Reactive oxygen species and oxidative burst: Roles in stress, senescence and signal transduction in plants

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The oxidative burst, during which large quantities of reactive oxygen species (ROS) like superoxide, hydrogen peroxide, hydroxyl radicals, peroxy radicals, alkoxy radicals, singlet oxygen, etc. are generated, is one of the earliest responses of plant cells under various abiotic and biotic stresses and natural course of senescence. In fact, reactions involving ROS are an inherent feature of plant cells and contribute to a process of oxidative deterioration that may lead ultimately to cell death. Sources of ROS include leakage of electrons from electron transport systems, decompartmentalization of iron which facilitates generation of highly reactive hydroxyl radicals, and also various biological reactions. The imposition of both abiotic and biotic stresses causes overproduction of ROS, which ultimately imposes a secondary oxidative stress in plant cells. Degradation of membrane lipids, resulting in free fatty acids, initiates oxidative deterioration by providing a substrate for enzyme lipoxygenase, causing membrane lipid peroxidation. Since lipid peroxidation is known to produce alkoxy, peroxy radicals as well as singlet oxygen, these reactions in the membrane are a major source of ROS.

Keywords: Abiotic stress, oxidative stress, senescence, signal transduction.

AN inevitable result of membrane-linked electron transport (chloroplastic, mitochondrial and plasma membrane) is the spilling of electrons onto molecular oxygen in plant cells, with the resultant generation of highly toxic reactive oxygen species (ROS)\(^1\)\(^\text{-}\)\(^4\). The imposition of abiotic and biotic stresses can further aggravate the production of ROS\(^\text{3,6}\). ROS are also generated during normal metabolic processes\(^\text{3,4}\). It has been estimated that 1% of O\(_2\) consumed by plants is diverted to produce ROS\(^7\) in various subcellular loci\(^8\)\(^-\)\(^9\).

The ROS capable of causing oxidative damage include superoxide (O\(_2\)), perhydroxyl radical (HO\(_2\)), hydrogen peroxide (H\(_2\)O\(_2\)), hydroxy radical (OH\_), alkoxy radical (RO\(_\cdot\)), peroxy radical (ROO\(_\cdot\)), organic hydroperoxide (ROOH\_), singlet oxygen (O\(_2\)), excited carbonyl (RO\(_\cdot\)), etc. During the reduction of O\(_2\) to H\(_2\)O, ROS namely O\(_2\)-, H\(_2\)O\(_2\) and OH\(^\cdot\) can be formed. The superoxide radical which is reactive in hydrophobic environment such as interior of membrane, is generated in plant cell at the onset of oxidative burst of cell. Protonated form of O\(_2\)-, HO\(_2\) is more reactive than superoxide itself, but in plant cells at physiological pH, a small proportion of O\(_2\)- would be in this form\(^8\). However, superoxide can dismutate to form H\(_2\)O\(_2\). And much more reactive OH\(^\cdot\) can be formed from O\(_2\) and H\(_2\)O\(_2\) through Fe catalysed Haber–Weiss reaction. Singlet oxygen, an electronically excited species of O\(_2\), is also toxic and its significance has been realized only recently, due to the development of methods for its generation, free from other contaminants as well as its detection\(^10\). In addition, peroxy and alkoxy radicals formed as intermediates in membrane lipid peroxidation are also toxic and pose a threat to several biomolecules.

O\(_2\)- is a moderately reactive, short-lived ROS with a half-life\(^11\) of approximately 2–4 µs. O\(_2\)- cannot pass through biological membranes as it is readily dismutated to H\(_2\)O\(_2\). O\(_2\) can either transfer its excitation energy to other biological molecules or continue with them, thus forming endoperoxides or hydroperoxides\(^12\). O\(_2\) can last for nearly 4 µs in water and 100 µs in polar solvent\(^13\). H\(_2\)O\(_2\), on the
contrary, is moderately reactive and has relatively long half-life (1 ms) and can diffuse some distances from its site of production\textsuperscript{11}. H\textsubscript{2}O\textsubscript{2} may inactivate enzymes by oxidizing their thiol groups\textsuperscript{14,15}.

So, any condition which disrupts redox homeostasis produces an oxidative stress in plants, where the redox steady state of the cell is altered in the direction of prooxidants that leads to accumulation of ROS. The manifestation of this state of cell ranges from membrane damage, metabolic and physiological impairment to genomic lesions associated with ageing and senescence of plant cells\textsuperscript{16–19}.

The ROS arise in plant cells via a number of routes. In fact most cellular compartments have the potential to become a source of ROS (Figure 1). Most ROS in plant cells are formed via dismutation of superoxide, which arises as a result of single electron transfer to molecular oxygen in electron transfer chains principally during the Mehler reactions in chloroplast\textsuperscript{20}. In case of photosynthetic electron transport, O\textsubscript{2} uptake associated with photoreduction of O\textsubscript{2} to superoxide (Figure 1) is called Mehler reaction, in honour of its discoverer\textsuperscript{21}. Although photoreduction of oxygen is an important alternative sink for the consumption of excess energy, it is always associated with the generation of toxic ROS. If the accumulation of ROS exceeds the capacity of enzymic and non-enzymic antioxidant systems to remove them, photodynamic damage to the photosynthetic apparatus ensues, which leads to cell destruction. The dearth of NADP\textsuperscript{+} in PSI due to redox imbalance triggers the generation of O\textsubscript{2}. The regulated activation of Calvin cycle and control of rate of electron flow in \textit{z}-scheme of photosynthesis is extremely important as the electron carriers of PSI have sufficient negative electrochemical potential to donate electrons to O\textsubscript{2}, resulting in O\textsubscript{2} formation. Majority of O\textsubscript{2} \textit{in vivo} is thought to be produced via electron spilling from reduced ferredoxin to oxygen. O\textsubscript{2} then undergoes dismutation either spontaneously or facilitated by SOD. Another potential source of generation of ROS H\textsubscript{2}O\textsubscript{2} in plant cells is during the oxidation of glycolate in the C\textsubscript{2} pathway of peroxisomes (Figure 1).

Mitochondrial electron transport system is also a potential source of ROS (Figure 1), including superoxide, hydrogen peroxide, and hydroxyl radicals. Direct reduction of O\textsubscript{2} to O\textsubscript{2} anions takes place in the flavoprotein region of NADH dehydrogenase segment of the respiratory chain. During mitochondrial electron transport, the oxygen radical is markedly enhanced in the presence of antimycin A, which blocks electron flow after ubiquinone (Figure 1). This results in the accumulation of reduced ubiquinone which may undergo autoxidation, resulting\textsuperscript{22,23} in the production of O\textsubscript{2}. Several observations reveal ubiquinone as a major H\textsubscript{2}O\textsubscript{2} generating location of the mitochondrial electron transport system.
chain in vitro and it would appear that O2 is a major precursor28,25 of H2O2.

Superoxides are known to be produced during NADPH-dependent microsomal electron transport25. Two possible loci of O2 production in microsomes are auto-oxidation of oxyctochrome - P-450 complex that forms during microsomal mixed function oxidase (MFO) reactions25 and/or auto-oxidation of cytochrome P-450 reductase27, a flavoprotein that contains both FAD and FMN.

Cell wall peroxidase is able to oxidize NADH and in the process catalyse the formation of O2. This enzyme utilizes H2O2 to catalyse the oxidation of NADH to NAD+, which in turn28 reduces O2 to O2. Superoxide consequently dismutate to produce H2O2 and O2.

Other important sources of ROS in plants that have received little attention are detoxification reactions catalysed by cytochrome P450 in cytoplasm and endoplasmic reticulum (ER)26. ROS are also generated in plants at plasma membrane level or extracellularly in apoplast. Plasma membrane NADPH-dependent oxidase (NADPH oxidase) has recently received a lot of attention as a source of ROS for oxidative burst, which is typical of incompatible plant-pathogen interaction. In phagocytes, plasma membrane localized NADPH oxidase was identified as a major contributor to their bacteriocidal capacity27. In addition to NADPH oxidase, pH-dependent cell wall-peroxidases, germin-like oxidase oxidases and amine oxidases have been proposed as a source of H2O2 in apoplast of plant cells28. pH-dependent cell-wall peroxidases are activated by alkaline pH, which in the presence of a reductant produces H2O2. Alkalization of apoplast upon elicitor recognition precedes the oxidative burst and production of H2O2 by a pH-dependent cell wall peroxidase has been proposed as an alternative way of ROS production during biotic stress28.

Lipid peroxidation: A potential source of ROS in plant cells

Peroxidation of lipids (primarily the phospholipids of cell membranes) is mechanistically important from free-radical production perspective as it is one of the few examples of carbon centred radical production in plant cells25. Peroxidation of lipids in plant cells appears to be initiated by a number of ROS. Essentially membrane lipid peroxidation involved three distinct stages, which include initiation, progression and termination. Initiation event involves transition metal complexes, especially those of Fe and Cu. The role of these metal complexes lies in the fact that either they form an activated oxygen complex that can abstract allylic hydrogens or act as a catalyst in the decomposition of existing lipid hydro-peroxides. Although O2 and H2O2 are capable of initiating the reactions but as OH is sufficiently reactive, the initiation of lipid peroxidation is mainly mediated by OH. Loosely bound Fe is also able to catalyse the decomposition of lipid peroxides resulting in the formation of alkoxy and peroxy radicals, which further stimulate the chain reactions of lipid peroxidations25,29. It is likely that physical structures of plant membranes which place the fatty acid side chains in close proximity facilitate autocatalytic propagation of lipid peroxidation.

\[
\begin{align*}
\text{RH + OH}^ & \rightarrow \text{R}^* + \text{H}_2\text{O} & \text{Initiation step} \\
\text{R}^* + \text{O}_2 & \rightarrow \text{ROO}^* & \text{(Lipid alkyl radical)} \\
\text{ROO}^* + \text{RH} & \rightarrow \text{ROOH} + \text{R}^* & \text{(Lipid peroxy radical)} \\
\text{ROOH} & \rightarrow \text{RO}^* \rightarrow \text{Epoxides, hydro-peroxides, glycol, aldehydes} & \text{Propagation step} \\
\text{R}^* + \text{R}^* & \rightarrow \text{R} + \text{R} & \text{(Fatty acid dimer)} \\
\text{R}^* + \text{ROO}^* & \rightarrow \text{ROOR} & \text{(Peroxide bridged dimer)} \\
\text{ROO}^* + \text{ROO}^* & \rightarrow \text{ROOR} + \text{O}_2 & \text{(Peroxide bridged dimer)} & \text{Termination step}
\end{align*}
\]

Lipid peroxidation in plant cells can also be initiated by the enzyme lipoxigenase. The enzyme is able to initiate the formation of fatty acid hydro-peroxides and ensuing peroxidation25.

ROS in senescence

ROS plays a critical role during the natural course of senescence30,31. Lipid peroxidation is an inherent feature of a senescing cell30,31 and a source of ROS, especially alkoxy, peroxy radicals and singlet oxygen, which are highly toxic. Peroxidation of lipid during plant cell senescence can be triggered either by ROS or lipoxigenase as it has been shown by some tissues, where lipoxigenase activity increases with advancing senescence30,31. Thus lipoxigenase plays a central role in promoting oxidative injury during senescence in that it not only initiates chain reaction of lipid peroxidation31, but it can also form O2. In some cases, the activity of the enzyme increases during senescence in a temporal pattern that is consistent with its putative role in promoting oxidative injury31,33. There is increasing evidence that mobilizing fatty acids from membrane phospholipid serve as substrate for lipoxigenase.

In particular, there is a dramatic decline in membrane phospholipid during early stages of senescence, which is manifested as an increase in the sterol:fatty acid ratio31. These observations imply a role for lipase in membrane deterioration and it has been established that there are three lipid degrading enzymes, phospholipase D, phosphatidic acid phosphatase, and lipolytic acyl-hydrolase associated directly with senescing microsomal membrane31. Under normal circumstances, transit pool of Fe that could be used to catalyse the formation of ROS is small. It is not known convincingly whether Fe pool during senescence increases due to decompartmentalization or proteolytic degradation of metalloproteins, which is likely to increase
the level of mobile catalytic Fe and is certainly an integral part of senescence, causing acceleration in the generation of ROS.

The ETC of chloroplast, mitochondrion, ER can all leak electron to \( \text{O}_2 \) resulting in the formation of ROS, at the outset of senescence\(^{31,33} \). Loss of photosynthetic competence during senescence is attributable, at least in part, to impairment of photosynthetic electron transport. There is also a significant decline in the activity of Rubisco \(^{34} \), that results in a decline in the regeneration of NADPH from NADP+ + H\(^+\). These conditions collectively are likely to endanger enhanced production of ROS. Little is known about the mitochondrial electron transport during senescence, apart from the observation that the structural and functional integrity of mitochondria persists to the very late stages of senescence\(^{35} \). Thus it is unlikely that there is any increased production of ROS attributable to changes in the integrity of the mitochondrial electron transport, at least in the early stages of senescence.

The following hypothesis of sequences of events during senescence involving ROS has been put forward. Initially the membrane lipids are degraded by lipid hydrolysing enzymes like phospholipase D, phosphatidic acid phosphatase, etc. causing the release of free fatty acids. Peroxidation of free fatty acids released containing cis, cis 1,4 pentadiene moiety enzymatically (by lipoxygenase), and non enzymatically (catalysed by free radicals), leads to the production of ROS, increase in ethylene levels and acceleration of senescence (Figure 2).

The effect of the rise in ethylene is, therefore, to accelerate the senescence process, since ethylene synthesis requires membrane deterioration so that ACC, a polar molecule, may approach the enzyme ACC oxidase responsible for \( \text{C}_2\text{H}_4 \) evolution.

**ROS and associated mechanisms of acclimatory stress tolerance and signalling**

Although accumulation of ROS or imposed oxidative stress is potentially a lethal situation, plant cells exploit the interaction with \( \text{O}_2 \). In fact, plant cells have embraced the potential interactions with \( \text{O}_2 \) for metabolic regulation\(^{36} \). Rapid and non-specific reactions of ROS include lipid peroxidation and damage to proteins and DNA, resulting in necrosis\(^{25,36} \). While \( \text{O}_2^- \) and \( \text{OF}^- \) have few well-characterized functions in plant cells except in stress-induced ageing and senescence, ROS like \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) have important metabolic roles\(^{37,38} \). \( \text{H}_2\text{O}_2 \) is selectively a stable intermediate of ROS metabolism and may act as a second messenger, since it could diffuse from the site of production. In incompatible plant-pathogen interactions \( \text{H}_2\text{O}_2 \) has been implicated in the elicitation of a variety of defence responses\(^{39} \). Increase in \( \text{H}_2\text{O}_2 \) production (as a result of oxidative burst) has been noticed by action of plasma-membrane associated NADPH-dependent superoxide synthase together with apoplastic SOD during hypersensitive response\(^{39} \). Since \( \text{H}_2\text{O}_2 \) is an endogenous oxidant which accumulates under stress situation and senescence, a central role of this metabolite as diffusible signal molecule for selective induction of defence genes has been envisaged. So, an important contribution to redox state of plant cells by \( \text{H}_2\text{O}_2 \) has been implicated, which ultimately plays a crucial role in stress tolerance.

The significance of \( \text{O}_2^- \) has been realized only recently, due to development of methods for its separation free from contaminants as well as its detection. Recent studies show that \( \text{O}_2^- \) in low concentration can also act as signalling molecule with several biological implications\(^{10,40} \).

There are several lines of evidence suggesting strongly that \( \text{H}_2\text{O}_2 \) initiates a signal transduction process for acquisition of tolerance to abiotic and biotic stresses. A transient increase in \( \text{H}_2\text{O}_2 \) was suggested to signal activation of protective mechanism for acclimation to chilling\(^{41} \). Exogenous application of \( \text{H}_2\text{O}_2 \) can induce tolerance to chilling, high temperature and biotic stress, all of which cause elevated endogenous \( \text{H}_2\text{O}_2 \) production\(^{43} \). Treatment of winter wheat with low concentrations of \( \text{H}_2\text{O}_2 \) and catalase inhibitor induced the synthesis of polypeptides similar to those found when plants were exposed to low temperature\(^{2} \). Prasad et al.\(^{41} \) also demonstrated that maize seedlings became more chilling-resistant following treatment with low concentrations of \( \text{H}_2\text{O}_2 \). A transient increase in \( \text{H}_2\text{O}_2 \) was suggested to

![Figure 2](image)

**Figure 2.** Sequence of events proposing possible role of ROS-mediated membrane lipid damages during senescence.
Figure 3. Redox sensing in chloroplast, where PS II receives more light (EPE), plastoquinone (PG) becomes predominantly reduced, resulting in the activation of LHC II kinase via structural changes around Riske centre (2Fe–2S) protein of cytochrome bf (Cyt bf) complex. The kinase in turn phosphorylates LHC II and PS II, resulting in migration of LHC II away from PS II and further reducing ligand absorption by PS II. Oxidation of PG reverses structural changes of Cyt bf and leads to kinase inactivation. Phosphatase-mediated dephosphorylation of mobile LHC II leads to reassociation of PS II from LHC II by degrading D1 proteins, thus reducing light energy absorption.

ROS and redox signalling

Plants can sense, transduce and translate the ROS signal into appropriate cellular responses. This process requires the presence of redox-sensitive proteins (RSPs), which can undergo reversible oxidation/reduction and may switch ‘on’ or ‘off’ depending on the cellular redox state. ROS can oxidize the RSPs directly or indirectly via glutathione or thioredoxin (redox-sensitive molecules). The best-studied example of involvement of RSP in redox signalling is the cytochrome-bf complex of photosynthetic electron transport chain located in chloroplast of higher plants (Figure 3). Because PS II and PS I acts in tandem in the z-scheme of electron flow of photosynthesis, the amount of excess photochemical energy (EPE) delivered to the two reaction centres must be controlled. When light harvesting is not balanced by utilization and dissipation, toxic radicals are formed leading to oxidative damages. If the accumulation of ROS under the condition of EPE exceeds the capacity to remove them, then oxidative damage to photosynthetic apparatus ensues, which leads to cell death. However, ROS may play a positive role in the response to EPE by initiating an increase in the rate of degradation of D1 protein component of PS II reaction centre. This causes photoinhibition of photosynthetic electron transport, which may be a protective mechanism in such conditions. There is also a process of controlling dissociation of light harvesting complex from PS II, which is controlled by phosphorylation. The kinase responsible for that phosphorylation is activated by reduced plastoquinone pool, a signal that is transduced to kinase activation via structural change of Fe–S protein associated with cytochrome bf complex. Additionally, by an yet unresolved mechanism, the redox state of plastoquinone controls the transcription of chloroplast genes encoding...
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PS II and PS I reaction centre apoprotein\(^5\) (Figure 3) as well as mRNA stability and translation rate\(^6\). The redox state of plastoquinone also controls nuclear gene expression\(^7\).

**H\(_2\)O\(_2\) as an inducer of biotic stress response**

Recognition of invading pathogen results in a coordinated activation of plant defence mechanisms\(^8\). In many incompatible plant-pathogen interactions, the hypersensitive response results in localized PCD\(^9,55\). The oxidative burst, during which large amounts of H\(_2\)O\(_2\) are generated at the cell surface, is now well established as one of the earliest responses of plant cells to attempted invasion of phytopathogenic microorganisms or in response to challenges by various elicitor molecules. H\(_2\)O\(_2\) generated during oxidative bursts under biotic stress has several effects: it may be directly microbiocidal; it can mediate excess linking of cell wall polymers; it induces the expression of genes encoding proteins involved in defensive and antioxidant processes, or it can induce PCD, characteristic of hypersensitive reactions\(^9\). Treatment of *Arabidopsis* suspension culture with harpin, induces expression of defence-related gene GST (glutathione-S-transferase), required for detoxification of lipid hydroperoxides generated during oxidative stress\(^4\).

H\(_2\)O\(_2\) is believed to play two distinct roles in pathogenesis. One involves the restriction of pathogen growth and other, induction of phytoalexins and PR proteins\(^56\). H\(_2\)O\(_2\) is a putative, selective signal for the induction of subset of defence genes. The expression of H\(_2\)O\(_2\)-induced enzymes in transgenic plants has provided an innovative approach to study the plant defence resistance. Expression of a gene encoding glucose oxidase in transformed potato led to H\(_2\)O\(_2\) accumulation and increased resistance to soft rot and potato late blight. Direct injection of H\(_2\)O\(_2\) enhanced expression of PR genes\(^57\), antioxidant enzymes\(^58\), phytoalexins\(^59\) and enhanced accumulation of signalling component, salicylic acids\(^60\).

As yet, there are little data\(^45,46\) regarding intracellular signalling processes mediating H\(_2\)O\(_2\) responses and oxidative stress that results in increased cytosolic Ca. H\(_2\)O\(_2\)-induced PCD in soybean cultures was dependent on Ca\(^2+\) influx and protein phosphorylation\(^39\). Mitogen activated protein (MAP) kinase were activated in response to a number of infectious conditions\(^61\). However, it still remains unclear whether the activities of protein kinases are essential for H\(_2\)O\(_2\)-mediated PCD and gene expression.

**ROS: Part of signalling network**

In order to exert such a profound effect on plant metabolism, ROS must utilize and/or interfere with other signalling molecules. H\(_2\)O\(_2\) was found to induce accumulation of stress hormones such as C\(_2\)H\(_4\) and salicylic acid\(^60,62\). Plant hormones are not only downstream of the ROS signal, ROS themselves are secondary messengers in many hormone mediated signalling pathways\(^57,63\). Therefore, it is conceivable that feedback/feedforward interactions between ROS and hormones occur.

Abscisic acid (ABA) is implicated in a number of abiotic stress responses associated with dehydration, such as drought, cold, salinity and heat shock\(^64\). One of the physiological ABA responses is stomatal closure that prevents excessive transpiration. As a consequence, availability of CO\(_2\) for fixation of the C\(_3\) cycle is limited, which may, in turn, enhance ROS production in chloroplasts (details described earlier). Despite this well known link between ABA and ROS, little attention has been drawn on the relationship between these two signalling molecules and their pathways. Earlier studies have shown that ABA can modulate activities of antioxidant enzymes and can affect expression of genes encoding them\(^65,66\). Both H\(_2\)O\(_2\) and ABA independently acclimate maize seedlings to chilling; however, the relative position of the messengers in this process is not yet clear. The antioxidant gene *Cat1* induction takes place independently both by ABA and ROS. Since H\(_2\)O\(_2\) levels increase rapidly upon ABA treatment, H\(_2\)O\(_2\) was proposed to be a downstream component of ABA signalling involving *Cat1* induction\(^57\). Therefore, a model for signalling pathway leading to downstream ABA responses starts to emerge and is in part based on the studies of regulation of stomatal conductance by ABA. Briefly, stomatal closure is mediated via a reduction of osmolyte concentrations (both inorganic and organic) in guard cells. ABA initiates the process via an increase in cytosolic Ca\(^2+\) level that can be released from internal stores via ADP-ribose and from external sources via influx across the plasma membrane\(^58\). ABA signalling pathway is negatively regulated by PP2C-like phosphatases that are transcriptionally activated by ABA, creating a negative feedback loop\(^69\). It was demonstrated that ABA induces generation of H\(_2\)O\(_2\) in stomatal guard cells and H\(_2\)O\(_2\) activated Ca\(^2+\) influx as well as stomatal closure. Alternatively, H\(_2\)O\(_2\) may modulate ABA responses by compromising the negative effect of PP2Cs on ABA pathway, because a tobacco homologue of PP2Cs, implicated in ABA signalling, is transcriptionally down-regulated by a number of oxidative stress stimuli\(^70\). Thus, the condition where ROS enhance and/or prolong downstream ABA responses or activate the pathway by decreasing the negative regulator PP2C may be envisaged. A simplified model of ABA signal transduction pathway, with depicted places of ROS intervention is presented in Figure 4.

Ethylene, another stress hormone is also found to interact with ROS in their signalling network. Tobacco plants with reduced peroxisomal catalase activity produce C\(_2\)H\(_4\) as an earliest response of high light irradiance\(^62\). Exogenous application of H\(_2\)O\(_2\) increases C\(_2\)H\(_4\) production in pine needles in a concentration-dependent manner\(^71\). Ozone, which is known to form ROS in apoplast, induces accumulation of C\(_2\)H\(_4\) in tobacco plants\(^72\). C\(_2\)H\(_4\) in all cases seems to originate from *de novo* synthesis, as ACC (precursor of C\(_2\)H\(_4\) biosynthesis) increases concomitantly with C\(_2\)H\(_4\) production. Recently, O\(_2\)-induced O\(_2\) accumulation and cell death
have been demonstrated to be substantially reduced in ethylene-insensitive *Arabidopsis* mutant ein-2, whereas ethylene-insensitive *Arabidopsis* mutant eto-1 is hyper-sensitive to ozone\(^7\). This strongly suggests that \(\text{C}_2\text{H}_4\) has a potentiating role in oxidative cell death by controlling \(\text{O}_2^–\) accumulation.

Apart from stress responses, ROS also modulate plant growth and development and a relationship between ROS and growth hormones or their signalling pathways can be anticipated. \(\text{H}_2\text{O}_2\)-induced MAPK cascade in *Arabidopsis* which represses auxin-inducible gene expression has been demonstrated\(^74\) (Figure 5). This antagonistic effect on auxin-repressive gene expression is in line with the opposite effect of \(\text{H}_2\text{O}_2\) and auxin on cell elongation\(^75\). Studies to find the link between ROS and other growth promoters like CKs and GAs are relatively scanty. Both the hormones modulate the expression of antioxidant enzymes and increase tolerance to oxidative stress\(^76\). Down-regulation by GA of the \(\text{H}_2\text{O}_2\)-scavenging peroxidation (*Per1*) gene in barley aleurone has been suggested as a signal for release from dormancy\(^77\).

Plant hormones are not only located downstream of ROS signal, but ROS themselves are also secondary messengers in many hormone signalling pathways\(^78,79\). Therefore, feedback or feedforward interactions may conceivably occur between different hormones and ROS\(^58,80\).

**Conclusion and perspectives**

The rationale of this review describing the physico-chemical basis for ROS formation in plant has its root in a myriad of physico-chemical conditions and aberrations in which ROS have been implicated. Among these are various stresses, both abiotic and biotic, ageing and natural courses of senescence. The enhanced titres of ROS in stressed and senescing tissues increase the prospect of oxidative damage to a range of essential macromolecules, including lipids, proteins, polysaccharides, nucleic acids, etc. that results in cell and tissue death.

Sublethal amounts of ROS acclimate plants to various abiotic and biotic stresses and reduce plant growth, probably as a part of the adaptational mechanism. Although substantial genome response and activity of many enzymes are known to be affected by ROS, the molecular mechanism underlying such adaptation is still in its rudimentary state. The task of identifying conditions under which ROS-responsive processes gives rise to successful resistance lies ahead of the workers of this field.

ROS like \(\text{H}_2\text{O}_2\) have multifunctional interactive roles in early stages of plant stress responses. \(\text{H}_2\text{O}_2\) being a strong oxidant can initiate localized oxidative damage leading to disruption of metabolic function. \(\text{H}_2\text{O}_2\) can also diffuse

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**Figure 4.** Model of ROS intervention with ABA signalling cascade. ABA stimulates an increase in cytosolic \(\text{Ca}^{2+}\) and subsequently promotes stomatal closure. Both processes are mediated by externally applied \(\text{H}_2\text{O}_2\). ABA-stimulated, \(\text{H}_2\text{O}_2\)-mediated stomatal closure is blocked by an inhibitor of NADPH oxidase (DPI) and catalase (CAT). Protein phosphatase 2C (PP2C) affects ABA signalling pathway negatively, either upstream or downstream of \(\text{Ca}^{2+}\) fluxes. PP2C may act via farnesyl transferase (ERAI) that negatively regulates ABA signalling and is genetically positioned downstream or parallel to PP2C. ABA increases PP2C mRNA, thus creating negative feedback loop.

**Figure 5.** Schematic illustration of crosstalk between auxin and \(\text{H}_2\text{O}_2\) signalling pathway. ABA activates a specific *Arabidopsis* mitogen activated protein kinase (MAPKK) ANPI, which initiates a phosphorylation of cascade process leading to activation of downstream mitogen activated protein kinases (MAPK). At MPK3 and At MPK6 and induction of specific stress responsive genes. The same cascade process represses auxin-induced expression of auxin responsive genes.
relatively long distances causing changes in redox state of surrounding cells and tissues, where at low concentration it initiates antioxidative response. There exists no specific evidence to suggest that plant cells can distinguish between $H_2O_2$ accumulation due to intracellular processes such as photorespiration and that due to activation of hypersensitive response. Accumulation of $H_2O_2$ will therefore signal ‘oxidative stress’ in compartments where it originates and lead to an appropriate response in cellular defence systems. Such signalling will lead to a degree of cross tolerance to several types of stress factors. Therefore, besides exacerbating cellular damage, ROS are also capable of acting as ubiquitous signal molecules in plants. While at low concentrations ROS induce defence genes and adaptive responses, at high concentrations cell death is initiated.

ROS communicate with other growth factors and the pathway forming part of the signalling network that controls responses downstream of ROS, ultimately influencing growth and development. Genetic analysis in addition to physiological studies will be required to ascertain the position of ROS in signal transduction pathway and also assess their proper status in plant cells.


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