hydrogen signaling cascade.

Melatonin is known to re-establish ion/redox homoeostasis. In *Helianthus annuus* seedlings melatonin eliminated the harmful effect of ROS and RNS by the modulation of two superoxide dismutases (SOD) Cu/Zn SOD and Mn SOD (Arora and Bhatla 2017). Melatonin also affects the catabolism of hormones like GA and ABA and increases the expression of genes down regulated by salt stress (Kaur et al. 2015; Reiter et al. 2015).

In response to biotic stress melatonin through its crosstalk with plant hormones activates pathogen related gene expression (Arnao and Hernandez-Ruiz 2018) NO is also produced by melatonin in response to stress (Shi et al. 2015a).

In *Arabidopsis thaliana* melatonin up-regulated the expression of C-repeat-binding factors, Drought Response Element Binding factors, a cold-responsive gene, *COR15a*, a transcription factor involved in freezing and drought-stress tolerance *CAMTA1* and transcription activators of reactive oxygen species (ROS)-related antioxidant genes, *ZAT10* and *ZAT12*, in a response to mitigate cold stress (Bajwa et al. 2014). In *Citrullus lanatus* melatonin treatment given to the roots improved cold tolerance of the leaves. Melatonin was detected in the xylem sap indicating that it is transported from roots to leaves via the xylem. Exogenous melatonin promoted cold-induced up-regulation of genes involved in signal transduction and transcriptional regulation in leaves, but not in roots suggesting that melatonin is involved in the sensing the cold signal and subsequent signal transduction (Li et al. 2017a, b). In *Arabidopsis* high temperature tolerance was achieved through endogenous melatonin production which led to the expression of heat shock factors and heat shock proteins (HSP90, HSP10; Shi et al. 2015b).

Abiotic stress leads to the production of ROS which acts as a second messenger in signal transduction (Baxter et al. 2014).

Melatonin is an antioxidant and can scavenge ROS by triggering the production of endogenous antioxidants or through activating redox—sensitive regulating pathways Melatonin is active in ROS scavenging which results in a good redox balance essential for the development of a robust root system (Yan et al. 2020; Shi et al. 2015c).

In plants, ROS signaling occurs through complex mechanisms and hormone response crosstalk via salicylic acid, jasmonic acid (JA) and ethylene (Et) genetic components (Mittler et al. 2011).

Serotonin is known to regulate developmental processes via ROS scavenging (Ramakrishna et al. 2011). Serotonin induced redistribution of ROS in the root tip mediated through the RCD1 locus and the JA—Et signaling pathway, is responsible for inhibition of primary root growth in *Arabidopsis thaliana.* (Pelagio–Flores et al. 2016). The inhibition of root growth is due to serotonin and not due to the conversion of serotonin to melatonin (Pelagio-Flores et al. 2016).

10 Is Melatonin a Phytohormone?

Melatonin regulates diverse plant processes. Its mode of action is similar to auxin in many aspects it influences root initiation and growth in a dose dependent manner through the establishment of a gradient. Like auxins melatonin can induce the gravitropic response. As a scavenger of ROS and RNS melatonin can mitigate stress through its interaction with downstream signaling molecules and crosstalk with hormones. Melatonin is now viewed as a master regulator or a new plant hormone (Arnao and Hernandez Ruiz 2018, 2020a, b).

The signaling pathway of melatonin and serotonin is not clear as receptors for the two indoleamines have not been discovered in plants. Based on the similarity of serotonin and IAA activity, it is proposed that serotonin may function through auxin receptors on the cell membrane in shared/similar signaling pathways (Mukherjee 2020). Wei et al. (2018), for the first time reported a phytomelatonin receptor (CAND2/PMTR1) in *Arabidopsis thaliana.* The membrane bound receptor mediates the phytomelatonin induced stomatal closure through a H_2 O_2 and Ca^{2+} signaling transduction cascade. Ca^{++} permeable ion channels exist in plants (Zimmermann et al.

1999). The role of calcium channels and calcium ionophore (A23187) in the perception of melatonin and serotonin induced responses has been reported (Ramakrishna et al. 2009).

However, a receptor for melatonin has not been detected in other plants. Lee and Back (2020) claimed that CAND 2 protein is located in the cytoplasm rather than the plasma membrane, is not involved in melatonin induced MAPK activation or in melatonin-mediated defense signaling pathway via G protein components and is therefore not a melatonin receptor. The authors concluded that it may be a melatonin binding protein that can decrease the free melatonin level in cells. So while the role of melatonin and serotonin on plant growth and viability is well established it is still not considered to be a plant hormone in the absence of a receptor.

The ability of melatonin and serotonin to induce root induction and its positive impact on root growth can be used for clonal propagation of improved varieties of plants. Seen in the perspective of their role in promoting growth, protecting photosystem II, delaying senescence and combating stress, melatonin and serotonin have the potential to improve yield and reduce dependence on synthetic chemicals to counter biotic and abiotic stress.

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