ROS as key players in plant stress signalling

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Abstract

Reactive oxygen species (ROS) play an integral role as signalling molecules in the regulation of numerous biological processes such as growth, development, and responses to biotic and/or abiotic stimuli in plants. To some extent, various functions of ROS signalling are attributed to differences in the regulatory mechanisms of respiratory burst oxidase homologues (RBOHs) that are involved in a multitude of different signal transduction pathways activated in assorted tissue and cell types under fluctuating environmental conditions. Recent findings revealed that stress responses in plants are mediated by a temporal–spatial coordination between ROS and other signals that rely on production of stress-specific chemicals, compounds, and hormones. In this review we will provide an update of recent findings related to the integration of ROS signals with an array of signalling pathways aimed at regulating different responses in plants. In particular, we will address signals that confer systemic acquired resistance (SAR) or systemic acquired acclimation (SAA) in plants.

Key words: Reactive oxygen species (ROS), respiratory burst oxidase homologue (RBOH), stress response, systemic acquired acclimation (SAA), systemic acquired resistance (SAR), temporal–spatial coordination.

Introduction

The reactive oxygen species (ROS) signalling network is highly conserved among aerobic organisms and controls a broad range of biological processes such as growth, development, and responses to biotic and/or abiotic stimuli (Mittler et al., 2011). Although early research involving ROS metabolism focused on the potential toxicity of ROS and the different ROS-scavenging mechanisms, more recent studies have focused on the role ROS play as signalling molecules. To utilize ROS as signalling molecules, non-toxic levels must be maintained in a delicate balancing act between ROS production, involving ROS-producing enzymes and the unavoidable production of ROS during basic cellular processes, and the metabolic counter-process involving ROS-scavenging pathways (Mittler et al., 2004). In plants, NADPH oxidases, respiratory burst oxidase homologues (RBOHs), play a key role in the network of ROS production (Torres and Dangl, 2005; Suzuki et al., 2011). In Arabidopsis, RBOHs constitute a multigenic family comprised of 10 genes (i.e. AtRBOHA–AtRBOHJ). In recent years, several studies have revealed that plant RBOHs are involved in a multitude of different signalling pathways including root hair growth, stomatal closure, pollen–stigma interactions, plant defence, and acclimation to different abiotic stresses (Torres et al., 2005; McInnis et al., 2006; Monshausen et al., 2007; Jammes et al., 2009; Miller et al., 2009; Nishimura and Dangl, 2010; Suzuki et al., 2011).

Various biological processes that occur in different tissue types, under a multitude of environmental conditions, might be regulated by temporal and spatial coordination between ROS and other signals. In response to stress stimuli, early signalling events in plants include increased flux of Ca2+ into
the cytosol, activation of mitogen-activated protein kinases (MAPKs), and protein phosphorylation (Benschop et al., 2007). These regulatory mechanisms can all be activated within seconds or minutes (Benschop et al., 2007; Miller et al., 2009; Finka et al., 2012). Following these early signalling events, long-term responses control phenotypic changes such as growth, development, and survival of cells (Torres and Dangl, 2005; Coupe et al., 2006; Muhlenbock et al., 2008; Pesaresi et al., 2009; Dubiella et al., 2013). The contribution of ROS to these rapid and long-term responses is thought to occur as burst of ROS, often occurring as two distinctive peaks, accompanying the different signalling events (Nishimura and Dangl, 2010; Mittler et al., 2011). In addition, the differential co-expression of assorted RBOHs in various tissue and cell types indicates that, to some extent, a high degree of signal specialization is attributable to the spatial coordination of ROS signals (Suzuki et al., 2011).

Recent findings highlight the significance of cell-to-cell communication to mediating temporal–spatial coordination of signals in plants. Being sessile organisms, plants evolved sophisticated acclimation and defence mechanisms that can be activated in the primary tissue(s) exposed to stress, as well as in distal portions not directly exposed to stress (Fig. 1). The activation of defence or acclimation mechanisms in systemic or non-challenged tissues is termed systemic acquired resistance (SAR) or systemic acquired acclimation (SAA), respectively, and both play an important role in preventing further infection or damage to the entire plant (Karpinski et al., 1999; Rossel et al., 2007; Carr et al., 2010; Szczyrbska-Hebda et al., 2010; Dempsey and Klessig, 2012; Spel and Dong, 2012; Shah and Zeier, 2013). The involvement of ROS in systemic signalling during plant immunity, wound response, and high light acclimation was initially addressed more than a decade ago by Alvarez et al. (1998), Orozco-Cardenas and Ryan (1999), and Karpinski et al. (1999), respectively. We recently uncovered the existence of an H₂O₂-dependent long-distance signal induced by various abiotic stimuli (Miller et al., 2009). RBOHD was shown to be required for the initiation and self-propagation of a rapid cell to cell systemic signal that is dependent upon H₂O₂ accumulation in the extracellular spaces to generate a ‘ROS wave’ (Mittler et al., 2011). In addition, we demonstrated the important biological function of the ROS wave in the SAA of plants to heat or high light stresses (Suzuki et al., 2013). Our studies suggested that the ROS wave functions as a general priming signal in plants, alerting systemic tissues to the occurrence of a localized abiotic stress stimulus. Moreover, we found that the SAA of plants to abiotic stress is mediated by temporal–spatial

![Fig. 1. Different types of systemic signalling in plants. (A) Systemic acquired resistance (SAR) triggered by pathogens (viruses, bacteria, and fungi). The signal enhances resistance of systemic tissues to pathogens. (B) Systemic wound response triggered by insects and mechanical injury. The signal activates defence mechanisms in systemic tissues against insect attack. (C) Systemic acquired acclimation (SAA) triggered by abiotic stimuli such as high light, UV light, heat, cold, or salinity. The signal enhances tolerance of systemic tissues to abiotic stress. (D) Systemic metabolic responses triggered by changes in the level of sugars, phosphate, and other metabolites. The signal alters the metabolic state in systemic tissues. (E) Systemic developmental responses activated by changes in light conditions and atmospheric CO₂. Growth and stomatal distribution are coordinated in new developing leaves.](https://academic.oup.com/jxb/article-abstract/65/5/1229/2884866)
interactions of the ROS wave with stress-specific hormone or amino acid signals activated in systemic tissues.

In this review, we will provide an update on findings related to the temporal and spatial coordination between ROS and other signals that mediate stress responses in plants, especially SAA and SAR. We will also attempt to address key questions related to the mechanisms that determine signal specificity in response to different stimuli.

Regulatory mechanisms of RBOH proteins

Plant RBOHs have cytosolic FAD- and NADPH-binding domains in the C-terminal region, and six conserved transmembrane-spanning domains that correspond to those in mammalian NADPH oxidases (Kobayashi et al., 2007; Lin et al., 2009; Gliyan’ko and Ischenko, 2010; Proels et al., 2010; Kimura et al., 2012). Unlike the mammalian counterpart, plant RBOHs have a cytosolic N-terminal extension comprised of two Ca$^{2+}$-binding EF-hand motifs and phosphorylation target sites that are important for their activity (Kobayashi et al., 2007; Oda et al., 2010; Kimura et al., 2012; Drerup et al., 2013). Once actuated, superoxide (O$_2^-$) is produced at the apoplast via the function of RBOH proteins, and dismutates to H$_2$O$_2$ spontaneously or catalytically by the action of superoxide dismutase (SOD) (Lin et al., 2009; Wi et al., 2012). Membrane-permeable H$_2$O$_2$ can then play a key role as a signalling molecule that regulates cellular metabolism involved in growth, development, and response to environmental stimuli (Sagi et al., 2004; Xia et al., 2009).

Previous studies in Arabidopsis have revealed several regulatory mechanisms of RBOH protein homologues (e.g. AtRBOHC, D, and F) (Fig. 2). These mechanisms depend on various signalling components including protein phosphorylation, Ca$^{2+}$, calcium-dependent protein kinases (CDPKs), and phospholipase Dα1 (PLDα1) (Lin et al., 2009; Monshausen et al., 2009; Zhang et al., 2009; Jakubowicz et al., 2010; Drerup et al., 2013; Dubiella et al., 2013). Mechanical stimulation of plant tissue can initiate an increase in cytosolic Ca$^{2+}$ via an influx from the extracellular space across the plasma membrane (Monshausen et al., 2009). The increased Ca$^{2+}$ then activates RBOHC-dependent ROS production followed by an amplification loop between Ca$^{2+}$ and RBOHC to regulate root hair development (Monshausen et al., 2007, 2009; Takeda et al., 2008). Recent studies uncovered different regulatory mechanisms of RBOHD and RBOHF. Ca$^{2+}$ binding and phosphorylation synergistically activate the ROS-producing activity of RBOHD and RBOHF in Arabidopsis (Ogasawara et al., 2008; Kimura et al., 2012). A Ca$^{2+}$ increase in the cytosol was found to be necessary for the activation of RBOHD, and this activation requires conformational changes in EF-hand motifs by Ca$^{2+}$ binding (Ogasawara et al., 2008). PLDα1 and its lipid product phosphatidic acid (PA) play an integral role in abscisic acid (ABA)-induced production of ROS in guard cells via the function of RBOHD and RBOHF (Zhang et al., 2009). The PA-binding motifs, arginine residues 149, 150, 156, and 157, in RBOHD are required for ROS production and stomatal closure. RBOHF was also found to be phosphorylated by OPEN STOMATA 1 (OST1) at Ser174 and Ser13 during ABA-dependent stomatal closure (Sirichandra et al., 2009). Although these findings indicate an integration between RBOHD and RBOHF in the regulation of ABA-dependent stomatal closure, the coordination between PA and OST1 needs to be addressed in future studies. In a recent study, RBOHD was shown to be phosphorylated by calcium-dependent protein kinase 5 (CPK5) during pathogen defence (Dubieda et al., 2013). RBOHD’s involvement along the path of the rapid systemic signalling is H$_2$O$_2$ dependent and requires CPK5, supporting the hypothesis that Ca$^{2+}$-dependent ROS production is involved in the propagation of the ROS wave over long distances (Miller et al., 2009). In addition, a recent finding demonstrated that the activity of RBOHF is regulated by direct Ca$^{2+}$ binding to its EF-hands and Ca$^{2+}$-dependent phosphorylation by CBL1/9–CIPK26 complexes (Drerup et al., 2013). Taken together, these findings indicate that the diverse functions of RBOH signalling in plants might be, at least partially, attributed to differences in regulatory mechanisms.

Regulatory mechanisms of RBOH proteins were also studied in other plant species and crops. A homologue of mammalian Rac in rice (OsRac1) was shown to be a positive regulator of OsRBOHB involved in pathogen defence (Wong et al., 2007; Oda et al., 2008). OsRac1 activates OsRBOHB by directly interacting with its N-terminal region that includes the EF-hand motifs in a Ca$^{2+}$-dependent manner. The significance of OsRac1 to ROS production was supported by the finding that constitutively active or dominant-negative forms of OsRac1 can stimulate or suppress ROS function, respectively (Ono et al., 2001; Wong et al., 2007). In potato, two Ca$^{2+}$-dependent protein kinases, StCDPK4 and StCDPK5, were found to activate StRBOHB-dependent ROS production (Kobayashi et al., 2007). Two phosphorylation sites, Ser82 and Ser97, were identified in the N-terminal region of StRBOHB, and phosphorylation at Ser82 was shown to be required for the oxidative burst during pathogen defence (Kobayashi et al., 2007). In addition, the N-terminal variable domain of StCDPK5, including the myristoylation and palmitoylation sites, confers subcellular localization which results in interaction and phosphorylation of StRBOHB in vivo (Asai et al., 2013). In pepper, Capsicum annum, receptor-like protein kinase 1 (CaRLK1) is induced by pathogen infection and exogenous application of H$_2$O$_2$ (Yi et al., 2010). Transgenic plants that constitutively express CaRLK1 exhibit enhanced resistance to pathogen infection and reduced cell death, as well as increased O$_2$ production and expression of RBOH genes (Yi et al., 2010). As described above, several regulatory mechanisms of RBOH proteins found in different crops seem to be slightly different from those found in Arabidopsis. It would be interesting to determine whether RBOH regulatory mechanisms are highly conserved among various plant species or evolutionarily diverse.

RBOH proteins are not the only source for ROS in plant cells. Numerous pathways for ROS production exist in plants, and include photosynthesis (via the electron transport chain and photosystems I and II), respiration (via the electron transport chain), glycolate oxidase, oxalate oxidase, xanthine...
oxidase, amine oxidase, excited chlorophyll, fatty acid oxidation, and peroxidases (Mittler, 2002). Different oxidases were found to play a role in the response of plants to various biotic and abiotic stresses. Oxalate oxidase was shown to be involved in ROS production in root cells during drought stress (Voothuluru and Sharp, 2013), whereas glycolate oxidase (GOX) has been shown to play a role in non-host pathogen defence in Arabidopsis and tobacco (Rojas and Mysore, 2012; Rojas et al., 2012). Peroxidases also play important roles in ROS production during defence responses in plants. PEROXIDASE33 (PRX33) and PRX34 have been identified as major contributors to ROS production in Arabidopsis during responses to a fungal cell wall elicitor or bacterial pathogens (Daudi et al., 2012; O’Brien et al., 2012b; Wrzaczek et al., 2013). Peroxidase-dependent ROS are also involved in callose deposition and expression of defence genes (Daudi et al., 2012; Wrzaczek et al., 2013). Peroxidases might be responsible for about half of \( \text{H}_2\text{O}_2 \) production that is induced.

Fig. 2. Regulatory mechanisms of RBOH proteins in Arabidopsis. (A) Basic structure of plant RBOH proteins. RBOHs have cytosolic FAD- and NADPH-binding domains in the C-terminal region, and six conserved transmembrane-spanning domains (blue cylinders). The N-terminal domain (box) contains two EF-hand motifs and phosphorylation target sites that are important for activity of RBOHs (B–F). (B) Binding of Ca\(^{2+}\) to EF-hand motifs is required for activation of RBOHs (Ogasawara et al., 2008; Drerup et al., 2013). (C) Activation of RBOHD by phosphorylation of Ser39, Ser148, Ser343, and Ser347 residues via the function of CPK5 (Dubiella et al., 2013). (D) Activation of RBOH by phosphorylation of Ser13 and Ser174 residues via function of OPEN STOMATA 1 (OST1) kinase (Sirichandra et al., 2009). (E) Activation of RBOH by binding of phosphatidic acids (PAs) produced via the function of phospholipase D\(\alpha\)1 (PLD\(\alpha\)1) to Arg149, Arg150, Arg156, and Arg157 residues (Zhang et al., 2009). PA also activates a Ca\(^{2+}\) channel located at the plasma membrane. (F) Activation of RBOHF by phosphorylation of the N-terminal domain via the function of CBL1/9–CIPK26 complexes and direct binding of Ca\(^{2+}\) to the EF-hand motifs (Drerup et al., 2013).
by bacterial pathogens (O’Brien et al., 2012b). Interestingly, PRX knockdown plants exhibited stronger dysfunction of defence gene expression and callose disposition compared with rbhD plants in response to fungal elicitor, suggesting that ROS production by peroxidases might not be functionally equivalent to ROS generated by RBOH proteins (Daudi et al., 2012; Wrzaczek et al., 2013). This hypothesis can be supported by the finding that stomatal closure and ROS burst induced by a yeast elicitor were not inhibited in rbhD and rbhF mutants in Arabidopsis (Khokon et al., 2010). In addition, recent studies uncovered a role for peroxidase-dependent ROS in the regulation of root growth and response to potassium deficiency (Kim et al., 2010; Jia, 2011; Kwasniewski et al., 2013). The integration between RBOH proteins and peroxidases should be addressed in future studies.

Functional differences between RBOH proteins and peroxidases may be partially attributed to differences in the types of ROS generated by these enzymes. Superoxide (O₂⁻), generated by RBOH proteins, can activate specific signalling pathways distinct from those activated by H₂O₂ (Suzuki et al., 2011). Another possibility is that diverse functions between these different types of enzymes might be due to differences in their respective reductants. NADPH serves as a reductant for the RBOH-dependent generation of superoxide. In contrast, different chemicals or compounds including phenols, organic acids, and auxin have been suggested as candidate reductants for the peroxidase-dependent generation of H₂O₂ (O’Brien et al., 2012a). Pathways involving these different reductants could be integrated with ROS signals activated via the different functions of these enzymes.

**Temporal coordination of ROS signals in plants**

Changes in environmental conditions are likely to cause rapid changes in the level, composition, and structure of different metabolites, proteins, and RNA molecules, that precede signal transduction or acclimation events in plants. Early signalling events including ion fluxes across the plasma membrane, increased Ca²⁺ levels in the cytosol, activation of MAPKs, and production of ROS can all be activated within minutes following application of biotic or abiotic stimuli (Benschop et al., 2007; Miller et al., 2009; Finka et al., 2012). For example, ROS production can be triggered in tobacco cells within 3 min following heat stress, and heat stress-induced ROS can be inhibited by an NADPH oxidase inhibitor (Konigshofer et al., 2008). A plasma membrane channel that initiates an inward calcium flux has been identified as one of the heat sensors (Saidi et al., 2009; Finka et al., 2012; Mittler et al., 2012). Ca²⁺ channels transiently open and induce an inward flux of Ca²⁺ into the cytosol within 10 min following heat stress (Saidi et al., 2009; Finka et al., 2012). This Ca²⁺ signal might be linked to regulatory mechanisms of ROS-producing enzymes (Mittler et al., 2012). Using phosphoproteomic approaches, researchers demonstrated that a pathogen elicitor, flg22, activates MAPKs and induces phosphorylation of membrane proteins including ion channels, calmodulins, protein kinases, protein phosphatases, and proteins associated with auxin signalling, as well as RBOHD in Arabidopsis suspension-cultured cells within 5–10 min (Benschop et al., 2007). In addition, CDPKs in plants are biochemically activated within a few minutes following exposure to biotic stimuli and participate in the induction of early defence responses (Ludwig et al., 2005; Kobayashi et al., 2007; Boudsocq and Sheen, 2013). CDPK-dependent in vivo phosphorylation of AtRBOHD can be induced within 15 min in leaves not directly challenged with a pathogen elicitor, flg22 (Dubiella et al., 2013).

Early responses of plants to high light have also been described in previous studies (Karpinski et al., 1999; Rossel et al., 2007; Muhlenbock et al., 2008; Szychsinska-Hebda et al., 2010; Gordon et al., 2012). Studies utilizing transgenic plants expressing a luciferase reporter gene under the control of an APX1, APX2, or ZAT10 promoter demonstrated the activation of acclimatory responses within 5–20 min following application of high light both in leaves locally exposed to the stimuli and in distal tissues that did not directly encounter the stimuli (Karpinski et al., 1999; Rossel et al., 2007; Szychsinska-Hebda et al., 2010). These high light responses are shown to be associated with redox changes in the plastquinone (PQ) pool, increased production of ROS and ethylene, reduction of maximal photochemical efficiency and non-photochemical quenching (NPQ), and changes in extra-cellular electric potential (Karpinski et al., 1999; Rossel et al., 2007; Szychsinska-Hebda et al., 2010).

Research has also demonstrated a rapid response in plants to mechanical wounding. In Arabidopsis, elevated levels of jasmonic acid (JA) accumulate in damaged tissues as well as undamaged systemic leaves within 30 s to 5 min in response to mechanical wounding (Glauser et al., 2009; Koo et al., 2009). The velocity of the long-distance signal leading to de novo synthesis of JA in systemic tissues was 3.4–4.5 cm min⁻¹ (Glauser et al., 2009; Koo et al., 2009). In a recent study, we demonstrated that the NADPH oxidase homologue RBOHD is required for the initiation and amplification of a rapid auto-propagating systemic signal that travels at the rate of ~8.4 cm min⁻¹, and is induced by various abiotic stimuli including mechanical wounding (Miller et al., 2009). In addition, the potential involvement of electric signals that propagate with similar rates was also implicated in RBOHD-triggered rapid systemic signalling during wounding (Zimmernann et al., 2009; Mittler et al., 2011; Suzuki and Mittler, 2012). These findings implicate the integration of JA and mobile signals such as ROS and electric signals.

We recently revealed that RBOHD-dependent long-distance signals play an important biological role in the SAA response of plants to heat or high light (Suzuki et al., 2013). SAA, which enhances the plant’s tolerance to heat stress, was correlated with activation of the ROS wave and occurred as early as 5–10 min following heat stress application. In addition, metabolome analysis revealed the rapid accumulation of glycine, serine, and glyceraldehyde in leaves directly exposed to high light within 60 s as well as in systemic tissues of plants at 15 min and 45 min following local high light treatment, suggesting that a portion of the photorespiratory machinery is involved in the early
response of plants to high light. Rapid local responses to high light were altered in the mutant lacking cytosolic APX1, demonstrating the involvement of H₂O₂ scavenging in this process.

Long-term responses to fluctuating environmental conditions regulate phenotypic changes such as growth, development, and survival of cells. For example, defence mechanisms associated with ROS signalling confer protection to plants hours or days following pathogen infection (Torres et al., 2005; Muhlenbock et al., 2008; Dubiella et al., 2013). ROS accumulation mediated by RBOH proteins is also observed hours or days following pathogen infection, and ROS accumulation has been shown to be accompanied by gradual necrotic symptoms (Kobayashi et al., 2007; Wi et al., 2012).

Developing leaves, not directly exposed to changes in light conditions and atmospheric CO₂ experienced by mature leaves, can alter their photosynthetic rate and tolerance to high light, as well as growth and development (Coupe et al., 2006; Araya et al., 2008; Jiang et al., 2012). Although alterations in photosynthetic rate and response to high light implicate ROS and redox signalling in the systemic regulation of long-term responses in new developing leaves, links between ROS signalling and these responses have yet to be conclusively established. ROS signalling can be modulated by the redox state of the PQ pool in chloroplasts and plays a key role in the response of plants to changes in fluctuating light conditions (Muhlenbock et al., 2008; Li et al., 2009; Mittler et al., 2011). Changes in light quality result in imbalances in energy distribution between the photosystems and induce altered thylakoid composition via the function of STN7 kinase within hours and days (Pesaresi et al., 2009).

How are rapid and long-term responses of plants to stress stimuli linked? Previous studies demonstrated that the biphasic production of ROS consists of a primary phase that occurs within minutes and a secondary phase that occurs within hours/days (Soares et al., 2009; Nishimura and Dangl, 2010; Kunihiro et al., 2011; Mittler et al., 2011). Such a biphasic ROS production accompanies several different signalling events in many biological systems (Soares et al., 2009; Nishimura and Dangl, 2010; Mittler et al., 2011). For example, mechanical wounding induced an initial burst of O₂⁻ within 3 min followed by later production of O₂ and H₂O₂ after 6 h (Soares et al., 2009). Inhibition of early ROS accumulation by an NADPH oxidase inhibitor suppresses later production of O₂⁻ and accumulation of wound response proteins. These results indicate that a rapid burst of ROS is required for the later phase of ROS production which regulates downstream pathways and acclimation of plants to stress stimuli. Our recent findings suggest that these two phases of the ROS burst are linked via the ROS wave that communicates the initial ROS burst in the local tissue to the systemic tissue via a cell to cell relay mechanism (Miller et al., 2009; Suzuki et al., 2013).

**Spatial coordination of ROS signals in plants**

Plant genomes, such as that of *Arabidopsis*, contain a host of RBOH homologues. In recent years, several studies have revealed that plant RBOHs perform a multitude of signalling functions in assorted tissue and developmental stages (Suzuki et al., 2011), implying that various biological processes are regulated by coordination between different ROS signals activated in individual plant tissue types.

To some extent, signals generated in plants during SAA are similar in local and systemic tissues. Rossel et al. (2007) compared the transcriptomes of local leaves, directly exposed to high light, and systemic leaves, not directly challenged by the stimulus. More than 70% of the transcripts up-regulated in local leaves in response to high light were also altered in systemic leaves, suggesting that similar signals exist between local and systemic tissues during high-light-mediated SAA. Similarities between local and systemic responses to high light are demonstrated by findings showing that alterations in ROS and redox signals occurred both in local and in systemic tissues (Muhlenbock et al., 2008; Miller et al., 2009; Szychynska-Hebda et al., 2010). Our recent study demonstrated that amino acids involved in the photorespiratory pathway such as glycine, serine, and glyceralate are similarly altered in both local and systemic tissues in response to local application of high light (Suzuki et al., 2013). Local application of heat or cold stimuli can also induce similar stress response proteins or transcripts in both local and systemic tissues (Gorsuch et al., 2010; Suzuki et al., 2013). In particular, induction of heat-responsive proteins in systemic tissue was shown to be ROS wave dependent (Suzuki et al., 2013).

Although signals generated in local and systemic tissues showed considerable overlap, previous studies have demonstrated differences in alterations of transcripts or metabolites between these types of tissues. Ethylene accumulated in both local and systemic tissue in response to local application of high light; nevertheless, the signal mediated by ethylene insensitive 2 (EIN2) was shown to be required for induction of APX2 only in systemic tissues, but not in leaves directly subjected to high light (Muhlenbock et al., 2008). In addition, SID2 delays induction of APX2 in leaves directly exposed to high light but not in systemic leaves. Expression of APX2 in local and systemic tissue, in response to high light, might be regulated by the coordination between ethylene and SA signalling during SAA. A recent study demonstrated spatial diversity in high light responses between different leaves of plants during SAA (Gordon et al., 2012). Thus, local high light treatment resulted in the accumulation of different transcript levels of ZAT10 and Redox Responsive Factor 1 (RRTF1) in systemic leaves, depending on leaf position (Gordon et al., 2012). Plants possess mechanisms to alter spatial distribution of metabolites in response to stress stimuli (Schwachtje and Baldwin, 2008; Simon et al., 2010). Metabolic profiling of pathogen-infected and adjacent uninfected leaf tissues demonstrated a quantitatively different distribution of secondary metabolites between these regions of leaf tissues (Simon et al., 2010). The distribution of secondary metabolites was altered in a mutant lacking CAT2, suggesting a role for ROS-scavenging mechanisms in determining the distribution of secondary metabolites in response to pathogen infection.

How are the signals generated in local and systemic tissues linked? The ROS wave may play a key role in propagating...
signals from local tissues to systemic tissues. The initial abiotic stress-induced burst of ROS in a local group of plant cells triggers a cascade of cell-to-cell communication events that propagate throughout different tissues of the plant and carries a systemic signal over long distances (Miller et al., 2009). Szechynska-Hebda et al. (2010) uncovered the pattern of spreading systemic changes in NPQ, H2O2 concentration, and APX1 expression during SAA response of plants to high light. Wave-like patterns of APX1 expression in systemic tissue of plants correlate positively with H2O2 accumulation, but negatively with NPQ (Szechynska-Hebda et al., 2010; Karpinski et al., 2013). The activation of systemic signals by local application of high light was recently shown to be accompanied by plasma membrane electrical signals in a light wavelength-specific manner (Szechynska-Hebda et al., 2010). Our recent finding that the RBOHD-dependent ROS wave is associated with the generation and/or propagation of systemic potential variations may demonstrate a link between electric signals in plants and ROS production (Suzuki et al., 2013). In addition, recent studies identified a number of different chemicals and compounds involved in pathogen-induced SAR in plants, and these signalling molecules might be transported from local tissue to systemic tissue (Dempsey and Klessig, 2012; Shah and Zeier, 2013). Integration of ROS signals and these metabolic cues could be a promising subject for future studies.

Previous studies have highlighted the signalling links between shoot and root during the defence response following insect attack (Soler et al., 2013; Wondafrash et al., 2013). For example, root herbivory elicits water limitation in the shoot, resulting in induction of ABA accumulation in leaves (Soler et al., 2013). Involvement of ROS signalling in mediating a connection of signals between different tissue types is suggested by the distribution of RBOH proteins from roots to reproductive tissues (Suzuki et al., 2011) and our finding that RBOHD-dependent long-distance signals propagate from leaves to the entire plant (Miller et al., 2009). In our recent study, grafting experiments between wild-type and rbohD seedlings demonstrated the significance of RBOHD to propagating a signal from leaves to roots (Suzuki et al., 2013). Local application of heat stress to the cotyledons (local tissue) of control grafted seedlings (wild type–wild type) resulted in the enhanced SAA response to heat stress in root tips (systemic tissue). In contrast, this SAA response was attenuated in all grafting experiments that involved a deficiency in RBOHD in the local or systemic tissues.

**Integration of ROS signals with chemicals, compounds, and hormones**

Numerous studies uncovered metabolic cues including hormones, amino acids, and chemical compounds activated by stress stimuli. Plants recognize various pathogens by utilizing extracellular surface receptors to decode pathogen-associated molecular patterns (PAMPs), and consequently initiate defence responses (Dempsey and Klessig, 2012). Defence responses in local and systemic tissues are characterized by accumulation of free salicylic acid (SA) and its 2-O-β-D-glucoside (SAG), as well as elevated expression of pathogenesis-related (PR) genes (Dempsey and Klessig, 2012). These metabolic changes, which may be induced by pathogens, can result in SAR (Dempsey and Klessig, 2012). Signals are initiated in infected tissue, translocated by the vascular tissue to distal portions of the plant, typically by the phloem, and perceived in systemic tissue (Dempsey and Klessig, 2012; Kachroo and Robin, 2013). Research in the area of phloem-mobile SAR signals identified several biologically active molecules in phloem sap, including methyl salicylate (MeSA) (Park et al., 2007), a glycerol-3-phosphate (G3P) derivative (Chanda et al., 2011), a lipid transfer protein (DIR1) (Maldonado et al., 2002), azelaic acid (AzA) (Jung et al., 2009), dehydroabietylaldehyde (DA) (Chaturvedi et al., 2012), jasmonic acid (JA) (Truman et al., 2007), and pipelic acid (Pip) (Dempsey and Klessig, 2012; Shah and Zeier, 2013). Current research has demonstrated that MeSA, AzA, DA, and G3P all induce systemic resistance when applied locally (Kachroo and Robin, 2013). Research has also demonstrated that primary pathogen infection results in rapid accumulation of AzA and G3P (Chanda et al., 2011; Kachroo and Robin, 2013). Neither AzA nor G3P induces SA accumulation; however, AzA is thought to prime the plant for future SA accumulation in response to a secondary infection (Dempsey and Klessig, 2012; Kachroo and Robin, 2013). In Arabidopsis, AtBSMT1, up-regulated by JA and NiSAMT1 expression in tobacco, converts a portion of accumulating SA to MeSA (Dempsey and Klessig, 2012; Kachroo and Robin, 2013). This biologically inactive molecule can then travel to systemic leaves via the phloem, and upon arrival is converted to SA by SABP2 (Dempsey and Klessig, 2012). DIR1 (defective in induced resistance1), which gains access to the phloem via the cytosol of companion cells, and AZI1 (AzA INDUCED 1) interact with each other, and are essential for AzA-, DA- (Dempsey and Klessig, 2012), and G3P-mediated SAR (Dempsey and Klessig, 2012; Kachroo and Robin, 2013). Additional putative SAR signalling molecules in the phloem include DA and Pip. CTR1 can be inhibited by TRIP 2009). Ethylene biosynthesis was found to be modulated by positive regulation via RBOH proteins and negative regulation by CTR1 (constitutive triple response 1) (Jakubowicz et al., 2010). In Arabidopsis, CTR1 can be inhibited by...
PA which positively enhances activation of RBOHD and RBOHF (Jakubowicz et al., 2010). Previous studies revealed the involvement of ethylene in the regulation of SAR and SAA to high light induced by local high light application (Muhlenbock et al., 2008; Karpinski et al., 2013). In response to high light, alterations in the redox state of the PQ pool can initiate a signal that induces production of 1-aminocyclopropane-1-carboxylate (ACC; the immediate precursor of ethylene), ROS, and the expression of ethylene-regulated genes (Muhlenbock et al., 2008). Increased ROS production results in bleaching of leaves, and programmed cell death that relies on regulation of EIN2 by LESION SIMULATING DISEASE 1 (LSD1) (Muhlenbock et al., 2008; Karpinski et al., 2013). Recent studies demonstrated the involvement of brassinosteroids (BRs) in SAR and SAA to high light in cucumber (Xia et al., 2009, 2011; Li et al., 2013). Although BRs are not directly involved in long-distance signalling, they affect other signals such as auxins and polyamines (Li et al., 2013). Local applications of 24-epibrassinosteroid (EBR) can induce SAR and SAA to oxidative stress accompanied by local and systemic expression of known defence/acclimatory genes such as APX and catalase (Li et al., 2013). Involvement of BR signalling in ROS-dependent stress responses was also supported by the finding that exogenous BR treatments

**Fig. 3.** Temporal–spatial coordination of the ROS wave in plants. (A) A hypothetical model for local and systemic stress response signal transduction in plants. Local stress stimuli can initiate rapid signalling events such as an increase in the cytosolic Ca$^{2+}$ level, production of ROS, and activation of stress-specific metabolic cues within seconds or minutes. These rapid signals that are propagated through the entire plant can activate defence or acclimatory mechanisms in systemic tissues. Long-term responses to fluctuating environments regulate phenotypic changes such as growth, development, and survival of cells. (B) Time-lapse imaging of rapid systemic signal transduction in *Arabidopsis* using a luciferase reporter gene expressed under the control of the Zat12 promoter (Zat12:Luc) in response to wounding (Miller et al., 2009). The wounding site is indicated with a white arrow.
resulted in enhanced tolerance to oxidative stress accompanied by induction of H$_2$O$_2$ production in the apoplast and expression of RBOH, MAPK1, and MAPK3 (Xia et al., 2009). A recent study indicated the involvement of auxin in the SAA response of plants to high light. Large portions of the transcripts that exhibited significant changes in the distal leaves overlap with auxin-responsive transcripts (Gordon et al., 2012), indicating a connection between SAA and developmental processes mediated by auxin. Integration between ethylene and BRs during SAR or SAA response to high light need to be elucidated in future studies.

Amino acids involved in the photorespiratory pathway such as glycine, serine, and glycerate were shown to be accumulated in systemic tissues at 15 min following local application of high light, but not heat stress or wounding (Suzuki et al., 2013). Signals regulated by these high-light-specific amino acids also need to be linked with hormone signals.

ABA is involved in a broad range of biological functions, and its integration with ROS has been revealed (Kwak et al., 2003; Sagi et al., 2004; Ma et al., 2012; Drerup et al., 2013). For example, RBOHD and RBOHF function synergistically in signalling cascades to regulate stomatal closure, seed germination, root elongation, and Na$^+$/K$^+$ homeostasis under salt stress (Kwak et al., 2003; Ma et al., 2012). In addition, ABA and SA treatment have been shown to result in transient increases in H$_2$O$_2$ production which induces tolerance to salt, high light, heat, and oxidative stress (Xia et al., 2009). Our recent study demonstrated that SAA of plants to heat stress was correlated with activation of the ROS wave and transient accumulation of ABA in systemic tissues, and these responses were suppressed in a mutant lacking RBOHD (Suzuki et al., 2013). The SAA response to heat stress was also attenuated in mutants deficient in ABA signalling. These results indicate that temporal–spatial interactions between RBOHD-dependent ROS and ABA mediate SAA to heat stress (Suzuki et al., 2013).

Cross-talk between response pathways to different stress stimuli

Cross-talk exists between complex signalling networks that regulate different stress response pathways. There is an extensive overlap between pathogen defence and response to high light (Mullineaux and Baker, 2010; Straus et al., 2010). Accordingly, local application of high light induces tolerance of plants to pathogen infection as well as SAA to oxidative stress in systemic tissues, indicating a cross-talk between high light acclimation and pathogen responses (Rossel et al., 2007; Muhlenbock et al., 2008; Karpinski et al., 2013). This process is accompanied by alterations in ROS and redox signals, and induction of glutathione and SA in both local and systemic leaves (Muhlenbock et al., 2008). During acclimation, plants express LESION SIMULATING DISEASE 1 (LSD1) that suppresses the ROS-/ethylene-dependent programmed cell death pathway by acting as a negative regulator of pathogen response genes, EDS1 and PAD4 (Karpinski et al., 2013). Another example of cross-talk between biotic and abiotic stress responses is insect attack and wounding. Systemic signalling induced by insect attack and wounding relies on biosynthesis of JA at the site of wounding and the ability to perceive a jasmonate signal in the systemic tissues (Suzuki and Mittler, 2012). JA could play a key role in the regulation of cell death associated with H$_2$O$_2$ and SA signalling during insect attack and wounding (Pasqualini et al., 2003; Zhou et al., 2009; Lin et al., 2011).

Conclusions

ROS play a pivotal role in regulating numerous responses to biotic and abiotic stresses in plants. The complexity in ROS responses to various environmental stimuli might be, at least partially, attributed to different regulatory mechanisms of ROS production via NADPH oxidases (RBOHs) that function in an array of tissue types and developmental stages under various environmental conditions. Plants have evolved different mechanisms that control temporal and spatial coordination between ROS and other signals activated in separate parts of the plant at different times (Fig. 3). A key mechanism in coordinating these complex spatial and temporal responses in plants is the cascade of cell to cell communication events that result in the formation of a wave of ROS production that rapidly propagates throughout the different tissues of the plant. The significance of this rapid systemic signalling to SAR and SAA in plants has been revealed by recent studies. How the ROS wave is integrated with different stress-specific signals is, however, an open question that requires further research.

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References


