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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Contents

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

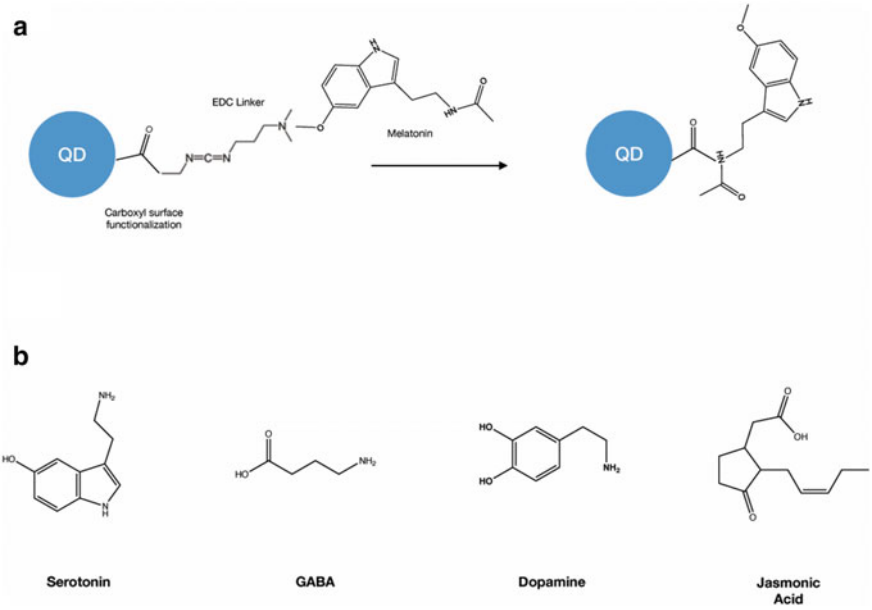

Fig. 1 **a** Depiction of the EDC-mediated conjugation process and **b** structures of some plant signalling molecules successfully conjugated to QD through an EDC-mediated conjugation

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,

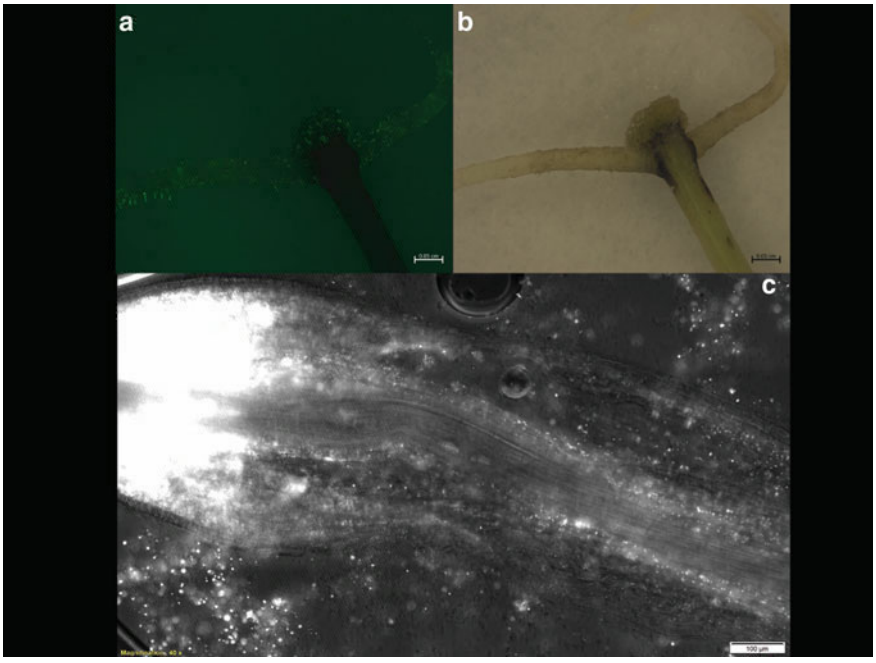

Fig. 2 Uptake of melatonin labelled QD by intact in vitro grown Hypericum perforatum plantlets **a** viewed under a GFP fluorescence filter and **b** under bright field microscopy and **c** localization of QD-MEL in the root of *H. perforatum* under physiological conditions using epifluorescence microscopy

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).

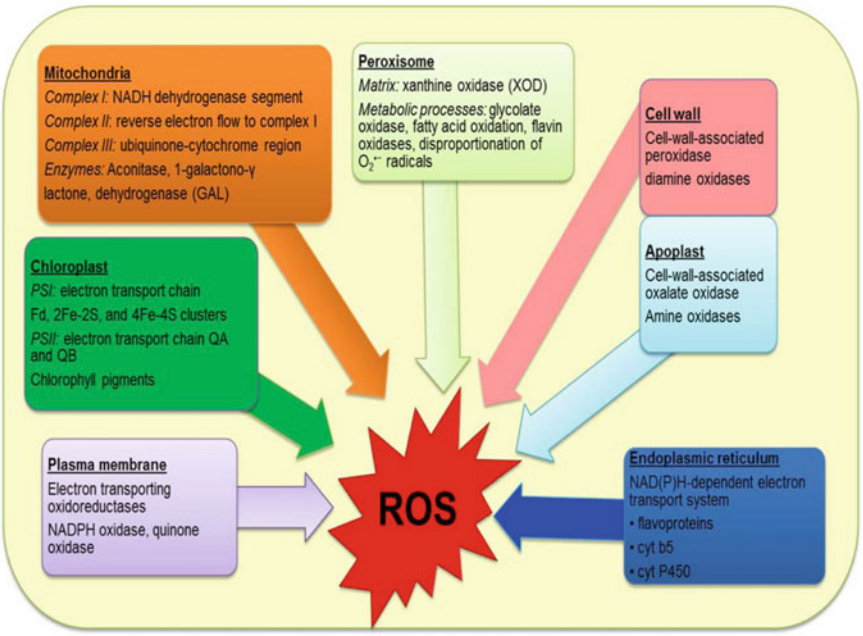

Fig. 1 Sites of production of reactive oxygen species (ROS) in plants

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