Signaling and Communication in Plants

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Neurotransmitters in Plant Signaling and Communication

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Preface

Physiological investigations across the past few decades have substantiated the fact that plants do imply the classical neurotransmitters in various signaling pathways. Plant neurotransmitters (serotonin, melatonin, dopamine, acetylcholine, and GABA) share biochemical similarities with those in animal system in terms of their chemical nature and biochemical pathways. Plant–environment interactions associated with abiotic stress management, growth modulation, flowering, circadian rhythm, fruit ripening, and allelopathic interactions are the major aspects of investigation for plant neurotransmitters. Recent advancements in genomic, transcriptomic, and metabolomic approach have resulted in deciphering the molecular mechanisms associated with various neurotransmitters in plants. According to various analytical investigations and reviews, phytomelatonin is likely to be considered as an upcoming putative phytohormone. Receptor-mediated signaling of plant neurotransmitters is a nascent area of research. The upcoming volume of the *Signaling and Communication in Plants* book series shall provide a comprehensive update on the recent developments of the role of plant neurotransmitters in signaling and communication. The book shall also collate the recently investigated molecular crosstalk mechanisms operative among various neurotransmitters and will update the current understanding of the physiology of plant signaling and communication with environment.

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Seeing is Believing: Quantum Dot Visualization Provides New Insights into Indoleamine Signalling Networks

Lauren A. E. Erland

Abstract Plants have evolved complex and sensitive signalling networks to perceive their environment and rapidly and dynamically respond. Plant signalling molecules, including plant neurotransmitters, control every aspect of a plant's life; however, despite an increasing wealth of knowledge on their roles, functions and mechanisms, it has not been possible to visualize these molecules in living tissues. Determination of the localization of plant neurotransmitters within cells and tissues can enhance our understanding of the functions and mechanisms of these compounds. Quantum dots are UV and fluorescence active nanoparticles which through relatively simple chemical conjugation can be attached to diverse biologically active molecules for fluorescence imaging. They can be used for single molecule or tissue-specific tracking, and conjugation offers one possible means by which direct visualization of these molecules can be achieved.

1 Introduction

Our understanding of plant signalling mechanisms and molecules is increasing exponentially, and the importance of plant neurotransmitters in plant life is becoming increasingly apparent. Molecules such as the indoleamines' melatonin (N-acetyl-5-methoxy-tryptamine) and serotonin (5-hydroxytryptamine) play critical roles in every aspect of plant life from protecting developing embryos during seed development and germination to promoting growth of young seedlings and protecting plants from abiotic and biotic stress (Erland et al. 2015; Arnao and Hernández-Ruiz 2019a). Melatonin and serotonin are potent antioxidants and have been found to have direct antioxidant functions as well as upregulating endogenous antioxidant systems and enzymes such as the ascorbate–glutathione cycle (Arnao and Hernández-Ruiz 2019b). The mechanisms of indoleamines' action continue to be elucidated; they have been found to interact with diverse signalling cascades including map kinase and calcium signalling, other plant growth regulatory pathways and primary

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and secondary metabolite networks (Ramakrishna et al. 2009; Lee and Back 2017; Zhang et al. 2017; Mukherjee 2018). Indoleamines have been found to modify not only the metabolome, but also the proteome, transcriptome and genome (Weeda et al. 2014; Xu et al. 2016). There is still much to uncover in relation to the mechanisms and signalling dynamics of the indolamines and other plant neurotransmitters. One approach to understanding the mechanisms of plant signalling molecules is to understand their location and transport dynamics through the use of fluorescence microscopy and labelling technologies.

Quantum dots (QD) are ultraviolet and fluorescence active nanoparticles which may be functionalized with reactive side chains such as amine, carboxyl and aldehyde functional groups to allow for easy conjugation to biologically active molecules. To date, the reports of the use of QD in the plant system are relatively limited; however, reports have been published both in the plant and animal system on neurotransmitter molecules as well as several other classes of signalling molecules. QD were developed for use in the animal system and have been used in applications ranging from cell tracking during development to monitoring cellular trafficking at synapses (Medintz et al. 2005; Wegner and Hildebrandt 2015). Mammalian neurotransmitters were among the first groups of compounds in which QD conjugation was utilized in the mammalian system, and quantum dot labelling of dopamine (Clarke et al. 2006) has been reported, as well as labelling of receptors for serotonin and glutamate for the study of synapse dynamics (Dahan et al. 2003; Chang et al. 2012; Bailey et al. 2018). Despite their diverse functions and broad applicability in the animal system, QD have been slower to be adopted in plant science applications. Though imaging in the plant system presents unique challenges compared to mammalian cell imaging; in some cases, the specific properties of QD can be exploited to overcome some of the more common challenges such as autofluorescence. With QD microscopy, samples can be allowed to photobleach to reduce background noise without reducing the emission of the QD. Additionally, the highly tunable wavelength of the QD and narrow emission spectra mean that filters of relatively narrow wavelengths can be used, which can avoid overlap with some of the most common plant pigments.

This chapter will (1) provide a brief introduction to quantum dots for the plant researcher, including methods for conjugation; (2) provide an overview of their applications in plants including challenges to their implementation in plants; (3) utilize the indoleamines' melatonin and serotonin as an example of a plant signalling system where QD are shedding new light on plant neurotransmitter function and (4) provide some insights into future directions for the use of QD to understand plant signalling.

2 Quantum Dots

QD represent a new technique to monitor in vivo and in real time the location, transport and trafficking of plant signalling molecules and plant neurotransmitters when coupled with imaging techniques such as electron and fluorescence microscopy. They are approximately 20 nm in diameter, and the most popular QD for imaging in the literature are the new core shell particles which typically are a ZnS/CdSe (or CdTe) complex. QD do not experience photobleaching and are up to 20 times brighter than organic dyes. The size of the QD determines their wavelength with blue or UV range emission being the smallest and red/far-red the largest. They possess wide excitation wavelengths but narrow emission spectra, making them good candidates for multichannel imaging (Medintz et al. 2005). Though early QD had issues with blinking (intermittent fluorescence) at the single dot level, this has largely been resolved in recent years through the use of core shell QD (Lane et al. 2014; Wegner and Hildebrandt 2015) enabling their use in single molecule tracking. QD are generally stable under physiological conditions (aqueous environment pH 6–10) and may be stable in tissues for up to months, though there are some indications that leaching of heavy metals at low levels may be possible from these nanoparticles in plants. There are many well-described published protocols for the synthesis of QD (see Goryacheva et al. 2015 for a review of current methods), as well as many readily available commercial preparations of functionalized and non-functionalized QD from a range of commercial suppliers, the latter of which provides an excellent option for plant and life science researchers. Commercial QD are commonly available in nine wavelengths ranging from UV to far-red. Imaging with combinations of short and long wavelength QD (e.g. blue and red) are the most easily adapted to multichannel images as there is no overlap in emission spectra, allowing the use of non-specific filter sets which may already be available in typical labs (e.g. mCherry, cy3, GFP, DAPI).

2.1 QD Conjugation

The conjugation reaction for functionalized quantum dot is relatively straightforward. Most reports use the same general protocol for conjugation of functionalized QD, which takes advantage of the presence of reactive amine or hydroxyl/carboxyl groups on plant neurotransmitters which can easily be reacted with amine or carboxyl functionalized QD. A simple condensation reaction can then be performed between the two groups through addition of the linker 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). Briefly, commercially available 0.8 mM QD-Carboxyl or QD-Amino (e.g. Thermo Fisher's QDot ITK™) are incubated with the 8 mM target molecule in 10 mM borate buffer (neutral to basic pH) with 1 mM EDC for 2 h. The reaction mixture is then washed through a series of buffer exchanges either through the use of spin filters (Erland et al. 2019b) or dialysis (Whiteside et al. 2009) to remove unconjugated target molecule, and EDC can then be stored at 4 °C in 50 mM borate buffer. Our lab has found the conjugates tested to date to be stable even up to one year from the initial conjugation reaction when stored in these conditions. Water, ethanol and dimethylsulfoxide (DMSO) have all been found to be suitable solvents for the target molecule stock solution and have not been found to interfere with the conjugation process, while presence of any halogenated solvent or functional group is not suitable as it causes crystallization of the QD. Slight modifications to this protocol may be required, depending on the pKa of the target molecule, as the

QD have a tendency to precipitate at low pH levels. The use of slightly basic reaction buffer or dropwise addition of 1N NaOH to the reaction buffer is required until the QD are brought back into solution. Addition of N-Hydroxysuccinimide (NHS) to the reaction solution has also been employed to further improve efficiency of the conjugation process to good effect in several reports (Gao et al. 2013; Wang et al. 2009). Beta-mercaptoethanol has also been used to increase dispersal in aqueous solutions and buffers and enhance stability (Yu et al. 2006). Table 1 provides an overview of ligands in the literature reported to be successfully conjugated to QD, and Fig. 1 provides structures of some of these ligands. Additionally, in our lab, we have been able to successfully conjugate several other plant metabolites including the phytohormone auxin. We have applied this method of conjugation successfully to diverse neuroactive compounds and plant metabolites including indole-3-acetic acid, brassinosteroids, indoleamines, non-protein amino acids and several neurotransmitter receptor agonists (Erland et al. 2019b) (Fig. 1). The frequent presence of reactive amine or hydroxyl groups on plant neurotransmitters makes them excellent targets for conjugation.

3 QD and the Importance of Location

Determining location and changes in location can provide valuable insights into the functions and mechanisms of diverse biological processes. However, despite a growing interest in plant signalling molecules and a growing body of literature, interest in the localization of these molecules in plants has been more limited. For example, a Web of Science search of the terms melatonin OR serotonin AND location OR localization yields 4,956 results; however, only seven of these are in the plant system. This provides an advantage to plant scientists studying these molecules as the techniques have already been well developed in animal or microbial systems, and therefore, require sometimes only slight modifications to be adapted for use in the plant system. QD localization and imaging is one such example.

In the case of understanding plant neurotransmitters, which may include diverse groups such as catecholamines, indoleamines, etc., amines are present almost universally across this class of plant signals which makes them good candidates for QD conjugation as QD conjugation has been found effective in a wide subsection of amino acids (Table 1). The first reports of the use of quantum dots for tracking of metabolites in plants were published by Whiteside et al. in 2009, which used QD conjugated to amino acids including glycine to examine the transfer of nitrogen in the symbiotic relationship between mycorrhizal fungi and plant roots. Later studies have used QD labelling to investigate the dynamics of other non-protein amino acids such as gamma amino butyric acid (GABA); QD-GABA were applied in *Nicotiana* and *Arabidopsis* cultures to determine membrane-binding sites (Yu et al. 2006).

Table 1 Summary of papers utilizing quantum dot (QD) labelling of biomolecules in the plant system

Ligand	Application	QD composition	Species	Imaging type	References
Glycine Arginine Chitosan	Nitrogen transfer between mycorrhizal fungi and plants	Commercial carboxyl functionalized	*Poa annua*, mixed roots from boreal forest	Fluorescence confocal microscopy, field imaging with UV lamp	Whiteside et al. (2009, 2012a)
Amino acids (Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Try, Tyr, Val)	Quantitative nitrogen transfer between mycorrhizal fungi and plants	Commercial carboxyl functionalized	*Sorghum bicolor*	Fluorescence quantification by microplate reader	Whiteside et al. (2012b)
Gamma amino butyric acid (GABA)	Determination of GABA binding sites and colocalization with calcium signalling	CdSe/ZnS	*Nicotiana tabacum* pollen protoplasts, *Arabidopsis thaliana* mesophyll cell protoplasts	Fluorescence confocal microscopy	Yu et al. (2006)
Glycine Mercaptosuccinic acid Cysteine	Uptake of conjugated and unconjugated QD from soils All three conjugates taken up; unconjugated shows little to no uptake	CdSe/ZnS	*Lolium perenne* *Allium cepa* *Chrysanthemum sp.*	Fluorescence microscopy	Al-Salim et al. (2011)
DNA	Plant chromosome mapping	CdSe/ZnS	*A. thaliana*	Fluorescence in situ hybridization (FISH)	Ma et al. (2008)
Calmodulin	Visualization of calcium signalling dynamics and calmodulin binding sites	CdTe	*Lilium longifolium* pollen protoplasts, *N. tabacum* pollen protoplasts, *A. thaliana* cell suspension culture protoplasts	Fluorescence confocal microscopy, transmission electron microscopy (TEM)	Wang et al. (2009)

(continued)

Table 1 (continued)

Ligand	Application	QD composition	Species	Imaging type	References
Melatonin Serotonin	Subcellular localization of melatonin and serotonin in vivo Localization under temperature (heat or cold) stress	CdSe/ZnS QDot ITK™ Carboxyl	*Hypericum perforatum*	Fluorescence microscopy	Erland et al. (2019b)
Jasmonic acid	Localization of jasmonic acid binding sites	CdTe	*A. thaliana* root tip, mung bean seedling	Laser scanning confocal microscopy	Gao et al. (2013)

(continued)

Table 1 (continued)

Ligand	Application	QD composition	Species	Imaging type	References
Lipopolysaccharide	Localization of binding site	CdSe/ZnS QDot ITK™	*A. thaliana* mesophyll protoplasts	Fluorescence microscopy	Mgcina et al. (2015)

[a] Results generated from a Web of Science search for the terms ‘quantum dot’ AND plant (84 results), further refined by type ‘article’ (68 results) and limited to the fields of cell biology, plant science, biochemistry and molecular biology, physiology, environmental science and ecology (22). Results further refined manually to exclude papers solely utilizing quantum dots in a methodology such as microRNA assays

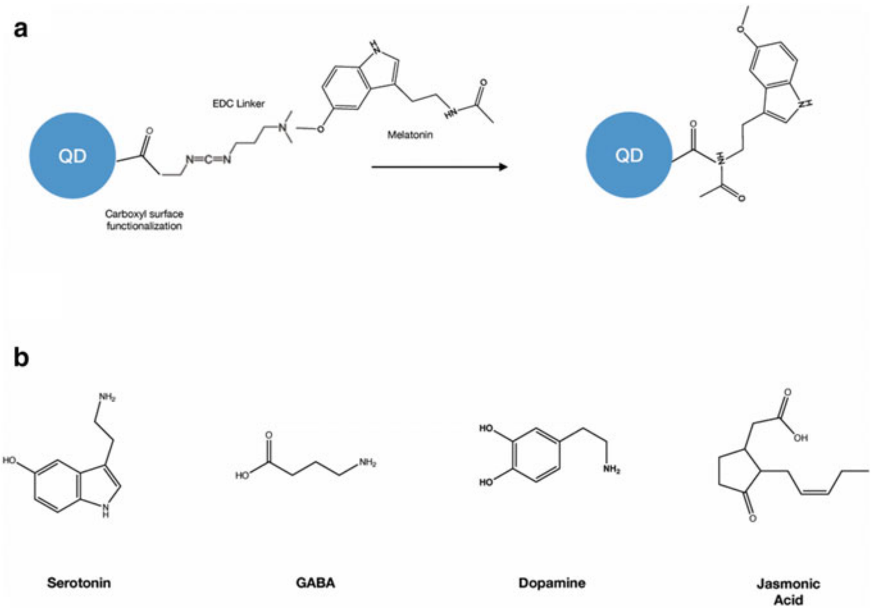

Fig. 1 **a** Depiction of the EDC-mediated conjugation process and **b** structures of some plant signalling molecules successfully conjugated to QD through an EDC-mediated conjugation

3.1 *Localization of Melatonin and Serotonin in Plant Roots by QD Labelling*

As our understating of the mechanisms of action of plant neurotransmitters continues to widen, the dynamic nature of these signals continues to be demonstrated. This is not surprising as signalling molecules by nature of their function must react rapidly. Biosynthesis of compounds is a relatively slow and costly process, and though it may be sufficient for response to longer stresses such as increased salinity, where a quick response to the changing conditions will determine the plant's survival at the time scale of days or weeks, it does not serve a plant well when faced with an immediate threat such as herbivory. Deactivation through conjugation or modification of signalling molecules may represent a less costly strategy. Inactive conjugates may be transported to locations of action of the active molecule without inducing physiological effects, thus creating a readily available pool. Such strategies are employed in auxin signalling (Enders and Strader 2015). Sequestration of the signalling molecule within the tissue at the location of action, either within vacuole or other compartment, may also occur so that it need only be released requiring no enzymatic action. This allows for an immediate and rapid response. It is likely that many plant neurotransmitters employ a combination of these different strategies. Monitoring localization of such compounds may help to elucidate some of these actions. The location of synthesis and the location of action of the indoleamines melatonin and serotonin has remained relatively elusive as the first plant melatonin receptor was not discovered until 2019, and a plant serotonin receptor has yet to be identified (Wei et al. 2018). PMTR1 was identified in stomatal guard cells and its expression has been shown in several other aerial plant tissues; however, no receptor has as of yet been identified in plant roots, despite the fact that melatonin levels have been found to be quite high in the roots of many species. Research in isolated chloroplasts has identified them as a site of biosynthesis (Zheng et al. 2017), and transgenic studies examining localization of melatonin biosynthetic enzymes have supported the chloroplastic location of biosynthesis, as well as suggesting that these enzymes may also localize to both the cytoplasm and mitochondria (Byeon et al. 2013, 2016; Wang et al. 2017). This research has thus provided information on the locations of synthesis, and the first indication of locations of action; however, transport mechanisms and dynamics, which are intrinsically linked to action in other plant signalling networks, such as auxin, cannot be fully elucidated.

Our recent work utilized QD labelling of melatonin and serotonin to examine their subcellular localization in axenic root cultures of the indoleamine model plant system *Hypericum perforatum* or St. John's wort (Fig. 2). While localization of the serotonin molecule directly had previously been undertaken in immature fruit,

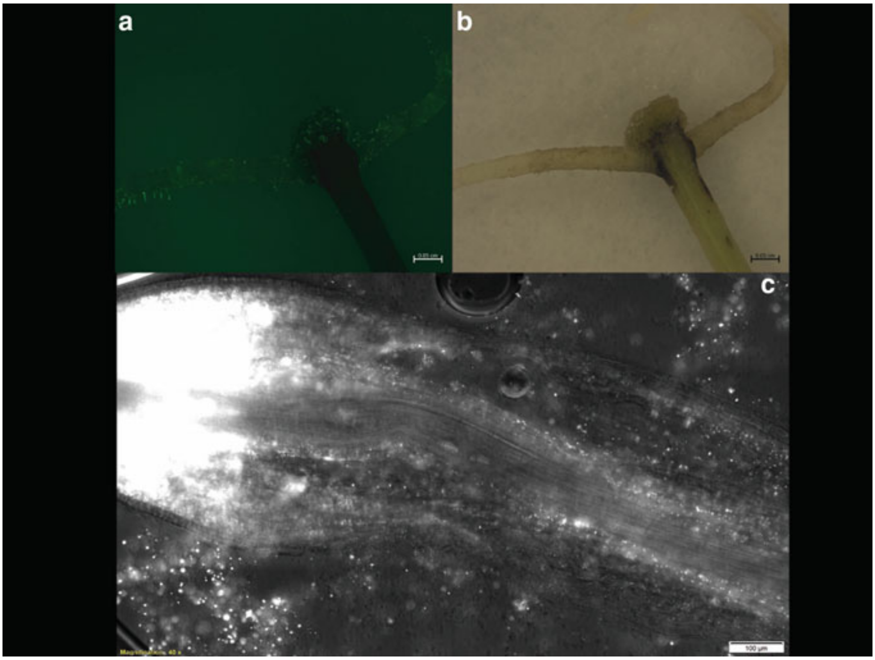

Fig. 2 Uptake of melatonin labelled QD by intact in vitro grown Hypericum perforatum plantlets **a** viewed under a GFP fluorescence filter and **b** under bright field microscopy and **c** localization of QD-MEL in the root of *H. perforatum* under physiological conditions using epifluorescence microscopy

somatic embryos and in vitro cultured plantlets of coffee (*Coffea canephora* P ex Fr) and found to localize to the vasculature (Ramakrishna et al. 2011), such experiments have not been undertaken for melatonin. Under optimal tissue culture growth conditions, melatonin and serotonin were found to have distinct and specific localization and transport patterns. Melatonin was found to be absorbed through epidermal cells, then travelled laterally, and accumulated in endodermal and rapidly dividing pericycle cells (Erland et al. 2019b). Serotonin in contrast was absorbed by cells proximal to the crown and moved through the vasculature similarly to that observed by Ramakrishna et al. (2011), via rapid polar movement towards the root tip (Erland et al. 2019b). In addition to their roles as plant growth regulators moderating processes such as root morphogenesis and shoot induction (Erland et al. 2019a), melatonin and serotonin have been shown to have diverse and potent effects in enhancing plant survival and adaptation to biotic and abiotic stresses. Thermal stress was found to disrupt the specific localization patterns of melatonin and serotonin in *Hypericum* roots and instead led to their uniform dispersal across cells. The mechanisms of melatonin and serotonin action in physiological conditions compared to stress are well documented, and often times their effects are much more significant when a plant is under stress (Erland et al. 2015). These results demonstrate a dual localization of the indoleamines which mirrors their dual functions and suggest a potential explanation for this via sequestration and mobilization depending on environmental conditions (Erland et al. 2019b). The role of serotonin in plant stress responses remains relatively under-investigated in comparison to its metabolite, melatonin. The loss of specific localization of serotonin in response to stress suggests that serotonin is being redirected either to serve as a precursor for melatonin, or indicates that serotonin itself may serve as an effective antioxidant to mitigate many more stresses than it has currently been examined in (Erland et al. 2016). Future studies examining colocalization of the indoleamines with other signalling molecules through multichannel imaging and across species, developmental stage and environmental conditions hold great promise for improving our understanding of these important molecules.

4 Challenges

A significant concern in the use of QD is the possibility for heavy metal leaching from the QD inducing stress responses or toxicity in the plant. Fortunately, Cd and Se leeching from QD has been found to be minimal even after seven days in culture solution (Navarro et al. 2012). As many labelling experiments occur on the time scale of minutes to hours and not days or months, leaching of heavy metals is unlikely to have a significant physiological effect. Additionally, only small concentrations are required for effective visualization, keeping levels of heavy metals, even if the total concentration were to be released, which does not represent a realistic scenario, relatively low. Tests examining the potential detrimental effects of quantum dots as environmental contaminants have shown that there are no detrimental effects on plant growth at levels below 40 μg/L (Das et al. 2015), a level much higher than the pg to ng levels needed for effective imaging (Erland et al. 2019b). A study from 2020 which focused on physiological and morphological effects of CdS QD in *A. thaliana* also found that QD treatment induced general stress responses, however the concentrations were again relatively high (60 mg/L) (Marmiroli et al. 2020).

In studies to determine environmental toxicity and persistence of other commercial QD, uptake by plant cells has been found to be dependent on the surface coating. Positive or anionic non-specific coatings on QD showing little to no uptake; only anionic surface chemistries showed any non-specific uptake (Zhao et al. 2012; Koo et al. 2014; Majumdar et al. 2019). Additionally, several reports using QD conjugates as labels in the plant system have found limited to no uptake of unconjugated carboxyl conjugated QD (Whiteside et al. 2009; Gao et al. 2013; Erland et al. 2019b); and those which have found uptake of non-specific anionic surface chemistries observed a uniform distribution of the QD throughout the tissues examined, using Arabidopsis as a model (Navarro et al. 2012). This provides a relatively simple method of exclusion for unconjugated QD and limits concern for non-specific growth effects which may be induced by QD labels themselves.

A common concern in the use of QD conjugates is that the QD will inhibit or modify function of the molecule due to the increased size of the molecule, conjugation to an active moiety or steric hindrance. Gao et al. (2013) addressed this concern for CdTe-JA conjugates by performing competition studies. Fluorescence of QD bound JA decreased as a function of increase JA concentration as non-fluorescence JA replaced QD-JA at the binding sites. The authors suggest that the results demonstrate that QD-JA competes with native JA for binding at target proteins, the biological activity of JA. Application of the CdTe probe alone, with no JA conjugate, showed negligible fluorescence, limited uptake and no activity in root sections (Gao et al. 2013). However, this also presents a potential challenge as co-application of QD-labelled compound with unconjugated compound will lead to suppression/competitive inhibition of the fluorescence signal and therefore needs to account for in experimental design.

5 Future Perspectives and Applications

QD labelling provides an exciting new tool for investigating localization of plant neurotransmitters. As we are better able to understand the locations and transport dynamics of these important plant signalling molecules, their mechanisms and our understanding of their importance in the plant life cycle is likely to only grow.

Coupling of QD labelling with other visualization systems may represent a new opportunity for understanding the signalling cascades induced by plant neurotransmitters. For example, whole-plant imaging of ROS signalling is a recently developed approach which has provided valuable insight into the importance of ROS beyond just stress metabolites (Fichman et al. 2019). The indoleamines in particular are believed to function in part through mediation of ROS signalling cascades. The indoleamines have been hypothesized to mediate this effect both through direct antioxidant capacity and through upregulation of other antioxidant mechanisms, as well as interaction with NADPH Oxidase (Chen et al. 2017; Gong et al. 2017). Colocalization of the indoleamines with locations of ROS signalling in response to diverse stresses may shed light on the specificity indoleamine mechanisms in these responses.

The coupling of live imaging chamber designs (Ruan et al. 2018; Kirchhelle and Moore 2017) with QD visualization is also an exciting application that is enabled by the stability of QD emission as QD-bioconjugates can be monitored for days at a time and therefore could be applied to understanding developmental or morphogenetic processes. This may open the door to understanding the function of plant neurotransmitters as inductive signals, as has been hypothesized to be the case for the indolamines (Erland and Saxena 2019). While imaging in systems such as Arabidopsis roots is fairly straightforward due to their lack of pigment and small size, a challenge in imaging of non-model and larger plant tissues is the presence of pigments and other light-scattering plant metabolites. However, integration of light sheet microscopy and transparency techniques may realize the potential for imaging of intact whole plants in three dimensions. Hasegawa et al. (2016) describe a clearing technique referred to as TOMEI (transparent plant organ method for imaging) which removes interference by pigments and cytoplasmic components while retaining cell morphological characteristic enabling imaging of depths of up to 200 μm using confocal microscopy and 3D imaging using optical sectioning.

The monitoring of QD-labelled metabolites by in vivo flow cytometry to monitor trafficking of small molecules in plant xylem and phloem also represents an exciting new technology which may allow for improved understanding of uninvestigated plant signals, such as plant neurotransmitters, where little is known about transport mechanisms (Nedosekin et al. 2011). These techniques allow for real-time monitoring of signals conjugated to the QD and could be monitored in response to external stimuli.

QD represent an exciting new development in small molecule labelling in plants and provide the possibility for understanding the transport, localization and dynamics of plant neurotransmitters. Enhanced understanding of these dynamics is likely to shed new light on the importance of plant neurotransmitters in mediating diverse plant processes, assist in the identification of transport proteins and receptors and improve our understanding of their mechanisms.

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Role of Signal Molecules Under Stressful Environments

Mona Gergis Dawood and Mohamed El-Sayed El-Awadi

Abstract Signal molecules are essential for the growth, development, and adaptation of plants, as well as for the activation of their antioxidant responses to a number of environmental stress factors. The plant sensing to abiotic stress conditions induces signaling cascades that activate production of reactive oxygen species (ROS), reactive nitrogen species (RNS), H_2O_2, calcium (Ca^{2+}), nitric oxide (NO), soluble sugar, secondary metabolites, melatonin, hormones such as abscisic acid, ethylene, jasmonic acid, and salicylic acid. In this chapter, we will focus on the role of H_2O_2, NO, and melatonin as signal molecules. H_2O_2 and NO can play a dual role in cells. During oxidative stress, H_2O_2 is a strong toxic oxidant causing cell damage or even cell death. At low levels, it serves conversely as a signaling molecule to activate a rescue/defense system for restoring the redox homeostasis in plant cells. Nitric oxide (NO) is an important signaling molecule that has diverse biological functions in plants, regulates different physiological processes and increasing abiotic stress tolerance depending on its concentration. Melatonin is considered as a central indoleamine neurotransmitter, largely involved in the diverse biological proceses and accepted as an important plant metabolite.

1 Introduction

The improvement of different crops to tolerate abiotic stresses such as excessive or inadequate supply of water, salinity, extreme temperatures, high winds, and frost is the main target to ensure food security for the coming decades. However, yield is not the only crop parameter affected by abiotic stress, but the impact of stress and climate change on crop composition is also important.

Abiotic stress has a harmful impact on plant metabolic activities and responsible for severe losses in the yield. The resulting growth reductions can reach >50% in most plant species (Wang et al. 2003; Shao et al. 2008) because of disruption in plant

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metabolism (Bolton 2009; Massad et al. 2012). Moreover, plants show different degrees of sensitivity to abiotic stress depending on the growth condition, the developmental stage of the plant and plant species (Mittler and Blumwald 2010) as well as intensity and duration of the stress (Ramegowda et al. 2013; Rasmussen et al. 2013). It is not clear whether stresses are antagonistic, synergistic, or additive, inducing more or less susceptibility to a specific kind of stress (Anderson et al. 2004; Asselbergh et al. 2008). Interestingly, when plants are exposed to multiple stresses, plants are able to defend themselves via facing one stress and can become more resistant to other stresses (Bowler and Fluhr 2000). This phenomenon is called cross-tolerance, showing that plants possess a powerful regulatory system that allows them to adapt quickly to a changing environment (Bowler and Fluhr 2000; Capiati et al. 2006; Suzuki et al. 2012). Thus, in response to stress, there are gradual and complex changes in plant metabolism. The plant molecular responses to abiotic stresses involve interactions and cross-talk with many molecular pathways (Takahashi et al. 2004).

2 Signal Molecules

Signal molecules are essential for the growth, development, and adaptation of plants, as well as for the activation of their antioxidant responses to a number of environmental stress factors such as extreme temperatures, light, drought, salinity, heavy metals, herbicides, pathogens, and others (Dmitriev 2003; Gururani et al. 2015). The study of their impact on plants is becoming more relevant in view of progressive climate changes and increasing pollution worldwide.

Stress signals are firstly perceived by receptors present on the membranes of the plant cells and followed by signal generation and transduction leading to the triggering of specific defense responses (Tuteja and Sopory 2008). Different signaling pathways can operate independently to each other and can modulate other pathways (Kaur and Gupta 2005). Sometimes, components of pathways are dependent on each other and can cross-talk among them.

The plant sensing to abiotic stress conditions induces signaling cascades that activate production of reactive oxygen species (ROS), reactive nitrogen species (RNS), H_2O_2, calcium (Ca^{2+}), nitric oxide (NO), soluble sugar, secondary metabolites, melatonin, hormones such as abscisic acid, ethylene, jasmonic acid, and salicylic acid. These signals ultimately induce expression of specific subsets of defense genes that lead to the assembly of the overall defense reaction (Jaspers and Kangasjärvi 2010). We can say the responses to environmental stress occur by stimulus-response coupling: the plant cell perceives a stimulus, a signal is generated and transmitted (signal transduction), and a biochemical change is instigated (the response) (Bowler and Chua 1994).

2.1 Reactive Species

One of the earliest signals in many abiotic stresses involves reactive oxygen species (ROS) and reactive nitrogen species (RNS), which modify enzyme activity and gene regulation (Wilkinson and Davies 2009; Mittler et al. 2011; Molassiotis and Fotopoulos 2011). ROS and RNS form a coordinated network that regulates many plant responses to the environment; there are a large number of studies on the oxidative effects of ROS on plant responses to abiotic stress, but only a few studies are documenting the nitrosative effects of RNS (Molassiotis and Fotopoulos 2011).

2.2 Reactive Oxygen Species

The evolution of oxygen (O_2) metabolism in higher plants led to the production of reactive oxygen species (ROS) in the organelles involved in aerobic process (mitochondria, chloroplasts, and peroxisomes) (Apel and Hirt 2004; Slesak et al. 2007; Corpas et al. 2001). A rapid generation of ROS is observed after stress sensing (Wojtaszek 1997; Foyer and Noctor 2005). ROS include superoxide ($O_2^{\bullet-}$), hydroxyl (HO) radicals, singlet oxygen ($1O\ _2$), and hydrogen peroxide (H_2O_2) (Gupta et al. 2016; Kalia et al. 2017). ROS at a high concentration are significantly harmful to organisms and affects a large variety of cellular, physiological, and biochemical functions, such as the disruption of plasma membrane, carbohydrate deoxidation, lipid peroxidation, protein denaturation, and the destruction of DNA, RNA, enzymes, and pigments (Bose et al. 2013; Martínez et al. 2017; Li et al. 2018a, b; Van Ruyskensvelde et al. 2018). ROS exhibit growth retardation under oxidative stress, affecting on flower and leaf abscission (Goldental-Cohen et al. 2017; Muñoz and Munné-Bosch 2018), root gravitropism (Mugnai et al. 2014), seed germination (Shi et al. 2014), polar cell growth (Mangano et al. 2016), lignin biosynthesis in cell wall (Chialva et al. 2018), cell senescence (Bu et al. 2017), and results in the loss of crop yield and quality (Guo and Gan 2014; Reshi et al. 2014; Petrov et al. 2015; Shahid et al. 2015; You and Chan 2015; Fulda 2016; Sharma et al. 2017).

Hence, it is important to remove these toxic molecules from cells to prevent stress-induced injuries. The plants possess a complex battery of antioxidant defense systems to regulate ROS production with beneficial effects. Where ROS-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and associated antioxidant enzymes, glutathione reductase (GR), and antioxidants such as “big three” antioxidants (ascorbic acid, glutathione, and the pyridine nucleotides) and many redox-active phenolics, carotenoids, and tocopherols are essential for ROS detoxification. Hence, the production of ROS is fine-modulated by the plant to avoid tissue damage (Apel and Hirt 2004; Foyer and Noctor 2011; Mittler et al. 2011; Bhattacharjee 2012; Xia et al. 2012; Choudhury et al. 2013; Pastor et al. 2013; Kissoudis et al. 2014).

When the level of ROS is low or moderate, they act as second messenger (Apel and Hirt 2004; Hancock et al. 2006; Meng et al. 2010; Spoel and Loake 2011) that mediates a series of reactions in plant cells and a number of regulated processes during plant growth and development, like cell elongation and differentiation (Foreman et al. 2003), stomatal closure, programmed cell death (PCD) (Petrov et al. 2015), gravitropism (Wassim et al. 2013), hormone signaling and acquisition of tolerance to both abiotic and biotic stresses (Saed-Moucheshi et al. 2014; Nath et al. 2017). Additionally, the production of ROS can act as a secondary messenger by modifying protein structures and activating defense genes (Spoel et al. 2010; Spoel and Loake 2011).

ROS respond to abiotic and biotic stress, but differently from one stress to another (Pastori and Foyer 2002). Where ROS may possibly be the central process mediating cross-tolerance between abiotic and biotic stressresponsive networks (Atkinson and Urwin 2012). Xia et al. (2012) mentioned that ROS are involved in stress-induced tolerance in *Arabidopsis thaliana* after infection with the vascular pathogen *Verticillium* spp. by increasing drought tolerance due to de novo xylem formation and the resulting enhanced water flow. Gechev et al. (2006) proposed that ROS are inducers of tolerance by activating stress response-related factors like mitogen-activated protein kinases (MAPKs), transcription factors, antioxidant enzymes, dehydrins, as well as heat shock and pathogenesis-related proteins.

It became clear that ROS play a dual role in plants as toxic compounds or as key regulators of many biological processes such as growth, cell cycle, hormone signaling, biotic and abiotic cell responses, programmed cell death (PCD), and plant development (Apel and Hirt 2004; Miller et al. 2008; Corpas et al. 2001). Moreover, reactive oxygen species (ROS) have been also shown to play an important role in plant defense mechanisms (Kreslavski et al. 2012; Saed-Moucheshi et al. 2014). It has been proposed that ROS participate as signaling molecules in the transduction of stress signals from chloroplasts to the nuclear genome and also the interactions between ROS and other signaling systems within the cell (Kreslavski et al. 2012) (Fig. 1).

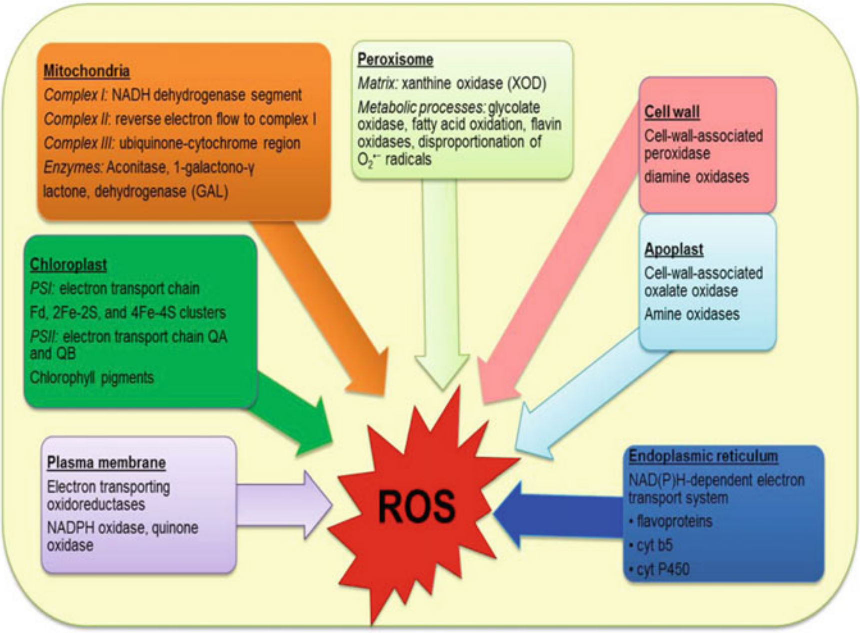

Fig. 1 Sites of production of reactive oxygen species (ROS) in plants

2.3 Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a part of cellular components referred to reactive oxygen species (ROS) that are formed by aerobic respiration and other oxidation-related processes within the plant (Orozco-Cárdenas et al. 2001; Slesak et al. 2007). Hydrogen peroxide is formed due to two-step reduction of molecular oxygen (the first step leading to superoxide radical) and has a relatively long lifespan in comparison to other ROS. The long half-life (1 ms) of H_2O_2 and its small size allow it to traverse across cellular membranes and migrate in different compartments, which facilitate its signaling functions (Bienert et al. 2006).

The H_2O_2 belongs to non-radical ROS, carries no net charge, stable in solution (cellular half-life ~1 ms, steady-state levels ~10 −7 M), and can diffuse across biological membranes (Upadhyaya et al. 2007). Due to its relative stability and diffusibility through membranes, hydrogen peroxide is more likely a long-distance signaling molecule (Vranová et al. 2002). Diffusion of H_2O_2 might be modulated by changes in lipid membrane permeability or by transport through aquaporins (Bienert et al. 2006, 2007).

Its endogenous concentration ranged between nmol and several hundred mmol/g FW. Normally, H_2O_2 generation is often maintained at a constant basal level in healthy cells, but their levels increase transiently or persistently in response to stress

(Desikan et al. 2003; Apel and Hirt 2004). Hydrogen peroxide—at high concentrations—results in the release of the factors that are responsible for programmed cell death (Dat et al. 2000). Whereas, hydrogen peroxide at low concentrations plays a biological role in the plant by sending chemical signals that lead to the resistance of the plant to stress and acts on the gene expression (Hung et al. 2005). It mediates various physiological and biochemical processes in plants (Niu and Liao 2016) and acts as a key regulator in several physiological processes (Uchida et al. 2002; Ashfaque et al. 2014).

The mutual relationship between positive and negative functions performed by H_2O_2 in biological systems depends on the H_2O_2 concentration, and on the specificities of processes affected by H_2O_2. There is a considerable challenge to separate the roles of H_2O_2 from those of other reactive oxygen species (ROS) such as superoxide anion (O2•−) and hydroxyl radical (•OH), which may coexist and be converted into one another through spontaneous and catalyzed reactions (Wojtyla et al. 2016).

In this concern, multiple antioxidant enzymes are involved in the scavenging of ROS. Superoxide dismutases (SOD) react with the superoxide radical to produce hydrogen peroxide (H_2O_2) that is scavenged by catalases (CAT) and peroxidases (POD). CAT reacts with H_2O_2 to produce water and oxygen. Among peroxidases, ascorbate peroxidases (APX) and glutathione peroxidase (GPX) which uses ascorbate and glutathione as electron donors, respectively, and leading to H_2O_2 detoxification in plants.

2.3.1 Role of H_2O_2 in Plant Growth and Development

Hydrogen peroxide has many basic roles in the metabolism of the plant and involved in a wide variety of interactions and the sequencing of signals necessary for all aspects of the growth. Hydrogen peroxide stimulates the division and elongation of cells and the formation of secondary walls (Abass and Mohamed 2011) and improves the dynamics of the roots, length, and number, leading to a high absorption of nitrogen that is reflected in the growth and plant yield (Hameed et al. 2004; Liao et al. 2004). Hydrogen peroxide induced nutrients absorption that are necessary for plant growth such as calcium and potassium (Desikan et al. 2004; Liu et al. 2004; Wendehenne et al. 2004).

It plays a vital role in the regulation of senescence process (Jajic et al. 2015), stomatal behavior (Rodrigues et al. 2017), cell wall cross-linking Li et al. (2017), regulation of the cell cycle (Pokora et al. 2017), photosynthesis (Exposito-Rodriguez et al. 2017), stress acclimation (Lv et al. 2018), and antioxidative defense (Liu et al. 2016). In addition, H_2O_2 can interact with other signal molecules such as abscisic acid (ABA), auxin, brassinosteroid (BR), and ethylene, which are important for plant development (Krishnamurthy and Rathinasabapathi 2013; Xia et al. 2015; Alqurashi et al. 2017).

Using cDNA microarray technology to carry out a transcriptomic analysis, Desikan et al. (2001) provided further evidence of H_2O_2 as a central signaling mediator. Their study showed that the expression of some genes is upregulated by H_2O_2

and others are repressed. Classified by their potential biological functions, these genes include heat shock proteins, heat shock transcription factors, mediators for calcium signal transduction, such as calmodulin, important signaling enzyme protein tyrosine phosphatases (PTPs), a blue copper-binding protein which is essential catalyst for redox reactions. Hung et al. (2005) stated that H_2O_2 is a regulator of the expression of some genes in cells including genes encoding antioxidants, cell rescue/defense proteins, and signaling proteins such as kinase, phosphatase, and transcription factors.

More studies have provided evidence that H_2O_2 itself is a key signal molecule mediating a series of responses (Desikan et al. 2003) and activating many other important signal molecules (Ca^{2+}, salicylic acid (SA), abscisic acid (ABA), jasmonic acid (JA), ethylene, nitric oxide (NO) of plants) (Desikan et al. 2004; Liu et al. 2004; Wendehenne et al. 2004).

In addition, Agarwal et al. (2005) hypothesized that the H_2O_2 produced as a result of the treatment with various signaling molecules could in turn induce the synthesis or activate various transcription factors, which are associated with the induction of antioxidative enzymes.

The generation of H_2O_2 is increased due to a wide variety of stresses, and some authors have suggested that H_2O_2 is a key factor mediating the phenomena of acclimation and cross-tolerance (Neill et al. 2002a, b). Al-Hayany (2019) mentioned that 15 mmol L^{-1} of hydrogen peroxide effectively increased the number of pods, the number of seeds per pod, the weight of 100 of mung bean seeds. Pretreatment of mung bean seedlings with low concentrations of H_2O_2 induces chilling tolerance (Yu et al. 2003). Hameed et al. (2004) showed that exogenous application of H_2O_2 provided more vigorous root system in wheat. AzevedoNeto et al. (2005) reported that addition of H_2O_2 to the nutrient solution induced salt tolerance by enhanced activities of antioxidants and reduced peroxidation of membrane lipids in leaves and roots of maize. Likely, He et al. (2009) stated that seed treatment with H_2O_2 could greatly alleviate the deleterious effects of drought on the membrane integrity and stability in the wheat seedlings through reducing membrane damage rate and MDA content. They added that H_2O_2 pretreatment enhanced the photosynthetic rate and attributed this enhancement to the positive role of H_2O_2 in inducing the expression of antioxidant system.

H_2O_2 treatments improved osmotic stress resistance of two cucumber varieties by activating antioxidant system (Liu et al. 2010). Abass and Mohamed (2011) observed that exogenous application of H_2O_2 to common bean enhanced the root growth and fresh weight under drought stress. In addition, Li et al. (2011) stated that exogenous H_2O_2 treatments prevent the increase of oxidative stress and endogenous H_2O_2 concentration in plants and enhance tolerance of plants to salt stress by enhancing the production of enzymatic and non-enzymatic antioxidants which can quench the ROS and decrease lipid peroxidation. They added that exogenous 100 nM H_2O_2 treatments decreased the deleterious effect of salt stress on growth of wheat than 50 nM H_2O_2. Furthermore, exogenous H_2O_2 may induce oxidative stress tolerance by enhancing the activities of POD and PPO under various biotic and abiotic stresses (Goud and Kachole 2011). Similarly, Goldani et al. (2012) reported that 5 mM H_2O_2 increased total chlorophyll and carotenoid content in salt-stressed oregano plant by

46.6 and 100.6%, respectively, compared to control plant. Furthermore, the exogenous application of H_2O_2 alone or in combination with drought stress caused significant increase in both IAA and GA_3 contents (Abass and Mohamed 2011). Terzi et al. (2014) mentioned that exogenous H_2O_2 treatment can enhance tolerance of maize seedlings to osmotic stress by increasing some metabolite and phytohormone levels. H_2O_2 is found to be involved in the acclimation and tolerance of plants grown under salt stress as mentioned by Li et al. (2011), Wang et al. (2013). Orabi et al. (2015) concluded that H_2O_2 at 0.1 mM has positive significant effect on growth, growth regulators (GA_3, IAA, and ABA), antioxidant enzymes (POD and PPO) activity, fruits yield quantity and quality (total soluble solids and antioxidant activity) of the two tomato cultivars grown under low-temperature conditions in sand-ponic culture. On the other hand, these treatments caused significant decreases in CAT enzyme activity, MDA and EL values. They added that endogenous H_2O_2 concentration depended on the balance between its production rates and its utilization by enzymatic and non-enzymatic levels to alleviate membrane damages and significantly decreased lipid peroxidation under stressed conditions. In order to keep H_2O_2 as a signaling molecule and non-toxic levels, a balance must be maintained between H_2O_2 productions and scavenging. Exogenous application of H_2O_2 can induce tolerance to salinity, drought, chilling, high temperatures, and heavy metal stress, all of which cause elevated H_2O_2 production (Ishibash et al. 2011; Hossain et al. 2013). Orabi and Sadak (2015) revealed that soaking grains of wheat plants with 50 and 100 μM of H_2O_2 led to significant increase in total soluble carbohydrate, free amino acids, and some enzymes such as CAT, PPO, APX, POX, and SOD activities. Semida (2016) noticed that H_2O_2 increased chlorophyll and photosynthesis activity in onion plants and resistance to abiotic stresses.

Several recent studies have demonstrated that the H_2O_2 priming can enhance abiotic stress tolerance by modulating ROS detoxification and by regulating multiple stress-responsive pathways and gene expression. Orabi et al. (2018) stated that the addition of H_2O_2 improved almost all growth and yield criteria, increased antioxidant enzymes activity in leaves and non-enzymatic antioxidants in seeds besides lowering oxidative damage in canola plants under different irrigation intervals. Application of low concentrations of H_2O_2 would act as a signal molecule to improve antioxidative defense system as well as the highest growth and yield. Thus, they concluded that the application of low concentration of hydrogen peroxide can help plants to resist drought stress induced from prolonging periods between irrigation of canola plants.

2.4 Nitrogen Monoxide or Nitric Oxide

Nitric oxide (NO) is an important signaling molecule that has diverse biological functions in plants, regulates different physiological processes and increasing abiotic stress tolerance (Freschi 2013). It is well known that plants release NO under normal growing conditions and NO can accumulate in the atmosphere from a variety of sources such as industrial pollution (Wildt et al. 1997). NO is a highly reactive molecule that rapidly diffuses and permeates cell membranes.

NO is mainly formed in actively growing tissue such as embryonic axes and cotyledons and the levels decrease in mature and senesced organs (Leshem et al. 1998; Caro and Puntarulo 1999). NO is a non-traditional regulator of plant growth and most of its functions are dependent on its amount (Beligni and Lamattina 2001).

Leshem and Haramaty (1996) stated that NO acts on the lipid double layer of membrane cell and other wall components directly by apoplastic, and then cell wall loosed and enhanced the fluidity of cell membrane and accelerated the expansion of cell. In addition, 25–100 μM SNP may increase the plant growth by decreasing the level of lignifications of cell wall and accelerating the expansion. However, high level of SNP may enhance the leakage of membrane due to the oxidative stress and the impaired cell wall, and thus inhibited the growth of plants.

Qiao and Fan (2008) stated that NO can provoke both beneficial and harmful effects, which depend on the concentration and location of NO in plant cells. (Anderson and Mansfield 1979); high concentrations (40–80 pphm) inhibited tomato growth, while low concentrations (0–20 pphm) enhanced it (Hufton et al. 1996). Moreover, Xiao and Zhang (2004) reported that 0.01–0.1 mmol/l SNP contradict with ROS and the exogenous NO (SNP) above 0.5 mmol/l can cooperate with ROS. The dual function of NO as a potent oxidant or effective antioxidant mostly depends on its concentration and on the status of the environments (Beligni and Lamattina 1999a).

Beligni and Lamattina (1999a) indicated that lower level of NO can protect plant directly by reacting with effectual molecule or indirectly by changing the redox electricity potential difference of cell and by participating in the signal transmit process of the growth and accommodation of plant. While high NO can show toxic effect because it can induce the generation of free oxygen (O^-) and oxide hydrogen and can further interact with oxide hydrogen in producing a mass of peroxynitrite, $ONOO^-$, resulting in the damage to structure and function of the big biological molecules (Beligni and Lamattina 1999b).

NO clearly perturbs plants normal metabolism when applied at a relatively high dose. It has been demonstrated that excess of NO has the capacity to injure membranes, proteins, and nucleic acids in plant cells (Yamasaki 2000). Photosynthesis in leaves of oats and alfalfa would decrease when exposed to NO (Hill and Bennett 1970). NO can also reduce the respiration in carrot cell suspensions (Zottini et al. 2002). A high concentration of NO is associated with potential impairment of photosynthetic electron transport, inhibition of shoot and root development, DNA damage, and cell death (Leshem et al. 1997, 1998; Pedroso et al. 2000a, b; Gould et al. 2003). It has been reported that photosynthesis can be affected not only by generation of reactive oxygen species (Asada 1999) but also by reaction with NO. In addition, under circumstances of a high NO steady-state concentration (i.e., high nitrite content in chloroplasts), generation of reactive nitrogen species (RNS) may lead to impairment of the photosynthetic machinery (Jasid et al. 2006).

However, NO promotes normal growth and development of plants at lower concentrations (Beligni and Lamattina 2001).

NO can cooperate with other signals either directly or with other signaling pathways to adjust cellular processes. ROS generation such as H_2O_2 is a regular companion of NO production.

2.4.1 Nitric Oxide Biosynthesis in Plants

It is necessary to investigate the different NO-producing enzymes in plants to understand endogenous NO synthesis, detailed signaling mechanism, and the chemical changes induced by this molecule. There are two main sources of NO production proposed in plants:

1. From nitrate by nitrate reductase enzymatically or non-enzymatically (Yamasaki 2000; Rio et al. 2004) in plants.
2. From arginine by NO synthase.

NO produced in each of these routes depends on the species, the cells and tissues, plant growth condition (Fig. 2).

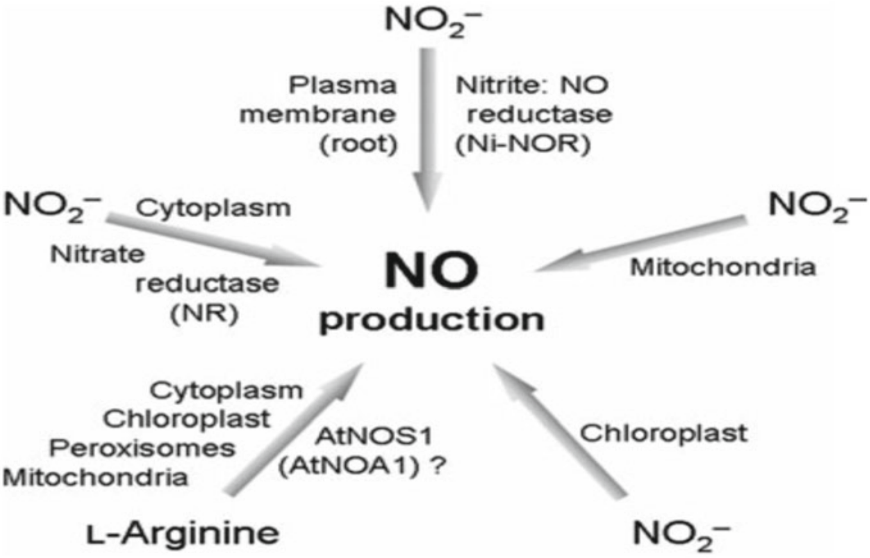

Fig. 2 The various routes of nitric oxide (NO) production in plants cells. NO can be synthesized enzymatically from nitrite (NO_2^-) by nitrate reductase (NR). There is also considerable evidence for l-arginine-dependent NO synthase (NOS) activity in plant cells, although the protein AtNOS1 is no longer considered to be a NOS and no other plant candidate for the role has been identified. Evidence also exists for the activity of a nitrite: NO reductase in roots and for the ability of both chloroplasts and mitochondria to convert NO_2^- to NO (Wilson et al. 2008)

2.4.2 Roles of Nitric Oxide in Plant Growth and Development

NO as a key signaling molecule has been involved in mediation of a variety of biotic and abiotic stress-induced physiological responses in plants (Delledonne et al. 1998; Leshem et al. 1998; Durner and Klessing 1999; Ribeiro et al. 1999; Beligni and Lamattina 2000; Pedroso et al. 2000b; García-Mata and Lamattina 2001). NO alleviates the deleterious effect of reactive oxygen species (ROS) in establishing stress resistance responses. In addition to its signaling roles, NO may act as a regulator for gene expression (Kopyra and Gwozdz 2004).

NO can involve in the regulation of physiological responses including photomorphogenesis, seed germination, de-etiolation, hypocotyl elongation (Leshem and Haramaty 1996; Beligni and Lamattina 2001), organ maturation, and senescence (Leshem 1996; Leshem et al. 1998; Tu et al. 2003). NO is also involved in many processes as antioxidant agent in plants in responses to abiotic stresses from heavy metals and UV-B-radiation (Beligni and Lamattina 1999b; García-Mata and Lamattina 2001; Uchida et al. 2002).

It could affect growth and development of plant tissue 7 NO PDF (Leshem and Haramaty 1996; Gouvea et al. 1997; Durner and Klessing 1999), and enhance plant cell senescence (Pedroso and Durzan 2000; Pedroso et al. 2000a; b).

NO can act together with cysteine and tyrosine amino acids in proteins and with thiol groups present in other molecules ubiquitous regulatory tri-peptide glutathione (Jia et al. 1996; Wendehenne et al. 2001).

Studies using different NO donors have shown that NO triggers the induction of root tip elongation and lateral adventitious root formation, which affects cell cycle regulatory gene expression (Pagnussat et al. 2002; Kopyra and Gwozdz 2003; Correa-Aragunde et al. 2004, 2006). Endogenous NO was involved in determining root branching in sunflower (*Helianthus annuus* L.) by regulating lignin composition (Corti Monzón et al. 2014).

Conclusive evidence proved that NO is involved in many plant physiological and metabolic processes, such as mitochondrial (Zottini et al. 2002) and chloroplast (Puntarulo et al. 2007) functionality, gravitropism (Hu et al. 2005), floral regulation (He et al. 2004), stomata closure (Lamattina et al. 2003), and senescence (Procházková and Wilhelmová 2011) and adaptation to environmental stresses (Uchida et al. 2002). It has been increasingly evident that NO also plays important roles in diverse physiological processes in plants, as suppression of floral transition (He et al. 2004), and involvement in light-mediated greening (Zhang et al. 2006a), to mediation of stomatal movement as an intermediate downstream of abscisic acid (ABA) signaling (Garcia-Mata and Lamattina 2001; Neill et al. 2002a; Desikan et al. 2004; Bright et al. 2006; Garcia-Mata and Lamattina 2007), and regulation of multiple plant responses toward a variety of abiotic and biotic stresses, such as drought (Garcia-Mata and Lamattina 2002), salt (Zhao et al. 2004, 2007a, b; Zhang et al. 2006b), heat (Uchida et al. 2002), and disease infection (Modolo et al. 2005; Floryszak-Wieczorek et al. 2007). Both biotic and abiotic stresses alter (promote or suppress) NO production, whereas externally applied NO donors enhance plant tolerance to specific stresses (Garcia-Mata and Lamattina 2002; Uchida et al. 2002; Zhao et al. 2007b).

NO regulates the expression of a number of genes involved in the synthesis of jasmonic acid (Orozco-Cárdenas and Ryan 2002; Jih et al. 2003), the synthesis and signaling of ethylene, the phenylpropanoid pathway, protein antioxidation mechanisms, photosynthesis, cellular trafficking, cell death, and other basic metabolic processes (Polverari et al. 2003; Wendehenne et al. 2004).

NO may be acting as a downstream signaling molecule of auxin-induced lateral and adventitious root formation (Pagnussat et al. 2003, 2004). Moreover, gravistimulation of roots not only induces auxin accumulation at the lower root flank, but also of NO (Joo et al. 2001, 2005; Hu et al. 2005), and a reduction in the NO level inhibits gravitropic bending of gravi-stimulated root apices (Joo et al. 2005). Therefore, auxin promotes the production of nitric oxide (NO) in roots, which is required for auxin-mediated root organogenesis (Pagnussat 2002; Correa-Aragunde et al. 2004). Development of root architecture including LR formation is a critical event for successful growth of plants (Woodward and Bartel 2005).

Cytokinin induced NO synthesis in tobacco, parsley, and Arabidopsis cell cultures (Tun et al. 2001). NO can imitate some cytokinin effects; NO donors induced betalaine accumulation in Amaranthus seedlings and NOS inhibitor inhibited cytokinin-induced betalaine accumulation (Scherer and Holk 2000).

Exogenous applications of NO to senescent pea leaves trigger a reduction in ethylene generation (Leshem and Haramaty 1996; Leshem et al. 1998), and NO can counteract the ABA-induced senescence of rice (*Oryza sativa*) leaves (Hung and Kao 2003). It was observed that Arabidopsis leaves exposed to 4 ppm NO gas show delayed senescence (Mishina et al. 2007). Additional data also indicate that NO interacts with the phytohormones SA and JA. SA treatment of Arabidopsis plants delays methyl jasmonate-induced leaf senescence by upregulating NOS-like activity and antioxidant defenses including catalase, APX, superoxide dismutase activity, and gene expression (Ji et al. 2016).

Moreover, NO has been identified as a mediator of guard cell ABA signaling. ABA induces the synthesis of NO in guard cells; NO induces stomatal closure via scavenging of NO or inhibition of NO synthesis reduces ABA-induced stomatal closure. NO synthesis is critical for ABA-induced stomatal closure (Neill et al. 2002a). This finding has been confirmed in *Vicia faba* (Garcia-Mata and Lamattina 2002). Tun et al. (2001) indicated the tissue specificity of ABA to induce NO synthesis in Arabidopsis suspension cultures.

Beligni et al. (2002) suggested that aleurone layers produce NO and endogenous NO plays a role in GA-induced programmed cell death.

In addition, researchers also investigated the NO relation with the plant stresses hormone ethylene. Low concentrations of NO either endogenously produced or exogenously applied in the 10^{-6} M range exert significant growth promoting and ethylene inhibiting effects, which are reversed by higher NO concentrations or equimolar applications of NOS inhibitor N6-methyl-arginine or NO-releasing compounds (Leshem 1996).

On the contrary, some reports revealed that treatment of Arabidopsis plants with NO raised the ethylene level and inhibition of NO biosynthesis did not affect the ethylene increment (Magalhãe et al. 2000). During fruit ripening ethylene formation increases and this occurs together with reduced NO release (Leshem and Pinchasov 2000). Moreover, treatment of fruits with NO also delayed the senescence and prolonged their postharvest period.

Probably, NO acts on stomata closing with other signaling molecules like H_2O_2. There is some indication that ROS and NO interact to induce ABA biosynthesis (Zhao et al. 2001).

The effects of osmotic stress on leaf water loss and ABA contents were partially reversed by NO scavengers or NOS inhibitors (Xing et al. 2004). Short-term heat stress caused an increase in NO production in alfalfa (Leshem 2001). NO treatment mediates for chilling resistance in different plants (Lamattina et al. 2001); this effect probably reflects the antioxidant properties of NO by inhibiting ROS following chilling or heat stress (Neill et al. 2002b). Tun et al. (2006) showed a relation between PA-mediated stress responses and other stress mediators using NO as an intermediate.

NO enhanced salinity tolerance of perennial ryegrass and associated with delayed leaf senescence, increased photosynthetic capacity, and reduced Na^+ toxicity. NO ameliorated salinity tolerance by improving plant growth, maintaining photochemical efficiency, or reducing Na+ concentration at 200 mM NaCl (Ji et al. 2019). Qian et al. (2009) showed that supplementation of low SNP (20 μM) increased chlorophyll content in *Chlorella vulgaris* but high level (100 μM) SNP inhibited the chlorophyll content. They also showed that the increase and decrease of chlorophyll content were associated with the enhanced- and inhibited-expression of photosynthesis genes, respectively.

Pretreatment with a NO donor, SNP, protected young rice seedlings, resulting in better plant growth and viability (Uchida et al. 2002), promoted seed germination and root growth of yellow lupine seedlings (Kopyra and Gwozdz 2003), and increased the growth and dry weight of maize seedlings (Zhang et al. 2006b) under salt stress conditions.

Zhang et al. (2004) reported that NO enhanced salt tolerance in maize seedlings, through increasing K^+ accumulation in roots, leaves, and sheathes, as well as decreasing Na^+ accumulation. Similarly, NO induced salt resistance of calluses from *Populus euphratica* under salt stress by increasing the K^+/Na^+ radio, and this process was mediated by H_2O_2 and was dependent on the increased plasma membrane H+-ATPase activity (Zhang et al. 2007). In addition, NO was observed to stimulate the expression of plasma membrane H+-ATPase in both salt-tolerant and salt-sensitive reed calluses (Zhao et al. 2004).

Exogenously applied sodium nitroprusside (SNP), a NO donor, reduced water loss from detached wheat leaves and seedlings subjected to drought conditions, decreased ion leakage, the transpiration rate, and induced stomatal closure, thereby enhancing plant tolerance to drought stress (Garcia-Mata and Lamattina 2001). The NO regulation of stomatal closure may be via modulating intracellular Ca^{2+} in guard cells. It has been reported that NO selectively activates intracellular Ca^{2+} channels in *V. faba* guard cells through a cGMP/cADPR-dependent signaling pathway, unraveling the involvement of NO as a signaling molecule in the ABA-induced stomatal closure (Garcia-Mata et al. 2003).

Hsu and Kao (2004) also detected that 100 μM SNP decreased the APX activity in rice leaves under cadmium toxicity, while Singh et al. (2009) found that 50 μM SNP increased APX activity in roots of *Oryza sativa*. Less reduction of plant dry weight and chlorophyll content and lower electrolyte leakage were found in maize seedlings irrigated with 100 mM NaCl solution amended with NO (Zhang et al. 2006a, b). Application of NO increased chlorophyll content and photosynthetic rate (Fatma et al. 2016; Kong et al. 2016; Liu et al. 2016) and antioxidant activity (Khan et al. 2012; Liu et al. 2016), and regulated K^+ and Na^+ concentration (Zhao et al. 2004; Kong et al. 2016) in plants exposed to salinity stress. Cechin et al. (2015) confirmed the role of NO as signal molecule on sunflower plant under drought stress. They stated that water-stressed plants treated with 1 μM SNP showed an increase in the relative water content compared with 0 μM SNP. When the water-stressed plants were treated with 10 μM SNP, the activity of PG-POD and the content of proline were increased and the level of MDA was decreased. The action of NO may be explained by its ability to increase the levels of antioxidant compounds and the activity of ROS-scavenging enzymes and dependent on the external NO concentration.

Liu et al. (2016) suggested that NO could increase salinity tolerance by maintaining growth, cell membrane integrity, and proper function of the photosynthetic system. Reduced Na^+ concentration might be partially due to SNP increasing plasma membrane H+-ATPase activity to decrease the uptake of Na^+ and increase uptake of K^+, as seen in two ecotypes of reed (*Phragmites communis*) at 200 mM NaCl (Zhao et al. 2004). Singh et al. (2009) have reported that the protective effects of SNP in wheat under Cd stress clearly ascribed to the release of NO, although they did not use any NO scavenger (or the metabolic products of SNP) to establish the protective role of SNP through the release of NO. The application of NO may contribute to the decrease of H_2O_2 level, since inhibition of NO synthesis can increase the hydrogen peroxide, superoxide anion, oxidized lipid, and oxidized protein levels in *Arabidopsis* (Guo and Crawford 2005). Wang et al. (2010) showed that 25–100 μM SNP could increase total chlorophyll contents and enhance the relative growth rate of *H. verticillata*, whereas 200–400 μM SNP decreased total chlorophyll contents and have no significant effect on the relative growth rate. The results indicating NO may be not the main cause of *H. verticillata* in entrophic water column, since 100 μM SNP can cause damage to algae *Chlorella vulgaris* (Qian et al. 2009). The increased plant growth rate is associated with the decreased H_2O_2 and MDA content. Where NO (100–200 μM SNP) application could decrease the activity of APX, CAT, and POD which contributed to the accumulation of H_2O_2 and thus induced the oxidative stress. Esim and Atıcı (2014) investigated the effects of nitric oxide on the activities of antioxidant enzymes (superoxide dismutase and peroxidase), hydrogen peroxide (H_2O_2) and superoxide anion (O2.−) contents, lipid peroxidation level on the leaves of maize (*Zea mays* L.). Sodium nitroprusidde (SNP), (a donor NO) concentrations of 0.0, 0.1, 1, and 100 μM were applied by spraying on the plant leaves at 10th day and after 14 days plants were harvested. H_2O_2, O2.−, and lipid peroxidation levels were reduced by application SNP concentration compared to the control. At the same time, SNP concentrations also increased the activities of antioxidant enzymes such as SOD, POD. Application of NO also maintained higher water content, chlorophyll content, and K+ to Na+ ratio, alleviating salinity damage on plant growth and ionic balance in bermudagrass (*Cynodon dactylon*) (Liu et al. 2016).

2.5 Melatonin

Melatonin (N-acetyl-5-methoxytryptamine) is a naturally occurring low-molecular-weight multiregulatory molecule that exists in all living organisms, including plants and animals (Wang et al. 2017; Arnao and Hernández-Ruiz 2018a, b). It is considered as a central indoleamine neurotransmitter, largely involved in the diverse biological process and accepted as an important plant metabolite (Manchester et al. 2000; Murch and Saxena 2002). Since its detection in plants, scientists' curiosity regarding melatonin has increased, because of its diversified biological role as a plant master regulator and defensive roles in capricious environmental conditions, such as extreme temperatures, salinity, drought, heavy metals, UV radiation, and oxidative stress (Wang et al. 2017; Arnao and Hernández-Ruiz 2018a, b; Li et al. 2018a, b; Wei et al. 2018). It can be speculated that melatonin is inexpensive and safe for animals and humans, its application as a biostimulator could be a good, feasible, and effective method used in agriculture to decrease environmental stress (Bonnefont-Rousselot and Collin 2010) as well as increase food quality. Melatonin is an indolic compound derived from serotonin (5-hydroxytryptamine). Both biogenic amines are synthesized from the amino acid tryptophan in a biosynthetic pathway that has been extensively studied in both animals and plants (Arnao and Hernandez-Ruiz 2006; Tan et al. 2015; Back et al. 2016). In plants, tryptophan is converted into tryptamine by tryptophan decarboxylase (TDC). Tryptamine is then converted into 5-hydroxytryptamine (commonly known as serotonin) by tryptamine 5-hydroxylase (T5H). The *N*-acetylation of serotonin is catalyzed by the enzyme serotonin *N*-acetyltransferase (SNAT). *N*-Acetylserotonin is then methylated by acetylserotonin methyl transferase (ASMT), a hydroxyindole-*O*-methyltransferase, which generates melatonin. In plants, the methylation of *N*-acetylserotonin can also be performed by a caffeic acid *O*-methyltransferase (COMT), a class of enzyme that can act on a diversity of substrates, including caffeic acid and quercetin (Byeon et al. 2014). Serotonin may also be transformed into 5-methoxytryptamine by ASMT (and by COMT) to generate melatonin through the action of SNAT. Also, melatonin can be generated through the formation of *N*-acetyltryptamine, which is converted into *N*-acetylserotonin.

Serotonin is also formed from 5-hydroxytryptophan after the action of tryptophan hydroxylase (TPH) and TDC, the latter step occurring mainly in animals but also, to a lesser extent, in plants. Moreover, melatonin can be generated through the formation of 5-methoxytryptamine, as proposed by several authors, suggesting that the melatonin biosynthesis pathway may follow various alternative routes compared with animal cells, with a greater capacity of adapt to metabolic changes in plants (Arnao and Hernandez-Ruiz 2014a, b; Tan et al. 2016). In short, melatonin in plants can be synthesized in many ways, the most relevant being the sequence: tryptophan → tryptamine → serotonin → *N*-acetylserotonin → melatonin. All the named enzymes have been detected and characterized in rice and arabidopsis, except TPH, which is well known in animals but not in plants. Nevertheless, some authors have proposed that T5H can act as a hydroxylase with low substrate specificity and is capable of acting in all the hydroxylation steps described (Arnao and Hernandez-Ruiz 2014a, b; Arnao and Hernandez-Ruiz 2015a). This same broad substrate specificity can also be attributed to SNAT, ASMT, and COMT enzymes. Melatonin intermediates are produced in various subcellular compartments, such as the cytoplasm, endoplasmic reticulum, and chloroplasts. Additionally, mitochondria and chloroplast are referred to the original site of melatonin synthesis in plants (Tan et al. 2013). In another study, mitochondria were pinpointed as a major generation site for NO and ROS (Jagadis Gupta et al. 2018; Igamberdiev and Hill 2018) and could be important in playing a key role in mitigating various stresses via NO accumulation and ROS regulation (Igamberdiev and Hill 2018; Mur et al. 2013; Mao et al. 2018). The mitochondria can be damaged due to the over-production of ROS under environmental stresses (Jagadis Gupta et al. 2018). However, melatonin was reported to recover the damaged mitochondria (Franco et al. 2018).

The chemical structure of melatonin (indole derivative) and its biosynthetic pathway (from tryptophan) indicated that the influence of melatonin and auxin on plant growth was similar (Arnao and Hernandez-Ruiz 2006). Melatonin at the low level (1 lM) caused auxinic response concerning the number and length of roots, but at the higher level (10 lM) it inhibited the root growth as in sweet cherry rootstocks (Sarropoulou et al. 2012). Moreover, Hernandez-Ruiz et al. (2004) reported that melatonin at high concentrations acts as an inhibitor (probably reaching the toxic level in tissues), while at lower concentration it induces the growth of *L. albus* hypocotyl segments. Similar to IAA, melatonin acts as growth promotors and stimulate growth in etiolated lupines (*Lupinus albus*), coleoptiles of canary grass (*Phalaris canariensis*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), and oats (*Avena sativa*); however, its activity in comparison with IAA ranged between 10 and 55%. Moreover, Melatonin inhibited root elongation in some monocots even at very low concentrations. For canary grass and oats, 0.01 mM melatonin inhibited root growth (Hernandez-Ruiz et al. 2005). Whereas, the maximum inhibitory effect of melatonin on wild leaf mustard roots occurred at 100 mM, which is considerably higher than that for canary grass and oat as reported by Manchester et al. (2000), Hernandez-Ruiz et al. (2004), (2005) mentioned that a higher concentration of melatonin (200 μM) had no significant effect or even inhibitory effect on seed germination. However, lower concentrations of melatonin (50 or 100 μM) promoted seed germination as mentioned by Wei et al. (2015).

2.5.1 Role of Melatonin in Plant Growth Regulation

Melatonin accelerates seed germination (Tiryaki and Keles 2012), influences root and plant architecture (Arnao and Hernández-Ruiz 2007), enhances growth vitality, ameliorates leaf senescence, regulates nitrogen metabolism (Zhang et al. 2017a, b), and alters physiological processes by inducing differential gene expression (Arnao and Hernández-Ruiz 2018a, b). The most important function of melatonin is ROS detoxification through the production of free radicle scavengers (H_2O_2, $O_2^{\bullet-}$) and modulation of both antioxidant enzyme activity and concentration (Rodriguez et al. 2004; Shi et al. 2015). Melatonin is a dynamic antioxidant (Manchester et al. 2015; Reiter et al. 2016) that extensively stimulates cellular redox homeostasis by enhancing the activity of enzymatic antioxidants, including SOD, CAT, POD, APX, GR, and non-enzymatic antioxidants, including AsA and GSH (Hardeland 2014; Arnao and Hernández-Ruiz 2015b; Reiter et al. 2015; Nawaz et al. 2018). Therefore, melatonin helps detoxify excess ROS, which helps plants to survive under stressful conditions.

Melatonin has a range of possible cellular and physiological effects, such as changes in intracellular Ca^{2+} and in the permeability of membranes mediated by ion transporters (Li et al. 2016a); changes in the opening and/or closing of stomata, in carbohydrate, lipid, and nitrogen metabolisms, and also in osmoprotector metabolites (Shi et al. 2015; Wei et al. 2015) as well as optimizing efficiency and leaf water/CO_2 exchange (Li et al. 2017) and regulates other processes, such as ripening and/or senescence, the internal biological clock and parthenocarpy (Liang et al. 2018; Liu et al. 2018; Arnao and Hernández-Ruiz 2018a, b). In some cases, there are sufficient data to show that melatonin exerts its function in parallel to other plant hormones. For example, in the auxinic response to plant growth, IAA and melatonin appear to regulate the process by independent pathways (Pelagio-Flores et al. 2012), and do so by regulating the differential expression of the auxin transporters (Weeda et al. 2014). Something similar appears to occur during the generation of root primordia and/or rooting and also in gravitropism (Arnao and Hernández-Ruiz 2017).

Melatonin appears to have a clear role in plant stress signaling, in combination with other plant hormones. Its relationship with ROS and RNS provides evidence that melatonin is a key component in the center of the redox network from which the different biochemical, cellular, and physiological responses are controlled. The melatonin–ROS–RNS triangle is self-regulating through the components directly interacting with each other as well as through regulating their own biosynthesis and that of their catabolic genes. The recent identification of the plant melatonin receptor in arabidopsis provides a cornerstone for understanding the responses and elements of the transduction chain. There are also some signal transduction elements shared by plant hormones and melatonin (Arnao and Hernández-Ruiz 2018a, b).

Melatonin acts as an effective free radical scavenger against hazardous reactive molecules, both reactive oxygen and reactive nitrogen species (ROS/RNS), among others. The excellent properties of melatonin as a natural antioxidant against ROS/RNS was mentioned by Tan et al. (2000), Teixeira et al. (2003), Reiter et al. (2014), Arnao and Hernandez-Ruiz (2015a).

Higher plant survival rates, higher shoot and root growth, and photosynthetic efficiency, accompanied by improved chloroplast and stomatic morphologies, and high sucrose and proline levels have been observed in melatonin-treated plants, together with lower levels of ROS/RNS, lipid membrane peroxidation, and cell damage (Kolar and Machackova 2005; Arnao and Hernandez-Ruiz 2009a, b, 2013, 2015b; Shi et al. 2016).

Generally, melatonin exerts multiple functions on plant development that can be recognized into three categories: (1) growth promoter as an auxin (acts as an independent plant growth regulator and it may mediate the activities of other plant growth regulators (Park 2011); (2) antioxidant for free radicals; (3) and other functions (signal molecule for circadian maintenance, regulation of flower development, or maintenance of developmental stage in fruits tissue) (Paredes et al. 2009).

Beneficial effects of melatonin may result from its signaling function, through the induction of different metabolic pathways and stimulate the production of various substances, preferably operating under stress (Tan et al. 2012).

2.5.2 Role of Melatonin in Mitigating Abiotic Stresses

The very amplitude of its biological actions in plants has led it to being called a multiregulatory molecule (Arnao and Hernandez-Ruiz 2018a, b). Such actions include the ability to act as a plant biostimulator against stress, both biotic and abiotic; the ability to regulate plant growth; the ability to regulate processes of plant vegetative development, such as rooting, leaf senescence, photosynthetic efficiency, and biomass yield; as well as a role as a potential regulator in the processes of flowering, and the formation and ripening of fruits and seeds (Arnao and Hernandez-Ruiz 2014a, b, 2015a, b; Hernandez-Ruiz and Arnao 2015; Nawaz et al. 2016).

In recent times, melatonin as a biostimulant and plant growth regulator attracts the interest of plant biologists (Arnao an Hernández-Ruiz 2015a, b). For instance, it provides physiological and molecular resistance against many abiotic stresses by means of its involvement in regulating stress signaling (Xu et al. 2016; Liang et al. 2018). Additionally, its beneficial effect on photosynthesis and other growth-related factors among different crops under the diverse abiotic stresses is another promising aspect of melatonin application (Meng et al. 2014; Wang et al. 2016). Exogenous melatonin treatments induced the level of endogenous abscisic acid (ABA) and gibberellic acid (GA) in cucumber seedling under the saline condition, due to improve the resistance against salinity (Zhang et al. 2014). While in plants affected by heat stress, the level of cytokinin (CK) was degraded gradually. Increases in the level of CK was observed after the plants were treated with exogenous melatonin. The study further reported that the resistance against heat stress was perceived in the melatonin-treated plants due to enhanced CK level (Zhang et al. 2017a, b).

The ability of melatonin to scavenge H_2O_2 and the induction of antioxidant enzymes activities by melatonin helps to recover plants from abiotic stresses (Li et al. 2012; Keunen et al. 2013; Liang et al. 2015). The melatonin was proposed to upregulate the expression of heat shock protein (HSP) to mitigate the high-temperature stress

(Xu et al. 2016). While for biotic stress, the melatonin was anticipated for activating the NO and salicylic acid (SA) mediated defense signaling pathway by expressing the PR-protein (pathogenesis-related protein) immediately (Shi et al. 2015; Zhao et al. 2015).

The reported advantages of melatonin under environmental stress include improved germination and growth at low temperatures (Han et al. 2017), heat stress prevention (Hernández et al. 2015), germination improvement in contaminated soil (Posmyk et al. 2008), increased tolerance in water and saline stress (Zhang et al. 2014), and disease resistance (Nawaz et al. 2016).

Mukherjee et al. (2014) reported that salt stress for 48 h caused increases in endogenous serotonin and melatonin content in roots and cotyledons of sunflower (*Helianthus annuus*) seedling. Accumulation of serotonin and melatonin under salt stress exhibits differential distribution in the vascular bundles and cortex in the differentiating zones of the primary roots, suggesting their compartmentalization in the growing region of roots. Moreover, serotonin and melatonin accumulation in oil body rich cells of salt-treated seedling cotyledons correlates with longer retention of oil bodies in the cotyledons. Exogenous serotonin and melatonin treatments (15 μM) regulate hypocotyl elongation and root growth of sunflower (*Helianthus annuus*) seedling under NaCl stress. Salt stress-induced root growth inhibition, thus pertains to partial impairment of auxin functions caused by increased serotonin biosynthesis. In seedling cotyledons, NaCl stress modulates the activity of N-acetylserotonin O-methyltransferase (HIOMT; EC 2.1.1.4), the enzyme responsible for melatonin biosynthesis from N-acetylserotonin.

Li et al. (2016b) reported that pretreated melatonin protects oxidative damage in cucumber through melatonin-mediated redox signaling pathways. Ke et al. (2018) determined that supplemental melatonin alleviates salinity stress in wheat seedlings by regulating PAs metabolism. Additionally, melatonin pretreatment alleviated chilling stress in harvested peach fruits (Cao et al. 2016) and cucumber seedlings (Zhao et al. 2017) which are closely related to PAs metabolism.

Melatonin might ameliorate the thermal-oxidative stress by interacting with the PAs and NO biosynthesis pathways (Jahan et al. 2019). The exogenous application of melatonin elevated the endogenous free PAs level. Similarly, expression levels of different PAs biosynthesis genes were also upregulated in melatonin-pretreated heat-stressed seedlings. Melatonin along with NO has the potential to combat different stress conditions through the L-arginine and PAs metabolic pathways (Gupta et al. 2011).

Jahan et al. (2019) observed that 100 μM exogenous melatonin treatment improved the thermal tolerance of tomato seedlings by lowering ROS (H_2O_2, $O_2^{\bullet-}$, MDA) production, enhanced antioxidant enzyme activity, AsA–GSH cycle modulation, and upregulation of antioxidant-related gene expression. Additionally, melatonin elevates endogenous PAs via upregulation of PAs biosynthesis genes. NO content along with NR and NOS activity was also increased with melatonin supplementation. Therefore, they concluded that heat stress-induced damage was suppressed by melatonin, which coordinates with the PAs and NO biosynthesis pathways, which helps to detoxify the over accumulated ROS. These findings provide novel insight into the cross-talk that exists among melatonin, PAs, and NO to inhibit thermal stress. Overall, melatonin enhanced mitigation of heat-induced damage through coordination with PA- and NO-mediated signaling pathways. It is considered primarily an antioxidant with important actions in the control of reactive oxygen and nitrogen species (ROS and RNS), among other free radicals, and harmful oxidative molecules present in plant cells (Arnao and Hernández-Ruiz 2019). Interestingly, pretreatment tea leaf with melatonin on leaves alleviated ROS burst, decreased malondialdehyde levels, and maintained high photosynthetic effciency. Moreover, 100 _M melatonin-pretreated tea plants showed high levels of glutathione and ascorbic acid and increased the activities of superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase under abiotic stress. Notably, melatonin treatments can positively upregulate the genes expression of antioxidant enzyme biosynthesis (Li et al. 2019).

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Neurotransmitters in Signalling and Adaptation to Salinity Stress in Plants

Mohsin Tanveer and Sergey Shabala

Abstract Soil salinity is a major threat to agricultural sustainability and global food security. While a major progress has been made in understanding the molecular identity of key ion transporters mediating plant adaptive responses to salinity, the modes of their post-translational regulation remain elusive. In mammalian systems, a broad class of chemical substances termed ‘neurotransmitters’ operates upstream of receptors and membrane transporters. Many of these substances have been also found in plants and shown to operate in various signalling cascades. In this chapter, we focus on several of such neurotransmitters and summarize the current knowledge of the roles and operational modes in plant adaptive responses to salinity. The above list includes melatonin; serotonin; GABA; dopamine; glutamate; NMDA and purines.

1 Introduction

Soil salinity is a major threat to agricultural sustainability and global food security. Currently, over 50% of the world’s population (4.03 Bn people) live in 13 countries most affected by soil salinity (Liu et al. 2020a). The current trends in population dynamics, urbanization and climate change will exacerbate the process of land salinization (Shabala 2013; Panta et al. 2014; Florke et al. 2019; Pan et al. 2020). All major staple crops (wheat, rice, maize) that provide over 50% of calories intake to human population are highly sensitive to salinity and show significant yield losses when grown even in moderately saline soils (Liu et al. 2020a). Thus, understanding the physiological and genetic basis of salinity tolerance in plants is of a paramount importance for incorporating the appropriate traits into high yielding varieties, to match predicted population growth and ensure global food security.

Salinity tolerance trait is highly complicated, both genetically and physiologically, and relies on an orchestrated operation of numerous complementary mechanisms and signalling pathways (Julkowska and Testerink 2015; Shabala et al. 2015; Huang et al 2019; Yang and Guo 2018; Manishankar et al. 2018; Koster et al. 2019; Zhao et al

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2020). Among these, efficient operation of membrane transporters regulating ion transport and intracellular homeostasis is of a critical importance. Over the last few decades, a major progress has been made in understanding the molecular nature of such transporters and their role in plant adaptive responses to salinity (see next section). What is less known, however, are the modes of their regulation.

In mammalian systems, a broad class of chemical substances termed 'neurotransmitters' operates upstream of a broad array of receptors and membrane transporters, to mediate transfer of the signal to the nerve or a muscle fibre, or some other structure. Such neurotransmitters are numerous (over 200 known substances listed in Wikipedia) and are broadly classified in several major categories such as amino acids, gasotransmitters, monoamines, trace amines, peptides, purines, catecholamines and some others. Many of these substances have been also found in plants and shown to operate in various signalling cascades. In this chapter, we focus on several of such neurotransmitters (Fig. 1) and summarize the current knowledge of their roles and operational modes in plant adaptive responses to salinity.

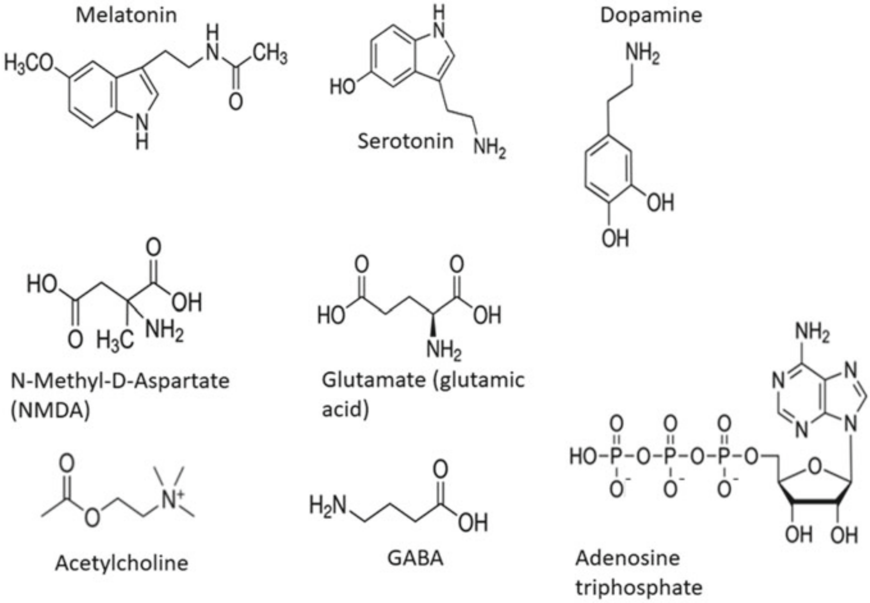

Fig. 1 The chemical structure of some known neuromodulators operating in plants and discussed in this review

2 Ion Transporters in Plant Sensing and Adaptation to Salinity

Traditionally, detrimental effects of salinity can be roughly divided into two major categories: osmotic stress and ion toxicity. To deal with each of them, plants employ a broad range of membrane transporters.

Na^+/H^+ exchangers. Two major types of Na^+/H^+ exchangers operate in plant cell; both belong to the cation proton antiporter (CPA) subfamily of proteins (Maser et al. 2001). SOS1 exchangers are located at the plasma membrane. They are located predominantly in the root apex (Zhu 2001; Zhao et al. 2020) and represent the key mechanism for Na^+ exclusion from uptake. SOS1 transporters are also highly abundant in the xylem parenchyma (Shi et al. 2002) and control xylem Na^+ loading and delivery to the shoot. Tonoplast-based Na^+/H^+ exchangers from the NHX family mediate vacuolar Na^+ sequestration (Bassil and Blumwald 2014) thus reducing toxic effects of Na^+ in the cytosol.

HKT transporters. HKT proteins belong to the HKT/Trk/Ktr-type superfamily of K^+ transporters (Zhao et al. 2020). Members of class I (HKT1) contain a Ser residue at the first pore-loop domain and are highly selective for Na^+ over K^+ (Horie et al. 2001). When expressed in *Xenopus laevis* oocytes and yeast, HKT1 transporters show a highly specific Na^+ influx (Uozumi et al. 2000; Xue et al. 2011). The functional role of HKT1 transporter is in Na^+ retrieval from the xylem (Maser et al. 2002; Munns et al. 2012; Zhang et al. 2018). Class II HKT transporters operate in root epidermis and represent one of the pathways for Na^+ entry into the root (Hauser and Horie 2010; Mian et al. 2011).

Non-selective cation channels. Non-selective cation channels (NSCCs) mediate passive fluxes of cations through plant membranes. Two major classes of NSCC are glutamate receptors (GLR) and cyclic nucleotide-gated channels (CNGC); 20 members each in group are found in Arabidopsis genome (Demidchik and Maathuis 2007). All these channels are present at the plasma membrane and are permeable to Na^+ thus representing a major pathway for Na^+ entry into the plant. NSCC also represents a major pathway for salt stress-induced K^+ leak (Wu et al. 2018) thus affecting cytosolic K^+ homeostasis (a key determinant of plant salinity tolerance; Shabala et al. 2016a, b, c; Rubio et al. 2020). NSCC also operates as Ca^{2+} -permeable channels and thus mediate salinity stress-induced cytosolic Ca^{2+} signalling (Demidchik et al. 2018; Demidchik and Shabala 2018) that plays a critical role in adaptation to salinity. Several other types of NSCC such as annexins (Laohavisit et al. 2013; Yadav et al. 2018) or TPC (two-pore cation channels; Hedrich et al. 2018; Pottosin and Dobrovinskaya 2018) are also involved in such signalling.

H^+ pumps. Two major types of H^+-pumps, fuelled, respectively, by ATPase and PPase, are present at cellular membranes. H^+-ATPases are found on both plasma (PM) and tonoplast membranes, while H^+-PPases operate at the tonoplast only. H^+ pumps are a major electrogenic source that generates an electric potential difference across the PM (Palmgren and Nissen 2011); they also create a proton motive force for the secondary active transport of both cations and anions (Shabala et al. 2016a, b, c). Salinity-induced activation of H^+-ATPase transport and hydrolytic activity was reported for both halophyte (Vera-Estrella et al. 2005; Bose et al. 2015) and glycophyte (Chen et al. 2007; Alvarez-Pizarro et al. 2009) species; this activation occurs at both transcriptional and post-translational levels (Fuglsang et al. 2011; Palmgren and Nissen 2011).

2.1 GORK K^+ Efflux Channels

Cytosolic K^+ homeostasis is a critical determinant of the cell's fate, with cytosolic K^+ loss causing the activation of the catabolic enzymes leading to programmed cell death (PCD) (Shabala 2009; Demidchik et al. 2010). The outward rectifying potassium efflux GORK channels are central to this stress-induced K^+ loss from the cytosol (Shabala and Pottosin 2014; Wu et al. 2018; Rubio et al. 2020). GORK channels show strong voltage gating and are activated by salt stress-induced membrane depolarization (Hosy et al. 2003). A recent bioinformatic analysis has also shown that GORK channels possess the binding motifs and domains that may enable them to operate as ligand-gated channels, with their activity being modulated by cyclic nucleotides, GABA, G proteins, protein phosphatases, inositol and ATP (Adem et al. 2020).

2.2 K^+ Uptake Channels and Transporters

While maintaining high level of cytosolic K^+ is essential for optimal operation of numerous metabolic enzymes (Dreyer and Uozumi 2011; Wu et al. 2018), inhibiting energy-consuming anabolic reactions and saving energy for adaptation and repair may give species a competitive advantage under energy-limiting conditions imposed by salinity (Rubio et al. 2020). In this context, salinity stress-induced K^+ efflux was suggested as a 'metabolic switch' that allows plants to redirect a large pool of ATP towards defence reactions (Demidchik 2014; Shabala 2017). However, the amount of K^+ lost for signalling purposes should not compromise plant's nutritional demand for this element. Plants solve this dilemma by using K^+ efflux as a signal in the root apex (Shabala et al. 2016a, b, c; Rubio et al. 2020) and compensating its loss by activation of high- and low-affinity K^+ uptake system in the mature root zone. Here, HAK/KUP K^+/H^+ cotransporters operate at lower micromolar range of external K^+ concentrations, and at concentrations above 0.5 mM low-affinity K^+ uptake prevails (mediated by AKT-type of channels; Nieves-Cordones et al. 2016).

2.3 Ca^{2+} ATPases and Exchangers

Rapid channel-mediated stress-induced elevation in the cytosolic Ca^{2+} ($[Ca^{2+}]_{cyt}$) is one of the earliest events observed in response to salt stress (Tracy et al. 2008). These stress-induced $[Ca^{2+}]_{cyt}$ elevations vary in magnitude, frequency and shape, depending on the severity of the stress as well as the type of stress experienced (Wilkins et al. 2016; Koster et al. 2019). This creates a unique stress-specific calcium 'signature' that is then decoded by signal transduction networks (Bose et al. 2011). However, once the signalling is over, the basal $[Ca^{2+}]_{cyt}$ levels have to be restored, to enable its operation as a second messenger in plant adaptive responses. Plants evolved two major mechanisms for transporting Ca^{2+} against its electrochemical gradient that is required for the removal of excess Ca^{2+} from the cytosol and reloading it into internal stores (Demidchik and Shabala 2018). The first one involves Ca^{2+}-ATPases, enzymes belonging to P-type ATPase superfamily (Bonza and De Michelis 2011; Huda et al. 2013). The second mechanism employs Ca^{2+}/H^+ exchangers (CAXs) (Manohar et al. 2011; Pittman and Hirschi 2016).

2.4 NADPH Oxidases

To adapt to salt stress, plants rely on ROS-mediated signalling network that operates upstream of a broad array of physiological and genetic alterations. A key player in this ROS signalling is the NADPH oxidase, a plasma-membrane-bound enzyme from the NOX family encoded by *RBOH* genes (Liu et al. 2020b). NADPH oxidases are activated by salt stress, both at the transcriptional and post-translational levels (Chung et al. 2008; Ma et al. 2012) and, together with ROS-activated Ca^{2+}-permeable channels, form the so-called 'ROS-Ca^{2+} hub' (Demidchik et al. 2003; Foreman et al. 2003; Demidchik and Shabala 2018) that operates as an efficient self-amplifying mechanism. Recent bioinformatic analysis suggested that the number of RBOH genes is smaller in halophytes compared with glycophytes, while their potential activation can be faster owing to additional phosphorylation sites in N-terminus (Liu et al. 2020b).

3 Neurotransmitters as Regulators of Cellular Ionic Homeostasis in Plants

3.1 Melatonin and Serotonin

Melatonin and serotonin are two major indoleamines derived from tryptophan (Benjamin et al. 2019) and found to be present in different plant families (Park and

Back 2012; Pelagio-Flores et al. 2012; Wei et al. 2015). Melatonin- and serotonin-induced salinity tolerance in plants is associated with their enhanced antioxidant activity and regulation of the transcripts of ion channels genes such as SOS, NHX or AKT (Li et al. 2012; Shi et al. 2015). Under salinity stress, elevation in the cytosolic Na^+ compromises plant metabolic activity (Pan et al. 2020; Khare et al. 2020). Both these neurotransmitters play a critical role in reducing this cellular Na^+ toxicity. Melatonin application increased K^+/Na^+ ratio and decreased Na^+ and Cl^- contents in roots under salinity stress (Dawood and El-Awadi 2015). To the large extent, these beneficial effects are attributed to the activation of the SOS pathway (Li et al. 2016a, b). Melatonin application increased the expression of *OsSOS1* transcript thus increasing plant's capacity for Na^+ export from roots and retention in stems and preventing Na^+ from reaching photosynthetic leaf tissues (Olías et al. 2009; Li et al. 2017).

Among different components of SOS signalling pathway, SOS2 and SOS3 are calcium-dependent proteins (CIPK24 and CBL4, respectively) that are activated upon Ca^{2+} binding following its elevation upon salinity stress (Almeida et al. 2017). Melatonin application induced increased Ca^{2+} uptake and reduced Na^+ accumulation in different plant tissues (Wu et al. 2019), further indicating the signalling role of melatonin in inducing Ca^{2+} dependent signalling cascades and activating SOS pathway. Overexpression of serotonin *N*-acetyltransferase gene (SNAT) in *Arabidopsis* resulted in a salt-tolerant phenotype, with an increase in salinity tolerance in transgenic lines being associated with the increase of SOS1 mediated Na^+-efflux and NHX- mediated Na^+ vacuolar sequestration (Zhao et al. 2019).

Maintaining optimal cytosolic K^+ content and reducing stress-induced K^+-leakage is an essential trait associated with salinity stress tolerance (Wu et al. 2018). The magnitude of salinity stress-induced K^+-efflux depends largely on two factors, namely plant's ability to manage ROS production and regulate the plasma membrane H^+-ATPase activity (Shabala and Pottosin 2014). Intrinsically higher H^+-ATPase activity is essential to avoid membrane depolarization and salinity stress tolerance (Wu et al. 2015). Upon salinity stress, uncontrolled transport of positively charged Na^+ ions across the plasma membrane induces membrane depolarization, leading to K^+-efflux mediated by depolarization-activated GORK channels (Shabala et al. 2016a, b, c). To overcome such depolarization, plants tend to increase the activity of plasma membrane H^+-ATPase activity and pump out H^+ across the plasma membrane (Jung et al. 2017). Melatonin has been observed to reduce K^+-efflux by increasing H^+-ATPase activity, thus increasing salinity stress tolerance (Yu et al. 2018). Direct measurements of H^+ pumping and ATP hydrolysis capacity proved that melatonin can also reverse the inhibitory effects of salinity on the activity of PM H^+-ATPase. Thus, melatonin-induced enhancement of PM H^+-ATPase activity may negate salinity-induced MP depolarization thus preventing activation of outward K^+ channels, thereby leading to a smaller reduction in cytosolic K^+ concentration under salinity stress (Yu et al. 2018). In rice plants, melatonin also enhanced the expression of both high- (*OsHAK1* and *OsHAK5*) and low-affinity (*OsAKT1*) potassium transporter genes (Liu et al. 2020c), with strongest effects confined to the root apex.

Melatonin has been found to actively regulate K^+ homeostasis by regulating the transcript of *NHX1, AKT1* and *HKT1* genes (Li et al. 2012, 2016a, b), most likely via SOS3–SOS2 pathway (Li et al. 2016a, b).

ROS production under salinity stress also triggers K^+-efflux by activating ROS-gated NSCC and/or GORK channels. Melatonin is known to be an important ROS scavenger (Arora and Bhatla 2017). Exogenous melatonin application improved oxidative stress tolerance and resulted in reduced K^+ loss in sweet potato exposed to H_2O_2 stress (Li et al. 2016a, b). The above effects were attributed to both higher ROS scavenging capacity and/or subsequent deactivation of ROS-dependent K^+ permeable channels (Yu et al. 2018). Similarly, exogenous application of melatonin reduced ROS production and improved salinity tolerance in *Malus hupehensis* (Li et al. 2012).

The causal link between melatonin and ROS scavenging/signalling was demonstrated in experiments with Arabidopsis *Rboh* knockout mutants. Exogenous supply of melatonin resulted in a significant reduction in Na^+ accumulation in wild type but not *AtrbohF*, suggesting *AtrbohF*-dependent regulation of ROS signalling is required for melatonin-induced salinity tolerance in *Arabidopsis* (Chen et al. 2017). Further genetic evidence suggested that the *SOS*-mediated Na^+ efflux triggered by *AtrbohF*-dependent ROS might be one of the effective strategies for plant survival in the presence of melatonin (Chen et al. 2017). Electrophysiological experiments on rice revealed that melatonin reduced sensitivity of the plasma membrane K^+-permeable channels to hydroxyl radicals, thus decreasing salt-induced K^+ efflux (Liu et al. 2020c). These beneficial effects of melatonin on plant phenotype and K^+ retention were abolished by NADPH oxidase blocker DPI. Transcriptome analyses also revealed that melatonin induced 585 differentially expressed genes in rice roots; of these, the most noticeable changes were related to increase in the expression of several DEGs encoding respiratory burst NADPH oxidase (*OsRBOHA* and *OsRBOHF*), calcineurin B-like/calcineurin B-like-interacting protein kinase (*OsCBL/OsCIPK*) and calcium-dependent protein kinase (*OsCDPK*) under salt stress (Liu et al. 2020c). A brief summary of potential role of melatonin in controlling intracellular ionic homeostasis under salinity stress is given in Fig. 2.

3.2 Dopamine

Dopamine (3,4-dihydroxyphenethylamine) is a non-protein amino acid that belongs to the catecholamine and phenethylamine families. In human and animal, dopamine acts as a neurotransmitter and plays multiple roles in numerous physiological processes (Melrose et al. 2010; Wang et al. 2018). In plants, dopamine is produced from tyrosine amino acid. Its content varies between different crops (Kulma and Szopa 2007), and it regulates numerous physiological processes such as activation of an antioxidant system, hormonal production and plant sugar metabolism (Szopa et al. 2001; Allen 2003; Skirycz et al. 2005). Dopamine has also found to regulate cell ion permeability and ion transport (Jiao et al. 2019). Exogenous application of dopamine significantly reduced Na^+ and Cl^- uptake and increased K^+ uptake in root

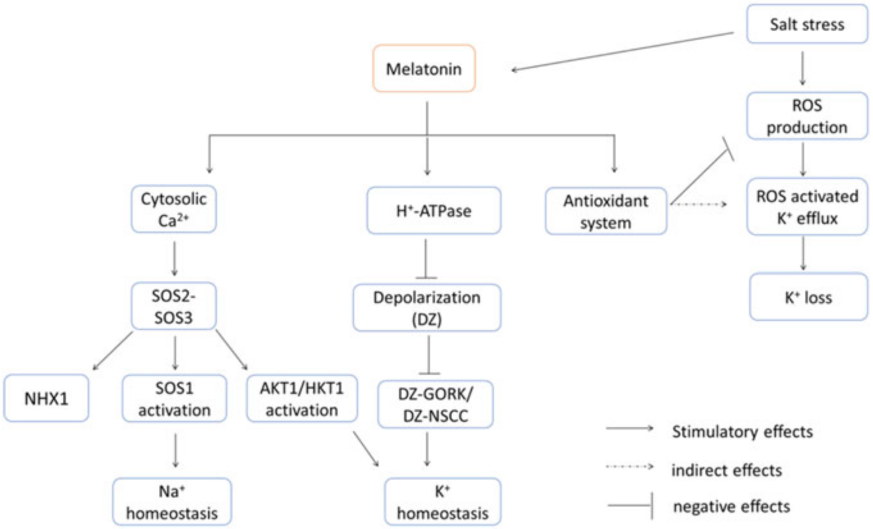

Fig. 2 A tentative model depicting the role of melatonin in mediating Na^+ and K^+ homeostasis in plants

and leaf tissues of *Malus hupehensis* (Li et al. 2015). Further molecular evidences revealed that dopamine-induced reduction in the cellular Na^+ was due to higher activation of *SOS1* at the plasma membrane and *NHX1* at vacuolar membrane while higher K^+ uptake was due to higher activation of *HKT1* in the presence of dopamine (Li et al. 2015).

Hyperosmotic stress is another constraint imposed by salinity stress on plants. Osmotic stress induces water deficit which results in imbalance in ion uptake and ROS production (Parida and Das 2005). Dopamine has been shown as down-regulator of ROS production, controlling ROS production via activating antioxidant defence system (Soares et al. 2014).

Recently, aquaporins have emerged as a possible pathway of Na^+ entry into plant cells (Byrt et al. 2017). In this context, dopamine has been shown to down-regulate expression levels of PIP*1;3* in salt-stressed rice and thus reduce Na^+ uptake and accumulation under saline conditions (Abdelkader et al. 2012). Dopamine may also help plants to cope with salinity-induced osmotic stress by sensing osmotic pressures changes by regulating or activating mechano-sensitive cation channels (MSCC). These channels could be possible candidates for sensing ionic toxicity or ionic deficiency in plant tissues, as they can convert mechanical changes (induced by turgor pressure) into electrical signals. MSCC are diverse in their conductivity ranging from non-selective to K^+ and Ca^{2+} selective channels (Monshausen and Haswell 2013; Shabala et al. 2015). While no direct evidence has been reported so far relating to the activation of MSCCs by dopamine, future studies should focus on this aspect as well. Dopamine and other catecholamines were shown to participate in the intercellular regulation of ion permeability and photophosphorylation of chloroplasts (Roshchina

2001). Pre-treatment with adrenaline and dopamine agonists and antagonists led to changes in verapamil-induced Ca^{2+} and K^+ fluxes in oat coleoptile parenchyma cells (Babourina et al. 2000).

3.3 Acetylcholine

Acetylcholine is one of the important neurotransmitters in human and animal brain (Hillmer et al. 2016; Salma et al. 2018). Acetylcholine has been found in all types of plants as well as in bacteria and fungi (Fluck and Jaffe 1976). In plants, acetylcholinesterase family is widely distributed across the plant kingdom (Sagane et al. 2005) suggesting that acetylcholine and its related molecules might play a role in plant response to environmental stimuli (Sugiyama and Tezuka 2011). In tobacco, exogenous acetylcholine application improved root hydraulic conductivity and increased the expression of numerous genes involved in chlorophyll biosynthesis, thus having beneficial effects on salinity tolerance (Qin et al. 2019). Acetylcholine appeared to regulate ion transport proteins such as NHX1, HKT1 and AKT1, leading to maintenance of high K^+ and low Na^+ (Qin et al. 2019).

Acetylecholine is also causally related to the long-distant electrical signalling in plants. Analysis of action potential characteristics in *Nitellopsis obtusa* cells showed that when H^+-ATPase activity was blocked using a H^+-ATPase blocker, acetylcholine application increased the membrane depolarization and prolonged repolarization and intracellular Ca^{2+} concentration, suggesting a link between acetylcholine signalling, membrane potential and Ca^{2+} signalling (Kisnieriene et al. 2012). Reported effects of acetylcholine on ion transport include the regulation of membrane permeability to K^+ (Kisnieriene et al. 2012), Ca^{2+} (Palee et al. 2016) and Cl^- (Gong & Bisson 2002; Yamada et al. 2011)—three ions are involved in a generation of plant action potentials (Sukhov et al. 2011). Also, choline acts as a precursor of acetylcholine biosynthesis and as a neurotransmitter (Zhao et al. 2001). Choline pre-treatment improves K^+/Na^+ ratio in salt-grown plants (Gao et al. 2020), as a result of a significant reduction in Na^+ and Cl^- uptake and increased K^+ and Ca^{2+} accumulation (Salama et al. 2011). Patch-clamp experiments also revealed that choline application exerted a potent block on slow vacuolar (SV) channel (Pottosin et al. 2014). Encoded by *TPC1* gene, this channel is critical to prevent Na^+ leak from the vacuole into cytosol and, therefore, enable efficient Na^+ sequestration and osmotic adjustment in the cell.

3.4 Purines (ATP)

Purines are bicyclic nitrogen-containing aromatic bases and an integral component of different important biomolecules such as ATP, GTP and NADH. ATP is a universal energy currency, and in plants cytosolic ATP is reported to range between 0.5 and

2 mM (De Col et al. 2017). Not surprising that early studies in 60 s and 70 s interpreted effects of purines as related to the energy supply and Ca^{2+} chelation but not as signalling molecules. Recent evidences, however, showed that low nano- and micromolar levels of ATP operate as potent signals and are unrelated to cell energetics (Demidchik 2014). This signalling involves release of ATP in apoplast (thus the term eATP) (Wu et al. 2011; Matthus et al. 2019). eATP accumulates to nanomolar concentration during growth (Dark et al. 2011; Zhu et al. 2017; Nizam et al. 2019). eATP levels are controlled by ATP-hydrolysing enzymes such as nucleotidases and apyrases (Massalski et al. 2015). Upon stress imposition, oxidative damage to plasma membrane induces the release of intracellular ATP into extracellular spaces (Dark et al. 2011), therefore, eATP has been termed as 'danger signal' in the literature (Choi et al. 2014). By analogy to the cytosolic free Ca^{2+}, certain basal levels of eATP are required for normal plant functioning (Jia et al. 2019).

Extracellular ATP induces accumulation of Ca^{2+}, ROS, NO and phosphatidic acid; all these molecules are involved in a complex signalling network (Clark and Roux 2018; Wang et al. 2019). These eATP-stimulated secondary messengers may be involved in changes in the gene expression and protein abundance (Lang et al. 2017; Tripathi et al. 2018; Jewell et al. 2019). As a result, eATP acts as an important signalling molecule in mediating plant growth and stress responses (Bonora et al. 2012). In this context, eATP was shown to play a role in root hair and cotton fibre growth (Clark et al. 2010a, b), auxin transport (Tang et al. 2003), root gravitropism (Tang et al. 2003, 2010), stomatal movement (Hao et al. 2012) and cell viability (Sun et al. 2012a, b). eATP has been also widely accepted as a signalling molecule that plays an important role in regulating ionic homeostasis under salinity stress. In plants, eATP can be sensed by purinergic receptors (e.g. P2X- Choi et al. 2014) and then activate downstream signalling cascades. Recently experiments with *Arabidopsis* revealed that a lectin receptor kinase (LecRK-1.9) is another eATP receptor (Balague et al. 2016). Downstream eATP signal transduction targets in the plasma membrane include heterotrimeric G proteins (Hao et al. 2012), Ca^{2+} channels (Wang et al. 2014) and NADPH oxidase (Hao et al. 2012; Wang et al. 2014). In *Arabidopsis*, eATP application increased cytosolic Ca^{2+} and ROS production; however, these responses were blocked in the presence of animal purinergic receptors (Song et al. 2006; Demidchik et al. 2009). Moreover, in *Arabidopsis*, a knockdown of the heterotrimeric G protein α subunit resulted in an impaired Ca^{2+} influx and PIN2 distribution, thus confirming the role of heterotrimeric G proteins in eATP sensing (Zhu et al. 2017).

The salinity stress-elicited eATP accumulation results in an abrupt ROS production via the activation of PM-NADPH oxidase (Demidchik et al. 2009; Sun et al. 2012a), which, as an early response, causes elevation of the cytosolic Ca^{2+} to initiate Ca^{2+} signalling cascades and stress adaptation. Cellular Na^+ toxicity is one of the major problem encountered during salinity stress, and role of eATP in mediating Na^+ homeostasis has been reported in numerous studies (Sun et al. 2012a, b; Lang et al. 2014). Pre-treatment with purines resulted in a reduced Na^+ concentration in a shoot while K^+ concentration was increased (Yasmeen et al. 2013; Bajwa et al. 2018). The molecular basis of eATP control over Na^+ transport and salinity tolerance remains elusive. It was shown that eATP interacts with the cytosolic Ca^{2+},

H_2O_2 and NO and increases the transcription of *SOS3, CIPK*, *RbohD* (respiratory burst oxidase homolog protein D), *NIR* (nitrate reductase), *MAPK3*, and *MAPK6* (the mitogen-activated protein kinases 3 and 6) and Na^+/H^+ antiporters, to confer salinity stress tolerance in plants (Zhao et al. 2016; Lang et al. 2017). The possible mechanism is summarized in Fig. 3. Upon salinity exposure, Na^+ enters into cytosol and causes membrane depolarization and ROS production which activate voltage and ROS-dependent K^+ outward rectifying channels to trigger K^+ efflux. To encounter this, eATP sensed by a purino-receptor at the plasma membrane activates NADPH oxidase to produce ROS. This elevates cytosolic Ca^{2+}, which then binds to SOS2–SOS3 complex and causes Na^+ efflux via the activation of Na^+/H^+ antiporter (SOS1) at the plasma membrane. To reduce PM depolarization and retain K^+, plants initiate

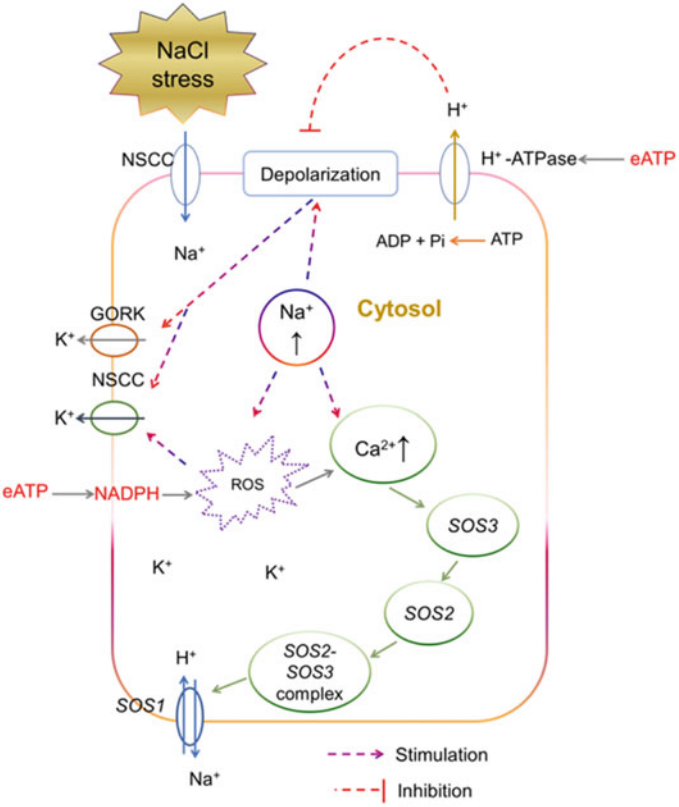

Fig. 3 A model depicting a signalling role of eATP in conferring salinity stress tolerance in plants. Upon salinity stress, Na^+ causes membrane depolarization that leads to a K^+ efflux via GORK and NSCC channels. eATP assists in reducing K^+ loss and induces salinity tolerance in plants by (1) activating H^+-ATPase activity at the plasma membrane thus preventing membrane depolarization and assisting in K^+ retention and (2) by the activation of NADPH oxidase at the plasma membrane to induce ROS production, which concomitantly increases cytosolic Ca^{2+} to initiate SOS pathway in order to induce Na^+ efflux

H^+ pumping, and eATP was found to increase the transcript levels of both S*OS1* and *AHA* to maintain higher cytosolic K^+/Na^+ ratio during salinity stress (Sun et al. 2012a; Lang et al. 2017).

3.5 N-Methyl-d-Aspartic Acid or N-Methyl-d-Aspartate (NMDA)

NMDA is an amino acid derivative that acts as a specific agonist and a neurotransmitter in humans. In plants, asparate has been shown to activate numerous physiological mechanisms including antioxidant defence system, ion transport and hormonal regulation and improving overall stress tolerance in plants (Kim et al. 2005; Surabhi et al. 2008). HKT-type transporters play a key role in keeping low cytosolic Na^+ concentrations thus maintaining higher K^+/Na^+ ratio and inducing high salinity stress tolerance (Suzuki et al. 2016; Wang et al. 2020). As commented earlier, there are two different types of HKT transporters. These share nucleotide and protein sequences, but still show differential ion selectivity and response to salinity stress (Ali et al. 2019). The ion selectivity of different HKT-type transporters depends on the presence of specific amino acid in their pore-loop domain. A comparative analysis of *AtHKT1* from *Arabidopsis* and *TsHKT1;2* from its halophytic relative *Thellungiella salsuginea,* found that *TsHKT1;2* was dramatically induced upon salt stress, whereas *AtHKT1* was down regulated (Ali et al. 2013). In plants, *TsHKT1;2* contains an aspartic acid residue in the second pore-loop domain that is replaced in all other subclass 1 HKT1 proteins by an asparagine. However, yeast *ScTRK1*, which is characterized by a strong specificity for K^+, also carries aspartate amino acid in this position (Ko and Gaber 1991). When replacing aspartate to asparagine in *TsHKT1;2*, the transporter lost its ability for significant K^+ uptake and acted as a dogmatic subclass 1 HKT1 protein (Ali et al. 2013; Almeida et al. 2014). Moreover, EpHKT1;2 from Eutrema parvula, a close relative to Arabidopsis, contains conserved aspartate residues in its second pore-loop domains (Aspartate-205) as well as in the adjacent transmembrane domain (Aspartate-236) (Ali et al. 2016) and substitution of Aspartae-205 by asparagine in *EpHKT1;2* did not enhance salt tolerance and rather resulted in a similar function to that of AtHKT1 (Na^+ influx but no K^+ influx), showing that the presence of aspartate or asparagine determines the cation selectivity of the HKT1-type transporters (Ali et al. 2018), and suggesting that substitution of aspartate results in the loss of ion selectivity of HKT1 (Ali et al. 2019).

Salinity tolerance is positively correlated with higher activation of Na^+/H^+ antiporter at plasma membrane (SOS1) and loss of SOS1 gene resulted in higher Na^+ toxicity and salt-sensitive phenotypes (Yue et al. 2012; Zhou et al. 2018). In plants, SOS1 contains aspartate residues and deletion or replacement of aspartate with any other amino such as glutamate or tyrosine results in an inactivation of SOS1 activity (Hamada et al. 2001). In salt-tolerant barley cultivar, Na^+ accumulation was negatively correlated with aspartate accumulation in leaves, suggesting that reduction in Na^+ uptake and accumulation of aspartate-like metabolites could be essential for developing salinity tolerance (Wu et al. 2013). Besides interacting with Na^+ uptake, aspartate also regulates Ca^{2+} uptake in plants (Geisler et al. 2000) and animals (Iwamoto et al. 2000). The latter study showed that single or multiple mutations of six residues including Asn-125 and conserved aspartates residues in the α repeat domains reduce the apparent affinity of the exchanger for extracellular Ca^{2+} by up to sixfold, indicating the important role of aspartate in regulating ion transport activity.

ROS production is an integral component of plant responses to salinity stress. These ROS disrupt ionic homeostasis in plants by interacting with different ion transporters and channels (Demidchik et al. 2014). Aspartate interacts with other amino acids and hormones and develops conjugates to maintain redox status and to improve salinity tolerance in plants. For instance, aspartate interacts with indole acetic acid (IAA) and develops IAA-asparate conjugates, which potentiated the activity of APX and GPX enzymes to scavenge ROS, thus improving salinity tolerance (Ostrowski et al. 2016). Likewise, the catalytic activity of several *Medicago truncatula* amidohydrolases releasing IAA from IAA-Asp also increased during interaction with symbionts (Campanella et al. 2008). IAA-Asp conjugate concentration increased by 2 folds under salinity stress in *Brassica rapa* and was positively correlated with higher activation of antioxidant defence system and lowering Na^+/K^+ ratio and root growth (Pavlović et al. 2018). Improvement in salinity tolerance in bermudagrass was attributed to the higher accumulation of aspartate and glutamate and reduced Na^+ accumulation in root and shoot tissues (Hu et al. 2015).

3.6 Gamma-Aminobutyric Acid

Gamma-aminobutyric acid (GABA) is a four-carbon non-protein amino acid. In mammals, GABA has been known as a major neurotransmitter; however, in plants the signalling role of GABA has been established only recently (Ramesh et al. 2016). In mammals, GABA acts as an inhibitory neurotransmitter via its activation of $GABA_i$ (ionotropic) and $GABA_m$ (metabotropic) receptors (Palacios et al. 1981). GABA induces the activation of Cl^- conductance that passes through $GABA_i$ receptors into mature neurons leading to membrane hyperpolarisation, thus causing calming effects in neurons (Watanabe & Fukuda 2015). In animals, the role of GABA as a signalling molecule has been extensively reviewed (Li and Xu 2008; Siucinska 2019). In plants, a number of reviews have been published in the past two decades, which have summarized plant GABA metabolism and its contribution to plant growth, development and stress adaptation (Bouche and Fromm 2004; Michaeli and Fromm 2015; Bown and Shelp 2016; Shelp et al. 2017). Generally, GABA affects numerous biological processes in plants such as N metabolism (Bouche and Fromm 2004), acidification of cytosol (Shelp et al. 2006), buffering of carbon (Bouche and Fromm 2004) and activation of plant defence systems (Maryam et al. 2020). GABA rapidly accumulates in different plants tissues under stress conditions (including salinity stress) and confers stress tolerance in plants (Xing et al. 2007; Allan et al. 2008; Renault et al. 2011, 2013; Mahmud et al. 2017; Salah et al. 2019).

GABA negatively regulates Aluminium (Al^{3+})-activated Malate Transporters (ALMTs), a family of plant anion channels, and thus can regulate plant tissue growth (Pineros et al. 2008; Long et al. 2020). This regulation has been suggested to transduce GABA metabolism into membrane signalling via an alteration of anion flux across cell membranes (Gilliham and Tyerman 2016). ALMT proteins encode voltage gates anion channels and at least one rapid activation anion channel (RAC type) (Meyer et al. 2010). An ALMT from *Arabidopsis* carries a R-type anion conductance across the plasma membrane, whereas other ALMTs are localized to the vacuolar membrane and are involved in the passage of malate and chloride across the tonoplast (De Angeli et al. 2013). In plants anion channels have been revealed as important signalling proteins in plants. Under stress conditions, anion channels open and allow the release of anions from cell to induce membrane depolarization (Barbier-Brygoo et al. 2011). ALMTs are activated in the presence of some anions on the efflux side of channel protein (Ramesh et al. 2015). Such transactivation is observed in vivo for the R-type anion channels of stomatal guard cells, vacuoles and hypocotyls (Colcombet et al. 2009). Transactivation may serve to keep anion efflux occurring through the channel in the face of a decreasing gradient. Upon salinity stress, activation of anion channels induces depolarization, which activates voltage-dependent outward rectifying K^+ channels activate to initiate K^+-efflux, which result in the loss of cytosolic K^+ and concurrent drop in turgor pressure (Kollist et al. 2011).

GABA has been also found to be a regulatory molecule which controls activation of other ion channels/transporters to confer stress tolerance in plants. For instance, pre-treatment with GABA resulted in lower Na^+/K ratio by the increasing the transcripts of several genes involved in genes encoding Na^+/K^+ transportation (HKT1, HKT8, HAL2, H^+-ATPase and SOS1) (Cheng et al. 2018). A electrophysiological analysis of two *Arabidopsis* mutants *pop2-5* and *gad1,2* to salt stress revealed that salinity tolerance in *pop2-5* mutant (which can over accumulate GABA) was associated with higher activation of H^+-ATPase to overcome depolarization, reduced rate of net Na^+ influx, higher Na^+ exclusion via SOS1 and better vacuolar Na^+ sequestration via NHX activation, and better K^+ retention due to lower expression of GORK channels in plant leaf tissues (Su et al. 2019). GABA can also regulate the activity of ROS-activated ion channels or transporters by reducing the ROS production under saline conditions (Jin et al. 2019; Su et al. 2019). However, the oversensitivity of loss-of-function *pop2-1* mutant to salinity stress was associated with the decline in K^+ accumulation not over-accumulation of Na^+ or Cl^- (Renault et al. 2010). Salinity treatments are known to trigger rapid elevation of cytosolic Ca^{2+} concentration (Bose et al. 2011; Tanveer and Ahmed 2020). Thus, GABA accumulation in the response to salinity exposure would mainly result from an activation of GAD activity by Ca^{2+} release in the cytosol; when stressful conditions are extended, GABA level control would implicate transcriptional regulation of GABA metabolism genes (Renault et al. 2010). The genetic variability in salinity tolerance in durum wheat was associated with higher accumulation of GABA and higher K^+/Na^+ ratio under salinity stress in tolerant genotypes (Borrelli et al. 2018). Salinity tolerance in *Cichorium spinosum* was also related to higher accumulation of GABA that led to higher K^+ accumulation (Ntatsi et al. 2017).

GABA cross- talks with other stress hormones and improves plant nutrition under salinity stress. Pre-treatment with 24-epibrassinolide resulted in higher GABA production, which concomitantly increased K^+ uptake root and shoot under salt stress (Nejad-Alimoradi et al. 2019). GABA also interacts with other important metabolites such as proline, glycine betaine and polyamines which are known to be shared between and commonly utilized in the aroma production and/or osmotolerance regulation pathways during environmental stress. For instance, GABA increased polyamine levels affecting Na^+/K^+ homeostasis and alleviating membrane lipid peroxidation under salinity-alkalinity stress tolerance (Xu et al. 2019). Spermine pre-treatment increased GABA production and expression of NHX genes, which resulted in a higher Na^+ vacuolar sequestration and thus confer salinity tolerance (Nejad-Alimoradi et al. 2018).

4 Conclusions and Prospects

In mammalian systems, neurotransmitters operate upstream of a broad array of receptors and membrane transporters, to mediate transfer of the signal to the nerve or a muscle fibre. While plants have no nerves in a classical meaning of this word, many ion transporters have been evolutionally conserved and may be sharing binding motifs similar to their mammalian counterparts. Thus, the physiological role of these 'neurotransmitters' as second messengers in plant adaptive and developmental responses is highly plausible. The biggest problem is that, with relatively few exceptions, most of the reported studies are observational and do not allow to make a clear causal relationship between stress-induced elevation in the content of a ligand (neurotransmitter), and operation of a specific membrane transporter. Future studies should shift a focus from the whole-plant physiological observations to the cellular/molecular level, revealing the mechanistic basis of operation of neurotransmitters in controlling key membrane transporters mediating plant adaptive responses to salinity. A combination of cell electrophysiology (e.g. patch-clamp) with molecular genetics seems highly promising in this respect. The field will also benefit from a comprehensive bioinformatic analysis of the structure and evolution of the known binding motifs in mammalian system and the search for their presence in plants. Finally, comparative analysis of the structure and function of appropriate receptors between halophyte and glycophyte species may be essential to reveal the role of such neurotransmitters in plant adaptive responses to salinity.

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Serotonin in Plant Signalling and Communication

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Abstract Serotonin (5-hydroxytryptamine; 5-HT) is an indoleamine neurotransmitter deriving from tryptophan and is present in almost every single living being on earth such as humans, animals and plants, and regulates numerous physiological functions. It is considered as a vital signalling molecule in plants and animals. 5-HT modulates both growth and developmental processes in plants through different mechanisms. This chapter focuses on the interaction of 5-HT with the signalling pathways known to modulate various aspects of growth and developmental processes in plants such as root architecture, shoot organogenesis, flowering and reproduction as well as pigmentation, senescence and defence responses. Special emphasis has been given to calcium signalling, auxin phytohormones interaction, antioxidant activity and reactive oxygen species signalling. Moreover, we also highlight the different techniques that are currently employed for the analysis, detection and quantification of 5-HT in plants. A brief overview of the history and biosynthesis of 5-HT is also presented.

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Abbreviations

5-HT	5-Hydroxytryptamine
T5H	Tryptamine 5-hydroxylase
ROS	Reactive Oxygen Species
TDC	Tryptophan decarboxylase
HPLC	High-Performance Liquid Chromatography
SNAT	Serotonin N-acetyltransferase
FW	Fresh Weight
H_2O_2	Hydrogen peroxide
ABA	Abscisic acid
JA	Jasmonic acid
EGTA	Ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid
RIA	Radioimmunoassay
TLC	Thin-layer chromatography

1 Introduction

Serotonin (5-hydroxytryptamine; 5-HT) is an important neurotransmitter in the peripheral and central nervous system in human and is derived from the tryptophan through 5-HT pathway (Moriarty et al. 2011). 5-HT is present in all organs of the body (for instance, kidney, gut, skin, testis, liver and lung, etc.) and in almost every single living species on earth (Azmitia 1999). It is considered as a vital signalling molecule in both plants and animals and acts as a precursor in the synthesis of another important neurotransmitter with regulatory functions, known as melatonin (Paredes et al. 2009; Pelagio-Flores et al. 2012; Arnao 2014).

5-HT is known to exert several physiological functions in animals and humans such as regulation of anxiety, sleep, appetite, mood, blood pressure, maintenance of energetic balance as well as social interactions and sexual behaviour (Leonard 1996; Veenstra-VanderWeele et al. 2000; Young and Leyton 2002; Tecott 2007; Watts et al. 2012; Voigt and Fink 2015). Similar to humans and animals, 5-HT also has a vital role in numerous physiological functions in plants such as the regulation of growth and developmental processes including organogenesis, seed germination, pollen growth, stimulation of root growth, flowering, reproduction and senescence as well as stress responses (Roshchina and Melnikova 1998; Murch et al. 2001; Roshchina 2001; Kang et al. 2009; Pelagio-Flores et al. 2011; Ramakrishna et al. 2011; Erland et al. 2015, 2019a). Moreover, 5-HT has also been spotted to have a role in plant pigmentation (Kanjanaphachoat et al. 2012; Bajwa et al. 2015).

In evolutionary perspective, 5-HT occurred in plants even before animals appeared, with the first detection being done in the fruit of cowhage (Bowden et al. 1954; Azmitia 2001). Up to date, 5-HT has been detected in more than 70 different plant families and now regarded as universally present phytochemical which can be found in different parts of the plant including the roots, fruits, flowers, seeds and leaves (Grobe 1982; Kang and Back 2006; Kang et al. 2007; Pelagio-Flores et al. 2011; Erland et al. 2016).

Studies indicate that 5-HT exerts its functions in plants through various mechanisms such as interaction with calcium (Ca^{2+}), phytohormones (auxin, for instance) and reactive oxygen species (ROS) signalling cascades as well as modulation of gene expression and ethylene action to list as few (Erland et al. 2015; Mukherjee 2018). This chapter sheds light on how 5-HT interacts with the signalling pathways known to modulate different aspects of growth and developmental processes in plants. Mechanistic overview of the interaction of 5-HT with auxin, ROS and Ca^{2+} signalling has been presented in detail.

2 Discovery and Biosynthesis of 5-HT

Szeitz and Bandiera (2018) recently proposed a brief history of the discovery of 5-HT. It was observed in 1868 that, in isolated muscle preparations, the clotted (defibrillated) blood augmented vascular resistance, whereas, the non-clotted blood didn't, therefore, suggesting that a compound was released or formed by clotting which stimulated the contraction of muscle. A research group, during the period of 1930s and 1940s, at the Cleveland Clinic worked to recognize the endogenous constrictive factors in blood, which may take part in hypertension. Vasoconstrictor substance was isolated and characterized from the beef blood in 1948 and was then named as 5-HT. Then in 1949, the molecular structure of 5-HT was determined. In the meanwhile, in 1937, an amine-containing vasoconstrictor was extracted from the gastrointestinal mucosa of rabbit and was named as enteramine, which was later, in 1952, demonstrated to have the same structure as 5-HT. Since then, 5-HT has been detected in numerous plant and animal species (Szeitz and Bandiera 2018). In plants, 5-HT was first identified in the legume *Mucuna pruriens* and afterwards, there exist numerous reports evidencing its distribution in plant kingdom, occurring in edible and wild plants in a wide range of concentrations (Bowden et al. 1954; Roshchina 2001; Ramakrishna et al. 2011).

5-HT biosynthesis has been extensively reviewed recently by Mukherjee (2018). The biosynthesis of 5-HT in plants is contingent upon two enzymatic stages. First, the tryptophan decarboxylase (TDC) enzyme converts tryptophan to tryptamine and then, with the help of tryptamine 5-hydroxylase (T5H), the tryptamine is hydroxylated into 5-HT (Fig. 1) (Schröder et al. 1999; Kang et al. 2007). Conversely, in human system, hydroxylation of L-tryptophan with the help of tryptophan hydroxylase results in the formation of 5-hydroxy-tryptophan, which is then enzymatically decarboxylated to form a 5-HT (Fig. 1). The rate-limiting enzyme in this reaction is tryptophan hydroxylase (Veenstra-VanderWeele et al. 2000; Jonnakuty and Gragnoli 2008). Not all the tryptophan in the body is used for 5-HT biosynthesis, i.e. the percentage of tryptophan employed in the synthesis of 5-HT is only 3%, of which 1% takes place in the brain (Richard et al. 2009).

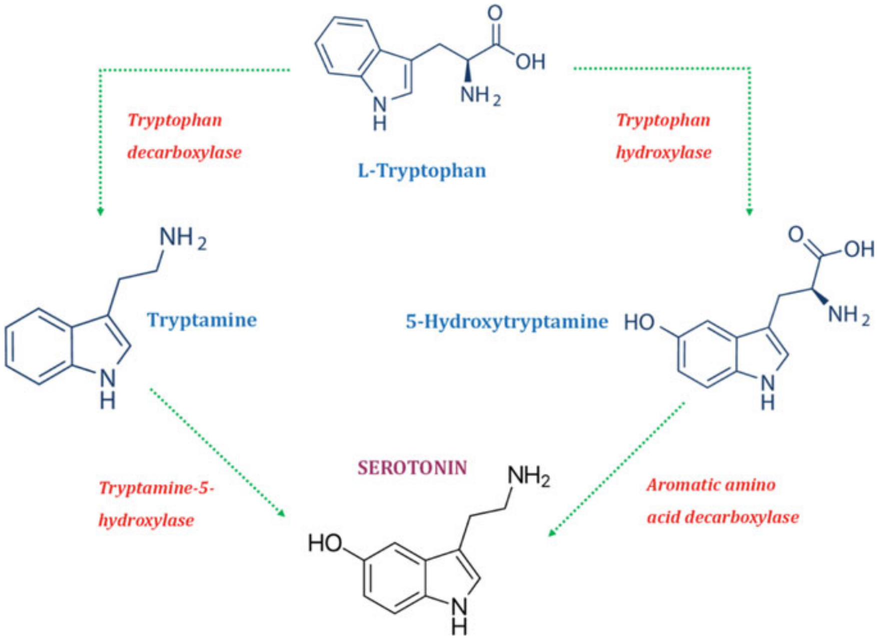

Fig. 1 Schematic of serotonin (5-HT) biosynthesis in plants (left) and animals (right)

Tryptophan decarboxylase appears to be the main rate-limiting enzyme of 5-HT synthesis (Kang et al. 2008). In plant cells, tryptophan is not present in abundance because it acts as a precursor of various metabolites such as phytoalexins, indole glucosinolates and alkaloids (Dharmawardhana et al. 2013; Mukherjee et al. 2014; Hildebrandt et al. 2015). Moreover, Kaur et al. (2015) suggested that the regulation of biosynthesis of tryptophan is subjected to different environmental factors. Studies on the *Sekiguchi lesion* gene in rice have shown that it encodes the enzyme cytochrome P450 mono-oxygenase that bears the activity of T5H (Fujiwara et al. 2010). This enzyme also catalyses the conversion of tryptamine to 5-HT in plants, respectively.

5-HT, however, is not the end metabolite and therefore several 5-HT derivatives including caffeosylserotonin, feruloylserotonin, cinnamoylserotonin and coumaroylserotonin also occur in plants (Kang et al. 2009; Dharmawardhana et al. 2013). Similarly, 5-HT is also converted to melatonin in plants (Arnao and Hernández-Ruiz 2006), thus limiting its concentration in plant species.

3 High-throughput Techniques for the Quantification of 5-HT

Currently, different methods are employed for estimating biogenic amines such as thin-layer chromatography (TLC), HPLC with ultraviolet detection (LC-UV), radioimmunoassay (RIA), HPLC coupled with mass spectrometry (LC–MS) and enzyme-linked immunosorbent assay. Nonetheless, the preferred method is still HPLC with fluorescence detection due to its cost-effectiveness, simple sample pre-treatment requirements, high accuracy and sensitivity and ease of use as compared to LC-UV (Wang and Chan 2014). RIA and LC-UV have been used for the estimation of 5-HT content in several plants species (Feldman and Lee 1985; Ly et al. 2008).

Similarly, electrochemical techniques also offer several advantages such as high sensitivity, easy operation, low-cost, simple instrumentation and fast responses. Carbon nanomaterial-based screen-printed electrodes have been employed for the simultaneous detection of 5-HT and melatonin. Graphene, multi-walled carbon nanotubes and single-walled carbon nanotubes have been used as carbon allotropes for the alteration of carbon screen-printed electrodes to determine 5-HT and melatonin simultaneously. The use of carbon nanomaterials, particularly the grapheme oxide nanoribbons on carbon screen-printed electrodes, denotes a disposable and excellent tool for sensing both the molecules in even minute sample volumes (Gomez et al. 2015).

The identification and concentrations of 5-HT in different plant species have been thoroughly reviewed by Erland et al. (2016). Recently, Yilmaz et al. (2019) developed the method of ultra-performance liquid chromatography-mass spectrometry for 5-HT determination in raw and roasted nuts (coconut, almond, pecan, cashew, Brazil nut, hazelnut, walnut, chestnut, pistachio, peanut, pine nut and Macadamia nut) as well as in other nut products. For nuts, 5-HT content varied from 0.05 ± 0.01 (for pine nut) to 155 ± 57.0 μg/g (for walnut) in raw nuts, whereas, in roasted nuts, the content ranged from 0.03 (Macadamia nut) to 15.3 ± 1.27 μg/g (pecan). Similarly, 5-HT content in nut products was between 0.09 ± 0.00 and 8.99 ± 0.92 μg/g (Yılmaz et al. 2019).

Islam et al. (2016) also estimated the levels of 5-HT along with its precursors, tryptamine and tryptophan, in thirty-eight (38) fruits and vegetables consumed commonly in Japan by employing a LC method with fluorescence detection. Highest levels of 5-HT were observed in cherry tomato (12.4470.19 μg/g FW), while potato and kiwi were found to contain highest levels of tryptophan and tryptamine, respectively, suggesting that these foods may act as excellent dietary sources of 5-HT (Islam et al. 2016).

In contrast to vegetative organs, 5-HT is mainly distributed in reproductive organs. For instance, the leaves of *Griffonia simplicifolia* contain 0.007 μg/g FW, whereas, the seeds contain 2000 μg/g of 5-HT (Fellows and Bell 1970). Moreover, in several species, the level of 5-HT rises as the fruit ripens, however, in case of pineapple fruit, the inverse is true (Udenfriend et al. 1959; Foy and Parratt 1960).

4 The Role of 5-HT in Plant Growth and Development

Both 5-HT and melatonin are vital signalling molecules that play important roles in plant growth and development. There exist a number of studies which show the evident role of 5-HT in regulation of growth and developmental processes in plants such as root architecture, shoot branching, flowering, reproduction, germination, senescence, protection against pathogens and adaptation to stress conditions of the environment (Csaba and Pál 1982; Odjakova and Hadjiivanova 1997; Murch et al. 2001; Roshchina 2001; Ishihara et al. 2008a, b; Kang et al. 2009; Pelagio-Flores et al. 2011). Modulation of root morphogenesis by 5-HT occurs via auxin-dependent or auxin-independent mechanisms (Csaba and Pál 1982; Murch et al. 2001).

4.1 Root Architecture

In *Arabidopsis thaliana*, application of 10–160 mM of 5-HT inhibited root hair formation and primary root growth, while stimulating the formation of lateral and adventitious root, possibly acting as a natural inhibitor of auxin (Pelagio-Flores et al. 2011). The application of 5-HT at a concentration above 160 mM resulted in the inhibition of both primary and lateral rooting, leading to adventitious roots formation; this activity happened autonomously of auxin-related loci (Pelagio-Flores et al. 2011). Similarly, modulation of rooting by 5-HT has been explored in several other plant species including *Hordeum vulgare*, *Helianthus annus*, *Hypericum perforatum* and *Juglans nigra* x *Juglans regia* (Csaba and Pál 1982; Gatineau et al. 1997; Murch et al. 2001; Mukherjee et al. 2014).

Studies indicate that the differential accumulation of ROS in root tips tightly regulate the growth of primary root (Tsukagoshi et al. 2010). The transcription factor UPBEAT1 (*UPB1*), known as a transcription factor modulating the balance between cellular proliferation and differentiation in root growth, regulates the distribution of O_2 and hydrogen peroxide (H_2O_2) in the meristem and elongation zones of roots via repression of peroxidase gene expression in roots, independently of auxin pathway (Tsukagoshi et al. 2010; Silva-Navas et al. 2016). Previously, it has been shown that 5-HT regulates the distribution of ROS in roots by ethylene and jasmonic acid signalling pathways (Pelagio-Flores et al. 2016). Nevertheless, further investigation is needed to comprehend how ROS accumulation is induced by 5-HT and how high ROS levels in 5-HT-treated seedlings regulate the plant root growth.

Recently, Wan et al. (2018a, b) explained the underlying molecular mechanisms of 5-HT-mediated primary root growth inhibition in *Arabidopsis*. 5-HT inhibited the elongation of primary root by influencing both the elongation and meristem zones. It repressed both the stem cell niche activity and potential of meristem cell division in the meristematic zone. 5-HT induced the over-accumulation of H_2O_2 in the elongation zone and decreased the accumulation of O_2 via UPB1 pathway in the meristem zone, thus disrupting ROS equilibrium in the root tips and subsequently resulting in the inhibition of primary root growth. The same authors also concluded that 5-HT regulates the distribution of auxin in root tips by reducing the expression of auxin-related gene and repressing the transport of auxin by modulating PIN2 (Auxin efflux carrier component 2, an auxin efflux carrier involved in the root-specific auxin transport, and mediates the root gravitropism) and AUX1 (Auxin transporter protein 1, a carrier protein involved in proton-driven auxin influx) abundances in the root tips. These results collectively reveal that elevated levels of 5-HT in plants induce stress responses by inhibiting the elongation of primary root through the regulation of O_2 and H_2O_2 distribution in primary root tips and also via auxin pathway by repressing the biosynthesis and transport of auxin (Wan et al. 2018a).

Erland et al. (2018) used quantum dot nanoparticles as a novel technique to directly visualize 5-HT and melatonin in axenic roots. Absorption of melatonin occurred through epidermal cells, after which it moved laterally and hoarded in endodermal and the rapidly dividing pericycle cells. On the other hand, 5-HT was absorbed by cells proximal to the crown and was then, in a polar manner, transported rapidly towards the root tip through the vasculature (Erland et al. 2019b). Similarly, another study also predicted the localization of 5-HT to the vascular tissues in the roots, stems and somatic embryos, as well as, the husk of immature fruits in *C. canephora* (Ramakrishna et al. 2012).

4.2 Shoot Morphogenesis

It has been found that 5-HT is effective in promoting the production of shoot in *Mimosa pudica*, where application of 5-HT significantly enhanced shoot height, shoot number and total fresh weight of the explants cultured in vitro. Treatment with fluoxetine (Prozac) and p-chlorophenoxyacetic acid (5-HT receptor inhibitors) reversed these effects (Erland et al. 2019a).

5-HT N-acetyltransferase (SNAT) is an enzyme that is associated in the conversion of 5-HT to melatonin. In transgenic rice that lacks the downstream SNAT, 5-HT content was enhanced and resulted in increased growth of coleoptile but slower overall growth of seedling (Byeon and Back 2016). 5-HT has been found to increase the somatic embryogenesis and shoot formation in culture. In *H. perforatum*, an enhancement in endogenous 5-HT levels correlated with increased shoot production was also observed by Murch et al. (2001). Likewise, the number of shoots was decreased with the use of human 5-HT inhibitors in cultures, while the application of *p*-chlorophenyl alanine (an inhibitor which prevents the conversion of 5-HT to melatonin) was found to enhance the endogenous 5-HT concentrations along with promotion of shoot production and inhibition of auxin-induced rooting (Murch et al. 2001; Erland et al. 2015). Similarly, the application of 5-HT in *M. pudica* and *C. canephora* was also found to increase shoot multiplication and somatic embryogenesis, respectively. Addition of fluoxetine or *p*-chlorophenyl alanine resulted in decreased somatic embryogenesis (Ramakrishna et al. 2009; Ramakrishna et al. 2012). Moreover, shoot multiplication was increased with the inclusion of Ca^{2+} while the application of Ca^{2+}

channel inhibitors or Ca^{2+} chelators inhibited the effects of shoot multiplication and somatic embryogenesis which shows that Ca^{2+} signalling and Ca^{2+} concentration are intricately connected to indoleamine response (Ramakrishna et al. 2009; Ramakrishna et al. 2012). 5-HT was also found to increase the elongation of hypocotyl in sunflower (*H. annus*) and to delay senescence in rice (*Oryza sativa*) (Kang et al. 2009; Mukherjee et al. 2014).

Recently, Wan et al. (2018b) integrated the physiological and transcriptome analyses to elucidate the roles of 5-HT and melatonin in modulating plant growth and defence using the model plant *Arabidopsis* (Wan et al. 2018b). Moderate concentrations of 5-HT and melatonin significantly induced lateral root formation but did not affect the growth of primary root. Both 5-HT and melatonin locally induced the expression of the cell wall-remodelling-related genes *LBD16* (Lateral organ boundaries-domain 16, a transcriptional activator involved in lateral root formation) and *XTR6* (xyloglucan endotransglucosylase/hydrolase protein 23), thus inducing the development of lateral root. These data supported the idea that 5-HT and melatonin are devoid of auxin-like activity. As supported by the transcriptome data, 5-HT in 50 μM concentration markedly improved the activity of photosystem II (PSII). These neurotransmitters also regulated the catabolism of numerous key amino acids, thus influencing the metabolism of carbon and energy. However, these two indoleamines slightly affected the TCA cycle and glycolysis. Moreover, 5-HT and melatonin also induced iron-responsive gene expression and thus improved the iron deficiency tolerance (Wan et al. 2018b).

Murch et al. (2001) observed enhanced shoot formation with increased 5-HT levels while increased level of endogenous melatonin was found to increase the formation of de novo root in *H. perforatum* explants, providing the evidence that a balance of endogenous levels of these two indoleamines may modulate plant morphogenesis in vitro (Murch et al. 2001). Overproduction of 5-HT may be deleterious to plants, for instance, rice plants overexpressing *TDC-1* and *TDC-3* had enhanced levels of 5-HT, low fertility and stunted growth (Kanjanaphachoat et al. 2012).

4.3 5-HT Plays a Key Role in Senescence and Defence Mechanism

5-HT accumulation in plants is induced by both senescence and pathogenic infection; nevertheless, the physiological roles of improving pathogen tolerance and delaying senescence by 5-HT fluctuate (Kang et al. 2009). Induction of senescence in the leaves of *Oryza sativa* with nutrient deprivation or detachment resulted in enhanced accumulation of 5-HT. Transgenic variety of rice overexpressing tryptophan decarboxylase accumulated 5-HT in higher levels as compared to the wild type and thus displayed delayed senescence of the leaves (Kang et al. 2009). Conversely, transgenic variety of rice with suppressed expression of tryptophan decarboxylase accumulated lower levels of 5-HT and thus senesced faster as compared to the wild type, which shows the possible role of 5-HT in attenuating leaf senescence. The high antioxidant activity of 5-HT may be responsible for its senescence-retarding activity. Moreover, 5-HT was found in higher levels in the vascular parenchyma cells which depicts that it has a function in maintaining the cellular integrity of these cells for the facilitation of efficient recycling of nutrient from senescing leaves to sink tissues during senescence (Kang et al. 2009).

Similarly, Lee and Back (2017) found that the overexpression of 5-HT N-acetyltransferase 1 (SNAT1) in the transgenic *Oryza sativa* plants confers resistance to senescence and cadmium along with enhancemenst in the grain yield. 5-HT has also been found to be associated with delayed senescence in the leaves of corn via maintenance of chlorophyll content, Ca^{2+} signalling and interaction with phosphatidylinositol (Huang and Kao 1992).

In addition to delaying senescence, 5-HT also confers protection against herbivores and pathogens (Ishihara et al. 2008a, b; Kang et al. 2009). The activation of tryptophan pathway is associated with the defence responses of rice plant infected with fungal pathogen (*Bipolaris oryzae*) through 5-HT production (Ishihara et al. 2008b). Similarly, the tryptophan-derived metabolites including 5-HT, *p*-coumaroylserotonin, tryptamine and feruloyltryptamine were also found to be increased in the larvae-fed leaves of rice (Ishihara et al. 2008a). Inhibiting the accumulation of 5-HT by either treating the seedlings with tryptophan decarboxylase inhibitor or by inducing mutation in the T5H gene devoid cell walls of accumulating the brown material and increased the disease susceptibility (Ishihara et al. 2008b, 2011; Fujiwara et al. 2010). Similarly, 5-HT accumulation has also been linked to the protection of plant tissue from ROS generated in interaction with the fungal pathogen, *Magnaporthe grisea* (Hayashi et al. 2016).

In addition to 5-HT, its derivatives, i.e. *p*-coumaroylserotonin and feruloyltryptamine were also detected in response to fungal pathogen infecting the plants (Ishihara et al. 2008a, b, 2011; Kang et al. 2009). Likewise, Servillo et al. (2015) also concluded that the simultaneous presence of 5-HT and its glycosylated and N-methylated derivatives potentiate the defensive response in citrus to biotic stress. Studies also depict that 5-HT synthesis corresponds to a detoxification mechanism, protecting the plants from concentrations of toxic ammonia (Schröder et al. 1999).

4.4 Other Functions of 5-HT in Plants

5-HT has also been found to play a key role in several other physiological processes in plants including morphogenesis, flowering, reproduction, embryogenesis and adaptation to the changes in environment (Erland et al. 2015, 2019a). Enhanced levels of 5-HT were observed after cold treatment in *Datura metel* flowers, suggesting its role to protect the reproductive structures from stress induced by cold. Similarly, the level of 5-HT varies during different developmental stages of flowers (Murch et al. 2009; Erland et al. 2019a), thus suggesting that it regulates the flowering as well as the reproductive stability in plants. Quantification of 5-HT during flowering in *H. perforatum* signified greater concentrations in the buds of flowers during tetrad stage of microspore development (Murch and Saxena 2002a). Similarly, 5-HT has been known to stimulate pollen germination in *H. perforatum* and *Hippeastrum hybridum* via Ca^{2+} signalling and distribution (Roshchina and Melnikova 1998; Murch and Saxena 2002a; Erland et al. 2015). Nevertheless, the effect of 5-HT on plant reproduction still needs further investigation.

Previous reports show that 5-HT has also an impact on pigmentation of plants. It prevented fruit browning in peach and apple by inhibiting the polyphenol oxidase enzyme (an enzyme involved in fruit browning) and the effect was significant as compared to ascorbic acid. Interestingly, in addition to its anti-browning effect, the antioxidant activity and the total phenolic content of apple homogenate also enhanced upon the application of 5-HT (Bajwa et al. 2015). Similarly, the overexpression of *TDC* in transgenic rice plant resulted in low fertility and stunted growth as well as the formation of dark brown phenotype upon the conversion of 5-HT to 5-HT dimer (Kanjanaphachoat et al. 2012). Keeping in mind that *TDC* overexpression leads to the accumulation of 5-HT.

Erland et al. (2015) reported that 5-HT and melatonin protect photosynthesis and direct morphogenesis in in vitro explants of *H. perforatum* by working in coordination with their metabolites in a cascade of phytochemical responses comprising the phytohormones network (Abscisic acid (ABA), cytokinine and salicylic acid signalling) and multiple pathways (Erland et al. 2018).

5 Mechanistic Overview of the Common Signalling Cascades

Various biochemical, physiological and molecular analyses have been done to investigate the plant signalling responses. Metabolomics and transcriptomic analysis have imparted sequential cues to the crosstalk of plant hormones as well as other signalling molecules accompanying the indoleamines (Weeda et al. 2014), which involves primary phytohormones like cytokinine, ethylene, auxin, ABA or gibberellins and other signalling molecules such as nitric oxide, ROS, hydrogen sulphide and brassinosteroids. Similarly, in numerous plant systems, the long-distance signalling response of indoleamines in relation with salicylic acid, jasmonic acid or nitric oxide has also been observed (Qian et al. 2015; Shi et al. 2015; Zhu and Lee 2015; Mukherjee 2018).

Over the past years, several possible mechanisms of action of 5-HT in plant growth and development as well as reproduction have been proposed which have been summarized by Erland et al. (2015) as follow: (i) interaction with Ca^{2+} calmodulin signalling cascades; (ii) interaction with auxin or other phytohormones signalling cascades; (iii) modulation of gene expression; (iv) direct antioxidant activity as well

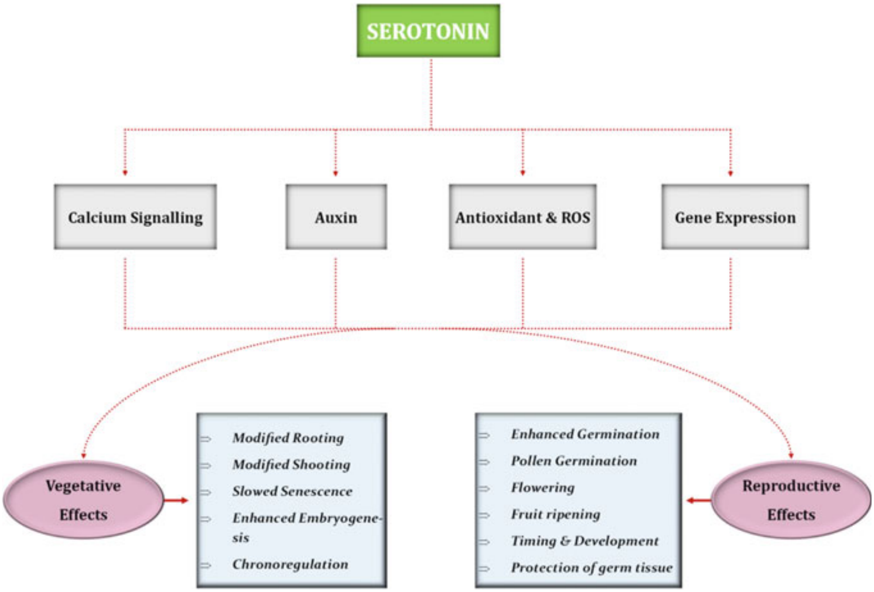

Fig. 2 Mechanistic overview of the effects of serotonin (5-HT) on vegetative and reproductive growth and development (Modified from Erland et al. (2015))

as upregulation of other antioxidant properties in cell; (v) presence of specific receptors which induce specialized signalling cascades. Similarly, Mukherjee (2018) critically reviewed the long-distance signalling responses of 5-HT in association with jasmonic acid, ABA, salicylic acid and auxin and came to the conclusion that 5-HT-induced signalling includes jasmonic acid, auxin, ROS and ethylene action. Figure 2 highlights the different effects of 5-HT on vegetative and reproductive aspects in plants.

5.1 Ca^{2+} Signalling

Ca^{2+} signalling pathway plays a vital role in several plant processes. Ca^{2+} is important for the growth and development of plant, particularly in the initiation of several signal transduction processes in higher plants' cells such as polar growth, bud formation and hormonal growth and development (White 2000; Sudha and Ravishankar 2002, 2003). Numerous cellular processes are triggered with the enhancement of cytosolic Ca^{2+} levels via modulation of ion channels, protein kinases as well as other cellular proteins (White 2000). A variety of cell types contain the Ca^{2+} permeable channels, as investigated in suspension cultures of capsicum and carrot (Sudha and Ravishankar 2002, 2003). Ca^{2+} ionophore can activate such type of channels, while the use of

Ca^{2+} channel inhibitors blocks the entry of Ca^{2+} into protoplast by binding to plant membranes (Graziana et al. 1988).

The effects of indoleamines (5-HT and melatonin), Ca^{2+} and Ca^{2+} ionophore were examined on somatic embryogenesis in *C. canephora,* and it was concluded that the addition of either of these indoleamines at a concentration of 100 μM increases the somatic embryogenesis (Ramakrishna et al. 2012). The application of either Ca^{2+} (5 mM) or Ca^{2+} ionophore (100 μM) also enhanced the number of somatic embryos. Additionally, the presence of Ca^{2+} chloride (5 mM) or Ca^{2+} ionophore with either 5-HT or melatonin (100 μM each) also promoted the somatic embryogenesis (Ramakrishna et al. 2012). On the other hand, the induction of somatic embryogenesis was inhibited individually with the addition of indoleamine inhibitors (Prozac or *p*-chlorophenylalanine), Ca^{2+} channel blocker (verapamil hydrochloride) and a Ca^{2+} chelator (EGTA), while also decreasing the levels of endogenous pools of 5-HT, indole-3-acetic acid and melatonin (Ramakrishna et al. 2012). Similarly, it has also been reported that the indoleamines, Ca^{2+} and Ca^{2+} ionophore induce morphogenesis in *Mimosa pudica* cultured in vitro, whereas, EGTA and verapamil hydrochloride suppress root induction and shoot multiplication, thereby suggesting that Ca^{2+} channels and indoleamines have a positive influence on shoot organogenesis (Ramakrishna et al. 2009).

5-HT is also known to stimulate pollen germination in several other plant species by interacting with Ca^{2+} signalling as well as Ca^{2+} distribution (Roshchina and Melnikova 1998; Murch and Saxena 2002a). Moreover, 5-HT has also been reported to be associated with delayed senescence in the leaves of corn via Ca^{2+} signalling, maintenance of chlorophyll content and phosphatidylinositol (Huang and Kao 1992).

5.2 Interaction with Auxins

Auxin and 5-HT share the common biosynthetic pathway and both of them display functional resemblances in promoting plant morphogenesis and growth. The main sites for biosynthesis of 5-HT and auxin in plants are the roots. Moreover, both of these molecules share structural similarities, offering the options of auxin receptors being substituted for the transport of 5-HT in plants (Pelagio-Flores et al. 2011; Kaur et al. 2015; Mukherjee 2018). The PIN proteins are mainly believed to be involved in the transport of auxin in plants (Petrášek et al. 2006); however, how 5-HT is transported, still needs to be explored. As previously described, 5-HT encourages the initiation of lateral root in *Arabidopsis*; this activity has been demonstrated to be autonomous of auxin-mediated action.

Furthermore, treating seedlings with 5-HT resulted in reduced auxin-inducible gene markers expression in developing lateral and adventitious root primordia and antagonized the effects of naphthalene acetic acid on auxin-inducible genes expression (Pelagio-Flores et al. 2011). The relationship between 5-HT, melatonin and auxin is still ambiguous but a possible coordinated regulation can be suggested from the common biosynthetic pathway of these molecules. How indoleamines interact with auxin is yet to be elucidated in a broader context. Similarly, Wan et al. (2018a) also reported that 5-HT regulates the distribution of auxin in root tips of *Arabidopsis* by reducing the expression of auxin-related gene and repressing the transport of auxin by modulating PIN2 and AUX1 abundances in the root tips (Wan et al. 2018a).

Auxin has an important role in regulating the development of root system in plants (Mähönen et al. 2014; Silva-Navas et al. 2016), and maintaining maximal accumulation of auxin in the quiescent centre and its steep gradient in the meristematic zone is critical for normal root growth as well as meristem cell activity (Liu et al. 2016). Similarly, root stem cell niche activity is also modulated by auxin by affecting the accumulation of PLETHORA (PLTs) in root tips. PLT is known to control both root development and meristem cell activity in a dose-dependent manner (Sabatini et al. 1999; Aida et al. 2004; Mähönen et al. 2014; Liu et al. 2016; Silva-Navas et al. 2016). 5-HT and melatonin also increased shoot organogenesis in *H. perforatum* root cultures by modulating auxin signalling (Murch et al. 2001; Murch and Saxena 2002b).

5.3 Antioxidant Activities and ROS Signalling

ROS including hydrogen peroxide, hydroxyl radical, singlet oxygen and superoxide are continuously generated in plants as a result of photosynthesis process, normal aerobic metabolism and in response to various endogenous and exogenous signals. Metabolic and signalling network strictly regulate ROS balance in cells, which include the production of ROS-producing enzymes, antioxidant enzymes and antioxidant compounds (Gechev et al. 2006; Mittler et al. 2011; Sharma et al. 2012; Wrzaczek et al. 2013).

Several studies suggest that 5-HT has antioxidant properties (Sarikaya and Gulcin 2013; Erland et al. 2015, 2019a). Kang et al. (2009) revealed that 5-HT delays senescence in rice leaves due to its antioxidant potential. The in vitro antioxidant capacity of 5-HT is high as compared to chlorogenic acid, tryptamine, tryptophan and other 5-HT derivatives, thus suggesting that it relieves the accumulation of tryptamine (toxic metabolite) and also modulates the reducing potential of cells in the senesced leaves due to its strong antioxidant activity. This in vitro antioxidant capacity of 5-HT was further confirmed in transgenic variety of rice plants producing either high or low levels of 5-HT (TDC overexpression and TDC RNAi lines, respectively). 5-HT-rich plants displayed slowed senescence phenotype while accelerated senescence was observed in 5-HT-deficient transgenic lines. These results clearly demonstrate that 5-HT, in addition to its synthesis in plants upon senescence, plays a practical role in slowing down the senescence process by efficiently scavenging ROS (Kang et al. 2009).

Studies suggest that 5-HT modulates root growth in *Arabidopsis* via modifications in ROS and JA-ethylene signalling (Pelagio-Flores et al. 2016). ROS act as signalling molecule and are considered as an essential player in the regulation of several growth and developmental processes in plants including stress tolerance, cell division and differentiation as well as defence responses (Foyer and Noctor 2013; Wrzaczek et al. 2013; Kangasjärvi and Kangasjärvi 2014). Similarly, 5-HT has also been shown to confer protection against herbivores and pathogens via antioxidant properties and cell walls reinforcement (Ishihara et al. 2008a, b).

6 Conclusion

In conclusion, 5-HT is a signalling molecule which displays various physiological functions in plants including the regulation of shoot organogenesis, root architecture, flowering, pigmentation, reproduction, germination as well as senescence and defence responses. These functions of 5-HT are due to diverse mechanisms like interaction with Ca^{2+} signalling, phytohormones, antioxidant activity and ROS signalling as well as modulation of gene expression and ethylene action. Although quite advancements have been made in the last two decades to elucidate its role in plant growth and developmental processes, particularly its function in the modulation of root and shoot morphogenesis as well as senescence and defence responses, nevertheless, the research regarding its role in other processes like germination and flowering is still at infancy and therefore needs further investigation.

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Serotonin Control of Root Growth via ROS and Hormone Signaling

Ramón Pelagio-Flores and José López-Bucio

Abstract The indoleamine biosynthetic pathway in plants involves two classical neurotransmitters, serotonin and melatonin, which are produced from the auxin precursor tryptophan. Serotonin reconfigurates root architecture through the control of primary root growth and root branching via a mechanism that antagonizes auxin signaling and involves jasmonic acid and ethylene crosstalk. The formation of serotonin dimers and reactive oxygen species is a hallmark of the plant response to pathogens mediating the hypersensitive response and cell wall softening during lateral root primordia maturation. Serotonin-jasmonic acid interaction influences iron nutrition, and acts independently of melatonin for regulation of primary root growth. Genes and proteins involved in serotonin response have been identified via transcriptomic approaches, which reveals their role in carbon metabolism and photosynthesis, opening the door for new agricultural applications.

1 Introduction

Serotonin (5-hydroxytrytamine) is a well-known indoleamine from mammals that functions as a neurotransmitter. Its widespread occurrence in plants and the many roles played in different species raises the speculation of whether it represents an emerging phytohormone or a versatile signaling molecule, which crosstalks with canonical growth regulating substances and/or second messengers, and in this manner affects growth, development, metabolism, and nutrition (Bajwa et al. 2015; Reiter et al. 2015; Wan et al. 2018a; Zia et al. 2019). The structural relationship between serotonin and indole-3-acetic acid (IAA), the main natural auxin, and the fact that both regulators are biosynthesized from the aminoacid tryptophan, led to

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the idea of an auxin-like activity, not only for serotonin but also for melatonin, to explain their effects in plants (Arnao and Hernández-Ruiz 2019). Nevertheless, the recent discovery of a putative melatonin receptor (CAND2/PMTR1) in *Arabidopsis thaliana*, which acts through heterotrimeric G protein and calcium signaling (Wei et al. 2018), and differs from the canonical auxin receptor(s) (Wang and Estelle 2014), as well as detailed analyses of root architecture and gene expression in *Arabidopsis thaliana* suggest that neither melatonin nor serotonin influence positively the auxin response (Pelagio-Flores et al. 2011, 2012; Wan et al. 2018a, b; Zia et al. 2019). Although it still remains to be clarified if serotonin and melatonin influence some common physiological and/or cellular programs for growth reconfiguration, recent transcriptomic, and molecular approaches demonstrated that indoleamines affect root morphogenesis in a differential and contrasting manner and that serotonin antagonizes auxin biosynthesis and transport while influencing jasmonic acid/ethylene signaling mediated by reactive oxygen species to orchestrate root morphogenesis.

2 Serotonin and the Indoleamine Biosynthetic Pathway in Plants

Serotonin is present in many plant families both in roots and in shoots, controlling basic functions from germination to senescence, developmental transitions, and adaptation to environmental stress (Erland et al. 2016; Mukherjee 2018). Serotonin is biosynthesized from tryptophan (Trp) an aromatic amino acid that serves as structural backbone for proteins, but also for other plant bioactive metabolites including the canonical phytohormone auxin (indole-3-acetic acid; IAA) (Woodward and Bartel 2005; Zhao 2010).

Serotonin act as a precursor in the indoleamine synthetic pathway that ends with melatonin production. Initially, tryptophan is converted to tryptamine by tryptophan decarboxylase (TDC), then tryptamine-5-hydroxylase (T5H) converts tryptamine to serotonin. Serotonin is then acetylated by serotonin-*N*-acetyltransferase (SNAT) to form *N*-acetylserotonin (NAS), which gives rise to melatonin by acetylserotonin-O-methyltransferase (ASMT) (Kang et al. 2007; Park et al. 2013, 2014). This suggests that the functions of tryptophan in plant signaling are complex as it acts as a common precursor for IAA, serotonin, and melatonin, each playing specific and/or overlapping functions (Mano and Nemoto 2012; Erland and Saxena 2019). Recent research has clarified how tryptophan supplementation increases the carbon flow to the auxin/melatonin biosynthetic pathways, thereby inducing morphogenesis. It appears that not only tryptophan, but IAA itself modifies indoleamine biosynthesis and the balance between serotonin and melatonin, which may contribute to the overall effects on plants (Erland and Saxena 2019). Moreover, an enhanced accumulation of serotonin and melatonin was reported to occur in *Helianthus annuus* roots under salt stress, indicating the link in their biosynthetic mechanisms (Mukherjee et al. 2014).

3 Serotonin Distribution in Plants

Serotonin can be taken up by roots and distributed to shoots, as determined through gas chromatography-mass spectrometry (GC–MS) analysis in *Arabidopsis thaliana* under standard growth conditions (Pelagio-Flores et al. 2011) and in salt stressed *Helianthus annuus* seedlings, where there is a correlation between root levels of serotonin and its accumulation in cotyledons (Mukherjee et al. 2014), thus indicating their long distance transport from roots to aerial organs. Direct visualization of serotonin via fluorescent and UV active quantum dot nanoparticles in *Hypericum perforatum,* in which serotonin entered the root through epidermal cells proximal to the crown, evidenced a serotonin gradient from the vasculature toward the root tip (Erland et al. 2019). These reports are consistent with immuno-detection of serotonin in *Helianthus annuus* that exhibit a differential distribution in the vascular bundles and cortex in the differentiation zone of the primary root (Mukherjee et al. 2014), suggesting their compartmentalization in the root growth zones. As such, plants posses the molecular machinery to spread serotonin, perhaps membrane transporters are responsible for both local and long distance distribution as occurring in the case of other phytohormones.

4 Serotonin Triggers a Dark-Brown Coloration in Plant Tissues

Pelagio-Flores et al. (2011) employed a pharmacological strategy to investigate the responses of roots of *Arabidopsis thaliana* plants grown in vitro, using agar plates supplemented with concentrations of up to 600 μM serotonin. A dark-brown color was evident in the vasculature of primary roots (Fig. 1) and in adventitious roots formed from hypocotyl explants, similar to that reported for rice seedlings that overexpress the tryptophan decarboxylase enzymes TDC-1 and TDC-3, in which exacerbated levels of serotonin caused formation of serotonin dimers, and this caused stunted growth and low fertility to plants (Kanjanaphachoat et al. 2012). In this context, engineered rice plants that overaccumulate lysine developed a characteristic dark-brown appearance (Yang et al. 2018), and it could be demonstrated through metabolomic and transcriptomic approaches that the dark-brown phenotype was caused by an exacerbated serotonin biosynthesis. Hayashi et al. (2016) while investigating the hypersensitive response (HR) of plants against pathogens found that rice plants challenged with the blast fungus *Magnaporthe oryzae* develop dark-brown dot lesions on leaves, and an induced accumulation of the oxidized form of serotonin was the cause of the browning halos, which mark the hyphal penetration sites.

The change from serotonin to its oxidized form was explained by the action of hydrogen peroxide, a highly reactive molecule produced during the hypersensitive reaction (Hayashi et al. 2016). These data add to the seminal work by Ishihara et al. (2008), which reported the accumulation of serotonin from tryptophan in

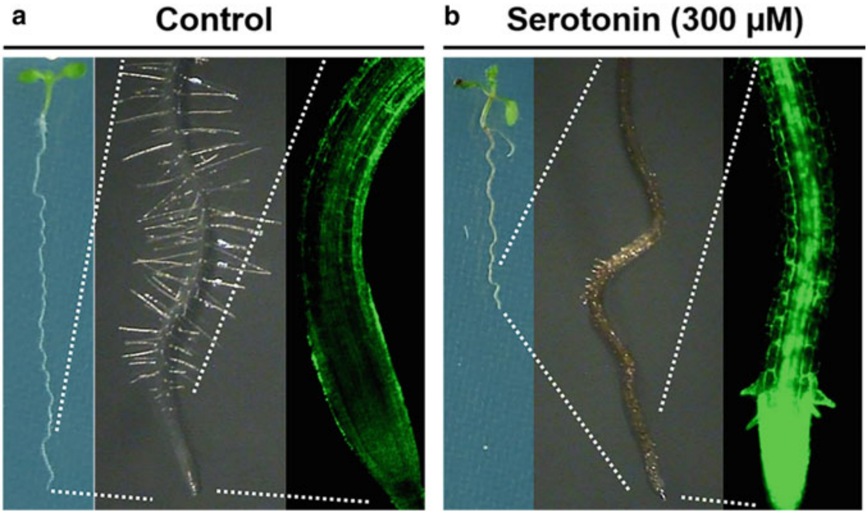

Fig. 1 Effects of serotonin in Arabidopsis roots. Serotonin supplementation causes dark-brown pigmentation and increased ROS levels in Arabidopsis primary roots. **a** Arabidopsis roots under standard growth conditions and **b** supplemented with 300 μM serotonin. The green color corresponds to the detection of ROS visualized by confocal microscopy using 2′,7′-dichlorofluorescein diacetate (H2DCF-DA), a probe to detect general ROS. Notice the dark-brown pigmentation and how ROS accumulates mainly in the primary root tip only in response to serotonin

rice leaves infected by the fungus *Bipolaris oryzae*, which also causes dark-brown halos. The authors performed high-performance liquid chromatography coupled with tandem mass spectrometry to identify the metabolites being accumulated during the hypersensitive reaction and found serotonin and its hydroxycinnamic acid amides, mostly incorporated into the cell walls around damaged tissue. It seems that via its high antioxidant activity, serotonin could suppress damage and protects DNA and proteins from oxidative stress by acting as a ROS scavenger. To the best of our knowledge, neither IAA nor melatonin trigger dark-brown coloration in plant tissues, which indicates highly specific signaling mechanism for each regulator, and it remains to be tested whether root pathogens, including fungi and bacteria, or if growth promoting microorganisms typically associated with the rhizosphere triggers serotonin accumulation in roots.

5 Serotonin Control of Root Architecture

The root system critically influences plant growth and productivity through water and nutrient uptake and anchoring the photosynthetic and reproductive tissues to the substrate. Following germination, the primary root is the first structure to emerge from the seed and explores the soil in search of valuable resources, later in development novel branches are generated via adventitious and lateral root formation, which extend the root absorptive capacity (Marhavý et al. 2016; Steffens and Rasmussen 2016). Many environmental and endogenous factors influence root development, including nutrients, pH, aminoacids, hormones, microbial metabolites, and volatile compounds, to mention a few (López-Bucio et al. 2003; Ruiz Herrera et al. 2015; Ortiz-Castro and López-Bucio 2019). Because its many and varied functions, the configuration of root architecture, including the initiation of new roots, their growth rate, and the expansion of surface area is considered a major trait in the search of a more efficient and environmentally friendly agriculture (Kochian 2016).

Considerable variation exists for the configuration of root architecture, which correlates with hormone levels, with auxin and cytokinin playing major roles in primary root growth and root branching (Lee et al. 2018). Nevertheless, an increasing number of growth regulating substances including jasmonic acid and ethylene, which were formerly thought to orchestrate plant defense, crosstalk to cell division, elongation and/or differentiation processes to guide root organogenesis (Raya-González et al. 2012; Liu et al. 2017; Barrera-Ortiz et al. 2018; Méndez-Bravo et al. 2019).

Recent findings on the identification of a receptor for melatonin have been instrumental to understand indoleamine activity (Wei et al. 2018). It is tempting to speculate that serotonin signaling across plant cell membranes may likely occur via protein receptor(s). This notion is supported from its dose-dependent effects on primary root growth (Pelagio-Flores et al. 2011; Wan et al. 2018a), the highly specific, dark-brown phenotype manifested in plants with increased endogenous serotonin levels (Kanjanaphachoat et al. 2012; Hayashi et al. 2016; Yang et al. 2018), and specific gene expression signatures of tissues upon sensing serotonin (Wan et al. 2018b; Zia et al. 2019).

5.1 Primary Root Growth

Two reports have examined the regulation of root development by serotonin in *Arabidopsis thaliana* seedlings grown in vitro. Pelagio-Flores et al. (2011) supplemented low (below 100 μM) or high (above 300 μM) serotonin concentrations to the growth media and root architectural traits were analyzed twelve days after germination. The activity of the indoleamine was much lower than IAA in modulating primary root growth, since auxin inhibited growth at nanomolar or low micromolar concentrations, whereas 300 μM serotonin was required to inhibit 50% the growth of primary roots. Comparable results were obtained in transfer experiments, in which five-day-old seedlings were supplied by 0, 120, 180, 240, or 450 μM serotonin, and primary root growth was inhibited in a dose-dependent manner starting at 180 μM (Wan et al. 2018a). This response may be reminiscent of the activation of a general stress response of the seedlings.

The primary root growth has been used as an experimental system to interpret how serotonin influences basic cellular behavior. Measurements of fully developed cortical cells from the differentiation region and the primary root meristems as well as analysis of expression of cell cycle, and meristem-related genes including *pPRZ1:uidA*, *CyCB1:uidA*, and *pCYCLINB1;1:CYCLINB3;1-GUS* showed that root growth inhibition by serotonin is caused by dynamic changes in cell division and elongation (Pelagio-Flores et al. 2011; Wan et al. 2018a). Moreover, the activity of the stem cell niche was greatly compromised in serotonin-treated roots, as the expression of *pPLT1:PLT1-GFP* and *pPLT2:PLT2-GFP* constructs was reduced upon 180 μM serotonin (Wan et al. 2018a). These constructs are used to monitor the PLETHORA transcription factors, a family of master regulators decoding the auxin signal for the control of stem cell niche. Since auxin is an inducer of PLT genes (Aida et al. 2004), these data show the opposite roles of serotonin and auxin, the former decreasing the cell proliferative potential of meristems and the activity of the stem cell niche.

5.2 Lateral and Adventitious Roots

Roots branch via lateral and adventitious root formation and the regulation of these processes by serotonin were examined in high detail in the report by Pelagio-Flores et al. (2011). Serotonin had a dual effect on lateral root formation, it stimulated root branching at 10–160 μM concentrations without affecting primary root growth. The resulting indoleamine-stimulated seedlings showed a dominant root axis with many lateral roots being formed along its surface. Higher (150–600 μM) concentrations, which compromise primary root growth, also reduced lateral root formation, but in contrast, led to the proliferation of adventitious roots in intact seedlings and in stem explants obtained from etiolated *Arabidopsis* seedlings. Determination of the initiation and stage distribution of lateral root primordia indicated that serotonin did not promote lateral root initiation, but the maturation of preformed primordia, which accelerate their growth to emerge from the primary root. From these data, we raise two important conclusions, first, serotonin increases root branching in *Arabidopsis* by inducing the maturation of lateral root primordia and second, it coordinates the developmental transition from lateral roots to adventitious roots, which may be of adaptive relevance for plants growing under adverse growth conditions.

6 Influence on Auxin Signaling

It was initially controversial whether serotonin and auxin act through similar, overlapping, or opposite signaling mechanisms. However, recent research helped to clarify the identity of the genes and proteins that are modulated by serotonin, which fail to support an auxin role for this indoleamine. Moreover, it appears that serotonin actually antagonizes the auxin action. In the research conducted by Pelagio-Flores et al. (2011), several lines of evidence were presented, which indicated that serotonin acts as a natural auxin inhibitor. First, serotonin promoted lateral root primordia maturation while decreasing auxin-regulated gene expression. Indeed, primary root growth, lateral root formation, and root hair elongation stimulated upon IAA application could be antagonized by serotonin. Additional genetic analysis performed to compare the sensitivity of the meristem to IAA and serotonin showed that serotonin exerts its bioactivity independently from at least three auxin-related loci, namely AXR2, AXR4, and AUX1, which are critically involved in auxin response or transport.

Recent efforts have been made toward elucidating how serotonin antagonizes auxin signal transduction. Wan et al. (2018a) analyzed auxin response in root tips using the auxin-sensitive Aux/IAA-auxin interaction domain II (DII VENUS) marker and *DR5:GFP* synthetic construct. Consistently, serotonin reduced auxin-responsive gene expression in root tips as revealed by both molecular markers, whereas auxin supplementation normalized primary root growth and lateral root formation at growth repressing serotonin concentrations. Serotonin-IAA antagonism was further confirmed by qRT-PCR analysis, where serotonin diminished the expression of genes encoding proteins involved in IAA-biosynthesis such as *TRYPTOPHAN AMINO-TRANSFERASE OF ARABIDOPSIS1* (*TAA1*), *SUPERROOT 1* (*SUR1*), *YUCCA2* (*YUC2*), *YUC3*, and *YUC9* (Stepanova et al. 2008; Dai et al. 2013), and auxin transporters including *AUX1* and *PIN2*. Thus, serotonin may affect the endogenous auxin content in root meristem, its transport to the cell elongation and differentiation regions and overall auxin-regulated developmental programs such as primary root growth and lateral root development.

7 Crosstalk with Jasmonic Acid and Ethylene

Serotonin plays key roles in plant tolerance to biotic and abiotic stress (Hayashi et al. 2016; Mukherjee et al. 2014). Jasmonic acid and ethylene have been traditionally considered the modulators of defense responses, environmental adaptation, and senescence (Wang et al. 2013; Yang et al. 2019), processes that mostly occur in leaves and flowers, but whose specific functions in the configuration of root architecture are just starting to be clarified.

Jasmonic acid is produced upon plant herbivory and organ injury in chloroplasts and peroxisomes (Wasternack and Hause 2019). It mediates plant signaling through binding to an F-box protein that is a component of E3 ubiquitin ligase, encoded by the *CORONATINE INSENSITIVE 1* (*COI1*) gene. COI1 associates with the SKP1, and cullin proteins to form the SCF-type E3 ubiquitin ligase, which targets the JAZ repressor proteins for degradation by the proteosome via ubiquitination. Downstream of the JA signaling pathway, the basic helix-loop–helix (bHLH) transcription factor MYC2 acts as a master regulator of gene expression (Ruan et al. 2019). Ethylene is a gaseous phytohormone highly released during climacteric fruit ripening and a triggering factor for senescence and adaptation to biotic and abiotic stress (Liu et al. 2015). In the absence of ethylene, the ethylene receptors, including ETR1 activate the CTR1 protein kinase, which represses EIN2 function. In the nucleus, the master transcription factors EIN3/EIL1 are degraded. When the levels of ethylene increases, and upon binding to the receptors, CTR1 fails to be activated, resulting in the proteolytic release of the EIN2 C-END, which inhibits protein translation of the F-box proteins EBF1/2. EIN3/EIL1 are consequently stabilized to activate transcription (Chang 2016). Very recently, the repressing effects of jasmonic on primary roots and the concomitant promotion of root branching was found to be controlled by EIN2, which represents a signaling element for jasmonic acid-ethylene modulation of root architecture (Barrera-Ortiz et al. 2018).

The jasmonic acid-ethylene crosstalk mediates the serotonin signaling in *Arabidopsis* seedlings. The JA-receptor COI1 was resistant to the inhibition of primary root growth by serotonin, a process that also involved the ethylene signaling elements ETR1, EIN2, and EIN3 (Pelagio-Flores et al. 2016). Supplementation of ethylene blocker $AgNO_3$ restored primary root growth to serotonin-treated plants, whereas roots of the ethylene overproducer mutant *eto3* were oversensitive to the indoleamine. Additionally, in high free lysine transgenic rice, RNA-seq and qRT-PCR analyses demonstrated that endogenous serotonin levels in seeds correlated with enhanced expression of acyl-CoA oxidase or b-oxidase (ACX) and lipoxygenase, two key enzymes involved in jasmonic acid biosynthesis, and with jasmonic acid content (Yang et al. 2018). Collectively, these data point to jasmonic acid and ethylene as mediators of the root architectural responses elicited by serotonin (Fig. 2), which apparently occurred independently of its conversion to melatonin.

8 Reactive Oxygen Species in Serotonin Signaling

Jasmonic acid-ethylene signaling proceeds through a range of second messengers including nitrosative and oxidative molecular species (Sewelam et al. 2016; Barrera-Ortiz et al. 2018). An *Arabidopsis* mutant defective at the *RADICAL-INDUCED CELL DEATH1* (*RCD1*) gene, which is insensitive to ROS-induced-stress showed decreased inhibition or root growth in response to serotonin (Pelagio-Flores et al. 2016). The indoleamine caused differential ROS accumulation in meristem and cell elongation zones (Fig. 1), which were altered in jasmonic acid and ethylene-related mutants. Thus, oxidative signaling explains the antagonistic auxin responses in plants treated with serotonin, because ROS accumulation suppresses auxin activity in *Arabidopsis* primary root meristems (Sanz et al. 2014) and impairs root growth (Dunand et al. 2007; Tsukagoshi et al. 2010).

The UPBEAT1 (UPB1) transcription factor controls the distribution of ROS in meristem and cell elongation region of the *Arabidopsis* primary root via repressing the expression of genes encoding peroxidases (Tsukagoshi et al. 2010), and mediates serotonin-induced H_2O_2 accumulation in root tips (Wan et al. 2018a). Serotonin could induce *UPB1* expression while *Arabidopsis upb1-1* mutants were less sensitive than the corresponding WT seedlings to the root growth repressing effects of serotonin. Thus, serotonin controls ROS redistribution within primary root tips via the UPB1 pathway.

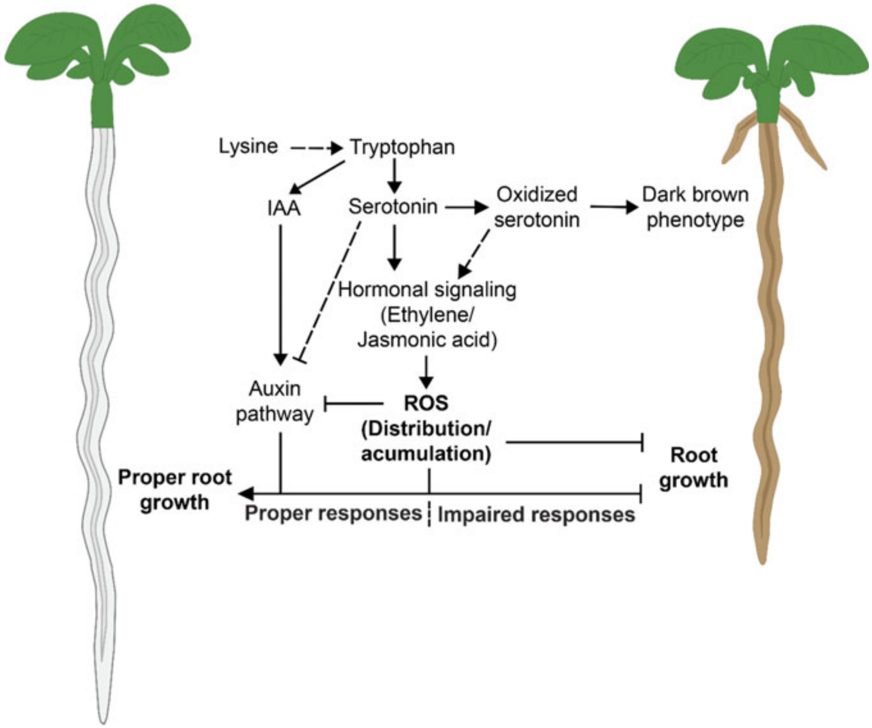

Fig. 2 Serotonin signaling in roots. Serotonin changes ROS levels and distribution in the root, through a crosstalk with the jasmonic acid and ethylene response pathways, which correlate with a dark-brown pigmentation. Continuous lines represent confirmed components in the signaling network, whereas discontinuous lines indicate possible targets

9 A Dynamic Regulator: Deleterious Versus Adaptive Effects

Serotonin levels in plants are low under standard growth conditions but dramatically increase upon attack by fungal pathogens, senescence, and salt stress. Its constitutive production in rice plants that overexpress tryptophan decarboxlyase caused halted growth and low fertility, comparable to plants experiencing strong stress symptoms (Kanjanaphachoat et al. 2012). Thus, serotonin has to be metabolized for normal growth to proceed. Supplementation of high concentrations of serotonin to *Arabidopsis* seedlings in vitro strongly repressed root growth and shoot biomass production (Pelagio-Flores et al. 2011), but these seedlings could recover upon transfer to soil yielding much more biomass and seed production than plants never exposed to the indoleamine (Pelagio-Flores and López-Bucio 2016). The growth repressing effects were observed for prolonged treatments, nevertheless, the analysis of transgenic seedlings expressing *AtHistH2B:YFP*, a marker of viable root cell nuclei and vital staining with propidium iodide to roots of wild type seedlings grown for ten days under high concentrations of serotonin failed to show any damage or cell death to the meristem or root elongation zone, indicating that growth repressing effects are not due to the toxicity of the compound (Pelagio-Flores et al. 2011).

The critical role of serotonin for plant adaptation to environmental stress is rather well supported. Nutrient deprivation and leaf detachment are factors that induce senescence and up-regulate serotonin biosynthesis in rice, which correlate with higher expression of tryptophan biosynthetic genes as well as tryptophan decarboxylase. The function of serotonin as an anti-senescence molecule has been explained via genetic approaches, since transgenic rice that accumulates serotonin showed delayed senescence, whereas suppression of its biosynthesis through RNA interference promoted senescence (Kang et al. 2009). The tryptophan-indoleamine pathway is strongly activated during plant defense against rice fungal pathogens including *Magnaporthe oryzae* and *Bipolaris oryzae*, leading to serotonin accumulation and plant protection (Ishihara et al. 2008; Hayashi et al. 2016). The response to *B. Oryzae* was typified by an enhanced anthranilate synthase activity, and along with serotonin yielded more anthranilate, indole, and tryptophan. Mukherjee et al. (2014) found that salt stress is an inducer of serotonin accumulation in *Helianthus annuus* roots and cotyledons and modulates the activity of *N*-acetylserotonin-O-methyltransferase, the enzyme responsible for melatonin biosynthesis from *N*-acetylserotonin. Perhaps, the conversion of serotonin into melatonin might represent a mechanism to abrogate its strong bioactivity.

10 Conclusions and Perspectives

The analysis of the bioactivity of serotonin and the comparisons made with melatonin and IAA indicate that each compound has specific signaling targets in plants. Serotonin appears to be much more active than melatonin in regulating primary root growth and lateral root formation and exerts its action both by acting as an anti-auxin molecule, and via crosstalk with jasmonic acid and ethylene as well as reactive oxygen species. The promotion of root branching by serotonin occurs through the stimulation of lateral root primordia maturation, an aspect that is specifically influenced by cell wall remodeling via ROS and is independent of auxin signaling (Orman-Ligeza et al. 2016; Wan et al. 2018b). The formation of serotonin dimers is responsible for the dark-brown color of roots and leaf halos and appears to be a critical response to environmental stress mediated by jasmonic acid. These findings clear the initial confusion in the literature regarding the relationship of the indoleamines with auxin and help unravel the very basic function of neurotransmitters in plant physiology (Fig. 2).

Recent transcriptomic data support a role of serotonin in energetic metabolism and iron (Fe) nutrition (Wan et al. 2018b) and coincidentally, endogenous concentrations of jasmonic acid rapidly increase in roots of cereals in response to Fe deficiency (Kobayashi et al. 2016). The fact that fungal and bacterial species can induce both physiological and morphological responses to Fe deficiency in roots via jasmonic acid also in dicot plants and that serotonin is an important mediator of the hypersensitive reaction indicates that their sensing mechanisms overlap (Ishihara et al. 2008; Hayashi et al. 2016; Hernández-Calderón et al. 2018). Serotonin is a ubiquitous molecule from prokaryots, animals, and plants (Erland et al. 2016) and thus its possible role as a cross-kingdom signaling molecule is exciting, the identification of a serotonin receptor and any transporter (s) for local and long distant transport is still a major challenge.

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Functional Correlation of Auxin and Serotonin in Plants: Possible Receptor Surrogacy?

Soumya Mukherjee

Abstract Among various chemo-signals produced by plants, auxin and serotonin share both supporting and contrasting nature in their physiological effects. Serotonin is an intermediate compound produced in the biosynthetic pathway of melatonin, which initiates from tryptophan (common precursor for auxin and serotonin). Various environmental cues can precisely regulate tryptophan levels in plant cells. The complex regulation of root development involves an interplay of several plant growth regulators, among which, auxin and serotonin share certain functional similarities. To date serotonin receptors are characterized in plants. However, evidence substantiate the fact that serotonin is an important regulator of auxin activity in plant tissues. Serotonin is transportable from roots, through the phloem, up to the aerial parts of plants. Thus, it is likely that the functional correlation of auxin and serotonin might be explained by its possible receptor surrogacy in plant cells. Although this hypothesis does not bear sufficient evidence, investigations from molecular data support evidence of serotonin–auxin interaction in plants. Serotonin is likely to repress or activate the auxin-inducible responses associated with plant growth and development. Serotonin accumulation and its biosynthesis regulate auxin-mediated root growth during abiotic stress. Auxin–serotonin interaction exerts precise control over root growth and regulation of root architecture. Serotonin-mediated growth promotive effects on plant organs are possibly mediated by a canonical auxin signaling pathway. Serotonin inhibits the expression of auxin-responsive elements at the sites of induction of primary and adventitious roots and in lateral root primordia. Interestingly, the action of serotonin on lateral root primordia generation is independent of AUX1 and AXR4 loci but dependent on AXR1 and AXR2 auxin-related loci. Investigations, therefore, provide a possibility to the fact that exogenous serotonin at higher concentrations might cause impairment of auxin activity in roots. Investigations for serotonin accumulation in the auxin-deprived cells during abiotic stress is likely to decipher the partitioning of tryptophan between auxin and serotonin biosynthesis. Endophytic colonization of microbes may play a substantial role in regulating auxin homoeostasis and initiating auxin–serotonin crosstalk during abiotic stress in plants.

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Interesting correlations are required to be drawn from future investigations on the impact of mycorhizal association or plant growth-promoting bacteria on serotonin metabolism in roots.

1 Introduction

Plant indoleamines exhibit a plethora of physiological effects manifested by growth induction, tissue differentiation, photomodulation, flowering, stress amelioration, and fruit ripening in plants (Odjakova and Hadjiivanova 1997; Murch et al. 2001; Roshchina 2001; Ishihara et al. 2008; Kang et al. 2009). It is important to understand the precise role of various neurotransmitters functioning in a diverse group of plants. Investigations across the past decade have established the role of plant neurotransmitters like serotonin, melatonin, dopamine, GABA, acetylcholine, and catecholamines in various aspects of plant development, signaling, and communication. Serotonin and melatonin are two major indoleamines in plants and are present in various plant organs to exhibit a diverse response to environmental stress, ontogenetic changes, and gene regulation. In this context, it is important to analyze the functional correlation of serotonin and auxin in plants. Serotonin is an intermediate compound being produced in the biosynthetic pathway of melatonin, which initiates from tryptophan. Tryptophan, therefore, functions as the common precursor to auxin and serotonin. Various environmental cues can precisely regulate tryptophan levels in plant cells. Tryptophan biosynthesis and its catabolism are tuned with various stages of plant development, temporal variation, and biotic or abiotic stress stimulus. Among various chemo-signals being produced by plants, auxin and serotonin share both supporting and contrasting nature in their functions. Auxin, predominantly in the form of IAA regulates wide aspects of plant development (Woodward and Bartel 2005). Auxin homoeostasis and signal perception in plants are mediated by its biosynthesis, spatiotemporal distribution, gravitropic changes, transport across membranes, and conjugation to its active or inactive forms (Ljung et al. 2002; Leyser 2006; Mockaitis and Estelle 2008). Serotonin (5-hydroxytryptamine) was originally discovered as an animal neurotransmitter to be associated with mitogenic functions, hormone signaling, and immunomodulatory response (Frazer and Hensler 1999). Phytoserotonin has been reported to be present in leaves, fruits, seeds, and roots of more than 42 plants species (Grobe 1982; Engstrom et al. 1992; Roshchina 2001) and regulate the diverse type of physiological responses (Odjakova and Hadjiivanova 1997; Murch et al. 2001; Roshchina 2001; Ishihara et al. 2008; Kang et al. 2009). Recent investigations report the involvement of serotonin in various aspects of signaling and crosstalk events associated with various plant growth regulators. Arabidopsis has been implied to investigate the molecular mechanisms of root development being regulated by the complex interaction of auxin and serotonin (López-Bucio et al. 2006, Contreras-Cornejo et al. 2009). The complex regulation of root development involves an interplay of several plant growth regulators, among which, auxin and serotonin share structural and functional similarities. The initiation of lateral roots

(LR) and growth of primary root (embryonic origin) are precisely tuned by the regulation of environmental factors (Malamy and Benfey 1997, Casimiro et al. 2001, López-Bucio et al. 2005a, 2005b, López-Bucio et al. 2003, Péret et al. 2009).

To date serotonin receptors have not been characterized in plants. However, evidence from various investigations substantiate the fact that serotonin concentrations regulate auxin activity in cells. Serotonin has been suggested to be transportable from roots, through the phloem, and up to the aerial parts of plants. Polar auxin transport (PAT) in plants is finely tuned by the coordinated activity of various auxin-binding proteins or receptors like 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. 2004; Mravec et al. 2008). Auxin signals are perceived by specific TRANSPORT INHIBITOR RESPONSE1/AUXIN SIGNALING F-BOX PROTEIN 1–3 (TIR1/AFB1-3) receptor family. TIR1 is a component of the ubiquitin–ligase complex SCFTIR1/AFB that ubiquitinates and degrades AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) proteins (Gray et al. 2001, Dharmasiri et al. 2005, Kepinski and Leyser 2005). Serotonin shares structural similarities to auxin and is, therefore, expected to function is mechanisms similar to the pathways of auxin signaling. Thus, it is likely that the functional correlation of auxin and serotonin might be explained by possible receptor surrogacy in plant cells. Although this hypothesis does not bear sufficient evidence, investigations from molecular data support evidence of serotonin–auxin interaction in plants. Serotonin is likely to either repress or activate the auxin-inducible responses associated with plant growth and development.

2 Tryptophan-Mediated Biosynthesis of Auxin and Serotonin is Programmed by Environmental Cues

Tryptophan appears to be one of the least abundant amino acids in plant cells (Hildebrandt et al. 2015). Tryptophan functions as the precursor molecule for various metabolic pathways in plants. Auxin and serotonin biosynthesis is, therefore, associated with the production and catabolism of tryptophan within the plant tissue (Dharmawardhana et al. 2013; Mukherjee et al. 2014). The role of serotonin as a regulator of circadian rhythm in plants is temporally regulated by tryptophan biosynthesis (Kang et al. 2007a, b, Kang et al. 2008; Schröder et al. 1999). Serotonin and melatonin biosynthesis initiates from their common precursor tryptophan. Tryptophan biosynthesis and activity of anthranilate synthase are regulated by feedback inhibition exerted by tryptophan (Kanno et al. 2004; Zhao and Last 1995). Furthermore, tryptophan synthetase also exhibit spatiotemporal regulation in maize and Arabidopsis (Last et al. 1991; Zhao and Last 1995; Kriechbaumer et al. 2008). The regulation of tryptophan biosynthesis is subject to modulation by various environmental cues (salinity, heat, cold stress, pathogen attack, nutrient stress, etc.). Auxin and serotonin biosynthesis is also precisely regulated by the modulation of tryptophan. Investigations using ^{14}C-tryptophan have been performed to decipher the biosynthetic pathway of serotonin and melatonin from their precursor—tryptophan (Murch et al. 2000). Among various plant organs, root tip and root stele are the major sites of auxin and serotonin biosynthesis. Serotonin biosynthesis is mostly initiated by tryptamine formation, which is catalyzed by 2-tryptophan decarboxylase (TDC; EC 4.1.1.28), and is further mediated by tryptamine 5-hydroxylase (T5H) activity which leads to the synthesis of serotonin (Schröder et al. 1999; Kang et al. 2007a). TDC functions as the regulatory enzyme associated with serotonin biosynthesis in plants. Analysis of TDC-overexpression transgenic lines in rice show almost 25-fold increase in serotonin accumulation in comparison with the wild strains (Kang et al. 2007b). Overexpression of TDC-1 and TDC-3 in rice resulted in higher accumulation of serotonin in transgenic rice, which was accompanied by stunted growth, reduced fertility, and brown pigmentation in leaves (Kanjanaphachoat et al. 2012). Auxin and serotonin biosynthesis are expected to be regulated by the diversion of the pathway from tryptophan, which leads to higher accumulation of serotonin. TDC is the rate-limiting enzyme for serotonin biosynthesis which exhibits high K_m value (690 mM) for tryptophan (Kang et al. 2008). Thus, it is likely that higher tryptophan levels in the cells may trigger increased serotonin biosynthesis. Transcriptional analysis in Arabidopsis reveals abiotic stress-induced regulation of tryptophan biosynthesis (Less and Galili 2008). Tryptophan serves as an important biomolecule or precursor for various secondary growth metabolites such as phytoalexins, indole glucosinolates, alkaloids, and serotonin. Spatiotemporal distribution of these biomolecules is precisely associated with plant acclimatization and abiotic stress tolerance. Enzymes of tryptophan biosynthesis pathway (anthranilate synthase-EC: 4.1.3.27 and tryptophan synthase-EC: 4.2.1.20) in rice and Arabidopsis show abiotic stress-induced modulation in their activity (Zhao and Last 1996; Kanno et al. 2004). An elevation in anthranilate synthase activity and subsequent increase in serotonin and other tryptophan-derived metabolites have been evidenced in leaves of rice plants subjected to biotic stress (Ishihara et al. 2008). Interestingly, TDC1 and TDC3 isoforms in rice are induced by abiotic and biotic stress factors in rice (Dharmawardhana et al. 2013). Metabolic pathway network database (RiceCyc) in rice (integrated bioinformatics analysis) revealed that abiotic or biotic stress-induced tryptophan biosynthesis show precise coordination with genes associated with serotonin and auxin biosynthesis (Dharmawardhana et al. 2013). Abiotic stress conditions such as salinity, osmotic changes, heat stress, and heavy metal stress have been reported to affect auxin biosynthesis, transport, and its conjugation.

The IPA (indole-3-pyruvic acid) pathway of auxin biosynthesis is more prevalent in higher plants (Mashiguchi et al. 2011; Won et al. 2011). However, the indole-3-acetaldoxime (IAOx) pathway is also operative during various stress-induced changes in plants (Julkowska et al. 2017; Lehmann et al. 2017). In this context, it is worth mentioning that tryptophan is an important regulatory molecule during various physiological conditions. The YUCCA family proteins are important regulators of the IPA pathway in plants. The YUCCA proteins are specifically expressed in shoot and root tissues. Arabidopsis has been reported with 11 isoforms of - YUC3, 5, 7, 8, and 9.

IPA pathway of auxin biosynthesis is modulated during salinity stress thus inducing tolerance (Kim et al. 2013; Ke et al. 2015; Yan et al. 2016). Microarray analysis reveals root-specific expression of YUCCA 3, 5, 8, and 9 (Dinneny et al. 2008; Kilian et al. 2007). The increase in epidermal auxin biosynthesis in response to salt stress exhibits changes in its spatial distribution in roots. Recent investigations reveal the involvement of IAOx pathway during stress conditions (Julkowska et al. 2017; Lehmann et al. 2017). Roots in their differentiation and elongation zone undergo auxin biosynthesis mostly by the IPA pathway of auxin biosynthesis.

3 Role of Serotonin in Plant Growth and Development

Serotonin (5-hydroxytryptamine; SER) exerts a plethora of physiological effects in plants associated with plant development, organogenesis and stress amelioration. Various investigations report the signaling role of serotonin associated with other plant hormones. Phytoserotonin was reported from a legume, *Mucuna pruriens,* for the first time (Bowden et al. 1954). Walnuts (*Juglans regia*) and hickory (*Carya* sp.) have been reported with the highest concentrations of serotonin ranging from 25 to 450 mg. Moreover, serotonin accumulation in *Griffonia simplicifolia* leaves varies in association with temporal changes of vegetative and reproductive period (Fellows and Bell 1971). Similar reports provide evidence of serotonin accumulation to be associated with senescence in rice roots (Kang et al. 2007a, b). Serotonin accumulation in the senescent rice roots has been observed by immunolocalization (Kang et al. 2007a, b). Furthermore, other plant systems like *Allium cepa* L. and *Musa paradisica* L. have been reported for serotonin localization (Kimura 1968). Abiotic and biotic stress conditions significantly alter serotonin levels in the tissue. Mukherjee et al. (2014) report NaCl-stress induced spatiotemporal differences in serotonin accumulation in etiolated sunflower seedling (roots and cotyledons). Serotonin associates with the oil bodies of cotyledons of *Juglans regia* (Grobe 1982). According to Erland et al. (2019), application of quantum dot nanoparticles provided evidence on the uptake and distribution of serotonin in axenic roots. Serotonin was observed to undergo polar transport toward the root tips which was later disrupted in the presence of thermal stress. The authors conclude that stress induction might result in the dissipation of the polar distribution of this indoleamine thus resulting in its antioxidative function in tissues. Apart from its function as an antioxidant, serotonin regulates flowering and ion homoeostasis in plants (Roshchina 2001). The growth stimulatory and morphogenetic response of serotonin has been essentially reported in roots and hooks of oat coleoptiles (9, 45). Seed (radish) and pollen germination (*Hippeastrum hybridum*) also involve the physiological activity of serotonin (Roshchina and Melnikova 1995; Roshchina 2001). Interesting observations reveal the morphogenetic role of serotonin in the presence of exogenous auxin in the culture media. Exogenous auxin application triggered an increase in serotonin level which in turn enhanced shoot organogenesis (Murch et al. 2001). These findings provide a clue to the concentration-dependent additive role of auxin and serotonin in tissue culture practices. Axenic cultures of

St. John's wort (*Hypericum perforatum* L.) reveal the integrative role of serotonin, melatonin and auxin in regulation of shoot and root organogenesis. Pharmacological investigations with various inhibitors of indoleamines revealed auxin-mediated regulation of endogenous serotonin and melatonin in John's Wort tissue. Reports (Murch et al. 2001; Ramakrishna et al. 2009a; Hernandez-Ruiz et al. 2004) depict the role of serotonin in the regulation of root growth and development in rice plants. In vitro growth modulation of *M. pudica* L. involve calcium-mediated signaling response exerted by serotonin (Ramakrishna et al. 2009b). Serotonin and melatonin have been reported to function in association with the calcium channels. The authors conclude on the synergistic role of serotonin and calcium in regulation of seed germination and its viability. *C. canephora* exhibit the precise involvement of serotonin during somatic embryogenesis (Ramakrishna et al. 2009b). Important insights have been obtained on the photomorphogenetic role of serotonin in plants. Serotonin regulates phosphoinositide (PI) turnover and also participates in a signaling route to red light sensing thus enhancing the nitrate reductase (NR) transcript levels in maize plants (Chandok and Sopory 1994). Thus, serotonin possibly mimics the effect of red light and modulates the levels of secondary messengers in cell. St. John's Wort tissues exhibit higher serotonin recovery in tissues subjected to dark condition than in comparison with illuminated conditions (Murch et al. 2000). The authors conclude the observations as an analogy to that in mammalian systems. Interestingly, in members of lower plant groups like *D. bardawil* indoleamine, serotonin levels vary as a function of photomodulatory changes (Ramakrishna et al. 2011). Serotonin levels were found to be higher at the day time (Ramakrishna et al. 2011). *Tetrahymena thermophile* was implied to investigate the effect of exogenous serotonin on biomass in dark (Leclercq et al. 2002). A high concentration of serotonin has been reported to be associated with motor activity in the pulvinus of *Albizzia julibrissin*, *P. sativum*, and *Mimosa* pudica (Collier et al. 1956). Metabolic derivatives of serotonin (phenylpropanoid amides of SER derivative, i.e., p-coumaroyl SER and N-ferulyl SER) play a pivotal role during biotic stress in rice and bamboo (Roschina and Melnikova 1995; Tanaka et al. 2003). Thus, serotonin exerts protective action to plant tissues subjected to pathogen attack. Serotonin accumulation in *Juglans regia* during fruit abscission has been reported by Bergmann et al. (1970). The authors report the association of serotonin with proteolysis and deamination of amino acids thus leading to ammonia accumulation in walnut seeds. Nevertheless, serotonin biosynthesis has been observed to associate with the reduction of ammonia toxicity in plant tissues. The predominant role of serotonin in senescent tissues is effectively associated with nutrient recycling from source to sink tissues (Kang et al. 2007a, b). Vascular bundle cells in rice show high serotonin accumulation associated with senescence. This finding has been attributed to the protective role of serotonin toward ROS detoxification and senescence delay (Kang et al. 2009). The antioxidative role of serotonin has been reported to be more intense than tryptophan, tryptamine, and SER derivatives. Serotonin and its derivatives (SER derivatives viz. N-(p-Coumaroyl) serotonin (CS) and N-feruroyl serotonin (FS) exhibit antioxidative properties in safflower oil (Hotta et al. 2002). *Datura* flowers subjected to cold stress exhibit serotonin accumulation associated with ROS homoeostasis and oxidative balance (Murch et al. 2009).

4 Regulation of Auxin Signaling and Auxin Homoeostasis in Plants

Brassica and other plants exhibit precise control of auxin gradient in developing region of plant organs (Sirrenberg et al. 2007; Vadassery et al. 2008; Schäfer et al. 2009; Hilbert et al. 2013; Lee et al. 2011; Dong et al. 2013). Changes in the auxin gradient are precisely regulated by various environmental cues. Investigations have deciphered the transcriptional control of various signaling pathways transduced by auxin activity. The auxin specific receptor, a F-box protein, called TIR1 and various other transcriptional repressors of Aux/IAA family regulate the cascade of auxin signaling. The Aux/IAA elements in turn regulate the expression of ARF family of transcription factors (De Rybel et al. 2010; Overvoorde et al. 2010). Poplar and Arabidopsis exhibit auxin conjugation associated with salt and anoxic stress (Junghans et al. 2006; Alam et al. 2010). Genetic manipulation of various regulatory factors associated with auxin and serotonin signaling during abiotic stress in plants shall provide broader merits to stress tolerance mechanisms in crops. Alterations in auxin levels are associated with factors like nutrient deficiency and perception of environmental changes by roots (López-Bucio et al. 2005a, b). The asymmetric division of cells in the root pericycle results due to the activity of AUXIN RESPONSE FACTORS (ARFs) and AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) proteins. Auxin/indole-3-acetic acid (AUX/IAA)-histone deacetylase (HDA) and auxin response factor (ARF)-histone acetyltransferase (HAT) involve in auxin signaling during root morphogenesis (Nguyen et al. 2020). IAA and phenols are important metabolites during the signaling cascades (Sofo et al. 2017). Nitrilase subfamily proteins (NIT1, NIT 2, and NIT 3) mediate the hydrolysis of organic cyanides during auxin biosynthesis. *NIT1* and *NIT 2* regulate primary root length, lateral root branching in response to biotic or abiotic stress (Lehmann et al. 2017). Auxin-inducible indole-3-acetic acid-amido synthetase (GH3 proteins) regulate auxin conjugation during abiotic stress in Arabidopsis (Park et al. 2007). The DNA-binding auxin response factors (ARFs) interact with auxin response DNA elements (AuxRE) in the promoters of auxin-regulated genes thus transducing stress-induced auxin signaling. The *TRANSPORT INHIBITOR RESPONSE 1* (*TIR1)* gene and AXR1 function in a synergistic manner in the auxin signaling pathways during abiotic stress. The F-box TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX PROTEIN (TIR1/AFB) auxin co-receptors, the Auxin/INDOLE-3-ACETIC ACID (Aux/IAA) transcriptional repressors, and the AUXIN RESPONSE FACTOR (ARF) transcription factors are vital elements in auxin signaling pathway (Lavy and Estelle 2016). The meristematic region of the roots undergoes transient changes associated with the gradient-specific distribution of PIN proteins. Endophytic colonization of microbes may play a substantial role in regulating auxin homoeostasis and initiating auxin–serotonin crosstalk during abiotic stress in plants. Interesting correlations are required to be drawn from future investigations on the impact of mycorhizal association or plant growth-promoting bacteria on serotonin metabolism in roots.

5 Serotonin and Auxin Interaction Regulate Plant Growth and Signaling: Possible Receptor Surrogacy?

Serotonin and auxin have been considered to exhibit functional similarities in terms of their morphogenic role associated with root and shoot development. The two biomolecules share a common biosynthetic pathway originating from tryptophan. Thus, the biosynthetic partitioning of these biomolecules in plant tissues is subject to regulation by various external factors associated with plant growth and development. Auxins function as primary growth regulators for polar and lateral growth of organs. Auxin activity, biosynthesis and its polar gradient are modulated during abiotic stress. To understand the functional correlation of auxin and serotonin, it is necessary to understand the mechanism of functioning of auxin transporters and auxin-responsive elements (AUXRE, PGP, and PIN, GH3, Aux/IAA, and SAUR). During environmental stress conditions such as heavy metal stress, temperature and salinity stress polar auxin gradient (AUX1) in shoot and root tissues are likely to be disturbed. Among various other biomolecules, nitric oxide and reactive oxygen species act as major regulators of AUX/IAA elements. Changes associated with auxin levels trigger various pathways of cell signaling and metabolism in the cell. Serotonin accumulation and its biosynthesis might regulate auxin-mediated root growth during abiotic stress. Auxin–serotonin interaction exerts precise control over root growth and architecture. IAA-conjugates are capable of modifying various peptides and proteins in the cells (Ludwig-Müller 2011). Ectopic expression of various intermediates of the auxin biosynthesis pathway has helped to decipher the mechanism of auxin-mediated modulation of root architecture. Subcellular and tissue-specific auxin homoeostasis controls growth and morphogenesis of plant organs. Specific auxin efflux transporters in plants regulate polarized growth of shoots and roots (Feraru and Friml 2008). PIN proteins and phosphoglycoproteins (PGPs) are auxin efflux carriers, which mediate auxin efflux across the cell membranes (Petrasek et al. 2006). The columella and elongation zone of roots contain ambient levels of auxin required to initiate primary root elongation and lateral root branching. PIN proteins are comprised of transmembrane, extracellular, and cytoplasmic domains. Roots function as an important location for the biosynthesis of auxins and serotonin plants. Moreover, the spatiotemporal distribution of auxin in the elongation and differentiation zone of roots is likely to be modulated by the polar gradient of serotonin and melatonin. Although the exact mechanism of transporter-mediated distribution of serotonin and melatonin in plant tissues still remains persuasive, pharmacological studies and gene expression analysis have revealed the additive or inhibitory effect of these biomolecules on the expression of auxin-responsive elements (Pelagio-Flores et al. 2011). Auxin homoeostasis in roots is accomplished by its conjugation and degradation processes triggered by various environmental cues. IAA oxidation is a major mechanism of turnover for free and conjugated auxin in cells (Zhang et al. 2017). IBA levels in roots prevalently function as a storage form of IAA (Tognetti et al. 2010) while the conjugate and the free form of IBA are necessary for auxin homoeostasis. Thus, it is likely that the prediction of auxin redistribution and homoeostasis in roots can be investigated by computational modeling. However, it may appear difficult to integrate several factors associated with the changes in auxin level in roots. Various root models have been prepared for the prediction of auxin distribution and transport which partially depict the mechanism of auxin homoeostasis. Auxin-mediated regulation of proton efflux pump and pH levels are important regulators in the process of root cell elongation. The auxin transporter-like protein 3 (LAX3) model is effective in analyzing the effects of exogenous auxin (Mellor et al. 2015). A diverse set of PIN family efflux proteins have been reported to be essential for establishing the polar gradient of auxin in plant tissues (Petrasek et al. 2006).

Serotonin exhibits dose-dependency in terms of its effect on plant growth. Exogenous serotonin promotes lateral root development in a concentration-dependent manner (Pelagio-Flores et al. 2011). However, higher concentrations of serotonin (>160 μM) promoted adventitious rooting but inhibited lateral root formation. Thus, this observation provide a clue to the fact that higher serotonin concentrations might cause an alteration in the spatial distribution of auxin in roots. It is likely that serotonin signaling across membranes might operate through receptor-mediated processes. Since auxin and serotonin share structural similarities, possibilities lie on the fact that auxin receptors might be surrogated by serotonin in plant cells. Serotonin-mediated growth promotive effects on plant organs are possibly mediated by a canonical auxin signaling pathway. Exogenous serotonin application (varying concentrations) might cause changes in the spatial distribution of auxin in roots. Arabidopsis has been investigated to decipher the inhibitory effect of exogenous serotonin on the expression of auxin-responsive gene elements. However, serotonin-mediated lateral root initiation in Arabidopsis has been observed to be independent of auxin action (Pelagio-Flores et al. 2011). Serotonin inhibits the expression of auxin-responsive elements at the sites of induction of primary and adventitious roots and in lateral root primordial. Interestingly, the action of serotonin on lateral root primordia generation is independent of AUX1 and AXR4 loci but dependent on AXR1 and AXR2 auxin-related loci (Dharmawardhana et al. 2013). This investigation thus provides a possibility to the fact that exogenous serotonin at higher concentrations might cause impairment of auxin activity in roots. Salt stress-induced increase in endogenous serotonin levels in sunflower roots has been reported by Mukherjee et al. (2014) where NaCl stress-induced inhibition in root growth has been observed to be ameliorated by exogenous application of serotonin. Various plant systems subjected to abiotic stress have been reported to exhibit a decrease in the auxin levels (Shen et al. 2010; Sun et al. 2017; Yuan et al. 2013). Investigations of serotonin accumulation in the auxin deprived cells during abiotic stress are likely to decipher the partitioning of tryptophan between auxin and serotonin biosynthesis. The functional correlation of auxin and serotonin during root growth and signalling has been summarized in Fig. 1. Table 1 enlists the various contrasting and supporting roles of auxin and serotonin in plants.

6 Future Perspectives

Serotonin exerts a plethora of physiological effects in plants associated with plant development, organogenesis, and stress amelioration. Abiotic and biotic stress conditions significantly alter serotonin levels in the tissue. Auxin and serotonin biosynthesis is associated with the production and catabolism of tryptophan within the plant tissue. Among various plant organs, root tip and root stele are the major sites of auxin and serotonin biosynthesis. Various environmental cues can precisely regulate tryptophan levels in the cell. Thus, it is likely that higher tryptophan levels in the cells trigger increased serotonin biosynthesis. Tryptophan biosynthesis and its catabolism are tuned with various stages of plant development, temporal variation, and biotic or abiotic stress stimulus. Tryptophan serves as an important biomolecule or precursor for various secondary growth metabolites such as phytoalexins, indole glucosinolates, alkaloids, and serotonin. Serotonin has been suggested to be transportable from roots through phloem up to the aerial parts of plants. Thus, it is likely that the functional correlation of auxin and serotonin might be explained by possible receptor surrogacy in plant cells. Serotonin is likely to either repress or activate the auxin-inducible responses associated with plant growth and development. Serotonin accumulation and its biosynthesis regulate auxin-mediated root growth during abiotic stress. Various aspects of auxin homoeostasis and signal perception in plants include its biosynthesis, spatiotemporal distribution, gravitropic changes, transport across membranes, and conjugation to its active or inactive form. Serotonin shares structural similarities to auxin and is, therefore, expected to function is mechanisms similar to the pathways of auxin signaling. Genetic manipulation of various regulatory factors associated with auxin and serotonin signaling during abiotic stress in plants shall provide broader merits to stress tolerance mechanisms in crops. Endophytic colonization of microbes may play a substantial role in regulating auxin homoeostasis and initiating auxin–serotonin crosstalk during abiotic stress in plants. Interesting correlations are required to be drawn from future investigations on the impact of mycorhizal association or plant growth-promoting bacteria on serotonin metabolism in roots.

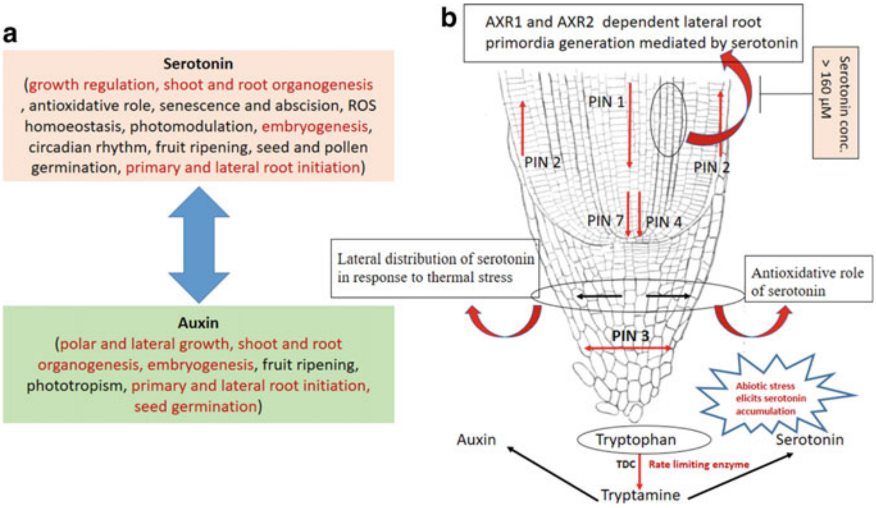

Fig. 1 **a** Functional correlation of serotonin and auxin. **b** Serotonin–auxin interaction associated with primary root growth and lateral root primordia initiation. Partitioning of auxin and serotonin biosynthesis from tryptophan (AXR—auxin-responsive elements; TDC—tryptophan decarboxylase)

Table 1 Physiological roles of serotonin and their functional correlation with auxin

Plant system	Physiological effects of serotonin and correlation of serotonin and auxin activity (+ additive/supporting;—contrasting)	References
	Role in circadian rhythm (−)	Kang et al. (2007a, b, 2008), Schröder et al. (1999)
Oryza sativa	Senescence regulation (−) Root growth regulation (+)	Murch et al. (2001), Hernandez-Ruiz et al. (2004), Kang et al. (2007a, b), Ramakrishna et al. (2009)
Griffonia simplicifolia	Regulation of vegetative and reproductive phase (−)	Fellows and Bell (1971)
Helianthus annuus	NaCl stress-alleviation	Mukherjee et al. (2014)
Juglans regia	Association with oil body biogenesis in cotyledons	Grobe (1982)
	Spatial distribution and antioxidative role in response to thermal stress	Erland et al. (2019)
Avena sativa	Growth stimulatory and morphogenetic response in root and coleoptile development (+)	Csaba and Pal (1982), Niaussat et al. (1958)
Raphanus sativus	Seed germination (+)	Roshchina (2001)

(continued)

Table 1 (continued)

Plant system	Physiological effects of serotonin and correlation of serotonin and auxin activity (+ additive/supporting;—contrasting)	References
Hippeastrum hybridum	Pollen germination (+)	Roshchina and Melnikova (1995)
Hypericum perforatum L	Shoot and root organogenesis (+)	Murch et al. (2001)
Mimosa. pudica L	Growth modulation and calcium signaling (+)	Ramakrishna et al. (2009a)
C. canephora	somatic embryogenesis (+)	Ramakrishna et al. (2009b)
Zea mays	Phosphoinositide signaling, modulation of nitrate reductase (NR) transcript expression	Chandok and Sopory (1994)
D. bardawil	Photomodulation (+)	Ramakrishna et al. (2011)
Tetrahymena thermophile	Regulation of biomass	Leclercq et al. (2002)
Albizia julibrissin, *Pisum sativum*, *Mimosa pudica*	Motor activity of pulvinus	Collier et al. (1956)
Bambusa sp.	Regulation of abiotic stress	Tanaka et al. (2003)
Juglans regia	Regulation of abscission (−), association with proteolysis, and deamination of amino acids	Bergmann et al. (1970)
Safflower	Antioxidant activity in seed oil (−)	Hotta et al. (2002)
Datura sp.	ROS homoeostasis and oxidative balance (−)	Murch et al. (2009)
Arabidopsis	Lateral root growth (+), inhibitory at high concentration Lateral root primordial generation (+)	Pelagio-Flores et al. (2011)

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Dopamine in Plant Development and Redox Signaling

Kiran Bamel and Prabhavathi

Abstract Several animal neurotransmitters are present in plants. They are also known to affect several aspects of plant growth and development. Owing to this a great enthusiasm is observed among investigators in finding the non-neuronal role and various components of these neurotransmitter systems in plants. One such biomediator that has generated inquisitiveness in the plant scientists is dopamine. It is a well-recognized animal neurotransmitter that belongs to a group of biogenic amines better known as Catecholamines. Several works have reported the exogenous and endogenous roles of dopamine. This review is an effort to discuss the overall position of the dopaminergic system in plants and unravel the role of dopamine in plant signaling.

1 Introduction

The past couple of decades have witnessed great enthusiasm of investigators in finding the non-neuronal role of neurotransmitters in animals and plants. This interest is ascribed to the discovery of these chemicals in significant amounts in plants. One such biomediator that has generated inquisitiveness in the plant scientists is dopamine. It is a well-recognized animal neurotransmitter that belongs to a group of biogenic amines better known as catecholamines. Other members in this group are norepinephrine, epinephrine, and their derivatives. They structurally possess a substituted 3, 4-dihydroxy phenyl ring (Wang et al. 2018).

There are a plethora of evidence that suggest the presence and synthesis of animal neurotransmitters such as acetylcholine and biogenic monoamines (e.g., adrenaline, dopamine, noradrenaline, serotonin) in plants even before the animals. Iriti (2013) justified this by logically considering the fact that the plants are primitive than the animal kingdom in the evolutionary timescale. Since the plants and animal groups belong to different evolutionary lineage, therefore the biosynthetic pathways of these chemicals in animals and plants are different. The catecholamines have very well

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documented functions in the animal world. Dopamine, epinephrine, norepinephrine, serotonin, and acetylcholine are known to play an important role in peripheral and central neurotransmission of animals. The aromatic monoamines perform a very specific role in animals. Dopamine and norepinephrine are neurotransmitters, and epinephrine is involved in rapid responses to stress (Darnell et al. 1990). In animals, absence of dopamine in nerve cells can causes of Parkinson disease. Though all these biogenic amines are found in plants as well (Smith 1977; Szopa et al 2001), their significance and mode of action are yet to be ascertained. But data is accumulating and suggests a possible involvement of dopamine in the regulation of several aspects of plant growth and development (Tretyn and Kendrick 1991; Kuklin and Conger 1995).

Literature reviews on dopamine in the plant system reflect the extensive research that has been carried out by several researchers. These works suggest that it exerts an array of physiological and regulatory roles in the plants. Since the present chapter explores the possible role of dopamine in plant growth and development, it is essential to summarize the current status of the role of dopamine in living organisms, animals as well as plants. In animals, dopamine influences several vital functions ranging from the commonly known neurotransmission to immune response, mental health, blood pressure, and motility (Konovalov 2018; Roshchina 2018a). An overview of various components of the dopamine system as well as their involvement in different processes has also been presented briefly.

2 Dopamine System in the Plants

The presence of the immediate precursor of dopamine, (3,4-Dihydroxyphenyl)-L-alanine, i.e., L-DOPA in plants was established several years ago (Daxenbichler et al. 1971). L-DOPA has been reported to be present in callus cultures of *Stizolobium hassjoo* and *Mucuna pruriens* and cell suspension cultures of *M. pruriens* (Obata-Sasamoto and Komamine 1983; Brain 1976; Huizing et al. 1985). Dopamine was also reported to be present along with DOPA in the later. Dopamine is detected in several other plant species like *Solanum tuberosum*, *Musa acuminata*, *Musa sapientum*, *Musa cavendishii*, *Plantago major*, and *Persea americana* (Kulma and Szopa 2007; Ponchet et al. 1982; Lundström and Agurell 1971; Kanazawa and Sakakibara 2000). The levels were high in the pulp of banana sps (*Musa acuminata*, *Musa sapientum* var. baracoa), plantain (*Plantago major*), and avocado (*Persea americana*). But Feldman and coworkers (1987) reported low levels in several others like apples (*Malus sylvestrus*), beans (*Phaseolus vulgaris*), eggplants (*Solanum melongena)*, oranges (*Citrus sinensis*), peas (*Pisum sativum*), spinach (*Spinacia aleracea*), and tomatoes (*Lycopersicon esculentum*). Intact plants of *Papaver somniferum* L. contain dopamine and L-DOPA as precursors of alkaloid (Battersby and Francis 1964; Battersby et al. 1975; Leete and Murrill 1964). The enzyme DOPA-decarboxylase that metabolizes DOPA to dopamine was also detected (Roberts and Antoun 1978; Roberts et al. 1983). L-DOPA and dopamine were also detected in leaf extracts of

M. pruriens. The presence of dopamine, epinephrine, norepinephrine, their precursors, phenylethylamine and tyramine, and other derivatives, has been detected in 44 families of plants, which include at least 29 species cultivated for human consumption (Smith 1980). Its presence is reported in many plants of medicinal importance (Konovalov 2018) and several edible plants (Bhattacharjee and Chakraborty 2018).

3 Biosynthesis of Dopamine

Dopamine is synthesized in plants from the amino acid tyrosine by any of the two pathways, either via tyramine or via L-DOPA (Kulma and Szopa 2007). In some plants, it is synthesized from L-DOPA which in turn is produced from tyrosine by the enzyme tyrosine hydroxylase (Tocher and Tocher 1972) just like it is synthesized in an animal cell. In others, tyrosine is decarboxylated by tyrosine decarboxylase to tyramine which is further hydroxylated by monophenol hydroxylase to produce dopamine (Kulma and szopa 2007).

Dopamine is synthesized in banana by hydroxylation of tyramine (Smith 1980) in banana. The enzymes L- tyrosine carboxylase and DOPA-decarboxylase which synthesizes catecholamine is reported in barley roots and *Cytisus scoparius,* respectively (Tocher and Tocher 1972; Hosoi 1974). In *Lophophora williamsii*, the peyote cactus, tyrosine is formed by hydroxylation of phenylalanine, tyrosine is then either decarboxylated to tyramine or hydroxylated to DOPA2 (Paul 1973). Tyramine can be also be obtained by p-hydroxylation of phenylethylamine (Smith 1977). Either tyramine or DOPA can act as a precursor for dopamine. In *Musa sapientum* hydroxylation of tyramine and in *Cytisus scoparius* and *Portulaca* callus (Endress et al. 1984) decarboxylation of DOPA results in dopamine formation (Smith 1980). In dark-grown callus, the biosynthesis and accumulation of catchcholamines is more than in light. Few plants produce high concentrations of dopamine. The site of production, i.e., the cell or organs varies in different species (Kulma and Szopa 2007) and the distribution and amount vary in different stages of development (Neubauer 1964).

4 Dopamine Receptor

Dopamine receptor in animals is known to have two major pharmacologic classes of dopamine receptor subtypes, D1-like and D2-like (O'Dowd 1993; Sokoloff and Schwartz 1995; Luedtke et al. 2003). The aqueous extract of the medicinal herb *Cestrum parqui*, belonging to Solanaceae family, showed the presence of a stable component behaving as an agonist at D1-like dopamine receptors and the extract also has intrinsic activity at D2-like dopamine receptors. Besides this species, no other *Cestrum sp* show dopaminergic activity. To date, no catecholamine receptor has been identified in plants. Few researchers have expressed the human dopamine receptors in transgenics.

Skirycz et al. (2005) expressed dopamine receptor in transgenic lines and showed more than twofold increase of dopamine, norepinephrine, and epinephrine. This increase was accompanied by a significant increase in direct precursors of dopamine, tyramine, and L-DOPA. The presence of components of animal catecholamine signaling pathway (G-proteins, cAMP, PKA homologs) in plants as well suggests that biogenic amines may play a role in plant signaling. The only component of the catecholamines signaling pathway that needs to be identified in plants is the receptor. The transgenic potato plants expressing the human dopamine receptor were characterized by an increase of all known tuber catecholamines (dopamine, norepinephrine, and normetanephrine). The scientists suggest that the exogenous receptor activates catecholamine action in potato plants.

5 Physiological Functions of Dopamine in Plants

Neurotransmitters are now known for their non-neuronal roles in animals and in plants. They play pivotal roles in a wide array of physiological processes. Likewise, dopamine protects against herbivores (Smith 1980; Van Alstyne et al. 2006), affects N-fixation, prevents oxidation of auxins (Gomes et al. 2014), anabolism of ethylene (Elstner EF 1976), regulation of ion permeability, and photophosphorylation in chloroplasts (Odjakova and Hadjiivanova 1997; Roshchina 1990).

5.1 Dopamine and Metabolism

Widrych et al. (2004) showed over-expressing tyrosine decarboxylase in transgenics. The accumulation of large amounts of catecholamines in transgenic plants over-expressing tyrosine decarboxylase, indicates a possible signaling effect on plant primary metabolism. Analogous with animals where catecholamines stimulate glycogen mobilization, an identical role for this group of chemicals is being suggested by various workers. It regulates a variety of metabolic activities in plant cells, such as active oxygen scavenging processes (Leng et al. 1999), plant sugar metabolism, regulation of ion permeability, and photophosphorylation of chloroplasts (Laukens et al. 2002; Fernie 2002; Geigenberger 2003).

Catecholamines have been suggested to be intermediate metabolites of anabolic and catabolic pathways (Applewhite 1973). For example, they are reported to be precursors of alkaloids of tetrahydrobenzylisoquinoline series. Dopamine is known to be a precursor for various alkaloids, papaverine, morphine, and mescaline (Lundstrom and Agurell 1971). But no significant metabolic role has been assigned to catecholamines in plants (Kuklin and Conger 1995). The possible role of dopamine in tyrosine metabolism is also suggested by Wichers and coworkers (1993). Alkaloids like morphine, thebaine, codeine, papaverine, narcotine were synthesized using exogenous DOPA that was converted to dopamine by the enzyme DOPA-decarboxylase (Khan et al. 2013). Dopamine influences sugar metabolism and coordinates with phytohormones to affect plant growth (Jung et al. 2000). The expression of human dopamine receptors HD1 and HD2 in potato along with a change in the enzymes (ADP-glucose phosphorylase, sucrose synthase, and sucrose phosphate synthase involved in carbon metabolism led Skirycz et al. (2005) to speculate that dopamine may affect carbon metabolism via the activation of the signaling pathway mediated by secondary messenger cAMP.

5.2 Dopamine and Allelopathy

Plants can release organic compounds into the environment. These secondary metabolites may accumulate in the soil environment and influence the growth and development of neighboring plants, with positive and negative effect. Generally, plants protect themselves through natural allelochemicals. Tocher and Meeuse (1966) detected dopamine in the only one species of alga *Ulvaria obscura.* Dopamine is the feeding deterrent toward crustaceans, urchins, and herbivorous snails (Van Alstyne et al. 2006). The exudates containing dopamine acts as an allelochemical and inhibits the embryo germination in *Fucus distichus* and growth of *U. lactuca.* The toxicity is due to the quinones formed from oxidation of dopamine. Earlier, it was reported by Nelson and coworkers (2003) that larvae of oyster showed cent percent mortality on exposure to the *U. obscura* exudates. The arm length of sea urchin is also regulated by dopamine (Adams et al. 2011). In *Periplanta* sp. exogenous dopamine stimulates non-proteinaceous saliva secretion (Troppmann et al. 2007). Roshchina (2018b) suggested that plant–animal interactions mediated by neurotransmitters offer a new insight into the field of allelopathy.

5.3 Dopamine in Stress

Dopamine is considered as stress hormones in animals. To draw an analogy, several investigators tried to explore and establish a similar role of dopamine in plants. Several studies have indicated the role of dopamine in plants under stress conditions. It is known to provide adaptation to environmental changes in some plants. Dopamine plays an important role in plant abiotic stress (Lawton et al. 1989; Katagiri et al. 1989).

5.3.1 Abiotic Stress

Wounding initiates a series of signaling events in a plant. These may be systemic, local, or both. The cascade of the signaling molecules is more often similar to the one activated in response to any other kind of stress. The endogenous level of some hormones like jasmonic acid and auxins also changes in response to stress. Dopamine levels are also observed to increase in the wounded leaves of potato plants (Szopa et al. 2001). The content of dopamine in cactus also increased after injury (Bruhn and Lundström 1976). Involvement of dopamine in the protection of the outer plant surface is reported in many species. Homeyer and Roberts (1984) showed that dopamine is released during wound healing process in some *Papaver* species. Similarly, an increase in dopamine was observed when wound tissue formed in the saguaro cactus (*Carnegiea gigantea)* (Kuklin and Conger 1995). Dopamine confers resistance to the fungus *Cercospora beticola* in sugarbeet (*Beta vulgaris*). Catecholamines and their derivatives role as deterrents to insect predators and foraging animals have already been proposed by Smith (1980).

Various stresses like elevated temperature (Dixon and Rees 1980) wounding (Hajirezaei 1994; Geigenberger et al. 1997) water stress (Geigenberger et al. 1998), and hypoxia (Geigenberger et al. 1998; Stitt et al. 1994) change the metabolite amounts in tuber. The conversion of sucrose to starch is decreased accompanied by an increase of sucrose synthesis The transgenic HD1 plants exhibited similar changes in carbohydrate metabolism thereby pointing toward the involvement of catecholamines in plant stress responses by influencing normal carbon metabolism in tuber (Skirycz et al. 2005).

It is a known fact that in response to any kind of stress there is a transient production of reactive oxygen species. Dopamine's role in redox signaling is gaining researchers attention. The antioxidative properties of dopamine enable organisms to modulate their stress responses (Kulma and Szopa 2007). It works in responses to abiotic stresses. The dopamine level is enhanced in *Solanum tuberosum* plants when exposed to various abiotic stresses like drought, ultraviolet light, and abscisic acid treatments. The activity of biosynthetic enzyme tyrosine decarboxylase increases under salinity stress (Swiedrych et al. 2004). Abdelkader and coworkers (2012) reported that exogenous dopamine regulates the expression of an aquaporin gene OsPIP1-3 in salt-stressed rice (*Oryza sativa*). Salt stress results in the release of L-DOPA into the medium without inducing dopamine formation. It also removes salt-induced (Li et al., 2015) and nutrient deficiency stress (Liang et al. 2017) in apple (*Malus hupehensis*). Not much is known about dopamine's possible role in the uptake and resorption of mineral elements by drought-challenged plants.

Jiao and coworkers (2019) demonstrated that exogenous dopamine (0.1 mM) effectively relieved the alkali stress in apple seedling and improved biomass accretion, net photosynthetic rate, chlorophyll content, and root morphology (Figs. 1 and 2). Earlier Zhang and coworkers (2016) and Kulma and Szopa (2007) have also reported alleviation of alkali stress on exogenous dopamine application. One of the most important chemical reactions is that DA produces melanin through the oxidation of lipoxygenase. Liang and coworkers (2018) observed that dopamine suppressed the chlorophyll degradation gene, pheide oxygenase, and senescence-associated gene-12. This effect of dopamine indicates its role in overall plant growth. it was also observed that the rate of photosynthesis was elevated and drought-induced senescence was retarded.

Fig. 1 Phenotypes of apple seedlings treated by dopamine (DA) application under alkali stress for 15 days. CK: control (pH = 6.0); AL: alkali stress (pH = 9.0). Jiao et al. 2019, Seedlings Plants 8: 580 (with permission)

Fig. 2 Phenotype of apple seedlings treated with dopamine (DA) application under alkali stress for 15 days. CK: control (pH = 6.0); CK + DA: control + 0.1 mM DA; AL + DA: alkali stress (pH = 9.0) + 0.1 mM DA; AL: alkali stress. Jiao et al. 2019, Seedlings Plants 8: 580 (with permission)

5.3.2 Biotic Stress and Defense

Although the main role of dopamine is in intra-organismal signaling, opportunistic organisms sometimes exploit dopamine signaling for interspecies interactions. For example, mammals release dopamine as part of their systemic response to infection, pathological organisms use this signal in an attempt to survive the immune response. Lyte and Ernst (1992) reported that Gram-negative bacteria respond to this dopamine signals by accelerating their division rate often overwhelming the host's defense. Catecholamine induced the growth of gram-negative bacteria. Some pathogenic fungi respond to this signal by synthesizing melanin, making them resistant to ionic oxidants released by the host's macrophages (Gómez and Nosanchuk 2003).

5.4 Dopamine and Ion Permeability

Odjakova and Hadjiivanova (1997) and Roshchina (1990) showed that adrenaline, dopamine, and noradrenaline regulate the intercellular regulation of ion permeability and photophosphorylation.

The photophosphorylation of chloroplast is due to its reduction power that ends with the scavenging of the free radicals. Dopamine is known to affect the ionic channels of the plasma membrane of *Chara corallina* (Zheralova et al. 2014). The action of dopamine is reversible and a complete restoration of various parameters of ionic currents was observed.

5.5 Dopamine in Plant Development

The role of dopamine in plants is poorly understood but it is evident from various research reports that they are part of many aspects of growth and development. Roshchina (1991, 2018a) suggested the role of biogenic amines present in the saliva in regulating plant growth and development after feeding of the plant. Dopamine affects growth of green alga *Ulva lactuca*, affects the germination of zygotes of brown alga, *Fucus dischia*. The survival, metamorphosis, and motility of crab larvae and juveniles are reported to be influenced by dopamine (Van Alstyne et al. 2014). In plants and other organisms, photosynthesizing protists have daily vertical migrations in the water column triggered by the presence of daylight. An antagonist dopamine-acetylcholine system has been shown to control this activity by affecting light sensitivity, with dopamine decreasing it (Forward 1997).

The concentrations of dopamine and its precursor DOPA varied in different organs and tissues and their different developmental stages of these tissues. Variations were noted in the alkaloid and dopamine concentrations during different developmental stages of poppy organs (Fairbairn and Steele 1981; Jindra et al. 1966; Neubauer 1964). The dopamine levels were reported to be high in the latex collected from plants in their floral bud stage in comparison to the latex collected some days after the plants had flowered. Cell-free extracts obtained from different organs and stages of plant growth showed variation in dopamine biosynthesis. It was more in leaf extracts of the rosette stage than the leaves of flowering plants. Maximum biosynthesis was in capsules which declined as the capsule matured.

Kamo and Mahlberg (1984) demonstrated that the leaves, capsules, and pedicels possessed dopamine biosynthetic activity. The conversion rates of the precursor DOPA to dopamine was maximum in capsules in comparison to in any other part of *Papaver somniferum* and it varied in the same organ at its different development stage. Earlier Robert and coworkers (1983) reported higher levels of dopamine in latex before flowering than in latex after flowering. This probably may be due to higher dopamine biosynthesis in the earlier case as has been indicated by Kamo and Mahlberg (1984) rather than enhanced dopamine translocation to the capsule prior to flowering or decline of dopamine levels in the latex after flowering due to dilution (Tables 1 and 2).

Several studies have indicated the importance of catecholamines in morphogenesis and reproductive organogenesis (Odjakova and Hadjiivanova 1997). High concentrations of dopamine and tyramine are reported to be present in spathes 'floral leaf' of Araceae inflorescences (Ponchet et al. 1982). Though the leaves, stems, adventious roots of members of Philodendroideae and Monsteroideae have high levels of catecholamines, the highest quantity was recorded in their inflorescences (Kuklin and Conger 1995; Tretyn and Kendrick 1991). The spathe develops before the sex organs and is of great significance in the reproduction of monocots. The abundance of these chemicals in the spathe suggests their importance in reproductive organogenesis. Cell proliferation in *Sacchromyces cerevisiae* is stimulated by dopamine (Malikina et al. 2010). It also affects the pollen–pistil interaction during fertilization (Roshchina and Melnikova 1998). Dopamine also promoted the callus growth and radial expansion in tobacco cultures. It also increased the fresh weight by more than double. But it inhibited the initiation of floral and vegetative bud. It can positively affect cell expansion synergistically with a growth medium supplemented with kinetin and indoleacetic acid (Protacio et al. 1992).

Table 1 Dopamine biosynthesis in cell-free extracts derived from various organs (The values listed represent the average of two samples, and each sample was collected from the pedicel-capsule of 40 plants). Adapted and modified from Kamo and Mahlberg (1984) J Natural Products 47(4) pp682-686 with permission

Origin of cell-free extract from *Papaver somniferum*	(%) conversion of DOPA to dopamine
Capsules	1.9
Pedicels	0.58
Capsules connected to pedicels	1.48
Leaves rosette stage	0.95
Leaves flowering stage	0.38

Table 2 Dopamine biosynthesis varied at different stages of plant growth (The values listed represent the average of two samples, and each sample was collected from the pedicel-capsule of 40 plants). Adapted and modified from Kamo and Mahlberg (1984) J Natural Products 47(4) pp 682–686 with permission

Developmental stage of *Papaver somniferum*	(%) conversion of DOPA to dopamine
Before flowering	2.1
1–2 days after flowering	1.3
5–6 days after flowering	1.7
13–14 days after flowering	0.8

6 Crosstalk of Dopamine with Other Plant Hormones

Several reports suggest that catecholamines may interact with plant hormones. This crosstalk with plant hormones affect the growth and development of plants (Fernie 2002; Lunn and MacRae 2003; Tauberger et al. 2000). DOPA and dopamine are precursors for various alkaloids (Guinaudeau and Bruneton 1993; Smith 1980) and are associated with processes such as ethylene production, nitrogen fixation, defense against herbivores, flowering, prevention of 3-indole acetic acid (IAA) oxidation and gibberellins signaling (Dai et al. 1993; Kuklin and Conger 1995).

Dopamine, earlier called cotyledon factor (Kamisaka 1973), present in the cotyledon of the lettuce acts synergistically with GA3 in the induction of hypocotyl elongation. It is shown to stimulate gibberellin (GA3) action in isolated lettuce hypocotyls (Kamishaka and Shibata 1982).

High concentration of the synthetic auxin 2,4-D is known to lower L-DOPA accumulation and alter the metabolism of L-tyrosine toward into protein synthesis (Obata-Sasamoto et al. 1981). On the contrary, dopamine level is significantly increased. It increases the concentrations of auxin by inhibiting indole acetic acid (IAA) oxidase thereby stopping the oxidation of IAA in vitro as well as in vivo. Since dopamine affected the root growth negatively in soybean seedling, two possible modes of action were suggested. Either the inhibition is brought out by its oxidation leading to the generation of reactive oxygen species (ROS), quinones, and semiquinones or alternatively by increasing the IAA levels due to its inhibitory effect on IAA oxidase.

Ethylene biosynthesis was also stimulated by exogenous dopamine in illuminated chloroplast lamellae from sugar beet leaves (Elstner et al. 1976). Dopamine acted as a cofactor for monovalent oxygen reduction that was important for ethylene formation. Dopamine-treated explants showed increased radial expansion, one of the triple effects attributed to ethylene (Goeschl et al. 1966) suggesting a direct relation between these two chemicals. Experimentally, it was shown that there was a threefold increase in the accumulation of ethylene in dopamine-treated cultures in comparison to the controls. Augmentation of the inhibitor of ethylene synthesis, aminooxyacetic acid (AOA), showed the opposite response. The explant growth with AOA-treated explant was the same as the controls. Hairy root cultures treated with dopamine and other catecholamines show a clear increase in ethylene release. But further experimentation showed that ethylene does not mediate the response. In many in vitro raised cultures, the growth-promoting effects of exogenous dopamine and other catecholamines were reasoned to be due to their interactions with endogenous plant hormones. The growth of hairy root cultures of *Acmella. oppositifolia* was influenced by catecholamines (Protacio et al. 1992). The researchers suggested that dopamine interacts with plant hormones and affects plant development.

7 Mode of Action of Dopamine

Dopamine and other catecholamines perform their physiological functions in animal cells by their interaction with G-protein coupled receptors. These receptors either stimulate or inhibit the adenylyl cyclase (AC) and in most animal cells cyclic AMP (cAMP) activates cAMP-dependent, serine–threonine protein kinase (PKA). Several recent reports have presented data that suggest a similar mechanism regulating the carbohydrate metabolism in plants. The only missing link is the identification of receptor for these chemicals. Evidence suggest the occurrence of cAMP (Richard et al. 2002; Ehsan et al. 1998), cAMP-binding enzymes (Laukens et al. 2002), cAMP response element-binding proteins (CREBs) (Katagiri et al. 1989), plant protein kinases, PPK (Lawton et al. 1989), and cyclic nucleotide gated cation channels (Leng et al. 1999) in higher plants. There are reports of involvement of cAMP in absiscic acid and gibberellin action (Duffus and Duffus 1969; Godoy et al. 1994). Guidotti and workers (2013) studied dopamine's role in enzyme activity and root growth in seedlings of soybean. They hypothesized two possible modes of action for dopamine. Dopamine may be performing its role either by interacting with other hormones or by generating quinones, semiquinones and ROS. Dopamine inhibits indole acetic acid (IAA) oxidase, which oxidizes IAA (Kuklin and Conger 1995). The inhibition of IAA oxidase thereby increases the endogenous level of IAA. In the later case, dopamine may produce quinones, semiquinones, and ROS like $^1O_2$, O_2^-, $HO^\bullet$, and H_2O_2 while itself being oxidized en route the melanin biosynthetic pathway. Dopamine may be auto-oxidized or may undergo enzymatic oxidation (Klegeris et al. 1995; Rosei et al. 1994; Matsumoto 2011; Gomes et al. 2014). These species may act upon the membrane components, lipids, proteins, nucleic acids, etc., and eventually cause damage to the cell.

8 Dopamine in Redox Signaling

All organism generate reactive oxygen species popularly called ROS. These creates an oxidative stress which is known to harm the cell. But the organisms counteract the ROS toxicity by the antioxidants which are naturally present in the organisms (Halliwell 2006). Nowadays, a new term oxidative signaling (Foyer and Noctor 2005) is being used to signify the importance of the ROS in cellular signaling. Research is growing in the field of ROS and evidences are accumulating that hints toward a bigger role of these species in plant growth and other physiological functions.

Plants contain various redox agents which may be interacting with dopamine. But how these redox chemicals interact with it is not yet known. Apart from the interaction with redox agents, dopamine itself generates various species that themselves act as redox agents (see Sect. 7). Dopamine undergoes autoxidation or oxidation by enzymes, leading to melanins-producing ROS like $^1O_2$, O_2^-, $HO^\bullet$, and H_2O_2 (Matsumoto 2011; Klegeris et al. 1995). These catecholamine oxidation products, ROS, semiquinone, and quinone, can interact with lipids, proteins, nucleic acids, and membrane components, and cause cell damage.

As previously mentioned exogenous dopamine relieved the alkali stress in apple seedling and improved biomass accretion, net photosynthetic rate, chlorophyll content, and root morphology (Jiao et al. 2019). This might be due to their enhanced antioxidant potential after treatment with the biomediator. Dopamine-treated seedlings showed increased antioxidant enzymes activity, accumulation of CHA and decrease of ROS production.

Tyrosinase enzyme oxidizes dopamine which in turn produces dopaminoquinone. The latter undergoes polymerization to form melanin (Rosei et al. 1994). During oxidation, both semiquinones and quinones are generated in a chain autoxidation process that also results in the production of reactive oxygen species (ROS) like hydroxyl radical (HO^{+}), hydrogen peroxide (H_2O_2) and superoxide anion (O_2-) can be produced. These will interact with proteins, lipids, nucleic acids, and membrane (Apel and Hirt 2004) causing damage and inhibited growth. Autoxidation without the involvement of enzyme can also form melanin. ROS (like O2, O_2-, HO^+ and H_2O_2), semiquinones, and quinones are produced during the oxidation processes (Matsumoto 2011; Klegeris et al. 1995). These ROS, as well as semiquinone and quinone, can interact with proteins, lipids, nucleic acids. The levels of these oxidation products increase during stress in comparison to the low levels under optimal conditions. Allen (2004) while working on chloroplasts isolated from spinach showed that photosynthetic oxygen reduction is dopamine and adrenaline mediated. The superoxide radical is the intermediate and not the product in this process.

On the contrary, dopamine is also reported to act as an antioxidant (Kanazawa and Sakakibara 2000; Yen and Hsieh 1997) and thus a great scavenger of ROS (Yen and Hsieh 1997). In fact, Kanazawa and Sakakibara (2000) reported that dopamine has higher antioxidant potential than glutathione and food additives and is similar to that of strong antioxidant ascorbic acid. Yen and Hsieh (1997) reasoned that dopamine is a stronger antioxidant probably due to a 1,2 position hydroxy group present on the phenolic ring. So dopamine is assumed to be a good ROS scavenger. Similarly, Gomes et al. (2014) concluded that the dopamine's toxicity was not due purely to ROS produced during its oxidation to melanin, as presumed by Guidotti et al. (2013). Rather the authors believed that both O_2- and H_2O_2 were eliminated mainly by the antioxidant activity of dopamine absorbed by the roots, and also by the melanin synthesized. Regarding the latter, a study exploring the gene products of soybean treated with dopamine would be remarkable. This advance may direct to a greater comprehension of the mechanism of action of dopamine in plants.

9 Conclusion

Dopamine is an important signaling molecule in plants as it is in animals. A wide range of activities are controlled and affected by this chemical. Dopamine confers protection to several species by feeding deterrence, and therefore as suggested by previous workers its role in agriculture may be explored. There are limited studies on the mechanism of action of dopamine in plants but there is no denying to the fact that it is mediating some very important events in plants. Besides, ongoing research on its role in redox signaling, plant growth and morphogenesis may be useful in giving a new insight into dopamine's exclusive role in plants. Development of an appropriate model system may help into a better understanding. Dopamine may be modulating its effects either via secondary messenger, plant hormones, and/or by generating reactive oxygen species. A better understanding would immerge when the missing component of the dopaminergic system is found in plants, and therefore the main focus of the researchers should be toward finding a plant receptor for dopamine. Another interesting area would be to unravel and elucidate the crosstalk between various signaling molecules.

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L-DOPA and Dopamine in Plant Metabolism

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Abstract Nonprotein amino acids are widely distributed in the plants. They constitute a numerous group of secondary metabolites and represent a relevant reservoir of nitrogen and carbon. Among them, L-3,4-dihydroxyphenylalanine (L-DOPA) and 3,4-dihydroxyphenethylamine (dopamine) possess relevant physiological and biochemical effects on plants. L-DOPA, a strong allelochemical released by *Mucuna* plants into soils, is a precursor of many alkaloids, melanin, and catecholamines such as norepinephrine (noradrenaline) and epinephrine (adrenaline). In plants, dopamine is produced via hydroxylation of tyramine or decarboxylation of L-DOPA. Because L-DOPA and dopamine reveal striking roles in plant communication and signaling, we revise in this chapter the state of current knowledge on their biosynthesis, oxidation, functions, and prooxidant and antioxidant activities.

1 Introduction

When environmental conditions become unfavorable for a living organism, usually the first behavior is to move: bacteria use their flagella, birds use their wings, and we use our legs. Although plants cannot run, they adopt a riveting survival strategy, i.e., they rely on the release of chemical compounds into the environment that help them tolerate abiotic (Iriti 2013) and biotic stress (Wink 2018). This incredible chemical arsenal that gives plants the ability to survive under unfavorable climatic conditions, pollutants, high irradiance, water stress, herbivory and infection by bacteria, fungi or viruses is based on products of secondary metabolism. The secondary metabolites can be released from aerial or subaerial parts of plants through exudation, volatilization, and leaching of the aerial parts. When accumulated in the soil environment, these compounds affect the growth and development of neighboring plants, an interaction named allelopathy (Einhellig 1995). This ecological phenomenon generates

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competition among plants for soil resources such as water and nutrients. Therefore, allelopathy constitutes a chemical contribution to the adaptation of plants to the environment. In addition, some secondary metabolites have important reproductive functions, giving colors and aromas to flowers and fruits that culminate in the attraction of pollinators and dispersers (Wink 2018).

More than 200,000 secondary metabolites are known. They are classified as amines, nonprotein amino acids, peptides, alkaloids, glucosinolates, cyanogenic glucosides, organic acids, terpenoids, quinones, polyacetylenes, and phenolics (Wink 2015, 2018). Among the thousands of allelochemicals, nonprotein amino acids such as L-3,4-dyhydroxyphenylalanine (L-DOPA) and 3,4-dihydroxyphenethylamine (dopamine) have attracted the attention of researchers. Given the importance of these compounds in plant communication and signaling, a detailed review is opportune. Thus, we aim to cover the following aspects in this chapter: the biosynthesis of L-DOPA and dopamine in plants, their oxidation, functions, and prooxidant and antioxidant activities.

2 Biosynthesis of L-DOPA, Dopamine, and Related Molecules

The shikimate pathway (Fig. 1) is responsible for the biosynthesis of most phenolic compounds in plants, but it is absent in animals. The process converts simple carbohydrate precursors derived from glycolysis (phosphoenolpyruvate) and the pentose phosphate pathway (erythrose 4-phosphate) into chorismate, the precursor of the aromatic amino acids phenylalanine, tyrosine, and tryptophan. The shikimate pathway acts directly in the formation of L-DOPA, because it is a result of the hydroxylation of tyrosine by the copper-containing enzyme tyrosine hydroxylase in the presence of molecular O_2. Also, the L-DOPA biosynthesis pathway in mammals is analogous to that in plants and equally important. It is a precursor of neurologically crucial molecules in animals, such as the neurotransmitters dopamine, norepinephrine (noradrenaline), and epinephrine (adrenaline), and it is an essential precursor in the biosynthesis of pigments such as melanin (Soares et al. 2014; Calabresi et al. 2015; Mosharov et al. 2015; Haddad et al. 2018; Ludin 2018).

Similar to L-DOPA, the biosynthesis of dopamine in plants uses the amino acid tyrosine as a precursor (Fig. 1) with two possible routes (Kulma and Szopa 2007). In the first one, tyrosine is hydroxylated by tyrosine hydroxylase producing L-DOPA, which is subsequently decarboxylated to dopamine by dopa decarboxylase. On the other hand, the second route is initiated by the decarboxylation of tyrosine to tyramine, which is catalyzed by tyrosine decarboxylase. Then, monophenol hydroxylase converts the tyramine into dopamine by hydroxylation. Although both routes are fully active, the predominant route is a species-specific feature (Kulma and Szopa 2007).

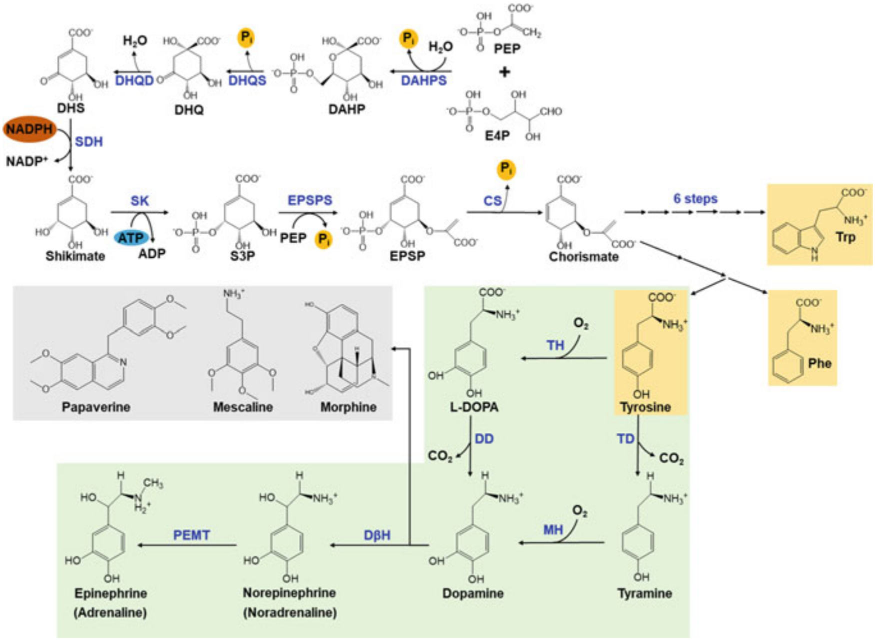

Fig. 1 Biosynthesis of L-DOPA, dopamine and related molecules in plants. Phosphoenolpyruvate (PEP); erythrose 4-phosphate (E4P); 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAHP); DAHP synthase (DAHPS); 3-dehydroquinate (DHQ); 3-dehydroquinate synthase (DHQS); 3-dehydroshikimate (DHS); 3-dehydroquinate dehydratase/shikimate dehydrogenase (DHQD/SDH); shikimate 3-phosphate (S3P); shikimate kinase (SK); 5-enolpyruvylshikimate 3-phosphate (EPSP); EPSP synthase (EPSPS); chorismate synthase (CS); phenylalanine (Phe); tryptophan (Trp); L-3,4-dihydroxyphenylalanine (L-DOPA); tyrosine hydroxylase (TH); L-DOPA decarboxylase (DD); tyrosine decarboxylase (TD); monophenol hydroxylase (MO); dopamine-β-hydroxylase (DβH); phenylethanolamine *N*-methyltransferase (PEMT)

For instance, dopamine in banana (*Musa sapientum*) originates from the hydroxylation of tyramine, but in the Scottish broom (*Cytisus scoparius*), it originates from the decarboxylation of L-DOPA (Kulma and Szopa 2007).

In addition, the hydroxylation of dopamine by dopamine β-hydroxylase produces norepinephrine, which can be methylated by phenylethanolamine *N*-methyltransferase to form epinephrine (Fig. 1). Norepinephrine and epinephrine are present in 44 plant families (Kuklin and Conger 1995), including those used for human feeding (for example, banana, broccoli, oranges, tomatoes, spinach, beans, and potato), in concentrations ranging from 0.5 to 6.9 μg g^{-1} fresh weight (Kulma and Szopa 2007). Also, dopamine is a precursor of important alkaloids such as papaverine, morphine, and mescaline (Lundström 1971). Papaverine and morphine are found in the opium poppy (*Papaver somniferum*). The first is a vasodilator and a phosphodiesterase inhibitor. It has been used for the treatment of cerebral vasospasm, erectile dysfunction in males, and as an antidepressant (Han et al. 2010); lately it has been suggested to be an antiviral agent (Aggarwal et al. 2020). Morphine is widely used in medicine for pain control (Mishra et al. 2013), while mescaline is a hallucinogen found in cactus *Lophophora williamsii* (Lem.), known as peyote (Longo and Musah 2020). Furthermore, L-DOPA and dopamine may undergo enzymatic and/or autooxidation to melanin, as described in detail later.

Interestingly, some conditions of stress can influence the biosynthesis of dopamine in plants (Świędrych et al. 2004). The activities of enzymes involved in the biosynthesis of dopamine (tyrosine decarboxylase, tyrosine hydroxylase, and L-DOPA decarboxylase) were increased in potato leaves treated with abscisic acid. Under salt stress, only tyrosine decarboxylase activity was increased, and under water stress, tyrosine hydroxylase and L-DOPA decarboxylase were activated. In addition, UV light increased the activity of L-DOPA decarboxylase. Leaves of plants cultivated in the dark and in red light were characterized by reduced activities of all three enzymes; while only L-DOPA decarboxylase activity decreased in plants cultivated in cold conditions (Świędrych et al. 2004). Also, wounds performed on potato leaves increased dopamine levels five minutes after the injury (Szopa et al. 2001). Drought, abscisic acid, and UV treatment significantly increased dopamine levels in potato plants (Świędrych et al. 2004). In contrast, the catecholamine levels decreased after treatment with red light, cold, and darkness.

More recently, the effects of drought stress and nitrogen fertilization rates on L-DOPA accumulation have been studied in faba bean (*Vicia faba* L.) (Etemadi et al. 2018). It has been demonstrated that drought stress increased the L-DOPA concentration in leaves, achieving 23 mg g^{-1} biomass. However, although L-DOPA is a nitrogen-containing compound, the nitrogen application rates (0–100 kg ha^{-1}) increased L-DOPA content only in the early stages of plant development (growth stages with 2, 4, and 6 leaves). In later stages of growth (plants with 8 leaves), nitrogen application rates did not significantly influence the L-DOPA content (Etemadi et al. 2018).

3 Oxidation of L-DOPA and Dopamine in Plants

Because the oxidation pathways of L-DOPA and dopamine are very similar, including the formation of reactive oxygen species (ROS), we describe herein only the sequence of reactions leading to L-DOPA (Fig. 2). The characterization of oxidation pathways for dopamine and other catecholamines can be found in Bolton et al. (2000), Kalyanaraman (1990), Kalyanaraman et al. (1987), and Pattison et al. (2002).

In animals, L-DOPA can be converted into melanin by enzymatic or nonenzymatic routes. By the first pathway, tyrosinase (also known as polyphenol oxidase; PPO) plays a crucial role. It is a copper-containing monooxygenase, with monophenolase and diphenolase activities, responsible for the transformation of tyrosine into L-DOPA and further into DOPA-quinone (Mapunya et al. 2012). Then, DOPA-quinone undergoes autooxidation and polymerization to produce melanin and melanin-like compounds (Hachinohe et al. 2004). Yet, the conversion of L-DOPA to DOPA-quinone in plants is catalyzed by PPO or peroxidase (POD) (Fig. 2). Experiments

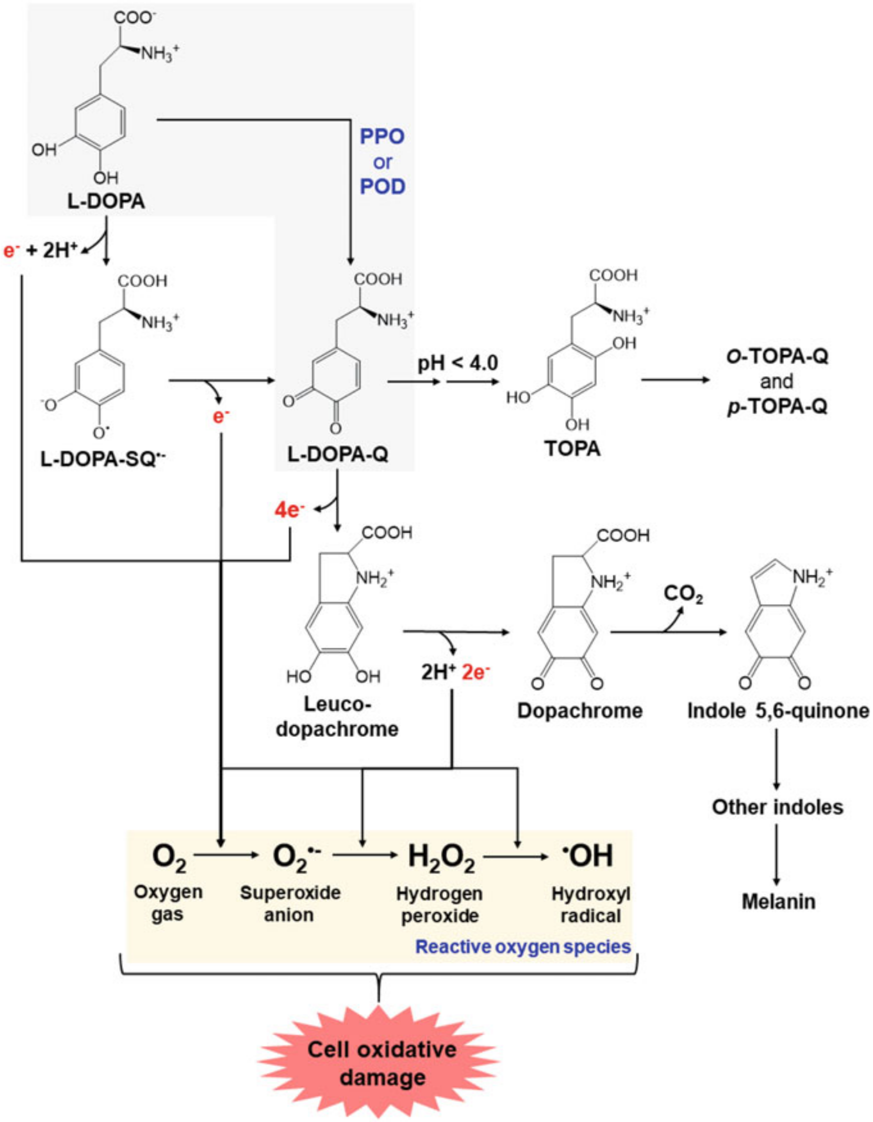

Fig. 2 Enzymatic (blue background) and autooxidation pathways of L-DOPA. Polyphenol oxidase (PPO); peroxidase (POD); L-3,4-dihydroxyphenylalanine (L-DOPA); L-DOPA-semiquinone radical (L-DOPA-SQ$^{-\bullet}$); L-DOPA-quinone (L-DOPA-Q); 6-hydroxy-DOPA (TOPA); TOPA-quinone (TOPA-Q)

in vitro revealed that L-DOPA and other catecholamines can be converted to melanin by soybean lipoxygenase in the presence of hydrogen peroxide (H_2O_2) (Rosei et al. 1994). Also, the application of H_2O_2 in young calli and protoplast isolated from faba bean led to the oxidation of L-DOPA to dopachrome in a reaction catalyzed by POD and not by PPO (Albrecht and Kohlenbach 1990).

By the nonenzymatic pathway, the autooxidation of L-DOPA to melanin produces ROS (Fig. 2), and its rate is increased by trace concentrations of Fe^{3+} and Cu^{2+} (Pattison et al. 2002; Soares et al. 2014). Initially, a semiquinone radical, L-DOPA-$SQ^{\bullet-}$, is originated by loss of an electron and two H^+ from L-DOPA. Subsequently, L-DOPA-$SQ^{\bullet-}$ is oxidized to L-DOPA-quinone, an intermediate in the L-DOPA oxidation pathway that also can be generated by the reaction of PPO with the loss of two electrons from L-DOPA. Then, closing the α-amino group in the L-DOPA-quinone ring leads to the production of leucodopachrome, a process that involves the loss of four more electrons (Bolton et al. 2000). Additional loss of more two electrons from leucodopachrome produces dopachrome (indole-5,6-quinone 2-carboxylic acid), an indolic compound whose decarboxylation yields indole 5,6-quinone. Subsequent reactions of indoles produce melanin. The rate of conversion of L-DOPA to melanin through these reactions is increased under alkaline conditions. On the other hand, under acidic conditions, L-DOPA-quinone can be hydroxylated to 6-hydroxy-DOPA (TOPA) and further oxidized to TOPA-quinone (Li and Christensen 1994).

Commonly, the toxicity of L-DOPA is related to the production of quinones and reactive radicals during its oxidation to melanin. For instance, quinones can make covalent bonds with proteins, inactivating them. Takasaki and Kawakishi (1997) proposed that the L-DOPA-$SQ^{\bullet-}$ can inactivate proteins through oxidation of their -SH group. Moreover, the formation of melanoproteins occurs by reaction of L-DOPA-quinone and cysteine residues of proteins, free cysteine, and low-molecular-weight species, as, for example, glutathione (Kalyanaraman et al. 1987; Pattison et al. 2002). The formation of complexes with cysteine and glutathione may be a part of the L-DOPA detoxification pathway, especially in humans, since cysteinyldopa is found in the urine of patients undergoing L-DOPA therapy (Kalyanaraman et al. 1987).

Furthermore, L-DOPA-$SQ^{\bullet-}$, which has an unpaired electron, is especially reactive and can cause damage to other molecules by removing hydrogen atoms or, preferably, by direct electron transfer. A superoxide anion ($O_2^{\bullet-}$) is formed when the electron acceptor is O_2 with subsequent rearrangement to H_2O_2 species. Particularly in the presence of transition metal ions, H_2O_2 can form a hydroxyl radical ($HO^{\bullet}$) (Pattison et al. 2002). The superoxide anion ($O_2^{\bullet-}$) rapidly destroys iron/sulfur complexes of metalloproteins (e.g., aconitase and fumarase) with inactivation of the enzymes (Asada 1999), besides leading to lipid peroxidation and membrane destruction. Therefore, L-DOPA oxidation can cause severe damage to cell proteins and lead to inhibition of various enzymes important during photosynthesis, peroxidation of lipids and pigments, mitochondrial dysfunction, and DNA fragmentation and apoptosis (Pattison et al. 2002).

The L-DOPA-$SQ^{\bullet-}$ and radicals on the target protein are produced when L-DOPA, or its oxidation products, are incubated with proteins (e.g., creatine kinase) in the presence of Fe^{3+}. Also, H_2O_2 reversibly inhibits various enzymes of the carbon fixation cycle and other metabolic pathways by oxidizing thiol functional groups and causing peroxidation of lipids and pigments (Dietz 2003).

4 The Role of L-DOPA

L-DOPA has many biological roles (Oviedo-Silva et al. 2018). Plants produce hundreds of nonprotein amino acids, and L-DOPA can be massively accumulated in species of the Fabaceae family. L-DOPA was first isolated from faba bean by Guggenheim in 1913 (Rijntjes 2019). Its content is very high in the pods of this plant species (6 and 6.75% of dry weight in *cv*. Diana and Alameda, respectively) (Burbano et al. 1995), which is one of the oldest crops in Europe, traditionally used for animal feed and human food (Soares et al. 2014). Also, L-DOPA is present in abundant amounts in leaves (1%) and seeds (4–7%) of the velvet bean [*Mucuna pruriens* (L.) *var. utiliz*], a legume that has a nutritional quality comparable to the soybean (Pugalenthi et al. 2005). One of the important reasons for the high content of L-DOPA in both plant species is that it acts as a feeding repellent. In fact, velvet bean is not attacked by small mammals or insects. Furthermore, larvae fed a diet containing seeds of velvet bean or synthetic L-DOPA have an increased mortality. Recently, it has been shown that L-DOPA accumulates in extrafloral nectaries of faba bean from predation by *Spodoptera exigua* (Hübner) caterpillars (Gish et al. 2015).

The toxicity of L-DOPA to insects is related to its possible misincorporation into proteins (due to competition with the protein amino acid tyrosine) (Gish et al. 2015), interference with neurotransmission (because it is a precursor of neurotransmitters) (Huang et al. 2011), and interference on tyrosinase activity, which is essential for sclerotization of insect cuticles (Rehr et al. 1973). However, the use of a nitrogen-rich compound as a defensive agent can be a dangerous strategy for plants due to the low nitrogen availability in the environment. Plants that accumulate nonprotein amino acids (L-DOPA, L-canavanine, L-canaline, L-mimosine, etc.) need to avoid their misincorporation into proteins, which would lead to autotoxicity (Huang et al. 2011). In some cases, this is achieved through high-affinity tRNAs that are able to discriminate protein and nonprotein amino acids (Peterson and Fowden 1965; Rosenthal 1990; Igloi and Schiefermayr 2009). On the other hand, nonprotein amino acids can act as a seed nitrogen reserve that is easy to transport, reuse, and integrate into primary metabolism (Huang et al. 2011).

As described earlier, L-DOPA is a precursor for the biosynthesis of melanin and neurotransmitters such as dopamine, norepinephrine, and epinephrine (Fig. 2). It has attracted much attention due to its preventive action against Parkinson disease in humans, which is characterized by a deficiency in the biosynthesis of the neurotransmitter dopamine in nerve cells. In contrast to dopamine, L-DOPA can cross the blood–brain barrier and enter nerve cells. Its misincorporation into the protein defense cells of a Parkinson patient seems to be the supposed mechanism of toxicity (Chan et al. 2012).

4.1 *Velvet Bean: Nutritional Composition, Properties, and Allelopathic Effects*

Velvet bean is a plant that originates from Malaysia, China, and India (Ceballos et al. 2011; Chinapolaiah et al. 2019; Rijntjes 2019). It has a nutritional quality comparable to soybean and has been touted as an alternative plant for developing countries to combat malnutrition (Arivalaga et al. 2014). It has significant contents (per 100 g dry weight) of total phenolics (27.73–103.5 mg), flavonoids (38.28–69.10 mg), total soluble sugars (4.92–9.64 g), potassium (1324–1903.3 mg), magnesium (94.7–157 mg), iron (5.56–12.47 mg), zinc (2.39–4.43 mg), copper (1.45–3.69 mg), and proteins (19.08–38.18%) (Arivalaga et al. 2014; Chinapolaiah et al. 2019). In addition, the flour from its seeds contains significant amounts of essential amino acids (Balogun and Olatidoye 2012).

Velvet bean also has interesting medicinal properties. It is widely used for the treatment of male infertility, nervous disorders, rheumatoid arthritis, diabetes, and as an aphrodisiac (Raina and Khatri 2011; Chinapolaiah et al. 2019). The L-DOPA used for the treatment of Parkinson's disease is largely obtained from its seeds and, therefore, the search for genotypes of *Mucuna* with high L-DOPA content is of commercial interest. A recent study has estimated the content of L-DOPA in 58 genotypes of velvet bean collected in different geographical regions of India (Chinapolaiah et al. 2019). The L-DOPA contents varied widely between genotypes. The genotype with the highest L-DOPA content was IIHR MP 62–1 (6.91%). Intriguingly, it was observed that wild-type genotypes with itchy trichomes have higher L-DOPA content in seeds than non-itchy-type genotypes. Unfortunately, the 7 genotypes with the highest L-DOPA content cannot be grown on a large scale, because the presence of the itchy trichomes makes harvesting infeasible. However, it may be useful in breeding programs to produce non-itchy genotypes with high L-DOPA content, which may be better suited for cultivation and commercialization. Similar results were found by Raina et al. (2012), who identified 6 accessions of velvet bean germplasm with high L-DOPA content in a screening of 38 accessions of *Mucuna* collected from various parts of India. The content of L-DOPA ranged from 2.39 to 5.44%. The genetic variability, geographical location, and environmental factors were responsible for the variability in L-DOPA content among the genotypes.

Velvet bean produces a significant amount of dry organic matter (7.0–9.0 ton ha^{-1}) (Ceballos et al. 2011; Chinapolaiah et al. 2019) with high digestibility. It is often used for soil cover or as silage (Soares et al. 2014), and its cultivation in tropical areas is aimed at enriching the soil due to its ability to fix nitrogen. The use of velvet bean is an interesting strategy for the development of sustainable agriculture, and it can reduce the use of pesticides on crops by controlling weeds and nematodes. In fact, it has been widely used in crop rotation or intercropping with maize, rice, sorghum, pearl millet (Bido et al. 2018), banana, cotton, and citric crops (Ceballos et al. 2011). Notably, when compared to monoculture, velvet bean crop rotation or intercropping with maize significantly increased the average yield of maize from $2.2 \pm 1.95\ \text{t ha}^{-1}$ to $3.2 \pm 2.39\ \text{t ha}^{-1}$ (Ceballos et al. 2011).

It is estimated that velvet bean can release about 100–450 kg ha^{-1} of L-DOPA into the soil (Soares et al. 2014). Allelopathic effects of velvet bean have been reported on several weed species (*Imperata cylindrical, Paspalum, fasciculatum, Striga hermonthica, S. asiatica, Cyperus rotundus, Spermacoce verticullata, Sphenostylis stenocarpa, Echinochloa crus-galli, Lolium perene*, among others) (Hachinohe et al. 2004; Nishihara et al. 2004; Eucharia et al. 2010; Ceballos et al. 2011; Vargas et al. 2018), and its effect is to decrease seed germination and root growth (Bido et al. 2018). In a laboratory study, L-DOPA in concentrations ranging from 5 to 50 mg L^{-1} reduced the growth of 23 plant species (Nishihara et al. 2004). In studies conducted by our research group, pure commercial L-DOPA and an aqueous extract of velvet bean seeds induced similar allelopathic effects on soybean plants, confirming that it is the main allelochemical present in the velvet bean (Marchiosi et al. 2016; Bido et al. 2018).

4.2 L-DOPA in Soils

The plant growth inhibitory effect of L-DOPA, and therefore the allelopathy of velvet bean as described earlier, is directly correlated to its soil availability. Adsorption on the soil, chemical transformation, and microbial degradation can reduce the amount of L-DOPA in a soil–water mixture (Furubayashi et al. 2005). However, the predominant process leading to the elimination of L-DOPA depends on the type of soil. Indeed, the amount of L-DOPA adsorbed was higher in volcanic ash than in calcareous and alluvial soils. On the other hand, the rate of soil chemical transformation of L-DOPA was higher in alluvial soil, followed by calcareous and volcanic ash soils. Nevertheless, calcareous soil showed a greater ability to reduce the plant growth inhibitory effect of L-DOPA than volcanic ash and alluvial soils.

Investigations on L-DOPA turnover kinetics have revealed that the amino acids phenylalanine and tyrosine were not adsorbed and transformed in the soil even in different pHs, in contrast to L-DOPA (Furubayashi et al. 2007). Thereby, the reactions of L-DOPA in the soil probably involve a chemical moiety, which is not present in these amino acids: the catechol group. In fact, the phenolic compound catechin, which also has the catechol group, was adsorbed and transformed into the soil in a manner similar to L-DOPA.

4.3 Phytotoxicity and Tolerance to L-DOPA

There is no doubt that L-DOPA is one of the strongest natural allelochemicals with significant physiological and biochemical effects on plants (Table 1). It can reduce the growth (especially radicle and hypocotyl) of many plants with an EC_{50} ranging from 5 to 50 mg L^{-1} (Nishihara et al. 2004). Although the toxicity of L-DOPA

Table 1 Effects and biological functions of L-DOPA

Species	Physiological/biochemical effects	References
Arabidopsis thaliana	Upregulated expression of genes associated with the homeostasis of metals, such as Zn, Cu, and Fe Downregulated photooxidative stress-induced and ferredoxin-nitrate reductase genes Influenced amino acid metabolism	Golisz et al. (2011)
Echinochloa crus-galli (barnyardgrass)	77-fold more tolerant to L-DOPA than lettuce Absorbed a larger amount of L-DOPA than lettuce Increased phenylalanine, tyrosine, and dopamine amounts, reduced ROS levels	Hachinohe et al. (2004) Hachinohe and Matsumoto (2007)
Glycine max (soybean): seedling	Root growth-inhibition associated with increased lignification Reduced ROS levels, cell viability, and CAD activity Increased PAL, POD, and SOD activities Increased phenylalanine, tyrosine and melanin contents	Soares et al. (2007, 2011, 2012)
Glycine max (soybean): seedlings	Reduced growth and lignin content in roots. Reduced water uptake, leaf area, and photosynthetic rate	Siqueira-Soares et al. (2013)
Glycine max (soybean): plants	Severe reduction of water uptake, leaf area, and plant growth Reduced photosynthetic rate, stomatal closure, and increased intercellular CO_2 concentration Changed chlorophyll fluorescence and affected carbon assimilation reactions	Marchiosi et al. (2016)
Glycine max (soybean): plants	Reduced PAL and CAD activities in roots and stems Reduced lignin and lignin monomer contents in roots Reduced CAD activity in leaves	Bido et al. (2018)

(continued)

Table 1 (continued)

Species	Physiological/biochemical effects	References
Lactuca sativa (lettuce)	High melanin content, oxidase activity, ROS production, and lipid peroxidation Increased L-DOPA in root but no other metabolites were observed	Hachinohe et al. (2004) Hachinohe and Matsumoto (2007)
Nicotiana tabacum (tobacco): thin cell layers *Acmella oppositifolia*: hairy root culture	Stimulated growth and in the rate of ethylene evolution Ethylene biosynthesis inhibitors reduced growth-promoting effect of dopamine Growth-promoting effect is not related to dopamine, as it was still observed after consumption of ethylene with mercury perchlorate	Protacio et al. (1992)
Southern armyworm larvae	Mortality increased, and abnormal pupae and adults At higher concentrations, L-DOPA acted as a feeding repellent	Rehr et al. (1973)
Spodoptera exigua (Hübner)	L-DOPA accumulates in extrafloral nectaries of faba bean from predation for *Spodoptera exigua*	Gish et al. (2015)
Zea mays (maize): seedlings	Growth-inhibition associated with increased lignification Reduced activities of PAL, TAL, and peroxidases Increased phenylalanine and tyrosine contents	Siqueira-Soares et al. (2013)
Herbivores and competitor plants	Misincorporation into proteins Interfered with neurotransmission Interfered with tyrosinase activity	Huang et al. (2011)
23 species from 6 different families	Suppressive herbicidal effect on weeds	Nishihara et al. (2004)
Other species	Root elongation more suppressed than shoot elongation	Hachinohe et al. (2004)

has been attributed to ROS formation and/or misincorporation into proteins, other aspects of its mode of action in plants still remain inconclusive.

It has been noted that the effects of L-DOPA on plants are species-specific. For instance, Gramineae (e.g., maize and wheat) and Leguminosae (e.g., soybean and barley) species are less susceptible to L-DOPA than Brassicaceae (e.g., cabbage) and Compositae (e.g., sunflower) species. Thus, the tolerance to L-DOPA is related to its metabolic destinations in plants (Fig. 3), but not with monocotyledonous and dicotyledonous or C3 and C4 species. This particularity enables the use of L-DOPA as a selective herbicide that is capable of controlling some weed species without

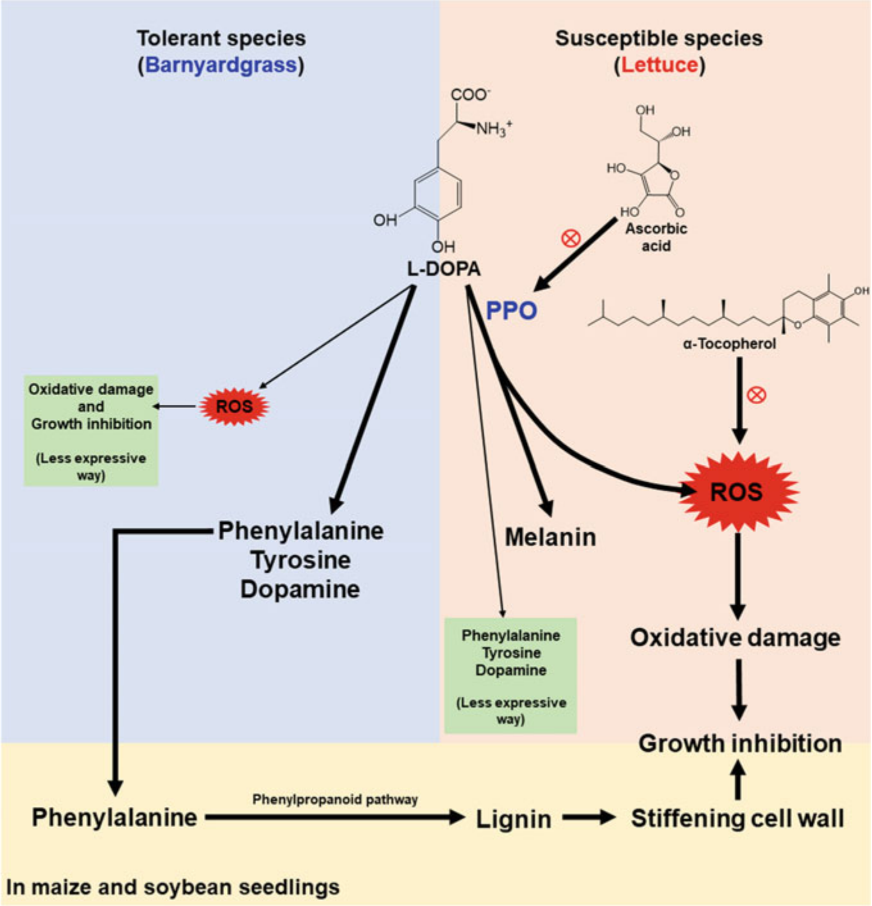

Fig. 3 L-DOPA tolerance mechanism in seedlings. The thick arrows indicate the predominant metabolic pathways occurring in L-DOPA susceptible and tolerant species. Tocopherol combats the reactive oxygen species (ROS) directly, while ascorbic acid inhibits their formation by reducing the activity of polyphenol oxidase (PPO). Inhibitory activities of ascorbic acid and tocopherol on PPO and ROS, respectively, are indicated by ⊗

affecting the growth of plants of interest. Indeed, L-DOPA showed a suppressive herbicidal effect on weeds without significantly affecting wheat and barley growth (Nishihara et al. 2004). Remarkably, some plants have more capacity to metabolize L-DOPA into phenylalanine, tyrosine, and dopamine, avoiding its oxidation to melanin and the associated ROS production (Fig. 3). As a consequence, there is a low production of ROS, protein degradation, and lipid peroxidation, and little influence on growth and development.

The first evidence of this came from the work of Hachinohe et al. (2004) who compared the toxicity of L-DOPA on barnyardgrass (a L-DOPA0-tolerant species) and lettuce (a susceptible species). Barnyardgrass absorbed more L-DOPA than lettuce. L-DOPA metabolism to phenylalanine, tyrosine, and dopamine was observed in both species, with an increase of their amounts in barnyardgrass but not in lettuce. These findings suggest that less L-DOPA undergoes enzymatic and/or autooxidation in barnyardgrass and, consequently, there is low ROS production (Fig. 3). Furthermore, these same authors demonstrated that exposure to L-DOPA induces more lipid peroxidation in lettuce than barnyardgrass, suggesting a higher ROS generation in susceptible species (Fig. 3) (Hachinohe et al. 2004; Hachinohe and Matsumoto 2007). In addition, lettuce plants showed higher melanin content and PPO activity, suggesting that the pathway of L-DOPA oxidation to melanin, responsible for ROS generation, is more active in susceptible species than in tolerant species (Fig. 3) (Hachinohe and Matsumoto 2007). Interestingly, antioxidant agents such as ascorbic acid, α-tocopherol, POD, catalase (CAT), and superoxide dismutase (SOD) may reverse or alleviate the growth inhibitory effect caused by L-DOPA, as shown for carrot cells, PC12 cells from rat adrenal medulla, and HeLa cells (Parsons 1985; Basma et al. 1995; Hachinohe and Matsumoto 2005). In the work of Hachinohe and Matsumoto (2007), ascorbic acid and α-tocopherol reduced melanin formation and lipid peroxidation by different mechanisms. Ascorbic acid inhibits the PPO activity, whereas tocopherol appears to act directly as a ROS scavenger.

Some evidence suggests that ROS production is not the only factor responsible for L-DOPA toxicity in plants. Soybean seedlings exposed to L-DOPA showed inhibition of root growth and increased PPO activity and melanin content, while the levels of ROS were significantly reduced (Soares et al. 2007, 2011). Seedlings exhibited high phenylalanine ammonia lyase (PAL) activity and high lignin, phenylalanine, and tyrosine content (Soares et al. 2007, 2012). Thus, at least in soybean, the possible metabolization of L-DOPA to phenylalanine may have stimulated the phenylpropanoid pathway and lignification, with consequent seedling growth-inhibition. Similarly, L-DOPA-induced growth-inhibition associated with increased lignification was also verified in maize (Siqueira-Soares et al. 2013). Unlike in soybean, the activities of PAL, tyrosine ammonia lyase (TAL), and soluble and bound peroxidases were significantly reduced. However, increases in the phenylalanine and tyrosine contents suggest that L-DOPA can be metabolized to aromatic amino acids and feed the phenylpropanoid pathway (Fig. 3).

In contrast to the results obtained with soybean seedlings (Soares et al. 2007), soybean plants exposed to L-DOPA and aqueous extracts of velvet bean seeds for 22 days showed a remarkable reduction of the lignin content in roots (Bido et al.

2018). Attractively, the monomeric composition of lignin was also altered with wide reduction of *p*-hydroxyphenyl, guaiacyl, and syringyl units of lignin. The activities of PAL and cinnamyl alcohol dehydrogenase (CAD), enzymes compromised with lignification, were reduced in roots and stems, although the lignin content and its monomeric composition were not changed in stems. Thus, under long-term exposure, the phytotoxicity of L-DOPA in soybean and consequent growth reduction does not appear to be related to high lignification.

To gain more information about the mode of action of L-DOPA in plants, Golisz et al. (2011) analyzed the gene expression profile in *Arabidopsis thaliana* plants exposed to the allelochemical. Microarray analysis revealed that L-DOPA upregulated the expression of 110 genes, many of which were associated with the homeostasis of metals, such as Zn, Cu, and Fe. Two genes involved in osmotic stress (alcohol dehydrogenase genes; At1g77120, At1g64710) were upregulated. In contrast, L-DOPA downregulated 69 genes, which are mainly categorized into 'subcellular localization' and 'metabolism'. Among the downregulated genes were photooxidative stress-induced and ferredoxin-nitrate reductase genes. The latter is associated with nitrate assimilation and its protein can act in the photosynthetic electron transport chain as an electron carrier. The authors concluded that L-DOPA phytotoxicity may be related to its influence on amino acid metabolism and homeostasis of metals such as iron.

Corroborating the findings of Golisz and coworkers, our research group identified characteristic symptoms (leaf chlorosis) of nitrogen deficiency in soybean plants grown for 22 days in hydroponic medium containing L-DOPA and an aqueous extract of velvet bean seeds (unpublished data). In addition, L-DOPA profoundly affected photosynthesis (Marchiosi et al. 2016). Among the main findings, we highlight the severe reduction in water uptake, leaf area, and photosynthetic rate. In the early stages of L-DOPA treatment, the remarkable reduction in the photosynthetic rate was shown to be related to stomatal closure. However, the increased intercellular CO_2 concentration after prolonged exposure time suggests interference with carbon assimilation reactions, categorizing a non-stomatal limitation for photosynthesis. This was reinforced by a decrease in the quantum yield of electron flow through photosystem II (PSII) in vivo, which suggests an impairment of carbon assimilation reactions. Plants treated with L-DOPA or an aqueous extract of seeds of velvet bean showed wax accumulation around the stomata. Probably, this was a mechanism triggered to prevent excessive water loss, as these plants had low capacity to absorb nutrient solution due to extensive root damage, especially to root meristem.

5 The Role of Dopamine

Dopamine is a catecholamine that has a 3,4-dihydroxy-substituted phenyl ring. It is widespread in animals and well known as a neurotransmitter. Dopamine has also been detected in many plant families. In fact, 44 plant species can biosynthesize catecholamines, 29 of which are present in the human diet (Kimura 1968). For instance, dopamine and epinephrine are important bioactive components of *Portulaca oleracea*, a traditional phytotherapic (Yue et al. 2005). Also, dopamine was found in leaves of 2–3 weeks old velvet bean, and their levels exceeded the content of L-DOPA (Wichers et al. 1993). However, dopamine was not detected in roots, stems, and seeds, at any stage of development (Wichers et al. 1993). Then, it is possible that in leaves of velvet bean, L-DOPA is metabolized to dopamine to avoid its toxicity (Matsumoto 2011; Guidotti et al. 2013). Other dopamine-rich plant species are banana-of-the-earth (*Plantago major*), potato (*Solanum tuberosum*), avocado (*Persea americana*), and peyote (*Iophophora williamsii*) (Kanazawa and Sakakibara 2000; Kulma and Szopa 2007; Ibarra-Laclette et al. 2015).

In humans, dopamine is a neurotransmitter and its absence in nerve cells causes Parkinson's disease. Insufficient levels of dopamine can lead to depression, loss of motor control, and lack of motivation to perform routine tasks (Belujon and Grace 2017). Despite this, the physiological mechanism of action of dopamine in plants is poorly understood. Some studies have evaluated the physiological and biochemical effects of dopamine on plants (Table 2), and they have revealed that it is associated with defense against herbivores (Van Alstyne et al. 2006), abiotic stress (Liang et al. 2018; Gao et al. 2020), carbon metabolism (Szopa et al. 2001; Skirycz et al. 2005), flowering (Okatani et al. 2010), hormonal balance (Elstner et al. 1976; Kamisaka 1979; Protacio et al. 1992), intercellular regulation of ions' permeability, and photosynthetic reduction of oxygen in chloroplasts (Roshchina 1990; Allen 2003).

5.1 Dopamine as a Defense and Anti-stress Compound

After the discovery of neurotransmitters in plants, it was proposed that they could function as compounds that would avoid herbivory by insects and animals. The first report that dopamine could act as a feeding deterrent came from the work of Van Alstyne et al. (2006). They performed a bioassay-guided fractionation method to verify why the algae *Ulvaria obscura*, which causes green tide blooms, is less attacked by echinoderms, mollusks, and arthropods. The data revealed that the high dopamine content (4.4% of the dry weight of the algae) accounted for decreased feeding by sea urchins *Strongylocentrotus droebachiensis*. According to the authors, dopamine contributes to the formation of persistent blooms of *U. obscura* in the North Pacific and North Atlantic coast's waters, with damage to marine communities, fisheries, and aquaculture.

Some evidence suggests that dopamine has an important role in tolerance to stress. In a recent study, the role of dopamine in drought resistance by apple trees was investigated. Water stress remarkably reduced the plant biomass, photosynthetic rate, stomatal opening, and chlorophyll content. However, when exogenously applied, 100 μM dopamine was able to alleviate the impacts of stress and improve photosynthesis, chlorophyll content, and stomatal opening. Dopamine also improved the uptake and transport of nutrients in plants under drought conditions. Lastly, dopamine suppressed

Table 2 Effects and biological functions of dopamine

Species	Physiological/biochemical effects	References
Beta vulgaris (sugar beet): leaves	Stimulated ethylene biosynthesis	Elstner et al. (1976)
Chara corallina	Influenced calcium, potassium and chloride ion channels Dropped the resting potential of the membrane, reduced membrane resistance, and stopped movement of cytoplast. All effects were reversed after dopamine removal	Kataev et al. 2018)
Glycine max (soybean): seedlings	Reduced root growth, PAL, and bound POD activities Increased SOD activity Loss of root cell viability	Guidotti et al. (2013)
Glycine max (soybean): roots	Increased root melanin content Reduced the levels of ROS, damaged membranes, and decreased CAT activity	Gomes et al. (2014)
Lactuca sativa (lettuce)	Necessary factor for the growth of hypocotyl in seedlings	Kamisaka (1979)
Malus hupehensis (tea crabapple)	Alleviated the effects of salt stress and improved the photosynthetic capacity Stimulated the uptake of K, N, P, S, Cu, and Mn and inhibited the uptake of Na and C Reduced the formation of H_2O_2 Upregulated genes encoding Na^+/K^+ antiporters	Li et al. (2015)
Malus hupehensis (tea crabapple)	Drought resistance, improved photosynthesis, chlorophyll content, and stomatal opening	Liang et al. (2018)
Malus hupehensis (tea crabapple)	Reversed the inhibitory effect of alkali stress on plant height, root length, chlorophyll levels, and photosynthetic capacity	Jiao et al. (2019)
Malus domestica (apple)	Alleviated drought stress. Inhibited the degradation of photosynthetic pigments and increased net photosynthetic rate. Activated Ca^{2+} signaling pathways and regulated transcription factors expression related to the drought tolerance	Gao et al. (2020)

(continued)

Table 2 (continued)

Species	Physiological/biochemical effects	References
Oryza sativa (rice)	Expression of aquaporin gene (*OsPIP-3*) in salt-stressed rice; ameliorated salt stress in rice plants	Abdelkader et al. (2012)
Solanum tuberosum (potato)	ROS scavenger	Świędrych et al. (2004)
Solanum tuberosum (potato)	Expression of human dopamine receptor altered tuber carbon metabolism Transgenic plants showed decreased starch and increased soluble sugar contents Changed activities of ADP-glucose phosphorylase, sucrose synthase, and sucrose phosphate synthase	Skirycz et al. (2005)
Spinacea oleracea (spinach): leaves	Mediated photosynthetic reduction of oxygen in chloroplasts	Allen et al. (2003)
Ulvaria obscura (green alga)	Anti-herbivore and defense compound	Van Alstyne et al. (2006)

the expression of a *pheide, a oxygenase* gene involved in chlorophyll degradation and *senescence-associated* gene 12, which was upregulated by drought (Liang et al. 2018). In addition, the same research group demonstrated that dopamine promotes alkaline tolerance by apple seedlings (Jiao et al. 2019). In Northwest China, the climate is arid or semiarid, and low rainfall and high evaporation lead to the accumulation of alkali salts and alkalization of the soil, compromising the production of high-quality apples. According to the authors, exogenously applied dopamine (0.1 mM) reversed the inhibitory effects of alkalinity on plant height, root length, chlorophyll content, and photosynthesis. Such reversibility may be related to broad activation of the antioxidant system, because increases were observed in SOD, POD, and CAT activities and a reduction in H_2O_2 and malondialdehyde contents.

The effects of dopamine on salt-stressed *Malus hupehensis* Rehd were recently evaluated in both hydroponics and field-pot experiments (Li et al. 2015). At 200 mM, NaCl reduced plant growth, the photosynthetic rate and the maximum quantum yield of PSII in the dark (Fv/Fm). However, the inhibitory effect of salt was alleviated by pretreatment of plants with 100 or 200 μM dopamine. In addition, the application of dopamine stimulated the uptake of K, N, P, S, Cu, and Mn but inhibited the uptake of Na and Cl. Furthermore, dopamine also reduced formation of H_2O_2 due to activation of the antioxidant system enzymes and improvement of the ascorbate–glutathione cycle. Surprisingly, the *MdHKT1*, *MdNHX1,* and *MdSOS1* genes were upregulated in roots and leaves of salt-stressed plants pretreated with dopamine. These genes encode Na^+/H^+ antiporters that are responsible for pumping back out most of the Na^+ that enters root cells; thus, alleviating the stress caused by salt. Corroborating these findings, the tyrosine decarboxylase activity, a key enzyme in dopamine biosynthesis, was stimulated by salt stress (Świędrych et al. 2004). Also, dopamine regulates the expression of *OsPIP-3,* an aquaporin gene, in *Oriza sativa* grown under salt stress (Abdelkader et al. 2012).

5.2 Dopamine's Effect on Flowering

For over four decades, we have known that catecholamines can influence plant flowering. For instance, epinephrine and norepinephrine can release the inhibition of flowering caused by other agents such as sugars in *Lemna gibba* (Oota 1974) or sucrose and ammonium ions in *Lemna paucicostata* (Ives and Posner 1982). Epinephrine and norepinephrine also promoted flowering (besides sustaining the flowers for a long time) in *Lemna paucicostata* grown under a photoperiod of 8 h light and 16 h darkness (Khurana et al. 1987). More recently, Okatani et al. (2010) reported that not only dopamine but also L-DOPA and norepinephrine induce the flowering in *Lemna paucicostata* 151 under short-day conditions when they have been heat-treated. The decomposition products of dopamine, L-DOPA, and norepinephrine that are generated by heating and which are responsible for flowering induction have not been identified.

5.3 Dopamine and Carbon Metabolism

In mammals, catecholamines such as epinephrine and norepinephrine regulate glycogen degradation. Interestingly, it was observed that potato tubers stored at 4 °C increased their levels of soluble sugar content and respiration through starch degradation (Hill et al. 1996) associated with a massive reduction in dopamine and norepinephrine levels (Szopa et al. 2001). This finding strongly suggested that dopamine and other catecholamines may influence carbohydrate metabolism in plants. Corroborating the discovery of Szopa and coworkers, transgenic plants of potato encoding human dopamine receptor (HD1) also showed decreased starch content (20–60%) accompanied by an increase in soluble sugar content (Skirycz et al. 2005). For unknown reasons, the presence of the dopamine HD1 receptor increased dopamine and other catecholamine levels in the tuber of plants, supporting a vision for the functioning of a system that involves the mobilization of sugars, like that found in mammals. In addition, the tuber of transgenic potatoes revealed changes in activities of ADP-glucose phosphorylase, sucrose synthase, and sucrose phosphate synthase, enzymes dedicated to the conversion of sucrose to starch and to sucrose biosynthesis, justifying the altered content of starch and soluble sugars. In mammals, the action of dopamine HD1 receptor is mediated by a G-protein that stimulates the adenylyl cyclase to produce cAMC; therefore, it is possible that catecholamine regulation of enzymes involved in carbohydrate metabolism acts by a similar pathway in plants.

5.4 Dopamine in Hormonal Balance

The identified DoH-CB proteins could mediate the action of catecholamines in plants. This class of proteins contains dopamine-β-hydroxylase activity and a cyt-B561 domain of electron transport (CB). It is relevant to note that some of the DoH-CB proteins are stimulated by auxin. These proteins are very good candidates to act as mediators of catecholamines and provide a link between the action of auxin and catecholamine (Verelst and Asard 2004).

Catecholamines affect the growth and development of plants by their interaction with phytohormones. In fact, dopamine was identified as a necessary factor for the growth of hypocotyl in lettuce seedlings (Kamisaka 1979). In this context, the growth of tobacco thin cell layers (TCLs) was remarkably increased after exposure to 25 μM dopamine (Protacio et al. 1992). Interestingly, ethylene biosynthesis inhibitors such as aminooxy-acetic acid and silver thiosulfate reduced the growth-promoting effect of dopamine. However, ethylene does not seem to mediate the growth-promoting effect of dopamine, as it was still observed after consumption of ethylene with mercury perchlorate. In addition, the activity of indoleacetic acid (IAA) oxidase, an enzyme of degradation of IAA, was significantly reduced in the crude extract from tobacco roots and maize coleoptiles. Due to its influence on IAA metabolism, dopamine potentialized the growth-promoting effect of IAA on TLCs. Feeding experiments with [1-^{14}C]-IAA revealed an expressive decrease of the IAA degradation rate in TLCs. Also, at 5–100 μM, exogenous dopamine stimulates ethylene biosynthesis after illumination of sugar beet leaves (Elstner et al. 1976).

6 L-DOPA and Dopamine: Prooxidant and Antioxidant Activities

Prooxidants are compounds that induce oxidative stress either by creating ROS or by inhibiting antioxidant defense systems. Antioxidants work by neutralizing free radicals, preventing the oxidation of cell molecules and cell damage. It has been suggested that both L-DOPA and dopamine possess prooxidant and antioxidant activities. Because the prooxidant activity of L-DOPA has been widely discussed earlier, we will describe herein its antioxidant activity.

At low concentrations, and in presence of iron-ADP (Halliwell and Gutteridge 1984) and iron-citrate (Grootveld et al. 1989), L-DOPA stimulates the iron-dependent $HO^{\bullet}$ generation from H_2O_2; by contrary, at high concentration this process is inhibited (Soares et al. 2014). In this way, the antioxidant activity of L-DOPA has been reported (Camp et al. 2000; Randhir et al. 2005; Longhi et al. 2011; Soares et al. 2011). This activity seems to be related to the presence of two hydroxyl groups at positions 3 and 4 of the aromatic ring. Marinova and Yanishliera (1992) compared the antioxidant properties of *p*-hydroxybenzoic, vanillic, syringic, 3,4-dihydroxybenzoic, *p*-coumaric, caffeic, ferulic, and sinapic acids in lard autoxidation at 100 °C. Caffeic and 3,4-dihydroxybenzoic acids demonstrated stronger antioxidative properties than compounds containing hydroxyl groups in the third and fourth positions, like L-DOPA.

A seed extract of velvet bean revealed more antioxidant activity than butylated hydroxytoluene (BHT), L-ascorbic acid, curcumin, quercetin, and α-tocopherol by decreasing the concentration of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, ROS, and nitric oxide (Spencer et al. 1996). Although L-DOPA is present in significant amounts in velvet bean, it was not determined whether the antioxidant activity was directly related to its presence in the seed extract. In an in vitro assay, L-DOPA and dopamine eliminated $DPPH^{\bullet}$, ROS, and lipid peroxidation with an efficiency comparable to the antioxidants butylated hydroxyanisole (BHA), BHT, α-tocopherol, and Trolox. Dopamine showed more pronounced antioxidant activity than L-DOPA, as it inhibited the peroxidation of ox-brain with an IC_{50} of 8.5 μM, while L-DOPA exhibited an IC_{50} of 450 μM. Furthermore, L-DOPA and dopamine reacted with trichloromethyl peroxyl radicals ($CCl_3O_2^{\bullet}$) with rate constants of $1.3 \times 10^7\ M^{-1}\ s^{-1}$ and $2.1 \times 10^7\ M^{-1}\ s^{-1}$, respectively.

Our research group has shown that L-DOPA can reduce ROS levels in soybean seedlings, probably due to the stimulation of SOD and POD activities (Soares et al. 2011) or by its direct action as an antioxidant (Spencer et al. 1996). SOD acts in the first line of defense against ROS, catalyzing the dismutation of $O_2^{\bullet-}$ and $^{\bullet}O_2H$ to H_2O_2. CAT converts H_2O_2 to H_2O and O_2. In turn, PODs eliminate H_2O_2, but it can also catalyze the formation of $O_2^{\bullet-}$. Therefore, these enzymes can act as ROS-scavengers. Additionally, soybean seedlings exposed to L-DOPA showed increased lignification (Soares et al. 2007, 2012). Because H_2O_2 is used as a substrate during lignin biosynthesis, its reduction in L-DOPA treated seedlings may be associated with the stimulus of lignification. In fact, there were also increases in the activities of PAL and CAD, enzymes compromised with lignin biosynthesis. In summary, the phytotoxicity of L-DOPA in soybean does not appear to be directly related to ROS production during its oxidation to melanin.

In agreement with findings of Spencer et al. (1996), there is evidence that, in fact, dopamine acts as an antioxidant since Yen and Hsieh (1997) suggested that it could be an effective ROS scavenger. Other studies indicate the participation of dopamine and catecholamines in fine adjustments to responses to stress conditions. The effect probably occurs due to the antioxidant properties of the compound and its derivatives, such as melanin (Świędrych et al. 2004; Kulma and Szopa 2007). In this way, Kanazawa and Sakakibara (2000) evaluated the effect of dopamine on the peroxidation of linoleic acid and the elimination of the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH). Dopamine presented greater antioxidant potential than glutathione and food additives, such as hydroxytoluene, flavone luteolin, quercetin flavonol, and catechin, and a potency similar to strong antioxidants, such as gallate and ascorbic acid. Yen and Hsieh (1997) observed that the antioxidant effects of dopamine and related compounds in the peroxidation of linoleic acid were in the following order: dopamine > α-tocopherol = tyrosine > tyramine > noradrenaline. Earlier, Spencer et al. (1996) reported that dopamine inhibited the peroxidation of phospholipids in the brain; however, the compound seems to possess both antioxidant and prooxidant activity depending on the concentration used.

In view of the apparent contradiction about the oxidative or antioxidant properties of dopamine, Gomes et al. (2014) and Guidotti et al. (2013) investigated the mode of action of this compound in soybean. In these studies, soybean seedlings were cultivated in a nutrient solution with 0.25–1.0 mM dopamine. The effects on several issues related to the condition of oxidative stress (production of the $O_2^{\bullet -}$ and H_2O_2 and lipid peroxidation) and enzymes of the antioxidant defense system (POD, SOD, and CAT) were analyzed. The production of melanin from dopamine was also quantified. The results revealed that the dopamine increased melanin production in roots while the contents of the $O_2^{\bullet -}$ and H_2O_2 decreased. These results were correlated with a significant reduction in the lipid peroxidation levels. The SOD activity increased, while the POD activity was not changed. The CAT activity was substantially lower after dopamine treatments. Still, these authors noted a significant loss of cell viability in soybean seedling roots, which was confirmed by the increased uptake of Evans blue. As the content of ROS in roots was dramatically reduced, the authors suggested that dopamine may have reduced root growth by influencing the metabolism of IAA. As previously described, Kuklin and Conger (1995) demonstrated that dopamine inhibits the oxidation of auxin in vitro as well as in vivo via the inhibition of IAA oxidase. In brief, the contradiction seems to be determined by the test conditions and the concentration of dopamine used.

6.1 Conclusions

Herein, we have reviewed the metabolic pathways that lead to the biosynthesis of L-DOPA and dopamine as well as their biological roles in plants. Some plants, such as velvet bean and faba bean highly accumulate these nonprotein amino acids in their tissues, and they can act as defensive agents against insects and/or as a seed nitrogen reserve. We also extensively reviewed the phytotoxicity of L-DOPA and dopamine on plants, which is mainly associated with the production of ROS during their oxidation to melanin. As discussed, some plants are less susceptible to L-DOPA because they can metabolize it to phenylalanine, tyrosine, and dopamine; thus, reducing ROS formation during its oxidation. In addition, we also addressed the influence of dopamine on hormonal balance, flowering, and responses to biotic and abiotic stress. Finally, the role of L-DOPA and dopamine as antioxidant agents was highlighted.

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Beyond a Neurotransmitter: Physiological Role of Dopamine in Plants

Kiran Bala

Abstract Plant cell produces a wide range of chemical compounds needed for its survival. Mostly secondary metabolite and phytochemicals including neurotransmitters are essential for the reallocation of resources in plants in response to changing environmental factors. Among them, dopamine which is a catecholamine neurotransmitter is found in plants as well as in animals. Many plants species of different families were reported to contain significant amounts of dopamine. It mediates many physiological processes in plants. However, the role of dopamine in plants is poorly documented. They are involved in much aspect of growth, development and their synthesis is regulated by stress condition. Studies have addressed the effect of dopamine on plants as allelochemical that provides defence against herbivore, processes such as nitrogen fixation, flowering and prevention against IAA oxidation, intercellular regulation of ion permeability and photophosphorylation of chloroplast. It has been proposed to be a precursor for various alkaloids benzylisoquinolines like papaverine and morphine or of the hallucinogenic alkaloid. In this chapter current knowledge on role of dopamine in plants are documented. Dopamine, noradrenaline and adrenaline were shown to participate in intercellular regulation of ion permeability and photophosphorylation of chloroplasts. Dopamine is involved in many functions like precursor for various alkaloids, antioxidative, sugar metabolism and coordinates with phytohormones to affect plant growth. In this chapter, current knowledge on role of dopamine in plants is documented.

Abbreviations

ABA	Abscisic acid
BHT	Butylated hydroxytoluene
Cu^{2+}	Copper
DOPA	(3,4-Dihydroxyphenylalanine)

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DOPA-Q	Dopaquinone
DOPA-SQ^{-}	Semiquinone radical of Dopamine
DPDH	1,1-Diphenyl-2-picryl-hydrazyl
Fe^{3+}	Ferric ion
H_2O_2	Hydrogen peroxide
HO	Hydroxyl Radicals
HPLC	High Performance Liquid Chromatography
IAA	Auxin
NO	Nitric Oxide
O_2	Oxygen
O^{2-}	Superoxide
OSPIP	Aquaporin gene
PPO	Polyphenol oxidase
ROS	Reactive oxygen species
SH	Group thiol group

1 Introduction

Consumption of various nutrient rich fruits and vegetables is suggested as a potential treatment for depression, due to the presence of psychoactive neurotransmitters such as serotonin, dopamine and melatonin in many plant foods. Plants produce a wide range of phytochemicals that mediate cell function, plant stress and many of these are human neuroregulatory molecules. Neurotransmitters found in plants to date include acetylcholine, epinephrine, dopamine, levodopa, γ-aminobutyrate, γ-aminobutyric acid (GABA), glutamate, indole-3-acetic acid, 5-hydroxyindoleacetic acid, melatonin and serotonin (Kulma and Szopa 2007). In animals, these compounds are stored in neurons and released into a narrow space between connecting cells, the synaptic space, in response to a stimulus. They are involved in many physiological roles in the plants. In plant cells, acetylcholine is produced in the stems, leaves, nodes and roots in response to heat shock (Momonoki and Momonoki 1991). The neurotransmitter GABA accumulates in plants following stress and is involved in pH regulation, nitrogen storage, plant development and act as antiherbivory compounds and provides defence against insects (Shelp et al. 1999, 2003; Kinnersley and Turano 2000). Indoleamines are a class of human neurotransmitters derived from tryptophan that includes serotonin and melatonin. Melatonin (N-acetyl-5-methoxytryptamine) was first isolated from the bovin epineal gland (Lerner et al. 1958). Low quantities of plant melatonin were found in foodstuffs and melatonin is absorbed and active in animal model systems (Hattori et al. 1995; Wurtman et al. 1963; Yu and Reiter 1993). Many studies are on the presence of melatonin in diverse species ranging from mammals, insects, planatians, mollusks, dinoflagellates and algae to grains, vegetables and medical plants. The first identifications of melatonin in plant tissues were made in 1995 (Dubbels et al. 1995; Hattori et al. 1995) and the discovery of melatonin in intact plants was fairly recent (Murch et al. 1997, 2000). More recently melatonin is reported in 108 plant species used in traditional Chinese medicines (Chen et al. 2003; Murch et al 1997). Melatonin is also found in sour cherries (Manchester et al. 2000), variety of foodstuffs (Badria et al. 2002) and walnuts (Reiter 2005). In plants, melatonin respond to light and dark cycle and photoperiod (Reiter et al. 2001). The highly conserved nature of indoleamines and the diversity of species containing melatonin may indicate an important role for the compound in the growth or survival of higher plants (Murch and Saxena 2002). Serotonin is a neurotransmitter that has been identified across all forms. In recent years the presence and function of serotonin in plants (phytoserotonin) are becoming an increasingly active area of research. Serotonin has been found to function as a plant growth regulator and a stress defence molecule. Through these functions serotonin has been implicated in mediation of morphogenesis, vegetative growth, reproductive development, seed germination and survival, abiotic and biotic stress survival and mediation of plant signalling and mediation of plant cycles life (Lauren et al. 2019).

Catecholamines are also found at elevated levels in plant tissues exposed to stress (Swiedrych et al. 2004). Neurohormones, catecholamines, promote flowering in short-day plants (Khurana et al. 1987) and their synthesis is regulated by stress conditions (Swiedrych et al. 2004; Bowen et al. 2018). They are found in 44 plant families including at least 29 species grown for human consumption (Smith 1980) but, little is known about their roles, modes of action, mechanism of detection or the associated biosynthesis and regulatory pathways (Kulma and Szopa 2007). Catecholamines are an amine derived from the amino acid tyrosine that acts as neurotransmitters. They include dopamine, norepinephrine (noradrenaline) along with epinephrine (adrenaline). These are a group of biogenic amines possessing a 3,4-dihydroxy-substituted phenyl ring and widely distributed in animals and well known neurotransmitter in the mammals. The best understood example of the hormonal action epinephrine and non-epinephrine in mammals is glycogen mobilizing function. Some studies with plants have demonstrated that catecholamine has an antioxidative capacity and precursor for various alkaloids (Guinaudeau and Bruneton 1993; Smith 1980). There are reports showing that mucuna metabolizes L-DOPA to dopamine in leaves as a protective mechanism against the toxicity of L-DOPA (Matsumoto 2011). Noradrenaline and dopamine are major bioactive components of *Portulaca oleracea,* a traditional herbal medicine (Yue et al. 2005). One of catecholamines, i.e. dopamine is a natural product of the catecholamine pathway, widespread in animals especially in mammals (Wang et al. 2018) and has also been detected in many plant families (Kulma and Szopa 2007). In contrast to the vast amount of knowledge about its role in mammals, few reports are found on the physiological significance of dopamine in plants. In plants, studies have shown that effects of dopamine are associated with defence against herbivores, processes such as nitrogen fixation, flowering and prevention of IAA oxidation, intercellular regulation of ion permeability and photophosphorylation of chloroplasts (Weir et al. 2004; Allen 2003; Van Alstyne et al. 2006; Kuklin and Conger 1995a, b; Khurana et al. 1987) (Table 1).

Table 1 Details of the functions involved with dopamine in plants

Substance	Functions	References
Dopamine	Allelochemical	Golisz et al. (2011), Van Alstyne et al. (2006), Wicher et al. (1993)
	Antioxidative	Yasunari et al. (2000)
	Defence against nitrogen	Weir et al. (2004)
	Prevention of IAA oxidation	Guidotti et al. (2013)
	Intercellular regulation of ion permeability	Roschina (1990)
	Photophosphorylation of chloroplast	Kuklin and Conger 1995a, b
	Sugar metabolism	Jung et al. (2000) Jung et al. (2000)
	Coordination with phytochrome	Jung et al. (2000)
	Defence against salt stress	Li et al. (2015), Liang et al. (2017)
	Ethylene Biosynthesis	Elstner et al. (1976)
	Catabolism of IAA	Khurana et al. (1987), Guidotti et al. (2013)

2 Dopamine

This water-soluble molecule was first identified in plants as having strong antioxidative capability that was greater than glutathione, catechin, the flavonol quercetin and the flavone luteolin, and similar to that of gallocatechin gallate and ascorbic acid (Kulma and Szopa 2007). Dopamine influences sugar metabolism and coordinates with phytohormones to affect plant growth (Jung et al. 2000). It can accelerate cell expansion on a growth medium supplemented with indole acetic acid and kinetin but is useless for cells incubated on a basal medium (Protacio et al. 1992). Dopamine is found at high concentration in potato (*Solanum tubersum*) plants, spathes of Araceae inflorescence (Kulma and Szopa 2007; Kanazawa and Sakakibara 2000), the pulp of yellow banana (*Musa acuminate*), red banana (*Musa sapientum* var *Baracoa*), plantain (*Plantago major*) and fuerte avocado (*Persea americana*) (Kanazawa and Sakakibara 2000; Fieldman et al. 1987). It has been proposed to be precursor for various alkaloids benzylisoquinolines like papaverine and morphine or of the hallucinogenic alkaloid mescaline (Lundström and Agurell 1971). Some studies have addressed the effect of dopamine on plants and have revealed that it has attributes typical of an allelochemical (Inderjit and Duke 2003).

3 Dopamine Biosynthesis in Plants

The shikmic acid pathway (Fig. 1) converts simple carbohydrate precursors derived from glycolysis and pentose phosphate pathway to the aromatic amino acid tyrosine, phenylalanine, tryptophan and it participates in the biosynthesis of most plant phenolics (Taiz and Zeiger 2012). L-DOPA is a catecholamine formed by the hydroxylation of tyrosine residues by the copper containing enzyme tyrosine hydroxylase in the company of molecular oxygen and is precursor of many neurologically important molecules such as dopamine, adrenaline and nonadrenaline (Kulma and Szopa 2007).

The compound can undergo tyrosine decarboxylation in presence of tyrosine decarboxylase, resulting in tyramine synthesis. Dopamine is produced via hydroxylation of tyramine or decarboxylation of L-DOPA and dopamine hydroxylation leads to norepinephrine production which in turn methylates to give rise to epinephrine (Kulma and Szopa 2007; Kong et al. 1998; Steiner et al. 1996; Smith 1980). Although

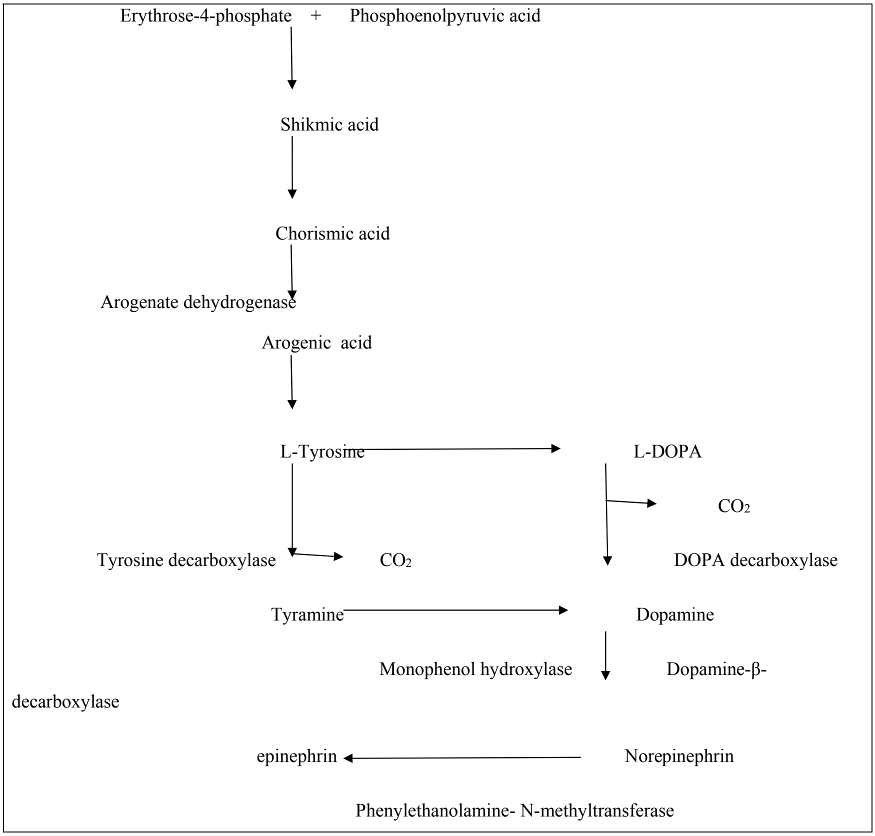


Fig. 1 Shikmic acid pathway in the plants

both initiating steps are fully active, different plants favour different synthetic routes. For example, in sweet banana (*M.sapientum*) dopamine originates from tyramine hydroxylation; but in Scotch broom (*Cytisus scoparius*), peyote cactus (*L.williamsi*) and callus of Portulacca dopamine formation occurs from DOPA (Lundstrom 1971; Smith 1980). L-DOPA precursor of dopamine is also an essential precursor in the biosynthesis of melanin which is present in much tissue of the plants (Guidotti et al. 2013). The physiological mechanism of action of dopamine is a well known neurotransmitter in mammals. Its synthesis in both plants as well mammals are analogous to each other and absence in nerve cells can cause Parkinson disease (Soares et al. 2007; Soares et al. 2014).

4 Metabolism of Catecholamines Including Dopamine

Methylation, oxidation and conjugation with other phenolic compounds are three pathways involved in metabolism of catecholamine. The methylated derivatives of catecholamine and both 3-methoxy-4-hydroxy mandelic acid and homovanillic acid are the final products of their catabolism in mammalian cell. Mandelic and homovanilc acids are absent in plants. Instead, it contains normethenephrine (Szopa et al. 2001). There is possibility that methylation can serve as a way for catecholamine deactivation. From study of animal cells it is known that methylation causes catecholamine inactivation (Li et al. 2005). Even though there were never extensive studies done, some data suggest that methylated compounds are no longer active in plants, at least in some aspects of their activity (Kamisaka 1979). Various derivatives also get synthesized by the methylation of catecholamine. Extensive studies of the catecholamine metabolism in Dona Ana cactus (*Coryphantha macromerix*) reveal production and accumulation of various methylated catecholamine derivative. Of these phenylamines normacromerine (N-methyl-3,4-dimethoxy-beta-hydroxyphenethylamine) is by far the most abundant (Keller and Yeary 1980). Dopamine in particular is an intermediate in alkaloid biosynthesis, most importantly of benzylisosoquinolines like paperverine and morphine of the hallucinogenic alkaloid mescaline, identified in many cactus species (Lundstrom 1971).

Studies of plants tissue cultures grown in presence of labelled tyramine and dopamine showed that catabolism of catecholamine also occurs via oxidation and oxidative polymerization (Meyer and Barz 1978). The plant amine oxidase (Medda et al. 1978) acts on monoamines oxidizing them to the corresponding aldehydes and thus participate in amine degradation. One of the more important chemical changes in dopamine oxidation is by lipoxygenase leading to melanin (Rosei et al. 1994). Catecholamine and their derivatives can also form conjugates with phenolic acids, i.e. p-coumaryladrenaline are involved in plant defence (Roepenack et al. 1974). Catecholamine also serves as substrate of other compounds active in plant cells.

Catecholamines have been shown to be precursors of a series of tetrahydrobenzylisoquinoline alkaloids. The peyote cactus (*Lophophora williams*) contains high concentrations of the hallucinogen mescaline which is synthesized after hydroxylation of tyrosine to L-dopa or by decarboxylation to tyramine (Smith 1980).

5 Plant Stress Modulation and Dopamine

Drought stress is often one of the most limiting factors for plant growth. Plant growth is affected by drought in several ways, e.g. reduced leaf water potential, altered plant water and nutrient relationships (Boomsa and Vyn 2008; Pugnaire and Chapin 1992). Studies have shown that drought treatment led to a decline in many parameters and exogenous application of dopamine eased those inhibitory effects (Liang et al. 2018). When potato (*Solanum tuberosum*) plants are exposed to drought, treatment with abscisic acid or ultraviolet light can significantly increase their concentration of dopamine (Swiedrych et al. 2004). Dopamine treatment mitigates the inhibitory effects of drought on plant growth and helps to maintain strong photosynthesis, chlorophyll level and stomata functioning. The interaction between drought and dopamine indicated that responses of these growth parameters to watering regime were significantly influenced by the application of dopamine. Dopamine has also been identified as a key factor in the growth of *Lactuca sativa* hypocotyls and its level in potato is also significantly increased under drought conditions (Swiedrych et al. 2004). Transcripts levels of a key cholorophyll degradation gene, pheide a oxygenase and senescence associate gene 12 were elevated upon drought treatment, dopamine significantly suppressed the up regulation of these genes under stress condition (Liang et al. 2018). Exogenous treatments of dopamine inhibit nutrition stress as well as salt-induced stress (Li et al. 2015; Liang et al. 2017). In salt-stressed rice (*Oryza sativa*), exogenous dopamine regulates the expression of the aquaporin gene OsPIP1-3 (Abdelkader et al. 2012). Dopamine can also alleviate salt-induced stress in apple (*Malus hupehensis*) (Li et al. 2015). Under salinity stress, activity of tyrosine decarboxylase, a key enzyme in the dopamine synthesis pathway, is enhanced (Swiedrych et al. 2004). Dopamine is coordinated with phytochrome activity to regulate growth and enable plants to fine-tune their stress responses (Kulma and Szopa 2007). These observations indicate that dopamine has an important anti-senescence effect that might be helpful for regulating nutrient uptake, transport and resortion and ultimately influencing overall plant growth. Understanding the role of dopamine in drought tolerance introduces new possibilities to use this compound for agricultural purpose. Some other reports also give the evidence of its function during stress conditions. For example, catecholamine synthesis is much higher in darkness in Portulacca callus. In contrast in the subantartic crucifer *Pringlea antiscorbutica*, levels of dopamine decrease during heat stress (Hennion and Martin-Tabguy 2000).

6 Dopamine as an Allelochemical

Due to their sessile way of life, plants cannot relocate when environmental conditions become unfavourable; they adopt much survival strategy among which one is allelopathy. They rely upon the release of chemical compounds such as cyanogenic glyocosides, glucosinolates, alkaloids, terpenoids, phenolics and nitrogen containing compounds for their defence strategy (Schoonhoven et al. 2005; Mithofer and Boland 2012). Allelochemicals typically inhibit seed germination and seedling growth. Moreover, they alter several physiological and biochemical processes including water utilization, mineral uptake, foliar expansion, photosynthesis, amino acid metabolism, protein synthesis, glycolysis, mitochondrial respiration and ATP synthesis among others (Weir et al. 2004). Dopamine is one of these compounds and has also been detected in many plant families (Kulma and Szopa 2007; Golisz et al. 2011; Topal and Kocacaliskan 2006). Data accumulated suggest that dopamine can be used as an antiherbivore defence compounds by the green alga *Ulvaria obscura* (Kathryn et al. 2005).

Velvetbean (*Mucuna pruriens*) is widely used in tropical regions for intercropping with maize, sorghum and millet and for providing benefits, such as suppression of the nematode population, weed smothering, symbiotic nitrogen fixation, nutrient recycling and control of erosion (Ananya 1999; Soares et al. 2012, 2014; Fuji 2013). Many secondary compounds are produced by velvetbean. Using HPLC coupled with mass spectrometry, dopamine in 2–3-week-old leaves of *Mucuna* were detected (Wichers et al. 1993). The dopamine content of the leaves even exceeded the content of L-DOPA, the most abundant allelochemical in *Mucuna* (Jander and de Vos 2011). However, in the roots, stems and seeds, no dopamine could be detected at any stage of development. Mucuna metabolizes L-DOPA to dopamine in leaves as a protective mechanism against the toxicity of L-DOPA (Matsumoto 2011). Dopamine has also been detected in many other plant families. Southern armyworn larvae fed a diet containing seeds of velvet bean or synthetic L-DOPA precursor of dopamine showed an increased mortality (Rehr et al. 1973). Non-protein amino acid accumulates massively in many plants and seems to play an important role in resistance to herbivores (Furstenberg et al. 2013; Huang et al. 2011). There are reports on catecholamines and their derivatives as deterrents to insect predators and foraging animals (Smith 1980; Nishihara et al. 2004). The resistance of sugarbeet (*Beta vulgaris*) to the fungus *Cercospora beticola* has been suggested to be due to the presence of dopamine (Odjakova and Hadjiivanova 1997). Catecholamines, and especially dopamine, are involved in the protection against penetration of the outer plant surface. Thus, wounds in some papaver species are sealed by a brown melanin-like substance accompanied by release of dopamine and polyphenolase (Homeyer and Roberts 1984). Wound tissue formation in the saguaro cactus *Carnegiea gigantea* is accompanied by increase in dopamine concentration (Kuklin and Conger 1995a, b). This compound is exuded from the roots, where its concentration can reach 1 ppm in water culture solution and 50 ppm in the immediate vicinity of the roots. This concentration is high enough to reduce the growth of neighbouring plants. This growth inhibition can even be seen in agar-medium culture in a mixed culture (Fujii et al. 1991). Investigation into kinetics of DOPA in volcanic ash soil, at various pH values showed that L-DOPA is easily absorbed and transformed in the soil. Thus the concentration of allelochemicals bearing catechol moiety in soil may decrease rapidly owing to adsorption and transformation reactions and this decrease will be faster in soil with high pH or high adsorption ability which can result in a reduction in its plant growth inhibitory activity (Furubayashi et al. 2007).

7 Interaction of Dopamine with Plant Hormones

Catecholamine is associated with ethylene production, prevention of 3-indole acetic acid oxidation and Gibberline signalling (Dai et al. 1993; Kuklin and Conger 1995a, b). A threefold increase in the rate of ethylene production from tobacco thin cell layers was observed after inclusion of catecholamines in the medium (Protacio et al. 1992). Exogenous dopamine at concentrations of 5–100 µM stimulates ethylene biosynthesis in illuminated chloroplast lamellae from sugar beet leaves (Elstner et al. 1976). Dopamine, noradrenaline and adrenaline stimulated ethylene production in potato suspension cultures (Dai et al. 1993). According to the studies, dopamine affects plant development by acting with hormones leading in elevated contents of auxin (Protacio et al. 1992). It was shown that dopamine can inhibit IAA oxidation in vitro as well as in vivo via the inhibition of IAA oxidase (Kuklin and Conger 1995a, b). It is known that auxins promote the growth of stems and coleoptile and inhibit the growth of roots. It is likely that roots may require a minimum concentration of auxin to grow, but growth is strongly inhibited by concentrations of auxin required to promote elongation of stems and coleoptiles (Taiz and Zeiger 2012) Thus, if dopamine actually inhibits IAA oxidase, thereby increasing the auxin content and high levels of this hormone in the roots inhibit growth (Guidotti et al. 2013), it is no exaggeration to suggest that this could be one of the modes of action of dopamine applied to the roots of soybean seedlings. Catecholamines have been suggested to play a role in the catabolism of indoleacetic acid (Khurana et al. 1987). As described by Kamisaka (1979) catecholamine stimulate gibberylin action that induces lettuce hipocotyl elongation. The activity of enzymes involved in catecholamine, i.e. tyrosine decarboxylase, tyrosine hydroxylase and L-DOPA decarboxylase was increased in potato leaves treated with abscisic acid (Kulma and Szopa 2007).

8 Dopamine and Photosynthesis

Dopamine, noradrenaline and adrenaline were shown to participate in intercellular regulation of ion permeability and photophosphorylation of chloroplasts due to its reduction power that ends with the scavenging of free radicals (Kuklin and Conger 1995a, b; Roshchina 1990). These experiments are indirect support for the existence of specific receptors for catecholamines. Studies on the effects of applying exogenous dopamine to water-depleted soil investigated its long-term effects on nutrient status and leaf senescence under drought conditions have shown that when stressed apple plants received supplemental dopamine (100 μM), they exhibited improved growth and photosynthesis (Liang et al. 2018). Dopamine regulates chlorophyll concentrations and stomatal behaviour, while also altering the uptake, transport, partitioning, and restoration of nutrients within the whole plant (Liang et al. 2018). Addition of dopamine significantly delayed the process of drought stress-induced leaf senescence. So it is anti-senescence and has a positive influence on drought tolerance and offers new opportunities for its use in agriculture, especially in regions that are challenged by such stress conditions in the field (Liang et al. 2017).

9 Dopamine and Organogenesis

The abundance of aromatic amines and especially of dopamine and tyramine in spathes of Araceae inflorescences (Ponchet et al. 1982) prompts for a role in reproductive organogenesis (Sharma et al. 1987). The spathe is a floral leaf developing before the sex organs and is thus important for reproduction of monocotyledonous plants. Tropical species of the families Philodendroideae and Monsteroideae mostly tropical creepers contain high concentrations of catecholamines in the leaves, stems, adventious roots but highest concentrations were estimated in their inflorescences (Kuklin and Conger 1995a, b). Changes in the growth of roots by dopamine have been reported in a few plant species. Catecholamines caused a stimulation of growth in root cultures of *Acmella oppositifolia* and *Nicotiana tabacum* cultures (Protacio et al. 1992). However there are reports on dopamine-induced inhibition in soybean roots via damage caused by reactive oxygen species (Guidotti et al. 2013).

10 Dopamine and Other Cellular Activities

In contrast to the vast amount of knowledge about its role and effects in mammals, little is known about the physiological significance of dopamine in plants. Since the early days of catecholamine discovery, plant researchers have been intrigued by the question of the physiological significance of these compounds. They alter several physiological and biochemical processes including utilization, amino acid metabolism, protein synthesis, glycolysis, mitochondrial respiration and ATP synthesis among others (Weir et al. 2004).

11 Pro-oxidant and Antioxidant Properties of Dopamine Precursor

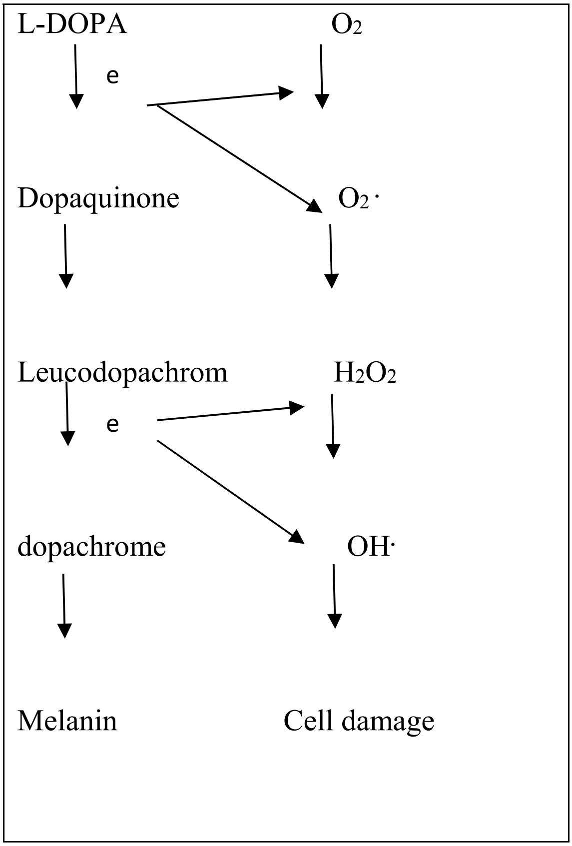
Catecholamines in animals are known to mediate toxicity by receptor-mediated and oxidative mechanism (Arno 2000). They may also be protective through their antioxidant properties. The balance of this dual nature, therefore, is dictated by ambient conditions such as concentration, pH, oxygen, content and localization.

12 Pro-oxidant Properties

Studies have shown that biosynthesis of melanin due to auto-oxidation of L-DOPA, one of the precursors of dopamine, generate ROS (Hachinohe and Matsumoto 2007, Hachinohe and Matsumoto 2005; Hachinohe et al. 2004; Pattison et al. 2002). As enzymes are not involved, the rate is enhanced by the trace concentrations of $Fe^{3+}$and Cu^{2+} ions. The loss of an electron from L-DOPA results the formation of semiquinone radical DOPA-SQ^- (Soares et al. 2014). This may be oxidized into dopaquinone (DOPA-Q), an intermediate in the L-DOPA oxidation pathway. DOPA-Q can also be generated by the direct loss of two electrons from L-DOPA by enzymatic reaction. In this way, it has been proposed that the oxidation of L-DOPA may result in damage to other molecule through either direct or indirect responses. DOPA-SQ^- can transfer electrons to other molecules or remove hydrogen atoms (Takasaki and Kawakishi 1997). Indirect damage may occur by production of ROS, direct reduction of peroxides or via reduction of molecule O_2 to O^{2-} and subsequent dismutation to H_2O_2 species. In the presence of certain transition metal ion, H_2O_2 can form $HO^{\cdot}$ Radicals. DOPA-Q can be oxidized and products of this process are indole compounds which can undergo further reactions to form melanin dependent (Pattison et al. 2002). (Fig. 2). Similar studies have indicated the toxic nature of dopamine. Dopamine can be enzymatically or spontaneously metabolized by molecular oxygen in physiological solutions to form ROS, leading to the formation of melanins. These ROS as well as semiquinone and quinine products of catecholamine oxidation can interact with protein, lipids. Nucleic acid and membrane components thus cause cell damage (Guidotti et al. 2013). Incubation of free DOPA, protein-bound DOPA (PB-DOPA) and related catecholamine with DNA, proteins and lipids has been shown to result in oxidative damage to the target molecule (Pattison et al. 2002). Antioxidant and pro-oxidant capacity of catecholamines and related compounds was also observed in pheochromocytoma PC12 cells (Sofic et al. 2001; Soares et al. 2012; Kruk et al. 1999; Pattison et al. 2002).

The ROS was also generated during L-DOPA oxidation and can also cause severe damage to cell proteins. For example, the iron/sulphur complexes of metalloprotein particularly Fe-S enzyme as aconitase and fumrase are rapidly destroyed by O^{2-} with inactivation of the enzymes in the carbon fixation cycle and other metabolic pathways by oxidizing thiol functional groups and also capable of causing peroxidation of

Fig. 2 Auto-oxidation of dopamine receptor



lipids and pigments (Dietz 2003). As mentioned above, L-DOPA can be oxidized towards melanin. In this context studies have shown that exposure to L-DOPA led to a greater accumulation of melanin in lettuce than in barnyard grass (Hachnohe and Matsumoto 2007). In barnyard grass, this compound is metabolized to phenylalanine, tyrosine and dopamine which were not observed in lettuce (Hachinohe et al. 2004). This reduces ROS formation and consequently membrane damage caused by lipid peroxidation. In this context reported that L-DOPA (0.1–1.0 mM) increased PPO activity and melanin synthesis (root become black) in soyabean (Soares et al. 2011). The results showed that the increase in the PPO activity was associated with browning root, suggesting that melanin synthesis came from the oxidation of L-DOPA.

13 Antioxidant Properties

Antioxidative properties of dopamine is well established in animals, whereas few reports on plants. High antioxidative potency of dopamine than glutathione, food additives such as butylated hydroxyanisole and hydroxytolune flavones, luteolin, flavonol, quercetin and catechin and similar potency to the strongest antioxidants gallocatechin gallate and ascorbic acid was found (Yasunari et al. 2000). Banana contained dopamine at high levels in both the peel and pulp. Dopamine levels ranged from 100 g in peel and 2.5–10 mg in pulp, even in ripened bananas, ready to eat. Banana is thus one of the antioxidative foods (Kanazawa and Sakakibara 2000). It enables organisms to fight with their stress responses, partly because of its antioxidative properties (Kulma and Szopa 2007). Whereas, L-DOPA has contradictory characteristics with respect to the formation of ROS. Some studies have reported an antioxidant activity of L-DOPA (Marinova and Yanishliev 2004). Importantly, L-DOPA has the structure of a phenolic acid similar to caffeic acid differing only in the presence of an amino group in the aliphatic chain of the latter. L-DOPA is the main phenolic of the seeds of Mucuna spp. When tested for their antioxidant activity it was noted that Mucuna extract showed strong antioxidant activity by decreasing the concentrations of the 1,1-diphenyl-2-picryl-hydrazyl (DPPH.) radical and ROS including nitric oxide (NO) when compared with different standards such as the antioxidant butylated hydroxytoluene (BHT), L-ascorbic acid, curcumin, quecetin and alpha-tocopherol (Rajeshwar et al. 2005).

14 Dopamine Receptors in Plants

Several experiments indirectly support the view that receptors for adrenaline or nonadrenaline are present in plants (Roshina 1990). Catecholamines were found to bind to membrane with same way as adrenoreceptors in mammals (Yasunari et al. 2000). Studies showed that propanol an antagonist of beta-adrenergic receptors in animals has been shown to suppress partially flowering of duckweed and this effect was relieved by the addition of adrenaline (Khurana et al. 1987; Högenauer 1978). Potato plants grown on alprenolol, a catecholamine agonist, were characterized by a bushy phenotype and yellowish leaves. Human dopamine receptor D1 was also expressed in potato plant that resulted in remarkable increase in catecholamine levels and changes in sugar metabolism (Skirycz et al. 2005). Newly identified DoH-CB proteins could mediate catecholamine action. This class of proteins contains both dopamine-beta-hydroxylase activity and a cyt b561 electron transport domain (CB) and thus combine in one protein with properties of two enzymes necessary for adrenaline production (Verelst and Asard 2004). In silico analysis of DoH-CB proteins from *Arabidopsis thaliana* shows that structural features of both CB and DoH domains are well conserved. It is interesting that some DoH-CB proteins were found to be inducible. The DoH domain was also identified in another auxin-inducible protein

AIR12. These proteins are very good candidates for mediators of catecholamine function in plants and can provide a link between auxin and catecholamine action since some of the proteins containing the dopamine binding domain are induced in response to auxin. Catecholamine receptor has been identified in transformed potato plants with a cDNA encoding human dopamine receptor (HD1) (Szopa et al. 2001).

15 Conclusion

Initially after the discovery of neurotransmitter substances in plants it was proposed that they might function as a deterrent to insect predator and foraging animals. Data accumulated showed that dopamine can be used as an antiherbivore defence compounds. It was suggested that dopamine might be simply products of synthesis and degradation pathway of other metabolite (Odjakova and Hadjiivanova 1997). Recent evidences on dopamine function have shown it is more complex. They influence many aspects of plant physiology. Involvement in oxidative stress, action in concert with phytohormone in regulation of plant growth, stress responses and regulation of sugar metabolism indicate that they have important regulatory functions. They display a rapid transient increase in plant leaves submitted to wounding, water stress and ABA treatment. Dopamine is required in very small quantities and they are readily modified (methylated) during course of action. The metabolic effect produced by plant dopamine is specific in regulating starch breakdown; Also characteristic is that they are produced mainly in leaves but affect specific physiological responses in another part (tubers) of the organism. Thus taken together the entire data presented make it conceivable that dopamine might play a general role in plant physiology. The molecular mechanism is yet poorly understood and further investigation will be helpful in final elucidation of their function in plants.

The study of dopamine as plant petrochemicals will lead to new understanding of both human health and plant physiology (Fig. 3). It is interesting that some of the same compounds that affect human brain function also affect the growth and development of higher plants. However studies on dopamine are at the initial stage when compared to other branches. Recently developed transgenic technology is a new tool for studying physiological relevance of this compound for plant physiology. A metabolic engineering approach has now provided direct evidence for the role of dopamine in carbohydrate metabolism and plant response to stresses. It is however not known in detail about the compound catabolism and the perspective of the use of plant overproduced dopamine for oral treatment of patients with Parkinson disease. Since dopamine biosynthesis is affected by stresses the potential coordination of their synthesis with other compounds of pathway might occur. Nothing is as yet known in detail on dopamine signal transduction in plant. By using genetic engineering approach the respective receptor and mediating signal transduction compounds can be finally identified. Much further research is required to fully understand the role of neurologically active compounds in plants.

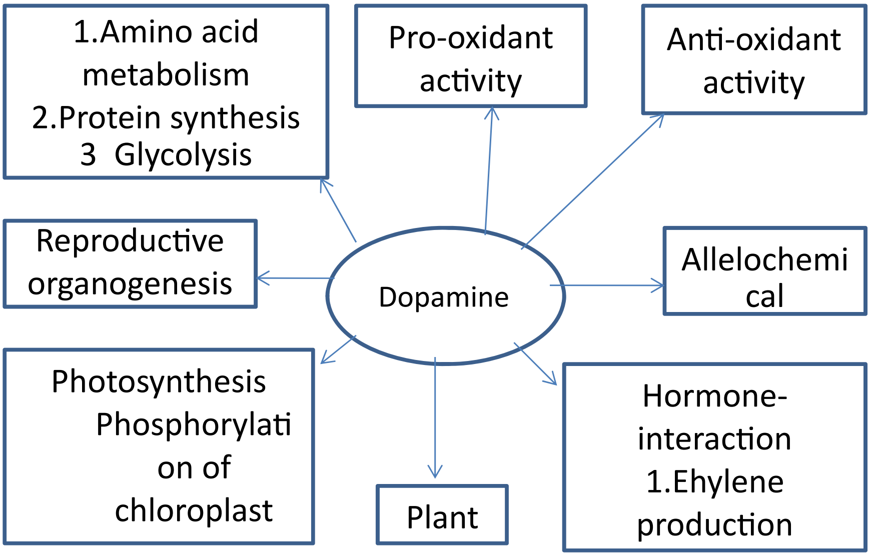


Fig. 3 Physiological role of dopamine

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Melatonin and the Transmission of Light and Auxin Signals in Plants

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Abstract The focus of this chapter is on an overview of the functional responses of plants associated with melatonin and auxin. The interaction of the signaling pathways of these substances, as well as their dependence on light, is shown. Melatonin (N-acetyl-5-methoxytryptamine, MEL) is an important neurotransmitter in mammalian cells. However, its presence in plants has been shown, and since MEL is synthesized not only in mitochondria, but also in chloroplasts, the question of its value for plant organisms arises. Identical precursor (L-tryptophan) and the structural similarity of MEL with indole-3-acetic acid (IAA), a phytohormone from the auxin group, suggests the existence of identical functions of it in the plant. It is known already about its participation in auxin-dependent reactions of growth (seed germination, formation of architecture shoot and root) and development (induction of embryogenesis and flowering, slowing down aging). MEL is an antioxidant that protects the plant from the effects of abiotic stressors (drought, salinity, high and low temperatures, ultraviolet radiation, heavy metals, and organic environmental pollutants). MEL increases the quantum yield of photosynthesis and is involved in the regulation of circadian rhythms. The relationship of MEL and IAA is shown. MEL alters expression of a large number of genes, including genes phytohormone signal transduction pathways, including IAA. The auxin response genes associated with carriers of IAA inflow and homeostasis are subject to change. Melatonin-mediated inactivation of the auxin response is associated with increased activity of the enzyme conjugating amino acids to IAA. Dynamics of the MEL, depending on the intensity, duration and quality of light, suggests its involvement in the COP1-dependent processes of regulation of development programs scoto- and photomorphogenesis. By analyzing the effects of different concentrations of MEL, the researchers came to the conclusion about dose-dependent signaling of MEL. Currently, the mechanisms of action of MEL and the light-dependent interaction of MEL-IAA in the plant organism have not been studied sufficiently.

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Abbreviations

IAA	Indole-3-acetic acid
MEL	Melatonin
SER	Serotonin

1 Introduction

Identical precursor (L-tryptophan) and the structural similarity of melatonin (MEL) with indole-3-acetic acid (IAA), a phytohormone from the auxin group, suggest the existence of identical functions of it in the plant. It is known already about its participation in auxin-dependent reactions of growth (seed germination, formation of architecture shoot and root) and development (induction of embryogenesis and flowering, slowing down aging). Currently, the mechanisms of action of MEL and the light-dependent interaction of MEL-IAA in the plant organism have not been studied sufficiently.

2 AUXIN

2.1 IAA Functions

One of the important regulators of the growth and development of auxin plants is indole-3-acetic acid (IAA). Due to the constantly existing gradient of IAA concentrations, uneven cell growth is supported in various plant organs. IAA regulates the apical dominance of shoots, rhizogenesis, tropism of shoots and roots, leaf arrangement on the shoot, cell stretching and division, etc. IAA can participate in the functioning of stress protection systems (Iglesias et al. 2011; Jung and Park 2011). During seed germination at Arabidopsis roots under salt stress, salt-mediated membrane-bound transcription factor NTM2 is mediated, which is closely linked through the *IAA30* gene to auxin signaling. At the same time, auxin acts as a negative regulator of seed germination at high salinity (Jung and Park 2011).

2.2 IAA Biosynthesis

The concentration and activity of IAA in a plant depend on the rate of de novo phytohormone biosynthesis and degradation processes, as well as the conjugation/deconjugation of IAA with proteins or sugar. Several pathways of IAA biosynthesis are known, among which the most studied and considered significant is L-tryptophan (L-Trp)-dependent IAA biosynthesis pathway (Tao et al. 2008; Mashiguchi et al. 2011; Cook and Ross 2016). It takes place in two stages. At the first stage of this pathway, L-Trp aminotransferases are converted to indole-3-pyruvate (IPA) using TAA family enzymes (TRYPTOPHAN AMINOTRANSFERASE ARABIDOPSIS) (Fig. 1a). In the second step, IPA is converted to IAA using enzymes from the family of flavin-containing monooxygenases YUC (YUCCA). Second L-Trp-dependent pathway is supposed to proceed in the following sequence: L-Trp—indole-3-acetaldoxime—indole-3-acetonitrile—IAA (Fig. 1b). CYP79B2 and CYP79B3 (monooxygenases cyt P450) are believed to be responsible for the conversion of L-Trp to indole-3-acetaldoxime. In the second–third steps, indole-3-acetaldoxime is converted to indole-3-acetonitrile, which using the NIT (nitrilase) enzyme is rotated into IAA.

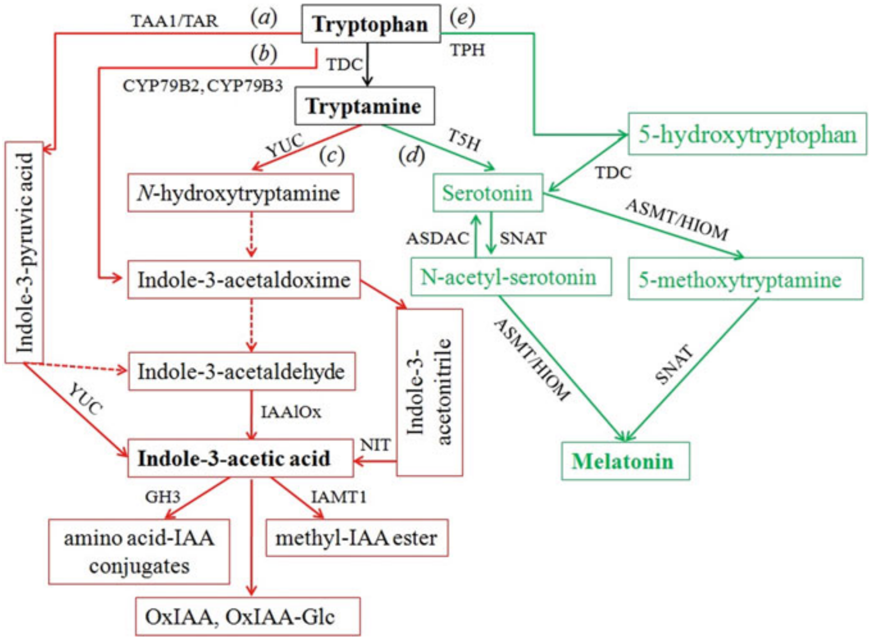

Fig. 1 Biosynthetic pathways of indole-3-acetic acid and melatonin in plants. The enzymes for the steps are ASDAC—N-acetylserotonin deacetylase, ASMT/HIOMT—N-acetylserotonin O-methyltransferase/hydroxyindole-O-methyltransferase, CYP79B2, CYP79B3—monooxigenases cyt P450, GH3—IAA amino synthase, IAAlOx—indoleacetaldehyde oxidase, IAMT1—IAA carboxyl methyltransferase, SNAT—serotonin N-acetyltransferase, T5H—tryptamine 5-hydroxylase, TAA—tryptophan aminotransferase Arabidopsis, TDC—tryptophan decarboxylase, TPH—tryptophan-5-hydroxylase, NIT—nitrilase, YUC—flavin-containing monooxygenases YUCCA (Murch et al. 2000; Qin et al. 2005; Arnao and Hernández-Ruiz 2006; Quittenden et al. 2009; Ludwig-Müller 2011; Ljung 2013; Matthes et al. 2019; Zhao et al. 2019)

Another way of biosynthesis of IAA depends on tryptamine. This pathway is supposed to proceed in the following sequence: L-Trp—tryptamine—N-hydroxytryptamine—indole-3-acetaldoxime—indole-3-acetaldehyde—IAA (Fig. 1c). YUCCA is believed to be responsible for the conversion of tryptamine to N-hydroxytryptamine. Other enzymes are less studied. Quittenden et al. (2009) succeeded in establishing an intermediate compound (indole-3-acetaldehyde) involved in the conversion of tryptamine to IAA in peas.

An important role in the regulation of IAA content or its activity is played by enzymes involved in auxin conjugation (GHs). Conjugation of IAA with amino acids using the GH3 enzyme, IAA amino synthase, leads to inactivation of the phytohormone (Ludwig-Müller 2011). The possibility of methylation of IAA by the enzyme IAMT1, IAA carboxyl methyltransferase, which converts IAA to methyl-IAA ester (MeIAA) has been shown (Qin et al. 2005).

2.3 IAA Transport

A major role in maintaining the IAA gradient in the plant and its homeostasis in individual cells is played by the transport of phytohormone from the site of its synthesis to the site of action. To date, four families of transporters of IAA transporters in plants are known: PIN (PINFORMED), PILS (PIN-LIKES), ABCB/MDR/PGP (ABC-B/multidrug resistance/P-glycoprotein) and AUX/LAX (AUXIN1/LIKE–AUX) (Cho and Cho 2013; Balzan et al. 2014; Grones and Friml 2015). Distinctive features between ABCB and auxin PIN transporters are their localization location, the specified phytohormone transport direction, and function. ABCBs are stably and symmetrically localized in the plasmalemma, function as basal auxin transporters, and regulate its cellular homeostasis. In turn, PIN transporters are asymmetrically located in the plasmalemma and change their localization in response to the action of internal and external factors, determining the directional transport of phytohormone to create local auxin gradients (Cho and Cho 2013).

2.4 AA Signal Transmission Pathway

The IAA coreceptors represented by TIR1/AFB proteins (TRANSPORT INHIBITOR RESPONSE1/AUXIN-RELATED F-BOX) and Aux/IAA, transcriptional co-repressors TPL (TOPLESS) and ARFs (AUXIN RESPONSE) act as transduction elements in the IAA signal transmission path (Matthes et al. 2019). Phytohormone signaling depends on its concentration in the cell. At low IAA levels in the cell, Aux/IAA physically interact with ARF and TPL, thereby preventing the expression of their target genes. High intracellular IAA levels promote binding between

Aux/IAA and TIR1, the latter is part of the SC3 E3 ubiquitin ligase complex and triggers Aux/IAA degradation, allowing ARF to bind to specific DNA elements called AuxRE (auxin-responsive elements) to activate downstream target genes.

3 Melatonin

3.1 MEL Functions

Melatonin (MEL) and serotonin (SER) are neurotransmitters in mammalian cells. It has now been established that these indolamines are present in plants of different taxonomic groups. The level of endogenous MEL depends on the type of plant, its organ, and stage of ontogenesis (Sharif et al. 2018). MEL concentrations measured in nine different plants by radioimmunoassay ranged from 0 to 862 pg MEL/mg protein (Dubbels et al. 1995).

Many physiological functions in a plant depend on the concentration of MEL. It affects mitosis and mitotic spindle formation, regulates ribosome biosynthesis, lipid metabolism, photosynthesis, and storage protein degradation (Murch and Saxena 2002; Arnao and Hernández-Ruiz 2015; Zhang et al. 2015). Wan et al. (2018) found that MEL induces changes in gene expression profiles associated with glycolysis, tricarboxylic acid cycle, glyoxylic acid cycle, primary nitrogen metabolism, and the catabolism of several key amino acids. The multidirectional effect of MEL on glycolysis enzymes was noted. Treatment is with 10 μM MEL repressed expression of phosphoglycerate mutases and pyruvate kinase, but enhanced expression of the *HKL1* and *HXK4* hexokinase gene. Exogenous melatonin stimulated the accumulation of anthocyanins in cabbage, increasing the expression levels of transcription factors MYB, bHLH, and WD40, which are responsible for the regulation of anthocyanin biosynthesis genes (Zhang et al. 2016).

MEL plays an important role in the regulation of plant growth and development. It controls the organogenesis of roots and shoots and the formation of plant tissues, participates in coordinating the internal rhythms of plants with the rhythms of alternating light and dark, and modulates reproductive development (Erland et al. 2015; Arnao and Hernández-Ruiz 2017). Indolamine regulates seed germination, root development (Wen et al. 2016), flowering (Huang et al. 2017), senescence, (Byeon et al. 2012) and plant stress responses in response to biotic and abiotic factors (Weeda et al. 2014; Mukherjee et al. 2014; Kholodova et al. 2018). The combined action of MEL and kinetin (KN) enhances the induction of somatic embryogenesis in *Coffea canephora* in vitro (Ramakrishna et al. 2011).

MEL is an antioxidant that protects plants from abiotic stressors: drought, salinity, high and low temperatures, intense light, ultraviolet radiation, heavy metals, and organic environmental pollutants (Arnao and Hernández-Ruiz 2015; Kholodova et al. 2018). Mukherjee et al. (2014) report the effect of NaCl stress on the accumulation of endogenous SER and MEL and their differential spatial distribution in *Helianthus* *annuus*, thereby indicating their participation in salt-induced signaling over long distances from roots to cotyledons. Inhibition of IAA-dependent root growth caused by salt stress can be attributed to partial dysfunction of IAA caused by enhanced SER biosynthesis. In seedling cotyledons, NaCl stress modulates the activity of N-acetylserotonin O-methyltransferase HIOM/ASMT, the enzyme responsible for the biosynthesis of MEL from N-acetylserotonin.

3.2 MEL Biosynthesis

It has now been established that MEL is synthesized in plant mitochondria and chloroplasts (Zheng et al. 2017). In a two-step process, SER is synthesized. In this case, L-Trp is decarboxylated to tryptamine using TDC (tryptophan decarboxylase), followed by SER biosynthesis catalyzed by T5H (tryptamine 5-hydroxylase) in plants (Fig. 1c, Zhao et al. 2019). Two subsequent biosynthesis steps result in the formation of the final product, MEL. The SNAT enzyme (serotonin N-acetyltransferase) plays a key role in the conversion of SER to N-acetylserotonin, while the ASMT enzyme (N-acetylserotonin O-methyltransferase, formerly known as hydroxyindole-O-methyltransferase, HIOMT) catalyzes the conversion of N-acetylserotonin. An alternative way of forming MEL is also possible. SER can be first methylated to 5-methoxytryptamine using AMST; thereafter, 5-methoxytryptamine is acetylated by NAT to form MEL (Fig. 1d). To maintain the optimal level of MEL in plants, reversible biosynthesis reactions function. The indolamine content is controlled by two enzymes, one of them SNAT promotes MEL biosynthesis, while the other ASDAC (N-acetylserotonin deacetylase) limits it (Lee et al. 2018). Huang et al. (2017) found an increase in the level of MEL in the roots and stems of transgenic plants overexpressing the homologous sheep arylalkylamine N-acetyltransferase and hydroxyindole-O-methyltransferase genes that catalyze the last two stages of MEL synthesis.

Another serotonin biosynthetic pathway exists in some plants, such as St. John's wort (*Hypericum perforatum*), and is similar to the melatonin biosynthesis pathway in animals, in which TPH (tryptophan 5-hydroxylase) converts tryptophan to 5-hydroxytryptophan and then 5-hydroxytryptophan is catalyzed into serotonin by TDC/AADC (aromatic-l-amino-acid decarboxylase) (Fig. 1e, Murch et al. 2000).

3.3 MEL Signal Transmission Pathway

The transmission of the MEL signal to intracellular processes can occur directly or indirectly when interacting with the receptor through the subsequent activation of secondary messengers. On the one hand, MEL, acting as an antioxidant, directly interacts with reactive oxygen species. On the other hand, the MEL-induced stomata closure of *Arabidopsis thaliana* is mediated by the $CAND_2$/PMTR1 receptor, which activates the Gα subunit, and this signal is transmitted via H_2O_2 and Ca^{2+} (Wei et al. 2018). MEL signal transduction can connect various signaling pathways, including cAMP, calcium/calmodulin (CaM) and mitogen-activated protein kinases (MAPK), NO (Murch et al. 2001; Chen et al. 2009; Ramakrishna et al. 2009; Lee and Back 2016; Mukherjee 2018; Fig. 2).

Among all signaling systems, calcium is crucial for the growth and development of plants, since it affects various biological processes. It was previously reported that an increase in cytosolic calcium triggers numerous cellular processes by modulating protein kinases, ion channels, and other cellular proteins (White 2000). Calcium-permeable channels were detected in the plasma membrane, tonoplast, endoplasmic reticulum, chloroplast, and plant cell nuclear membranes. Calcium channels are involved mainly in signal transmission. Their discovery leads to an increase in the cytoplasmic concentration of Ca^{2+}, followed by spatial and temporal changes in the Ca^{2+}pool, causing specific physiological reactions in response to various biotic and abiotic stimuli.

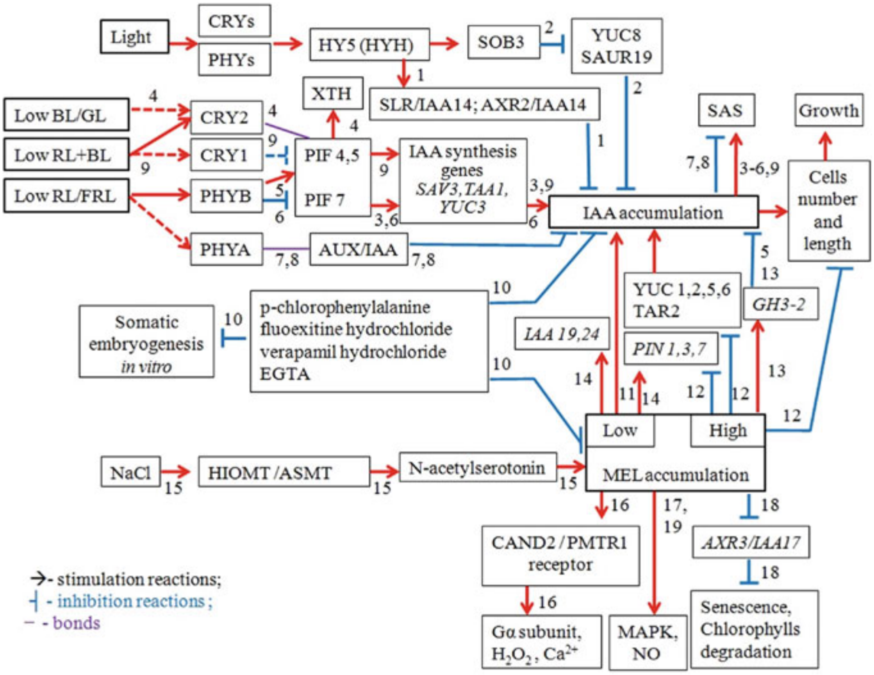

Fig. 2 The interaction of melatonin, indole-3-acetic acid, and environmental factors. References: 1—Sibout et al. (2006); 2—Favero et al. (2016); 3—Tao et al. (2008); 4—Sellaro et al. (2010); 5—Lorrain et al. (2007); 6—Li et al. (2012); 7—Yang et al. (2018); 8—Colón-Carmona et al. (2000); 9—Fankhauser and Batschauer (2016); 10—Ramakrishna et al. (2011); 11—Chen et al. (2009); 12—Wang et al. (2016); 13—Ludwig-Müller (2011); 14—Wen et al. (2016); 15—Mukherjee et al. (2014); 16—Wei et al. (2018); 17—Lee and Back (2016); 18—Shi et al. (2015); 19—Wen et al. (2016)

MEL has a high affinity for Ca^{2+}-activated CaM, which inhibits intercellular Ca^{2+}/CaM-dependent functions. In this regard, the negative effect of high MEL concentrations on root elongation may result from MEL-related changes in CaM antagonism (Chen et al. 2009). The simultaneous addition of MEL and kinetin (KN) to the culture medium led to increased induction of somatic embryogenesis in *Coffea canephora* in vitro (Ramakrishna et al. 2011). In contrast, the addition of such indolamine inhibitors (p-chlorophenylalanine, fluoexitine hydrochloride) and calcium channel inhibitors (verapamil hydrochloride and ethylene glycol-bis (b-amino ethylether) −N, N, N0, N0-tetra acetic acid (EGTA)) individually inhibits somatic embryo induction while reducing endogenous MEL and IAA levels. Ramakrishna et al. (2011) showed that in vitro*C. canephora* plants, when treated with calcium and calcium ionophore A23187, induce rooting, while the addition of calcium channel blocker effectively reduces root induction.

Another signaling system is a MAPK cascade. The involvement of this signaling system in the transduction of the MEL signal was detected. Treatment with 1 μM Arabidopsis MEL quickly activated two MAPKs (mitogen-activated protein kinase), MPK3 and MPK6 (Lee and Back 2016). Four MKKs (MAP kinase kinase), namely MKK4, MKK5 MKK7, and MKK9, were responsible for their activation.

The effect of MEL on growth processes is manifested in a change in the expression of genes that control the modification and growth of the cell wall. Of the 60 genes associated with the cell wall, 45 were suppressed and 14 were increased at least twofold, and one gene was completely suppressed (Weeda et al. 2014). Down-regulated genes include genes encoding 8 expansions and 4 pectin lyases or pectin methyltransferases. Among the genes with increased regulation, two xyloglucan endotransglucosylases were identified. Another study showed that MEL locally induces the expression of genes associated with cell wall remodeling of *LBD16* (*LATERAL ORGAN BOUNDARIES-DOMAIN 16*) and *XTR6* (*XYLOGLUCAN ENDOTRANSGLYCOSYLASE 6*), thereby causing the development of lateral roots (Wan et al. 2018).

As expected, the MEL signal transduction system included 183 genes involved in phytohormone signaling (Weeda et al. 2014). MEL alters stress-induced gene expression. During stress, most auxin-sensitive genes were suppressed in response to 1 mM MEL. While most of the genes involved in signal transduction of abscisic (ABA), salicylic (SA), and jasmonic (JA) acids and ethylene (ET) were amplified. Many of the genes sensitive to ABA, SA, JA, and ET induced by MEL are also induced in response to biotic and abiotic stresses. These results further confirm the critical role of MEL in protecting against stress in plants.

The regulatory role of MEL in increasing proline and chlorophylls levels and in modulating enzymes that absorb ROS, ROS/RNS, and glutathione levels has been shown (Kholodova et al. 2018; Mukherjee 2018). Melatonin acts also through ROS absorbers cascade, which allows elimination of up to 10 free radicals per melatonin molecule by formation of consecutively formed metabolites with scavenging properties (Rosen et al. 2006).

The ubiquitin proteasome system has been proposed as a possible mechanism of the multiple actions of MEL in the light (Sanchez-Barcelo et al. 2016). The hypothesis is based on the general functional aspects of MEL and COP1 and COP9, including their dependence on light, metabolic regulation, and tumor growth control.

The different role of MEL in the regulation of plant growth and development under the action of low (100 pM) and high (1 mM) indolamine concentrations was shown. The different gene expression profiles in Arabidopsis plants corresponded to this (Weeda et al. 2014). Treatment with 100 pM MEL significantly affected the expression of only 81 genes with 51 reduced and 30 increased. However, 1 mM MEL significantly altered 1308 genes with 566 increased and 742 reduced. Not all genes altered by low MEL activity were affected by high MEL.

4 Interactions Between Melatonin and Indole-3-Acetic Acid Pathways

The identical precursor and the structural and functional similarities of the MEL and IAA molecules (Arnao and Hernández-Ruiz 2006) suggest the possibility of their interaction in plants. Currently, studies of the growth processes and signaling pathways of MEL and IAA on various plant systems (whole plants, individual organs, and in vitro cell culture) have been carried out. The common points of MEL and IAA are found.

First of all, IAA-mediated MEL signaling is mainly via the activity of IAA transcription factors. AtIAA17 is known to be a positive modulator of natural leaf senescence in Arabidopsis; therefore, overexpression of the *AtIAA17* gene accelerates this process. Exogenous MEL treatment reduces the expression level of *AXR3/IAA17* (*AUXIN RESISTANT 3/INDOLE-3-ACETIC ACID INUCIBLE 17*), slowing the destruction of chlorophyll and delaying the natural senescence of leaves in Arabidopsis (Shi et al. 2015).

There is evidence of a similar effect of MEL and IAA on dose-dependent plant growth (Tan et al. 2012; Park and Back 2012; Pelagio-Flores et al. 2012). For example, MEL regulates the architecture of the rice root in the meristematic zone, modulating the IAA response (Liang et al. 2017). The physiological effect of exogenous MEL in the regulation of root growth depends on its concentration. When treated with low concentrations (0.1 mM), MEL increases the endogenous content of free IAA and increases root elongation, while higher concentrations (100 mM) of MEL have an inhibitory effect on hormone levels and growth (Chen et al. 2009). The inhibitory effect of high MEL concentrations is manifested not only on the extension of the main roots but also on the proliferation of the lateral roots (Park 2011). In contrast to Park (2011), treatment with MEL, despite significant inhibition of embryonic root growth, contributes to the formation and development of lateral roots. It was noted that high concentrations of MEL up to 1 mM suppress the number and length of root meristem cells, thereby reducing primary root growth (Wang et al. 2016).

At the molecular level, MEL induces changes in the expression of IAA-associated transcription factors WRKY, NAC, and MYB. In addition, MEL in high concentrations negatively regulates the biosynthesis of IAA and the expression of PIN protein genes in Arabidopsis (Wang et al. 2016). When exposed to 600 μM MEL, the levels of YUC1, YUC2, YUC5, YUC6, and TAR2 transcripts (auxin biosynthesis gene elements) are reduced, and *PIN1*, *PIN3*, and *PIN7* genes encoding IAA carrier proteins are suppressed.

Arabidopsis transcriptome analysis showed that the expression of 52 genes that control responses and auxin signaling was altered by melatonin with 29 down-regulated and 23 up-regulated (Weeda et al. 2014). Most IAA-responsive genes that have been suppressed in response to MEL are involved in IAA transport and homeostasis. It is no accident that the influence of MEL manifests itself at the level of regulation of IAA transport, since it was found that the polar IAA gradient is necessary for the growth and development of roots in Arabidopsis caused by MEL (Wang et al. 2016).

The overlapping effects of MEL and IAA in the regulation of rhizogenesis and root gravitropism due to the similar action of two molecules on the processes are noted. However, MEL demonstrates both positive and negative regulation of IAA activity depending on its concentration. The mechanism for transmitting IAA signals induced by MEL primarily works through its biosynthesis, conjugation, transport, and further expression of IAA-sensitive elements downstream in the cascade. For example, MEL-mediated inactivation of the IAA response may be due to the activation of *GH3* genes that encode the IAA amino synthase enzyme, which catalyzes the conjugation of amino acids with IAA (Ludwig-Müller 2011).

Another process in the plant—random root formation (AdRF)—is also caused by IAA and MEL (Wen et al. 2016). Exogenous MEL treatment enhances the transmission of IAA signals inducing AdRF in de-rooted tomato explants by increasing NO production. Evidence of the effect of NO as a downward signal of MEL can be provided by data on the reaction of plants with NO deficiency. Indeed, the creation of conditions for reducing NO accumulation in plants through overexpression of the *GSNOR* gene (GSNO reductase) or the use of an NO absorber [2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, potassium salt; cPTIO] reduces the MEL-induced AdRF process. Under similar conditions, the action of MEL on changes in the level of IAA also occurs. In addition, the use of exogenous MEL affects the expression of IAA transporter genes (*PIN1*, *PIN3,* and *PIN7*) and IAA signal transduction genes (*IAA19* and *IAA24*). The signaling cascades of other plant hormones (Wang et al. 2016; Arnao and Hernández-Ruiz 2018) can also participate in the regulation of growth processes through the NO signaling molecule.

Since indolamines have IAA-like activity, the pair of IAA and KN hormones necessary for the initial stage of in vitro embryogenesis can be exchanged for another pair of MEL/SER and 6-benzyladenine compounds (Ramakrishna et al. 2009).

In response to 1 mM MEL, expression levels of the following genes involved in IAA signaling pathways change: *ACS8* (*Encodes an auxin inducible ACC synthase*), *MJB20*.6 (*Auxin efflux carrier family protein*), *GH3*.3 (*Indole-3-acetic acid-amido synthetase*), *SAUR72* (*Small Auxin up-regulated 72*), *AT4G38840*, *AT4G38860*, *AT1G29500,* and *AT4G00880* (*SAUR-like auxin-responsive protein family*), *WAG1* (*Auxin-induced protein kinase*), *LAX2* (*Like auxin resistant 2*), *AXR3* (*Auxin resistant 3*), *SAUR68* (*Small auxin up-regulated 68*), *AUX1* (*Auxin resistant 1*), and *PIN5* (*Auxin efflux carrier component 8*) genes (Weeda et al. 2014). From identified 14 genes, the first four were up-regulated and ten were down-regulated.

The physiological effect of pre-sowing seed treatment of MEL, IAA, and their predecessor (L-trp) on the quantity and quality of chickpea plants was investigated. The most effective in regulating the parameters of vegetative growth of plants was treatment of MEL in comparison with seed treatment IAA and L-trp (El-Awadi et al. 2017). Treatment with IAA at 20 mg L^{-1} and all MEL treatments (0.25, 0.50 and 1.0 mM) showed a positive effect on increasing seed yields and yield characteristics. MEL treatment at 0.5 mM was most effective because it led to a significant increase in oil content, carbohydrate content, phenolic acid content, and antioxidant activity (as DPPH-radical scavenging capacity) compared to control.

It should be underlined that the actions of melatonin and auxins are transmitted by different signal transduction pathways, which finally converge at some checkpoints (for example, the involvement of cytosolic calcium in both melatonin and IAA signaling) (Hardeland 2015). Similar effects of MEL and IAA are associated with common transducers of their signal, among which the molecule of nitric oxide (NO) is called. With the combined action of IAA and MEL, NO levels are double-controlled to fine-tune responses through IAA transporter proteins (PIN1, PIN3 and PIN7) and signal transduction proteins IAA (IAA19 and IAA24), regulating growth, rhizogenesis, and tropism. IAA-mediated MEL signaling is primarily via the activity of IAA transcription factors (AXR3/IAA17, WRKY, NAC and MYB) and the level of transcripts YUC1, YUC2, YUC5, YUC6, and TAR2 (auxin biosynthesis gene elements).

5 Effects of Light on Indole-3-Acetic Acid and Melatonin Pathways

It is known that light has a different effect on plants. On the one hand, the energy of light is transformed into chemical energy during photosynthesis. On the other hand, light has a regulatory role in photomorphogenesis. Light accelerates seed germination, leaf expansion, flowering, slows stem elongation, triggers circadian rhythms in the plant and other reactions (Ahmad et al. 1995; Sullivan and Deng 2003; Kami et al. 2010). The specific perception of light helps the plant regulate processes such as deethiolation, phototropism, avoiding shade, opening stomata. These morphogenetic light responses are induced by the absorption of light by photoreceptor pigments that interact with other molecules that convert and transform light signals through changes in the expression of a large number of genes, and subsequently metabolic pathways of plants. Phytohormones are one of the systems involved in the conversion of light signals into a physiological function, since the influence of light of different quality on leaf growth in both monocotyledonous and dicotyledonous plants correlates with changes in the levels of endogenous phytohormones (Karnachuk et al. 1990; Golovatskaya 2005).

Plants use 13 highly sensitive photoreceptors for regulatory purposes. Among which there are five red/far red light (RL/FRL) phytochromes (PHYA-E), 7 blue-light (BL) phototropins (PHOT1 and PHOT2), cryptochromes (CRY1 and CRY2) and members of the Zeitlupe ZTL/FKF1/LKP2 (ZEIT_LUPE/FLAVIN_BINDING, KELCH, F_BOX1/LOV KELCH PROTEIN2) family, 1 receiving UV-B radiation UVR8 (Ahmad and Cashmore 1993; Briggs and Olney 2001; Briggs and Christie 2002; Banerjee et al. 2007; Kami et al. 2010; Ito et al. 2012; Tilbrook et al. 2013). CRY1 and CRY2 in addition to BL absorb UV-A radiation. Specific regulatory pigments for the perception of green light (GL) are not yet known (Golovatskaya and Karnachuk 2015); however, this mid-wave portion of the spectrum can inactivate cryptochromes (Fig. 3).

Regulatory photoreceptors allow the plant to trigger responses specific to the corresponding wavelength of light. Estimating the magnitude of the change in

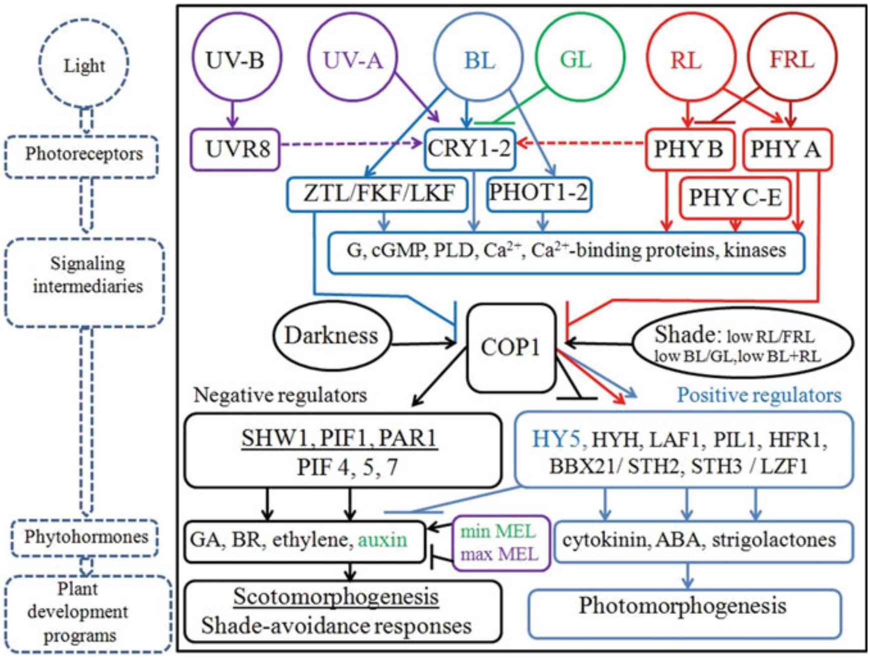

Fig. 3 Light signaling pathway model in the plant. Morphogenetic light responses are induced by the absorption of selective light by photoreceptor pigments that interact with other molecules (signaling intermediaries) that transform light signals through changes in the expression of a large number of genes, and subsequently metabolic pathways of plants. Phytohormones are one of the systems involved in the conversion of light signals into a physiological function (photomorphogenesis, shade avoidance reaction)

physiological processes with respect to the content of sensory pigments in plants, it was suggested that light signal amplification systems exist (Neuhaus et al. 1993; Dubovskaya et al. 2001; Malec et al. 2002; Kabachevskaya et al. 2004). It is believed that G-proteins, cGMP, phospholipase D (PLD), Ca^{2+}, Ca^{2+}-binding proteins (calmodulin, etc.), and kinases transmit the light signal inside the cell. At the tissue level, phytohormones act. The quality of light ambiguously changes the level of phytohormones and, accordingly, the growth of Arabidopsis seedlings in mutant lines according to CRY1 (Karnachuk et al. 2001, 2002, Fig. 3).

Photoreceptors perceive light waves of various lengths and transmit light signals downstream through a series of proteins. Proteins associated with photomorphogenesis are usually classified as positive or negative regulators of photomorphogenesis according to their effects (Fig. 3). Photomorphogenesis positive regulators are transcription factors HY5 (ELONGATED HYPOCOTYL 5), LAF1 (LONG AFAR FAR RED LIGHT 1), PIL1 (PHYTOCHROME-INTERACTING FACTOR 3 (PIF3)-LIKE 1), HFR1 (LYG HYPY-FYPOT), BBX21/STH2 (B-box (BBX) protein 21/SALT TOLERANCE HOMOLOG 2), STH3/LZF1. Negative regulators of photomorphogenesis are transcription factors SHW1 (SHORT HYPOCOTYL IN WHITE LIGHT 1), PIF1 (PHYTOCHROME-INTERACTING FACTOR 1), PAR1 (PHYTOCHROME RAPIDLY REGULATED 1). Associated with the development of light-sensitive proteins post-translationally controlled with activity of E3 ubiquitin ligase COP1 (CONSTITUTIVE PHOTOMORPHOGENESIS PROTEIN 1) (Kim et al. 2017). Functionality and localization of COP1 are regulated by light. In the dark, COP1 is localized in the nucleus, but in the light this protein translocates to the cytoplasm. COP1 is the central switch for the transition from plant growth in the dark (skotomorphogenesis) to growth when exposed to light (photomorphogenesis).

HY5 combines light and hormonal signaling pathways (Gangappa and Botto 2016). It promotes photomorphogenesis through the coordinated regulation of various hormonal signaling pathways. Hormones such as gibberellin (GA), brassinosteroid (BR), ethylene, and auxin contribute to scotomorphogenesis, while cytokinin, abscisic acid (ABA), and strigolactones promote photomorphogenesis.

In studies by Sibout et al. (2006) found that HY5 and its homolog HYH are important general negative regulators of the auxin signaling pathway, modulating the level of IAA at the stage of embryogenesis and seedlings. Light-mediated HY5 activation directly suppresses the auxin hormone pathway by activating the key negative regulators SLR/IAA14 (SOLITARY ROOT/INDOLE ACETIC ACID 14) and AXR2/IAA7 (AUXIN RESISTANT 2/INDOLE ACETIC ACID 7).

Under RL, IAA-binding activity localized on membranes of endoplasmic reticulum (ER) mesocotyl *Zea mays* L., begins to decline to 50–60% of that control in the dark (Walton and Ray 1981). At the same time, the activity of NADH: cytochrome c reductase localized on the ER decreases, and the elongation of mesocotyl segments in response to the action of IAA is inhibited. It is believed that the loss of binding occurs due to a decrease in the number of binding sites, without changing their affinity for IAA (Walton and Ray 1981).

A major role in the light modulation of the development of *A. thaliana* seedlings by suppressing hypocotyl lengthening is played by SOB3 (SUPPRESSOR

PHYTOCHROME B4- # 3) and other members of the AHL transcription factor family (AT-HOOK MOTIF CONTAINING NUCLEAR LOCALIZED). The subordinate targets of SOB3 are YUC8 (YUCCA8), as well as members of the SAUR19 subfamily (SMALL AUXIN UP-REGULATED RNA19) associated with auxin signaling (Favero et al. 2016). SOB3 represses genes associated with auxin signaling to modulate hypocotyl growth.

In dense phytocenoses, competition between plants for light arises, since the quality of light and its intensity change. The leaves of higher plants intensively absorb RL and BL, therefore, under the plants canopy a green-far red shade appears (the level of RL + BL decreases, the ratios RL/FRL, and BL/GL decrease). These changes in light quality trigger a series of reactions commonly known as the shade avoidance syndrome (SAS) (De Wit et al. 2016). While avoiding shade, the plants lengthen the stems, stretch the leaves, and suppress branching (Tao et al. 2008). Multiple changes in body structure due to SAS are associated with activation of the IAA biosynthesis pathway regulated by the SAV3/TAA1 aminotransferase (Tao et al. 2008). SAS formation at low RL + BL levels depends on CRYs photoreceptors and transcription factors PIF4 and PIF5 (De Wit et al. 2016). CRY2 is a sensor of the low blue/green light ratio (Sellaro et al. 2010). It binds to PIF4 and PIF5, regulating shade-induced genes expression.

Phytochrome inactivation induced by a low RL/FRL ratio leads to the accumulation and activation of transcription factors PIFs (PHYTOCHROME-INTERACTING FACTORs) 4, 5, and 7 and subsequent expression of their growth-mediated targets (De Wit et al. 2016). Lorrain et al. (2007) showed that PIF4 and PIF5 act in the early stages of PHYs signaling pathways to stimulate the shadow avoidance response. PIF4 and PIF5 accumulate to high levels in the dark, selectively decrease in response to RL, and remain at high levels when simulated by shadow. It is believed that proteasome-dependent degradation of these transcription factors is preceded by phosphorylation and interaction with activated light PHYB. Another basic helix–loop–helix (bHLH) transcription factor PIF7 (phytochrome-interacting factor 7) interacting with PHYB, accumulates in its dephosphorylated form in the shade, which allows it to bind auxin biosynthesis genes and increase their expression (Li et al. 2012). A new auxin synthesized via an adjustable PIF7 pathway is necessary for growth-induced shading, which directly relates growth to a quick response to the perception of a light quality signal. Shade-accumulating PHYA can release AUX/IAA (auxin/indole-3-acetic acid) auxin signal suppressors from auxin receptor SCFTIR1 to attenuate auxin signaling and negatively control the shading response (Yang et al. 2018). This is confirmed by the studies of Colón-Carmona et al. (2000), showing that recombinant AUX/IAA proteins from Arabidopsis and *Pisum sativum* interact in vitro with recombinant PHYA from *A. sativa*, and recombinant SHY2/IAA3, AXR3/IAA17, IAA1, IAA9, and Ps-IAA4 are phosphorylated by PHYA recombinant in vitro. From the data obtained, it follows that the PHY-dependent phosphorylation of proteins AUX/IAA and others serve as a molecular mechanism that integrates the transmission of auxin and light signals in the process of plant development.

The integration of phytochrome and cryptochrome signals determines the growth of plants under conditions of competition for light, and is described by the model proposed by De Wit et al. (2016). At low RL/FRL ratios, PHYBs are inactivated and localized in the cytosol (Sakamoto and Nagatani 1996). This contributes to the accumulation of PIF in the nucleus and subsequent transcription of both positive and negative shadow avoidance regulators, such as HFR1, which forms heterodimers with PIF that do not bind to DNA. At low BL + RL, PIF can accumulate, since CRY inactivation removes direct inhibition of PIF-mediated transcription. As a result, the combination of low RL/FRL and BL + RL provides transcription of PIFs, increasing the number of PIFs and their activity. In addition, the combined effect of low RL/FRL and low BL + RL leads to a decrease in the accumulation of negative shading avoidance regulators such as HFR1, many of which are targets for E3 ubiquitin ligase COP1. Low RL/FRL and low BL + RL induce nuclear translocation of COP1 (Pacín et al. 2013), while inactivation of CRY and PHY weakens their repression in combination with COP1/SPA. This enhances the degradation of COP1 targets.

Light is a key environmental signal that inhibits the extension of hypocotyl cells through the blue and red/far red light photoreceptors, including CRY and PHY-mediated signaling pathways in Arabidopsis. On the contrary, the main endogenous phytohormone auxin promotes lengthening of the hypocotyl through the degradation of AUX/IAA proteins mediated by TIR1/AFB auxin receptors. Light inhibits auxin signaling by stabilizing AUX/IAA via blue and red light-dependent CRY1 and PHYB interactions with AUX/IAA, respectively. Blue-light-induced interactions of CRY1 with AUX/IAA inhibit the association of TIR1 with AUX/IAA, leading to repression of auxin-induced degradation of these proteins.

Auxin hormone stimulates growth by affecting gene expression. Studying *A. thaliana* mutants with ARFs deficiency (AUXIN RESPONSE FACTORS), Reed et al. (2018) identified three ARF proteins that are necessary for auxin-sensitive extension of the hypocotyl. Plants deficient in these factors reduce the response to light enriched in red light, increasing auxin levels. AUX/IAA proteins encoded by IAA genes interact with ARF proteins to suppress auxin response.

Proposed by Xu et al. (2018) model illustrates light inhibition of auxin signaling via CRY1 and PHYB. In the dark, CRY1 localized in the nucleus is inactive, and PHYB is in the form of Pr and localized in the cytoplasm. They are unable to regulate auxin signaling because they cannot interact with AUX/IAA. In the light, CRY1 is activated, and PHYB undergoes a conformational change in Pr into Pfr and enters the nucleus, and they can compete with TIR1 for interaction with AUX/IAA to inhibit their degradation, thereby inhibiting ARF activity and auxin signaling. Therefore, antagonistic regulation of the stability of AUX/IAA protein using photoreceptors and auxin receptors allows plants to balance light and auxin signals to optimize their growth.

The content and activity of IAA were studied in the leaves of *Rhaponticum carthamoides* Willd. plants. Iljin., *Lychnis chalcedonica* L. and *Avena sativa* L. grown under monochromatic light of various spectral composition (RL, BL, and GL) Karnachuk and Golovatskaya (1998). In leaves of *A. sativa* under RL, the level of free IAA decreases, while the level of bound forms of IAA remains high. Under BL, a decrease in the activity of free IAA is also observed, while under GL this activity remains at the control level in the dark (Karnachuk and Golovatskaya 1998).

The quantitative determination of free IAA in oat leaves irradiated with RL, BL, and GL for 30 min revealed the absence of a free IAA form in leaves irradiated with RL and its high amounts in leaves irradiated with BL and GL. Studies have also been conducted on the dependence of the hormonal status of leaves of different ages *Rh. carthamoides* and *L. chalcedonica* from selective light during prolonged cultivation. Under BL, the activity of free IAA was high in young leaves of *L. chalcedonica* and mature leaves of *Rh. carthamoides*, i.e., BL, created favorable conditions for enhancing the synthesis of IAA or its release from bound forms (Karnachuk and Golovatskaya 1998). RL reduced the level of free IAA. The data obtained allow us to assume the participation of different photoreceptors and the functioning of different signaling pathways in selective light.

When studying the role of MEL in the germination of negatively photoblastic and heat-sensitive seeds of *Phacelia tanacetifolia* Benth. MEL has been shown to alter the inhibitory effect of light and heat on seed germination (Tiryaki and Keles 2012).

A study of the influence of the spectral composition of light (RL, BL, and white light —WL, and UV-B radiation 280–315 nm) on the MEL biosynthesis was performed on *Glycyrrhiza uralensis* plants (Afreen et al. 2006). The maximum accumulation of MEL in root tissues relative to the tissues of seeds, leaves, and stems was shown. At the same time, a different sensitivity of the process to the quality of light was noted. After 3 months of growth under selective light, MEL concentrations were highest in plants exposed to RL and decreased depending on the wavelength of the light spectrum in the following order: RL−BL−WL. MEL concentrations increased with plant age by 4, 5, and 3 times, respectively, on RL, BL, and WL. The highest concentration of MEL in the root was observed in plants exposed to UV-B radiation, which could be due to its protective role against oxidative damage caused by exposure to ultraviolet radiation.

Our studies show that the direction and magnitude of the effect of 1 μM MEL on the growth of *A. thaliana* seedlings depend on the lighting conditions and IAA signal transmission (Golovatskaya et al. 2017). In the dark, MEL inhibits hypocotyl elongation in *axr1-3* mutants, while under WL it increases the size of wild-type cotyledons. Under WL, MEL reduces the root size of the wild and mutant lines, but increases the root size under RL. The efficiency of MEL in the regulation of seedling growth in *axr1-3* mutant was higher than in wild-type Col. Comparison of the effects of MEL and IAA shows that exogenous MEL under WL has a stimulating effect on the growth of *A. thaliana* seedlings at lower concentrations (0.1 pM) than IAA (1 nM). MEL restores axial organs growth of the *axr1-3* mutant and the content of photosynthetic pigments to wild-type levels, probably by compensating for impaired IAA signal transmission. A study of the regulation of growth of *T. aestivum* coleoptile segments showed that in the absence of IAA MEL does not affect their elongation, while when combined with IAA MEL, it enhances the effectiveness of the phytohormone in regulating coleoptile stretching. It follows that the mechanism of action of MEL is not associated with the activation of the H^+-ATPase of the coleoptile plasma membrane, but mediates the effectiveness of the action of IAA (see Sect. 3).

The ubiquitin proteasome system has been proposed as a possible mechanism of the multiple actions of MEL in the light (Sanchez-Barcelo et al. 2016). The hypothesis is based on the general functional aspects of MEL and COP1 and COP9, including their dependence on light, metabolic regulation, and tumor growth control.

Light is a powerful incentive for regulating the biosynthesis of MEL in the pineal gland and the circadian system in humans. It has now been shown that not only very bright light stimuli (>2500 lx) can suppress MEL night secretion and cause other circadian reactions, but lower illumination values (<200 lx) can sharply suppress these processes (Brainard et al. 1997). The circadian rhythms in humans, measured by the rhythm of the functioning of the pineal gland and the plasma MEL content, also depend on the wavelength of light. Exposure to monochromatic light with a wavelength of 460 nm causes a twofold delay in the circadian phase than with a wavelength of 555 nm of the same photon density. At the same time, a twofold decrease in the content of MEL was noted. The same spectral dependence was observed for the circadian pacemaker of people (Lockley et al. 2003). The effect depends on the duration of exposure to light, as well as on the duration of pre-illumination with dim light (Jasser et al. 2006). Monochromatic light in the range from 446 to 477 nm provides the maximum suppression of MEL accumulation in humans (Brainard et al. 2001). An analysis of the above data indicates that a CRY-dependent system is required to regulate the MEL level, which is similar to plant photoregulation, but differs from the sensitivity of visual photoreceptors (rhodopsins), which have a maximum absorption in the green region of the spectrum.

The regulation of the MEL level by the human pineal gland occurs in the same way in response to polarized and non-polarized light with intensity in the range from 20 to 3200 lx through the visual receptor (Brainard et al. 2000). Similar MEL-dependent plant responses to light have not been investigated.

New data have appeared that testify to the strong influence of circadian rhythms on plant metabolic pathways producing IAA and SER/MEL (Beilby 2016). The dynamics of the MEL content in apple leaves shows two peaks (14:30 and 5:30), which correspond to an increase in the level of malondialdehyde (Zuo et al. 2014), and the daily maximum in the 24-h light/dark cycle is of high light intensity. Such dynamics indicate the dependence of the biosynthesis of MEL in leaves on oxidative stress. Exogenous MEL is also able to influence the early stages of photoperiodic induction of flowering and/or flower development in the *Chenopodium rubrum* short-day plant (Kolar et al. 2003). The data on the optimal daily sensitivity of plants to MEL show that the use of MEL was effective only before turning off the light or during the first half of the dark period.

El-Esawi et al. (2017), show that H_20_2 and ROS accumulate in the plant cell nucleus after cryptochrome activation in response to blue light. It is also known that MEL removes ROS, and it can be suggested that there is a possibility of interaction of MEL signaling pathway with light signaling pathway at the ROS level.

6 Conclusion

The analysis of the available information shows that MEL affects the growth processes of plants through a change in the IAA signal transmission path. The effects of different concentrations of MEL indicate about dose-dependent signal transmission of MEL. It should be underlined that relatively low concentrations of melatonin are required for auxin-like actions. It can be suggested that the low content MEL is signal molecule, whereas the high content MEL is antioxidant. It is well known than the physiological functions of MEL in a plant are influenced by the intensity and quality of light mediated by the functioning of the corresponding photoreceptors.

Currently, however, the mechanisms of action of MEL and the light-dependent interaction of MEL-IAA in the plant organism have not been studied sufficiently. It remains to be explored.

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Prospective Role of Melatonin in Signaling and Alleviation of Stress in Plants

Piyush Mathur and Subhendu Pramanik

Abstract Melatonin has long been known for its role in animals, but in the past few years extensive studies have been carried out to delineate its role in plants. Presently, melatonin is in the list of most studied bioactive chemical in plant systems. It is present in roots, stems, leaves, and in fruits also. Melatonin has profound effect in number of physiological and biochemical processes in plants, such as growth, photosynthesis, germination, rooting, fruit ripening, circadian rhythms, photoperiodism, senescence, fruit ripening, and many more. Moreover, melatonin is exploited well for its application in alleviation of stress in plants. Vast studies have been put forward to exhibit the dynamic role of melatonin in mitigation of salinity, drought, cold, heavy metal, and pathogen stresses. Exogenous melatonin application led to increased expression of genes involved in endogenous melatonin biosynthesis pathway. One of the key functions of melatonin under stress is to increase production of enzymatic antioxidants and non-enzymatic antioxidants that will shield the plants from oxidative stress in lieu of increased accumulation of ROS and RNS. At the same time, it increases photosynthetic rate, protects chloroplast damage, maintains redox balance, and so on. It has been observed that melatonin interacts with a number of other hormones and signaling molecules (auxin, ethylene, ABA, JA, SA, NO) and transcription factors (NAC, WRKY, MAPK, etc.) during stress and activates signal transduction of events involved in expression of stress responsive genes. This chapter will provide valuable information on melatonin-mediated stress signaling and deduce its role in mitigation of abiotic and biotic stresses in plants.

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Abbreviations

ABA	abscisic acid
APX	ascorbate peroxidase
CAT	catalase
Cd	cadmium
GR	glutathione reductase
GPX	glutathione peroxidase
GST	glutathione-S-transferase
HSPs	heat shock proteins
HSFs	heat shock factors
JA	jasmonic acid
MAPK	mitogen activated protein kinases
NO	nitric oxide
POD	peroxidase
ROS	reactive oxygen species
RNS	reactive nitrogen species
SA	salicylic acid
SOD	superoxide dismutase

1 Introduction

Melatonin (N-acetyl, 5-methoxytryptamine) and its role in stress amelioration has been investigated, both in plant and animal systems; however, it was first discovered from mammalian bovine pineal gland in 1958 (Lerner et al. 1958). In animals, it is found to be involved in number of physiological processes, such as mood control, inducing sleep, and seasonal reproduction, as well as regulates day/night cycles (Pandi-Perumal et al. 2006; Hardeland et al. 2011). On the other hand, the melatonin was first discovered in 1993 in plant *Pharbitis nil*, commonly known as Japanese morning glory (Kolář and Macháčková 2005). Since then, it has been extensively studied in whole plant kingdom and its presence is much observed in every part of plant, starting from root, stem, leaves, and fruits (Debnath et al. 2019; Zhao et al. 2019a). However, the concentration of melatonin varies within different parts of plants and also in fruits of different species and cultivars. Enormous studies have reported on the role of melatonin in plant growth, development, and biochemistry (Tan et al. 2012; Erland and Saxena 2018; Sharif et al. 2018; Zhao et al. 2019a). Melatonin aids in seed germination, induces rooting, stimulates growth, adjusts tropic movements, maintains circadian rhythms, and is involved in fruit ripening and senescence (Arnao and Hernández-Ruiz 2006, 2015; Debnath et al. 2019) (Fig. 1). It is reported for its own antioxidant properties. A number of melatonin metabolites, such as 3-orthohydoxymelatonin, 2-hydoxymelatonin, AFMK, act as potential antioxidants (Debnath et al. 2019). It can effectively reduce the oxidative properties

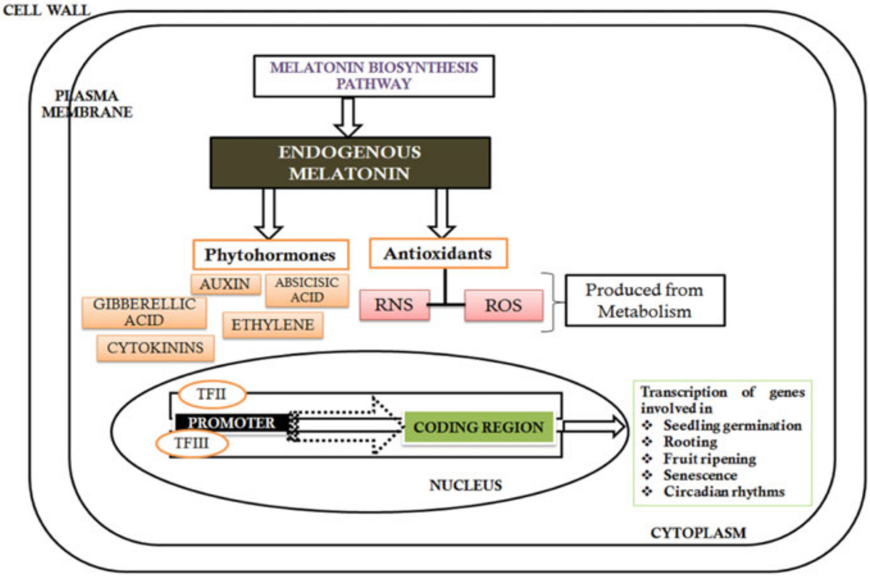

Fig. 1 Melatonin in regulating normal physiological processes in plants. Melatonin is commonly being synthesized inside plant cell and interacts with other phytohormones such as auxin, cytokinins, gibberellins, ethylene, abscisic acid and regulates large number of physiological and biological processes by inducing transcription of respective genes. At the same time, melatonin keeps a balance between levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced from day to day metabolic pathways and prevents their accumulation

of reactive oxygen species (ROS) and reactive nitrogen molecules (RNS) (Arnao and Hernandez-Ruiz 2019a) (Fig. 1). Melatonin is also found invariably in large number of medicinal herbs and has a high value in nutritional crops (Salehi et al. 2019). It is well known to interact with other phytohormones, such as auxins, cytokinins, gibberellins, abscisic acid (ABA), ethylene (ET) to bring about large number of physiological and biochemical responses of plants such as growth, fruit ripening, and senescence (Arnao and Hernández-Ruiz 2018; Kanwar et al. 2018). Extensive literature is now available that exemplifies the crucial role played by melatonin in mitigation of a number of abiotic and biotic stresses (Arnao and Hernández-Ruiz 2015; Hardeland 2016; Fan et al. 2018; Kanwar et al. 2018; Debnath et al. 2019; Kul et al. 2019). The present chapter will focus on the role of melatonin in signaling, as well as its efficacy in alleviating both abiotic and biotic stress. In addition, the chapter will also provide insights into the interaction of melatonin with other signaling molecules that play an influential role in melatonin-mediated stress mitigation.

2 Biosynthesis of Melatonin in Plants

Melatonin is a biologically active compound and its biosynthesis pathway is now well characterized both in plant and animal systems (Arnao and Hernández-Ruiz 2006, 2015; Nawaz et al. 2016; Erland et al. 2018; Debnath et al. 2019) although the differentiation between melatonin biosynthetic pathway in plants and animals was proposed by Murch et al. (2000). Tryptophan, a very important aromatic amino acid, acts as a precursor for the synthesis of melatonin and various other important biochemically active compound structurally similar to melatonin like auxin, serotonin, and so on (Murch et al. 2000). Melatonin is known to be synthesized by amalgamation of various pathways but the central step in each of the pathway is the conversion of tryptophan to tryptamine via action of enzyme tryptophan decarboxylase (TDC). This tryptamine is straightway converted into 5-hydroxy tryptamine (referred to as serotonin) by the functioning of enzyme tryptophan-5-hydroxylase (T5H) and it is the rate limiting enzyme in plants that controls the flux of this pathway. Serotonin is another major indole that shares the same biosynthetic pathway as of melatonin and also acts as a key molecule for assembly of melatonin. This serotonin undergoes *N*-acetylation and leads to production of *N*-acetylserotonin following the action of enzyme serotonin *N*-acetyl transferase (SNAT). Concurrently, this *N*-acetylserotonin gets converted to melatonin by the enzyme acetylserotonin methyl transferase (ASMT), which is also being referred to as hydroxyindole-O-methyltransferase (HOMT) (Fig. 2). Serotonin also gets converted into melatonin by various other procedures, like serotonin gets converted into 5-methoxytryptamine by the activity of enzyme HOMT and further by the action of SNAT on 5-methoxytryptamine it is converted to melatonin (Fig. 2). Melatonin can also be formed from *N*-acetyltryptamine which in turn gets converted to N-acetylserotonin. Studies have revealed that all the enzymes of these pathways have been well studied in rice and *Arabidopsis* (Erland and Saxena 2018).

3 Melatonin and Stress Signaling

Melatonin is now being considered as a major signaling molecule in plants during stress as the levels of endogenous melatonin highly fluctuate between stressed and unstressed condition. Studies have demonstrated that due to upsurge in the concentration of endogenous levels of melatonin under stress conditions will help in timely activation of signal transduction events to combat the different stresses (Shi et al. 2016; Erland et al. 2018) (Fig. 3). Wei et al. (2018) reported the presence of first melatonin receptor in *Arabidopsis thaliana* plants, that is, CAND2/PMTR1 and it regulates stomatal closure by interacting with Ca^+ ions. Therefore, it will be judicious to call melatonin as a phytohormone that comes into action by binding to its surface receptors and regulates number of physiological and biochemical processes in plants (Arnao and Hernández-Ruiz 2019a). Melatonin unregulated the expression

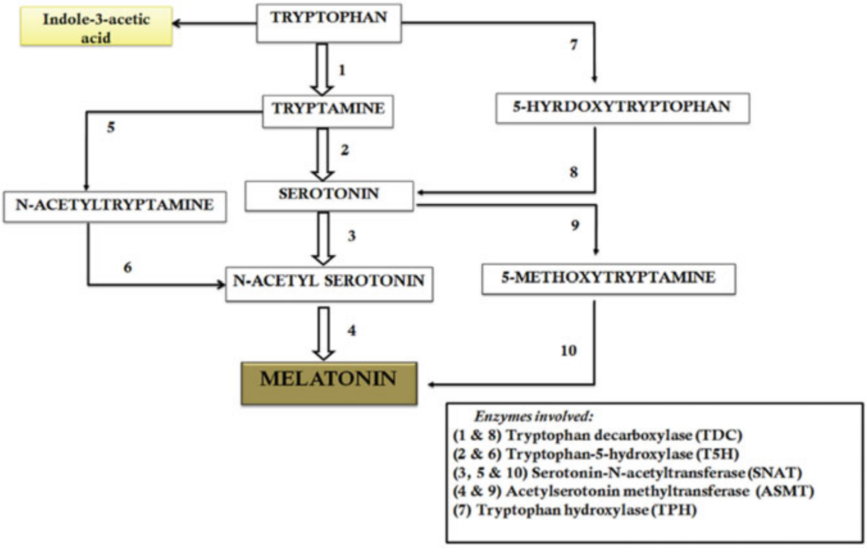

Fig. 2 Flowchart for melatonin biosynthesis pathway in plants. Melatonin is synthesized from aromatic amino acid tryptophan that initially forms serotonin and through series of pathways it gets converted into melatonin. Tryptophan is also a major precursor for synthesis of important phytohormones i.e. indole-3-acetic acid (IAA) commonly referred to as auxin. Figure also lists the name of enzymes that participate in melatonin biosynthesis pathway

of genes involved in the activation of receptors/kinases/Ca^+ ions that help in stress signal perception in plants during any environmental stress (Weeda et al. 2014). Once signal get perceived, melatonin interacts with transcription factors, such as C-repeat binding factors (CBF), DREB, NAC, MYB, ethylene responsive transcription factors (ERF), and WRKY under different abiotic and biotic stresses (Shi et al. 2015b; Fan et al. 2018) (Fig. 3). This interaction acts downstream in melatonin-mediated signaling pathway and will bring out expression of stress-responsive genes that will benefit the plant in overcoming stress. Another important signaling molecule that plays a vital role in melatonin-induced signaling is mitogen-activated protein kinases (MAPKs). MAPK cascade involves different MAPKs such as MAPKKK (MAPK3), MAPKK (MAPK2), MAPK4, and MAPK6, and is very well known for its significance in both abiotic and biotic stress-induced signaling in plants (Cristina et al. 2010) (Fig. 3). Melatonin led to activation of different MAPKs in response to pathogen and elevates plant innate defense in *Arabidopsis* and tobacco plants (Lee and Back 2016). Recently, Zhang et al. (2020) showed the stimulation of MAPK3, MAPK4, MAPK6 by exogenous melatonin and subsequent alleviation of salt stress in cucumber. During stress, apart from phytohormones (auxin, ethylene, abscisic acid), melatonin also interacts with signaling molecules in plants such as nitric oxide (NO), salicylic acid (SA), and jasmonic acid (JA), and this interaction is responsible for the production of various defense-related compounds that assist the plant in overcoming the harmful effects of stress (Fig. 3). A detailed transcriptomic investigation

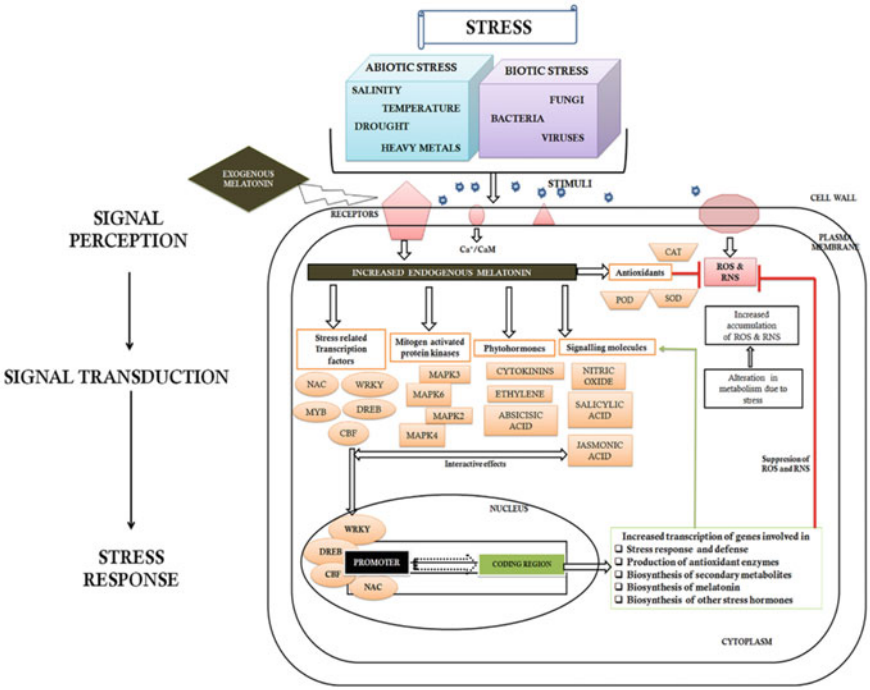

Fig. 3 Signal transduction pathway of melatonin in activating cascade of events for alleviation of stress in plants. Both abiotic and biotic stress sends some stimuli that are perceived by receptors present on plant cell wall surface. Concentration of endogenous melatonin gets significantly increased under the effect of these stresses as well through the exogenous application of melatonin. A number of stress responsive genes are up regulated due to interaction of melatonin with various transcription factors (such as DREB, WRKY, CBF etc.) and mitogen activated protein kinases (MAPKs). Melatonin also interacts with salicylic acid (SA), jasmonic acid (JA), nitric oxide (NO) to escalate the transcript expression of genes that are functional only during stress conditions as observed from literature. Due to altered metabolism in plants during stress increased accumulation of both reactive oxygen species (ROS) and reactive nitrogen species (RNS) has been observed. These increased ROS and RNS also acts as stress signals for plants and melatonin considerably balance the concentration of these toxic species by up regulating transcript expression of genes for production of various enzymatic and non-enzymatic antioxidants

was carried out by Weeda et al. (2014) in Arabidopsis and it was observed that melatonin considerably upregulated the expression of genes responsible for increasing production of ABA, SA, JA, and ET and their associated downstream signaling and stress-responsive genes. Molecular studies carried out on *Arabidopsis thaliana* have shown that melatonin significantly affects expression of genes involved in chief pathways of amino acid and carbon metabolism (Wan et al. 2018). Melatonin is also referred to as bio-stimulator due to their wide effects in inducing expression of genes for various physiological and biochemical pathways under stress (Arnao and Hernández-Ruiz 2019a).

4 Melatonin and Reactive Oxygen and Nitrogen Species

Plants are being exposed to number of stresses that has considerably affected their growth and subsequently their interaction with pathogens. Both abiotic and biotic stress also led to increased production as well as accumulation of ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyradical (OH^-), singlet oxygen ($^1O_2$) (Asada 2006; Tuteja and Singh Gill 2013), and RNS such as nitroxyl anion (NO^-), nitrosonium cation (NO^+), nitrous acid (HNO_2), and so on, although the exact source of generation of ROS and RNS is quite different (Del Río 2015). Both these ROS and RNS are highly toxic for the cell and cause oxidative as well as nitro-oxidative stress that is highly destructive for the plants. The magnitude of damage directly depends on the redox balance of these ROS/RNS molecules. These ROS and RNS can hamper membrane structure through lipid peroxidation, degrade protein structure via its oxidation, alter protein structure, modify enzyme activity, and can cause damage to nucleic acid (DNA or RNA) (Del Río 2015).

Accumulation of both ROS and RNS in stressed plants acts as a signal to increase biosynthesis of melatonin via increased expression of genes of enzymes involved in melatonin synthesis pathway (Arnao and Hernández-Ruiz 2019a). Nevertheless, detailed literature is available that unveiled application of exogenous melatonin under stress conditions and also promote similar increase in endogenous levels of melatonin and successive control of reactive species (Hernández-Ruiz and Arnao 2018; Debnath et al. 2019). Melatonin tends to control the levels of these toxic ROS and RNS, thereby defending plants from the deleterious effects of these reactive species and prevent from oxidative damage (Kaur et al. 2015; Zhao et al. 2019a). Melatonin balances the levels of ROS and RNS by converting these toxic species into non-toxic form by production of number of antioxidant enzymes such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) (Kolář and Macháčková 2005; Khan et al. 2020) (Fig. 3). Concurrently, melatonin also stimulates the synthesis of non-enzymatic antioxidants compounds such as ascorbate, polyphenols, tocopherols, glutathione, and carotenoids (Khan et al. 2020). All of these antioxidant molecules assist in scavenging these toxic species and convert them into non-toxic forms and maintain their ideal concentration for its beneficial role in growth and development.

5 Melatonin and Its Association with Various Signaling Molecules During Stress

5.1 *Abscisic Acid (ABA)*

A study by Yang et al. (2020b) showed that both melatonin and ABA when exogenously sprayed onto grape showed increase in concentration of flavonoids demonstrating that both of them shared in-between some relationship. Melatonin-treated

Lolium perenne under the influence of heat stress decreased expression of genes *(LpZEP, Lp NCED1)* for ABA biosynthesis and protects the plant from ABA-induced leaf senescence under heat stress (Zhang et al. 2017a). Melatonin pre-treatment to two *Malus* species under drought stress triggers the expression of ABA catabolism genes (*MdCYP707A1* and *MdCYP707A2)* as well as decrease the expression of ABA biosynthesis gene (*MdNCED3*), thereby decreasing the ABA levels and preventing the plant from stress (Li et al. 2015). Similar results were observed by Tan et al. (2019b) that exogenous melatonin treatment to Chinese flowering cabbage downregulates expression of transcription factors and downstream genes for ABA synthesis which in turn prevents the onset of senescence. Endogenous levels of melatonin and ABA showed significant increase in response to exogenous treatment of melatonin in drought primed barley plants and augment them with better tolerance to cold stress by maintaining water status, improve their growth, higher photosynthetic efficiency, and increased activity of antioxidant enzymes (Li et al. 2016b). Recently, Yoon et al. (2019) indicated that roots of maize seedlings when dipped in melatonin solution led to its translocation from roots to leaves by transpiration flow mechanism where it gets accumulated. However, when roots were dipped with both melatonin and ABA, no accumulation of melatonin in leaves was observed due to ABA-induced stomatal closure and low transpiration pull. Melatonin and ABA synchronize their levels during seed germination as observed in case of cucumber seedlings grown under salt stress. There was decreased expression of genes involved in ABA biosynthesis while increase in ABA catabolism genes (Zhang et al. 2014).

Similar observations were observed when low exogenous concentration of melatonin (20 μM) has a promoted seed germination of cotton and enhances the levels of gibberellins while concentration of ABA showed decrease (Xiao et al. 2019). Interactive effects of melatonin and ABA were seen in watermelon plants where melatonin led to increased accumulation of ABA under drought stress and ABA tends to have reformed the surface waxes so as to maintain water loss from plants (Li et al. 2020). ABA is actively involved in melatonin-mediated chilling tolerance to cucumber seedlings (Zhao et al. 2017). Conversely, it has been reported that in *Elymus nutans*, mitigation of cold stress by melatonin is persuaded by ABA dependent and by ABA independent pathway (Fu et al. 2017).

5.2 *Ethylene*

Ethylene is one of the important plant hormones involved in number of physiological processes in plants such as fruit ripening and nutritional quality of fruits, and also play a significantly role in plant defense against pathogens. Melatonin and its interaction with other hormones in fruit ripening process have also been observed (Tijero et al. 2019). Application of melatonin is well known to enhance shelf life of fruits and increase their storage capacity as well maintain their nutritional quality via controlling ET concentration in plants (Arnao and Hernández-Ruiz 2018, 2020). Melatonin-treated tomato fruits showed enhanced concentration of ET and they attain their climacteric peak earlier than non-melatonin-treated fruits. Concurrently, melatonin affects expression of ET biosynthesis gene as well as their receptor genes (NR and ETR4) and the various transcription elements EIL1, EIL3, and ERF2 in tomato fruit (Sun et al. 2015). In a different set of experiments, melatonin was being used in order to delay senescence of pear fruits (*Pyrus communis*) and showed downregulation of genes, that is, *PcACC* synthase (1-aminocyclopropane-1-carboxylate synthase) and the *PcACO* oxidase responsible for ET production in pear fruits (Liu et al. 2019). Similar results were observed by Zhai et al. (2018) that melatonin led to decreased ET production in *P. communis* fruits and delays their ripening for commercial use. There are reports stating that during fruit ripening, along with melatonin and ET, there is interplay of nitric oxide also (Mukherjee 2019). It has been observed that salt-stressed plants treated with melatonin showed increased production of the ET via better transcriptional activity of *MYB108A,* a transcriptional factor bound to promoter of *ACS1* gene responsible for ET production (Xu et al. 2019). Melatonin provides protection to *Medicago* plants from detrimental effects of waterlogging by decreasing ET biosynthesis genes, that is, ACS and ERF, a transcription factor (Zhang et al. 2019). Melatonin-supplemented plants showed increased ET concentration and intervened defense gene expression (Moustafa-Farag et al. 2020). It was observed that pre-treated melatonin plants challenged with bacterial pathogen improve plant resistance via ET-mediated defense pathway. This was demonstrated by the experiments of Lee et al. (2014), where *Arabidopsis* plants challenged with *Pseudomonas syringae* DC3000 in the presence of melatonin showed increased transcript expression of genes, that is, 1-aminocyclopropane-1-carboxylate synthase 6 (ACS6) involved in ET biosynthesis pathway and further induce genes for defense against the pathogen.

5.3 Salicylic Acid (SA)

Salicylic acid is a key molecule that activates plant innate immune defense responses and increases the transcript expression of genes involved in plant's resistance against pathogens. It is produced from chorismic acid that is produced from shikimate pathway and this chorismic acid on the other side directs the production of melatonin. Chorismic acid is responsible for the synthesis of crucial aromatic amino acids, that is, tryptophan, phenylalanine, and tyrosine. Tryptophan is a precursor for the synthesis of melatonin while phenylalanine led to synthesis of SA in plants. A number of articles have shown the role of melatonin and SA in alleviation of plants from large number of abiotic stresses (Hernández-Ruiz and Arnao 2018); however, very few studies have been able to delineate their relationship in biotic stresses. Exogenous application of melatonin to *Arabidopsis* led to increase in transcript expression of *isochorismate synthase 1* (ICS1) gene that facilitates increased production of SA and defense response alleviates against bacterial pathogen (Lee et al. 2014). In a similar type of study in *Arabidopsis*, *nahG* mutants that are unable to synthesize SA on their own if sprayed with melatonin too, no induction of defense response can be observed (Lee et al. 2015), indicating the involvement of SA in melatonin-mediated defense.

5.4 Nitric Oxide (NO)

Nitric oxide is considered as a major signaling molecule in plant systems. It is gaseous, highly diffusible, and can cross cell membranes very easily, and thus sometimes also acts as a secondary messenger during stress in plants. It has been involved in various physiological processes in plants and also in disease resistance (Delledonne et al. 1998). Melatonin and NO works in a coordinative manner and there is also a crosstalk between them for performing a number of functional roles in plant systems (Mukherjee 2019; Zhu et al. 2019). Melatonin led to adventitious root formation in seedlings of *Solanum lycopersicon* via increased transcript expression of genes (*NO*) involved in NO biosynthesis. Melatonin-treated tomato seedlings that have high NO accumulation showed better response to auxin response factors and auxin signaling that promoted adventitious root formation (Wen et al. 2016). Earlier it has been reported by Noda et al. (1999) that melatonin and some of its precursors play a significant role in scavenging of reactive NO species. At present, the studies demonstrated that melatonin through its interaction with NO controlled the damaging effects of RNS and ROS (Arora and Bhatla 2017) in salt-stressed sunflower seedlings.

Nitric oxide is also involved in melatonin-enhanced salt tolerance to *Brassica* seedlings by increased activity of antioxidant enzymes, and balances redox homeostasis and Na^+/K^+ ratios (Zhao et al. 2018). Melatonin and NO regulate the content of glutathione, reduce glutathione (GSH), oxidized glutathione (GSSG) and GSH/GSSG ratio along with enzyme GR, which in turn guard the sunflower seedlings from salt stress (Kaur and Bhatla 2016). Iron (Fe) deficiency of *Arabidopsis* plant has been overcome by melatonin through increased accumulation of polyamine (PA) and further increase in concentration of NO that help in upregulation of expression of genes involved in Fe acquisition (Zhou et al. 2016). Alleviation from cadmium (Cd) stress in wheat plants through increased production of antioxidant enzymes has been facilitated by NO. Positive effects of melatonin were reversed when NO inhibitors were applied to the plants as there was increased ROS production and oxidative stress (Kaya et al. 2019).

6 Melatonin and Alleviation of Abiotic Stress

6.1 Drought Stress

Jaleel et al. (2009) reviewed the effects of drought stress on morphological, physiological, and biochemical characteristics of plants that led to decreased yield and productivity. Melatonin mitigates drought stress in plants by bringing about number of physiological and biochemical changes (Sharma and Zheng 2019; Sadak et al. 2020). Exogenously sprayed melatonin also exhibits positive responses of plants in overcoming drought stress. Foliar spray of melatonin (100 μM) ameliorates *Dracocephalum moldavica* plants from drought stress via increased production of antioxidant enzymes (Kabiri et al. 2018). Similar concentration of melatonin, that is, 100 μM curbed the deleterious effects of drought in seedlings of kiwi fruit, promoted improved root morphology, lower lipid peroxidation, and increase in transcript expression of genes involved in carbon dioxide fixation (Liang et al. 2019). Melatonin effectively mitigated negative effects of drought stress in maize seedlings and showed better rate of photosynthesis, increased activity of both enzymatic and non-enzymatic oxidants, optimal water balance, decreased oxidative stress in plants and so on (Ye et al. 2016). Another study on maize reported that melatonin protects the plants from drought stress by employing protection to PSII and maintains F_V/F_m ratio (Fleta-Soriano et al. 2017). The study also stated positive correlation between drought stress and endogenous melatonin levels in maize plants. Huang et al. (2019) stated that melatonin application improves the PSII machinery of drought-stressed maize plants by increasing the activity of antioxidant enzymes, preventing membrane damage, and managing positive turgor pressure. Melatonin persuades activation of genes involved in MAPKs and transcription factors in seedlings of oat under drought stress (Gao et al. 2018). Recently, Cao et al. (2019) also revealed that melatonin alleviates soybean seedlings from drought-induced stress and led to improved photosynthetic rate and accelerates activities of antioxidant enzymes that prevent the plants from oxidative damage. Priming of seeds with melatonin cope up the rapeseed seedlings from drought-induced effects by increasing enzymatic and non-enzymatic antioxidants, osmoprotectants (Khan et al. 2019). Melatonin also preserved stomatal and chloroplast structure that were distorted due to drought stress. Under drought stress, alfaalfa (*Medicago sativa*) plants when supplied with 10 μM melatonin at rhizospheric region defend the plants from oxidative stress that arose from both NO and ROS (Antoniou et al. 2017). Melatonin significantly defends *Agrostis stolonifera* (creeping bent grass) from drought-mediated harmful effects like decreased growth, leaf senescence, membrane damage, and so on through increased chlorophyll production, higher activity of antioxidant enzymes, increase in expression of genes involved both in chlorophyll and cytokinin production (Ma et al. 2018).

6.2 Salinity Stress

A number of studies have shown the significant role played by melatonin in amelioration of salinity stress in plants. Exogenous melatonin was found to increase dry and fresh weight of salt-stressed *Malus hupehensis* seedlings, reduced chlorophyll degradation, and maintained low cellular Na^+, thus decreased cytotoxicity (Li et al. 2012). The studies also showed that melatonin induces the transcription of vacuolar Na^+/H^+ antiporter NHX1 and K^+ influxer AKT1 genes. Significant effect of melatonin in growth under salt stress was observed by Wang et al. (2016) in cucumber that showed increased photosynthetic rate, high concentration of chlorophyll, increased antioxidant enzyme activity, and so on. Another study by Zhang et al. (2014) on cucumber (*Cucumis sativa*) revealed positive role of melatonin in mitigating the effect of salt stress on seed germination. Molecular analysis revealed that exogenous melatonin induced the genes *Cu–Zn SOD*, *Fe–Zn SOD*, *POD*, and *CAT* and were found upregulated by several fold in plants exposed to salt stress. Melatonin also reduces the transcription of ABA biosynthetic genes *CsNCED1*, *CsNCED2* and alleviates ABA catabolism genes *CsCYP707A1*, *CsCYP707A2*. Melatonin-treated maize seedlings subjected to salt stress showed significantly better germination rate, improved relative water content, increased production of proline, phenols, high activity of antioxidant enzymes, and so on (Jiang et al. 2016). Melatonin helped the rubber plants to overcome the negative effects of salt stress by increased transcript expression of genes that plays a role in metabolism of ROS, antioxidant production, and also involved in growth and accumulation of flavonoids and increased biosynthesis of melatonin (Yang et al. 2020a). Comprehensive study on seed germination of cucumber with treatment of melatonin under salt stress was carried out by Zhang et al. (2017b) and it was revealed that melatonin variably affects genes that are fundamental pathways of plant system for energy production such as glycolysis, tricarboxylic acid cycle (TCA), and glyoxylate. This extra energy demand is utilized for enhanced growth of plants under stress. Transcriptomic data analyzed salt-stressed rapeseed seedlings treated with melatonin and revealed that a number of genes involved in cytokinin, gibberellic acid, lignin biosynthesis were differentially upregulated both in leaves and roots to combat salt stress (Tan et al. 2019a). In addition, melatonin-treated seedlings showed improved growth as compared to control. Melatonin-treated sweet potato seedlings have shown decreased accumulation of toxic Na^+ ion and maintain K^+/Na^+ ratio by inducing plasma membrane H^+-ATPase activity. The H^+-ATPase activity was stimulated by breakdown of triacylglycerol (TAG), fatty acid degradation, that is, β-oxidation (Yu et al. 2018). Melatonin prevents leaf senescence and enhances tolerance of rice plants over salt stress through increased activity of antioxidants that counteract H_2O_2 accumulation and scavenge other free radicals (Liang et al. 2015). Mitigation of salt stress by melatonin was observed by priming seeds of *Vicia faba* and it was observed that primed seed showed better growth in terms of relative water content, N^{a+}, Ca^+, and their respective ratios. Plants from melatonin primed seeds showed better accumulation of compatible solutes that maintained osmotic balance in salt-stressed plants than control (Dawood 2014).

6.3 Temperature Stress

Melatonin has been reported to ameliorate the heat induced damages in plants (Arnao and Hernández-Ruiz 2015). Melatonin application affirmed thermo tolerance to heat stressed *Arabidopsis* plant, and it was observed that the transcription of master regulator *heat shock factor HSF1As (At HSF1a, b, d, e)* was upregulated that further induces the transcription of heat shock proteins HSFA2, HSA32, HSP90, and HSP101 (Shi et al. 2015c). Heat shock proteins prevent protein misfolding and degradation (Shi et al. 2015c). Similar induction of HSPs (HSP17.4, HSP20, HSP21, HSP70, and HSP90) was seen in heat stressed tomato plants supplemented with melatonin that prevent protein degradation (Xu et al. 2016). Recently, Wang et al. (2020) also demonstrated interaction of HSP40 (a chaperone protein) with SlSNAT roused thermo tolerance to tomato via increased melatonin-induced ROS scavenging and other growth-promoting effects. Effect of melatonin on heat stressed tomato seedlings was studied by Jahan et al. (2019); exogenous melatonin treatment induces PA accumulation and NO production in heat stressed plants. Melatonin treatment induces transcription of SOD, POD, CAT, glutathione-S-transferase (GST), and GR genes in response to heat stress to counter the oxidative stress-induced damages (Jahan et al. 2019). Increased thermo tolerance due to melatonin was also observed in cherry radish (*Raphanus sativus* L. var. *radculus pers*) via increased activity of antioxidant enzymes, higher chlorophyll content, increased biomass, increased endogenous hormone such as auxin, and ABA (Jia et al. 2019). Melatonin improves growth of seedlings of kiwi fruit grown under high temperature by increased activity of number of antioxidant enzymes (CAT, POD, SOD) as well the enzymes of ascorbate glutathione cycle, like monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and GR (Liang et al. 2018).

Apart from the above studies, melatonin also performs a central role in protecting plants from chilling stress. Zhao et al. (2016) described application of melatonin alleviates cucumber seedlings from chilling stress by increasing activity of antioxidant enzymes involved in ascorbate glutathione cycle and prevents plants from ROS and oxidative stress. Further study by Zhao et al. (2017) in cucumber revealed that melatonin treatment led to increase in expression of *CsZat12*, a zinc finger transcription protein that prevents plants from chilling induced oxidative damage. At the same time, there was upsurge in concentration of putrescine (Put) and spermidine (Spd) that helped to maintain structure and protect integrity of proteins Lee and Back (2019). infer the prospect of 2-hydroxymelatonin in overcoming both drought and cold stress in cucumber, tomato, and tobacco. Melatonin helps the tea plants to tolerate the stress caused by drought, salt, and cold by increased activity of antioxidant enzymes that protects the plant from ROS and associated damage (Li et al. 2019a). It has been previously reported that melatonin maintains storage and quality of fruits during post-harvest; simultaneously it also protects the peach fruit from chilling stress by modulating the activity of enzymes and proteins responsible for maintaining cell wall disassembly (Cao et al. 2018).

6.4 Heavy Metal Stress

It is well known that melatonin plays a vital role in phytoremediation, as reviewed by Arnao and Hernández-Ruiz (2019b). Melatonin application to tomato plants grown under cadmium (Cd) stress induces defense activity with enzymatic and non-enzymatic antioxidant properties. Concurrently, melatonin increases the expression of *SlPC*s and *SlMT2* and *SlABC1* genes responsible for the synthesis of phytochelatins (PCs), metallothioneins (MTs), and ABC transporters (Hasan et al. 2015). The ABC transporter helps in compartmentalization of Cd-PC into vacuole and reduces the toxic effects of Cd. Another study reported that both melatonin and selenium mediate protection to tomato plants under Cd stress. Selenium led to synthesis of *TDC* gene that is a precursor of melatonin biosynthesis gene and alleviates Cd stress by increasing expression of PCs (Li et al. 2016a). A study by Cai et al. (2017) reported association of *HsfA1a* in Cd-induced transcription of phytomelatonin biosynthetic genes *TDC*, *T5H*, and *caffeic acid O-methyltransferase (COMT)* and further production of PCs. Tang et al. (2018) demonstrated enhancement of Cd phytoremediation capacity of two hyper accumulator plants *Malachium aquaticum* and *Galinsoga parviflora* attributed to exogenous melatonin. Melatonin significantly induces the transcripts of ABC transporter PDR8 while suppresses the expression of *Nramp6* to reduce the accumulation of toxic Cd in alfaalfa and Arabidopsis plants (Gu et al. 2017). Meanwhile, the study also reported the involvement of micro RNA *miR398a* and *miR398b* in maintaining redox homeostasis during Cd absorption.

Nawaz et al. (2018) reported that exogenous melatonin to watermelon seedlings exposed to vanadium stress induces the expression of a number of antioxidant enzymes, that is, SOD, POD, APX, GPX, and GST genes and prevents them from potential oxidative stress and improve their growth. Seed priming with melatonin at lower concentration (1–10 μM) proved to be effective for alleviation of cooper ion stress due to increased production of antioxidant enzymes that protected the plants from oxidative stress and membrane damage (Posmyk et al. 2008). Most of the studies that suggested the significant role of melatonin in alleviation of plant from abiotic stresses during the last five years have been highlighted in Table 1.

7 Melatonin and Alleviation of Biotic Stress

7.1 Bacteria

Melatonin activates SA and ET mediated defense gene expression in *Arabidopsis* and tobacco plants infected with *Pseudomonas syringae* DC3000 (Lee et al. 2014). A number of genes involved in defense such as isochorismate synthase 1 (*ICS1*), 1-aminocyclopropane-1-carboxylate synthase 6 (*ACS6*), glutathione-S-transferase 1 (*GST1*), ascorbate peroxidase 1 (*APX1*), and vegetative storage protein 1 (*VSP1*) were significantly increased upon melatonin treatment (Lee et al. 2014). At the same time, transcript expression of genes for pathogenesis-related proteins (PR) such as *NPR1*, *PR1*, *PDF. 1.2* was also increased in response to melatonin. This was confirmed in further studies by Lee et al. (2015) where melatonin did not induce defense response in *nahG* (SA-biosynthesis deficient) mutant lines of *Arabidopsis* plants inoculated

Table 1 Studies depicting the role of melatonin in alleviation of various abiotic stresses in different crop plants during last five years

Crop	Effective concentration of melatonin	Mode of application	References
Drought			
Malus prunifolia *M. hupehensis* (Apple)	100 µM	Irrigation	Li et al. (2015)
Zea mays (Maize)	100 µmol L^{-1}	Foliar spray	Ye et al. (2016)
Medicago sativa (Alfa-alfa)	10 Mµ	Irrigation	Antoniou et al. (2017)
Zea mays (Maize)	1 mM	Irrigation	Fleta-Soriano et al. (2017)
Brassica napus (Rapeseed)	50 µM	Foliar spray	Li et al. (2018)
Dracocephalum moldavica (Moldavian balm)	100 µM	Foliar spray	Kabiri et al. (2018)
Agrostis stolonifera (Creeping bentgrass)	20 µM	Foliar spray	Ma et al. (2018)
Avena nuda (Oat)	100 mM	Foliar spray	Gao et al. (2018)
Actinidia chinensis (Kiwi)	100 µM	Irrigation	Liang et al. (2019)
Glycine max (Soybean)	100 mg L^{-1}	Foliar spray	Cao et al. (2019)
Brassica napus (Rapeseed)	500 µM	Seed priming	Khan et al. (2019)
Zea mays (Maize)	100 mM	Foliar spray	Huang et al. (2019)
Salinity			
Vicia faba (Faba bean)	500 µM	Seed priming	Dawood and El-awadi (2015)
Cucumis sativus (Cucumber)	100 µM	Pre-treatment of seedlings	Wang et al. (2016)
Zea mays (Maize)	1 µM	Irrigation	Jiang et al. (2016)
Cucumis sativus (Cucumber)	1 µM	Seed priming	Zhang et al. (2017b)
Ipomoea batatas (Sweet potato)	1 µM—root 100 µM—leaf	Spray to roots and leaves	Yu et al. (2018)

(continued)

Table 1 (continued)

Crop	Effective concentration of melatonin	Mode of application	References
Brassica napus (Rapeseed)	1 µM	Irrigation	Zhao et al. (2018)
Hevea brasiliensis (Rubber)	100 µM	Pre-treatment of seedlings	Yang et al. (2020a)
Brassica napus (Rapeseed)	50 µM	Irrigation	Tan et al. (2019a)
Cucumis sativus (Cucumber)	100 µmol L^{-1}	Pre-treatment of seedlings	Zhang et al. (2020)
Temperature *(C)-Cold stress* *(H)-Heat stress*			
Arabidopsis thaliana (Arabidopsis)	20 µM	Pre-treatment of seedlings	Shi et al. (2015c) (*H*)
Cucumis sativus (Cucumber)	200 µM	Irrigation	Zhao et al. (2016) (*C*)
Cucumis sativus (Cucumber)	200 µM	Irrigation	Zhao et al. (2017) (*C*)
Solanum lycopersicum (Tomato)	10 µM	Foliar spray	Xu et al. (2016) (*H*)
Prunus persica (Prunus)	100 µM	Pre-treatment of fruits	Cao et al. (2018) (*C*)
Actinidia deliciosa (Kiwi)	200 µM	Pre-treatment of seedlings	Liang et al. (2018) (*H*)
Raphanus sativus (Cherry radish)	29 mg L^{-1}	Irrigation	Jia et al. (2019) (*H*)
Solanum lycopersicum (Tomato)	100 µM	Pre-treatment of seedlings	Jahan et al. (2019) (*H*)
Heavy metals *(Cd)-Cadmium stress* *(V)-Vanadium stress*			
Solanum lycopersicum (Tomato)	100 µM	Foliar spray	Hasan et al. (2015) (*Cd*)
Solanum lycopersicum (Tomato)	1 µM	Pre-treatment of roots	Li et al. (2016a) (*Cd*)
Arabidopsis thaliana (Arabidopsis)	100 µM	Pre-treatment of seedlings	Gu et al. (2017) (*Cd*)
Malachium aquaticum *Galinsoga parviflora*	200 µMol L^{-1} 100 µMol L^{-1}	Spraying to plant	Tang et al. (2018) (*Cd*)
Citrullus lanatus (Watermelon)	0.1 µMol L^{-1}	Irrigation	Nawaz et al. (2018) (*V*)

with *Pseudomonas syringae* DC3000. Concurrently, exogenous melatonin application to *snat* (serotonin N-acetyltransferase (SNAT) melatonin biosynthesis knockout) mutant lines showed resistance against *P. syringae* DC3000. As reported earlier, melatonin activates salicylic acid-induced defense signaling pathway and its downstream genes of signaling as well as defense (such as *NPR1*, *PR1*, *PDF. 1.2*) (Lee et al. 2015). Nitric oxide (NO) substantiates the melatonin-induced defense of *Arabidopsis* plants upon *P. syringae* DC3000 infection (Shi et al. 2015a). Endogenous levels of both melatonin and NO were significantly increased in plants upon pathogen infection, leading to activation of SA-mediated defense pathway. In a different study, Zhao et al. (2015) showed that melatonin application regulates the sugar metabolism pathways to induce defense responses in *Arabidopsis*. Exogenous melatonin promotes higher activity of cell wall invertase (CWI) in *Arabidopsis* infected *P. syringae Pst* DC3000 that led to adequate accumulation of cellulose, xylose, galactose, and callose in cell wall of infected plants and in turn enhances defense against pathogen through structural barrier. Role of sugar (fructose, glucose, cellulose) and glycerol in melatonin-induced defense pathway was shown by Qian et al. (2015). Infection of *Arabidopsis* plants with *P. syringae Pst* DC3000 enhances endogenous melatonin levels that led to significant accumulation of the fructose, glucose, cellulose, and glycerol that further increased NO and SA production, followed by increased expression of downstream resistance genes.

Melatonin is reported to provide resistance to rice plants infected with *Xanthomonas oryzae* pv. *oryzae* by restricting fungal cell proliferation and division as observed from TEM studies and altered expression of genes involved in cell division and amino acid metabolism (Chen et al. 2018). Recently, Chen et al. (2019b) showed reduction in incidence of another bacteria *Xanthomonas oryzae* pv. *oryzicola* (Xoo) infecting rice due to melatonin. It downregulated transcript expression of genes involved in cell division and decreased the virulence that may also be associated with lower expression of toxin genes.

7.2 Fungi

A number of reviews have suggested that melatonin has been reported to modulate defense of plants against fungal attack pathogens (Arnao and Hernández-Ruiz 2015, 2018; Shi et al. 2016; Sharif et al. 2018; Agathokleous et al. 2019; Moustafa-Farag et al. 2020). Yin et al. (2013) reported that application of exogenous melatonin to roots of apple tree infected with *Diplocarpon mali* shows induced activity of POD, APX, CAT, and phenylalanine ammonia lyase (PAL) enzyme activity to counter the adverse effects of ROS generated due to pathogen attack. Concurrently, melatonin also induced the expression of pathogenesis-related proteins. Defensive role of melatonin can be clearly seen from the studies of Wei et al. (2017) in *Musa acuminata* where exogenous application of melatonin led to increased expression of HSP90 and decreased disease severity of *Fusarium* wilt in banana. Their studies revealed that nine HSP90 genes were strongly regulated by exogenous melatonin, out of which seven HSP90s are reported to be involved in providing protection to *Fusarium* wilt.

A fine correlation between melatonin effect on plant pathogens was studied by Zhang et al. (2017b), where melatonin completely inhibited mycelial growth of *Phytopthora infestans* under in vitro. Transcriptomic studies were carried out in melatonin-treated hyphae of *P. infestans*, and it was observed that melatonin affects genes involved in amino acid metabolism, stress tolerance, and virulence of the fungus. In contrast to the above study, Zhang et al. (2018) investigated the effect of melatonin for control of pathogen *Phytopthora nicotianae* that causes black shank disease on tobacco. The study revealed that entirely no mycelial growth of *P. nicotianae* was observed under potato dextrose agar (PDA) medium supplemented with melatonin, and in addition, melatonin also led to decreased virulence of this pathogen as evident by low cell viability. In-depth role of melatonin in reducing disease severity was carried out by Mandal et al. (2018) in cucurbits. There was decrease in hyphal growth and lesion size and diameter of *Phytophthora capsici* and *P. xanthi* with melatonin treatment under in vitro and in detached leaf assay, respectively. Furthermore, it was postulated that melatonin brings about modulation of genes involved in SA, JA, and ET signaling involved in defense signaling pathway of plants.

Remarkably, melatonin has shown providing resistance to fruits during post-harvest from fungal attack as evident from the studies of Li et al. (2019b) where tomato fruit is protected well from *Botrytis cinerea* infection. Melatonin-treated fruits showed less hyphal growth of fungus over the fruit compared to non-melatonin-treated fruits. It was concluded that melatonin led to increased activity of POD and also the activity of defense enzymes such as chitinases (Chi), glucanases (Glu), PAL, 4-coumarate-coenzyme A ligase (4CL). PAL and 4CL further enhanced the production of secondary metabolites generated from phenylpropanoid pathway and boost defense of fruits. A different type of study showed that exogenous application of melatonin did not affect fungal growth of *Penicillium digitatum* under in vitro and over the citrus fruit surface (Lin et al. 2019).

Conversely, the concentration of endogenous melatonin that has increased due to H_2O_2 treatment and *Penicillium digitatum* inoculation improved the resistance of citrus fruits via suppression of ROS. Simultaneously, qRT-PCR revealed increased expression of genes that play a role in ROS metabolism upon melatonin-treated citrus fruits (Lin et al. 2019). A detailed study of signal transduction events at molecular levels associated with application of melatonin and reduced disease incidence of anthracnose in banana was carried out by Li et al. (2019c); melatonin upregulated genes involved in receptor proteins essential for activation of ROS, phytohormones such as auxin, ET, and MAPK pathway. These all, in turn, are responsible for the switching on genes of various defense-related processes of plants such as increase in wax content, cell wall thickening, increased concentration of secondary metabolites, increased expression of WRKY transcription factors involved in disease signaling, and so on.

Melatonin in combination with arbuscular mycorrhizal fungi (AMF) showed lower disease severity of *Fusarium* infection in cucumber plants. Melatonin-treated plants have great colonization of AMF, increased photosynthetic rate, higher antioxidant production, high concentration of secondary metabolites, and so on. These all, in turn, increased tolerance of plants against *Fusarium* infection (Ahammed et al. 2020).

7.3 Viruses

Melatonin prevents the in vitro shoots of apple cultivar gala from viral infection. This was demonstrated by growing infected shoots of apple in shoot proliferating medium (SPM) containing melatonin under in vitro, and it was observed that shoot regrowth rate was higher as well as increased percentage of virus free shoots in comparison to shoots regrown in SPM medium without melatonin (Chen et al. 2019a). Not only melatonin, nitric oxide is also involved in resistance against viral infection. A study was conducted by Lu et al. (2019) where exogenous treatment of melatonin to rice showed lower incidence of rice stripe virus. Disease incidence was linked to increased levels of melatonin and nitric oxide in melatonin pre-treated rice plants that modulated expression of *OsPR1b* and *OsWRKY45* involved in SAR pathway (Lu et al. 2019). Melatonin significantly increased defense of *Nicotiana glutinosa* and *Solanum lycopersicon* against *tobacco mosaic virus (TMV)* via SA-mediated defense pathway and increased expression of PR1, PR5. At the same time, exogenous melatonin treatment considerably increased levels of endogenous melatonin and nitric oxide that hinted close association of NO in melatonin-induced defense (Zhao et al. 2019b). Some of the studies carried out in last five years suggested the significant role played by melatonin in modulation of plant's defense against biotic stresses and have been depicted in Table 2.

8 Future Perspectives

The significant impact of melatonin in influencing plant growth and development has been well established through a number of studies. Concomitantly, melatonin-mediated stress signaling and alleviation in plants has been extensively discussed in the above chapter. Most of the studies related to melatonin and stress alleviation have been carried out under laboratory conditions and with seedlings, and very less authors have attempted to evaluate melatonin's tangible potential in natural agricultural fields and crops. Investigations should be carried out by studying multiple stresses at a time and in real-field-based environments. Future research efforts should focus on understanding the role of melatonin in modulation of host and pathogen relationship and their effects on associated defense pathway. Melatonin is known to alter transcription of various stress-responsive genes through interaction with various transcription factors although the exact molecular mechanism is still an enigma to scientists. Melatonin can be a prospective alternative solution in agriculture sector

Table 2 Studies depicting the role of melatonin in alleviation of biotic stresses in different crop plants during last five years

Crop	Biotic agent	Effective concentration of melatonin	Mode of application	References
Bacteria				
Arabidopsis thaliana (Arabidopsis)	*Pseudomonas syringae pv. Tomato DC3000*	20 µM	Foliar spray	Shi et al. (2015a)
Arabidopsis thaliana (Arabidopsis)	*Pseudomonas syringae pv. Tomato DC3000*	50 µM	MS growth media	Zhao et al. (2015)
Arabidopsis thaliana (Arabidopsis) *Nicotiana tabacum* (Tobacco)	*Pseudomonas syringae pv. Tomato DC3000*	1 mM	Foliar spray	Lee and Back (2016)
Oryza sativa (Rice)	*Xanthomonas oryzae pv. oryzicola*	200 µg L^{-1}	Foliar spray	Chen et al. (2019b)
Fungi				
Musa acuminata (Banana)	*Fusarium oxysporum f. Sp. Cubense*	100 µM	Pre-treatment of roots	Wei et al. (2017)
Solanum tuberosum (Potato)	*Phytophthora infestans*	3–10 mM	Foliar spray	Zhang et al. (2017c)
Citrullus lanatus (Watermelon)	*Podosphaera xanthii* *Phytophthora capsici*	1 mmol L^{-1}	Foliar spray	Mandal et al. (2018)
Nicotiana tabacum (Tobacco)	*Phytophthora nicotianae*	2 mmol L^{-1}	Pre-treatment of leaves	Zhang et al. (2018)
Lycopersicon esculentum var. cerasiforme	*Botrytis cinerea*	100 µM	Fruit dipping	Li et al. (2019b)
Musa acuminate (Banana)	*Colletotrichum musae*	10 mM	Fruit dipping	Li et al. (2019c)
Citrus reticulate (Citrus)	*Penicillium digitatum*	50 µM	Fruit dipping	Lin et al. (2019)
Solanum tuberosum	*Phytophthora infestans*	10 mM	Pre-treatment to leaf and tuber	Zhang et al. (2019)

(continued)

Table 2 (continued)

Crop	Biotic agent	Effective concentration of melatonin	Mode of application	References
Viruses				
Malus x domestica cv (Apple)	*Apple stem grooving virus* (ASGV)	15 µM	Supplemented with growth medium	Chen et al. (2019a)
Nicotiana glutinosa	*Tobacco mosaic virus* (TMV)	100 µM	Irrigated to root	Zhao et al. (2019b)
Oryza sativa (Rice)	*Rice stripe virus* (RSV)	10 µM	Pre-treatment of plants	Lu et al. (2019)

for increasing growth and productivity as well for plant disease management of crop plants in this current climate change scenario. Therefore, the need of the hour is to pursue a collaborative research so as to interpret the mechanisms behind melatonin amended stress tolerance in plants and substantiate these results in various other food crops to meet our food security challenges in the scenario of global climate change.

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Melatonin: An Alternative Signal to Antioxidant Enzyme Modulation in Plants

Phalguni Bhattacharya and Suchisree Jha

Abstract Melatonin (N-acetyl-5-methoxytryptamine) is a dynamic signaling molecule exhibiting a plethora of physiological effects in plants. It is commonly present in different plant parts and can stimulate several physiological responses toward adverse environmental conditions. This chapter reviews the role of melatonin as a potent antioxidant and scavenger of reactive oxygen species (ROS) and reactive nitrogen species (RNS) under abiotic and biotic stress conditions in plants. Accumulation of melatonin in plants is found to be modulated by its biosynthesis and catabolism under stress conditions. Melatonin, with its precursors and derivatives, acts as a powerful growth regulator, bio-stimulator, and antioxidant, which delays leaf senescence, reduces photosynthetic inhibition, and improves redox homeostasis. In addition, exogenous melatonin improves plant tolerance against drought, unfavorable temperatures, salinity, heavy metals stress, acid rain, and pathogen attack. Nitric oxide-melatonin and phytohormone crosstalk provides new avenue of research which might decipher molecular mechanisms of melatonin signaling in plants.

Abbreviations

Melatonin	N-acetyl-5-methoxytryptamine
5-MT	5-Methoxytryptamine
C_3OH M	Cyclic 3-hydroxymelatonin
AFMK	N1-acetyl-N2-formyl-5-methoxykynuramine
AMK	N1-acetyl-5-methoxykynuramine
IDO	Indoleamine 2,3-dioxygenase
EPO	Eosinophil peroxidase

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HRP	Horseradish peroxidase
MPO	Myeloperoxidase
CYP	Cytochrome P450
OHM	6-Hydroxymelatonin,
NAS	N-acetylserotonin, or AFMK
TDC	Tryptophan decarboxylase
T5H	Tryptamine 5-hydroxylase
SNAT	Serotonin N-acetyltransferase
AANAT	Non-homologous aralkylamine N-acetyltransferase
ASMT	N-acetylserotonin O-methyltransferase
HPLC	High-performance liquid chromatography
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
H_2O_2	Hydrogen peroxide
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
APX	Ascorbate peroxidase
CLH1	Chlorophyllase

1 Introduction

Melatonin (N-acetyl-5-methoxytryptamine) is a low molecular weight pleiotropic molecule with indole ring present both in plants and animals (Hardeland et al. 2011). It acts by membrane and nuclear receptors (Shiu et al. 2010; Imbesi et al. 2009). Interestingly, melatonin with its bioactive metabolites acts through the exchange of reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Schaefer and Hardeland 2009). Melatonin is an amphiphilic or amphipathic molecule which easily penetrates through the cell membrane and accumulates in the cytosol, nucleus, and mitochondria (Acuña-Castroviejo et al. 2001). It is a non-receptor-mediated active molecule which prevents cells, tissues, and organisms from oxidative stress by scavenging ROS or/and RNS and simultaneously improves antioxidant capacity of the organism (Nopparat et al. 2010; Um and Kwon 2010; Xu et al. 2010). Therefore, it serves as a first-line of defense against any hazardous conditions (Tan et al. 2014). Melatonin is largely distributed in various crops, namely tomatoes, cucumbers, bananas, apples, onions, rice, and it was reported that melatonin is widely scattered within different plant parts, probably in roots, stems, leaves, flowers, fruits, bulbs, and seeds (Nawaz et al. 2016). The abundance of melatonin varies with the variation in cultivars, species, tissue types, growth, and developmental processes (Erland et al. 2015).

Initially, melatonin was discovered in the bovine pineal gland of cows in 1958. Now, it has been reported as a widely explored natural molecules in living organisms ranging from bacteria to mammals (Hardeland et al. 2011; Arnao and Hernández-Ruiz 2015). The effects of melatonin in endosperm and epidermal cells of bulbous plants (Jackson 1969) and onions (Banerjee and Margulis 1973) opened a new avenue of research. It is found in single-celled dinoflagellates as methoxyindole (Poeggeler et al. 1991). Melatonin has also been reported in Japanese morning glories (***Pharbitis nil***) and subsequently in higher plants (Dubbels et al. 1995; Hattori et al. 1995). It is vastly distributed in all herbs at the nanograms levels to subsequent concentrations in per gram of tissue (Chen et al. 2003). It has been extracted from bacteria, fungi, algal members (pheophyceae, rhodophyceans, and chlorophyceans) and from wide range of angiosperm families, namely Apiaceae, Asteraceae, Brassicaceae, Fabaceae, Lamiaceae, Rosaceae, Solanaceae, Alliaceae, Poaceae, and Zingiberaceae along with Magnoliphyta division (Hardeland et al. 2011).

Melatonin improves physiological processes by protecting plant tissues against injury and stress caused due to environmental hazards (Meng et al. 2014; Arnao and Hernández-Ruiz 2015; Erland et al. 2015; Debnath et al. 2018; Cui et al. 2017; Li et al. 2017). The activity of melatonin in plants with its mode of action and its availability in different parts of plants and its stress ameliorative properties have been reviewed in the current chapter.

2 Biosynthetic Regulation of Melatonin in Plants

Melatonin is converted into other metabolites such as 5-MT (5-methoxytryptamine), C_3OH M (cyclic 3-hydroxymelatonin), AFMK (N1-acetyl-N2-formyl-5-methoxykynuramine), and AMK (N1-acetyl-5-methoxykynuramine) by enzymatic and/or non-enzymatic and also by pseudo-enzymatic actions (Tan et al. 2010; Hardeland et al. 2009). Melatonin is enzymatically cleaved into AFMK by IDO (indoleamine 2,3-dioxygenase), EPO (eosinophil peroxidase), HRP (horseradish peroxidase), MPO (myeloperoxidase), CYP (cytochrome P450) sub-forms, and NQR2. Reports on other pathways of AFMK and AMK formation and subsequent CYPs action on melatonin and its formation into OHM (6-hydroxymelatonin), NAS (N-acetylserotonin), or AFMK (Tan et al. 2010) are also available. These biologically active metabolites of melatonin increase its mode of action in plants (Schaefer and Hardeland 2009).

The pathway of melatonin biosynthesis (Fig. 1) from tryptophan in plants is categorized into four steps: decarboxylation by tryptophan decarboxylase (TDC); amine hydroxylation by tryptamine 5-hydroxylase (T5H) to serotonin, N-acetylation formation through activity of SNAT (serotonin N-acetyltransferase), activating AANAT (non-homologous aralkylamine N-acetyltransferase) of vertebrates; and lastly O-methylation by ASMT (N-acetylserotonin O-methyltransferase) to form melatonin (Tan et al. 2014; Hardeland 2014).

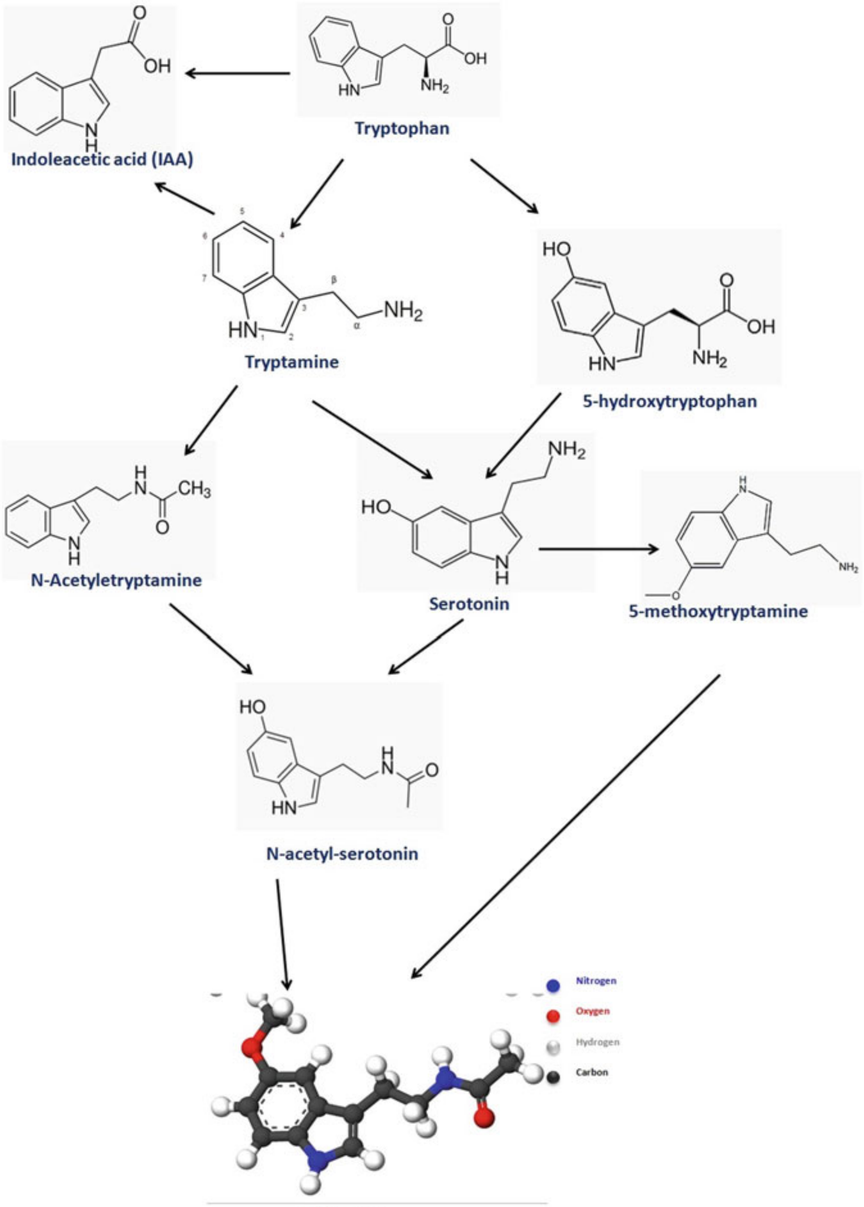

Fig. 1 Biosynthetic routes of melatonin in plants

3 Melatonin Stimulates Antioxidant Responses in Plants

Plants adapt to any adverse environmental conditions accordingly with respect to their physiological situations. Melatonin can modulate the physiological activity against adverse environments being an effective antioxidant compound. Melatonin can act as potent free radical scavenger (Ianăş et al. 1991). It has an ability to move freely via cell membranes to the cytoplasm. Sub-cellular partitions can be easily crossed due to its amphipathic nature with indole ring structure (Zhang et al. 2014). Cyclic 3-hydroxymelatonin with antioxidant properties counteracts the extremely toxic hydroxyl radical ($OH^{\bullet}$) and is also known to scavenge the superoxide (O^{2-}) (Poeggeler et al. 1996). Melatonin reacts with hydrogen peroxide to yield an antioxidative product (Tan et al. 2000). These scavenging activities exhibit supportive response in vivo (Kolar and Machackova, 2005). It increases the activity of a number of antioxidant enzymes, thereby augmenting the efficiency as an antioxidant (Shi et al. 2015a, b, c, d, e). Melatonin acts as a direct antioxidant and effectively lowers the levels of reactive oxygen compared to ascorbic acid. Various melatonin metabolites, such as 3-Ω, AFMK, and 2-hyxdroxymelatonin, are capable antioxidants (Tan et al. 2014). Melatonin is a good mediator in different antioxidant pathways like the glutathione ascorbate cycle, peroxidases, superoxide dismutase, and catalase modulating abiotic and biotic stress responses in the plant (Erland et al. 2018). It can detoxify reactive nitrogen species like nitric oxide (Shi et al. 2015a, b, c, d, e; Lee and Back 2017; Liang et al. 2019). Melatonin reimburses ROS and RNS in cells (Manchester et al. 2015). The melatonin and ROS coordination establishes signaling molecule within the plants, thus increasing ROS concentration (Mittler et al. 2011; Dietz et al. 2016). The dynamic coordination between melatonin and other biomolecules related with electric, ionic, and chemical signaling pathways responsible for developing tolerance to adverse environments is summarized in Fig. 2.

Transgenic plants for melatonin biosynthesis alter phytohormone activity in cells in hostile atmosphere as it is a boosting activity during the root growth in transgenic rice plants (Park and Back 2012). The involvement of melatonin was confirmed in rice ASMT-mRNA expression. It is due to melatonin activation of genes due to treatment of abscisic acid and methyl jasmonic acid otherwise creating situations for stress tolerance (Lee and Back 2017).

To combat with the oxidative damage, plants possesses defense mechanism of antioxidants (enzymatic/non-enzymatic), which includes SOD, POD, CAT, and APX (Wang et al. 2016; Cui et al. 2017). The negative impact of drought stress was recuperated by the application of melatonin, which induces the activities of these antioxidant enzymes. The concentration of melatonin applied as a foliar spray and/or soil drench in various crops enhanced their stress tolerance by protecting the photosynthetic apparatus, increasing their antioxidant capability, and improving the water-holding capacity (Wei et al. 2014; Cui et al. 2017). The decrease in ROS and reduced MDA enhances the activities of antioxidant enzymes to eliminate the excessive ROS, so it is considered as a broad-spectrum antioxidant and free radical scavenger (Tan et al.

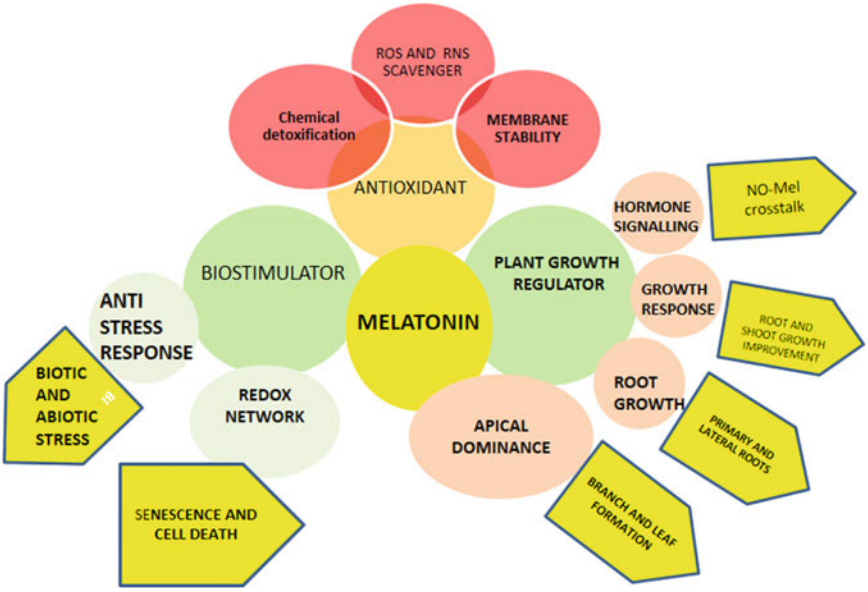

Fig. 2 Mode of action of melatonin in plants. It coordinates the physiological processes as growth regulator, antioxidant activator, and biological development stimulator. Mechanism of melatonin activities in plants as a growth regulator, bio-stimulator, and antioxidant

2000). Cui et al. (2017) and Li et al. (2017) also reported the role of antioxidant enzymes in reducing ROS under abiotic stresses.

4 Nitric Oxide and Melatonin Signaling Operates Through Modulation of Antioxidative Defense

Nitric oxide (NO) is essentially a potent antioxidant which controls physio-metabolic response in plants as a signaling molecule, increasing salinity tolerance during stress response. Several enzymatic pathways as a source of NO are nitrate/nitrite-dependent pathway and an L-Arg-dependent. Interestingly, melatonin weaves NO-dependent S-nitrosylation responses in plants, and it was documented that melatonin and NO governs plant tolerance against NaCl stress in sunflower seedlings and rapeseed seedlings. However, experimental evidences proved that NO is involved in melatonin signaling as a second messenger in nia1/2 and noa1 *Arabidopsis* mutants (Li et al. 2017). Mukherjee in the year 2019 suggested possible role of NO and melatonin response to be associated with redox signaling and long-distance communication. Recently, Jha et al. (2014) have suggested their opinions about elicitor-mediated NO management through antioxidant-rich low-molecular weight peptide (s) from mulberry, and further investigations are being carried out on melatonin-based NO responses in mulberry plant and its relationship with silk production by silkworm.

Soil salinity influences global agricultural production, where high salinity (mainly NaCl) provokes ionic and oxidative stress. High NaCl stress increases Na^+/K^+ ratio disturbing the ionic environment of plant cells. Plants usually remove excessive Na^+ by Na^+/H^+ antiporters, by activating antiporter genes, thereby improving salt tolerance. Na^+/H^+ antiporter as Na^+/H^+ exchanger (NHX) family can increase Na accumulation in vacuoles enhancing salt tolerance. A number of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydroxyl radicals, largely get induced during salinity conditions. To deal with such adversity of salt-induced oxidative stress, the enzymatic antioxidant system provides a highly efficient and specific ROS scavenging approach for plants. Superoxide dismutase (SOD), catalase (CAT), and guaiacol peroxidase (POD) are effective instruments of this enzymatic system, and generally plants decrease ROS by rising activities of these enzymes. Melatonin (N-acetyl-5-methoxytryptamine) in these situations improved the salt tolerance in *Arabidopsis*, soybean, Chinese crab apple, rice, cucumber, and Bermuda grass (Zhao et al. 2018). The same mode of action was observed under heat and cold stress. Melatonin upgrades the transcripts of ion channel genes, or sugar and glycolysis metabolism-related genes. However, actions of melatonin as an antioxidant can be grouped into four categories: (a) direct free radical scavenging; (b) stimulation of activities of antioxidant enzymes; (c) increasing the efficiency of mitochondrial oxidative phosphorylation and reducing electron leakage; and (d) augmenting the efficiency of other antioxidants (Zhao et al. 2018). Cold stress with melatonin applications induces on specific activities of antioxidant enzymes and on non-enzymatic antioxidants in rice seedlings especially the activities of antioxidant enzyme (SOD, CAT, and POD) (Zhao et al. 2018; Han et al. 2017). Reactive oxygen species are the byproducts of photosynthesis, respiration, and other normal metabolic processes, and play an important role in stress tolerance (Chen et al. 2017). ROS are also involved in programmed cell death and abiotic stress response (Shi et al. 2015a, b, c). H_2O_2 increased significantly under cold stress conditions. However, pretreatments with melatonin by leaf spraying method, seed soaking, and root immersion methods significantly decreased ROS levels in leaf tissues. SOD is a key enzyme that degrades O^{2-} to O_2 and H_2O_2, while CAT and POD can break down H_2O_2 to H_2O through different pathway in plant cells. Exogenous melatonin application crucially uplifts enzymatic antioxidant system or the non-enzymatic antioxidant system to maintain the redox balance. Shi et al. (2015a) found enhancement of antioxidative enzymes in Bermuda grass. GSH as a main component of non-enzymatic antioxidant system in plants is involved in direct and indirect regulations to ROS levels (Debnath et al. 2018). Melatonin applications promoted in both of GSH and GSSG contents under the cold stress.

Melatonin has the ability to neutralize the highly toxic hydroxyl radical ($OH^\bullet$) to produce effective antioxidant cyclic 3-hydroxymelatonin and also scavenge superoxide (O^2) and $NO^\bullet$ radicals in a better way and increases the activities of several antioxidant enzymes: superoxide dismutase (SOD, both MnSOD and CuSOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GRd), and glucose-6-phosphate dehydrogenase. Melatonin stimulation of glutathione (GSH) synthesis reacted by c-glutamylcysteine synthase increases intracellular concentration of GSH. Sublethal oxidative stress induced by different agents was also counteracted by melatonin (Han 2017). Various genes related to nitrogen metabolism, carbohydrate metabolism, tri-carboxylic acid transformation, transport, hormone metabolism, metal handling, and redox processes are expressed in melatonin responsive plants. Several specific stress-related genes, for example, chlorophyll content preservation via melatonin, and the activity of chlorophyllase (CLH1), the light-regulating enzyme associated with chlorophyll degradation, were expressed with melatonin treatment in *Arabidopsi*s (Weeda et al. 2014). Melatonin, in accordance with antioxidant enzymes, improves photosynthesis, delays leaf senescence, slows alterations in the leaf ultrastructure, delays biosynthesis of metabolites, and modulates stress genes. It maintains a well-known system that protects plants from harsh environments (Zhang et al. 2014).

5 Conclusion

Melatonin is an active molecule known to be instrumental against adverse situations that a plant faces. It increases the anti-oxidative capacity and is largely distributed in many common plant families with economic importance. The dynamic coordination between melatonin and other bio-molecules related with electric, ionic, and chemical signaling pathways is responsible for developing tolerance to adverse environment. Different antioxidant enzymes and their activities are enhanced by melatonin-nitric oxide crosstalk and it eliminates excessive ROS levels. The relationship between NO and melatonin was considered on the basis of redox signaling and long-distance communication. Role of melatonin in abiotic stress amelioration and modulation of stress enzymes are well investigated in the context of several antiporters or sodium–hydrogen ion exchanger. Several specific stress-related genes and their expression to conserve or protect the metabolic units are influenced by melatonin, thus maintaining plants in harsh environments.

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Glutamate: Physiological Roles and Its Signaling in Plants

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Abstract Glutamate (Glu) is a protein amino acid and a well-known neurotransmitter in human body, but little is known about the role of glutamate in plants. Recent investigations suggest that Glu has been found to play important roles, such as seed germination, root architecture, pollen germination and pollen tube growth, senescence, wound response, pathogen resistance, and adaptation to environmental stress. Many reports have shown that the exogenously supplied glutamine can be beneficial for in vitro culture, increasing the regeneration rate and biomass of the explants. Under stress conditions, Glu participates in various abiotic stresses such as salt, cold, heat, and drought, and triggered long-distance signaling transduction. This chapter describes the possible physiological functions of glutamate in plant growth and development and adaptation to various environmental stresses.

Abbreviations

Glu	Glutamate
GLRs	Glutamate receptors
GABA	γ-Amino butyric acid

1 Introduction

Glutamate (Glu), known as α-aminoglutaric acid, is a key excitatory neurotransmitter in humans. The important signaling role of Glu in animal systems has been known, but little is known about the role of glutamate in plants (López-Bucio et al. 2019;

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Ramamrishna and Mukherjee 2020; Ramakrishna and Roshchina 2018; Forde and Lea 2007). Glu is commonly found in the phloem sap, and perform a long-distance signaling role. Glutamine may enter the plant cell through amino acid transporters and can serve as a critical nitrogen source for plant growth and development. Recently, Glu is found in plants, and emerged as a novel signaling molecule and exert possible physiological functions in plants such as seed germination (Kong et al. 2015), root architecture (Forde 2014; López-Bucio et al. 2019), pollen germination and pollen tube growth (Michard et al. 2011; Wudick et al. 2018), wound response and pathogen resistance (Manzoor et al. 2013; Mousavi et al. 2013; Nguyen et al. 2018; Toyota et al. 2018; Jin et al. 2019), and adaptation to abiotic stress (Cheng et al. 2018; Zheng et al. 2018; Li et al. 2019a, b; Philippe et al. 2019). Various reports have showed that Glu usually exerts signaling role by its glutamate receptors (GLRs), similar to iGluRs in animals (Lam et al. 1998; Wudick et al. 2018; López-Bucio et al. 2019). The glutamate receptor-like (GLR) genes in plants were first identified in *Arabidopsis thaliana* as homologues of mammalian ionotropic glutamate receptors (iGluRs) (Weiland et al 2016). Glutamate may have a role similar to an elicitor or the exogenous glutamate may affect the cell wall and trigger an elicitor-like response in the plant cell. In soybean crop, foliar or seed application of Glu in both greenhouse and field could enhance the antioxidant capacity by activating antioxidant enzymes catalase, peroxidase, superoxide dismutase, polyphenol oxidase, and phenylalanine ammonia lyase (Teixeira et al. 2017) and improved nitrogen metabolism and productivity (Teixeira et al. 2018). Glu can act as a precursor of other protein amino acids (such as proline, arginine, and cysteine), nonprotein amino acids (like aminobutyric acid: GABA), antioxidants (e.g., glutathione: GSH), and polyamines, which in turn governs osmotic adjustment, protein synthesis, redox balance, and other cellular metabolism, followed by maintaining cellular homeostasis under heat stress conditions (Forde and Lea 2007; Brosnan and Brosnan 2013; Yoshida et al. 2016). This chapter summarizes the possible physiological functions of glutamate in plant growth and development and adaptation to environmental stress.

2 Role of Glutamate in Plant Cell Tissue Culture

Glutamine is a common organic nitrogen source used in higher plant tissue culture media (Franklin and Dixon 1994). It has been widely used in tissue culture for de-differentiation and re-differentiation processes. Many reports have shown that the exogenously supplied glutamine can be beneficial for in vitro culture, increasing the regeneration rate and biomass of the explants (Shetty et al. 1992; Ogita et al. 2001). In conifer, glutamine has been shown to be an effective compound which assists in the induction, maintenance, and maturation of somatic embryos (Guevin and Kirby 1996; Barrett et al. 1997). Furthermore, glutamine also plays an important role in conifer zygotic embryo development (Feirer 1995). Glu was one of the major components in embryogenic cell lines of *Cryptomeria japonica* (Ogita et al. 2000).

High concentrations of Gln have also been observed in embryogenic cultures of carrot (Kamada and Harada 1984; Joy et al. 1996) and white spruce (Joy et al. 1997).

In *C. japonica*, the results clearly demonstrated the importance of glutamine in maintaining the embryogenic property of the tissues (Ogita et al. 2001). Glu was found to induce seed germination (Kong et al. 2015), root architecture (López-Bucio et al. 2019), pollen germination, and pollen tube growth (Wudick et al. 2018). Addition of proline and glutamine in the medium reported a positive effect on frequency of callusing and regeneration in rice (Chowdhry et al. 1993; Ge et al. 2006; Shahsavari 2011). In bromeliads, Gln was an excellent nitrogen source to improve production of dry shoot mass. Glutamine enhances competence for organogenesis in pineapple leaves cultivated in vitro and also enhances embryogenesis cultures of *C. japonica* (Ogita et al. 2001). Moreover, Walch-Liu et al. (2006) reported that exogenous application of glutamate (50 μM) significantly affected the growth and various developmental processes of Arabidopsis. Moreover, glutamine application promotes nitrogen and biomass accumulation in the shoot of seedlings of the maize hybrid ZD958. Encina et al. (2014) reported somatic embryogenesis enhancement in avocado (*Persea americana* Mill.) using a two-step culture system, including glutamine in the culture medium.

3 Effect of Glutamine in Root Growth

Glutamine was detected at much higher levels in root tip zones when compared to those of root elongation zones. Glu is the amino acid whose effects on root development in a number of plant species are most distinctive (Walch-Liu et al. 2006; Walch-Liu and Forde 2008). It has been shown that exogenously applied Glu elicits specific changes in root system architecture that have the characteristics of a signaling effect (Walch-Liu et al. 2006; Walch-Liu and Forde 2008). In Arabidopsis, where these effects have been studied in most detail, Glu inhibited primary root growth while at the same time stimulating the outgrowth of lateral roots (lateral roots) near the primary root tip, producing a shorter and more branched root system (Walch-Liu et al. 2006). Besides, Glu treatments alter root architecture inhibiting primary root through reducing meristem mitotic activity and induce lateral root proliferation (Walch-Liu et al. 2006). Moreover, Glu and its crosstalk with other factors affect the root development, such as phosphorous, auxin, abscisic acid (ABA), and nitrate. Ion channels of the *GLUTAMATE RECEPTOR-LIKE* family act as sensors that convert this signal into an increase in intracellular calcium ion concentration (Toyota et al. 2018). A further possibility, and one that does not exclude the potential for glutamate to act as an endogenous signal, is that roots have evolved the capacity to respond to variations in glutamate concentration in the external environment (Walch-Liu et al. 2006). Synergistic action of Ca^{2+}, auxin, and Glu signaling in the structure of root architecture in Arabidopsis (Singh and Chang 2018). Similarly, Glu inhibited the growth of primary root and stimulated the outgrowth of lateral roots, forming a shorter and more branched root system in Arabidopsis (Walch-Liu et al. 2006). Role of glutamate under various abiotic stresses has been depicted in Table 1.

4 Role of Glutamate During Abiotic Stress Tolerance in Plants

Abiotic stress factors such as salt, heat stresses, flooding, drought, and cold limit the plant growth and reduce crop yield (Ramakrishna and Gill 2018; Ramakrishna and Ravishankar 2013, 2011). Glu as a signaling molecule is involved in the response and adaptation to salt, cold, heat, drought, pathogen, and wound stress in many plants species (Cheng et al. 2018). In plants, Glu receptors are reported to play a role in Ca^{2+} influx. It is well known that the addition of Gln/Glu induces a substantial transcriptional change in plants (Gutiérrez et al. 2008). Exogenous L-Glu treatment could induce resistance against *Penicillium expansum* in pear fruit (Jin et al. 2019).

5 Heat Stress

High temperature is a key stress factor affecting cellular metabolism, seed germination, plant growth, development, yield, geographical distribution, and even survival (Al-Whaibi 2011).

Heat tolerance has been studied at physiological, biochemical, molecular, and even omics levels in many plant species, such as Arabidopsis (Lam et al. 1998), rice (Li et al. 2006), *M. truncatula* (Philippe et al. 2019), and pear (Fabrice et al. 2018). In maize seedlings, treatment with Glu enhanced the survival percentage of seedlings under heat stress (Li et al. 2019b). GLRs-mediated Ca^{2+} signaling triggered by Glu was involved in the acquisition of heat tolerance in plants. Hassanein et al. (2013) reported that increasing temperature stress increased the level of endogenous Glu in wheat plants, which further increased the tolerance of wheat plants against temperature stress, with enhanced growth parameters and yield components.

6 Drought Stress

Under drought stress conditions, foliar or seed application of Glu increased relative water content, leaf and root dry weight, and plant productivity (Teixeira et al. 2020). In *Brassica napus*, Glu treatment triggered calcium signaling (mainly calcium-dependent protein kinase), which in turn increased the synthesis of salicylic acid. Also enhanced drought induced proline accumulation, thus improving drought tolerance by regulating cellular redox potential (La et al. 2019). Accumulation of Glu has been

Table 1 Roles of glutamate under various abiotic stresses

Plant species	Abiotic stress	Amelioration effect	Reference
Arabidopsis thaliana	Salt	Regulation of seed germination	Cheng et al. (2018)
Arabidopsis thaliana	Cold	Regulation of jasmonate signaling	Zheng et al. (2018)
Solanum lycopersicum	Cold	H_2O_2, redox homeostasis	Li et al. (2019a)
Zea mays	Heat stress	Calcium signaling	Li et al. (2019b)
Medicago truncatula	Drought	Production of nitric oxide	Philippe et al. 2019
Brassica napus	Drought	Ca^{2+} signaling, SA, proline	La et al. (2019)
Triticum aestivum L.	Drought stress	Increase in yield	Gupta and Thind (2017)
Glycine max L.	Salt stress	Increase in the contents of nonessential amino acids	Farhangi-Abriz and Ghassemi-Golezani (2016)
Triticum aestivum L.	Salinity stress	Enhanced growth attributes	Khan et al. (2016)
Oryza sativa L.	Salt stress	Improved yield	Maziah and Teh (2016)
Zea mays L.	Osmotic stress	Improving effects on growth, photosynthetic pigments, biochemical attributes	Moharramnejad et al. (2015)
Triticum aestivum L.	Drought stress	Improvement in biochemical attributes	Gupta et al. (2014)
Moringa oleifera	Salt stress	Increased chl. *a*, chl. *b* and carotenoids concentration	Hussen et al. (2013)
Triticum aestivum L.	Temperature stress	Increased total free amino acid concentration, growth parameters and yield components	Hassanein et al. (2013)
Triticum aestivum L.	Water stress	Improvement in growth	Aldesuquy et al. (2013)
Triticum aestivum L.	Salt stress	Improvement in yield	Rao et al. (2013)
Zea mays L.	Drought stress	Improving effects on growth, photosynthetic pigments, grain yield	Miri and Armin (2013)
Brassica napus L.	Salinity stress	Improvement in yield	Sakr et al. (2012)
Glycine max L.	Salinity stress	Yield attributes	Rezaei et al. (2012)

(continued)

Table 1 (continued)

Plant species	Abiotic stress	Amelioration effect	Reference
Gossypium barbadense L.	Drought stress	Improvement in growth attributes	Shallan et al. (2012)
Zea mays	Drought stress	Nutritional quality	Ali and Ashraf (2011)
Oryza sativa L.	Water stress	Growth pigment and biochemical attributes	Kathuria et al. (2009)
Zea mays	Drought stress	Osmoregulation by maintaining osmolyte accumulation	LiXin et al. (2009)
Brassica napus L.	Salt stress	Growth improvement	Athar et al. (2009)
Sporobolus stapfianus	Water stress	Increase in concentration of Glu 20–30%	Martinelli et al. (2007)
Glycine max L.	Water stress	Increased accumulation of amino acids under water stress	Ramos et al. (2005)
Oryza sativa	Salt, cold, heat stress	Improvement in growth	Kishitani et al. (2000)
Coleus blumei Benth	Salt stress	Accumulation of nitrogen containing compounds and amino acids	Gilbert et al. (1998)
Arabidopsis thaliana	Water stress	Accumulation of free amino acid contents	Nambara et al. (1998)

shown in detached leaves of rice due to water stress (Thakur and Rai 1982). Shelp et al. (1999) reported that, under abiotic stresses, Glu is converted into GABA, a stress indicator, through the activity of an enzyme, γ-aminobutyrate (GDC). Ramos et al. (2005) reported an endogenous increase in soybean plants due to drought stress. It was reported that water stress resulted in endogenous increase in *Arabidopsis thaliana*, which further increased accumulation of free amino acids (Nambara et al. 1998). An overexpression of the two glutamate receptors, OsGLR1 and OsGLR2, of *O. sativa* was found to increase drought tolerance (Lu et al. 2014). Martinelli et al. (2007) reported that, during water stress, endogenous Glu concentration increased from 20 to 30% in *Sporobolus stapfinus* plants.

7 Salt Stress

Glu can induce action potentials (long-distance electrical signals), which can be modulated by osmotic and salt stresses in *Helianthus annuus* seedlings (Stolarz and Dziubinska 2017).

Previous reports suggest that phenotyping of *glr3.7* mutants showed that *AtGLR3.7* is involved in salt stress response in *Arabidopsis* (Wang 2019). Stolarz and Dziubinska (2017) reported that exogenous application of Glu (50 μM) increased osmotic and salt stress tolerance in sunflower. Farhangi-Abriz and Ghassemi-Golezani (2016) reported the effects of salt stress on cellular Glu levels in soybean plants. In moringa plants, the biosynthesis of Glu was increased due to salt stress which increased the biosynthesis of photosynthetic contents (chl. a, chl. b and carotenoids) and also the content of total amino acids (Hussen et al. 2013). Gilbert et al. (1998) reported an increased accumulation of Glu induced by salt stress in *Caleus blumei.* Sadak et al. (2015) reported that exogenous application of Glu (7.24–9.12%) as foliar spray on faba beans was found to decrease the harmful effects of salinity and improve plant growth and yield. Moreover, Stolarz and Dziubinska (2017) reported that exogenous application of Glu (50 μM) increased osmotic and salt stress tolerance in sunflower.

8 Cold Stress

Cold acclimation is associated with remarkable physiological, biochemical, and metabolic re-programming in plants. To cope with cold stress, plants have developed sophisticated molecular regulatory mechanisms, which display similarities and differences in various plant species. Cold stress stimulates AtGLR3.4 expression in a calcium-dependent manner, which is associated with Glu and cold-induced elevation of cytosolic calcium (Meyerhoff et al. 2005; Weiland et al. 2016). Recently, cold acclimation at 12 °C upregulated the expression of GLR3.3 and GLR3.5, which in turn increased the resistance of tomato plants to a subsequent chilling stress at 4 °C. Plant GLRs (mainly GLR1.2, GLR1.3, GLR3.3, and GLR3.5) positively regulate the cold tolerance of plants by accumulating endogenous JA and subsequently activating GLR-H_2O_2-GSH cascade (Li et al. 2019a).

9 Summary

The recent investigations on the roles of Glu in the metabolism, physiology, and different developmental processes of higher plants showed their potential for the induction of stress tolerance. Perhaps, the relation between Glu and phytohormones will open up new perspectives in the possible role of Glu in plant morphogenesis, flowering, seed dormancy, and stress-amelioration. The relationship of Glu signaling with other signaling such as NO, H_2S, and methylglyoxal signaling needs to be studied. More studies are necessary to elucidate the relationship between GABA and other signaling molecules. Identification of GABA receptors will open new vistas in understanding the exact mechanism of action.

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The Saga of Catecholamine and GABA Through Prospecting Stress Tolerance in Plants

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Abstract Plants are subjected to various abiotic stress factors. They respond to these circumstances or in long term response, they change their metabolic systems with the help of newly explored inherent biochemicals. The production of certain primary and secondary metabolites, which are called as neurotransmitters like in animal system induces tolerance to stress situations in plants. Catecholamine and GABA play biochemical and molecular roles to overcome the adverse situations like drought or salinity reported in various plants systems. Stress responses also ignite perception in plant at molecular level. Action potentials, voltage gated channels, a vesicular trafficking apparatus sensitive to calcium signals, plasmodesmata for direct cell–cell transport are some of the preliminary ways in for the easy monitoring of environmental changes. A new field of research, Plant neurobiology, where workers of various disciplines are engaged in simulating the perception and responses of plants in different circumstances and also to environmental input thereby testing the alternative definition of plant intelligence. The chapter reviews the action of tswo group of chemicals, catecholamines and Gamma-amino butyric acid, GABA, their molecular pattern augmenting tolerance against detrimental climatic effect on plants. These biomolecules are not exclusive chemicals in plants to combat with adverse situations, rather in animals they have their origin.

Abbreviations

GABA	Gamma amino butyric acid
GAD	Glutamic acid decarboxylase
CK	Cytokinin
ABA	Abscisic acid
(TH)	Tyrosine hydroxylase
(L-dopa)	Dihydroxyphenylalanine

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(TD)	Tyrosine decarboxylase
(CB)	Cyt b561 electron-transport domain
(GABA-T; EC 2.6.1.19)	GABA transaminase
(SSADH; EC 1.2.1.16)	Succinic semialdehyde dehydrogenase
(ALMT)	Aluminium-activated malate transporter
PGRs	Plant growth regulators
Glu	Glutamic acid
Pas	Polyamines
Pro	Proline
EDTA	Ethylenediamine tetraacetic acid
PMSF	Phenylmethylsulfonyl fluoride
ProTs and AAP3 (GABA transporters)	Proline transporter family protein and Amino acid permease 3
CuAO	Cupric amine oxidase
PAO	Polyamine oxidase
ROS	Reactive oxygen species
AtGABP	GABA permease,
H_2O_2	Hydrogen peroxide
HO	Hydroxyl Radicals
Auxin-IAA	Auxin-indole acetic acid
NO^-	Nitric Oxide
O_2	Oxygen
O^{2-}	Superoxide

1 Introduction

Plants are capable of perceiving various environmental changes associated with biotic and abiotic factors in different circumstances. Plants are adversely affected by environmental stresses like desertification and water scarcity. According to recent data by FAO, around 3.6 billion of the world's 5.2 billion hectares of fertile dryland suffer from erosion, soil degradation, and salinity. Plant growth, biomass, and productivity are affected by several environmental conditions. This triggers a series of physiological responses in plants associated with altered gene expression and molecular crosstalk events. Certain transient changes are associated with variations in action potentials, voltage gated channels, vesicular trafficking, calcium signals, etc. Plant neurobiology has emerged as a new field of research where workers of various disciplines investigate plant perception and responses in different circumstances (plant intelligence). The present chapter reviews the physiological signficance of two groups of neurotransmitters in plants namely—catecholamines and Gamma-amino butyric acid (GABA). Furthermore, the molecular pattern of these two biomolecules in augmenting tolerance against detrimental climatic effect on plants have been elaborated. GABA is effectively involved in modulating various physiological responses to abiotic stress conditions. Various investigations have depicted increased accumulation of GABA in plants exposed to various adverse conditions, such as hypoxia, drought, cold, high temperature, low light, and high salinity. GABA in plant cells acts as an effective osmolyte operating salt-induced dehydration and thus functions as ROS scavenger under stressful environmental condition. GABA exerts a plethora of signaling effects including pH regulation. GABA-mediated physiological response is associated with rapid stress-induced kinetics in plant cells. In plants, GABA was initially discovered in potato (*Solanum tuberosum*) tubers (Steward et al. 1949). Subsequently, various investigations reported the effects of abiotic stress on endogenous GABA accumulation. Transporters and receptors of GABA have been reported in response to drought and it could increase the activities of ProTs and AAP3 (GABA transporters) which in turn regulate GABA transport across cell membranes (Ramesh et al. 2017). The identification of receptors and transporters associated with catecholamine functioning appears to be a key area of focus in future. Furthermore, it is important to understand the signaling pathways of GABA-induced stress tolerance associated with various phytohormones. ROS homoeostasis and modulation of antioxidative enzymes has been reported to be the major mechanism of stress amelioration being exerted by GABA. Localization studies for tissue specific spatial distribution of catecholamines using appropriate fluorescent probes might appear beneficial in deciphering its temporal distribution in various tissues during abiotic stress conditions.

2 The Catetecholamine Bioynthesis Pathway and Its Effect on Modulation of Stress Response

Plant-derived catecholamines are a group of amines possessing 3, 4-dihydroxy-substituted phenyl ring. Catecholamines accumulation in plants is largely regulated by biotic and abiotic stress factors. Catecholamines mainly include dopamine, norepinephrine (noradrenaline), and epinephrine. Catecholamines are known to regulate the activity of various plant hormones, carbohydrate metabolism, alkaloid biosynthesis, melanin synthesis and induce nitrogen detoxification.

Similar to that in animal systems, the catetecholamine biosynthesis pathway in plants is initiated from tyrosine which functions as the primary precursor. This molecule is hydroxylated by the activity of tyrosine hydroxylase (TH) to produce dihydroxyphenylalanine (L-DOPA). L-DOPA further undergoes substrate decarboxylation by the activity of tyrosine decarboxylase (TD) to produce tyramine. Thus, dopamine is produced via hydroxylation of tyramine or decarboxylation of L-DOPA. Dopamine hydroxylation is catalyzed by dopamine hydroxylase activity which further leads to norepinephrine synthesis. Certain plants like *Musa sapientum*, *Cytisus scoparius,* and *Portulacca* exhibit varied response of environmental factors which affects catecholamine biosynthesis. Dopamine acts as a precurosor of alkaloid biosynthesis namely—benzylisoquinolines like papaverine and morphine, and mescaline. These compounds have been identified in various species of cactus. More than 44 plant species (including 29 important) in human diet have been reported to possess active levels of catecholamines. Dopamine has been reported in plants of family Araceae mainly in spathes of inflorescence. Catecholamine, especially dopamine undergoes oxidation to produce a free radical scavenger—melanin. Melanin has been reported to possess strong antioxidative properties. Among various photomodulatory and stress-induced effects, catecholamine biosynthesis is higher in dark conditions in *Portulacca* callus. In *Pringlea antiscorbutica* dopamine levels show decrease during heat stress. Interestingly, wound or mechanical stress in *Carnegiea gigantean* results in increased levels of dopamine. Dopamine, epinephrine, and normetanephrine are all the more increased in potato leaves after 5 min of wound induction; the highest rise being recorded for norepinephrine. Various other stress conditions such as drought stress, ABA treatment, and UV applications also increased the dopamine levels in potato plants. In certain plants increased norepinephrine accumulation was associated ABA, drought, and NaCl treatment. In contrast to such observations catecholamine levels were low in the presence of red light, cold induction, and darkness. A novel protein DoH-CB has been reported to mediate signaling events associated with catecholamine action in plants. This protein exhibits dopamine-beta-hydroxylase activity and also possess a cyt b561 electron-transport domain (CB). Recent investigations involving in silico analysis of DoH-CB proteins from *Arabidopsis thaliana* reveal structural features of both CB and DoH domains to be well conserved. Interestingly DoH-CB proteins also exhibited auxin inducible activity. The DoH domain of the protein was identified to be present in another auxin-inducible protein—AIR 12. These proteins function as effective mediators of catecholamine function in plants. The proteins therefore, cascade auxin-catecholamine crosstalk auxin and catecholamine in plants (Kulma and Szopa 2007).

3 GABA Action and Its Stress-Related Kinetics

GABA (4-aminobutyrate) is a non-protein amino acid widely distributed throughout plant kingdom. Among various physiological actions mediated by GABA it has been reported to associate with stress through cytosolic Ca^{+2} stimulation. GABA is synthesized by the action of a cytosol-localized glutamate decarboxylase (EC 4.1.1.15; GAD), which is also a Ca^{2+}-calmodulin-dependent protein. GABA activity triggers rapid changes in cytosolic Ca^{2+} concentration. GABA is metabolized through glutamate decarboxylation to produce succinic acid (GABA shunt). The GABA shunt operating in the mitochondrion is regulated by a GABA transaminase using either α-ketoglutarate or pyruvate as amino acceptor (GABA-T; EC 2.6.1.19), and a succinic semialdehyde dehydrogenase (SSADH; EC 1.2.1.16). Proline transporters such as AtProT2 and LeProT1 have been reported to bind to GABA in cells. GABA probably functions as a sensor of nitrogen status in plant tissues and regulates C:N balance.

Table 1 Stress-related kinetics of GABA accumulation in plants (Kinnersley and Turano 2000)

Plant	Stress	GABA % of Control	Time	References
Asparagus cells	Acidosis	300	15 s	Crawford et al. (1994)
Soybean leaves	Mechanical damage	1800	1 min	Ramputh and Bown (1996)
Soybean leaves	Mechanical damage	2700	5 min	Wallace et al. (1984)
Soybean leaves	Cold (6 °C)	2000	5 min	Wallace et al. (1984)
Asparagus cells	Cold (10 °C)	200	15 min	Cholewa et al. (1997)
Radish leaves	Anoxia	10,000	4 h	Streeter and Thompson (1972)
Tea leaves	Anoxia	4,000	12 h	Tsushida and Murai (1987)
Tomato leaves	Viral	130	13 d	Cooper and Selman (1974)

GABA, therefore, functions as a long-distance signal molecule during various stress-induced conditions. GABA synthesis associated with low pH results in cytosolic acidification. GABA exerts a plethora of signaling effects including pH regulation. GABA-mediated physiological response is associated with rapid stress-induced kinetics in plant cells (Table 1).

Cellular accumulation of GABA results in a decrease in the water potential in the cell thus equating a balance in the system. In view of such protective role GABA protects the lipid constituents of biological membranes thus preventing its inactivation during freezing. Gamma-aminobutyric acid (GABA) has been reported to undergo transient and rapid accumulation in plant tissues in response to biotic and abiotic stress thus regulating plant growth. Further investigations are required to decipher the effects of GABA associated with regulation of carbon metabolism and its associated signaling pathway. Aluminium-activated malate transporter (ALMT) proteins are specifically activated by anions and are passively regulated by GABA action. Site-directed mutagenesis of selected amino acids within ALMT proteins results in abolition of GABA efficacy without altering its transport properties. GABA-induced modulation of ALMT activity is manifested by altered root growth and modulation of root tolerance to alkaline pH, acid pH and also to aluminium ions. Authors have proposed that GABA might exert multiple physiological effects in plants via the activity of ALMT, which involve regulation of pollen tube, root growth, and stress tolerance (Kinnersley and Turano 2000).

4 Role of GABA in Redox Homoeostasis

GABA is effectively involved in modulating various physiological responses to abiotic stress conditions. Various investigations have depicted increased accumulation of GABA in plants exposed to various adverse conditions, such as hypoxia, drought, cold, high temperature, low light, and high salinity. GABA in plant cells acts as an effective osmolyte operating salt-induced dehydration and thus functions as ROS scavenger under stressful environmental condition. Furthermore, physiological concentration of exogenous GABA effectively promotes plant growth, regulates antioxidant metabolism, and modulates the transcript levels of antioxidant enzyme genes. Thus, GABA signaling potentiates alleviation of stress-caused oxidative damage in plants and maintains redox balance in cells. Exogenous application of GABA regulates osmotic balance in plant cells thus contributing to the enhancement of physiological mechanisms associated with stress tolerance. Exogenous GABA results in the inhibition in the production of H_2O_2 thus reducing oxidative damage in *Caragana intermedia* roots under salt stress. Imbibed seeds primed with GABA application increase seed germination percentage and also reduce the salt stress-induced injury during seeds germination in wheat and maize plants. Furthermore, exogenous GABA also improves shoot–root ratio and fresh weight of maize (*Zea mays* L.) seedling under salt stress. A triangular relation exists among acidosis, trivalent aluminium ions (Al^{3+}), and GABA accumulation in plants. ALMT effectively functions as a large multigenic anion channel present exclusively to plants and exerts multiple physiological roles with discrete expression patterns. GABA-modulated ALMT activity in wheat roots has been reported to increase during aluminium stress. Thus, GABA-induced regulation of ALMT proteins triggers the onset of a novel signaling pathway that potentially translates changes in the concentration of stress-induced metabolites. Investigations through functional genomics in Arabidopsis thaliana suggest that GABA acts as a novel signaling molecule in crosstalk with phytohormones such as ABA and ethylene. Metabolomic analysis in transgenic *Agrostis stolonifera* plants shows elevated cytokinin (CK) content under drought stress to be associated with GABA accumulation. This finding is supportive to studies in barley (*Hordeum vulgare)* which showed higher expression of GABA receptor genes in drought-tolerant cultivars. Exogenously applied GABA might modulate antioxidant responses in *Prunus persica* fruit in response to chilling stress of the fruit. Both ethylene and CK are involved in crosstalk with GABA metabolism under abiotic stress. Interestingly, various GABA-related genes are also regulated by the application of 6-benzyl adenine in Arabidopsis seedlings grown in presence of 10 mM GABA. This finding suggests that CK could possibly play a major role in regulation of GABA metabolism. Increased GABA Transaminase (GABA-T) activity associated with rice leaf senescence and GABA-T could reduce ROS levels (Kinnersley and Turano 2000).

5 Exogenous Application of GABA and Its Effect on Stress Amelioration

Exogenous application of GABA can positively regulate the antioxidant defense mechanism and photosynthesis in pepper (*Capsicum annuum*) seedlings under low light stress. GABA pretreatment alleviates the damage induced by chilling stress in tomato (*Lycopersicon esculentum*) seedlings, peach (*Primus persica*) fruit as well as in wheat (*Triticum aestivum*) seedlings. Exogenous GABA effectively improves salinity resistance in muskmelon (*Cucumis melon*) by protecting the photosynthesis apparatus and by reducing photo inhibition. GABA application is effective in significantly enhancing drought tolerance mediated through lipid peroxidation and inhibition of photosynthetic and mitochondrial activity in black pepper (*Piper nigrum*). A considerable reduction in GABA content results in a decrease in resistance to drought in *Arabidopsis* mutants by the reducing the expression of the glutamate decarboxylase gene (Mekonnen et al. 2016). In the GABA shunt pathway, loss of GABA transaminase (GABA-T) in *Arabidopsis pop2* mutant lines resulted in increased sensitivity to various abiotic stresses. Moreover, the functions of GABA are closely linked with polyamine (PAs) signaling in plants in response to abiotic stress. The catabolism of PAs is an important regulation of GABA production. Hu et al. (2015) have demonstrated that exogenous GABA application positively facilitates PA biosynthesis thus enhancing endogenous GABA level, during $Ca(NO_3)_2$ stress in muskmelon. However, these investigations decipher partial relationships between GABA and other PGRs (Glu, PAs, or Pro).

6 Transporters and Receptors of GABA in Relation to Stress

In plants, GABA was initially discovered in potato (*Solanum tuberosum*) tubers (Steward et al. 1949). Subsequently, various investigations reported the effects of abiotic stress on endogenous GABA accumulation. Transporters and receptors of GABA have been reported in response to drought and it could increase the activities of ProTs and AAP3 (GABA transporters) which in turn regulate GABA transport across cell membranes (Ramesh et al. 2017). In addition, GABA also regulates the release of Ca^{2+} from the intracellular Ca^{2+} store by binding to its receptors (GLRs and ALMTs) thus modulating the activity of GAD. Increased EL and MDA accumulation have been considered as prime indicators of membrane damage and lipid peroxidation being induced by excessive ROS generation during abiotic stress. Pretreatment with GABA can effectively avoid drought-induced leaf wilting by improving membrane stability (decreases in EL and MDA content) under drought stress.

7 GABA, Polyamines and Stress Tolerance

PAs are ubiquitously distributed in all eukaryotic cells and mainly include putrescine, spermidine, and spermine which exist in free, conjugated, and bound form in plant cells. A direct link of GABA and PAs has been observed in relation to PAs catabolism which produces GABA via CuAO or PAO catalysis. Investigations have revealed that PAs degradation led to increase in approximately 30% of GABA content in fava beans (*Vicia faba*) under hypoxic stress. GABA could enhance PAs synthesis, but exerted an inhibitory effect on PAs catabolism thus resulting in a higher total PAs content in GABA-treated plants under drought condition. This finding implied that increased endogenous GABA could exert a negative feedback on PAs degradation (Signorelli et al. 2014; Li et al. 2017). Extensive accumulation of free proline in plant tissues during abiotic stress is possibly associated with scavenging of hydroxyl radicals (·OH). Signorelli et al. (2014) has reported a non-enzymatic pathway of GABA formation via Pro reaction with·OH under oxidative stress thus indicating a direct connection between Pro and GABA. However, recent investigations affirmed that a balance between Pro synthesis and its catabolism plays a pivotal role in plant tolerance against drought stress. Therefore, a positive effect of exogenous GABA on alleviation of oxidative stress is associated with Pro accumulation and ROS homeostasis in white clover under drought condition (Signorelli et al. 2014; Li et al. 2017) (Fig. 1).

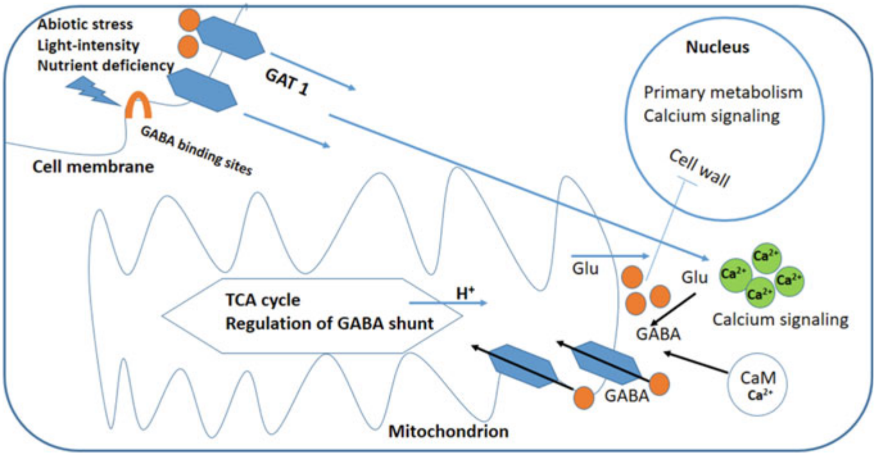

Fig. 1 A schematic model of GABA transport, metabolism and signaling in plant cells. Abiotic stress and light intensity variation leads to cascading of GABA signaling associated with glutamine transport, calcium signaling and gene expression associated with stress tolerance

8 Conclusions

Catecholamines and GABA are neuroprotective chemicals in plants which essentially function as inducer of stress tolerance. Their binding to transporter or receptor proteins increases the potentiality and expression of stress-related enzymes, antioxidants, polyamines, and other stress-related proteins However, unpublished data of the authors laboratory depict the role of low molecular weight leaf peptide(s) from mulberry which can increase polyamine activity associated with exogenous GABA treatment thereby expressing a probable role in stress sensitivity or tolerance. Further investigations are necessary to decipher the molecular mechanism of GABA signaling and its crosstalk with other biomolecules. The identification of receptors and transporters associated with catecholamine functioning appears to be a key area of focus in future. Furthermore, it is important to understand the signaling pathways of GABA-induced stress tolerance associated with various phytohormones. ROS homoeostasis and modulation of antioxidative enzymes has been reported to be the major mechanism of stress amelioration being exerted by GABA. Localization studies for tissue specific spatial distribution of catecholamines using appropriate fluorescent probes might appear beneficial in deciphering its temporal distribution in various tissues during abiotic stress conditions. Complex metabolomics analysis of various plant systems treated with exogenous GABA during abiotic stress shall help in deciphering catecholamine-induced metabolic regulations.

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