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REVIEW PAPER

Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance

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Abstract

As a consequence of a sessile lifestyle, plants are continuously exposed to changing environmental conditions and often life-threatening stresses caused by exposure to excessive light, extremes of temperature, limiting nutrient or water availability, and pathogen/insect attack. The flexible coordination of plant growth and development is necessary to optimize vigour and fitness in a changing environment through rapid and appropriate responses to such stresses. The concept that reactive oxygen species (ROS) are versatile signalling molecules in plants that contribute to stress acclimation is well established. This review provides an overview of our current knowledge of how ROS production and signalling are integrated with the action of auxin, brassinosteroids, gibberellins, abscisic acid, ethylene, strigolactones, salicylic acid, and jasmonic acid in the coordinate regulation of plant growth and stress tolerance. We consider the local and systemic crosstalk between ROS and hormonal signalling pathways and identify multiple points of reciprocal control, as well as providing insights into the integration nodes that involve Ca^{2+}-dependent processes and mitogen-activated protein kinase phosphorylation cascades.

Key words: Calcium, glutathione, hydrogen peroxide, mitogen-activated protein kinase, redox signalling, *RESPIRATORY BURST OXIDASE HOMOLOG*, stress acclimation.

Introduction

Aerobic metabolism has embraced the generation and effective utilization of reactive oxygen species (ROS) in a plethora of metabolic pathways and functions. ROS such as $O_2^{\bullet-}$ and H_2O_2, and also possibly $OH^{\bullet}$ in cell compartments such as the cell wall are generated by metabolism. Photosynthesis is the major producer of singlet oxygen, $O_2^{\bullet-}$ and H_2O_2 in the chloroplasts. Through photorespiration, photosynthetic carbon metabolism also supports a high flux of H_2O_2 production in the peroxisomes, particularly in C_3 plants. Mitochondrial electron transport also produces $O_2^{\bullet-}$ and H_2O_2. Although these oxidants are rapidly removed by the antioxidant systems in these organelles, an accumulation of H_2O_2 is often observed in chloroplasts and mitochondria in plants exposed to abiotic and biotic stresses (Foyer and Noctor, 2003). Moreover, any kind of physical, chemical, or metabolic shock activates the plasma-membrane-bound NADPH oxidases and apoplastic peroxidases, leading to an oxidative burst. For example, the pathogen-induced oxidative burst in the apoplast/cell-wall compartment is particularly well documented (Laloi *et al.*, 2004). ROS accumulation in plants

Abbreviations: ABA, abscisic acid; BR, brassinosteroid; BRZ, brassinazole; DPI, diphenyleneiodonium; ET, ethylene; GA, gibberellin; GR, glutathione reductase; GRX, glutaredoxin; JA, jasmonic acid; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PAMP, pathogen-associated molecular pattern; PCD, programmed cell death; QC, quiescent centre; ROS, reactive oxygen species; SA, salicylic acid; SAA, systemic acquired acclimation; SAR, systemic acquired resistance; SL, strigolactone; TRX, thioredoxin.

exposed to stress is a linchpin of the plant stress response, leading to changes in gene expression that allow acclimation to stress. However, excessive accumulation will trigger genetically programmed cell suicide pathways (Foyer and Noctor, 2005). A complex antioxidants network has evolved in plant cells to scavenge ROS and so regulate their levels according to the requirements of cell signalling. Plants accumulate large amounts of low-molecular-weight antioxidant metabolites such as ascorbate, glutathione, and tocopherol, and they have a large network of enzymatic antioxidants such as superoxide dismutases, ascorbate peroxidases, catalases, glutaredoxins (GRXs), and peroxiredoxins (Mittler *et al.*, 2004). The evolution of this complex oxidant (ROS)/antioxidant network allows a flexible control of cellular ROS levels. In this way, ROS can be generated as powerful oxidative signals with antioxidants either limiting the lifetime of the signal or in the case of thiol antioxidants propagating ROS signals within plant cells. The ability to harness the chemical reactivity of ROS so that they can act as powerful signalling molecules is a common feature of aerobic organisms, including plants, which have mastered the ability to control ROS during evolution in order to confer a high degree of adaptability in response to changing environmental conditions.

Many plant hormones generate ROS as part of the mechanism that regulates plant growth and development. Moreover, regulated ROS production as an intrinsic regulator of cell-cycle progression in plants has been suggested to occur in a similar manner to that in animal cells (Vivancos *et al.*, 2010). It is likely that there is spatial and temporal regulation of ROS production and accumulation during the plant development programme and stress response, but the specificity of such responses remains poorly documented. Molecular genetics approaches have shown that a block in ROS production by plasma membrane-bound NADPH oxidases leads to impaired root hair formation (Foreman *et al.*, 2003), altered shoot branching (Sagi *et al.*, 2004) and decreased stress resistance (Marino *et al.*, 2012). It is perhaps not surprising, therefore, that the accumulation of ROS in different cellular compartments can cause opposing physiological responses (Dat *et al.*, 2000; Foyer and Noctor, 2000). By analogy to another secondary messenger, Ca^{2+}, the spatial and temporal regulation of ROS dynamics encodes specificity, allowing proteins undergoing thiol redox regulation in specific compartments to simultaneously detoxify and sense ROS, and to transduce the signal in order to activate downstream transcriptional regulation (Dietz, 2008; Mittler *et al.*, 2011). The regulation of master regulator of disease resistance NONEXPRESSOR OF PATHOGENESIS-RELATED PROTEINS1 (NPR1) is a classic example of this type of regulation, in which specifically regulated changes in cellular redox status lead to regulation of gene expression and consequently a physiological response to pathogen attack (Mou *et al.*, 2003). In recent years, more and more redox regulators of plant development and stress tolerance have been identified including glutathione, GRXs, glutathione reductases (GRs), and thioredoxins (TRXs), and similar redox-active proteins (Ndamukong *et al.*, 2007; Bashandy *et al.*, 2010; Yu *et al.*, 2013). While the precise mechanisms that facilitate ROS/redox regulation of plant growth and stress tolerance remain to be elucidated, an increasing body of evidence supports the concept that ROS are critical second messengers in hormone signalling that co-ordinately regulate plant development and stress tolerance (Table 1).

There is now a substantial body of literature concerning the mechanisms by which hormones regulate plant development and stress tolerance. Intrinsic to this regulation is the hormone-dependent activation of ROS production, often through the activation of NADPH oxidases, which are encoded by *RESPIRATORY BURST OXIDASE HOMOLOG* (*RBOH*) genes in plant genomes (Sagi and Fluhr, 2006). While the specific roles of ROS in hormone-mediated regulation of plant development and stress tolerance remain to be characterized, the following discussion seeks to provide a critical appraisal of the current literature and personal perspectives concerning the integration and crosstalk between ROS and hormone signalling pathways.

Auxin

Auxin, which plays a fundamental role in plant growth and development, is transported through the plants by both passive and active mechanisms. Polar auxin transport establishes an auxin gradient across tissues to create centres of accumulation called auxin maxima, which determine polar cell growth and morphogenesis (Grunewald and Friml, 2010). This is achieved by the differential localization of PIN-FORMED (PIN) proteins in cell membranes. PIN proteins are rate-limiting auxin efflux transporters. Conversely, AUXIN RESISTANT 1 (AUX1) plays a key role in mediating auxin import (Benjamins and Scheres, 2008). The key aspect of PIN and AUX protein functions is their polar localization in the plasma membranes, which is feedback regulated by auxin (Sauer *et al.*, 2006).

The action of auxin in the regulation of plant growth and development is closely associated with ROS. Auxin-induced ROS are directly involved in cell-wall loosening and play a role in cell elongation (Schopfer, 2001b). Overexpression of the cell-wall-localized enzyme ascorbate oxidase results in increased oxidation of the apoplast and mimics auxin-mediated effects on plant growth (Pignocchi *et al.*, 2003). Auxin-induced ROS production and associated changes in the cellular redox state act as signals in auxin-mediated developmental processes. Asymmetric ROS accumulation mediates auxin-regulated root gravitropism (Joo *et al.*, 2001). Low NADPH oxidase activity in *RBOH* antisense tomato plants results in a highly branched phenotype, a determinate growth habit, and fasciated reproductive organs that are reminiscent of altered auxin homeostasis (Sagi *et al.*, 2004). Moreover, the increased cellular oxidation state associated with auxin maxima is thought to arrest the cell cycle in the quiescent centre (QC), which is important for the maintenance of the root meristem (Jiang *et al.*, 2003; Heyman *et al.*, 2013). Cellular redox status is an intrinsic regulator of the plant cell cycle (Vernoux *et al.*, 2000; Vivancos *et al.*, 2010). Moreover, low activities of GR in the plastids of root cells leading to an accumulation of glutathione disulphide (GSSG) caused a

Table 1. *Hormonal regulation of ROS and physiological functions*

Hormones	ROS sources	Regulator of ROS production	Physiological functions of ROS	References
Auxin	NADPH oxidase	Rho-like GTPase; FERONIA	Cell elongation; QC quiescence; root gravitropism; shoot branching	Duan *et al.* (2010); Foreman *et al.* (2003); Jiang *et al.* (2003); Joo *et al.* (2001); Sagi *et al.* (2004); Schopfer *et al*. (2001b)
BR	NADPH oxidase	Ca^{2+}; cGMP; MAP kinase	Cell elongation; photosynthesis; stress tolerance; stomatal closure or opening	Nie *et al.* (2013); Jiang *et al.* (2012b); Kwezi *et al.* (2007); Xia *et al.* (2009, 2014); Zhao *et al.* (2013); Zhou *et al.* (2014)
GA	NADPH oxidase?	DELLA	Seed germination; root growth; stress tolerance; disease resistance	Achard *et al.* (2006, 2008); Bethke and Jones (2001); Fath *et al.* (2001); Leymarie *et al.* (2012); Liu *et al.* (2010); Navarro *et al.* (2008); Schopfer *et al.* (2001a)
ABA	NADPH oxidase; chloroplast; mitochondria;	PP2Cs; OST1; PA; MAP kinase	Stomatal closure; seed dormancy; root growth; stress tolerance	He *et al.* (2012); Jiao *et al.* (2013); Kwak *et al.* (2003); Mustilli *et al.* (2002); Sirichandra *et al.* (2009); Xing *et al.* (2008); Zhang *et al.* (2006); Y.Y. Zhang *et al.* (2009); Zhou *et al.* (2014)
ET	NADPH oxidase; peroxidase	MKK9?; EIN2/3/4	Stomatal closure; salinity and nutrient stress tolerance; disease resistance; PCD	Bouchez *et al.* (2007); Desikan *et al.* (2006); He *et al.* (2011); Jiang *et al.* (2012a, 2013); Mersmann *et al.* (2010); Muhlenbock *et al*. (2007); Overmyer *et al.* (2000); Shin and Schachtman (2004); Steffens and Sauter (2009); Yoo *et al.* (2008)
SL	NADPH oxidase?	MAX2?; FHY3	Shoot branching; lateral root development; nutrient deficiency response; stress tolerance	Bonneau *et al.* (2013); Bu *et al.* (2014); Ha *et al.* (2014); Ouyang *et al.* (2011); Stirnberg *et al.* (2012)
SA	Chloroplast; mitochondria; peroxidase	Redox status of chloroplast; MAP kinase	Disease resistance	Chen *et al.* (1993); Chamnongpol *et al.* (1998); Han *et al.* (2013a, b); Liu *et al.* (2007); Miura *et al.* (2013); Muhlenbock *et al*. (2008); Khokon *et al.* (2011)
JA	NADPH oxidase	MAP kinase	Wound response; insect resistance	Orozco-Cardenas and Ryan (1999, 2001); Kandoth *et al.* (2007)

loss of QC identity and root meristem integrity (Yu *et al.*, 2013), a response that can partly be explained by disruption of auxin signalling. Such findings raise important questions concerning the role of the redox gradient between QC cells and the surrounding stem-cell initials. Cell division in the QC is activated via ERF115, a member of the ethylene response factor family of transcription factors (Heyman *et al.*, 2013). It seems likely that QC cells are activated by a stress-induced shift in the redox gradient between the QC and adjacent stem-cell initials. Interestingly, the balance between proliferation and differentiation in the root meristem and elongation zones appears to be controlled by the equilibrium between $O_2^{\bullet-}$ and H_2O_2 accumulation in the root tip, which could involve short-range cell–cell communication (Tsukagoshi *et al.*, 2010).

Although auxin-mediated processes are closely associated with ROS, the mechanism underlying auxin-induced ROS production is still obscure. RAC/ROPs, a plant-specific family of Rho-like small GTPases, are mediators for auxin-regulated gene expression, auxin signalling, and polar auxin transport (Wu *et al.*, 2011). Auxin can activate RAC/ROPs and determined the spatially regulated formation of RAC/ROP patches (Tao *et al.*, 2002; Fischer *et al.*, 2006), whereas RAC/ROPs relay the auxin signal to control cytoskeletal organization, vesicle trafficking, and feedback auxin polar transport (Xu *et al.*, 2010; Chen *et al.*, 2012; Lin *et al.*, 2012). Recently, the FERONIA receptor-like kinase has been shown to be essential for the activation of RAC/ROPs and is an upstream regulator of auxin- and NADPH oxidase-dependent ROS accumulation in roots (Duan *et al.*, 2010). While the mechanisms of regulation of NADPH oxidase by RAC/ROPs during development are still lacking, a study on ROS-mediated defence responses in rice showed that RAC interacts with an EF-hand motif containing N-terminal region of NADPH oxidase in a Ca^{2+}-dependent manner (Wong *et al.*, 2007). These studies implicate RAC/ROPs as direct regulators of NADPH oxidase during auxin-mediated developmental processes.

When plants are exposed to environmental stresses, ROS can attenuate auxin signalling, leading to altered plant growth and acclimation (Potters *et al.*, 2007). The *tir1/afb* mutants that are impaired in auxin perception display enhanced tolerance to oxidative stress (Iglesias *et al.*, 2010), which indicates that inhibition of auxin-regulated growth and development is an adaptive strategy that enables plants to withstand stressful environments. The stress-induced accumulation of ROS may alter auxin signalling through oxidative inactivation or degradation of auxin, and also by the decreased expression of genes involved in auxin signalling and polar auxin transport

(Blomster *et al.*, 2011; Peer *et al.*, 2013). Specific mitogen-activated protein kinase (MAPK) cascades such as ANP1–MPK3/6 and MEKK1–MPK4 participate in ROS-mediated downregulation of auxin signalling (Kovtun *et al.*, 2000; Nakagami *et al.*, 2006). In addition, the regulation of auxin signalling by ROS could also involve glutathione homeostasis (Bashandy *et al.*, 2010).

However, it is important to note that, in certain situations, auxin is a positive regulator of stress tolerance. The expression of genes involved in Trp-dependent auxin biosynthesis is induced by oxidative stress (Woodward and Bartel, 2005). Catalase-deficient mutants show altered auxin signalling in a light-dependent manner, as well as enhanced biotic stress tolerance (Han *et al.*, 2013*a*, *b*). Moreover, transcripts for the auxin UDP-glucosyltransferases are strongly upregulated in catalase-deficient mutants under photorespiratory conditions. The ectopic expression of UDP-glucosyltransferases led to increased levels of indole-3-butyric acid, together with perturbations in indole-3-acetic acid homeostasis, leading to enhanced drought tolerance (Tognetti *et al.*, 2010). Although the mechanisms of the auxin-dependent protective response are not clear, auxin appears to promote stress tolerance by regulating the abundance of photosynthetic components and chloroplast structure (Tognetti *et al.*, 2012). However, as yet, no auxin-regulated components that adjust chloroplast processes in response to environmental stresses have been identified.

Brassinosteroids

Brassinosteroids (BRs) function in conjunction with auxin to enhance growth and regulate gene expression in a synergistic manner (Nemhauser *et al.*, 2004). BRs have now become established as important regulators of a wide range of processes, such as cell division and cell elongation, vascular differentiation, photomorphogenesis, photosynthesis, and stress tolerance (Haubrick and Assmann, 2006). BR binding to the specific receptor BRASSINOSTEROID INSENSITIVE 1 (BRI1) induces a phosphorylation cascade, which inactivates BRASSINOSTEROID INSENSITIVE2 (BIN2), a negative regulator of BR signalling (Kim *et al.*, 2009). BRASSINAZOLE-RESISTANT 1 (BZR1) and BRI1-EMS-SUPPRESSOR 1 (BES1) are key regulators of BR-mediated transcriptional responses. Conversely, BIN2 and protein phosphatase 2A (PP2A) modulate the phosphorylation state and hence activity of the BZR1 and BES1 proteins.

In addition to BZR1- and BES1-mediated regulation of gene transcription, BR also initiate a metabolic signalling pathway, in which Ca^{2+} and ROS can act as second messengers. Stimulus-specific guard-cell Ca^{2+} oscillations and stomatal movements are altered in partially BR-insensitive *det3* mutants (Allen *et al.*, 2000). It has recently been shown that BR induces a receptor-dependent increase in cytosolic Ca^{2+}, which can be blocked by genetic mutation or chemical inhibition of the cyclic guanosine monophosphate (cGMP)-gated Ca^{2+} channel (Zhao *et al.*, 2013). However, the mechanism by which BR regulates Ca^{2+} fluxes is unknown and the nature of the interplay between Ca^{2+} signalling and BRI1-mediated phosphorylation cascades remains to be determined. cGMP produced by a putative guanylyl cyclase domain in BRI1 (Kwezi *et al.*, 2007) may play a role in the BR-induced cytosolic Ca^{2+} increase (Zhao *et al.*, 2013).

Ca^{2+} regulates NADPH oxidase-dependent ROS production by binding directly to the EF-hand motif in the N terminus of RBOH protein (Ogasawara *et al.*, 2008) and/or by regulating the Ca^{2+}-dependent protein kinase (CDPK), which induces the phosphorylation of RBOH (Kobayashi *et al.*, 2007). The regulation of Ca^{2+} signal by BR implies that BR can induce ROS production through Ca^{2+} signalling. Interestingly, BR was shown recently to enhance auxin signalling through regulation of the cytoskeleton and by control of polar PIN2 localization (Lanza *et al.*, 2012). Since ROP-GTPases have been shown to regulate cytoskeleton and PIN2 localization (Xu *et al.*, 2010; Lin *et al.*, 2012) and are involved in the regulation of NADPH oxidases (Wong *et al.*, 2007; Duan *et al.*, 2010), it is also likely that BR activates ROP proteins and the activated ROPs then regulate NADPH oxidase. Consistent with the role of BR in the regulation of cytoskeleton and ROS, BR induces expression of a gene encoding MAP65-1a, a microtubule-associated protein, which interacts with a MAPK and is involved in regulating the expression of NADPH oxidase genes (Zhu *et al.*, 2013). It is possible that MAPK cascades control ROS production in BR-mediated responses (A. Zhang *et al.*, 2010). BR induces MPK3/6 signalling by alleviating BIN2-mediated inhibition of the MAPK module (Kim *et al.*, 2012). Loss of *MKK4* function resulted in reduced BR signalling and an inhibition of cell proliferation in rice (Duan *et al.*, 2014). These results suggest that ROS form an integration node for the BR signalling pathway with other developmental and/or hormonal signalling pathways.

NADPH oxidase activity is correlated with endogenous BR levels in cucumber (Xia *et al.*, 2009). Moreover, NADPH oxidase-mediated ROS production plays an important role in regulating gene expression (Xia *et al.*, 2009). BR also regulates the activities of the thiol-modulated enzymes of the Calvin cycle (Yu *et al.*, 2004; Jiang *et al.*, 2012*b*). Low BR levels trigger transient ROS production in epidermal strips, followed by a change in cellular redox state and stomatal opening. In contrast, high BR levels induce prolonged ROS accumulation, which leads to a stress response involving abscisic acid (ABA) biosynthesis and stomatal closure (Xia *et al.*, 2014). Overexpression of the BR biosynthetic gene *CYP85A1* in tomato led to phenotypic epinasty, which is related to altered ROS levels (unpublished data). BR-induced ROS production and ABA biosynthesis (Xia *et al.*, 2009, 2014), together with BR-induced feedback repression of BR biosynthetic genes (Bancos *et al.*, 2002), may provide a mechanism for maintaining balanced BR signalling.

BR is considered to regulate tolerance to a wide range of abiotic and biotic stresses in plants, but the mechanisms underlying BR-induced stress tolerance are not known. Mutants in BR biosynthesis and signalling show the morphological and developmental changes that complicate the analysis of stress response phenotypes (Kagale *et al.*, 2007). Hence, the

precise functions of BR in plant stress responses remains to be elucidated. Brassinazole (BRZ) is a specific inhibitor of BR biosynthesis that decreases endogenous BR levels in plants. BRZ-dependent inhibition of BR accumulation has provided evidence that BR signalling is an essential part of the plant stress response (Xia *et al.*, 2009). BR signalling was enhanced in plants subjected to heat stress or endoplasmic reticulum stress induced by the inhibition of protein glycosylation (Che *et al.*, 2010). In addition, constitutive activation of BR signalling in the *bes1-D* mutant resulted in enhanced stress tolerance, providing further evidence that BR coordinately regulates plant growth and stress responses (Che *et al.*, 2010). Target genes for BZR1/BES1 signalling have been identified, but there is as yet no evidence to suggest that these pathways are involved in BR-mediated stress tolerance (Sun *et al.*, 2010). BR signalling pathways share common components with other developmental and defence pathways. For example, BRI1-associated receptor kinase 1 (BAK1) is a critical regulator of plant basal immunity and plant cell death programmes as well as BR signalling (Chinchilla *et al.*, 2007; Kemmerling *et al.*, 2007). The role of BRI1-mediated regulation of BAK1 in plant stress tolerance remains controversial. Different models have been proposed to explain how BRI1 might compete with defence pathways for BAK1, leading to negative regulation of plant defence pathways (Albrecht *et al.*, 2012). Alternatively, BRI1 may co-regulate stress signalling by facilitating BAK1 phosphorylation, thereby increasing plant defence responses (Belkhadir *et al.*, 2012). It is probable that BR regulates plant stress tolerance at multiple levels. Using virus-induced gene silencing together with pharmacological approaches, we demonstrated that NADPH oxidases mediate BR-induced ROS production, which plays a critical role in BR-regulated stress tolerance (Xia *et al.*, 2009; Nie *et al.*, 2013). Moreover, BR-mediated stress tolerance is dependent on ABA biosynthesis, and ROS production was shown to be upstream of BR-induced ABA accumulation (Zhou *et al.*, 2014). Taken together, these results demonstrate that NADPH oxidase-dependent ROS production is a convergence point of BR-mediated developmental and stress response pathway (Fig. 1). A BR-regulated balance of growth and stress tolerance facilitates appropriate growth responses to changing environmental conditions.

Gibberellins

Gibberellins (GAs) are key regulators of plant growth and development. The identification and characterization of mutants with altered height and sensitivity to GAs led to the establishment of the pathway of GA synthesis and signalling (Daviere and Achard, 2013). More recently, GAs were shown to function via the regulation of DELLA proteins, which are negative regulators of GA signalling. Binding of GAs to the receptor GA INSENSITIVE DWARF1 (GID1) leads to the formation of the GA–GID1–DELLA complex, enabling the release of transcription factors from DELLA-mediated suppression. In addition, DELLA proteins are targeted for destruction by the proteasome in the presence of GA via ubiquitination by a SCF (Skp1–Cullin–F-box) complex that is specified by SLEEPY1 (SLY1).

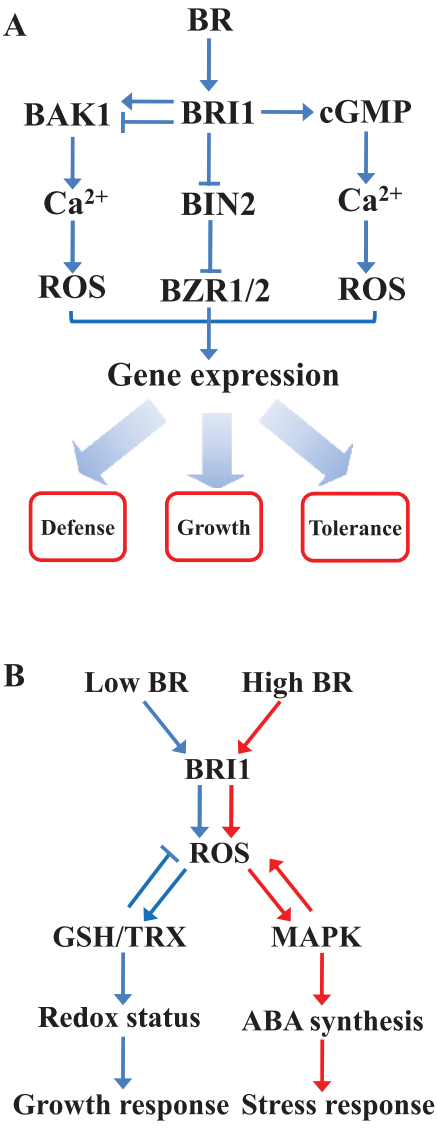

Fig. 1. (A) A model that integrates the current mechanism of BRI1-dependent plant growth, BR-induced ROS signalling that regulates stress tolerance, and BAK1-mediated plant basal immunity. BR binding to its receptor, BRI1, induces a phosphorylation cascade, which inactivates GSK3-like kinase BIN2, the negative regulator of BR signalling that mediates phosphorylation and inactivation of BZR1/2. Meanwhile, the guanylyl cyclase domain in BRI1 produces a second messenger, cGMP, which activates a cytosolic Ca^{2+} influx. Ca^{2+} binds to the EF-hand motif in the N terminus of RBOH and induces production of ROS. BAK1 mediates production of Ca^{2+} and ROS signals, which trigger a downstream defence response. BR activation of BRI1 seems to antagonistically or synergistically regulate BAK1-mediated defence signalling, which depends on the developmental stage and/or BR homeostasis. BZR1/2-mediated transcriptional regulation together with ROS- and BAK1-mediated signalling regulate BR-dependent plant growth, defence, and stress tolerance. (B) Model for the concentration-dependent BR-mediated plant growth and stress response. BR activation of BRI1 leads to the production of ROS through RBOH. However, the spatial/temporal changes of ROS differ at low and high BR levels. A low level of BR induces a transient production of ROS, which stimulate the GSH/TRX-dependent thiol reducing system, and finally leads to a reduced cellular redox status, which acts as a signal to regulate photosynthesis and other growth-related response. In contrast, a high level of BR induces a prolonged accumulation of ROS, which induce a MAPK phosphorylation cascade. In this case, ROS and MAPK form a positive amplification loop, which is potentially involved in the biosynthesis of ABA, the critical signal in the regulation of stomatal closure and stress tolerance.

DELLA proteins are central regulators of plant growth and stress responses that allow integration of environmental signals with developmental programmes (Achard *et al*., 2006, 2008; Navarro *et al.*, 2008; Yang *et al.*, 2012). GA signalling mediates stress tolerance through the control of cellular redox homeostasis. DELLA proteins appear to regulate ROS levels by controlling the expression of a subset of antioxidant genes. DELLA-mediated enhancement of ROS scavenging capacity promotes survival under abiotic stress conditions (Achard *et al.*, 2008). The increased abundance of DELLA proteins as a result of GA deficiency leads to reduced levels of ROS in roots, shorter root lengths, and a decreased sensitivity of root growth to diphenyleneiodonium (DPI), an inhibitor of NADPH oxidase (Achard *et al.*, 2008). In contrast, DELLA deficiency enhances root ROS accumulation and root growth.

ROS are also involved in GA signalling during seed germination. Cereal grain aleurone cells, which secrete hydrolytic enzymes for the remobilization of endosperm reserves during germination in order to support the growth of the embryo, undergo a form of ROS-mediated programmed cell death (PCD) (Fath *et al.*, 2001). PCD is a developmental programme that is indispensable for plant development, as well as being an important strategy in plant responses to biotic and abiotic stresses. GA induces upregulation of α-amylase and ROS-mediated PCD in aleurone cells in barley (Fath *et al.*, 2001). GA sensitizes the aleurone cells to ROS and hence PCD by suppression of ROS scavenging genes (Bethke and Jones, 2001). ROS generation may also be increased during the germination of dicotyledonous seeds as a result of activation of NADPH oxidases (Schopfer *et al.*, 2001a; Leymarie *et al.*, 2012). Germination in the dark was accompanied by a significant increase in DPI-sensitive ROS production in the embryo and seed coat in radish (Schopfer *et al.*, 2001a).The addition of DPI significantly inhibited seed germination in *Arabidopsis*, whereas freshly harvested seeds of *rbohD* knockout mutants that are defective in NADPH oxidase were found to be in a state of deep dormancy (Leymarie *et al.*, 2012). In non-dormant seeds, ROS accumulation is temporally and spatially regulated in a manner that appears to be under strict developmental control (Schopfer *et al.*, 2001a; Leymarie *et al.*, 2012). While the role of GA signalling in the regulation of localized ROS production during germination remains to be clarified, GA biosynthesis and signalling are known to be enhanced by ROS during germination (Liu *et al.*, 2010; Bahin *et al.*, 2011; Leymarie *et al.*, 2012). These findings suggest a complex crosstalk between ROS and GA signalling in different cell types during the initiation and organization of early seedling development.

DELLA proteins increase the susceptibility of plants to the hemi-biotrophic pathogen *Pseudomonas syringae* by suppression of salicylic acid (SA)-mediated pathways. In contrast, DELLA proteins increase tolerance to necrotrophic pathogens such as *Alternaria brassicicola* by enhancing jasmonic acid (JA) signalling pathways in a CORONATINE INSENSITIVE 1 (COI1)-dependent manner (Navarro *et al.*, 2008). Recent studies have shown that DELLA proteins enhance JA signalling by inhibitory interactions with JASMONATE-ZIM DOMAIN (JAZ) proteins, which function as negative regulators of JA signalling (Yang *et al.*, 2012). The pathogen-induced oxidative burst is associated with resistance to biotrophic pathogens. Conversely, ROS accumulation has a negative effect on resistance to necrotrophic pathogens (Yoshioka *et al.*, 2009). As with GA-mediated abiotic stress tolerance, the differential regulation of resistance to biotrophic and necrotrophic pathogens by GA signalling might be achieved by changes in ROS homeostasis. Recently, light-dependent nitric oxide (NO) accumulation was shown to play a role in the stabilization of DELLA proteins during photomorphogenesis (Lozano-Juste and León, 2011). NO can stimulate antioxidant production and so inhibit ROS-mediated PCD in GA-treated aleurone cells (Beligni *et al.*, 2002). As in most stress situations, it is likely that there is considerable crosstalk between ROS and NO signalling pathways during interactions with GA.

Abscisic acid

ABA is a stress hormone that plays a general role in promoting dormancy, such as inhibition of seed germination and root development, as well as being a key regulator of plant responses to abiotic stresses (Xiong *et al.*, 2002). While the pathway of ABA biosynthesis is well established, the mechanisms of ABA perception and signalling have proved to be more elusive due to the extraordinary complexity of the network, which involves a large number of components. ABA is perceived by the Pyrabactin Resistance Protein (PYR) or PYR-Like proteins (PYR1/PYLs) (Fujii *et al.*, 2009; Park *et al.*, 2009). ABA binding to the PYR1/PYL receptor complex leads to suppression of protein phosphatase 2Cs (PP2Cs), which inhibit the positive regulator of the ABA response sucrose non-fermenting1 (SNF1)-related protein kinase 2 family (SnRK2s), and then facilitates downstream ABA signalling.

NADPH oxidase-dependent ROS production in guard cells plays an important role in ABA-mediated stomatal closure (Kwak *et al.*, 2003). ABA-induced ROS production can activate Ca^{2+} channels and induce increases in cytosolic Ca^{2+} in guard cells, and so mediate ABA-induced stomatal closure (Pei *et al.*, 2000; Kwak *et al.*, 2003). Nitrate reductase-mediated NO production acts downstream of H_2O_2 in ABA-induced stomatal closure (Bright *et al.*, 2006). OPEN STOMATA 1 (OST1) is a member of the SnRK2 family that acts upstream of ROS signalling to mediate ABA-induced stomatal closure (Mustilli *et al.*, 2002). OST1 is considered to regulate ROS generation through a direct interaction and phosphorylation of the NADPH oxidase subunit of RBOHF in *Arabidopsis* (Sirichandra *et al.*, 2009). In turn, OST1 is regulated by PP2C proteins (Vlad *et al.*, 2009). NADPH oxidase may be regulated directly by the core ABA signalling module PYR–PP2C–SnRK2. The second messenger phosphatidic acid, which is produced by phospholipase D (PLD) could also play a role in the regulation of NADPH oxidase activity during ABA-mediated responses in guard cells. Phosphatidic acid binds to and activates NADPH oxidases (Y.Y. Zhang *et al.*, 2009). Moreover, PLDα1 deficiency results

in lower ROS accumulation and decreased stomatal closure in response to ABA.

In addition to the roles in ABA-induced stomatal closure, ROS production is also critical for ABA-mediated stress tolerance of seedlings. Water stress or ABA treatment triggers ROS accumulation in maize, together with increased expression of genes encoding antioxidant enzymes and higher antioxidant enzyme activities (Jiang and Zhang, 2002). Conversely, ROS scavengers completely block ABA-induced increases in antioxidant activities (Zhang *et al.*, 2006). Several lines of evidence show that NADPH oxidase plays a role in ABA-induced ROS accumulation (Jiang and Zhang, 2003; Zhang *et al.*, 2006). Our recent results in tomato suggest that ABA can induce ROS accumulation in chloroplasts (Zhou *et al.*, 2014). However, the DPI-dependent inhibition of NADPH oxidase in tomato or silencing of *RBOH1* only partially blocked ABA-induced ROS accumulation and associated increases in antioxidant enzymes (Zhou *et al.*, 2014). These findings suggest that regulation of photosynthetic electron transport and/or carbon metabolism in the chloroplasts plays a role in ABA-induced ROS production and in the stress response. During the ABA-mediated stress response, MAPK cascades may act both upstream and downstream of ROS production (Zhang *et al.*, 2006; Xing *et al.*, 2008), possibly because MAPK and NADPH oxidase activities form a positive feedback loop. However, there is still no strong evidence showing the direct regulation of NADPH oxidase activity by MAPK.

In contrast to the situation in seedlings, ABA decreased ROS accumulation in imbibed seeds, particularly in the embryo (Ye *et al.*, 2012), leading to inhibition of seed germination. This observation may be explained at least in part by increases in antioxidant enzyme activities (Fath *et al.*, 2001). H_2O_2 was shown to mediate upregulation of ABA catabolism during imbibition in *Arabidopsis* seed (Liu *et al.*, 2010). In barley seeds, however, H_2O_2 suppressed the expression and activity of an ABA-responsive protein kinase (PKABA) (Ishibashi *et al.*, 2012). PKABA regulates the expression of a GA-regulated Myb transcription factor (GAmyb), which is a regulator of the expression of α-amylase (Ishibashi *et al.*, 2012). Therefore, H_2O_2 may act as a signal that antagonizes ABA signalling during seed imbibitions. Subsequently, ABA-mediated inhibition of primary root growth is associated with the activation of NADPH oxidases and ROS accumulation (Jiao *et al.*, 2013). Interestingly, ROS homeostasis in *Arabidopsis* can be affected by the ROS produced at both the plasma membrane and in the organelles. Mitochondria ROS accumulation resulting from impaired splicing of complex I genes impinges on the ABA signalling, leading to a reduced auxin response and inhibition of primary root growth (He *et al.*, 2012). ABA may arrest lateral root growth by a similar mechanism. The inhibition of lateral root growth by salt stress is dependent on an endodermis-specific ABA signalling pathway (Duan *et al.*, 2013). The endodermal cells may play a role in sensing sodium ions (Duan *et al.*, 2013), whereas the NADPH oxidase subunits, RBOHD and -F, are involved in maintaining homeostasis of Na^+ and K^+ ions in plants (Ma *et al.*, 2012). It is likely that NADPH oxidase-dependent ROS production is involved in ABA-mediated arrest of lateral root growth. Compared with the lateral roots, primary root growth is much less sensitive to ABA-mediated inhibition (Duan *et al.*, 2013). In addition, ABA is required in certain situations to maintain root growth (Spollen *et al.*, 2000; H. Zhang *et al.*, 2010). These findings illustrate the complexity of ROS/ABA interactions in the regulation of root architecture, particularly under stress conditions.

Ethylene

Ethylene (ET) is essential for the regulation of growth, senescence, and stress responses (Lin *et al.*, 2009). Autocatalytic and feedback mechanisms control ET production through differential transcriptional and/or post-transcriptional regulation of 1-aminocyclopropane 1-carboxylate synthase (ACS). In addition, ET stabilizes the activities of some ACS forms through regulation of the protein phosphorylation state by MPK6 (Liu and Zhang, 2004) or by effects on proteasome-mediated degradation through the ETHYLENE OVERPRODUCER (ETO)/ETO-like protein (Wang *et al.*, 2004). The ET receptor (ETR) forms a high-molecular-weight complex with CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1), a Raf-like MAPKKK, and ETHYLENE INSENSITIVE 2 (EIN2), which is a critical positive regulator of ET signalling. ET promotes conformational changes in this complex that lead to the release and activation of EIN2, which then regulates the activity of EIN3 and downstream ET responses (Lin *et al.*, 2009).

Accumulating evidence suggests that ROS mediate a variety of ET-induced responses in plants. ET induces stomatal closure via H_2O_2 produced by RbohF in *Arabidopsis* (Desikan *et al.*, 2006). Moreover, ET mediates UV-B-induced stomatal closure through peroxidase-dependent H_2O_2 production in *Vicia faba* (He *et al.*, 2011). In addition, ETR1- and EIN2-mediated signalling is required for flagellin-induced *RBOHD*-dependent ROS accumulation, which is essential for stomatal closure and associated immunity against bacterial pathogens (Mersmann *et al.*, 2010). Both exogenous sources of ET and endogenous increases in ET in *eto1* mutants have antagonistic effects on ABA-induced pathways of stomatal closure (Tanaka *et al.*, 2005). Similarly, ET mediates the effects of ozone, leading to the suppression of stomatal closure during drought stresses (Wilkinson and Davies, 2009). It is likely that spatial–temporal changes in ROS production and accumulation dictate the specificity of these responses allowing different signalling transduction pathways to operate according to perceived environmental triggers and the physiological status of the plants.

Molecular genetics approaches have provided evidence indicating that ET and NADPH oxidases act in sequence to regulate the responses of *Arabidopsis* to salinity stress. Forward-genetics screens have identified mutants such as *soil-salinity sensitive1-1* (*sss1-1*) and *soil salinity tolerant1-1* (*sst1-1*) that have altered sensitivity to salinity (Jiang *et al.*, 2012a, 2013). Further analysis showed that *sss1-1* is a novel mutant in an *AtRBOHF* allele (Jiang *et al.*, 2012a). *SSS1-1* is

expressed in the root vascular tissue and is involved in reducing Na^+ influx in the root stelar region, where it operates to restrict the transport of Na^+ from the root to the shoot through the xylem-based transpiration stream (Jiang *et al.*, 2013). Crucially, *sst1-1* is a loss-of-function allele of *ETO1* (Jiang *et al.*, 2013). The *sst1-1* mutant had higher ET levels, which are thought to confer salinity tolerance through improved Na^+/K^+ homeostasis, which is partially dependent on AtRBOHF activity. The salinity tolerance conferred by *sst1-1* is also associated with the induction of high-affinity K^+ transporter (Jiang *et al.*, 2013). H_2O_2 acts downstream of ET, mediating the regulation of transcriptional and morphological changes in roots deprived of K^+ (Shin and Schachtman, 2004). Salt stress impairs the Na^+/K^+ homeostasis in a manner that may lead to a physiological deficiency in K^+. These findings suggest that H_2O_2-mediated ET signalling is a conserved mechanism of response to salinity and K^+-deficiency stresses.

The mechanisms by which ET enhances and regulates ROS production remain to be established. It is known that ET inhibits CTR1 function leading to the activation of the MKK9–MPK3/6 signalling cascade (Yoo *et al.*, 2008). Moreover, MPK6 phosphorylates ETHYLENE RESPONSE FACTOR6 (ERF6), which mediates the transcriptional regulation of ROS-responsive genes (Wang *et al.*, 2013). ET-induced MAPK cascades may regulate ROS production by transcriptional regulation of *RBOH* genes. When plants are exposed to environmental stimuli, ET and ROS may form an amplification loop that mediates cell death. Screens for mutants with altered sensitivity to ozone have identified *radical-induced cell death1* (*rcd1*), which shows transient spreading lesions in response to ozone fumigation (Overmyer *et al.*, 2000). $O_2^{\bullet-}$ and ET accumulation precede cell death in *rcd1* mutants, whereas the block in the ET signalling pathway in *ein2* mutants or *rcd1/ein2* double mutants substantially decreased $O_2^{\bullet-}$ production and cell death in response to ozone. Similarly, the lesion mimic mutant *vascular associated death1-1* (*vad1-1*), which exhibits the light-dependent appearance of propagative hypersensitive response-like lesions along the vascular system, showed an enhanced expression of genes associated with ET synthesis and signalling after light and pathogen treatments (Bouchez *et al.*, 2007). The *vad1-1* mutant exhibits high levels of ROS accumulation through activation of AtRBOHD. However, crosses of the *vad1-1* mutant with the ET-insensitive mutants drastically reduced expression of the *AtRBOHD* gene and cell death. These results demonstrate that ET potentiates the accumulation of ROS and that ET and ROS together regulate cell death during plant stress responses.

ET and ROS are central regulators of aerenchyma and adventitious root development, which involves PCD, during flooding and water logging in order to facilitate an increased supply of oxygen to the roots. ET treatment induces PCD in the epidermal cells immediately above the adventitious roots in the stem nodes of rice (Steffens and Sauter, 2009). The ET-dependent regulation of PCD in the epidermal cells is partially dependent on ROS production. Moreover, ROS can amplify the ET signalling cascade through the regulated expression of *ETO1-like* and similar genes associated with ET biosynthesis. The mechanisms controlling runaway cell death triggered by high light stress, as well as aerenchyma formation triggered by hypoxia, are genetically linked (Mühlenbock *et al.*, 2007). The formation of lysigenous aerenchyma is negatively regulated by the plant defence regulator LESION SIMULATING DISEASE1 (LSD1) in *Arabidopsis*, whereas ET and ROS act downstream of LSD1 to regulate development of lysigenous aerenchyma. The induction of aerenchyma formation in hypocotyls is accelerated when plants are exposed to high light, suggesting that a ROS/ET-mediated systemic signal originating in leaves is also involved in the development of lysigenous aerenchyma (Mühlenbock *et al.*, 2007).

Strigolactones

Strigolactones (SLs) are a group of carotenoid-derived hormones that are involved in regulation of shoot and root architecture, allowing a plasticity of growth responses to changing environmental conditions (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008; Kapulnik *et al.*, 2011; Ruyter-Spira *et al.*, 2011). SLs are produced primarily in roots. They act as long-distance signals that inhibit axillary bud outgrowth by triggering the expression of *BRANCHED1* (*BRC1*), which is a class II TCP transcription factor regulating dormancy (Aguilar-Martinez *et al.*, 2007). SLs also regulate bud growth indirectly through crosstalk with auxin. SLs dampen polar auxin transport by increasing the rate of removal of the PIN1 protein from the plasma membrane (Shinohara *et al.*, 2013). Conversely, auxin positively regulates the synthesis of SLs by activating the expression of genes encoding enzymes of the biosynthetic pathway (Hayward *et al.*, 2009). Current concepts suggest that auxin and SLs function together in a dynamic feedback loop.

MAX2 is an F-box protein that is required for SL signalling (Nelson *et al.*, 2011). The *max2 Arabidopsis* mutants show enhanced shoot and root branching. A mutation in *FHY3* in the *max2* mutant background suppresses the shoot-branching phenotype (Stirnberg *et al.*, 2012). FHY3 is a transposase-related transcription factor that is involved in the response to far-red light (Lin *et al.*, 2007). This finding suggests that FHY3 is a convergence point for light- and SL-mediated signalling pathways. FHY3 has a wide range of targets for binding including the *RBOH* genes (Ouyang *et al.*, 2011). Increased expression of *RBOH* genes arising from inactivation of *FHY3* may be responsible for the suppression of the branching phenotype in both the wild-type and *max2* backgrounds. This hypothesis is consistent with the role of the *RBOH* genes in the control of shoot branching as observed in transgenic tomato plants with antisense *RBOH* expression that showed increased shoot branching (Sagi *et al.*, 2004). In addition to the control of root and shoot branching, SLs play a role in the control of leaf senescence and stress tolerance that is dependent on MAX2 signalling (Bu *et al.*, 2014; Ha *et al.*, 2014). The *max2* mutants show an increased sensitivity to drought and salt stress associated with

an impaired ABA response that includes effects on stomatal closure and the expression of stress-responsive genes. If we accept that ROS are second messengers of ABA signalling, then it is possible to suggest that RBOH proteins are involved in SL-dependent regulation of shoot and root branching and also in stress responses.

Whereas SLs negatively regulate lateral root formation under optimal growth conditions, they positively regulate lateral root production in conditions of nutrient deprivation (Ruyter-Spira *et al.*, 2011). These differential effects might be explained by changes in auxin sensitivity to SLs resulting from SL-dependent regulation of the expression of the auxin receptor gene *TIR1* (Mayzlish-Gati *et al.*, 2012). SL levels in the roots of plants suffering from phosphate limitation can be increased by as much as 100 000-fold (Yoneyama *et al.*, 2012). The nutrient deprivation response is also associated with ROS production (Shin and Schachtman, 2004). Nutrient deprivation induces the activation of NADPH oxidases together with the coordinate expression of genes involved in SL biosynthesis in roots (Bonneau *et al.*, 2013). However, little is known about the relationship between SLs and ROS production and about the root response to nutrient deprivation stresses.

Salicylic acid

SA is a key signal in plant defences that is associated with the perception of pathogens, either through pathogen-associated molecular patterns (PAMPs) or through pathogen effectors. SA is required for the initiation of PAMP-triggered immunity or effector-triggered immunity. Both processes induce molecular events leading to the expression of pathogen resistance genes that involve SA synthesis, the production of ROS and NO, and the activation of MAPK cascades, as well as an increase in intracellular Ca^{2+} levels (Jones and Dangl, 2006). It has been suggested that SA disturbs cellular redox homeostasis by inhibiting catalase in the peroxisomes (Chen *et al.*, 1993) and the subsequent increase in ROS accumulation activates thiol signalling pathways involving glutathione and thioredoxin (Mou *et al.*, 2003; Tada *et al.*, 2008). However, SA and ROS interactions are complicated. The demonstration that altered H_2O_2 signalling initiated in catalase-deficient mutants and transgenic plants stimulates synthesis of SA together with the induced expression of *pathogenesis-related* (*PR*) genes suggest that ROS also function upstream of SA biosynthesis (Chamnongpol *et al.*, 1998; Han *et al.*, 2013a). Similarly, generation of ROS by the photosynthetic electron transport chain or changes in the redox state of the plastoquinone pool also activate defence responses (Liu *et al.*, 2007; Mühlenbock *et al.*, 2008). Such observations suggest that ROS and SA function together in a self-amplifying feedback loop, in which ROS induce SA accumulation and SA subsequently enhances ROS accumulation.

NPR1 protein functions downstream of SA in the plant defence response, sensing SA-induced cellular redox changes and undergoing a change from an oligomeric state to the monomeric form (Mou *et al.*, 2003; Tada *et al.*, 2008). The redox-activated NPR1 translocates to the nucleus where it acts in concert with TGA transcription factors to regulate the expression of *PR* genes. NPR1 is sequestered in the cytoplasm as an oligomer and *S*-nitrosylation of the protein facilitates its oligomerization, maintaining protein homeostasis upon SA induction (Tada *et al.* 2008).

Whereas PAMP-triggered immunity is the basal form of plant immunity, effector-triggered immunity is an elicited response that leads to a hypersensitive response, which involves an accelerated and amplified interaction between SA and ROS. Mutant such as *lsd1* shows a runaway cell death phenotype as a result of impaired control of cellular redox status and/or SA-dependent regulation (Jabs *et al.*, 1996). The introduction of the superoxide-producing xanthine/xanthine oxidase (X/XO) system to the extracellular environment mimics extracellular ROS generation following pathogen perception, and initiates the runaway cell death phenotype in *lsd1*. In contrast, runaway cell death is abolished in mutants that are impaired in SA biosynthesis in the presence of the X/XO system (Torres *et al.*, 2005). Runaway cell death was not observed in the *rbohD* knockout mutant in the presence of the X/XO system or when supplied with an appropriate pathogen trigger, but the *rbohD*/*lsd1* double mutant showed an accelerated cell death phenotype (Torres *et al.*, 2005). These findings highlight the different roles of SA and NADPH oxidases in the regulation of ROS related to the control of cell death in plant defence responses. Whereas NADPH oxidase produces ROS in the extracellular environment, SA induces changes in metabolic ROS accumulation in chloroplasts, mitochondria, and peroxisomes (Khokon *et al.*, 2011). Accumulating evidence suggests that the SA-mediated regulation of ROS production is independent of NADPH oxidase action (Miura *et al.*, 2013), whereas ROS produced by NADPH oxidases can trigger protective systems in situations where chloroplast and/or mitochondrial ROS increase as a result of pathogen attack (Pogany *et al.*, 2009).

Jasmonic acid

JA and its derivatives are important regulators of wound and environmental stress responses in higher plants. Mechanical stress is sensed by stretch-activated Ca^{2+} channels and also by a variety of receptors, which initiate downstream signalling through Rho-GTPases, NADPH oxidases, MAPK cascades, and JA biosynthesis (Wolf *et al.*, 2012). Oligogalacturonides produced during wounding induce JA biosynthesis through the octadecanoid pathway (Doares *et al.*, 1995). In addition, extracellular ATP has recently been shown to be a signal mediating the wound response, leading to the induction of ROS through NADPH oxidase activation and the induced expression of JA biosynthesis genes (Song *et al.*, 2006). The production of Ca^{2+} and ROS, together with the activation of MAPK cascades during the early phase of the wound response is important in the regulation of JA biosynthesis. However, the inhibition of ROS production (Orozco-Cárdenas *et al.*, 2001) or silencing of *MAPK* genes (Kandoth *et al.*, 2007) attenuated the expression of late wound-induced genes but did not affect the expression of JA biosynthesis genes in

tomato. Furthermore, both cytoplasmic alkalization and ROS accumulation are required for JA-induced stomatal closure (Suhita *et al.*, 2004). While the functions of JA-induced ROS remain controversial, it appears that very high JA levels cause ROS accumulation and cell death. In contrast, low JA concentrations induce the production of NO, which has an antagonistic effect to H_2O_2 on the activation of wound-inducible defence genes (Orozco-Cárdenas and Ryan, 2002). It would appear, therefore, that the cellular response to JA is distinct from the wound response and that JA acts in concert with other signals, such as Ca^{2+}, ROS, oligogalacturonides, and oxidized lipids, to regulate the wound response.

Systemic signalling pathways

Long-distance signalling is a concept that local exposure of a plant to an environmental stimulus induces a physiological and/or molecular response systemically in organs that are not yet exposed to the stimulus (Heil and Ton, 2008). Long-distance signalling provides a general mechanism for the coordination of responses in different plant parts and appears to be part of a more general sensing system for environment and/or nutrient status (Forde, 2002; Davies *et al.*, 2005; Dempsey and Klessig, 2012).

Systemic acquired resistance (SAR) is a type of long-distance signalling response following exposure to pathogens (Durrant and Dong, 2004). Whereas SA is required for the establishment of SAR, grafting experiments have shown that SA is not a mobile signal (Vernooij *et al.*, 1994). Putative phloem-mobile systemic SAR signals include methyl salicylate, glycerol-3-phosphate, the lipid-transfer protein DIR1, and JA (Dempsey and Klessig, 2012). ROS may also be involved in the long-distance mediation of SAR responses (Alvarez *et al.*, 1998). Recognition of virulent pathogens triggers a localized oxidative burst and cell death in the hypersensitive response, which after a short delay is followed by a systemic oxidative burst and a micro-hypersensitive response. Changes in the levels of ROS in the local and systemic tissues are required for the expression of SAR. ROS appear to mediate SAR by regulating *de novo* synthesis of SA in systemic leaves. The local accumulation of BR, which functions as an immobile hormone, induces a form of stress tolerance that is independent of SA (Xia *et al.*, 2011). While BR-induced systemic resistance appears to be somewhat different from the classic SAR response, the inhibition of ROS accumulation in BR-treated leaves abolished systemic responses.

Reciprocal grafting experiments in tomato have demonstrated that, similar to SAR, local mechanical damage induces systemic defence responses in undamaged tissues (Li *et al.*, 2002). JA signalling but not JA synthesis is required for the initiation of a systemic wound response in the scion. 12-Oxophytodienoate reductase 3 (OPR3) catalyses the rate-limiting step of the octadecanoid pathway of JA synthesis. Wounding induces a rapid systemic signal in the leaves of *opr3 Arabidopsis* mutants, showing that the presence of functional JA synthesis in wounded leaves is not required for the initiation of a systemic signal (Koo *et al.*, 2009). Since the *spr2* mutants that have been used in such studies are deficient in all oxylipins derived from trienoic fatty acids, the precise nature of the mobile signal is unknown. Signal(s) other than JA are rapidly transmitted from the local stress perception site to facilitate a systemic wound response (Koo *et al.*, 2009).

Wounding rapidly induces ROS accumulation in local and systemic tissues (Orozco-Cardenas and Ryan, 1999). RBOHD-dependent ROS production is required for rapid systemic signalling triggered by various stresses including wounding (Miller *et al.*, 2009). Inhibitors of NADPH oxidase block elicitor-induced JA synthesis in ginseng cell-suspension cultures (Hu *et al.*, 2003). While ROS production in response to wounding has been associated with the pathway of JA synthesis and ROS act as second messengers of JA responses following wounding (Orozco-Cardenas and Ryan, 1999; Orozco-Cárdenas *et al.*, 2001), the relationship between ROS accumulation and the activation of JA synthesis in systemic tissues remains unclear. It is possible that, like the SA/ROS signalling system, ROS and JA form a similar signal amplification loop. The propagation of waves of ROS signals may be transmitted from cell to cell in order to facilitate long-distance transmission of ROS signals, which activate JA synthesis in systemic tissues. Similarly, JA signalling could enhance ROS levels and initiate wound responses in systemic tissues.

By analogy to the classic SAR response, a long-distance signalling response called systemic acquired acclimation (SAA) is considered to operate following perception of abiotic stresses. Currently, hormones such as ABA and ET are widely considered to be mediators of long-distance signalling in response to drought, nutrient deprivation, and submergence stress (Forde, 2002; Davies *et al.*, 2005; Jackson, 2008). Root-sourced ABA, which is transported through xylem sap, has long been considered a mobile signal to regulate stomatal closure in response to drought (Davies *et al.*, 2005). However, grafting experiments using wild-type and ABA-deficient tomato indicated that the stomatal closure response in the shoot is independent of the ABA biosynthesis capacity in the stock during drought stress (Holbrook *et al.*, 2002). Interestingly, the redox status of the plastoquinone pool and ROS production via photorespiration and/or the Mehler reaction is implicated in the initiation of SAA (Karpinski *et al.*, 1999; Fryer *et al.*, 2003). Furthermore, ABA synthesized in the vascular parenchyma induces a burst of ROS accumulation in the apoplast, which facilitates retrograde signalling from chloroplasts (Galvez-Valdivieso *et al.*, 2009). However, ABA accumulation in systemic tissues that are distant from the perceived heat stress is abolished in *Arabidopsis rbohD* mutants (Suzuki *et al.*, 2013). It is likely that, as is the case with SA- and JA-mediated systemic signalling, ABA accumulation and signalling may participate in the cell-to-cell propagation of ROS waves and participate in the positive amplification loop that propagates SAA responses. The interplay between ROS and several plant hormones, including SA, JA, and ABA, provides a more general mechanism for systemic signalling responses (Fig. 2). The balance between the different hormones in such responses will therefore determine the nature of the transcriptional signatures that facilitate appropriate responses to specific stresses.

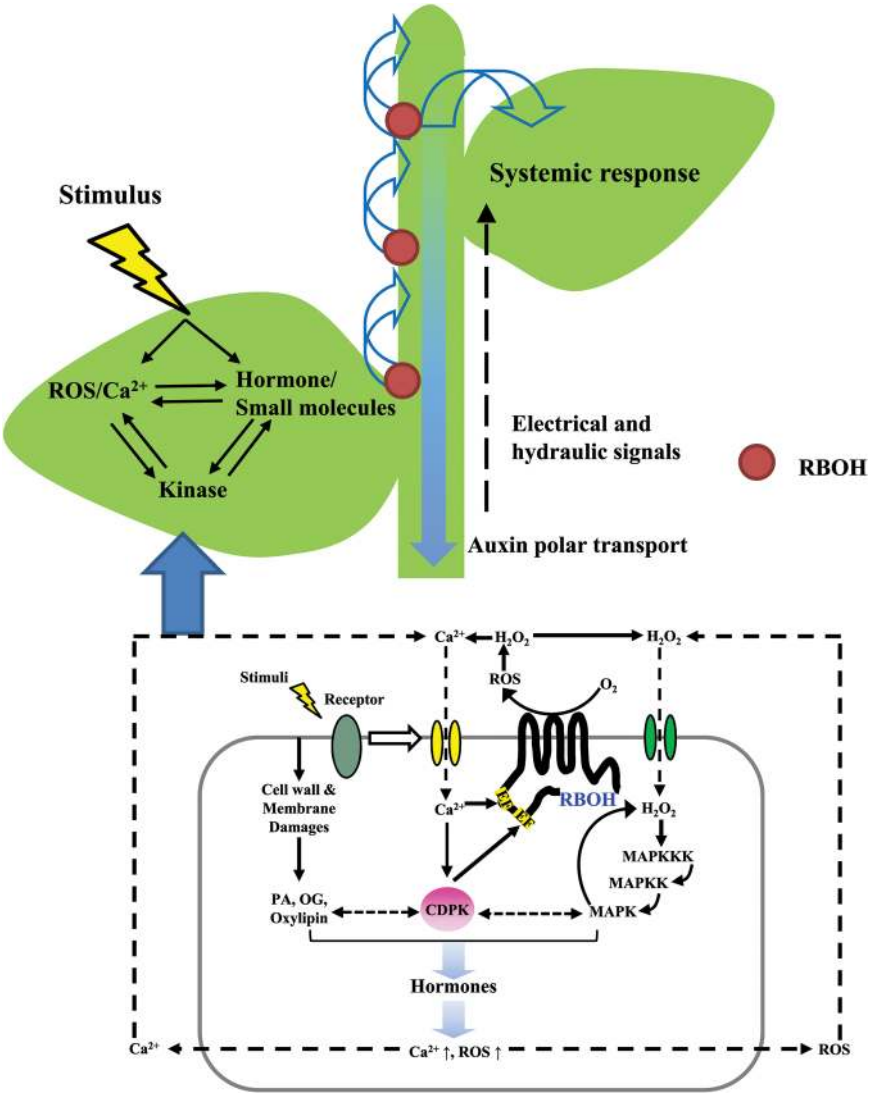

Fig. 2. A general model of systemic signalling. When local parts of a plant are exposed to specific stimuli, e.g. pathogen attack, wounding or high light, the receptor activates Ca^{2+} channels, which lead to a rise in concentration of Ca^{2+} in the cytoplasm. Meanwhile, stresses cause damage to the cell wall and/or membrane, which leads to the generation of small molecules such as phosphatidic acid (PA), oligogalacturonide (OG), and oxylipins. The temporally and spatially regulated Ca^{2+} together with Ca^{2+}-dependent protein kinase (CDPK) activates RBOH, whereas the increase in extracellular ROS in turn activates Ca^{2+} channels. ROS produced by RBOH activate a MAPK phosphorylation cascade, which can form a positive amplification loop with ROS. CDPK, MAPK, and the small molecules may work together to regulate the biosynthesis of hormones, which can initiate a second round of production of Ca^{2+} and ROS. The Ca^{2+} and ROS signature, which contains stimuli/hormone-specific information, may trigger a similar signalling process in neighbouring cells after crossing the plasma membrane. The ROS/Ca^{2+}, hormone/small molecules, and kinase mutually regulate each other and form a positive amplification loop. In this way, the stimulus-specific signal is propagated throughout the plants. The shoot meristem may sense the local changes in the plant and regulate the performance of plant growth by regulating the polar transport of auxin. We hypothesize that interaction of ROS/Ca^{2+} and hormone/small molecules rather than a specific mobile chemical signal mediates the systemic signalling, whereas RBOH plays an important role in amplifying the strength of signalling. The signalling loop induces Ca^{2+} flux across the membrane and induces a hyper- or depolarization of the membrane, which acts as an electrical signal. Physical signals, e.g. electrical or hydraulic signals, produced following the local stimuli dependently or independently of the signalling loop are potentially involved in systemic signalling.

Crosstalk between hormone signalling pathways

Literature evidence demonstrates that the interaction between stress signalling pathways and developmental stimuli is not linear but involves a complex network of interactions between different hormone signalling pathways, with extensive metabolic crosstalk and points of reciprocal control. The above discussion has highlighted the roles of the different plant hormones in ROS production and propagation of signals leading to responses in gene expression. Moreover, temporal and spatial changes in ROS accumulation serve as hubs for integration of signals from different hormone pathways. For example, a paradigm for this type of control is the hormone-mediated changes in regulation of ROS production and processing in the mediation of the antagonistic control of seed germination by GA and ABA. In this situation, GA promotes ROS accumulation and sensitizes the aleurone cells to oxidant-triggered PCD in barley grains, whereas ABA enhances the antioxidant capacity and essentially desensitizes the aleurone

cells to GA-induced oxidant production (Bethke and Jones, 2001; Fath *et al.*, 2001; Schopfer *et al.*, 2001*a*). ROS promote the end of seed dormancy in *Arabidopsis* by inducing ABA catabolism and suppressing ABA signalling (Liu *et al.*, 2010). However, NADPH oxidase-mediated ROS accumulation in the apoplast could also trigger germination through activation of GA signalling pathways (Leymarie *et al.*, 2012). The extent of ROS accumulation seems to be a determinant of the balance between GA and ABA signalling. However, our current knowledge of the mechanistic details of the regulation of ROS levels during seed germination is far from complete. The action of DELLA proteins as repressors of GA signalling pathways is also important in the crosstalk between GA signalling and other pathways, and also for regulating the levels of ROS during stress responses (Achard *et al.*, 2008). The DELLA protein called RGL2 inhibits seed germination by stimulating ABA synthesis and ABI5-mediated signalling (Tyler *et al.*, 2004; Piskurewicz *et al.*, 2008). The RGL2 protein is degraded by the F-box protein called SLY1 following GA treatment. Since *RGL2* is expressed at high levels in germinating seeds (Tyler *et al.*, 2004), it is possible that repression of seed germination by ABA is persistent but that it is overcome by the GA signal. In this way, ROS produced via NADPH oxidases could mediate the regulation of DELLA protein levels and act as secondary messengers following the perception of an appropriate environmental stimulus that is sensed by the seeds. Hence, ROS would serve to change the existing balance between ABA and GA pathways in order to promote seed germination.

ABA also antagonizes the action of BR, serving to dephosphorylate BZR1 and BES1 and release the repression of the GSK3-like kinase BIN2 (Kim *et al.*, 2009). ABA promotes phosphorylation of BES1 in the presence of BR through activation of BIN2 (S. Zhang *et al.*, 2009). DELLA proteins can interact directly with BZR1, inhibiting its transcriptional activity and serving to restrain plant growth (Gallego-Bartolomé *et al.*, 2012). Upon exposure to abiotic stresses, ABA signals stabilize DELLA proteins (Achard *et al.*, 2006). It appears that ABA suppresses BR signalling at multiple levels. ROS may also be important in the interplay between ABA and BR signalling pathways through regulation of the action of DELLA proteins that control ROS levels. ROS production by NADPH oxidases plays a critical role in the BR-dependent activation of ABA biosynthesis (Xia *et al.*, 2014; Zhou *et al.*, 2014). The strength and duration of ABA induction depends on the concentration of BR applied in such experiments. DELLA proteins positive regulate ABA synthesis and so it would be interesting to investigate whether DELLA proteins are involved in BR-mediated regulation of ABA synthesis. If this is the case, then BR-induced ROS accumulation could serve to stabilize DELLA proteins, which in turn could suppress BR signalling. In this way, ROS levels may function in the negative feedback regulation for BR signalling.

The concept that SA and JA signalling pathways can function in an antagonistic manner is well established in the literature, although the mechanisms that mediate such interactions remain unresolved. The relationship between SA and JA signalling pathways is dependent on the relative concentrations of each hormone. Low SA concentrations can also act synergistically with JA in the regulated induction of defence genes, such as *PR-1* and *PDF1.2*, which are markers for SA and JA signalling pathways, respectively (Mur *et al.*, 2006). An antagonistic relationship between SA and JA signalling is observed when these hormones are present at high concentrations, and this often results in the establishment of an oxidative burst and cell death (Mur *et al.*, 2006). Thus, regulation of ROS production may play a role in the interactions between SA and JA signalling pathways. SA suppresses the JA-dependent induction of defence genes, but only once JA signalling has been established (Koornneef *et al.*, 2008). In this interaction, SA induction could lead to a transient perturbation in cellular redox homeostasis leading to thiol-dependent signalling that regulates the expression of both JA- and SA-mediated pathways (Han *et al.* 2013*a*, *b*). The addition of inhibitors of glutathione synthesis after induction of the JA signalling pathway prevents the antagonistic interactions between the SA and JA signalling pathways. The glutaredoxin GRX480 is important in the SA-dependent suppression of JA-responsive genes (Ndamukong *et al.*, 2007). Taken together, these findings demonstrate that modulation of cellular redox homeostasis and thiol signalling pathways play critical roles in SA–JA crosstalk.

NPR1 can function as a sensor of SA-mediated changes in cellular redox homeostasis (Mou *et al.*, 2003; Tada *et al.*, 2008). NPR1 is also required for the negative effect of SA on the JA signalling pathways (Spoel *et al.*, 2003). The intracellular localization of NPR1, which determines its differential interactions with other proteins, may contribute to the dynamics of SA–JA interactions. The TGA2/5/6 factors are positive regulators of *PDF1.2* expression (Zander *et al.*, 2010), whereas MYC2, which is a major regulator of JA signalling, has a negative effect on *PDF1.2* expression (Lorenzo *et al.*, 2004). The antagonistic effects of SA on the JA signalling pathways are at least partly dependent on the opposing effects of the TGA and MYC2 transcription factors on the expression of *PDF1.2*. It is still unclear whether NPR1-mediated SA suppression of JA-dependent defence genes involves competition for TGA factors that activate SA-dependent genes. However, following the induction of ET synthesis during the abiotic stress response, SA can suppress JA signalling in the absence of NPR1 (Leon-Reyes *et al.*, 2009). This finding is somewhat surprising given that ET and JA can synergistically regulate the expression of *PDF1.2*. The regulation of ROS production and accumulation is likely to be involved in the fine-tuning of the crosstalk of the ET, SA, and JA signalling pathways. In conditions where SA levels are high, ET may serve to trigger further increases in ROS accumulation, which could influence the balance of crosstalk between the different hormone-regulated pathways.

Perspectives

The above discussion has emphasized the central role of ROS production and accumulation in plant hormone-mediated signalling and action in response to developmental and environmental stimuli. ROS signalling coupled to the action of Ca^{2+} signals and MAPK cascades form a flexible feedback

loop that can serve to amplify the effects of hormonal signalling (Fig. 3). The spatial and temporal accumulation of ROS also has a strong impact on hormone synthesis, transport, and localization, as well as signalling, and thereby impacts on hormone-mediated growth and stress tolerance responses.

The mechanisms by which changes in ROS production and the relative levels in different cellular compartments function to control hormone-dependent processes are poorly understood. Unlike the situation in yeast, where ROS sensing is mediated by redox regulation of the Yap1 transcription factor, the receptor for ROS sensing mechanisms in plants are still unidentified. However, glutathione, TRXs, and thiol-regulated proteins are considered the most probable receptors and mediators of ROS signalling, functioning in a compartment-specific manner.

The rapid, reversible, and flexible redox modification of proteins is finely tuned by an intricate balance between ROS production and scavenging. Many redox-sensitive proteins exist in plants and each has the potential to fulfil a role in redox sensing and signal transduction. Redox regulation of integral pathway proteins provides a rapid and simple mechanism for the regulation of plant development and defence pathways.

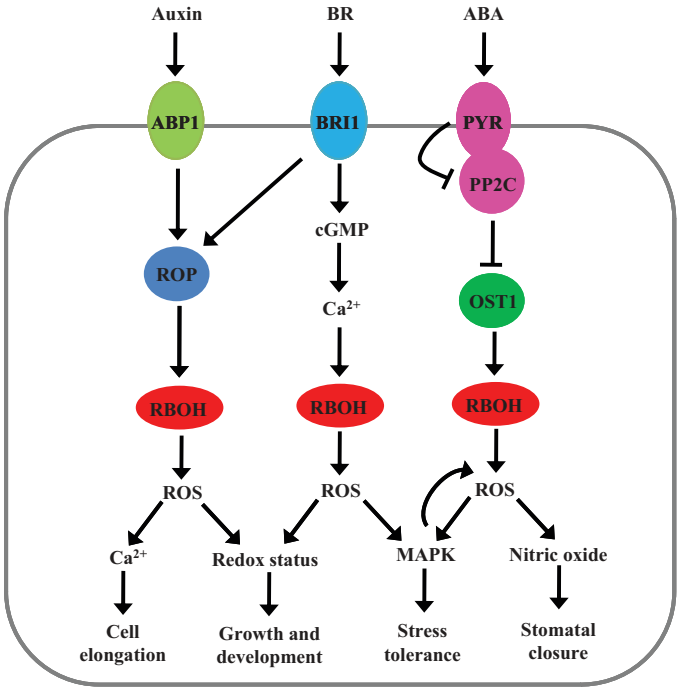

Fig. 3. A model for the involvement of ROS, redox, MAPK, and nitric oxide (NO) (nitric oxide) in signalling of auxin, BR, and ABA. Auxin-binding protein 1 (ABP1) is required for auxin-dependent activation of ROP-GTPases, which mediate ROS production through regulating RBOH, whereas RBOH-dependent ROS control cell elongation through the activation of Ca^{2+} channels. In addition, auxin-modulated ROS seems to play a role in regulating the TRX- and GSH-dependent redox status, which control plant growth and development potentially through regulating auxin homeostasis. BR activation of BRI1 leads to a sequential generation of second messengers: cGMP, Ca^{2+}, and ROS. Depending on the level of BR, different dynamics of ROS can induce a reduced redox status, which is associated with growth and development, or can lead to activation of MAPK cascades, which are involved in regulating stress tolerance. When ABA is perceived by the receptor PYR or PYR-like proteins, the phosphatase activity of PP2C is inhibited, leading to the release of OST1 from the suppression of PP2C. OST1 is required for the production of ROS through phosphorylation and activation of RBOH. ABA-induced ROS can form a positive feedback loop with MAPK, which regulates stress tolerance. Meanwhile, NO acts downstream of ROS in mediating ABA-induced stomatal closure.

There are many examples of redox regulation of proteins involved in plant metabolism, such as the thiol-modulated enzymes of carbon assimilation, metabolism, and transport. Within the domain of plant development and stress tolerance, the redox-mediated regulation of NPR1 leads to activation of transcription factors regulating the expression of defence genes. The redox regulation of NPR1 provides a classic example of how changes in intracellular compartmentation can be facilitated by redox regulation in order to elicit appropriate defence responses. The ABA-mediated stomatal closure is negatively regulated by NO through *S*-nitrosylation of the OST1 kinase (Wang *et al.*, 2014). Heat-shock factors are also potential targets of redox regulation. The application of redox proteomics within the context of a physiologically relevant background will help to identify potential redox-regulated proteins that perform key functions in plant development and environmental stress responses. However, further technical advances are required in order to probe the cellular pool of redox-regulated proteins for the full range of possible redox-regulated modifications. Measurements of processes such as protein glutathionylation remain technically challenging.

Whereas the concept that the plasma membrane NADPH oxidases are important components of ROS signalling pathways is firmly established in the literature, in many cases the exact molecular mechanisms that regulate NADPH oxidase activity are unknown. Similarly, it is unclear how NADPH oxidases are regulated to achieve specificity in ROS signalling. At present, only the RAC/ROP and CDPK proteins are known regulators of NADPH oxidase activity, for example in auxin and pathogen defence signalling. Similar mechanisms of direct NADPH oxidase regulation may exist with regard to other hormone signalling pathways. Alternatively, the activity of NADPH oxidases may be modulated in response to the highly dynamic Ca^{2+} signal oscillations that occur in response to stress.

The literature evidence discussed above demonstrates that dynamic spatial and temporal changes in ROS production and accumulation transmit specific information that is decoded by ROS-sensitive proteins in order to activate protein kinase cascades that in turn orchestrate downstream transcriptional responses. A new generation of improved *in situ* imaging techniques that use ROS-responsive reporters is required to detect the dynamics of spatial and temporal changes in ROS accumulation. ROS produced by metabolism in different organelles undoubtedly play important roles in the regulation of plant development and stress tolerance. However, the hormone-mediated regulation of plasma-membrane NADPH oxides appears to be extraordinarily important in the hormone crosstalk that underpins this control, as well as in systemic signalling associated with plant stress responses.

The plant genome encodes a large number of membrane-localized receptors and receptor-like kinases, many of which are localized in the plasma membrane. The plasma membrane is a hub of crosstalk between different signalling pathways. Regulation of the cellular localization of NADPH oxidases may be important in this integration. In pollen-tube tips, for

example, these enzymes are localized preferentially in polarized lipid microdomains. It is likely that interactions between NADPH oxidases and other membrane proteins also serve to modulate the specificity of ROS signalling. Clathrin- and membrane microdomain-mediated pathways cooperatively regulate endocytosis involving NADPH oxidases under stress conditions, providing a further layer of complexity.

ROS production and associated redox processing are an integral part of hormone regulation and function in the control of plant development and stress tolerance. We have only recently begun to understand the complex network of interactions and mechanisms that facilitate crosstalk between ROS and hormone signalling that underpin this control. Much remains to be discovered, particularly regarding the spatial–temporal regulation of ROS production as well as the identification of the proteins that sense changes in ROS and use this information to facilitate crosstalk between the different hormone signalling pathways.

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