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Reactive oxygen species homeostasis and signalling during drought and salinity stresses

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ABSTRACT

Water deficit and salinity, especially under high light intensity or in combination with other stresses, disrupt photosynthesis and increase photorespiration, altering the normal homeostasis of cells and cause an increased production of reactive oxygen species (ROS). ROS play a dual role in the response of plants to abiotic stresses functioning as toxic by-products of stress metabolism, as well as important signal transduction molecules. In this review, we provide an overview of ROS homeostasis and signalling in response to drought and salt stresses and discuss the current understanding of ROS involvement in stress sensing, stress signalling and regulation of acclimation responses.

Key-words: abiotic stress; osmotic stress; ROS metabolism; ROS signalling; scavenging.

INTRODUCTION

Acclimation of plants to changes in their environment requires a new state of cellular homeostasis achieved by a delicate balance between multiple pathways that reside in different cellular compartments. This coordination may, however, be disrupted during water and salt stresses, especially when the cell or the entire plant is exposed to a rapid decrease in water potential, or when additional environmental parameters are involved (Mittler *et al.* 2006). When different pathways are uncoupled, electrons that have a high-energy state are transferred to molecular oxygen (O_2) to form reactive oxygen species (ROS; Takahashi & Asada 1988; Mittler 2002). ROS, such as $^1O_2$, H_2O_2, $O_2^{\cdot-}$ and $HO^{\cdot}$, are toxic molecules capable of causing oxidative damage to proteins, DNA and lipids (Apel & Hirt 2004). Under optimal growth conditions, ROS are mainly produced at a low level in organelles such as chloroplasts, mitochondria and peroxisomes. However, during stress, their rate of production is dramatically elevated.

ROS accumulation during stress greatly depends on the balance between ROS production and ROS scavenging (Mittler *et al.* 2004), that in turn depends on changes in growth conditions (light intensity, temperature, etc.), as well as the severity and the duration of the stress and the ability of the tissue to rapidly acclimate to the energy imbalance. In chloroplasts, limitation of CO_2 fixation coupled with over-reduction of the electron transport chain is the main cause of ROS production. Over-reduction of the electron transport chain in mitochondria is also a major mechanism of ROS generation during stress (Davidson & Schiestl 2001; Fig. 1). In peroxisomes, H_2O_2 is produced when glycolate is oxidized to glyoxylic acid during photorespiration (Mittler *et al.* 2004; Fig. 1). Essential for ROS detoxification during normal metabolism, and particularly during stress, are antioxidants such as ascorbic acid (AsA) and glutathione (GSH), and ROS-scavenging enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX) and peroxiredoxin (PrxR) (Takahashi & Asada 1988; Apel & Hirt 2004; Mittler *et al.* 2004; Dietz *et al.* 2006). These mechanisms have been found in almost all cellular compartments, demonstrating the importance of ROS detoxification for cellular survival (Mittler *et al.* 2004; Fig. 1).

Osmotic stress and salinity were shown to enhance the production of ROS and cause ROS-associated injury (Serrato *et al.* 2004; Borsani *et al.* 2005; Miao *et al.* 2006; Abbasi *et al.* 2007; Zhu *et al.* 2007; Giraud *et al.* 2008). In accordance, ROS-scavenging mechanisms were shown to have an important role in protecting plants against osmotic stresses and a combination of high light and temperature stresses (Rizhsky *et al.* 2004b; Wang *et al.* 2005; Leshem *et al.* 2006; Abbasi *et al.* 2007; Koussevitzky *et al.* 2008). An additional contributor to cellular damage during different abiotic stresses is high light. High-light intensity stress has the potential to enhance the production of ROS in cells and cause oxidative damage to chloroplasts (Niyogi 1999; Davletova *et al.* 2005a; Møller, Jensen & Hansson 2007; Triantaphylides *et al.* 2008).

While ROS have the potential to cause oxidative damage to cells during environmental stresses, recent studies have shown that ROS play a key role in plants as signal transduction molecules involved in mediating responses to pathogen infection, environmental stresses, programmed cell death and different developmental stimuli (Mittler *et al.* 2004; Torres & Dangl 2005). The rapid increase in ROS production, referred to as 'the oxidative burst', was shown to be essential for many of these processes, and genetic studies have shown that respiratory burst oxidase homolog (*Rboh*) genes, encoding plasma membrane-associated

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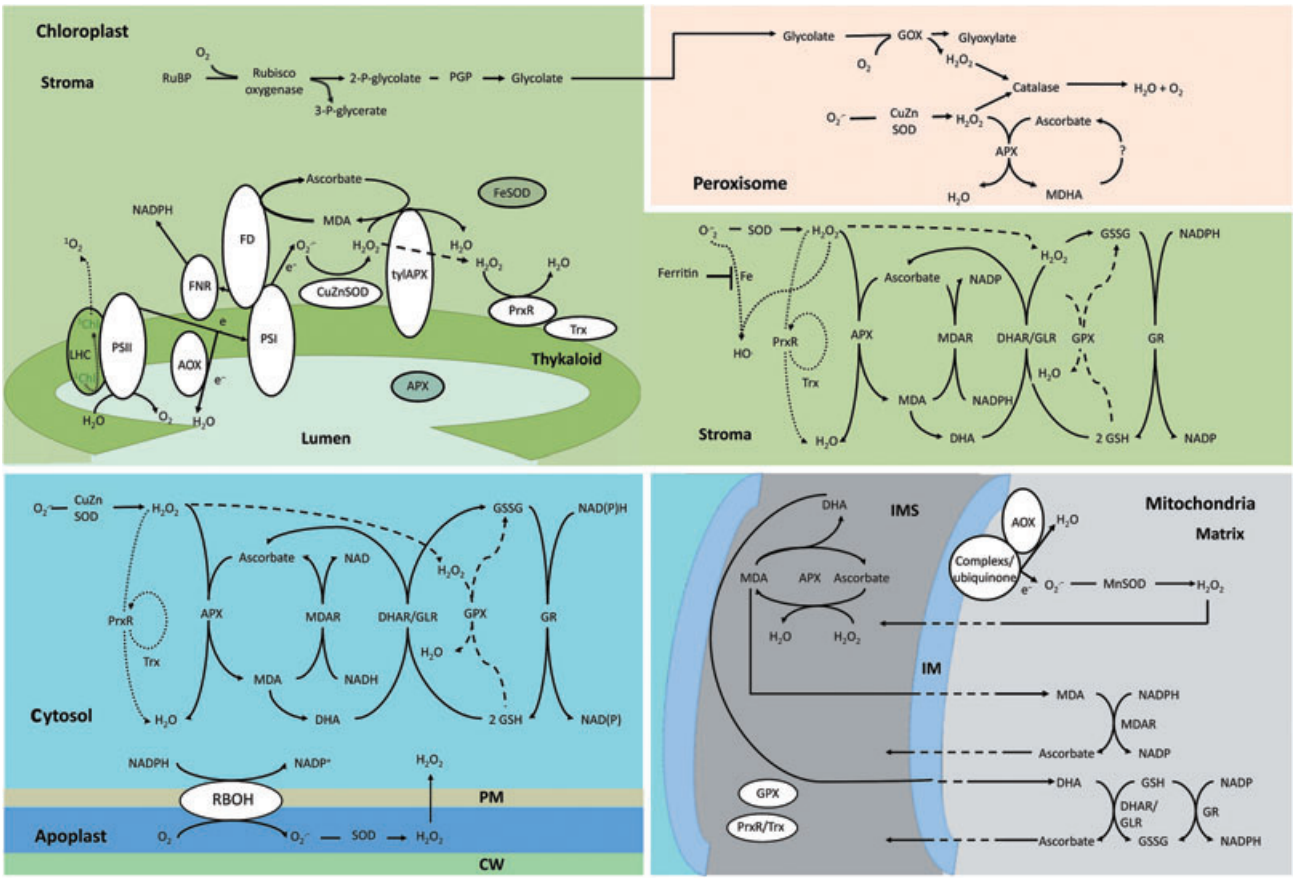

Figure 1. Localization of reactive oxygen species (ROS) generation and scavenging pathways in plant cells. The water–water cycle detoxifies $O_2^{\cdot-}$ and H_2O_2, and alternative oxidase (AOX; Immutans) reduces the production rate of $O_2^{\cdot-}$ in thylakoids [top left; in some plants iron superoxide dismutase (FeSOD) might replace CuZnSOD in the chloroplast]. ROS that escape this cycle and/or are produced in the stroma undergo detoxification by SOD and the stromal ascorbate–glutathione cycle. Peroxiredoxin (PrxR) and glutathione peroxidase (GPX) are also involved in H_2O_2 removal in the stroma (middle right). Excited chlorophyll (Chl) in its triplet state at the light-harvesting complex (LHC) can generate $^1O_2$ when the electron transport chain is over-reduced. ROS produced in peroxisomes during fatty acid oxidation, photorespiration or other reactions are decomposed by SOD, catalase (CAT) and ascorbate peroxidase (APX) (top right). SOD and other components of the ascorbate–glutathione cycle are also present in mitochondria. In addition, AOX prevents oxidative damage in mitochondria (bottom right). In principle, the cytosol contains the same set of enzymes found in the stroma (bottom left). NADPH oxidases [respiratory burst oxidase homologs (RBOHs)] are the major producers of ROS-associated signals required in a wide range of biological activities. The enzymatic components responsible for ROS detoxification in the apoplast and cell wall (CW) are only partially known, and the ROS-scavenging pathways at the vacuole and nucleus are unknown. Membrane-bound enzymes are depicted in white, GPX pathways are indicated by dashed lines and PrxR pathways are indicated by dotted lines in the stroma and cytosol. Although the pathways in the different compartments are mostly separated from each other, H_2O_2 can easily diffuse through membranes and antioxidants such as glutathione and ascorbic acid can be transported between the different compartments. DHA, dehydroascrobate; DHAR, DHA reductase; FD, ferredoxin; FNR, ferredoxin NADPH reductase; GLR, glutaredoxin; GR, glutathione reductase; GOX, glycolate oxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; IM, inner membrane; IMS, IM space; MDA, monodehydroascorbate; MDAR, MDA reductase; PGP, phosphoglycolate phosphatase; PM, plasma membrane; PSI, photosystem I; PSII, photosystem II; RuBP, ribulose-1,5-bisphosphate; Rubisco, RuBP carboxylase oxygenase; Trx, thioredoxin; tyl, thylakoid.

NADPH oxidases, are the main producers of signal transduction-associated ROS in cells during these processes (Mittler *et al.* 2004; Torres & Dangl 2005; Fig. 1).

In addition to active generation of ROS signals by the plant, for example, by NADPH oxidases, ROS generated due to metabolic imbalances during stress could also be channelled by the plant to serve as a stress signal to activate acclimation and defence mechanisms that would in turn counteract stress-associated oxidative stress (Mittler *et al.* 2004; Davletova *et al.* 2005a; Miller, Shulaev & Mittler 2008). The two, somewhat opposing, 'faces' of ROS, that is, on the one hand, the damaging toxic molecule, and on the other hand, the beneficial signal transduction molecule, underscore the need to control the steady-state level of ROS in cells during normal metabolism, as well as in response to different stresses. Elucidating the mechanisms that control ROS signalling in cells during drought and salt stresses could therefore provide a powerful strategy to enhance the tolerance of crops to these environmental stress conditions.

ROS REGULATION IN DIFFERENT ORGANELLES DURING DROUGHT AND SALINITY

Chloroplast

The reaction centres of photosystem I (PSI) and photosystem II (PSII) in chloroplast thylakoids are a major site of ROS generation. The photoproduction of ROS is largely affected by physiological and environmental factors and its rate is enhanced under conditions where photon intensity is in excess of that required for CO_2 assimilation (Asada 2006). Under water stress conditions, reduced CO_2 availability due to stomata closure and exposure to continuous excessive light direct electron transfer toward molecular oxygen, generating superoxide ions at PSI by the Mehler reaction (Asada 2006). A membrane attached copper/zinc superoxide (Cu/ZnSOD) in the vicinity of PSI converts the superoxide radicals into hydrogen peroxide and a membrane-bound thylakoid-ascorbate peroxidase (tylAPX) converts the H_2O_2 to water; this is also referred to as the water–water cycle (Fig. 1). In this process, AsA is oxidized to monohydroascorbate radical (MDA) that is reduced back to AsA by either reduced ferredoxin (Miyake & Asada 1994; Rizhsky, Liang & Mittler 2003), or by NAD(P)H, catalysed by MDA reductase (MDAR) (Sano *et al.* 2005; Asada 2006). Dehydroascorbate (DHA) is produced when MDAR fails to reduce MDA to AsA, and is reduced to AsA by DHA reductase (DHAR) using reduced GSH as an electron donor. PrxRs associated with the thylakoid membrane together with thioredoxin (Trx) can also provide antioxidative protection enabling an alternative water–water cycle for detoxification of photochemically produced H_2O_2 in chloroplasts (Dietz *et al.* 2006; Fig. 1). Peroxiredoxin (Prx) and Trx were shown to play an important role during drought and oxidative stress (Rey *et al.* 2005; Dietz *et al.* 2006; Vieira Dos Santos & Rey 2006).

Singlet oxygen ($^1O_2$) is generated at PSII by excited triplet-state chlorophyll at the P680 reaction centre and in the light-harvesting complex when the electron transport chain is over-reduced (Asada 2006; Fig. 1). H_2O_2 has a positive role in reducing the likelihood of $^1O_2$ formation and treatment with exogenous H_2O_2 was shown to promote the oxidation of quinone A (QA), the primary plastoquinone (PQ) electron acceptor, which increases the photosynthetic electron transport flow and decreased the generation of $^1O_2$ during the stress; thus, the water–water cycle functions also as a relaxation system to suppress the photoproduction of $^1O_2$ (Karpinska, Wingsle & Karpinski 2000; Asada 2006; Møller *et al.* 2007). $^1O_2$ can activate a genetic programme leading to growth inhibition and lethality through the EXEUTER1 and EXECUTER2 pathways (Wagner *et al.* 2004; Lee *et al.* 2007). On the other hand, the cytotoxicity of $^1O_2$ was shown to causes lipid peroxidation and extensive tissue damage in leaves under photo-oxidative condition that could directly lead to cellular death (Møller *et al.* 2007; Triantaphylides *et al.* 2008). Photo-oxidative stress induced an excessive lipid peroxidation in *Arabidopsis* leaves that was almost exclusively mediated by $^1O_2$, leading to cell death (Triantaphylides *et al.* 2008).

Controlling ROS production and scavenging in the chloroplast was shown to be essential for tolerance to drought and salinity in transgenic plants and in drought- or salinity-tolerant cultivars (Van Camp *et al.* 1996; Hernandez *et al.* 2001; Mittler & Berkowitz 2001; Tseng, Liu & Yiu 2007). In contrast, deficiency in chloroplastic ROS-scavenging mechanisms enhanced the stress sensitivity to both salinity and drought (Serrato *et al.* 2004; Wang *et al.* 2005; Supporting Information Table S1).

Peroxisomes

Peroxisomes produce H_2O_2 and O_2^- at high rates through several metabolic processes and the amount of ROS in peroxisomes is regulated by a delicate balance between production and scavenging. Reduced water availability and stomatal closure decrease CO_2 to O_2 ratio in mesophyll cells and increase photorespiration and production of glycolate in chloroplasts. Oxidation of glycolate by glycolate-oxidase in peroxisomes accounts for the majority of H_2O_2 production during photorespiration (Noctor *et al.* 2002; Karpinski *et al.* 2003; Fig. 1). Additional sources of H_2O_2 production in peroxisomes include fatty acid β-oxidation, the flavin oxidase pathway and the dismutation of superoxide radicals (Corpas, Barroso & del Río 2001; Palma, Corpas & del Río 2009). CATs localized mainly in peroxisomes are the major antioxidative enzymes that detoxify H_2O_2, under increased photorespiration conditions (Mittler *et al.* 2004; Vandenabeele *et al.* 2004). APX and the AsA–GSH cycle can also contribute to the scavenging of H_2O_2 in peroxisomes (Jiménez *et al.* 1997; Fig. 1). O_2^- is generated by xanthine oxidase (XOD) in the matrix of leaf peroxisomes, and SOD converts O_2^- to O_2 and H_2O_2 (del Río *et al.* 1996; Corpas *et al.* 2001). Furthermore, environmental stresses, such as desiccation and salinity, were shown to perturb the redox state, inhibit antioxidant mechanisms and increase ROS production in peroxisomes (del Río *et al.* 1996; Mittova *et al.* 2003; Nyathi & Baker 2006). For example, in tomato, salinity decreases AsA and GSH contents and induces lipid peroxidation in peroxisomes (Mittova *et al.* 2003).

Polyamine catabolism has also been shown to regulate ROS accumulation and ROS-scavenging enzymes in cells (Moschou *et al.* 2008; Mohapatra *et al.* 2009). Interestingly, a recent study demonstrated that expression of drought-responsive genes is altered in plants that are deficient in root peroxisomal polyamine oxidase (POX) that is involved in polyamine catabolism (Kamada-Nobusada *et al.* 2008). These results raise a possibility that peroxisomal POX contributes to the regulation of drought-responsive genes by balancing ROS generation and scavenging.

Mitochondria

Mitochondria are also known as a source of ROS production, although, they generate smaller amounts of ROS compared with chloroplasts and peroxisomes (Foyer & Noctor

2005; Rhoads *et al.* 2006). In mitochondria, complex I and complex III in the electron transport chain (mtETC) are major sites of ROS production (Møller 2001; Rhoads *et al.* 2006; Møller *et al.* 2007). Ubisemiquinone intermediate formed at complex I and III donates electrons to oxygen and generates O_2^- that is, in turn, reduced to H_2O_2 (Raha & Robinson 2000; Rhoads *et al.* 2006; Fig. 1). Increased mitochondrial respiration during water stress could potentially contribute to the generation of ROS during stress by transferring electrons from the cytochrome electron transport system to O_2 (Norman *et al.* 2004).

ROS production in mitochondria has been shown to increase under abiotic stress conditions, especially drought and salinity (Alscher, Donahue & Cramer 1997; Bartoli *et al.* 2004; Pastore *et al.* 2007). Perturbation of mtETC function results in over-reduction of the ubiquinone (UQ) pool and increased ROS production (Rhoads *et al.* 2006). Atkin & Macherel (2009) suggested that respiration rate increases under severe drought because the demand for mitochondrial ATP increases to compensate for the reduced rate of chloroplast ATP synthesis, causing enhanced production of ROS in the mitochondria. Changes in ROS levels caused by the perturbation of complex I were proposed to trigger a retrograde signal (Rhoads *et al.* 2006). Mitochondrial alternative oxidase (AOX) and manganese SOD (Mn-SOD) are key enzymes that function in controlling this signalling pathway (Foyer & Noctor 2005; Fig. 1). AOX acts to maintain the reduction state of the UQ pool and lower ROS production in mitochondria, while Mn-SOD converts O_2^- to H_2O_2 and O_2 in the initial step of the ROS detoxification (Møller 2001; Arnholdt-Schmitt, Costa & de Melo 2006; Rhoads *et al.* 2006). Inducing AOX 1 (AOX1) diverted electron transport from the cytochrome pathway at the UQ pool and uncoupled ATP production, preventing programmed cell death induced by down-regulation of the cytochrome pathway (Norman *et al.* 2004). Recent studies demonstrated that *Arabidopsis* plants with loss of function in mitochondrial AOX1a are sensitive to a combination of drought and moderate light stress, and exhibit altered expression of transcripts involved in antioxidant mechanisms in the chloroplast, as well as the mitochondria (Giraud *et al.* 2008). In addition, a salt-tolerant tomato cultivar showed higher activity of mitochondrial SOD compared with a salt-sensitive cultivar during salinity (Mittova *et al.* 2003).

Apoplast

Recent studies indicated that the apoplast is an important site for H_2O_2 production in response to abscisic acid (ABA) and adverse environmental conditions such as drought and salinity (Hernandez *et al.* 2001; Hu *et al.* 2005; Hu *et al.* 2006; Jubany-Marí *et al.* 2009). *AtRbohD* and *AtRbohF* encode two major NADPH oxidases expressed in guard and mesophyll cells in *Arabidopsis*. They have been shown to be responsible for apoplastic ROS generation that is required for ABA-induced stomatal closure (Kwak *et al.* 2003; Torres & Dangl 2005). Other apoplastic ROS-forming enzymes are cell wall-associated oxidases and peroxidases and polyamine oxidases (Mittler 2002; Moschou *et al.* 2008). Accumulation of H_2O_2 in the apoplast is thought to be involved in acclimation responses of plants to drought and salt stresses, such as growth and cell wall strengthening (Hernandez *et al.* 2001; Rodriguez *et al.* 2004; Ros Barceló 2005; Jubany-Marí *et al.* 2009). Generation of apoplastic ROS was shown to have a positive effect on leaf elongation in maize during salinity stress, and reduction in stress-induced apoplastic ROS formation was associated with a decrease in leaf elongation, specifically in response to NaCl treatment but not during osmotic treatment (Rodriguez *et al.* 2004). In addition, two pea cultivars with different degrees of salt tolerance exhibited changes in apoplastic ROS-scavenging activities, such as SOD, which positively correlated with salt tolerance (Hernandez *et al.* 2001).

NON-ENZYMATIC ROS SCAVENGING DURING DROUGHT AND SALINITY

Low-molecular-weight antioxidants such as AsA, GSH and tocopherols can also affect gene expression associated with abiotic stresses, altering acclimation responses. Antioxidants function as redox buffers that interact with ROS and act as a metabolic interface that modulate the appropriate induction of acclimation responses or programmed cell death (Halliwell & Foyer 1976; Takahashi & Asada 1988; Foyer & Noctor 2005).

AsA is an important antioxidant that serves as an electron donor to many important reactions (Smirnoff & Pallanca 1996; Noctor & Foyer 1998; Asada 1999; Smirnoff 2000; Mano, Hideg & Asada 2004; Ivanov *et al.* 2005; Shao *et al.* 2008). AsA was shown to play an important role in the protection of photosynthesis during salt stress in *Arabidopsis*. During salinity stress, the AsA-deficient mutant *vtc-1*, which has 30–60% of the AsA content of wild-type plants, accumulated a much higher level of H_2O_2 than wild type, which coincides with a greater decrease in the ratio of reduced AsA to total AsA and with reduced activity of the AsA–GSH cycle enzymes. This higher accumulation of H_2O_2 preceded the decrease in chlorophyll content, CO_2 assimilation and decrease in PSII activities (Huang *et al.* 2005). Supplying whole tomato seedlings with exogenous AsA enhanced the resistance of seedlings to salt stress and decreased lipid peroxidation (Shalata & Neumann 2001).

GSH is a tripeptide found abundantly in all cell compartments in its reduced form (Foyer & Noctor 2005). The ratio of GSH to its oxidized form, GSSG, plays an important role in maintaining redox equilibrium in the cell during H_2O_2 degradation and other processes (Shao *et al.* 2008). Additionally, GSH plays a key role in the regeneration of reduced AsA in the AsA–GSH cycle (Halliwell & Foyer 1976). Total GSH was increased during water deficit in sunflower seedlings (Sgherri & Navari-Izzo 1995), and in a salt-treated groundnut cell line (Jain *et al.* 2002). Maintaining a high ratio of GSH/GSSG that functions as a redox couple was shown to play an important role in salt and drought tolerance as observed in tomato, *Myrothamnus*

flaberllifolia, and wheat (Shalata & Neumann 2001; Kocsy, Szalai & Galiba 2002; Kranner *et al.* 2002).

α-Tocopherol (vitamin E) is a lipid antioxidant that can scavenge ROS and protect lipids from oxidation (Munné-Bosch & Alegre 2002; Asada 2006; Li *et al.* 2008). Several studies conducted with different plants including soybean leaves, rosemary and Mediterranean shrubs showed that drought stress resulted in an increase in α-tocopherol levels (Munné-Bosch & Alegre 2000; Shao *et al.* 2008; Munné-Bosch *et al.* 2009). Additionally, over-expressing *Arabidopsis* tocopherol cyclase (VTE1), an enzyme required for vitamin E synthesis, in tobacco enhanced both vitamin E level and tolerance to drought stress (Liu *et al.* 2008).

HORMONAL REGULATION AND ROS DURING DROUGHT AND SALINITY

Plant hormones plays a protective signalling role in responses to drought and salinity by activating acclimation responses such as stomatal closure, hydraulic conductivity responses to drought and salinity and regulation of developmental processes that affect stress tolerance such as senescence and abscission (Schroeder, Kwak & Allen 2001; Finkelstein & Gibson 2002; Norman *et al.* 2004; Miao *et al.* 2006; Zhang *et al.* 2006; Rivero *et al.* 2007; Boursiac *et al.* 2008; Sakamoto *et al.* 2008). ABA was shown to increase the expression and the activity of ROS network genes such as *CAT1*, *APX1*, *glutathione reductase 1* (*GR1*) (Zhang *et al.* 2006) and cytosolic Cu/ZnSOD, as well as APX and GR in leaves of maize (Hu *et al.* 2005), but was also shown to increase H_2O_2 levels in maize embryos, seedlings and leaves (Guan & Scandalios 2000; Zhang *et al.* 2001; Hu *et al.* 2005). ABA-induced stomata closure is partially dependant on NADPH oxidase activity (Pei *et al.* 2000; Kwak *et al.* 2003; Torres & Dangl 2005). Deficiency in mitochondrial AtGPX3 enhanced the production of H_2O_2 in guard cells and the sensitivity of plants to drought. *Arabidopsis gpx3* plants were also disrupted in ABA activation of calcium channels and in the expression of ABA-responsive genes, suggesting that redox state regulation is important in the response to ABA during drought (Miao *et al.* 2006). ABA and drought have been shown to induce the activities of cytosolic aldehyde oxidase (AO) and xanthine dehyrogenase (XDH) that produce respectively H_2O_2 and O_2^- (Yesbergenova *et al.* 2005). Furthermore, ABA-deficient mutants of *Arabidopsis*, tomato and tobacco plants lack AO and XDH activities (Leydecker *et al.* 1995; Sagi, Fluhr & Lips 1999; Schwartz *et al.* 1997). Both genes were able to produce ROS following ABA treatment suggesting that drought stress can increase ROS accumulation in plants through XDH and AO in an ABA-dependent manner (Yesbergenova *et al.* 2005).

During drought stress, cytokinin (CK) levels decrease increasing shoot responses to ABA, leading to stomatal closure (Davies & Zhang 1991; Goicoechea, Antolin & Sánchez-Díaz 1997). These stress-induced changes in CKs and ABA promote early leaf senescence leading to leaf abscission, thus decreasing the plant's canopy and reducing water loss (Pospíšilová, Synková & Rulcová 2000). Interestingly, transgenic tobacco plants expressing the *isopentenyltransferase* (*IPT*) gene, encoding an enzyme that catalyses the rate-limiting step in CKs biosynthesis, under the control of a drought-induced senescence-associated-receptor-protein-kinase (SARK) promoter, displayed improved water efficiency and enhanced drought tolerance (Rivero *et al.* 2007). SARK-IPT transgenic plants displayed increased expression of ROS metabolism genes especially AsA–GSH cycle genes (Rivero *et al.* 2007). Increased photorespiration during drought together with increased presence of CAT in peroxisomes was recently suggested to contribute to this tolerance (Rivero, Shulaev & Blumwald 2009).

Salicylic acid (SA), jasmonic acid (JA) and ethylene are key regulators of the signalling network inducing defence responses against pathogens and insect attack and the induction of systemic acquired resistance (SAR; Pieterse & Van Loon 2004). The presence of SA-, JA- and ethylene-responsive *cis* elements, in addition to other elements, in the promoter regions of ROS-responsive genes such as *Zat7*, *Zat12 WRKY25* and *Apx1* (Rizhsky *et al.* 2004a) indirectly suggests a broader role for these hormone-mediated responses, beyond plant–pathogen interaction. NaCl and SA treatments similarly induced the accumulation of ROS in roots of *Arabidopsis* and inhibit water uptake capacity (i.e. hydraulic conductivity, Lp_r). It was shown that SA-induced Lp_r inhibition depends on ROS formation and can be partially counteracted by the scavenging H_2O_2 (Boursiac *et al.* 2008). SA could potentially alter mitochondrial ROS formation by increasing AOX activity, which in turn could bypass ROS production by the cytochrome pathway at the UQ pool (Norman *et al.* 2004). Methyl jasmonate (MJ) can promote H_2O_2 production in guard cells causing stomata to close; furthermore, MJ-induced stomata closure is impaired in *AtrbohD/F* double mutant (Suhita *et al.* 2004).

Ethylene response factors (ERFs), a subfamily that belongs to the ERF/AP2 super family, are GCC-box-, DRE-/CRT- and CE1- *cis* elements-binding proteins that were shown to enhance plant tolerance to multiple stresses suggesting a role for these proteins in abiotic stress responses (Wu *et al.* 2008). Transgenic tobacco expressing *JERF3*, an osmotic- and oxidative-stress responsive ERF, showed enhanced adaptation to drought and salinity, including increased expression of ROS-detoxifying enzymes and the concomitant decreased accumulation of ROS (Wu *et al.* 2008).

Abscission occurs as part of normal plant development, but could also be induced by environmental stresses including salinity and drought (Taylor & Whitelaw 2001). Auxin (IAA) and ethylene display an antagonistic relationship in the regulation of abscission in many plant species where IAA from the growing part of the leaf prevents the sensitivity to ethylene and suppresses ethylene-initiated abscission signalling (Lewis, Leslie & Liljegren 2006; Sakamoto *et al.* 2008). Production of H_2O_2 was recently shown to be required for ethylene-dependent salt stress-induced

abscission, whereas IAA antagonized abscission by inhibiting generation of H_2O_2 (Sakamoto *et al.* 2008).

COMPATIBLE SOLUTES IN THE REGULATION OF ROS LEVELS DURING DROUGHT AND SALINITY

Plants accumulate compatible solutes, such as proline (Pro) and glycinebetaine (GB), in response to drought and salinity to facilitate water uptake (Hare & Cress 1997; Ashraf & Foolad 2007). In addition to osmotic adjustments, these osmolites were suggested to be important for protecting cells against increased levels of ROS accumulation under stress conditions.

Pro accumulates in the cytosol and the vacuole during stress (Aubert *et al.* 1999; McNeil, Nuccio & Hanson 1999) and was shown to protect plant cells against damages caused by $^1O_2$ or HO· (Matysik, Alia, Bhalu & Mohanty 2002). By quenching $^1O_2$ and directly scavenging HO·, Pro might be able to protect proteins, DNA and membranes (Smirnoff & Cumbes 1989; Matysik *et al.* 2002). In addition to directly scavenging of HO·, Pro might bind to redox-active metal ions and protect biological tissues against damages caused by HO· formation (Matysik *et al.* 2002). In a recent study, transgenic wheat plants that accumulated higher Pro than wild type exhibited less lipid peroxidation of membranes during drought, indicating a role for Pro in reducing ROS damages during drought (Vendruscolo *et al.* 2007). *Arabidopsis* mutants deficient in salt-induced siRNA that suppresses the expression of pyrroline-5-carboxylate dehydrogenase (functions in Pro degradation) were impaired in Pro accumulation, enhanced the accumulation ROS and consequently enhanced plant sensitivity to salinity stress (Borsani *et al.* 2005). Pro synthesis from glutamate, previously documented in the cytosol, was recently shown to occur, at least in part, in the chloroplast (Szekely *et al.* 2008). During Pro synthesis, NADPH is used for the reduction of glutamate, which increases $NADP^+$ availability, required for relieving of PSI over-reduction during stress, as well as increasing $NADP^+$/NADPH ratio that prevents perturbation of redox-sensitive pathways during drought and salt stresses (Hare & Cress 1997). Accordingly, Pro was shown to protect photochemical efficiency of PSII and prevent lipid peroxidation during drought (Molinari *et al.* 2007). These results indicate that Pro functions to indirectly protect PSII as well as directly scavenge ROS during drought.

In contrast to Pro, GB is not thought to directly scavenge ROS during abiotic stresses (Smirnoff & Cumbes 1989; Chen & Murata 2008). However, GB has been shown to protect cells against oxidative damage during abiotic stresses (Park *et al.* 2007; Chen & Murata 2008). GB accumulates mainly in the chloroplast and is involved in the maintenance of PSII efficiency under stress conditions (Génard *et al.* 1991; Ashraf & Foolad 2007; Ben Hassine *et al.* 2008). A previous study showed that accumulation of GB in the chloroplast is more effective than that in other cellular compartments in protecting plants against oxidative stress and salinity (Park *et al.* 2007). In addition, exogenous GB treatment prevents salt-induced structural damages to ROS-producing organelles, such as chloroplasts and mitochondria (Ashraf & Foolad 2007). These results suggest that GB acts to prevent excess ROS production by protecting chloroplasts during salinity. Activation of antioxidant mechanisms by Pro and GB during salinity has been studied using tobacco bright yellow-2 suspension cultured cells (Hoque *et al.* 2007; Banu *et al.* 2009). Salinity significantly inhibited the amount of reduced AsA, reduced GSH and the activity of AsA–GSH cycle enzymes, and exogenous application of Pro or GB increased the activity of these enzymes (Hoque *et al.* 2007). These results suggest a role of Pro and GB in the regulation of antioxidant enzymes during salinity.

Soluble sugars also contribute to the regulation of ROS signalling as well as osmotic adjustments during abiotic stresses (Vinocur & Altman 2005; Seki *et al.* 2007). Soluble sugars are involved in the metabolism and protection of both ROS-producing and ROS-scavenging pathways, such as mitochondrial respiration, photosynthesis and oxidative-pentose-phosphate pathway (Couée *et al.* 2006). A number of studies have suggested that mannitol can protect against photo-oxidative damages to the chloroplastic apparatus caused especially by HO· during stress. Transgenic tobacco plants with increased mannitol production targeted to the chloroplast showed increased scavenging capacity of HO· enhancing their resistance to oxidative stress (Shen, Jensen & Bohnert 1997a,b). Because the increase in mannitol content was insufficient to account for osmotic adjustments in transgenic wheat plants expressing the *Escherichia coli* mannitol biosynthetic gene *mtlD* during drought and salinity, mannitol function in enhancing stress tolerance in these plants was mainly attributed to its potential for scavenging of HO· and O_2^- (Abebe *et al.* 2003).

The disaccharide trehalose is known as a signalling molecule that regulates carbon and ABA metabolism under stress conditions (Avonce *et al.* 2004). In previous studies, over-expression of trehalose biosynthetic genes enhanced tolerance of transgenic plants to drought and salinity (Garg *et al.* 2002; Penna 2003; Miranda *et al.* 2007), suggesting the involvement of trehalose in the response of plants to these stresses. Transgenic rice plants that express *E. coli* trehalose biosynthetic genes showed less photo-oxidative damage to PSII during drought and salinity compared with wild-type plants (Garg *et al.* 2002).

GAIN- AND LOSS-OF-FUNCTION ALTERATIONS IN ROS METABOLISM AND THEIR EFFECT ON DROUGHT AND SALINITY TOLERANCE

Because many abiotic stresses are accompanied by oxidative stress, several groups in recent years have taken the straightforward approach of attempting to improve stress tolerance in plants by modifying their ability to scavenge ROS that are generated during stress. Supporting Information Table S1 summarizes the effect of altered expression of several ROS-scavenging enzymes, as well as

ROS-response regulatory genes, on tolerance to drought, osmotic and salt stresses in over-expressing or deficient plants. As it unfolds from these studies, over-expression of one or more ROS-scavenging enzyme in various compartments can relieve oxidative stress potentially increasing tolerance to drought, salt or both stresses (Supporting Information Table S1). For example, ectopically expressing genes such as *APXs* and *SODs* that function in the Halliwell–Asada–Foyer cycle, or the water–water cycle in chloroplasts, were shown to improve photosynthesis under hyperosmotic conditions (Yan *et al.* 2003; Eltayeb *et al.* 2007; Lu, Liu & Liu 2007; Tseng *et al.* 2007). Increased activity of these cycles can maintain close to normal levels of PSII and PSI activity during stress, decrease the inhibition of photosynthesis and reduce ROS levels. Targeting *Zm*CuZnSOD or *Zm*CAT that is normally expressed in peroxisomes to chloroplasts in transgenic *Brassica campestris* increased PSII activity and reduced damage to leaves under salt stress conditions compared with wild type. The co-expression of both transgenes further increased the tolerance of the transgenic plant (Tseng *et al.* 2007). Accordingly, deficiency in ROS scavenging in AsA-deficient *vtc1* mutants resulted in increased sensitivity to salinity (Huang *et al.* 2005) and deficiency in total tocopherol level in NtHPT(VTE2)-RNAi tobacco plants, as well as knockout NADPH Trx reductase, increased sensitivity to drought and salinity (Serrato *et al.* 2004; Abbasi *et al.* 2007, respectively; Supporting Information Table S1).

Another strategy that proved beneficial in enhancing tolerance to drought or salinity is to increase stress tolerance by over-expressing ROS-responsive regulatory genes that regulate a large set of genes involved in acclimation mechanisms, including ROS-scavenging enzymes (Supporting Information Table S1). Over-expression of transcription factors such as *Zat10*, *Zat12* or *JERF3* enhanced the expression of ROS-scavenging genes and tolerance to salt, drought or osmotic stresses (Sakamoto *et al.* 2004; Davletova *et al.* 2005b; Wu *et al.* 2008, respectively). Furthermore, over-expression of mitogen-activated kinase kinase 1 (MKK1) in *Arabidopsis* enhanced the activity of MAPK cascade, which is also activated by ROS (Yuasa *et al.* 2001; Teige *et al.* 2004), reduced stress-associated ROS levels and increased tolerance to salt and drought stresses (Teige *et al.* 2004; Xing, Jia & Zhang 2008). Conversely, deficiency in MKK1 resulted in increased ROS production and increased stress sensitivity (Xing *et al.* 2008).

Nevertheless, as it unfolds from the case of other ROS-scavenging genes, the situation is far more complex. Over-expression of *Nt*107, a tobacco gene with both glutathione-s-transferase (GST) and GPX activities, increased GSSG content and resulted in improved ability of tobacco seedlings to grow under salt stress (Roxas *et al.* 1997). However, a later attempt to over-express *Nt*107 in cotton plants did not enhance the GPX activity over that of the wild-type plant during salt stress and failed to increase the stress tolerance of seedlings (Light *et al.* 2005). The authors suggested that the endogenous antioxidant system in cotton may be disrupted by the ectopic expression of *Nt*107 (Light *et al.* 2005). In contrast to VTE2-RNAi, which resulted in hypersensitivity to salt, osmotic and oxidative stresses, a 95% decrease in α-tocopherol in γ-TMT(VTE4)-RNAi tobacco lines resulted in hypersensitivity to salt but dramatically enhanced tolerance to osmotic and oxidative stress in plants grown on high sorbitol concentrations (Abbasi *et al.* 2007). The reduction in α-tocopherol was compensated by γ-tocopherol accumulation in plants, revealing that γ-tocopherol is more potent than α-tocopherol in protecting against desiccation (Abbasi *et al.* 2007).

In recent years, ROS-scavenging enzymes were shown to be involved in signalling in addition to their more traditional function in cellular protection. Cytosolic APX1-deficient *Arabidopsis* plants had constitutively higher levels of H_2O_2 than wild-type plants, and induced the expression of many stress-responsive genes when subjected to a moderate level of light stress (Davletova *et al.* 2005a). Knockout APX1 plants were shown to grow better than wild-type plants under hyperosmotic or salinity condition (Ciftci-Yilmaz *et al.* 2007; Miller *et al.* 2007). These results were surprising because *apx1* plants show increased sensitivity to photo-oxidative- as well as paraquat-induced oxidative stress (Davletova *et al.* 2005a; Miller *et al.* 2007). Similarly, reduced expression of tylAPX in *Arabidopsis* led to increased tolerance to both osmotic and salt stresses but did not affect growth under oxidative stress conditions. However, in the double mutant *apx1/tylapx*, deficiency in both genes caused an increased sensitivity to sorbitol treatment while maintaining salt tolerance (Miller *et al.* 2007). Likewise, in the case of antisense APX1 and antisense CAT1 in tobacco, each were constantly subjected to oxidative damage, but the double antisense lines became more tolerant (Rizhsky *et al.* 2002).

Over-expression of Zat7, that was identified in knockout *Apx1* plants, resulted in growth suppression, enhanced expression of defence transcripts, such as WRKY70, AOX1, NHX1, Cor78, and dramatic tolerance to salinity and cold stresses (Rizhsky *et al.* 2004a; Ciftci-Yilmaz *et al.* 2007). Conversely, Zat7 RNAi lines showed decreased osmotic stress tolerance (Ciftci-Yilmaz *et al.* 2007). In contrast to their enhanced salt resistance, *Zat7* OE plants were more sensitive to hyperosmotic conditions imposed by sorbitol (Ciftci-Yilmaz *et al.* 2007).

MPK3 and MPK6, two ROS-responsive MAP kinases that are activated by H_2O_2 (Yuasa *et al.* 2001; Moon *et al.* 2003), were recently shown to be activated by MKK9. However, transgenic *Arabidopsis* that expresses a constitutively active MKK9 showed increased activation of the endogenous MPK3 and MPK6 and were rendered more sensitive to salt stress, whereas the *mkk9* mutant was more tolerant (Xu *et al.* 2008). Interestingly, MPK6, is activated by MKK1 as well as by MKK9 during exposure to salt; however, MKK1 over-expressing plants were more tolerant than the wild types to salt stress (Xing *et al.* 2008), whereas MKK9 transgenic plants where more sensitive (Xu *et al.* 2008).

A SPECIFIC FINGERPRINT FOR ROS REGULATORY GENES IN THE RESPONSE OF *ARABIDOPSIS* TO OSMOTIC AND SALT STRESSES

To further examine the relationship between ROS and salinity or drought stresses, we compared the osmotic and salinity response of 27 ROS-response transcripts encoding transcription factors and other signalling-related proteins up-regulated in *apx1* plants during photo-oxidative stress, as well as in wild-type plants following treatment with H_2O_2 (Davletova *et al.* 2005a,b), using Genevestigator Meta-analysis tool (Zimmermann *et al.* 2004; Fig. 2). All 27 transcripts showed significant induction (≥2) in at least one time point during osmotic or salinity stresses; however, the differences in the response pattern between the two treatments were striking, as emerged from the heat map. Up-regulation under osmotic conditions was almost exclusively in shoots, whereas during salinity, it was almost exclusively in roots (Fig. 2).

Several transcripts displayed an exclusive or a predominant stress specificity, for example, *CERK1* and *WRKY70* for salt stress, *CML38* (calcium-binding protein), and *ANAC053* (*Arabidopsis* NAC domain containing protein 53) for osmotic stress (Fig. 1). Little is known about the function of these ROS-responsive regulators during drought, osmotic stress or salinity, but they may play an important role in the specific acclimation to each type of stress in accordance with the typical oxidative homoeostasis under each condition. For example, CML38 is a calmodulin-like protein elevated especially during osmotic stress in both shoots and roots and transiently down-regulated in shoots in response to salt (Vanderbeld & Snedden 2007; Fig. 2).

NAC domain-containing proteins are plant-specific transcription factors implicated in a wide range of responses including development, hormone signalling and abiotic stress responses, including cold, dehydration and salinity (Pinheiro *et al.* 2009). CERK1 is a receptor-like kinase, which is essential for chitin elicitor signalling in *Arabidopsis*. Interestingly, *cerk1* mutants completely lost their ability to respond to a chitin elicitor including loss of ability to accumulate ROS, induce *RbohD* and *RbohF* and activate MAPK3 and MAPK6 (Miya *et al.* 2007). *WRKY70* and *Zat7* are co-expressed in knockout *Apx1* plants (Pnueli *et al.* 2003; Davletova *et al.* 2005a) and both interact through the ERF-associated amphiphilic repression (EAR) motif of ZAt7 as shown in yeast two-hybrid assay (Ciftci-Yilmaz *et al.* 2007). It is likely that WRKY70 and Zat7 are involved in rendering APX1-deficient plants more salt stress tolerant (Ciftci-Yilmaz *et al.* 2007; Miller *et al.* 2007).

Given that ionic stress in shoots of salt-stressed plants is likely to develop slowly over many days, long after the rapid

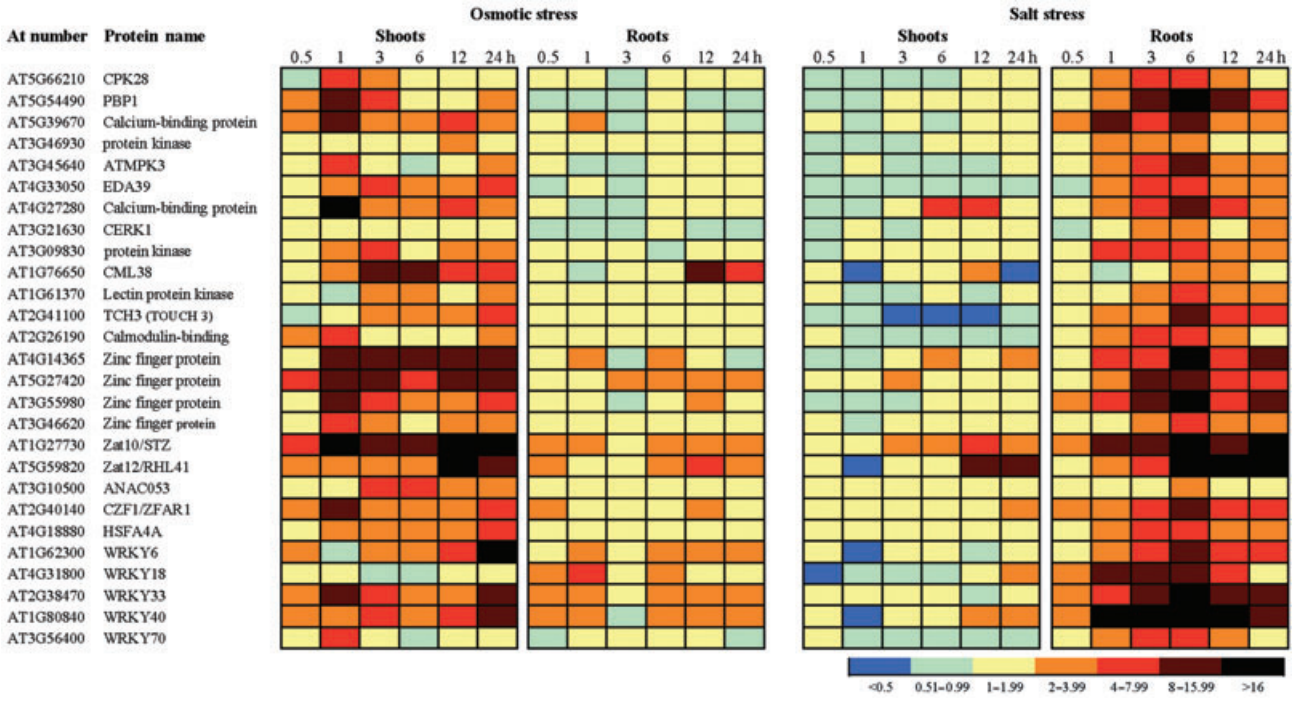

Figure 2. Change in expression of hydrogen peroxide-responsive transcripts in roots and shoots of *Arabidopsis* subjected to osmotic or salt stresses. Relative abundance of transcripts encoding hydrogen peroxide-related transcription factors and other regulatory genes in shoots and roots of *Arabidopsis* plants subjected to osmotic or salinity stresses. The different hydrogen peroxide-responsive transcripts were identified in wild-type plants subjected to hydrogen peroxide treatment, as well as in knockout APX1 plants subjected to light intensity stress (Davletova *et al.* 2005a,b). Values are colour coded to represent the fold change in expression relative to control treatment at each time point. All data were obtained from Genevestigator microarray database using the 'digital northern' tool (Zimmermann *et al.* 2004).

osmotic phase (Munns & Tester 2008), the changes in the expression of the ROS-responsive transcripts (Fig. 2) that occur as early as 30 min to 24 h, are presumed to be in response to the osmotic phase of both stresses. Therefore, the dramatic difference in the expression of these transcripts points to the differences in the immediate perception of the two stresses in plants and could suggest that under each condition, a distinct ROS signature is developed.

INVOLVEMENT OF ROS IN THE REGULATION OF NaCl RESPONSES

Plants respond directly and specifically to Na^+ within seconds by a mechanism not yet identified, and a fast increase in cytosolic [Ca^{2+}] (Munns & Tester 2008). One of the best-characterized signalling pathways specific to salinity involves sensing calcium by the calcineurin B-like protein (CBL) CBL4/SOS3 and its interacting protein kinase CIPK24/SOS2 (Zhu *et al.* 2007; Munns & Tester 2008; Luan 2009). NaCl stress-induced SOS2/SOS3 complex is targeted to the plasma membrane enabling the phosphorylation and activation of the membrane-bound Na^+/H^+ antiporter, SOS1 (Munns & Tester 2008). SOS2 kinase is an especially important regulatory compound because of its interaction with other signalling molecules. SOS2 was found to interact with nucleoside triphosphate kinase 2 (NDPK2) that is involved in ROS signalling, as well as with CAT2 and CAT3 (Verslues *et al.* 2007). NDPK2 is induced in response to oxidative stress and was shown to be important for the H_2O_2-induced activation of MPK3 and MPK6 (Moon *et al.* 2003). Deficiency in AtNDPK2 increased the plant salt sensitivity (Moon *et al.* 2003; Verslues *et al.* 2007) and further enhanced the sensitivity of *sos2* mutants to salinity (Verslues *et al.* 2007). A recent study suggested that the chloroplastic protein ENH1, containing a rubredoxin domain, which is thought to be involved in the reduction of $O_2\cdot^-$ in some bacteria, is involved both in ROS and ion homeostasis. Deficiency in ENH1 led to increased ROS levels in response to NaCl rendering the plant sensitive to salt and further enhancing the salt-sensitive phenotype of *sos3* mutant (Zhu *et al.* 2007). This study also suggested that ENH1 may function in a salt tolerance pathway that involves SOS2 (Zhu *et al.* 2007).

Intracellular trafficking through vesicles plays an important role in salt tolerance in plants. NaCl treatment caused an immediate osmotic and ionic stress and induced the formation of endosomes containing high levels of H_2O_2 in *Arabidopsis* cells (Leshem *et al.* 2006; Leshem, Seri & Levine 2007). Deficiency in AtVAMP711, a family member of N-ethylmaleimide-sensitive factor attachment protein receptors (v-SNAREs) that carries out vesicle fusion with the tonoplast, resulted in formation of mega-vesicles containing H_2O_2 that did not fuse with the tonoplast and increased salt tolerance (Leshem *et al.* 2006). In another study by Leshem *et al.* (2007), suppression of the salt-specific induction of NADPH oxidase-mediated ROS production within endosomes in the phosphatidylinositol 3 kinase mutants (*pi3k*) caused a reduction in oxidative stress but resulted in enhanced sensitivity to salt. Recently, Boursiac *et al.* (2008) shed light on the regulatory role that ROS formation in roots plays in response to salt in inhibiting hydraulic conductivity Lp_r. This work suggested a new role for ROS in regulating the gating of aquaporins in *Arabidopsis* roots during salt stress through a cellular signalling mechanism (Boursiac *et al.* 2008).

COORDINATION OF STRESS-INDUCED ROS SIGNALS WITHIN THE PLANT CELL

ROS serve as signalling molecules that regulate stress responses, as well as growth and development (Foyer & Noctor 2005). The oxidative stress that accompanies drought and salt stresses should not necessarily be viewed as a harmful event needed to be avoided or alleviated, but could also be viewed as a perquisite for the plant to adequately respond and induce proper acclimation mechanisms. As previously demonstrated, deficiency in either cytosolic APX1 or tylAPX in *Arabidopsis* resulted in ROS accumulation that in turn generated a signal that enhanced the plant's tolerance to both osmotic and salt stresses (Miller *et al.* 2007). Additionally, salt-induced ROS accumulation in endosomes of AtVAMP7C antisense plants gave rise to a cytosolic signal that enhanced salt tolerance (Leshem *et al.* 2007). As these cases demonstrate, in response to decreased water availability and/or increased salinity under light conditions, plants are subjected to increased production of ROS at different tissues and at different sub-cellular compartments. Under these circumstances, numerous divergent ROS signals could be generated, which calls for a high degree of control and coordination in the plant cells.

ROS signals originating at different organelles have been shown to induce large transcriptional changes and cellular reprogramming that can either protect the plant cell or induce programmed cell death (Op den Camp *et al.* 2003; Davletova *et al.* 2005a; Foyer & Noctor 2005; Umbach, Fiorani & Siedow 2005; Vanderauwera *et al.* 2005; Gadjev *et al.* 2006; Rhoads *et al.* 2006). These types of reprogramming suggest the involvement, at least in part, of organellear retrograde signalling in mediating ROS signals in the coordination of the stress response between ROS-generating organelles to the nucleus, and perhaps directly between the organelles themselves. Retrograde signalling is largely divided into two categories: (1) developmental control of organelle biogenesis; and (2) operational control, that is, rapid adjustments in response to environmental and developmental constraints (Pogson *et al.* 2008). ROS generated in these organelles are considered to be important signalling molecules that are involved in retrograde signalling under stress conditions (Rhoads & Subbaiah 2007; Pogson *et al.* 2008; Woodson & Chory 2008).

Mutants deficient in mitochondrial AOX1a showed higher sensitivity to a combination of drought and moderate light stress (Giraud *et al.* 2008). Interestingly, these mutants exhibited altered expression of transcripts

involved in stress acclimation such as anthocyanin synthesis, chloroplastic antioxidant system, transcription factors, chloroplastic and mitochondrial components, cell wall synthesis and sugar metabolism. Pastore *et al.* (2007) suggested the importance of the cooperation between the chloroplast, cytosol and mitochondria to modulate cell redox homeostasis in durum wheat exposed to drought. Mitochondria might act to prevent over-reduction of chloroplast and cytosol via oxidation of NAD(P)H by malate/oxaloacetate shuttle and NAD(P)H dehydrogenases. The photorespiratory glycolate oxidase reaction in peroxisomes contributes to the signal transduction that regulates stomata conductance under certain stresses such as drought, osmotic stress and salinity (Foyer & Noctor 2005).

A recent study demonstrated that all the chloroplast-to-nuclei retrograde signalling, including ROS signalling, converges into a pathway regulated by GUN1 (Genome Uncoupled 1) and ABI4 (ABA Insensitive 4) in *Arabidopsis* seedlings (Koussevitzky *et al.* 2007). The osmotic and salinity tolerance of *gun1* and *abi4* mutants were not yet determined; however, they were shown to be sensitive to heat stress, underlining the importance of retrograde signalling to abiotic stress responses (Miller *et al.* 2007). To uncover the role of organelle-to-nuclei retrograde signalling, as well as intra-organelle coordination during drought and salinity, the tolerance and transcriptome reprogramming of mutants deficient in these key regulators to drought and salinity should be tested.

CONCLUDING REMARKS

ROS signalling was shown to be an integral part of the acclimation response of plants to drought or salinity stresses. It is used to sense stress due to enhanced ROS production caused by metabolic imbalances, as well as to actively send different signals via enhanced production of ROS at the apoplast by different RBOH (respiratory burst oxidase homolog) proteins. ROS signalling during drought and salinity stresses is highly integrated into many of the other signalling networks that regulate plant acclimation, including calcium, hormone and protein phosphorylation. In light of this view of the integrated signalling network of plants, which is responsible for the timely activation of different acclimation pathways, it is easy to see why some changes in ROS metabolism were found to cause enhanced tolerance to stress, whereas other changes were found to cause enhanced sensitivity. It is also not easy to predict how different changes in ROS metabolism, engineered by different genetic manipulations, will affect crop tolerance to abiotic stress. It is likely that identifying master regulators that control stress response activation (e.g. Baena-Gonzalez *et al.* 2007; Baena-Gonzalez & Sheen 2008) will provide promising avenues to enhancing crop tolerance to abiotic stress. How these alterations would affect ROS homeostasis and signalling will be an exciting aspect of these studies.

In considering the relationships between ROS metabolism and signalling and plant responses to drought or salinity, we should also remember that these stresses almost always occur in nature or in the field together with other abiotic stress conditions. Such combinations could include drought and heat, or drought and cold, and may even include triple combinations such as drought, salinity and heat. How these different stress combinations affect ROS metabolism and signalling is a subject of active research (Keles & Oncel 2002; Rizhsky *et al.* 2002, 2004; Hewezi, Leger & Gentzbittel 2008; Koussevitzky *et al.* 2008) that should be taken into consideration when attempting to engineer crops to become more tolerant to field growth conditions (Mittler *et al.* 2006).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. A summary of salt and osmotic stress tolerant plants with altered expression of genes that function in ROS scavenging and signalling. The table represents several studies made over the years as a case study, and do not attempt to cover all the studies made in this field. Abbreviations: T, tolerant; S, sensitive; ND, no data; OE, overexpressing; AS, antisense; KD, knockdown; KO, knockout.

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