The hydroxyl radical in plants: from seed to seed

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Abstract

The hydroxyl radical (OH⁻) is the most potent yet short-lived of the reactive oxygen species (ROS) radicals. Just as hydrogen peroxide was once considered to be simply a deleterious by-product of oxidative metabolism but is now acknowledged to have signalling roles in plant cells, so evidence is mounting for the hydroxyl radical as being more than merely an agent of destruction. Its oxidative power is harnessed to facilitate germination, growth, stomatal closure, reproduction, the immune response, and adaptation to stress. It features in plant cell death and is a key tool in microbial degradation of plant matter for recycling. Production of the hydroxyl radical in the wall, at the plasma membrane, and intracellularly is facilitated by a range of peroxidases, superoxide dismutases, NADPH oxidases, and transition metal catalysts. The spatio-temporal activity of these must be tightly regulated to target substrates precisely to the site of radical production, both to prevent damage and to accommodate the short half life and diffusive capacity of the hydroxyl radical. Whilst research has focussed mainly on the hydroxyl radical's mode of action in wall loosening, studies now extend to elucidating which proteins are targets in signalling systems. Despite the difficulties in detecting and manipulating this ROS, there is sufficient evidence now to acknowledge the hydroxyl radical as a potent regulator in plant cell biology.

Key words: Calcium, germination, hydroxyl radical, peroxide, pollen, spoilage, stress, wall.

Introduction

Reactive oxygen species (ROS) are acknowledged regulators of plant development and immunity, participating also in signalling of environmental change (Mittler et al., 2011; Wrzaczek et al., 2013). Hydrogen peroxide (H₂O₂) has received the vast majority of experimental attention and is firmly implicated in immunity, cell death (Chaouch and Noctor, 2010; Balazadeh et al., 2011), regulation of stomatal aperture (McAinsh et al., 1996; Kwak et al., 2003), and environmental stress responses (Li et al., 2011). With a lifetime of approximately 10⁻⁹ s and an ability to damage anything in its immediate environment and generate further radicals, the hydroxyl radical (OH⁻) has received far less attention. But this most reactive and short-lived of the ROS radicals has now been found to play fundamental and positive roles in development and adaptation. Furthermore, studies in which H₂O₂ is applied exogenously may fail to take into consideration the conversion to OH⁻ that may occur in the plant cell wall or within the cell. Therefore, we may still be underestimating the roles played by OH⁻. Since the call by Mittler and Berkowitz (2001) to look beyond H₂O₂ in ROS studies, OH⁻ has been identified as a potent effector in calcium homeostasis (Demidchik et al., 2003; Foreman et al., 2003; Zepeda-Jazo et al., 2011; Laohavisit et al., 2012) and
stress signalling (Chung et al., 2008; Laohavisit et al., 2013). This radical acts positively in reproduction, germination, and growth (Schopfer et al., 2002; Müller et al., 2009a,b; Duan et al., 2014; Smirnova et al., 2014), whilst also playing a part in cell death (Demichik et al., 2010). As such, OH’ will have played a key part in the catastrophic 19th century Irish potato famine by destroying tubers (Rastogi and Pospíšil, 2012). Today, understanding OH’ generation is also important for maintaining post-harvest fruit quality in terms of structural and nutritional properties (Ruenvongklin et al., 2009). There is even potential for an impact on atmospheric methane levels and nutritional properties (Arantes et al., 2012; Rineau et al., 2012).

Detection methods for OH’ are far more limited than for other ROS (Halliwell and Gutteridge, 1999), which has restricted research. Nevertheless, it is clear that enzymes usually thought of as having an antioxidant role can be pro-oxidants for the generation of OH’. Moreover, the presence of transition metal ions can enable OH’ production. The Haber-Weiss reaction (eqn 1) sees H2O2 and superoxide anions form OH’, catalysed by transition metals (Halliwell and Gutteridge, 1999; Chen and Schopfer, 1999):

\[
\text{H}_2\text{O}_2 + \text{O}_2^- \rightarrow \text{OH}' + \text{OH}^- + \text{O}_2
\]  

In the Fenton reaction, transition metals catalyse OH’ formation from H2O2 (eqn 2):

\[
\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}’ + \text{OH}^- + \text{Fe}^{3+}
\]  

The availability of such metal ions, down to their level of displacement from protein-binding sites caused by oxidation, will have a profound influence on OH’ formation. Unlike H2O2, there are no enzymes to dispose of OH’. Rather, plants have evolved to restrict the formation of OH’ and limit the possible damage using a range of antioxidants, so enabling positive roles for this radical to emerge. Here we review how OH’ may be generated in vivo, and how it plays a part in the life and death of a plant.

**Extracellular generation of OH’ – of PODs, SODs, NOX, and metals**

Electron paramagnetic resonance (EPR) spectroscopy has revealed extracellular OH’ production, particularly by roots (e.g. Demidchick et al., 2010). It is becoming clear that production is in the wall and at the plasma membrane, with metal ions and ascorbates playing important regulatory roles (Fig. 1). Plants contain multi-gene families for secreted class III haem-containing peroxidases (PODs; EC1.11.1.7) and these are now known to be soluble or bound in the wall where they can participate not only in H2O2 catalysis but also in OH’ generation (Chen and Schopfer, 1999; Schopfer et al., 2001; Hadži-Tašković Šukalović et al., 2010; Miura, 2012). This capacity varies between PODs (Chen and Schopfer, 1999). In their compound III form (perferryl form converted from ferric form by O2’), PODs can reduce H2O2 and O2’ to OH’ and although this relies on Fe, the mechanistic basis is not yet fully resolved (Chen and Schopfer, 1999; Miura, 2012). More OH’ is thought to be produced when PODs exhibit steady-state dynamics than during the oscillatory activity of the enzyme (Olsen et al., 2003), and the low pH of the apoplast would also favour OH’ production (Chen and Schopfer, 1999). The resultant high [OH’] could lead to enzyme inactivation (Olsen et al., 2003).

Kukavica et al. (2009) have argued that PODs work with wall-bound superoxide dismutase (SOD) to generate OH’. Their work on pea wall fragments suggested that auto-oxidation of wall hydroxycinnamonic acids could generate O2’ which could then be converted to H2O2 by wall-bound Mn-SOD, with PODs then using the H2O2 for OH’ production. Apoplastic polyamine oxidases and copper amine oxidases have also been shown to be involved in H2O2 production (Wu et al., 2010; Planas-Portell et al., 2013) that could fuel OH’ formation. In addition, walls contain CuZnSODs (Kim et al., 2008), which in animals have been proposed to generate OH’ from H2O2, with the Cu as a catalyst (Yim et al., 1990).

Apoplastic levels of ascorbate and Cu’ are sufficient to generate OH’. Uptake, mobilization and sequestration of Cu is therefore tightly controlled in plants whilst accumulation to toxic levels is associated with OH’ generation, chlorosis, and reduced biomass (Drążkiewicz et al., 2004). It is envisaged that ascorbate non-enzymatically reduces O2 to H2O2 and Cu2+ to Cu’, and that the resultant H2O2 and Cu’ generate OH’ through a Fenton reaction (Fry, 1998; Schopfer, 2001). The root is a major repository of Cu and its binding to wall polymers would localize the Fenton reaction (Fry et al., 2002). However, at high concentrations wall Cu can inhibit OH’ generation by wall PODs (Hadži-Tašković Šukalović et al., 2010), which is perhaps due to greater Cu-catalysed OH’ production promoting enzyme inactivation by OH’ (Olsen et al., 2003).

Extracellular OH’ generation has also been detected from isolated plasma membrane (Mojović et al., 2004; Heyno et al., 2011). This may be mediated in part by membrane-bound PODs although in vitro their activity is low compared to their wall counterparts (Heyno et al., 2011). Plasma membrane NADPH oxidases ([Nicotinamide Adenine Dinucleotide (Phosphate) Hydrate] Oxidases: NOX) transport an electron from cytosolic NADPH through flavin adenine dinucleotide (FAD) to the outer side of the plasma membrane and are an important source of O2’ production. Impairment of NOX can lower OH’ generation (Renew et al., 2005; Heyno et al., 2011), but the requirement for extracellular O2’ in OH’ generation is disputed (Mojović et al., 2004; Heyno et al., 2011). Plants contain multiple NOX isoforms [encoded by the Respiratory Burst Oxidase Homologue (RB0H) genes] with transcription showing distinct spatio-temporal patterns (reviewed by Mittler et al., 2011 and Wrzacek et al., 2013). NOX can be localized in detergent-resistant microdomains (‘lipid rafts’) which potentially allows for the fine-tuning of OH’ production at highly specific membrane locations. NOX-generated O2’ is readily converted to H2O2 (non-enzymatically or by SOD) which raises the possibility of Fenton-mediated OH’ production by Cu sorption at the extracellular...
plasma membrane face (Kudo et al., 2011). Proteins that could position Cu at the extracellular membrane face to permit fine spatial control of OH\(^{\ast}\) production now need to be identified. In addition, the possibility of NOX and PODs acting together to generate OH\(^{\ast}\) also merits consideration.

**Intracellular generation of OH\(^{\ast}\)**

Cu can also be held at the intracellular plasma membrane face, for example by PCaPl in Arabidopsis (Nagasaki-Takeuchi et al., 2008). The role of such Cu in catalysing OH\(^{\ast}\) production, for example from apoplastic H\(_2\)O\(_2\) entering the cytosol, remains to be determined. However, a role for plasma membrane Cu uptake proteins in cytosolic OH\(^{\ast}\) formation has recently been proposed (Rodrigo-Moreno et al., 2013). Release of O\(_2^{-}\) or H\(_2\)O\(_2\) from ROS-generating organelles such as the chloroplast or mitochondrion (Schwarzländer and Finkemeier, 2013) could also potentially be involved in OH\(^{\ast}\) generation at their cytosolic face or in the cytosol if suitable metal catalysts were present. The chloroplast has long been acknowledged as a key site of organelle OH\(^{\ast}\) formation through O\(_2\) production at Photosystem II (PS II). Mn and Fe in PSII are all clearly implicated in OH\(^{\ast}\) generation that can lead to photoinhibition (reviewed by Pospišil, 2009). Under stress conditions electron leakage from the mitochondrial electron transport chain results in H\(_2\)O\(_2\) production that can then generate damaging OH\(^{\ast}\) through the Fe\(^{2+}\) and Cu\(^{2+}\) in the system (reviewed by Keenen et al., 2011). High concentrations of mitochondrial H\(_2\)O\(_2\) can displace the Fe-S cluster of mitochondrial aconitase, leading to inactivation and Cu binding (Tan et al., 2010). In animal mitochondria, the displaced Fe can catalyse OH\(^{\ast}\) formation (Cantu et al., 2009). Whether this occurs in plants and whether Cu is involved is unknown.

 Peroxisomes are adept at ROS disposal but can become highly oxidized, at which point they are targeted for autophagic degradation (Shibata et al., 2013). Recently, peroxisomes of Arabidopsis were found to generate peroxynitrite [ONO\(_2\)\(^{-}\), derived from nitric oxide (NO)] with production increasing in response to Cd stress (Corpas and Barroso, 2014). This is an interesting development because protonation of ONO\(_2\)\(^{-}\) to peroxynitrous acid could lead to fission generation of OH\(^{\ast}\) and nitrogen dioxide radicals (Halliwell and Gutteridge, 1999; Chen et al., 2012). Overall, OH\(^{\ast}\) production is spatially complex with clear scope for interacting mechanisms of production and interplay with metal nutrients. Maintaining Fenton catalysts at non-damaging levels is
therefore central to the positive roles that OH’ plays in plant life.

Germination – radicles need radicals

Seed germination requires release from dormancy. Treatment of dormant seeds with methylviologen (as a generator of ROS including OH’) breaks dormancy (Whitaker et al., 2010) and produces similar patterns of protein carbonylation to natural after-ripening (Oracz et al., 2007, 2009). It has previously been suggested that radicals originate from protein glycation or non-enzymatic lipid auto-oxidation (reviewed by Bailly, 2004). However, Arabidopsis RBOHB is now implicated in the generation of ROS upstream of protein carbonylation (Müller et al., 2009a). Interestingly, generation of OH’ by application of Fenton reagents in the weed Bidens pilosa has been shown to reduce the need for after-ripening for germination, whilst increasing the concentration of H2O2 as a Fenton component inhibits germination suggesting that different ROS may have opposing actions in controlling dormancy (Whitaker et al., 2010).

Following imbibition, extracellular OH’ functions in cell wall loosening by catalysing scission of polysaccharides (pectins, xyloglucans; Fry, 1998; Schweikert et al., 2000; Fry et al., 2001, 2002; Miller and Fry, 2001; Schopfer, 2001; Scopfer, et al., 2001; Messenger et al., 2009; Müller et al., 2009b) thus placing these reactive molecules as important components in germination. Wall peroxidases, Cu, and Fe are all held to be catalytically competent (Fry, 1998; Schweikert et al., 2000; Schopfer, 2001; Fry et al., 2002). An OH’ burst has been observed in the seed coat and embryo during germination in radish which could be inhibited by application of OH’ scavengers (Schopfer et al., 2001). In addition, OH’ production in the endosperm cap and radicle of Lepidium sativum (cress), determined by EPR spectroscopy (detecting apoplastic cell wall OH’), has been shown to correlate with endosperm cap weakening and less force is required by the radicle to puncture the endosperm cap when incubated with OH’ for 1 h (Müller et al., 2009b). It is envisaged that OH’ generation is tightly controlled at specific sites. For example, it is suggested that OH’ is downstream of NOX (Renew et al., 2005; Heyno et al., 2011). The NOX inhibitor diphenylene iodonium (DPI) eliminates formation of O2’ during germination in radish (Schopfer et al., 2001) and inhibits germination in barley, Arabidopsis, and cress seeds (Müller et al., 2009a,b; Ishibashi et al., 2010). OH’ production and cap weakening are also inhibited by abscisic acid (ABA) (which can be lowered by H2O2: Barba-Espin et al., 2011) whilst this effect was reversed when gibberellins (GAs) were applied. Moreover, ethylene has also been shown to promote OH’ formation in radicles, counteracting inhibitory ABA effects (Graeber et al., 2010). Taken together, these data suggest that apoplastic OH’ production and cell wall loosening are under hormonal control.

Seed ageing has been linked to impaired ability to generate O2’ during germination (Kraner et al., 2010). It has also been linked to free radical production although it is not known whether OH’ is involved (Roqueiro et al., 2010). In addition, it has been suggested that stress conditions may inhibit germination through over-production of OH’ (Li et al., 2008). This is in keeping with the concept of an ‘oxidative window’ within which any level of ROS leads to germination whilst ROS levels outside the window lead to non-germinating seeds, with the latter due to oxidative damage (Bailly et al., 2008). Furthermore, some seeds are sensitive to desiccation and here extracellular O2’ production contributes to seed death (Roach et al., 2010). It is not known whether OH’ is involved in this; however, if seeds are able to germinate then early seedling growth involves OH’.

Elongative growth

An extracellular burst of O2’ is associated with radical elongation during early seedling growth that could be generated by NOX, extracellular PODs, or lipoygenases (Kraner et al., 2010). This O2’ may be the source of the extracellular OH’ that is now firmly implicated in the cell wall loosening to allow cell elongation (Fry, 1998; Schweikert et al., 2000; Fry et al., 2001, 2002; Schopfer, 2001). The generation of OH’ with Cu (or Fe), ascorbate, and H2O2 can cause wall extension in vitro and in vivo and is favoured by the low pH of the apoplast (Schopfer, 2001; Schopfer et al., 2002; Liszkay et al., 2004). Importantly, the level and position of apoplastic ascorbate are likely to play a critical role in regulating elongative growth. In its more familiar guise as an antioxidant it can suppress wall OH’ formation (Veljovic-Jovanovic et al., 2005), which may help explain the low levels found in the maize root elongation zone (Cordoba-Pedregosa et al., 2003). Ascorbate release to the apoplast can be triggered by extracellular H2O2 (Parsons and Fry, 2010), raising the possibility of complex and dynamic regulation of wall extensibility.

It has been speculated that auxin-induced increases in OH’ production may be involved in cell wall elongation, stiffening, and lignification depending on the concentration of auxin. Increases in the rate of OH’ production induced by auxin in maize coleoptiles and their auxin-induced elongation can be inhibited by OH’ scavengers (Schopfer, 2001; Schopfer et al., 2002). Moreover, growing zones of maize and barley roots produce more extracellular OH’ than mature regions (Liszkay et al., 2004; Tamas et al., 2009). In contrast, auxin concentrations that inhibited pea root elongation increased OH’ production (Kukavica et al., 2007). Whether auxin-induced increases in OH’ result from stimulation of O2’ production as a precursor is unclear; 1 μM active auxin suppresses production by maize roots (Liszkay et al., 2004), but 10 μM stimulates production by soybean hypocotyl plasma membranes, as do inactive auxin analogues (Heyno et al., 2011). The stimulation by inactive analogues suggests that auxins may have non-hormonal effects.

Genetic and inhibitor studies point to both NOX and (wall- and plasma membrane-bound) POD activities contributing to OH’ production in a variety of elongative growth systems, including cotton fibres, hypocotyls, and roots (Schopfer et al., 2002; Liszkay et al., 2004; Renew et al., 2005; Passardi et al., 2006; Mei et al., 2009; Heyno et al., 2011). For example, the
AtrbohC NOX mutant produces approximately half of wild-type root extracellular OH' and its roots and root hairs are shorter (Foreman et al., 2003; Renew et al., 2005). Two PODs are also implicated in the production of extracellular OH' for softening trichoblast cell wall to permit root hair outgrowth (Fig. 2A; Kwansiewski et al., 2013).

There is potential for crosstalk between extracellular OH' in cell wall loosening and in Ca^{2+} uptake for growth. A key observation is that fusicoccin (a stimulator of plasma membrane H^+-ATPase activity) increased maize root OH' generation (Liszkay et al., 2004). H^+-ATPase-mediated H^+ efflux would acidify the plasma membrane/apoplast interface potentially favouring Cu- or Fe-catalysed OH' production. It would also tend to hyperpolarize the plasma membrane thereby stimulating electron efflux (and hence extracellular O_2^{-}\bullet) production by NOX (Mortimer et al., 2008). This is in keeping with the finding of lower OH' generation by the AtrbohC NOX mutant (Renew et al., 2005). Membrane-bound POD activity, albeit at low levels, has recently been reported for plasma membrane vesicles isolated from growing regions of soybean hypocotyls (Heyno et al., 2011), raising the possibility of their working in compound III mode with O_2^{-}\bullet} sourced by NOX (Mika et al., 2008).

The lower OH' generation by the AtrbohC NOX mutant also results in the impaired elongation of root hairs and the main roots at normal soil pH due to an inability to generate ROS to activate a plasma membrane Ca^{2+} channel required for Ca^{2+} influx and growth (Fig. 2A; Foreman et al., 2003). The channel, recently identified as being formed by an annexin (AtANN1; Laohavisit et al., 2012), is activated by extracellular OH' (generated experimentally by physiological Cu, ascorbate, and H_2O_2 levels), can also mediate K^+ efflux, and is present at the root hair apex and main root epidermis, with greater activity in elongating cells (Demidchik et al., 2003; Foreman et al., 2003). Similar channels, activated by extracellular OH', have been identified in pea and barley root epidermal plasma membrane. Their activities can be potentiated by extracellular polyamines, possibly by polyamines binding to channel-related components and sensitizing the transport pathway to OH' (Zepeda-Jazo et al., 2011; Velarde-Buendia et al., 2012). In growing pollen tubes, H_2O_2 production by a polyamine oxidase appears necessary for activation of a plasma membrane Ca^{2+} influx channel for growth (Wu et al., 2010), but it is not yet known if extracellular OH' is involved.

As mentioned earlier, Rodrigo-Moreno et al. (2013) proposed that Cu influx across the plasma membrane could generate cytosolic OH' to activate channels. Therefore, although it

Fig. 2. Involvement of OH' in polar growth and stress signalling. (A) Schematic diagram of events at the root epidermis for polar growth. Cell wall softening by extracellular PODs permits root hair outgrowth (indicated by arrow) from barley trichoblasts (Kwansiewski et al., 2013) while at the root hair apex (enlarged inset) NOX permits elongative growth (Foreman et al., 2003). Enlarged inset: as in Fig. 1, the plasma membrane (PM) NOX generates extracellular O_2^{-}\bullet} (Renew et al., 2005) to source H_2O_2 formation that could occur non-enzymatically or through SOD. This H_2O_2 could generate OH' by a metal-based Fenton reaction (Fry, 1998; Schopfer, 2001; Foreman et al., 2003; Kudo et al., 2011). Extracellular OH' causes Ca^{2+} influx for growth (Foreman et al., 2003), mediated by an annexin in Arabidopsis (Lachavisit et al., 2012). In pollen tubes, NOX are involved in elongation (Potocky et al., 2012) and polyamine oxidases can also be involved in activating Ca^{2+} influx (Wu et al., 2010). (B) Schematic of events in salt stress signalling. In salt stress of Arabidopsis roots, ingress of Na^+ across the plasma membrane results in elevation of cytosolic free Ca^{2+} ([Ca^{2+}]_{cyt}) which should promote plasma membrane NOX activity to increase extracellular OH'. The extracellular OH' produced by salt stress (Demidchik et al., 2010) activates Ca^{2+} influx through the annexin 1 pathway (ANN1). This activates the salt overly sensitive (SOS) signalling pathway for adaptive growth (Lachavisit et al., 2013). It is feasible that a positive feedback loop is set up by Ca^{2+} entry through ANN1 to activate NOX further.
remains to be seen whether annexins are the only transporters that could link OH• to Ca2+ transport for growth or signalling, it is nevertheless intriguing that modelling of channel structures predicts Cu binding by two cyclic nucleotide-gated channels, bringing the possibility of their regulation by a ‘self-sourced’ Fenton reaction (Demidchik et al., 2014).

Reproduction, senescence, and recycling

Levels of OH• must be tightly controlled in anther and pollen development. Although the source and target of OH• are unknown there is evidence that MT-1-4b, a cysteine-rich metal-binding protein, is a key regulator of OH• during male reproductive development; in its absence, anther and pollen structures are abnormal (Hu et al., 2011). Recently, Smirnova et al. (2014) have found opposing roles for OH• and H2O2 in tobacco pollen germination. While OH• loosens the inner cell wall at the germination pore, H2O2 strengthens the remaining wall through polysaccharide oxidation. In common with root hairs, pollen tube elongation involves ROS sourced by NOX activity (Potocky et al., 2012), but H2O2 from polymain oxidase is also required (Wu et al., 2010). However, the role of extracellular OH• in pollen tube elongation remains to be determined. In contrast, once the pollen tube reaches the entrance of the female gametophyte, OH• is the predominant ROS produced by the female to cause tube rupture (involved wall loosening) and sperm cell release. Rupture is a Ca2+-dependent process, implicating an OH•-activated plasma membrane Ca2+ channel that now needs to be identified (Duan et al., 2014).

Recently, Liu et al. (2014) found high levels of OH• in walls of juvenile seed pods of pea and Brassica chinensis, which correlated with high POD activity and are likely to be involved in elongation. Mature pods also had high wall OH• levels that were proposed to relate to increasing wall thickness during lignification. Pod or fruit abscission (or abscission of any organ) has been proposed to involve extracellular OH• to weaken cell walls but as yet there is no direct evidence (Cohen et al., 2014). Fruit softening in pear and fruit senescence in longan is mediated by OH• attack on polysaccharides (Fry et al., 2001; Duan et al., 2011) while OH• causes anthocyanin degradation in the pericarp of stored litchi fruit (Ruenroengklin et al., 2009). All can cause significant loss of post-harvest crop value.

Developmental senescence involves ROS production and membrane lipid peroxidation. Senescence of soybean root nodules involves OH• generated with catalytic Fe (Becana and Klucas, 1992), but detection of H2O2 and O2•− as possible precursors varies with species. Intriguingly, ascorbate-deficient Arabidopsis has a delayed senescence phenotype (Pavet et al., 2005) that could implicate ascorbate-catalysed OH• production in this last developmental stage. Once dead, OH• plays a significant part in the breakdown of plants. Saprotrophic and probably mycorrhizal fungi can deploy an extracellular Fe-based Fenton reaction to generate OH• for breakdown of cellulose and lignin (Arantes et al., 2012; Rineau et al., 2012). This can involve secretion of small glycoproteins that reduce Fe3+ to Fe2+ and bind to it to generate a Fenton catalyst (Tanaka et al., 2007).

Biotic induction of OH•

It has recently been hypothesized that ratios of specific ROS will determine whether defence or death is initiated (Sabater and Martin, 2013), whilst the spatio-temporal dynamics of the production of specific ROS could also be important. Certainly, there is now evidence for OH• acting as messenger and executioner. In biotic interactions, roots exposed to allelopathic furanic and p-coumaric acids generate OH•, implicating this ROS in root competition (Gmerek and Politycka, 2010). Wounding increases OH• in Brassica napus hypocotyls (van Doorslaer et al., 1999). Fungal attack or elicitors also elevate OH• (Rhizoctonia solani, Singh et al., 2011; Aspergillus, Malencic et al., 2012; Alternaria, Chen et al., 2010; N-acetylchitooligosaccharides, Kuchitsu et al., 1995). Fanelli et al. (1992) found evidence for OH• involvement in potato tuber hypersensitive response (HR) while Rastogi and Pospišil (2012) discovered that the necrotrophic phase of Phytophthora infestans attack on tubers relied on OH•-mediated lipid peroxidation. Indeed, OH•-mediated cell death may be a characteristic of necrotrophic fungal attack as Govrin et al. (2006) found that HR necrosis induced by Botrytis infection could be lessened by Fe chelation, implicating OH• involvement. However, Deng et al. (2010) reported that OH• production by photoactivated riboflavin limited the HR of tobacco leaves triggered by the elicitin protein ParA1 from Phytophthora (a chloroplast-dependent HR). This was not the case for the HR triggered by a hairpin elicitor, which is mitochondria-dependent (Deng et al., 2010). They hypothesized that the OH• production by riboflavin was acting in a discrete signalling pathway to HR cell death and changed the fatty acid hydroperoxide signals (that command the death response) to achieve suppression. The specific combination of pathogen and environmental conditions is therefore critical to the response observed.

Abiotic induction of OH•

OH• production is implicated in numerous abiotic stress responses. Light-induced wilting has been attributed to OH• generation in the epicotyls of dark-grown peas (possibly through the Fenton reaction) (Hideg et al., 2010). However, OH• appears capable of negatively regulating water flow. OH• induced aquaporin closure in the green alga Chara and reduced water flow in high light was similar to that induced by Fenton reagents in maize leaf parenchyma (Henzl et al., 2004; Kim and Steudle, 2009). This apparent contradiction may be explained by etiolated tissues being more sensitive to ROS and water loss may have been the result of membrane damage. Chilling in the light also causes damaging OH• generation in chloroplasts (Wise, 1995). UV can cause apoplastic OH• production (Mesenger et al., 2009) that can (in common with exogenous application of Fenton reagents) cause the formation of peroxules, (transient extensions of the
peroxisome) that appear aligned with ER tubules and in this case extend around chloroplasts (Sinclair et al., 2009). These authors have suggested that the subsequent fission of the peroxisomes is a way to increase the peroxisomal population as an anti-stress measure.

Lethal OH⁻ production in chloroplasts is induced by the bipyridyl herbicides paraquat and diquat. The herbicides accept electrons from PSI to form bipyridyl radicals that generate O₂⁻ from O₂ for OH⁻ formation (Babbs et al., 1989; Halliwell and Gutteridge, 1999). The gaseous pollutant ozone (O₃) also generates ROS (including OH⁻) in the apoplast of the sub-stomatal cavity (Kadono et al., 2006). O₂ enters through stomata initiating a damaging oxidative burst that can resemble that of a pathogen attack and involves NOX activity (Joo et al., 2005; Vahisalu et al., 2010). ROS production has been linked to stomatal closure, and in Arabidopsis involves the plasma membrane Guard Cell Outwardly Rectifying K⁺ (GORK) efflux channel (Vahisalu et al., 2010). This channel has been shown to be activated by extracellular OH⁻ in roots (Demidchik et al., 2010), although this has not been tested in guard cells. Nevertheless, low levels of exogenous OH⁻ (generated by Fenton reagents) have been shown to promote stomatal opening whilst high levels promote closure (Hao et al., 2012). Native OH⁻ production is proposed to be downstream of extracellular ATP signalling but as yet this has not been directly measured. However, this would be consistent with current models for ABA-induced stomatal closure in which NOX are activated (Kwik et al., 2003).

Soil conditions may have a profound influence on OH⁻ formation. Drought increases Fe and Cu availability for Fenton reactions and could cause increased OH⁻ generation (Moran et al., 1994). Nitrogen deficiency has been shown to cause OH⁻ production, lipid peroxidation, and senescence in wheat (Stopario and Maksimovic, 2008). Uptake of toxic metals also causes deleterious OH⁻ formation. Aluminium induces an oxidative burst through the action of cell wall-bound PODs (Achary et al., 2012). Mn can inflict severe damage through its ability to catalyse OH⁻ formation although in cucumber leaves this can be ameliorated by silicon, which decreases POD abundance and Mn levels (Maksimovic et al., 2012). Cadmium is a potent soil contaminant that causes ROS generation that can signal adaptation or inflict damage. Surprisingly, however, Heyno et al. (2008) found that while Cd induced H₂O₂ formation in cucumber and soybean roots, it inhibited OH⁻ production and this could be ameliorated by Ca²⁺. Inhibition of OH⁻ production by Cd was also observed in barley roots by Tamas (2009). This may in part be due to the ability of Cd to inhibit NOX activity and suppress expression (Groppa et al., 2012). Finally, salt stress has been shown to promote OH⁻ formation by Arabidopsis roots (Demidchik et al., 2010). This is reliant on NOX activity (Chung et al., 2008) and has a signalling role, activating Ca²⁺ influx at the extracellular plasma membrane face through the AtANN1 transport pathway to trigger adaptive responses at transcriptional and growth levels (Laohavisit et al., 2013; Fig. 2B). It can also lead to programmed cell death, with K⁺ loss from cells being triggered by extracellular OH⁻ activating the GORK efflux channel (Demidchik et al., 2010). Salt stress also causes accumulation of the extracellular polyamines that can potentiate OH⁻-activated K⁺ loss from roots. Importantly, salt-sensitive barley loses more K by this route than salt-tolerant barley, highlighting the potential significance of elucidating the molecular mechanisms underpinning OH⁻ effects for agricultural sustainability and global food security (Velarde-Buendia et al., 2012).

Conclusions and future directions

To date, the study of ROS has been hampered by technical challenges of which the detection of OH⁻ has proved particularly intractable. However, the recent advent of ratiometric indicators for real-time imaging of OH⁻ offers exciting possibilities for elucidating further the spatio-temporal dynamics of OH⁻ production and improving our understanding of where, when, how, and why OH⁻ forms (Yuan et al., 2010). Although it is envisaged that OH⁻ is involved wherever walls require loosening, there are key questions left unanswered. What regulatory pathways are involved in OH⁻ production during normal and adaptive development? Which genes respond specifically to OH⁻? We also need to know more about the mechanisms of OH⁻ action on lipids and proteins operating in signalling and development. Together, this will enable better exploitation or defence against the effects of ROS. Furthermore, an improved understanding of when plants evolved to use OH⁻ ‘positively’ and whether it was an early event that permitted branching will be important. Studying ancestral systems may aid in resolving the problems we face studying the angiosperms.

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