Nitric Oxide and Hydrogen Peroxide in Root Organogenesis

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Abstract Nitric oxide (NO) and reactive oxygen species (ROS) are central messengers in the way plants respond to environmental and hormonal stimuli and for the configuration of root architecture. ROS determine the boundaries between the meristem and cell elongation zone of the primary root and act in concert with NO to promote lateral root primordia maturation and epidermal cell differentiation. Overall, the capacity of roots to acquire nutrients such as phosphate, nitrate, and sulfate is determined by NO and ROS via their effects on root hair development and expression of genes for improving nutritional responses or orchestrating the activities of proteins of all major hormonal pathways, including auxin, ethylene, jasmonic acid, brassinosteroids, and abscisic acid. Specifically, ROS target phosphatases and transcription factors of two main families, MYB and BHLH, these later being probably recruited by the *mediator* complex to the promoters of genes for transcription. Here, we review the information about the functions and mechanisms of NO and ROS modulated-root organogenesis, including growth, patterning, and differentiation.

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1 Root System Architecture and Patterning

The root system provides support to photosynthetic and reproductive organs of plants and explores the soil to acquire water and nutrients. Its tridimensional configuration commonly referred to as root architecture depends upon cell division, elongation and differentiation in the primary root, and branching patterns that develop postembrionically, all these aspects being fine-tuned regulated by the growth conditions.

The model plant *Arabidopsis thaliana* develops a taproot system typical of dicots, in which the primary root sustains indeterminate growth and forms a dominant axis. In contrast, multiple embryonic primary and seminal roots are formed in cereals such as maize, sorghum, and wheat (Martínez-de la Cruz et al. 2015; Rogers and Benfey 2015). Adventitious and/or lateral roots increase the overall root absorptive capacity since every branch can develop specialized types of epidermal cells termed root hairs, which are directly involved in water and nutrient uptake (Salazar-Henao et al. 2016; Du and Scheres 2018; Stoeckle et al. 2018).

Roots are very sensitive and respond to light, gravity, acidity or alkalinity, temperature, and salinity (Mo et al. 2015; Ruiz-Herrera et al. 2015; Ha et al. 2018). In actively growing root tips, three main regions can be distinguished: the root apical meristem, an elongation zone, and a differentiation region. In the meristem, cells proliferate at a high rate through mitosis, which is sustained by the root stem cell niche (RSCN), composed by the quiescent center (QC) and peripheral initials or stem cells that divide asymmetrically for self-renewing (Cederholm et al. 2012). Later on, the daughter cells enter into the elongation zone where mitosis ceases, increase in size, and begin to differentiate (Tsukagoshi 2016). The differentiation region is typified by the formation of root hairs from epidermal cells termed trichoblasts, vascular tissue formation occurs, and lateral roots initiate from pericycle cells (Salazar-Henao et al. 2016; Du and Scheres 2018). All major root developmental transitions are related to specific NO and ROS signatures. This chapter updates the knowledge on the functions and mechanisms of NO and ROS in the signaling network that orchestrates root system development.

NO plays critical roles in plant growth and in all major developmental transitions, including embryogenesis, seed germination, root system configuration, flowering, fruit maturation, and leaf senescence (Domingos et al. 2015). Biochemical and cellular analyses show the presence of NO and NO-derived molecules in roots of several species, for instance, using fluorescent indicators, like 4,5-diaminofluorescein diacetate (DAF-2DA), which is a cell-permeable compound that when nitrosylated by NO emits fluorescence (Airaki et al. 2015; Corpas and Barroso 2015; Yamasaki et al. 2016), has revealed that in *Arabidopsis*, NO accumulates in the primary root tip, trichoblast cells, and lateral root cap, and its levels

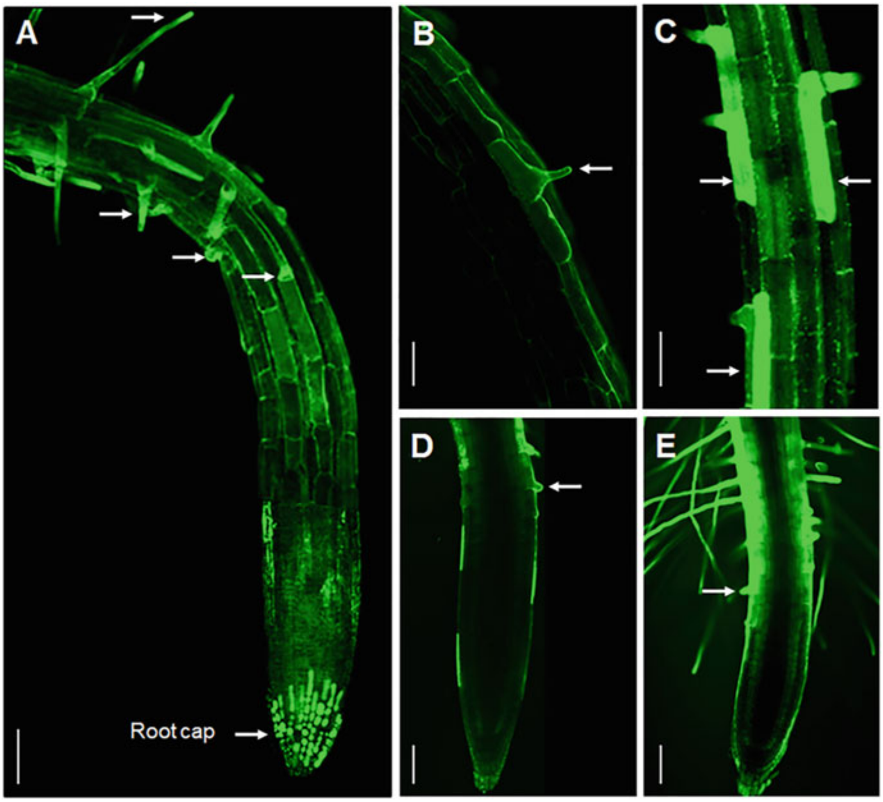

Fig. 1 NO detection in distinct cell types and zones of the *Arabidopsis* primary root. Representative confocal images of primary roots from 7-day-old *Arabidopsis* (Columbia-0 ecotype) seedlings that were stained with the specific NO indicator DAF-2DA for NO detection. (**a**) Primary root. (**b**) Root hair initiation in differentiation zone. (**c**) NO accumulation in trichoblast cells. (**d**, **e**) Root tips under standard growth conditions or supplemented with NO donor SNP, respectively. Arrows in (**a**) show NO accumulation in several stages of root hair development and in root cap cells, in (**b**) mark a trichoblast that just started to elongate, and in (**d**) and (**e**) show the first root hair developed. Fluorescence signal was detected using a confocal laser scanning microscope and monitored with an argon laser with an excitation line from 488 to 568 nm and an emission window from 585 to 610 nm. Scale bar = 70 μm in (**a**, **d**, **e**) and 50 μm in (**b**, **c**). The green color corresponds to the detection of NO. Note the correlation between root hair size and NO fluorescence in SNP-treated seedlings

increase upon treatment with NO donors (Fig. 1). Recent advancements further support the importance of NO cross talking with hormonal pathways for modulation of growth and patterning.

ROS comprise superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radical ($^{\cdot}OH$). These reactive molecules are generated at organelles, including mitochondria, chloroplasts, and peroxisomes, but also in the plasma membrane by ROS-generating enzymes, such as the plant homologs of

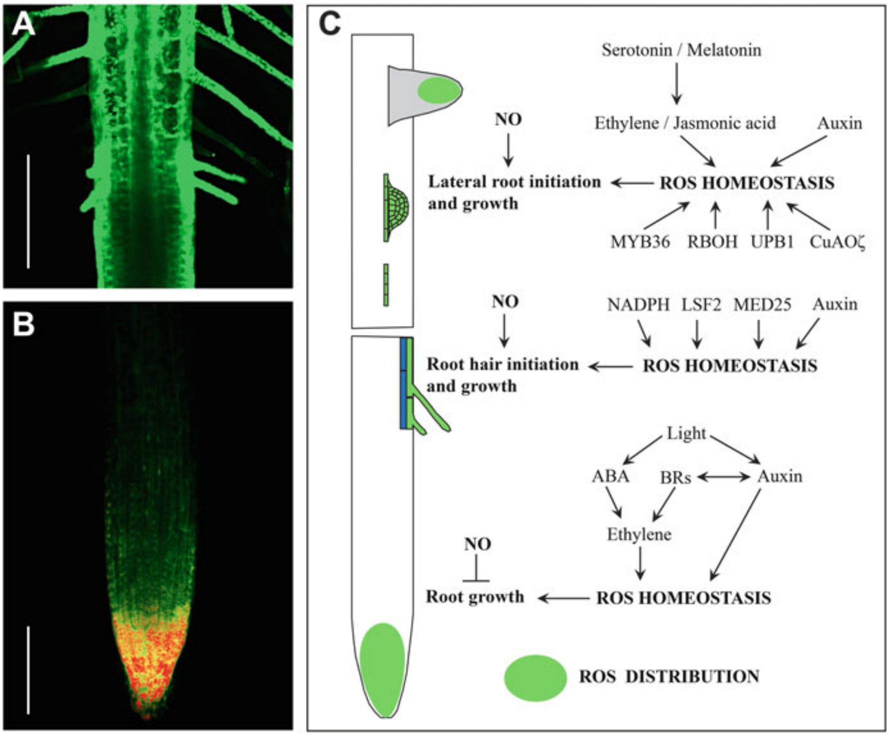

Fig. 2 Roles of NO and ROS in root organogenesis. ROS detection by confocal microscopy using: (**a**) fluorescent dye staining H2DCF-DA (2′,7′-dichlorofluorescein) in the differentiation zone and (**b**) the H_2O_2-specific sensor 35*s:Hyper:YFP* in the meristem. The image in (**b**) was acquired at 405 nm wave lengths for Hyper-H_2O_2-independent excitation and 485 nm for Hyper-H_2O_2-dependent excitation and an emission line of 530 nm. (**c**) NO and ROS signaling modulate primary root growth, root branching, and root hair production. Red/yellow color shows the greater H_2O_2 concentration within the primary root tip. Scale bars in **a** and **b** = 100 μM

respiratory-burst NADPH oxidases and class III peroxidases (Apel and Hirt 2004; Marino et al. 2012; Corpas et al. 2015; Tsukagoshi 2016). Plants accumulate ROS in specific root domains and/or in response to physiological or environmental stimuli (Fig. 2; Xia et al. 2015; Mittler 2017). ROS target DNA, proteins, lipids, and carbohydrates, causing conformational changes (Stadtman and Levine 2003; Nowicka et al. 2013; Tian et al. 2018), and their effects on cellular processes are balanced by the rate of production and cell detoxification via antioxidants or scavenging enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). ROS are not inherently toxic, such that the oxidation of regulatory proteins may improve their function and all types of oxidative modifications are important for cell, tissue, and organ specification (Foyer et al. 2017; Tian et al. 2018).

2 Primary Root Growth

Application of sodium nitroprusside (SNP), a NO donor to tomato, cucumber, and *Arabidopsis* plants, reduces primary root growth, whereas the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) blocks the action of NO donors and increases root elongation. Characterization of the *Arabidopsis* NO-hypersensitive mutant, *no overproducer/chlorophyll a/b binding protein underexpressed* 1 (*nox1/cue1*), showed the correlation between SNP hypersensitivity and formation of a shorter primary root (Correa-Aragunde et al. 2004; Fernández-Marcos et al. 2011).

In *Arabidopsis*, low NO levels cause programmed cell death (PCD), whereas NO accumulation induces DNA damage and cell cycle arrest at G1 and G2/M phases, restraining root growth (Bai et al. 2012). Shen et al. (2013) showed that the expression of CYCD3;1 is modulated by NO levels, NO production mutant *Atnos/noa1* defective at *Arabidopsis thaliana nitric oxide synthase 1/nitric 101 oxide associated 1* have repressed the *CYCD3;1* gene, whereas its constitutive expression complements the mutant phenotype and restores normal root development. The undifferentiated status of root stem cells requires expression of *WUSCHEL-related homeobox 5* (*WOX5*) in the quiescent center. Sanz et al. (2014) showed that application of NO synthesis inhibitors reduces WOX5 expression and this effect could be reverted by SNP supplementation. In addition, the development of the *Arabidopsis* triple mutant *nia1 nia2 noa1* defective on the nitrate reductases 1 and 2 confirmed that decreased NO production and signaling lead to small root meristems with abnormal divisions. The mentioned evidences point to NO as a key player in regulating stem cell activity and root meristem maintenance.

ROS determine the balance among cell proliferation and differentiation in the *Arabidopsis* primary root. ROS are distributed within the root tip, particularly in the meristem and elongation zone (Fig. 2; Tsukagoshi et al. 2010; Hernández-Barrera et al. 2015). Considerable advances have been made toward identifying the proteins orchestrating ROS homeostasis in roots. An *Arabidopsis* P-loop NTPase (APP1) protein located in mitochondria of root meristems displays ATPase activity and hydrolyzes nucleoside triphosphates. Mutation of *APP1* gene causes a reduction in ROS levels and increases cell division rate in the QC, which leads to stem cell differentiation (Yu et al. 2016). Treatments with methyl viologen (MV) and H_2O_2 normalized root patterning, implying that both an increased rate of cell division in the QC and stem cell differentiation can be attributed to a low level of ROS. APP1 acts upstream of the key transcription factors *scarecrow* (*SCR*) and *short root* (*SHR*) to control the undifferentiated status of the meristem and to fine-tune root stem cell niche activity. Impairment of root meristem proliferation could also be observed in *Arabidopsis* mutants lacking the mitochondrial protease AtFTSH4, for which exposure to high temperatures caused the precocious cessation of root growth, which correlates with oxidative stress and progressive mitochondria dysfunction (Dolzblasz et al. 2018).

Kong et al. (2018) characterized the *Arabidopsis* prohibitin PHB3, which belongs into a highly conserved family of proteins that restrict cell proliferation in all three domains of life. *phb3 Arabidopsis* mutants developed a short root with higher rate of QC division and meristem differentiation that correlates with both $O_2^{\cdot-}$ and H_2O_2 accumulation. RNA-seq analysis was performed to compare the root transcriptomes of WT and *phb3* mutants, in which the expression of ethylene response factors (ERF) 115, 114, and 109 was highly increased in *phb3* roots and was antagonized by diphenyleneiodonium (DPI), an inhibitor of NADPH oxidases. Tissue-specific gene expression analysis further indicated that PHB acts through phytosulfokine (PSK) peptide hormones and independently of the *plethora* (PLT) transcription factors. Taken together, these data indicate that ERF115, ERF114, and ERF109 mediate ROS signaling downstream of PHB3 to control root stem cell niche maintenance.

ROS mediates root meristem function during sensing of pathogens or molecules derived from microbes. Mabuchi et al. (2018) identified genes regulated by H_2O_2 in the meristem and elongation zone, including the transcription factor with basic helix-loop-helix (BHLH) domains termed *upbeat1* (UPB1), which controls the expression of a set of peroxidases that establish the ROS gradient correlated with root tip zonation. ROS-induced expression of MYB30, a transcription factor responsive to pathogens and targets genes involved in the transport of very-long-chain fatty acids (VLCFAs) in the epidermis and cortex cell layers of the root tip. Comparison of root growth of wild-type and *myb30-2* seedlings to treatment with Flg22, a microbe-associated molecular pattern (MAMP) that represses growth revealed the resistance of the mutants, suggesting that a MYB30-dependent regulatory network links root growth and immunity. This indicates that growth and defense trade-offs are orchestrated via a single regulatory node, and to this respect, roots colonized with the plant pathogen *Pseudomonas aeruginosa* or treatment with the bacterial virulence factor pyocyanin caused a strong reduction of cell division and elongation (Ortiz-Castro et al. 2014). In this case, however, pyocyanin modulation of the primary root growth required the gaseous hormone ethylene for signaling, since the *Arabidopsis etr1-1*, *ein2-1*, and *ein3-1* ethylene-related mutants were less sensitive to pyocyanin-induced root stoppage. Not only bacterial toxins modulate ROS levels in roots, Pelagio-Flores et al. (2016) while characterizing the role of the neurotransmitter serotonin as a plant signaling molecule found that the ethylene inhibitor $AgNO_3$ antagonized its growth-repressing effects on *Arabidopsis* primary roots, whereas *ethylene overproducer 3* mutants were oversensitive to this compound. Thus, ethylene and ROS are mediators in transducing serotonin and pyocyanin bioactivity.

3 Root Branching

Root branching occurs via the formation of lateral roots, structures formed by de novo organogenesis from pericycle, an inner tissue within mature roots. Overall, lateral root formation comprises two main programs: (1) initiation, through which a lateral root primordium is formed from lateral root founder cells, and (2) emergence, which allows the passage of the primordium through at least three overlying cell layers, the endodermis, cortex, and epidermis, in order to emerge (Du and Scheres 2018; Stoeckle et al. 2018).

NO promotes lateral and adventitious root maturation (Liao et al. 2012; Ma et al. 2014). Early studies demonstrated that application of NO donors, SNP and SNAP, to hypocotyl explants of cucumber activated de novo root organogenesis. The cellular components sensitive to NO included Ca^{2+}-dependent protein kinases (CDPKs), phosphatidic acid (PA), and mitogen-activated protein kinases (MAPKs), which involve cGMP and Ca^{2+} as second messengers (Lanteri et al. 2006). Environmental stimuli, such as CO_2, promote lateral root initiation by increasing the production of NO, which subsequently increases cytosolic Ca^{2+} concentration-activating plasma membrane and/or intracellular Ca^{2+}-permeable channels. Besides, NO acts downstream of plant hormones to control root organogenesis, including auxin, jasmonic acid, and ethylene (Fig. 2c). In cucumber, *Arabidopsis*, and rice, NO donors mimic the effect of auxin in adventitious root formation, and NO accumulation in lateral root primordia promoted its maturation (Kolbert et al. 2008; Chen and Kao 2012). NO is indispensable for crown root primordia formation, whereas a reduction of intracellular levels blocks this process in rice seedlings (Xiong et al. 2009).

A few *Arabidopsis* mutants have been identified, which represent valuable tools for investigating NO biosynthesis and/or signaling, including *argh1/2* (*arginine amidohydrolase 1/2*), which is defective in an enzyme involved in arginine (Arg) biosynthesis (Flores et al. 2008). Mutations of either *Arabidopsis ARGH1*, two genes result in increased formation of lateral and adventitious roots, accompanied with NO accumulation. *argh1/2* double mutants show higher sensitivity on lateral root formation in response to auxin and increased expression of the auxin-responsive reporter *DR5:GUS* in root tips, suggesting that arginine or its derivatives are potential NO sources to control root morphogenesis (Flores et al. 2008).

The cell wall remodeling of pericycle overlying tissues correlates with ROS deposition in the apoplast, which overlaps with the expression domains of extracellular ROS donors of the *Respiratory burst oxidase homologs* (RBOH), and disrupting or enhancing expression of *RBOH* promotes or antagonizes lateral root emergence (Orman-Ligeza et al. 2016). Thus, RBOH-mediated ROS production establishes the lateral root primordium boundary that facilitates lateral root outgrowth, and it was recently found to depend on the activity of the MYB36 transcription factor (Fernández-Marcos et al. 2017). MYB36 is expressed in the endodermis of primary roots and in developing lateral root primordia, and in *myb36-1* mutants, these structures manifest defective progress after stages IV–V leading to a flat appearance in contrast to the dome-shaped form of the wild type. MYB36 controls a set of peroxidase genes, which maintain the ROS balance in cell tissues overlying the primordium and in this manner fine-tunes its emergence through the parent layers.

ROS are specifically involved in lateral root outgrowth, since H_2O_2 supplementation accelerates lateral root development from newly formed primordia. The effects of ROS is independent of auxin signaling, because H_2O_2 could restore lateral root formation in auxin-related mutants that are defective in cell wall softening and remodeling (Orman-Ligeza et al. 2016). ROS may also act downstream of auxins as reported for the function of the *Arabidopsis* peroxisome-localized copper amine oxidase ζ (CuAOζ), which controls the ROS production essential for lateral root development. Mutation of CuAOζ results in deficient auxin-induced ROS generation and *pinformed2* (PIN2)-mediated auxin transport (Qu et al. 2017). The UPB1 transcription factor responsible of ROS distribution within the primary root tip is critical in the branching process as *upb1-1* mutants develop a higher number of emerged lateral roots and, conversely, UPB1 overexpressing roots accumulate more primordia, which nowadays failed to emerge (Manzano et al. 2014). In addition, hormonal stimuli that trigger the root branching program rely on ROS to break lateral root primordium quiescence. Treatment of *Arabidopsis* roots with neurotransmitters serotonin and melatonin increased lateral root development, which correlated with higher H_2O_2 levels (Pelagio-Flores et al. 2011, 2012; Chen et al. 2018). The compounds modulated expression of genes responsible for G2-M cell cycle transition, including CDKB1;1, CDKB2;1, CDKB1;1, and CDKB2;1, in a process that involves ethylene and jasmonic acid signaling (Pelagio-Flores et al. 2016; Chen et al. 2018).

4 Root Hair Development

Root hairs are tubular outgrowths from epidermal cells termed trichoblasts that are specialized in form and function to take up water and nutrients and represent an important niche for bacteria inhabiting the rhizosphere. Root hairs develop from a bulge in the trichoblast, which elongates via tip growth and reaches its maximum size (up to 1 mm in *Arabidopsis*) depending upon the plant hormonal status and nutrient availability in the soil and proceeds through the generation of a high Ca^{2+} gradient (Shin et al. 2005; Bhosale et al. 2018; Dindas et al. 2018). For instance, phosphate starvation increases up to threefold the overall root surface through inducing extra cell files of root hairs and increasing the growth of these structures in a NO- and ROS-mediated genetic program (López-Bucio et al. 2003; Ruiz-Herrera et al. 2015; Gutiérrez-Alanís et al. 2018).

NO promotes epidermal cell differentiation during root hair development of lettuce (*Lactuca sativa*) and *Arabidopsis* (Fig. 1a, d, and e; Lombardo et al. 2006). SNP application to lettuce plants resulted in almost all rhizodermal cells to be differentiated into root hairs. Treatment with the synthetic auxin 1-naphthyl acetic acid (NAA) increased root hair formation that was prevented by the NO scavenger, cPTIO (Lombardo et al. 2006). Two *Arabidopsis* mutants associated to NO production, namely, *Atnos1/noa1* and *nia1 nia2* single and double mutants, respectively, are affected in root hair growth, which could be phenocopied in wild-type plants by cPTIO (Lombardo et al. 2006). Interestingly, NO was detected inside the vacuole of root hairs and acts as a critical component for endocytosis, vesicle formation, and trafficking, nucleus migration, and vacuolar development during root hair growth, and NO application restored vesicle formation and trafficking in *nia1 nia2* mutants.

Root hair development is increased in *Atgsnor1-1* and reduced in *Atgsnor1-3* plants defective on *S-nitrosoglutathione (GSNO) reductase* (*AtGSNOR*), which catalyzes the posttranslational modifications of proteins via the addition of an NO moiety to a reactive cysteine thiol, to form an S-nitrosothiol (Kwon et al. 2012). Thus, nitrosylation can be regarded as an instructive signal for tip growth of root epidermal cells.

Different molecules have been reported to affect ROS homeostasis principally during root hair development. For instance, treatments with vanadate increases root hair density and length, and this correlated with induced ROS production. Lin et al. (2015) investigated the pathways involved in vanadate-induced root hair formation in *Arabidopsis* by supplying diphenylene iodonium (DPI), an inhibitor of NADPH oxidase, and using the NADPH oxidase mutant *root hair-defective* mutant 2 (*rhd2*) that encodes a NADPH oxidase (AtrbohC). Vanadate changed the levels of transcripts related to cell wall formation and ROS signaling and required the NADPH oxidase. Taken together, these studies support the important role of ROS homeostasis in regulating root hair growth in response to environmental stress.

Several screens identified *Arabidopsis* mutants lacking root hairs or producing short-root hairs that helped clarifying the roles of ROS in polar growth. The RHD (*root hair defective*) /RSL (*root hair defective like*) transcription factors control both the initiation and elongation phases during root hair development. Foreman et al. (2003) showed that *rhd2* regulates root hair growth through the activation of Ca^{2+} channels (Foreman et al. 2003). On the other hand, RHD6 activates the RSL4/RSL2 transcription factors, which act downstream of auxin to release *auxin response factors* (ARFs) ARF5, ARF7, ARF8, and ARF19 from Aux/IAA proteins. Auxin activation of RSL4 expression was related to changes in ROS homeostasis through the RBOHC, H, and J proteins and four type III-secreted peroxidases (Mangano et al. 2017, 2018).

Auxin response involves several components of the *mediator* (MED) transcriptional complex, which acts as a bridge between ARFs and the RNA polymerase II. *Phytochrome and flowering time1* (PFT1) corresponds to the MED25 subunit and its loss of function renders plants oversensitive to auxin (Raya-González et al. 2014). Global gene expression analysis revealed the activation of class III peroxidases by PFT1, while the corresponding *Arabidopsis* mutants had an altered $O_2^{\cdot -}$ and H_2O_2 distribution, indicating that PFT1 is critical to maintain redox homeostasis. Normalization of ROS levels rescued the *pft1* mutant phenotype, suggesting its essential prerequisite for root hair patterning through cell wall remodeling genes (Sundaravelpandian et al. 2013). These results link the MED complex via PFT1/MED25 to the transcriptional machinery orchestrating ROS distribution.

In plants, protein phosphatases regulate a myriad of cellular processes via dephosphorylation reactions that affect ROS homeostasis. The *starch excess4* (SEX4) and *like sex four2* (LSF2) are two glucan phosphatases controlled by the redox status. LSF2 is located in the chloroplast and cytoplasm and is related to starch metabolism. Zhao et al. (2016) characterized the *lsf2-1* mutant, which shows reduced rates of $O_2^{\cdot -}$ generation and higher levels of H_2O_2 in response to oxidative stress, which correlates with root hair growth. LSF2 interacts with mitogen-activated protein kinase 8 (MPK8), a known component of ROS homeostasis pathways in the cytoplasm. Thus, a MAPK cascade may integrate LSF2 function, ROS homeostasis, and root hair development.

5 Shoot-to-Root Long-Distance Signaling

Roots respond to local soil conditions as well as to systemic signal cues, and this is important for adaptation and survival to the dynamic environment (Raya-González et al. 2017). Light is required for photosynthesis and reconfigures plant architecture, such that different wave lengths are perceived in stems and leaves through red/far-red photoreceptor phytochromes, or cryptochromes, which mediate primary root elongation, gravitropism, and hormone responses (Lee et al. 2016). Abscisic acid (ABA) accumulates in shoots following light exposure and is transported long distance to roots where it triggers developmental plasticity linked to ROS, antioxidants, and ROS-detoxifying enzymes, specifically during mitosis (de Tullio et al. 2010).

Two reports have clarified the mechanisms by which ABA, imported from shoots or locally produced in roots, influence meristematic activity, and both involved ROS as second messengers. Yang et al. (2014) identified a recessive, ABA-oversensitive *Arabidopsis* mutant with retarded growth named *abo8-1*, which is defective in a pentatricopeptide repeat (PPR) protein responsible for the correct functioning of the mitochondrial complex I. Interestingly, *abo8-1* mutants accumulated more ROS in root tips than the wild type, and this effect was exacerbated by ABA treatment. High ROS levels reduced root meristem activity through affecting the expression of genes that determine stem cell niche identity, whereas the normal growth could be reversibly recovered by treatment with the reducing agent GSH. In the other works, Ha et al. (2018) showed that in *Arabidopsis* plants exposed to light, the phyB photoreceptor stimulates ABA synthesis in shoots and then the hormone moves to roots and triggers a peroxidase-mediated ROS detoxification.

UV light may cause damage to DNA and the residing mutations often result in cell death. Genetic screens aimed at identifying ABA-related genes found the MED18 subunit of the transcriptional *mediator* complex, because the *med18* loss-of-function mutant is oversensitive to root growth inhibition by ABA (Zhu et al. 2017). Noteworthy, the *med18* mutants show delayed root growth, related to cell death in the root meristem, which exacerbates with age and/or exposition to DNA damaging agents (Raya-González et al. unpublished). Cell death was reduced in *med18* seedlings grown in darkness but remained when only the shoot is exposed to light, suggesting that MED18 acts to protect root meristem cells from local cell death, and/or in response to root-acting signal (s) such as ABA and/or ROS emitted by the shoot in response to light stimuli. *med18* mutants overexpress the cell regeneration factor ERF115, which triggers cell division and replenishes the stem cell pool during root tip regeneration in a similar manner to animal limb recovery, and in such case the lost part of the body could be replaced through conversion of normal cells into stem cells that act as progenitors of the missing tissues (Efroni et al. 2016; Heyman et al. 2016). As mentioned above ERF115 overexpression is found in *phb3* mutants that displayed a short-root phenotype dependent of ROS deregulation. These data evidences the break point between root growth and adaptation to stress, which is integrated by a complex but fine-tuned pathway in which ABA, MED18/ERF115 and ROS are key components.

6 Hormone Cross Talk

NO and ROS interact with most signaling pathways underlying hormonal and nutritional responses in plants, which influence, at least to some degree, the endogenous levels of these reactive molecules (Freschi 2013; Sanz et al. 2015; Liu et al. 2018; Sun et al. 2018). Brassinosteroids-auxin-ethylene cross talk activates NO- and ROS-dependent mechanisms for growth modulation, which occur in a concentration and tissue-dependent manner. Brassinosteroid (BR) synthesis and signaling enable root growth and development, but their alteration by either pharmacological or genetic means induces a short-root phenotype through decreased cell division and elongation (Wei and Li 2016; Lv et al. 2018). The response of roots to BRs application correlated with enhanced NO levels and was blocked by cPTIO, suggesting that NO is required for BR-induced changes in root system architecture (Tossi et al. 2013). Indeed, the promoting effect of ethylene on adventitious rooting in cucumber explants could be reverted by cPTIO and NO synthesis inhibitors (Xu et al. 2017).

Tian et al. (2018) showed that BRs binding to its receptor kinase BRI1 promoted dephosphorylation of the transcription factor *brassinazole-resistant1* (BZR1), and increased intracellular levels of H_2O_2, which in turn caused oxidation of BZR1 at a conserved cysteine residue. This modification promoted the interaction with *auxin response factor6* (ARF6) and *phytochrome interacting factor4* (PIF4), which act as regulators in the auxin and light-signaling pathways, respectively. A genetic screen of *Arabidopsis* mutants producing short primary roots identified the *det2-9* mutant defective in a steroid 5α-reductase from the BR synthesis pathway. The *det2-9* root phenotype correlated with reduced cell number in meristem and decreased cell size at the maturation zone, which was caused by an enhanced rate of ethylene biosynthesis and was recovered in the *det2-9/acs9* double mutant and *det2-9/ein3/eil1-1* triple mutant, which have defects either in ethylene synthesis or ethylene signaling, respectively. These data indicate that ethylene signaling acts downstream of BRs for the modulation of cell processes that determine primary root growth. Interestingly, the *det2-9* mutant produced more $O_2^{\cdot-}$ than wild type plants through the peroxidase pathway (Lv et al. 2018).

The alkamides comprise a group of fatty acid amides, which have emerged as modulators of root development (López-Bucio et al. 2006). *Arabidopsis* root explants treated with *N*-isobutyl decanamide showed higher adventitious root number and an increase in NO accumulation in zones of adventitious root formation

(Campos-Cuevas et al. 2008). Later on, Méndez-Bravo et al. (2010, 2011) found that morphogenetic effects of alkamides decreased by cPTIO application. Interestingly, *Arabidopsis* mutants defective at the *DRR1* (*decanamide resistant root 1*) locus were less sensitive in both primary root reduction and lateral root promotion to NO treatments and bacterial quorum-sensing perception and had decreased senescence (Morquecho-Contreras et al. 2010), suggesting its role as a modulator in small lipid amide and NO sensing.

7 Conclusions

NO and ROS production by plants has been traditionally related with adaptation to stress and defense against pathogens. The emerging view is that accumulation and/or distribution of these reactive molecules support the basic cellular programs defining tissue and organ shape (Fig. 2c). Their fundamental role underlies root growth and development and goes beyond the polarized tip growth of the primary root, lateral roots, and root hairs.

Major roles of NO have been defined during lateral root formation, and its alteration causes root apical meristem defects and growth inhibition while reducing auxin transport. It also orchestrates root architecture configuration in response to bioactive metabolites such as alkamides, bacterial quorum-sensing signals, and cross talks with most phytohormone signaling pathways including auxin, ethylene, and jasmonic acid. The recent characterization of NO-related mutant *drr1* of *Arabidopsis*, unraveled its critical function in plant senescence, whereas NO production through nitrate reductases, NOS, and NOS-related enzymes supports a direct link among nutrition and metabolism that should influence all major plant phase transitions.

The environmental and hormonal long-distance communication between shoots and roots are orchestrated by ROS acting in the meristems. The ongoing characterization of *Arabidopsis* mutants has proven to be useful toward identifying the signaling players in ROS accumulation/detoxification, for which phosphatases and MYB and BHLH transcription factors orchestrate gene expression, probably being recruited by the *mediator* complex to the promoters of genes for transcription.

A very interesting perspective is that an ABA-ROS signaling could inform the root of the light quality in leaves to fine-tune cell division and elongation and, even more, to support regeneration of damaged tissues. Global gene expression analysis demonstrated that the regulatory network orchestrated by ROS is dynamic and specific and that the phytohormones auxin, ethylene, jasmonic acid, and brassinosteroids influence positively or negatively ROS levels in the meristem and elongation zones and determine the rate of growth of primary and lateral roots. Characterization of the activities of the proteins and other macromolecular targets of ROS may confirm that nitrosylation and oxidation have fundamental roles in organogenesis and in the way plants react to the provision of mineral nutrients, such as nitrate, phosphate, and sulfate, which are required in high amounts to support agriculture.

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