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Review article

Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms

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Abstract

Reactive oxygen species (ROS) are a by-product of normal cell metabolism in plants; however, under stress conditions, the balance between production and elimination is disturbed. ROS rapidly inactivate enzymes, damage vital cellular organelles in plants, and destroy membranes by inducing the degradation of pigments, proteins, lipids and nucleic acids which ultimately results in cell death. In addition to degrading macromolecules, ROS act as a diffusible signal in signal transduction pathways and also as a secondary messenger in various developmental pathways in plants. Plants possess a complex battery of enzymatic and non-enzymatic antioxidative defense systems that can protect cells from oxidative damage and scavenge harmful ROS that are produced in excess of those normally required for various metabolic reactions. The mechanism by which ROS is generated in aerobic organisms is poorly understood. This review paper describes the generation, origin, and role of ROS in signal transduction and cell death, and the removal of ROS by antioxidative defense systems in plants during various developmental pathways.

Keywords: Antioxidative defense system, Hydrogen peroxide, Oxidative damage, Reactive oxygen species, Signal transduction.
Abbreviations: AA-ascorbic acid; APX-ascorbate peroxidase; CARs-carotenoids; CAT-catalase; Chl-chlorophyll; DHA-dehydroascorbate; DHAR-dehydroascorbate reductase; ET-ethylene; GPX-guaiacol peroxidase; GR-glutathione reductase; GSH-glutathione; GSSG-oxidized glutathione; GST-glutathione-*S*-transferase; H_2O_2-hydrogen peroxide; $HO_2^{\cdot}$-hydroperoxyl radical; HR-hypersensitive response; JAs-jasmonates; LOX-lipoxygenase; LP-lipid peroxidation; MDA-malondialdehyde; MDHA-monodehydroascorbate; MDHAR-monodehydroascorbate reductase; $^1O_2$-singlet oxygen; $O_2^{\cdot-}$-superoxide radical; $OH^{\cdot}$-hydroxyl radical; PCD-programmed cell death; POX-peroxidase; RO^*-excited carbonyl; $RO^{\cdot}$-alkoxy radical; $ROO^{\cdot}$-peroxy radical; $ROOH^{\cdot}$-organic hydroperoxide; ROS-reactive oxygen species; SA-salicylic acid; SOD-superoxide dismutase; TOCs-tocopherols.

Introduction

The accumulation of molecular oxygen (O_2) in Earth's atmosphere allows aerobic organisms to use O_2 as the terminal electron acceptor during cellular respiration, which provides a higher yield of energy than fermentation (Dismukes et al., 2001). Ground state O_2 is relatively unreactive. However, during normal metabolic activity, and as a consequence of various environmental perturbations O_2 is capable of giving rise to fruitful reactive excited states, such as reactive oxygen species (ROS) and their derivatives (Fig. 1, Mittler et al., 2004; Scandalios, 2005). ROS are a product of normal cellular metabolism, but under stress conditions, the balance between the production and elimination of ROS is disturbed in cellular components of plants (Apel and Hirt, 2004; Munne-Bosch and Alegre, 2004; Karuppanapandian et al., 2006a,b,c, 2008, 2009, 2011; Karuppanapandian and Manoharan, 2008; Vellosillo et al., 2010). ROS include the superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$), hydroperoxyl radical ($HO_2^{\cdot}$), hydrogen peroxide (H_2O_2), alkoxy radical ($RO^{\cdot}$), peroxy radical ($ROO^{\cdot}$), singlet oxygen ($^1O_2$) and excited carbonyl (RO^*), all of which are cytotoxic to plants (Dismukes et al., 2001; Karuppanapandian et al., 2006a, b, c, 2009, 2011; Karuppanapandian and Manoharan, 2008; Vellosillo et al., 2010). ROS can attack virtually all macromolecules, which results in serious damage to cellular components, DNA lesions and mutations, and this often leads to irreparable metabolic dysfunction and cell death (Karuppanapandian et al., 2011). Under steady state conditions, the ROS are scavenged by various antioxidative defense systems (Foyer and Noctor, 2005; Navrot et al., 2007). In plants, various environmental perturbations (e.g., high light intensity (HL), salinity, drought, heat, chilling, wounding, ozone (O_3), herbicides,

heavy metals, pathogens, atmospheric pollutants, and photosensitizing toxins) induce the overproduction of ROS, which causes oxidative cellular damage (Mittler, 2002; Torres et al., 2002; Mittler et al., 2004; Karuppanapandian et al., 2006a,b,c, 2009, 2011; Karuppanapandian and Manoharan, 2008; Mafakheri et al., 2010; Vellosillo et al., 2010). Increasing evidence also indicates that ROS can also act as a signaling molecule involved in the regulation of various physiological and developmental processes and in pathogen defense (i.e., the HR: hypersensitive response) in plants (Guan and Scandalios, 2000; Pei et al., 2000; Mittler et al., 2004; Foyer and Noctor, 2005; Vellosillo et al., 2010). The delicate balance between ROS production and scavenging that allows this duality in function to exist in plants is thought to be orchestrated by a large network of genes termed the 'ROS gene network', which includes more than 152 genes in Arabidopsis tightly regulating ROS production and scavenging (Mittler et al., 2004). In this review, we describe the origin and generation of ROS, the role of ROS in signal transduction and cell death, and ROS detoxification mechanisms in plants.

Biochemical properties of ROS

Plants require O_2 to produce the energy needed for their own developmental processes. During normal cellular metabolism, ground state O_2 is reduced to water (H_2O) and ROS which include $O_2^{\cdot-}$, H_2O_2, $HO_2^{\cdot}$, $OH^{\cdot}$ and $^1O_2$ (Fig. 1, Mittler, 2002; Scandalios, 2005; Halliwell, 2006). It has been estimated that 1-2% of O_2 consumed by plants is sidetracked to produce ROS in various subcellular loci (Blokhina et al., 2003). ROS is generated from O_2 either by energy transfer or electron transfer reactions. Initially, the reaction cascade requires an energy input, whereas subsequent steps are exothermic and occur spontaneously. Acceptance of excess energy by O_2 can additionally lead to the formation of $^1O_2$, a highly reactive molecule compared to O_2 (Mittler, 2002; Halliwell, 2006). $^1O_2$ can interact with target other biomolecules either by transferring its excitation energy or chemical combination. Preferential targets for chemical reactions are double bonds; e.g., in polyunsaturated fatty acids (PUFAs) or guanine bases in DNA. In biological systems, $^1O_2$ is produced under UV stress or in chloroplasts due to photosensitization of chlorophyll (Chl) molecules (Chalapathi Rao and Reddy, 2008). A single electron reduction of O_2 results in the generation of the $O_2^{\cdot-}$. $O_2^{\cdot-}$ is a moderately reactive, short-lived ROS with a half-life of approximately 1 µs (Table 1); therefore, it cannot cross biomembranes and is easily dismutated to H_2O_2. $O_2^{\cdot-}$ can also react with another very influential signaling free radical species, $NO^{\cdot}$ to give rise to peroxynitrite ($OONO^-$). $HO_2^{\cdot}$ is formed from $O_2^{\cdot-}$ by protonation in aqueous solutions. $HO_2^{\cdot}$ can cross biomembranes and subtract hydrogen atoms from PUFAs and lipid hydroperoxides, thus initiating lipid auto-oxidation (Halliwell and Gutteridge, 2000). H_2O_2 is moderately reactive and a relatively long-lived molecule (half-life, 1 ms; Table 1) that can diffuse short distances away from its generation site. H_2O_2 may inactivate enzymes by oxidizing their thiol groups. H_2O_2 can travel freely across membranes, which enables it to diffuse the damage and possibly also act as a messenger in the stress signaling response (Halliwell, 2006; Moller et al., 2007). H_2O_2 can also lead to the production of $OH^{\cdot}$, the most reactive oxidant in the ROS family, via the so-called Haber-Weiss/Fenton reactions, which use suitable transition metals, especially, iron (Fe), at neutral pH and ambient temperatures and is considered as one of initiation radicals for lipid peroxidation (LP, Fig. 1, Halliwell and Gutteridge, 2000; Lee et al., 2007). $OH^{\cdot}$ is not considered to have signaling function, although the products of its reactions can elicit signaling responses, and cells sequester the catalytic metals to metallochaperones efficiently avoiding $OH^{\cdot}$ (Halliwell, 2006; Moller et al., 2007). $OH^{\cdot}$ can potentially react with all biomolecules like, pigments, proteins, lipids and DNA, and almost any constituent of cells. Since plant cells are unable to scavenge this highly reactive ROS, its production in excess results in programmed cell death (PCD, Vranova et al., 2002; Manoharan et al., 2005; Karuppanapandian et al., 2011).

Sources of ROS in plant cells and their reactivity in various cellular components

In plants, ROS are normal byproducts of various metabolic pathways and are also produced under stress conditions in various cellular compartments, including chloroplasts, mitochondria, peroxisomes, the endoplasmic reticulum (ER), and plasma membranes (Fig. 2, Corpas et al., 2001; del Rio et al., 2002; Mittler, 2002; Asada, 2006; Navrot et al., 2007). ROS production in each of these compartments is described below.

Chloroplasts

Oxidative stress in chloroplasts originates from several locations and occurs in a variety of forms. Chl associated with the electron transport chain (ETC) is the primary source of $^1O_2$, which may also arise as a byproduct of LP, which is catalyzed by lipoxygenase (LOX, Fig. 2, Asada, 2006; Foyer and Noctor, 2009). Limited CO_2 fixation due to stress conditions leads to a decrease in carbon reduction by the Calvin cycle and to a decrease in oxidized $NADP^+$ to serve as electron acceptor in photosynthesis when ferredoxin (Fd) is over reduced during photosynthesis electron transfer, electron may be transferred from photosystem I (PSI) to O_2 to form $O_2^{\cdot-}$ by the process called Mehler reaction. This trigger chain reaction that generates more aggressive oxygen radicals. Environmental stress disturbs the balance between light harvesting and energy utilization and extends the half-life of singlet chlorophyll ($^1Chl^*$), thereby increasing the likelihood that $^1Chl^*$ will undergo intersystem crossing to form triplet chlorophyll ($^3Chl^*$). $^3Chl^*$ is longer lived than $^1Chl^*$ and reacts with ground state triplet oxygen ($^3O_2$) to produce $^1O_2$, which liberates O_2 from the spin restriction that normally limits its reactivity with single biomolecules (Zolla and Rinalducci, 2002). $^1O_2$ that arises in the chloroplast most likely affects membrane proteins and lipids located near the site of $^1O_2$ production, placing the reaction centre of photosystem II (PSII) in jeopardy (Zolla and Rinalducci, 2002). $^1O_2$, which has been detected by $^1O_2$-specific fluorescent probes in leaves exposed to HL, is probably generated in PSII even in the absence of environmental stress (Fryer et al., 2002). $^1O_2$ can be added directly to the double bonds of PUFA to form lipid hydroperoxides. The close packing of PUFA molecules in the chloroplast membrane favors the initiation of LP chain reactions that emanate from the initiation site and propagate until scavenged by antioxidants. $^1O_2$ is more likely to be produced in PSII, where the lifetime of $^1Chl^*$ is generally longer than in PSI. However, the high reduction potential of the acceptor site of PSI has been hypothesized to reduce O_2 to $O_2^{\cdot-}$, particularly when the concentration of CO_2 is limiting (Asada, 2000). $O_2^{\cdot-}$ accumulation in HL has been observed in mesophyll

Table 1. Key reactive oxygen species (ROS), their properties, and main scavenging systems in plant cells

ROS	Half-life and mobility	Mode of action	Cellular sources	Main scavenging systems
Superoxide radical ($O_2^{\cdot-}$)	1 µs, 30 nm	Reacts with double bond-containing compounds such as iron-sulphur (Fe-S) clusters of proteins; reacts with nitric oxide (NO) to form peroxynitrite ($ONOO^-$)	Formed in many photooxidation reactions (flavoprotein, redox cycling), Mehler reaction in chloroplasts, mitochondrial electron transport chains (ETCs) reactions, glyoxisomal photorespiration, peroxisomes, and plasma membrane. NADPH oxidase in membranes. Xanthine oxidase and membrane polypeptides in peroxisomes. Reactions of ozone (O_3) and $OH^{\cdot}$ in apoplastic space	Superoxide dismutases (SODs)
Hydroxyl radical ($OH^{\cdot}$)	1 ns, 1 nm	Extremely reactive with protein, lipids, DNA, and other macromolecules	Reaction of H_2O_2 with $O_2^{\cdot-}$ (Haber-Weiss reaction), reactions of H_2O_2 with Fe^{2+} (Fenton reaction). Decomposition of O_3 in apoplastic space	Flavonoids, prevention of $OH^{\cdot}$ formation by sequencing Fe
Hydrogen peroxide (H_2O_2)	1 ms, 1 µm	Oxidizes proteins; reacts with $O_2^{\cdot-}$ in a Fe-catalyzed reaction to form $OH^{\cdot}$	ETCs of mitochondria, chloroplasts, endoplasmic reticulum, and plasma membrane. Photorespiration, fatty acid β-oxidation, urate oxidase, and MnSOD in peroxisomes	Catalases, various Peroxidases, peroxiredozins, and flavonoids
Singlet oxygen ($^1O_2$)	1 µs, 30 nm	Directly oxidizes protein, polyunsaturated fatty acids, and DNA	Photoinhibition, photosystem II electron transfer reactions in chloroplasts	Carotenoids and α-tocopherols

tissue; however, $O_2^{\cdot-}$ is rapidly converted to H_2O_2 either spontaneously or with the aid of superoxide dismutases (SODs, Asada, 2000; Fryer et al., 2002). The destructive properties of H_2O_2 and $O_2^{\cdot-}$ result primarily from their contribution to $OH^{\cdot}$ production, rather than from direct damage.

Mitochondria

Mitochondria generate cytotoxic and harmful ROS, such as H_2O_2 and $O_2^{\cdot-}$ as well as the ROS targets in plant cells (Jezek and Hlavata, 2005; Navrot et al., 2007). The mitochondrial ETC harbours electrons with sufficient free energy to directly reduce O_2 which is considered as a primary source of ROS generation, a necessary accompaniment to aerobic respiration (Fig. 2). However, ROS production in mitochondria takes place under normal respiratory conditions but in response to various biotic and abiotic stresses, the production can be enhanced. Two pathways of O_2 consumption potentially exist in isolated plant mitochondria and submitochondrial particles; namely, (1) O_2 consumption via cytochrome oxidase to produce H_2O, a process that accounts for more than 95% of O_2 consumption under normal conditions; and (2) direct reduction of O_2 to $O_2^{\cdot-}$ in the flavoprotein region of the NADH dehydrogenase segment of the respiratory chain (Jezek and Hlavata, 2005). The flavoprotein or iron-sulphur (Fe-S) centre of either the internal or external dehydrogenase is likely to be responsible for the consumption of O_2 (Fig. 2). O_2 is reduced to $O_2^{\cdot-}$ in the ubiquinone (UQ) cytochrome region of the respiratory chain, and this process may be identified by its insensitivity to salicylhydroxamic acid and antimycin A, its sensitivity to potassium cyanide (KCN, Fig. 2). In the enzymatic pathways presented in Figure 2, fully reduced UQ donates an electron to cytochrome c_1 (Cyt c_1) and leaves an unstable, highly reducing semiquinone species, which would normally reduce cytochrome *b* (Cyt *b*). Cyt *b* is a closely interacting species that reduces the O_2 to $O_2^{\cdot-}$. In aqueous solution, $O_2^{\cdot-}$ is moderately reactive, but this $O_2^{\cdot-}$ can further reduced by SOD dismutation to H_2O_2 (Moller, 2001). It has been estimated that about 1-5% of mitochondrial O_2 consumption leads to H_2O_2 production (Moller, 2001). This H_2O_2 can react with reduced Fe^{2+} and Cu^+ to produce highly toxic $OH^{\cdot}$, and these uncharged $OH^{\cdot}$ can able to penetrate membranes (Moller, 2001; Rhodas et al., 2006). The alternate oxidase (AOX) catalyses the O_2-dependent oxidation of ubiquinol, limiting the mitochondrial generation of ROS. Lack of AOX induction caused increased ROS production (Jezek and Hlavata, 2005). Consistent with this, tobacco cells lacking AOX had increased PCD in response to H_2O_2 and tobacco plants overexpressing AOX developed HR lesions in response to virus infection (Robson and Vanlerberghe, 2002). H_2O_2-treatment of Arabidopsis cells and H_2O_2 accumulation in catalase (CAT)-deficient tobacco lead to induction of antioxidant defenses and increased AOX levels in the mitochondria (Sweetlove et al., 2006).

Peroxisomes

Peroxisomes are probably major sites of intercellular harmful H_2O_2 production, and occur almost all eukaryotic cells (Fig. 2). H_2O_2 is typically generated in the peroxisomal respiratory pathway by different flavin oxidases. Like chloroplasts and mitochondria, plant peroxisomes also produce $O_2^{\cdot-}$ as a consequence of their normal metabolism (Corpas et al., 2001). Production of this ROS was attributed to the matrix-localized enzyme, xanthine oxidase (XOD), which catalyses the oxidation of xanthine or hypoxanthine to uric acid, and produces $O_2^{\cdot-}$ in the process. An NAD(P)H-dependent $O_2^{\cdot-}$ production site, which appears to be formed by a small ETC is composed of a flavoprotein NADH and Cyt *b*, and uses O_2 as an electron acceptor, is present in the peroxisomal membrane and releases the $O_2^{\cdot-}$ into the cytosol (López-Huertas et al., 1999; del Rio et al., 2002). Monodehydroascorabte reductase (MDHAR) participates in $O_2^{\cdot-}$ by peroxisomal membranes (Corpas et al., 2001). Previously, three peroxisomal membrane polypeptides (PMPs) with molecular masses of 18, 29 and 32 kDa have been characterized and demonstrated to be responsible for the production of $O_2^{\cdot-}$ were purified from the membranes of pea

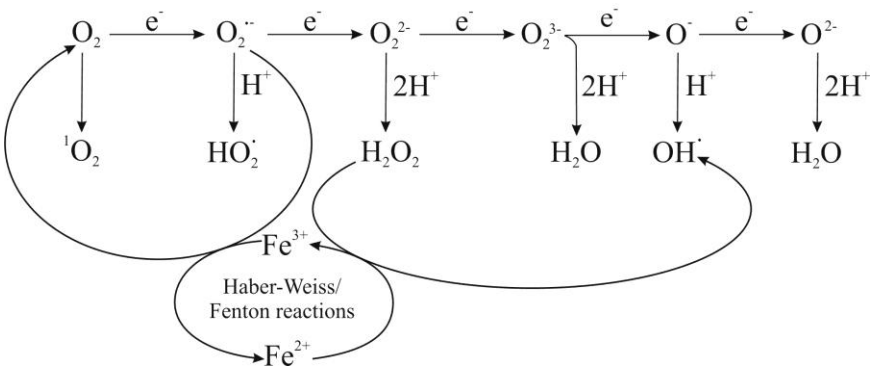

Fig 1. Metabolic pathways in the univalent reduction of molecular oxygen (O_2) to water (H_2O) leading to generation of various intermediate reactive oxygen species (ROS) in plants (Halliwell and Gutteridge, 2000; Dismukes et al., 2001; Mittler et al., 2004; Scandalios, 2005; Halliwell, 2006). The single electron (e^-) reduction of O_2 results in the generation of the superoxide radical ($O_2^{\cdot-}$). At low pH, dismutation of $O_2^{\cdot-}$ is unavoidable, with one $O_2^{\cdot-}$ giving up its added electron to another $O_2^{\cdot-}$, and then with protonation resulting in the generation of hydrogen peroxide (H_2O_2). Furthermore, $O_2^{\cdot-}$ can be protonated to form hydroperoxy radical ($HO_2^{\cdot}$). Additionally, $O_2^{\cdot-}$ can also donate an electron to iron (Fe^{3+}) to yield a reduce form of iron (Fe^{2+}) which can then reduce H_2O_2, produced as a result of dismutation of $O_2^{\cdot-}$ to hydroxyl radical ($OH^{\cdot}$). The reductions through which $O_2^{\cdot-}$, H_2O_2 and iron rapidly generate $OH^{\cdot}$ is called the Haber-Weiss reaction, whereas the final step which involves the oxidation of Fe^{2+} by H_2O_2 is referred to as the Fenton's reaction. H_2O is formed when $OH^{\cdot}$ is further reduced. Singlet oxygen ($^1O_2$) is another form of ROS, but here there is no addition of an extra electron to O_2, rather an electron is elevated to a higher energy orbited, thereby freeing oxygen forms its spin-restricted state. Abbreviations: O_2^{2-}: peroxide ion; O^-: oxene ion; O^{2-}: oxide ion.

leaf peroxisomes, including two NADH-dependent proteins (i.e., PMP18 and PMP32) and a NADPH-dependent protein (i.e., PMP29; López-Huertas et al., 1999). The $O_2^{\cdot-}$ produced has a half-life of 1 μs (Table 1) and is subsequently converted into H_2O_2 and O_2 by SOD, the final result of these stress conditions will be an increase of H_2O_2 in the plant cell (del Rio et al., 2002). During photorespiration, the oxidation of glycolate by glycolate oxidase in peroxisomes accounts for the majority of H_2O_2 production (Fig. 2, Noctor et al., 2002; Mittler et al., 2004). Additional sources of H_2O_2 production in peroxisomes include fatty acid β-oxidation, the flavin oxidase pathway and the disproportionation of $O_2^{\cdot-}$ (Fig. 2, Corpas et al., 2001).In peroxisomes from plants subjected to stress conditions by xenobiotics, and the herbicide, an oxidative stress mechanism mediated by ROS has been demonstrated (del Rio et al., 2002). In peroxisomes of leaves and roots from salt-tolerant tomato plants, there was an up-regulation of the antioxidative systems in response to salt-induced oxidative stress (Mittova et al., 2004). In Arabidopsis, treatment with 100 μM $CdCl_2$ produced a significant increase in speed, which was dependent on endogenous ROS and Ca^{2+}, but was not related to actin cytoskeleton modifications (Rodríguez-Serrano et al., 2009). In cells that metabolize fatty acids, glyoxysomes, which contain the β-oxidation cycle enzymes, generate ROS via fatty acyl coenzyme A (acyl-CoA) oxidase, a flavoprotein that reduces O_2 directly to H_2O_2 (Fig. 2). Thus, glyoxysomes, like peroxisomes of plant cells, contain high concentrations of CAT. Because plant membranes are permeable to H_2O_2, the H_2O_2 produced by both the glyoxysome and the mitochondrion (during electron transport) will influence the cytosolic concentration of ROS.

Endoplasmic reticulum

ROS production in the ER could facilitate the transmission of toxic Ca^{2+} ions at the ER-mitochondria interface. In addition, H_2O_2 could diffuse out of the ER and attack the membranes of neighboring mitochondria, bypassing the protection conferred by mitochondrial SOD that is located in the mitochondrial matrix. Mixed function oxygenases that contain a heme moiety add an oxygen atom to an organic substrate using NAD(P)H as the electron donor in a generalized reaction catalyzed by cytochrome P_{450} (Cyt P_{450}). The best-characterized Cyt P_{450} in plants is cinnamate-4-hydroxylase, which functions in flavonoid and lignin biosynthesis, but also participates in a plethora of biochemical pathways, including gibberellin and sterol biosynthesis (Shi et al., 2010). Activation of oxygen by these pathways is required for the oxygen addition reactions in the synthesis of Cyt P_{450} metabolite complex. $O_2^{\cdot-}$ is produced by microsomal NAD (P) H-dependent electron transport involving Cyt P_{450} (Mittler, 2002). After the univalent reduction of the substrate (RH) and the addition of $^3O_2$ to form the P_{450}-RHOO complex and further reduced by a flavoprotein to form a radical intermediate that can readily react with $^3O_2$, because both the intermediate and $^3O_2$ bear one unpaired electron. This oxygenated complex may be reduced by Cyt *b* or, occasionally, the complex may decompose to P_{450}-RH by releasing $O_2^{\cdot-}$ (Fig. 2). Recently, many important genes mediated PCD have been identified in plant cell, and ER stress-mediated PCD has been found to be involved in cell death progression induced by various biotic and abiotic stress factors (Watanabe and Lam, 2006; Shi et al., 2010). For example, BAX inhibitor-1 (BI-1) located in the ER was identified as a key attenuator of cell death in eukaryotes. In plants BI-1 mRNA level is increased during leaf senescence and under several types of environmental stimuli, such as heat, salt, etc. (Watanabe and Lam, 2006).

Apoplast

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; Zhu, 2001). *AtRbohD* and *AtRbohF* encode two major NADPH oxidases that are expressed in guard and mesophyll cells in Arabidopsis. They have been shown to be responsible for the apoplastic ROS generation that is required for ABA-induced stomatal closure (Kwak et al., 2003).

Table 2. Most important enzymatic and non-enzymatic antioxidative systems, their localization and scavenging in reactive oxygen species (ROS) in plant cells

Enzymatic antioxidants	Reaction catalyzed	Enzyme code number	Subcellular localization
CAT	$2H_2O_2 \rightarrow O_2 + 2H_2O$	1.11.1.6	Per, Gly, and Mit
APX	$H_2O_2 + AA \rightarrow 2H_2O + DHA$	1.11.1.11	Cyt, Per, Chl, and Mit
GPX	$H_2O_2 + DHA \rightarrow 2H_2O + GSSG$	1.11.1.7	Chl, Cyt, Mit, and ER
SOD	$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow 2H_2O_2 + O_2$	1.15.1.1	Cyt, Chl, Per, and Mit
MDHAR	$2MDHA + NADH \rightarrow 2AA + NAD^+$	1.6.5.4	Chl, Mit, and Cyt
DHAR	$DHA + 2GSH \rightarrow AA + GSSG$	1.8.5.1	Chl, Mit, and Cyt
GR	$GSSG + NADPH \rightarrow 2GSH + NADP^+$	1.6.4.2	Cyt, Chl, and Mit
Non-enzymatic antioxidants			
AA	Substrate for APX. Detoxifies H_2O_2		Chl, Cyt, Mit, Per, Vac, and Apo
GSH	Substrate for various POXs, GSTs and GR. Detoxified H_2O_2, other hydroperoxidases and toxic compounds		Chl, Cyt, Mit, Per, Vac, and Apo
TOCs	Protects membrane lipids from peroxidation, detoxifies lipid peroxides, and quenching $^1O_2$		Membranes
CARs	Quench $^1O_2$. Photosystem assembly, key components of the light harvesting complex, precursors for abscisic acid (ABA)		Chl, chromoplast, elaioplast, and amyloplast
Flavonoids	Can directly scavenge H_2O_2 and $OH^{\cdot}$		Vac

Abbreviations: $^1O_2$: singlet oxygen; AA: ascorbic acid; Apo: apoplast; APX: ascorbate peroxidase; CARs: carotenoids; CAT: catalase; Cyt; cytosol; Chlo: chloroplast; DHA: dehydroascorbate; DHAR: dehydroascorbate reductase; ER: endoplasmic reticulum; Gly: glyoxisomes; GPX: guaiacol peroxidase; GR: glutathione reductase; GSH: glutathione; GSSG: oxidized glutathione; GSTs: glutathione-*S*-transferases; H_2O: water; H_2O_2: hydrogen peroxide; MDHA: monodehydroascorbate; MDHAR: monodehydroascorbate reductase; Mit: mitochondria; O_2: oxygen; $O_2^{\cdot-}$: superoxide radical; $OH^{\cdot}$: hydroxyl radical; Per: peroxisomes; POXs: peroxidases; SOD: superoxide dismutase; TOCs: tocopherols; Vac: vacuole.

Other apoplastic ROS-forming enzymes include cell wall-associated oxidases, pH-dependent cell wall POXs, germin-like oxalate oxidases and polyamine oxidases have been proposed to generate ROS at the apoplast (Fig. 2, Mittler, 2002). H_2O_2 accumulation in the apoplast is thought to be involved in the acclimation responses of plants, such as modulation of growth rate and cell wall strengthening, to drought and salt stresses (Hernandez et al., 2001; Zhu, 2001; Rodriguez et al., 2004). Apoplastic ROS generation was shown to have a positive effect on leaf elongation in maize during salinity stress and a 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 (e.g., due to changes in SOD activity), which positively correlated with salt tolerance (Hernandez et al., 2001).

Plasma membranes

Recently, the plasma membrane NADPH-dependent oxidases, similar to the mammalian calcium-regulated NADPH oxidase have gained much attention in regard to its gene expression, protein structure, and different homologs upon various environmental stresses (Apel and Hirt, 2004). Molecular and physiological data indicate functional and mechanistic similarities between animal and plant NADPH oxidase, and this enzyme has been proposed to play a key role in the production and accumulation of ROS in plants under stress conditions (Pei et al., 2000; Torres et al., 2002; Vranova et al., 2002; Kwak et al., 2003; Apel and Hirt, 2004; Laloi et al., 2004). NADPH oxidase transfers electrons from cytoplasmic NADPH to O_2 to form $O_2^{\cdot-}$, followed by dismutation of $O_2^{\cdot-}$ to H_2O_2. It has been well documented that NADPH oxidase is involved in plant defense reactions to pathogen attack (Sagi and Fluhr, 2001; Torres et al., 2002) and in response to various abiotic stresses (Orozco-Cardenas et al., 2001; Kwak et al., 2003) including deficiency or excess of cadmium (Cd), copper (Cu), and nickel (Ni) (Quartacci et al., 2001; Hao et al., 2006). Some studies have shown that, diphenylene iodinium (DPI), an important inhibitor of NADPH oxidase, have been shown to block or impair H_2O_2 production during stress conditions in plants (Overmyer et al., 2003; Laloi et al., 2004). NADPH oxidases-dependent $O_2^{\cdot-}$ production is involved in peroxidation of vital cell constituents and PCD (Neill et al., 2002). It is generally accepted that $O_2^{\cdot-}$-generating NADPH oxidases play a major role in cell damage and associated decreases in growth by abiotic stresses. For example, preventing NADPH oxidation or NADPH-dependent $O_2^{\cdot-}$ generation in cucumber plants under chilling stress reduced chilling-induced leaf necrosis and LP (Shen et al., 2000). In the roots of wheat seedlings, NADPH oxidase could hold up oxidative stress induced by Ni (Hao et al., 2006). These results imply that the expression of NADPH oxidase is necessary for the development of stress resistance. Actually, it has been suggested that during oxidative burst, NADPH oxidase can trigger Ca^{2+}, and mitogen-activated protein kinase (MAPK) signaling pathways as well as depress the hormone signal transfer routes such as salicylic acid (SA), jasmonates (JA), and ethylene (ET, Overmyer et al., 2003; Evans et al., 2005).

Cell walls

Cell walls are sites of active metabolism and O_2 activation. Whereas some of these reactions are involved in defense mechanisms against pathogens and in the degradation or compartmentalization of xenobiotic chemicals, most are biosynthetic. For example, the phenylpropanoid precursors of lignin are cross-linked by H_2O_2-dependent reactions that randomly link subunits to form lignin (Higuchi, 2006). NADH is generated by a cell wall malate dehydrogenase and then gives rise to H_2O_2, possibly by the action of an NADH oxidase localized to the plasmalemma. Diamine oxidases are

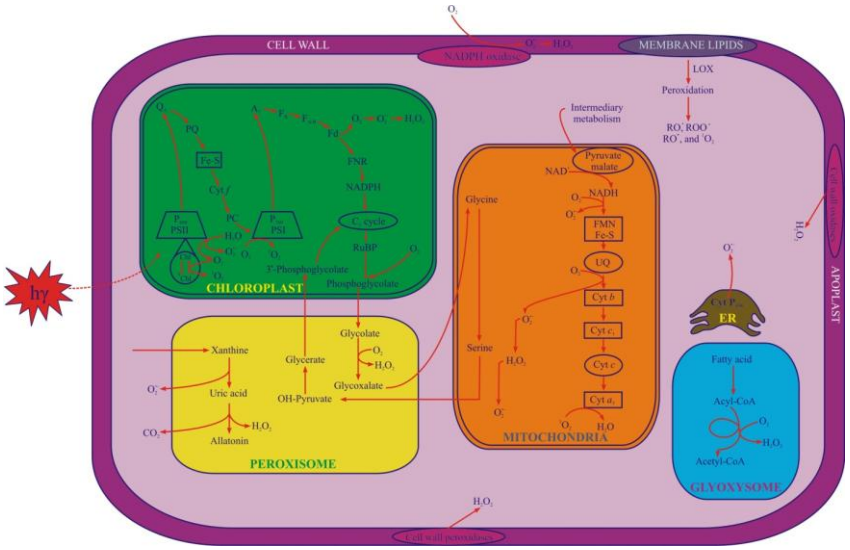

Fig 2. Sources of reactive oxygen species (ROS) in plant cells and their reactivity in various cellular components. The model is based on results described by various authors (Corpas et al., 2001; Hernández et al., 2001; Quartacci et al., 2001; Mittler, 2002; Apel and Hirt, 2004; Mittler et al., 2004; Asada, 2006). In chloroplast, molecular oxygen (O_2) uptake associated with photoreduction of O_2 to superoxide radical ($O_2^{\cdot-}$). Singlet oxygen ($^1O_2$) is produced by energy transfer to triplet oxygen ($^3O_2$) from the excited triplet chlorophyll ($^3Chl^*$) in photosystem II (PSII). Excitation of PSII results in the oxidation of water (H_2O) to O_2. The reductant formed by this process donates electrons (e^-) to plastoquinone (PQ), which passes them, on to the cytochrome *f* (Cyt *f*) complex. This complex passes electrons to plastocyanin (PC), a small protein that can act as a mobile of electron carrier to photosystem I (PSI). PSI have sufficient negative electrochemical potential to donate electrons to O_2, resulting in $O_2^{\cdot-}$ formation. Majority of $O_2^{\cdot-}$ *in vivo* is thought to be produced via electron spilling through a series of ferridoxin (Fd) carriers to O_2. $O_2^{\cdot-}$ formed then undergoes dismutation to hydrogen peroxide (H_2O_2) either spontaneously or facilitated by superoxide dismutase (SOD). In the presence of transition metal like iron, $O_2^{\cdot-}$ and H_2O_2 can give rise to the production of hydroxyl radical ($OH^{\cdot}$). H_2O_2 was generated during the oxidation of glycolate in the C_2 cycle of peroxisomes. Xanthine oxidase catalyses the oxidation of xanthine and hypoxanthine to uric acid and is a well-known producer of $O_2^{\cdot-}$. In mitochondria, direct reduction of O_2 to $O_2^{\cdot-}$ takes place in the flavoprotein region of NADH dehydrogenase segment. During mitochondrial electron transport, the oxygen radical is markedly enhanced in the presence of antimycin A, which blocks electron flow after ubiquinone (UQ). This results in the accumulation of reduced UQ which may undergo autooxidation, resulting in the production of $O_2^{\cdot-}$. $O_2^{\cdot-}$ is a major precursor of H_2O_2 production in UQ location of the mitochondrial electron transport chain (ETC). O_2 reduction to $O_2^{\cdot-}$ in the UQ-cytochrome region of the respiratory chain. In these schemes, fully-reduced UQ donates an electron to cytochrome c_1 (Cyt c_1) and leaves an unstable, highly reducing semiquinone species, which would normally reduced cytochrome *b* (Cyt *b*), which reduces the O_2 to $O_2^{\cdot-}$. Alternate oxidase (AOX) can divert electrons flowing through ETCs and use them to reduce O_2 to H_2O. The acyl coenzyme A (acyl-CoA) oxidase of glyoxisomal β-oxidation avoids that by transferring electrons from reduced form of flavin adenine dinucleotide ($FADH_2$) directly to O_2 resulting in H_2O_2. Plasma membrane-bound NADPH oxidase as well as cell wall-associated extracellular peroxidases (POXs) and oxidases are the main H_2O_2 and $O_2^{\cdot-}$ producing enzymes in the apoplast. pH dependent cell-wall POXs are activated by alkaline pH, which in the presence of a reductant produces H_2O_2. In addition, lipoxygenase (LOX) catalyses the peroxidation of membrane lipids to produce alkoxy radical ($RO^{\cdot}$), peroxy radical ($ROO^{\cdot}$), excited carbonyl (RO^*) and $^1O_2$. Abbreviations: A_1: phylloquinone, secondary electron acceptor in PSI; Acetyl-CoA: Acetyl coenzyme A; Chl: chlorophyll; CO_2: carbon dioxide; Cyt a_3: cytochrome a_3; Cyt *c*: cytochrome *c*; Cyt P_{450}: cytochrome P_{450}; ER: endoplasmic reticulum; Fe-S: the Rieske iron-sulphur centre; FMN: flavin mononucleotide; FNR: ferredoxin-NADP reductase; F_X and $F_{A/B}$: iron-sulfur acceptors in PSI; hγ: gamma irradiation; P_{680}: primary electron donor in PSII; P_{700}: primary electron donor in PSI; Q_A: primary quinone acceptor in PSII; RuBP: ribulose-1,5-bisphosphate.

also involved in the production of ROS in the cell wall; diamines or polyamines (e.g., putrescine, spermidine, and cadaverine) are used to reduce a quinone that will then auto-oxidize to form peroxides (Spiteller, 2003; Higuchi, 2006). The LOX reaction, which catalyzes the hydroperoxidation of PUFA is involved in response to stresses, is another possible source of ROS such as $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$ and $^1O_2$ (Spiteller, 2003). Generation of $OH^{\cdot}$ was noted *in vivo* and *in vitro* with cell wall of several plant species (Schopfer, 2001; Spiteller, 2003). Liszkay et al. (2004) have been demonstrated that production of $O_2^{\cdot-}$ and H_2O_2, in cell wall of maize roots using specific histochemical assays and electron paramagnetic resonance spectroscopy. $OH^{\cdot}$ and $^1O_2$ can react with the methylene groups of PUFA to form conjugated dienes, lipid peroxy radicals, and hydroperoxides. The lipid hydroperoxides (PUFA-OOH) produced can undergo reductive cleavage by reduced metals, such as Fe^{2+}. The lipid $RO^{\cdot}$ produced, PUFA-O, can initiate additional chain reactions. Cell wall localized POX contributes to ROS production during potassium (K) deficiency stress in Arabidopsis (Kim et al., 2010). It has also been noted that plants exposed to various abiotic stresses an increase in LP in cell wall due to the generation of ROS. Treatment with chromium (Cr), and aluminium (Al) significantly increased the accumulation of lipid peroxides in different plants (Karuppanapandian et al., 2006a,b,c, 2009; Karuppanapandian and Manoharan, 2008).

Factors involved in the generation of ROS in plant cells

In plants, ROS generation can be induced by various environmental factors, including heavy metals, pathogen attack

attack (which triggers the HR), herbicides, atmospheric pollutants, and drought (Dangl and Jones, 2001; Mittler, 2002; Vollenweider et al., 2003; Mittler et al., 2004; Karuppanapandian et al., 2006a,b,c, 2008, 2009, 2011; Gunes et al., 2008; Karuppanapandian and Manoharan, 2008; Xiao et al., 2008). The effects of these factors on ROS production are discussed below.

Heavy metals

ROS, such as $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$, are commonly generated under stress conditions and these represent intermediates that emerge during the successive reduction of O_2 to H_2O (Mittler, 2002; Karuppanapandian et al., 2006a,b,c, 2009, 2011; Karuppanapandian and Manoharan, 2008). One of the most deleterious effects induced by heavy metals exposure in plants is LP, which can directly cause biomembrane deterioration. Malondialdehyde (MDA), one of the decomposition products of PUFAs of membrane is regarded as a reliable indicator of oxidative stress (Karuppanapandian and Manoharan, 2008; Karuppanapandian et al., 2009, 2011). Plants exposed to certain heavy metal ions shift the balance of ROS metabolism towards the accumulation of H_2O_2 (Karuppanapandian et al., 2006a,b,c, 2009; Karuppanapandian and Manoharan, 2008). In the presence of redox active transition metals, such as Cu^{2+} and Fe^{2+}, H_2O_2 can be converted into the highly reactive $OH^{\cdot}$ via the metal-catalyzed Haber-Weiss/Fenton reaction. The oxidized metal ions undergo a re-reduction in a subsequent reaction with $O_2^{\cdot-}$ (Fig. 1). Alternatively, $OH^{\cdot}$ can be formed directly from H_2O_2 with tetrachlorohydroquinone (TCHQ) via the metal-independent Haber-Weiss reaction (Zhu et al., 2000). The $OH^{\cdot}$ molecule is one of the most reactive species because it is able to initiate radical chain reactions, which are thought to be responsible for the irreversible chemical modification of various cellular components in plants (Overmyer et al., 2003; Mittler et al., 2004). Another ROS that might be involved mainly in LP is the protonated form of $O_2^{\cdot-}$ to the $HO_2^{\cdot}$. High level of Hg^{2+} interfere the mitochondrial activity and induces oxidative stress by triggering the generation of ROS. In addition, Hg^{2+} ions inhibit antioxidative enzyme activity, especially the activity of glutathione reductase (GR), and also transiently deplete reduced glutathione (Overmyer et al., 2003; Mittler et al., 2004). As Hg^{2+} is not a transition metal, it cannot replace Cu^{2+} and Fe^{2+} in the Fenton reactions.

Pathogens/herbivores

The production and accumulation of ROS in plants as a defense response to pathogen attack are well documented (Dangl and Jones, 2001; Torres et al., 2002). However, the enzymatic origin of these inducible ROS is still under discussion. Various potential sources have been described in different plant species, including apoplastic amine, diamine, and polyamine oxidase-type enzymes, a cell wall-localized POX that directly forms H_2O_2, and a plasma membrane-localized NADPH oxidase. This latter enzyme represents the most widely studied mechanism for the synthesis of ROS (Sagi and Fluhr, 2001; Torres et al., 2002). The purpose of NADPH oxidase activity is likely to produce $O_2^{\cdot-}$, which is converted into the more stable ROS forms of H_2O_2 and O_2 spontaneously or by a SOD reaction. The origin of ROS in response to herbivory is not clear. However, insect feeding causes wounding and thus the production of ROS in the damaged tissue. As shown for soybean (*Glycine max*), herbivory by the insect Helicoverpa tea induced a shift in the oxidative status of the plant, causing an increase in $O_2^{\cdot-}$ and $OH^{\cdot}$ formation. In a more direct way, insect salivary gland-derived enzymes, such as H_2O_2-generating glucose oxidase, might contribute to the increase in ROS concentrations at the site of herbivore attack (Dangl and Jones, 2001).

Herbicides

Several classes of herbicides have been found to result in ROS generation, either by direct involvement in radical production or by inhibition of biosynthetic pathways (Hess, 2000; Garcia-Plazaola et al., 2002; Kim and Lee, 2005; Artetxe et al., 2006). These herbicides exacerbate the production of ROS, causing a rapid photo bleaching when treated plants are exposed to light (Hess, 2000; Artetxe et al., 2006). The herbicide bipyridinium generates ROS directly in light (Kim and Lee, 2005). Compounds such as paraquat (methyl viologen) induce light-dependent oxidative damage in plants. The di-cationic nature of these compounds facilitates their reduction to radical cations. The PSI-mediated reduction of the paraquat di-cation results in the formation of a mono-cation radical, which then reacts with O_2 to produce $O_2^{\cdot-}$. The $O_2^{\cdot-}$ then gives rise to other ROS, such as H_2O_2 and $OH^{\cdot}$ through a process that can be accelerated by traces of transition metals, such as Cu and Fe (Halliwell and Gutteridge, 2000; Kim and Lee, 2005). The diphenyl ethers, cyclic imides, and lutidine derivatives inhibit biosynthetic pathways in the plant, thereby resulting in the accumulation of reactive, radical-forming intermediates. The efficiency of these herbicides is based on their ability to induce the abnormal accumulation of photosensitizing tetrapyrroles, specifically protoporphyrin. It is somewhat anomalous that the reaction product, protoporphyrin IX, accumulates in conditions where the enzyme that catalyses its formation is expected to be inhibited. O_2 and light interact with protoporphyrin IX to produce $^1O_2$. This ROS initiates LP of PUFAs (Hess, 2000). Other compounds, such as acifluorfen methyl, which blocks photosynthetic electron transport, and norflurazon, which inhibits carotenoid (CAR) biosynthesis, initiate photo-oxidative processes most probably via the generation of $^1O_2$ (Artetxe et al., 2006). Herbicides that block photosynthesis cause increased excitation energy transfer from $^1O_2$ to O_2, while those that inhibit CAR biosynthesis eliminate important quenchers of $^3Chl^*$ and $^1O_2$ (Garcia-Plazaola et al., 2002).

Air pollutants

Air pollutants, such as O_3 and sulphur dioxide (SO_2), have been implicated in ROS formation and are considered to be one of the major factors contributing to modern forest decline (Langebartels et al., 2002). Li et al. (2009) suggested that the phytotoxicity of O_3 is due to its oxidizing potential and the consequent formation of radicals, which induce free radical chain reactions. The concentration of O_3 in the intercellular air spaces of leaves is close to zero. Thus, O_3 is unlikely to reach the chloroplast, but it nevertheless causes pigment degradation and LP. PSII-D1 synthesis and degradation was stimulated in spruce (*Picea excelsa*) trees following O_3-treatment, and the activity and quantity of Rubisco was found to decrease in poplar following exposure to O_3 (Vollenweider et al., 2003). Exposure to SO_2 results in tissue damage and release of ET from both photosynthetic and non-photosynthetic tissues. Fumigation with SO_2 causes a shift in cytoplasmic pH. The concentration of protons in the cytoplasm is a key factor in the regulation of cellular activity. When cells are exposed to SO_2, an appreciable acidification of the cytoplasm occurs because this gas reacts with H_2O to

form sulphurous acid, which may then be converted into sulphuric acid. The oxidation of sulphite to sulphate in the chloroplast, which is initiated by light and mediated by photosynthetic electron transport, gives rise to $O_2^{\cdot-}$. This ROS inhibits the activity of SH-containing, light-activated enzymes of the chloroplast, resulting in the loss of photosynthetic function (Vollenweider et al., 2003). O_3 exposed plants includes a bimodal oxidative damage that has some similarities to the pathogen-induced oxidative damage during plant immune response (Overmyer et al., 2003). In Arabidopsis, O_3 exposure leads to production of ROS in adjacent cells, and influence intercellular ROS signaling (Kwak et al., 2003).

Drought

The plant's response to drought stress is a complex phenomenon that appears to involve the synthesis of polyamines and a novel set of proteins whose function is largely unknown. Abscisic acid (ABA) plays a central role in the drought response, which minimizes water loss by stimulating stomatal guard cell closure (Pei et al., 2000). This process reduces the availability of CO_2 for photosynthesis, which can misdirect electrons in the photosystem and lead to the formation of ROS. Hence, mechanisms that reduce oxidative stress may play an important role in drought tolerance. Ascorbate peroxidase (APX), SOD and GR activity was elevated in drought-stressed cowpea and *Reaumuria soongorica* (Pall.) Maxim plants and it was proposed that, in addition to removing H_2O_2, this elevation might minimize $O_2^{\cdot-}$ formation by producing NADP, which accepts electrons from Fd (Contour-Ansel et al., 2006; Bai et al., 2009). In drought-tolerant *Hordeum* species, levels of GR and APX increased in response to drought, but SOD activity was not examined (Ramanjulu and Bartels, 2002). Drought-stressed cotton was found to be resistant to a subsequent challenge with paraquat, which may indicate the existence of a common protective mechanism against these stresses. In an earlier study, drought-tolerant and -intolerant maize inbred lines were analyzed and resistance was found to correlate with increased Cu/Zn-SOD and GR activities; interestingly, increased levels of one of these enzymes alone did not confer drought tolerance (Pan et al., 2006; Gunes et al., 2008; Moussa and Abdel-Aziz, 2008; Xiao et al., 2008). In many plants, H_2O_2 scavenging systems, as represented by APX and CAT, are more important in imparting tolerance against drought-induced oxidative stress than SOD (Chen et al., 2010; Hojati et al., 2010). The relative tolerance of a genotype to drought stress, as reflected by its comparatively lower LP, higher membrane stability index, and higher Chl and CARs content, was closely associated with its enzymatic antioxidative system (Ramanjulu and Bartels, 2002; Munne-Bosch and Alegre, 2004).

ROS-scavenging antioxidative systems in plants

ROS are generated by normal cellular metabolism, and it's production is controlled by various enzymatic and non-enzymatic antioxidative systems. Enzymatic antioxidants include CAT, APX, guaiacol peroxidase (GPX), SOD, and enzymes that detoxify LP products and non-enzymatic antioxidants include ascorbic acid (AA), glutathione (GSH), tocopherols (TOCs), CARs, and phenolic compounds (Table 2). In addition, an array of enzymes, such as MDHAR, dehydroascorbate reductase (DHAR), and GR, is needed for the regeneration of the active forms of the antioxidants (Apel and Hirt, 2004; Munne-Bosch and Alegre, 2004; Karuppanapandian et al., 2006a,b,c, 2009, 2011; Karuppan-apandian and Manoharan, 2008; Vellosillo et al., 2010).

Enzymatic antioxidative systems in plants

Catalase (EC 1.11.1.6)

CAT (H_2O_2 oxidoreductase) is a heme-containing enzyme that catalyses the dismutation of H_2O_2 into H_2O and O_2 (Table 2, Fig. 2). The enzyme occurs in all aerobic eukaryotes and its function is to remove the H_2O_2 generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration, purine catabolism and during oxidative stress (Mittler, 2002; Vellosillo et al., 2010). This is also due to the fact that there is proliferation of peroxisomes during stresses, which might help in the scavenging of H_2O_2 that diffuses from the cytosol (Lopez-Huertas et al., 2000). Various isoforms of CAT have been described in several plant species (Dat et al., 2001; Vandenabeele et al., 2004). The three isoforms present in Arabidopsis, i.e., cat-1, cat-2, and cat-3, have been cloned and were found to occur on separate chromosomes and to be differentially expressed and independently regulated (Frugoli et al., 1996). It is important to note that CAT is highly sensitive to light and has a rapid turnover rate, similar to that of the D1 protein of PSII. This may be a result of light absorption by the heme group or perhaps of H_2O_2 inactivation. Nonetheless, stress conditions that reduce the rate of protein turnover, such as salinity, drought, and heavy metals, reduce CAT activity (Karuppanapandian et al., 2006a,c; Karuppanapandian and Manoharan, 2008; Chen et al., 2010; Hojati et al., 2010). This reduction in CAT activity may limit the plant's tolerance to environmental stress. Various researchers has been investigated the role of CAT in pathogen defense by either overexpressing or suppressing CAT in transgenic plants (Dat et al., 2001; Vandenabeele et al., 2004). Increase in CAT activity is supposed to be an adaptive trait possibly helping to overcome the damage to tissue metabolism by reducing toxic levels of H_2O_2.

Ascorbate peroxidase (EC 1.11.1.11)

Whereas CAT reduces H_2O_2 levels in peroxisomes, APX performs this function in chloroplast and cytosol of plant cells. APX uses ascorbate as a hydrogen donor to break down H_2O_2 to form H_2O and monodehydroascorbate (MDHA, Table 2, Asada, 2000). APX has two cytosolic forms, which have defensive roles, and a membrane-bound form, in addition to its role in H_2O_2 scavenging, which modulates quantum efficiency and controls electron transport in conjunction with the ascorbate-glutathione (AsA-GSH) cycle (Foyer and Noctor, 2005). In chloroplasts, SOD and APX enzymes exist in both soluble and thylakoid-bound forms (Asada, 2000, 2006). $O_2^{\cdot-}$ generated at the membrane surface can thus be trapped and converted immediately to H_2O_2, which is scavenged by membrane-bound APX (Asada, 2000, 2006). Isolated intact chloroplasts rapidly metabolize exogenously applied H_2O_2, indicating that, *in situ*, chloroplasts may eliminate H_2O_2 that is generated both internally and externally (Smirnoff, 2000). The mRNA of cytosolic APX showed up-regulation during drought stress in the alfalfa nodule (Naya et al., 2007). Hossain et al. (2009) noted that during waterlogging, APX activity increased significantly in citrus plants. Transgenic Arabidopsis plants overexpressing cytosolic APXs exhibited increased tolerance to salt stress compared to wild-type plants (Lu et al., 2007).

Zhang et al. (2008) reported that transgenic tobacco overexpressing 9-*cis*-epoxycarotenoid dioxygenase (NCED) gene *SgNCED1* showed increased activity of APX and the transgenic plants improved growth under abiotic stress. Giacomelli et al. (2007) observed that *A. thaliana* deficient in two chloroplast APXs (stromal APX and thylakoid APX) showed accelerated light-induced necrosis when levels of cellular AA are low. Simultaneous over expressing of Cu/Zn-SOD and APX genes in chloroplasts of transgenic tall fescue plants showed tolerance to abiotic stresses (Lee et al., 2007).

Guaiacol peroxidase (EC 1.11.1.7)

GPX is a heme-containing protein, which are monomers of approximately 40 to 50 kDa, oxidize certain substrates at the expense of H_2O_2, and rid the cell of excess peroxide produced by metabolism under both normal and stress conditions (Table 2). GPX decomposes indole-3-acetic acid (IAA) and has a role in the biosynthesis of lignin and defence against biotic stresses by consuming H_2O_2 in the cytosol, vacuole, and cell wall as well as in extracellular space. GPX prefers aromatic electron donors such as guaiacol and pyragallol usually oxidizing ascorbate at the rate of around 1% that of guaiacol (Table 2, Asada, 2000; Jebara et al., 2005). Amongst the various antioxidants GPX can be considered that as one of the key ones, since both of its extra- and intra-cellular forms are participating in the breakdown of H_2O_2. GPXs are widely accepted as 'stress enzymes'. Induction in GPX activity has been reported in common bean (*Phaseolus vulgaris*) nodules under salinity stress conditions (Jebara et al., 2005). The GPX activity varies considerably depending upon plant species and stresses condition. It increased in Cd-exposed plants of *Ceratophyllum demersum* L. (Aravind and Prasad, 2003). An initial increase in GPX activity in both the leaf and root tissues of green gram (*Vigna radiata*) (Panda, 2001), cowpea (Cavalcanti et al., 2007), and rice (*Oryza sativa*) (Koji et al., 2009) has been reported under salinity stress. In some previous studies, reported the increased GPX activity under drought stress conditions in various plants, like liquorice (Pan et al., 2006), sun flower (Gunes et al., 2008), and polar (Xiao et al., 2008). Very recently, Haluskova et al. (2009) also noted that an enhanced activity of GPX was observed in metals (Cd, Cu, Ni, mercury (Hg), and lead (Pb)-treated barley root tips. Under sublethal salinity conditions, level of GPX activity has been used as potential biomarker to evaluate the intensity of stress. An enhancement in the activity of GPX, suggesting that this enzyme serves as an intrinsic defense tool to resist stress-induced oxidative damage in plants (Cavalcanti et al., 2007; Koji et al., 2009).

Superoxide dismutase (EC 1.15.1.1)

The SOD family of metalloenzymes catalyzes the disproportionation of $O_2^{\cdot-}$ into H_2O_2 and O_2 and is present in all aerobic organisms and subcellular components susceptible to oxidative stress (Table 2, del Rio et al., 1996; Halliwell and Gutteridge, 2000; Moussa and Abdel-Aziz, 2008; Chen et al., 2010). SODs are classified into three types based on their metal cofactor, two of which are similar, i.e., Fe-SOD (localized to chloroplasts) and Mn-SOD (localized to mitochondria), and one of which is structurally unrelated, i.e., Cu/Zn-SOD (localized to chloroplasts, peroxisomes, and cytosol) (del Rio et al., 1996). Although these isozymes differ in their sensitivity to H_2O_2 and potassium cyanide (KCN), all three are encoded by the nucleus, and SODs are sensitive to various abiotic stresses, presumably as a consequence of increased ROS production (Apel and Hirt, 2004; Jebara et al., 2005; Nagesh Babu and Devaraj, 2008). The effect of the metal stress on SOD transcription has been demonstrated a tri- and hexa-valent Cr-treatment in green gram, and black gram, resulted in a significant increase in MDA, membrane permeability, and the production of $O_2^{\cdot-}$ and H_2O_2 in leaves, and in the accumulation of $O_2^{\cdot-}$ due to reduced SOD activity (Karuppanapandian et al., 2006a; Karuppanapandian and Manoharan, 2008). SOD activity under various abiotic stress conditions, such as drought, salinity, extreme temperature, water-logging, and the presence of heavy metals, suggests that different mechanisms may be involved in various oxidative stress injuries (Jebara et al., 2005; Karuppanapandian et al., 2006a, b, c, 2009; Karuppanapandian and Manoharan, 2008; Moussa and Abdel-Aziz, 2008; Nagesh Babu and Devaraj, 2008). Oxygen activation may proceed through various mechanisms and does not necessarily produce a substrate for ROS, whereas changes in the electronic configuration of O_2 can lead to the formation of highly reactive $^1O_2$. A comparison of the effects of drought and water stress on wheat genotypes suggests that different mechanisms participate in ROS detoxification. For example, water stress did not affect SOD activity, whereas drought resulted in a significant increase in SOD activity (Moussa and Abdel-Aziz, 2008). ROS-detoxifying enzymes (e.g., CAT, APX, and SOD) have been shown to be inefficient in plants subjected to drought-induced oxidative stress (Chen et al., 2010).

Monodehydroascorbate reductase (EC 1.6.5.4)

Two enzymes are involved in the regeneration of reduced ascorbate, namely MDHAR, which uses NAD(P)H directly to recycle ascorbate, and DHAR (Table 2). However, MDHA is itself an efficient electron acceptor (Noctor and Foyer, 1998; Asada, 2000). MDHA is reduced directly to ascorbate using electrons derived from the photosynthetic ETC. Accompanying APX, MDHAR is also located in mitochondria and peroxisomes, where, it scavange H_2O_2 (del Rio et al., 2002; Mittler, 2002). Schutzendubel et al. (2007) have noted increased MDHAR activity in *Pinus sylvestris* and a declined MDHAR activity in poplar hybrids (*Populus* x *Canescens*) under Cd stress. Sharma and Dubey (2005) reported that the activities of enzymes involved in regeneration of ascorbate i.e., MDHAR, DHAR and GR were higher in drought stressed rice seedlings. It has also been reported that the increase in MDHAR activity contribute towards chilling tolerance in tomato (Stevens et al., 2008). Overexpression of MDHAR in transgenic tobacco increased the tolerance against salt and osmotic stresses (Eltayeb et al., 2007). In our laboratory, we reported that increased MDHAR activity was observed in one-week-old green gram and black gram seedlings upon treatment with tri- and hexa-valent Cr (Karuppanapandian et al., 2006a; Karuppanapandian and Manoharan, 2008). This increased MDHAR activity is thought to scavenge harmful ROS.

Dehydroascorbate reductase (EC 1.8.5.1)

AA is a major antioxidant in plants that detoxifies ROS and maintains photosynthetic function. DHAR catalyzes the regeneration of AA from its oxidized state and serves as an important regulator of AA recycling. The univalent oxidation of AA leads to the formation of MDHA, which is converted to the divalent oxidation product dehydroascorbate (DHA) via spontaneous disproportionation or further oxidation (Table 2). DHA is then reduced to AA by DHAR in a

reaction that requires GSH (Eltayeb et al., 2007). DHAR is expressed in rate-limiting amounts and contributes to the regulation of the symplastic and apoplastic AA pool size and redox state (Chen and Gallie, 2006). DHAR overexpression in tobacco and Arabidopsis had been shown to enhance tolerance to environmental stress (Chen and Gallie, 2006; Eltayeb et al., 2007).

Glutathione reductase (EC 1.6.4.2)

The ubiquitous tripeptide GSH, which occurs mostly as a low molecular weight thiol compound in almost all cells, acts as a disulphide reductant to protect the thiol groups of enzymes, regenerate ascorbate, and react with $^1O_2$ and $OH^{\cdot}$ (Table 2). GSH detoxifies herbicides by conjugation, either spontaneously or by the activity of a glutathione-*S*-transferase (GST), and regulates gene expression in response to environmental stress and pathogen attack (which induces the HR). GSH also participates in the regeneration of ascorbate from DHA via the enzyme DHAR (Table 2, Noctor and Foyer, 1998). GR catalyses the NADPH-dependent formation of a disulphide bond in glutathione disulphide (GSSG) and is thus important for maintaining the reduced pool of GSH. The role of GSH and GR in H_2O_2 scavenging has been well established in the Halliwell-Asada pathway (Noctor and Foyer, 1998; Asada, 2000). GR catalyses the rate-limiting last step of the Halliwell-Asada pathway. An increase in GR activity in plants results in the accumulation of GSH and ultimately confers stress tolerance in plants. Various studies have demonstrated that abiotic stress increases GR activity in pea (Hernández et al., 2001), cowpea (Contour-Ansel et al., 2006), French bean (*Phaseolus vulgaris*) (Nagesh Babu and Devaraj, 2008) and *Reaumuria soongorica* (Pall.) Maxin. (Bai et al., 2009), and, as shown by our laboratory, heavy metal stress increases GR activity in black gram and green gram (Karuppanapandian et al., 2006a,b,c, 2009; Karuppanapandian and Manoharan, 2008). Increase in GR activity has been reported in a tolerant cultivar of wheat (*Triticum aestivum* L.) (Sairam and Srivastava, 2002). Expression of GR is unregulated under stresses such as HL, mechanical wounding, high temperature, chilling and exposed to heavy metals and herbicides (Apel and Hirt, 2004; Karuppanapandian et al., 2011).

Non-enzymatic antioxidative systems in plants

Ascorbic acid

AA is one of the most powerful antioxidants and is present in most plant cell types, organelles, and the apoplast (Horemans et al., 2000; Smirnoff, 2000). Under physiological conditions, AA exists mostly in the reduced form (90% of the ascorbate pool) in chloroplasts (Smirnoff, 2000). The ability of AA to donate electrons in a wide range of enzymatic and non-enzymatic reactions makes AA the main ROS-detoxifying compound in the aqueous phase. AA can directly scavenge $O_2^{\cdot -}$, $OH^{\cdot}$, and $^1O_2$, and can reduce H_2O_2 to H_2O via the APX reaction (Table 2, Noctor and Foyer, 1998). In chloroplasts, AA acts as a cofactor of violoxanthin de-epoxidase, thereby dissipating excess excitation energy (Smirnoff, 2000). AA regenerates TOC from the tocoperoxyl radical ($TOC^{\cdot}$), which provides membrane protection (Horemans et al., 2000; Smirnoff, 2000). Thus, elevated levels of endogenous AA in plants are necessary to offset oxidative stress in addition to regulating other plant metabolic process (Smirnoff, 2000). Oxidation of AA occurs in two sequential steps. First, MDHA is produced and, if this compound is not rapidly re-reduced to ascorbate, it disproportionates into AA and DHA. The regeneration of AA within the chloroplast provides a putative mechanism for the regulation of electron transport. In addition to being a potent antioxidant, AA is also implicated in the pH-mediated modulation of PSII activity, and its down-regulation is associated with zeaxanthin formation. This is a potent mechanism for preventing photo-oxidation.

Glutathione

GSH is an abundant tripeptide (γ-glutamylcysteinglycine) in plant tissues, occurring in virtually all cellular components such as chloroplasts, mitochondria, ER, vacuoles, and cytosol and performing multiple functions (Noctor and Foyer, 1998). In combination with its oxidized form (GSSG), GSH maintain redox equilibrium in the cellular compartments. This latter property is of great biological importance because it allows for the fine-tuning of the cellular redox environment under normal conditions and, upon the onset of stress, provides the basis for GSH stress signaling (Wang et al., 2008). A central nucleophilic cysteine residue is responsible for the high reductive potential of GSH, which scavenges cytotoxic H_2O_2 and reacts non-enzymatically with other ROS, such as $^1O_2$, $O_2^{\cdot -}$, and $OH^{\cdot}$ (Noctor and Foyer, 1998; Wang et al., 2008). The central role of GSH in the antioxidative defense is due to its ability to regenerate another powerful water-soluble antioxidant, AA, via the AsA-GSH cycle (Noctor and Foyer, 1998; Halliwell, 2006). GSH is a precursor of phytochealatins (PCs), which plays important role in controlling cellular heavy metal concentration. The concentration of cellular GSH has a major effect on its antioxidant function and it varies considerably under abiotic stresses. Furthermore, strong evidence has indicated that an elevated GSH concentration is correlated with the ability of plants to withstand metal-induced oxidative stress. It has been found that enhanced antioxidant activity in the leaves and chloroplast of *Phragmites australis* Trin. (cav.) ex Steudel was associated with a large pool of GSH which resulted in protecting the activity of many photosynthetic enzymes against the thiophilic bursting of Cd (Pietrini et al., 2003). Increased concentration of GSH has been observed with the increasing concentration of Cd, Cr, and Al in *Pisum sativum* (Metwally et al., 2005), green gram (Karuppanapandian et al., 2006a,b), and black gram (Karuppanapandian and Manoharan, 2008). Recently, in our laboratory we reported an increase in GR activity and GSH content under 2,4-dichlorophenoxyacetic acid (2,4-D)-induced leaf senescence in mung bean leaves (Karuppanapandian et al., 2011). Xiang et al. (2001) observed that Arabidopsis with low levels of GSH were highly sensitive to even low levels of Cd^{2+} due to limited PC synthesis.

Tocopherols

TOCs are lipophilic antioxidants synthesized by all plants and also essential components of biological membranes (Kiffin et al., 2006). In higher plants, chloroplast membranes containing TOCs were also known to protect lipids and other membrane components by physically quenching and chemically reacting with O_2 in chloroplasts, thus protecting the PSII structure and function (Igamberdiev et al., 2004). α-TOC is a chain-breaking antioxidant, i.e., it is able to repair oxidizing radicals directly and thereby prevent the chain propagation step during lipid autooxidation. α-TOC reacts

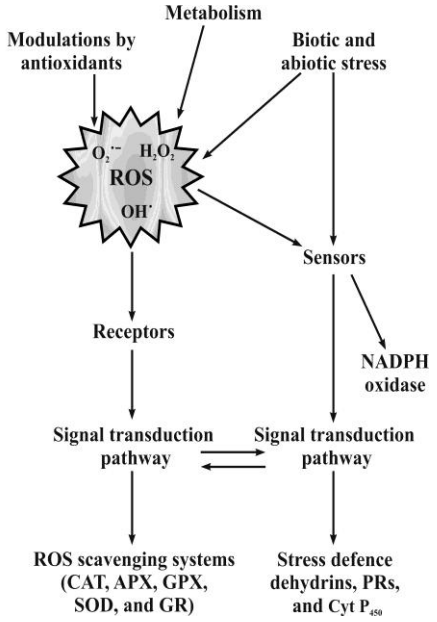

Fig 3. A hypothetical model extended reactive oxygen species (ROS) cycle in plants. The cycle operates in plant during biotic and abiotic stresses. ROS production in plant cells as a consequence of myriad stimuli ranging from biotic and abiotic stress, and modulation by antioxidants, as well as cell metabolism. ROS overproduction during stress can pose a threat to plant cells, and many stress conditions enhance the expression of ROS-scavenging enzymes. However, it is also thought that during stress ROS are actively produced by cells (e.g., by NADPH oxidase), and act as signals for the induction of defense pathways and eliciting specific cellular responses. The influence of these molecules on cellular processes is mediated by both the perpetuation of their production and their amelioration by scavenging enzymes such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), superoxide dismutase (SOD), and glutathione reductase (GR). Abbreviations: Cyt P_{450}: cytochrome P_{450}; PRs: pathogenesis-related proteins.

with $RO^{\cdot}$, $ROO^{\cdot}$, and RO^{*} derived from PUFA oxidation. The reaction between α-TOC and lipid radicals occurs in the membrane-water interface, where α-TOC donates hydrogen atom to lipid radicals, with the consequent formation of $TOH^{\cdot}$ that can be recycled back to the corresponding α-TOC by reacting with AA or other antioxidants (Igamberdiev et al., 2004). Regeneration of $TOH^{\cdot}$ back to its reduced form can be achieved by GSH and AA, in addition, α-TOC acts as chemical scavengers of ROS, especially of $^1O_2$, and as physical deactivators of $^1O_2$ by change transfer mechanism. α-TOC was found to modulate membrane fluidity in a similar manner to cholesterol and also to alter the permeability of digalactosyldiacyl glycerol (DGDG) vesicles to glucose and protons. Complexation of TOC with free fatty acids and lysophospholipids protects membrane structures against the deleterious effects of TOCs. Researchers reported that α-TOC levels increase in plant tissues in response to a variety of abiotic stresses (Ledford and Niyogi, 2005). Oxidative stress activates the expression of genes responsible for the synthesis of α-TOC in higher plants (Havaux et al., 2005). Antioxidants including α-TOC and AA have been reported to increase following triazole-treatment in tomato, and these may have a role in protecting membranes from oxidative damage, thus contributing to chilling tolerance (Havaux et al., 2005; Ledford and Niyogi, 2005).

Carotenoids

CARs are lipophilic organic compounds located in the plastids of both photosynthetic and non-photosynthetic plant tissues. CARs play a multitude of function in plant metabolism including the role in oxidative stress tolerance. They also referred to as antenna molecules as they absorb light in the region from 450-570 nm of the visible spectrum and pass the captured energy on to the Chl. In chloroplasts, CARs function as accessory pigments in light harvesting; however, perhaps a more important role is their ability to detoxify various forms of ROS. CARs can exist in a ground state or in one of two excited states after the absorption of light energy. In terms of their antioxidant properties, CARs can protect photosystems in one of four ways: (1) by reacting with LP products to terminate chain reactions, (2) by scavenging $^1O_2$ and dissipating the energy as heat, (3) by reacting with $^3Chl^*$ or excited chlorophyll (Chl^*) molecules to prevent the formation of $^1O_2$, or (4) by dissipating excess excitation energy through the xanthophyll cycle. The main protective role of β-carotene in photosynthetic tissue may be accomplished via direct quenching of $^3Chl^*$, which prevents $^1O_2$ generation and thereby inhibits oxidative damage (Collins, 2001). During quenching of $^3Chl^*$, energy is transferred from Chl to CAR, which subsequently dissipates the energy in a non-radiative form (i.e., heat). Thus, CARs act as a competitive inhibitor of $^1O_2$ formation and this is aided by their proximity to Chl in the light-harvesting complex. This method of protection is especially critical when light intensity increases above saturating levels (Collins, 2001). Another form of CAR, zeaxanthin, has been implicated in the dissipation of thermal energy, but the precise mechanism underlying this dissipation has not been resolved. Zeaxanthin appears to facilitate the conversion of $^3Chl^*$ to $^1Chl^*$ more efficiently than does β-carotene (Mortensen et al., 2001).

Phenolic compounds

Phenolics, including flavonoids, tannins, hydroxycinnamate esters, and lignin, are diverse secondary metabolites that are abundant in plant tissues (Jung et al., 2003). Polyphenols posses an ideal structural chemistry for free radical scavenging, and they have been shown to be more effective antioxidants *in vitro* than TOCs and AA. The antioxidative properties of phenolics arise from their high reactivity as electron donors and from the ability of the polyphenol-derived radical to stabilize and delocalize unpaired electrons (i.e., their chain-breaking function), and from their ability to chelate transition metal ions (by terminating the Fenton reaction) (Jung et al., 2003). The roots of many plants exposed to heavy metals exude high levels of phenolics (Winkel-Shirley, 2002). Another mechanism underlying the antioxidative properties of phenolics is the ability of the polyphenolic compounds, flavonoids, to alter peroxidation kinetics by modifying the lipid packing order to decrease the fluidity of the membranes (Arora et al., 2000). These changes could sterically hinder the diffusion of free radicals and restrict peroxidative reactions. Phenolic compounds acting as antioxidants may function as terminators of free radical chains and as cheaters of redox-active metal ions that are capable of catalyzing LP. Moreover, phenolic compounds can participate in the ROS scavenging cascade in plant cells (Winkel-Shirley, 2002; Jung et al., 2003). Arora et al. (2000) show that flavonoids are able to alter peroxidation kinetics by modifying the lipid packing order. They stabilize membranes by decreasing membrane fluidity and hinder the diffusion of

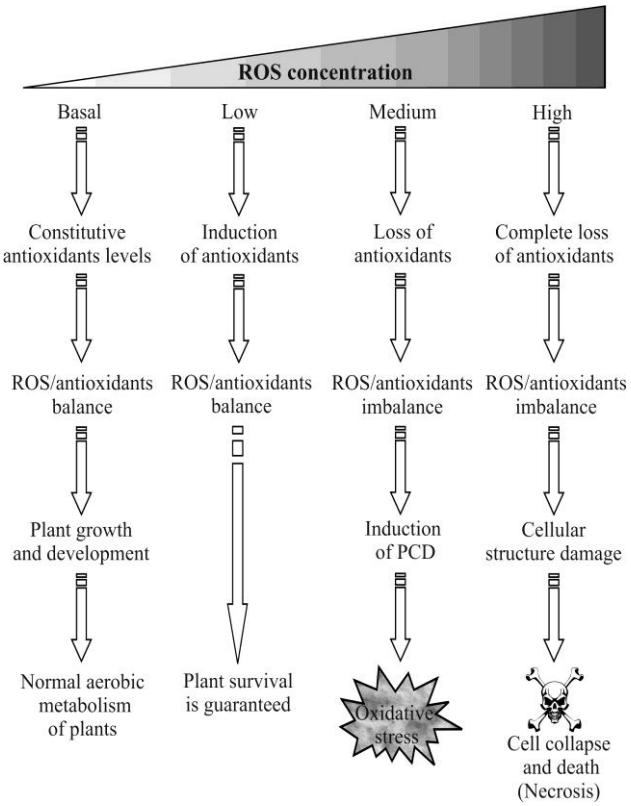

Fig 4. Schematic representation of the cellular concentration of reactive oxygen species (ROS) and their effects in plants. ROS are produced in plants and normally exist in the plant cell when the amount of ROS accumulation is maintained at normal levels by a series of antioxidative enzymes and molecules to maintain the redox equilibrium. Plant grow and develop under optimal conditions when the constitute level of antioxidative systems to control the basal production of ROS, which is the result of normal aerobic metabolism in plants. When plants exposed to various stresses in which plant survival is guaranteed by a proper induction of antioxidant defenses to control the low levels of ROS and maintain redox equilibrium in the plant cell. Oxidative stress occurs when this redox equilibrium is disturbed by excess levels of ROS or depletion of antioxidative defense systems or both, and finally this leads to cell collapse and death (necrosis) in plants. Abbreviations: PCD: programmed cell death.

ROS and restrict peroxidative reaction (Arora et al., 2000; Blokhina et al., 2003).

The role of ROS in plant cell death

Cell death is an essential process in the plant's life cycle. Two modes of cell death have been described in plants: PCD and necrosis. PCD and necrosis may be two extreme cases of the same process that is initiated by ROS (Figs. 3, 4, Manoharan et al., 2005; Gadjev et al., 2008; Karuppa-napandian et al., 2011). PCD is controlled genetically and has features that are characteristic of apoptotic cell death in plant cells, such as cell shrinkage, condensation of the cytoplasm, chromatin, and nucleus, and DNA fragmentation (Manoharan et al., 2005; Gadjev et al., 2008; Karuppanapandian et al., 2011). Necrosis results from severe and persistent trauma that is considered not to be genetically orchestrated (Gadjev et al., 2008). Moreover, PCD and necrosis can be halted by administering either high concentrations of antioxidants or inhibitors of both the translation and transcription of signal transduction components known to be involved in ROS generation, such as kinases and phytohormones. Accordingly, transgenic plants with low or high levels of several antioxidants (i.e., CAT, APX, GPX, SOD, and GR) also exhibit an altered response to O_3-driven PCD and 2,4-D-induced senescence, again demonstrating the importance of a tightly orchestrated redox balance (Apel and Hirt, 2004; Mittler et al., 2004; Manoharan et al., 2005; Karuppanapandian et al., 2011). Plant cell death is best studied during the HR, which is a typical incompatible plant-pathogen interaction (Dangl and Jones, 2001). During the HR, oxidative damage coincides with the induction of cell death. The source of oxidative damage is considered to be an NADPH oxidase complex. However, modulation of antioxidant enzyme activity probably contributes to ROS production during the HR. In tobacco cells that undergo HR upon infiltration with fungal elicitors, the CAT, *Cat1*, and *Cat2* mRNA and protein levels decrease, and CAT activity is suppressed, and this is paralleled by H_2O_2 accumulation (Overmyer et al., 2003). Similarly, virus-induced HR, like cell death, is accompanied by the suppression of cytosolic APX expression. This suppression probably contributes to the accumulation of H_2O_2 and the activation of the cell death program (Mittler et al., 2004). In addition to the presence of several PCD hallmarks, this HL-driven cell death could, like PCD, be blocked by infiltration with various antagonists of HR (Guan and Scandalios, 2000; Dangl and Jones, 2001). The existence of a dose-dependent ROS threshold below which PCD will be triggered and above which necrotic cell death will prevail might be the reason for the overwhelming effect that phytotoxic ROS levels have on PCD hallmarks. Alternatively, similarly to what is reported in animals, high doses of oxidants might inhibit components of the PCD pathway (Kazzaz et al., 1996).

The role of ROS in signal transduction

ROS must be utilized and/or interfere with other signaling pathways or molecules to affect plant growth and cell metabolism. There is evidence that plant hormones are positioned downstream of the ROS signal; for example, H_2O_2 induces the accumulation of plant stress hormones, such as JAs, SA, and ET (Fig. 5, Pei et al., 2000; Orozco-Cardenas et al., 2001). Plant hormones are not only located downstream of the ROS signal, but ROS themselves are also secondary messengers in many hormone signaling pathways (Pei et al., 2000; Orozco-Cardenas et al., 2001). Therefore, feed-back or feed-forward interactions may occur between ROS and hormones (Mittler et al., 2004). ROS can act as ubiquitous signal molecules in plants, which is a central component in stress responses. ROS have dual functions that depend upon their concentration; at low concentrations, ROS induce defense genes and adaptive responses, and at relatively high concentrations, ROS initiate cell death (Neill et al., 2002). The numerous sources of ROS and a complex system of oxidant scavengers provide the flexibility needed for their functioning. How these systems are regulated to achieve the temporal and spatial control of ROS production is still poorly understood. Sub-lethal concentrations of ROS acclimate plants to biotic and abiotic stress conditions and reduce plant growth, probably as part of an acclimatory mechanism (Neill et al., 2002; Torres et al., 2002 Karuppanapandian et al., 2006a, b,c, 2008, 2009, 2011; Karuppanapandian and

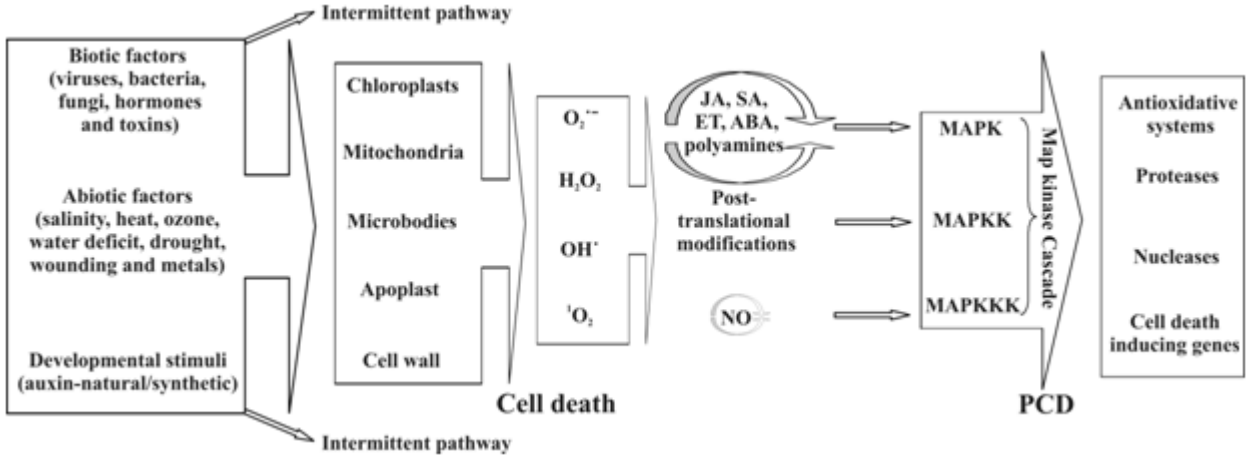

Fig 5. Schematic representation of reactive oxygen species (ROS)-dependent cell death pathways in plants. Confluence of discrete biotic and abiotic stress stimuli onto mitogen-activated protein kinase (MAPK) pathways via ROS, leading to awakening of antioxidant defense genes. Intermittent pathways have also been associates. Environmental and developmental changes stimulate ROS production through various enzymes and organelles. This initial increase in ROS may be further enhanced by various broadcast and production centers. Transient increases in ROS may initiate the signal transduction cascades that involve cross-talk with jasmonates (JAs), salicylic acid (SA), ethylene (ET), abscisic acid (ABA), polyamines, and nitric oxide (NO). This cross-talk can amplify the ensuing cascade through transducer sensors and targets of ROS-dependent and cell death-related gene expression. There is also cross-talk with the plant-pathogen signal transduction pathway, which might depend on pathogen recognition by the gene-for-gene mechanism and can result in an inverse effect on the regulation of ROS production and ROS scavenging mechanisms, as well as on the activation of programmed cell death (PCD). Besides MAPK-driven phosphorylation cascades, other regulatory post-translational modifications, such as protein oxidation and nitrosylation, might be involved in ROS-dependent cell death pathways. Abbreviations: $^1O_2$: singlet oxygen; H_2O_2: hydrogen peroxide; MAPKK: mitogen-activated protein kinase kinase; MAPKKK: mitogen-activated protein kinase kinase kinase; $O_2^{\cdot -}$: superoxide radical; $OH^{\cdot}$: hydroxyl radical.

Manoharan, 2008; Benderradji et al., 2011). Although the activity of many enzymes and a substantial genome response are known to be affected by ROS, the biochemical and molecular mechanisms of acclimation are still not understood. ROS communicate with other signal networks that control the downstream response of ROS. Recently, information on the role of ROS as signal molecules in growth and morphogenesis has emerged that suggests that ROS are not only stress signal molecules but also an intrinsic signal in plant growth and development. Other important transducers of the stress signal include MAPKs that act upstream of the oxidative burst during O_3-treatment and the HR (Fig. 5, Samuel and Ellis, 2002). However, MAPKs might also work in ROS-dependent cell death events. The primary ROS-activated tobacco MAPK is the SA-induced protein kinase, which is required during harpin-dependent PCD (Fig. 5, Samuel et al., 2005). A MAPKKK of alfalfa (*Medicago sativa*) activates cell death induced by H_2O_2 through a specific MAPK-scaffolding action (Nakagami et al., 2004). The overlapping features of animal and plant PCD have inspired various functional approaches, such as the introduction of animal genes into plants to gather mechanistic insight into oxidative stress-dependent cell death events. No obvious homologs of the BCL-like animal cell death suppressors have been identified in plant genomes to date; nevertheless, protection against mitochondrial- and chloroplast-derived ROS-dependent cell death is conferred by the overproduction of these suppressors in tobacco (Samuel et al., 2005; Kang et al., 2006). Although functional plant homologs protect against ROS-mediated PCD in the transgenic lines (Chen and Dickman, 2004). However, an evolutionarily conserved Arabidopsis BCL2-associated athano protein was shown to be induced by H_2O_2 and to be capable of provoking PCD in both yeast (*Saccharomyces cerevisiae*) and plants (Kang et al., 2006).

Conclusion and future perspectives

ROS are not only produced as toxic by-products but are also an important component of the plant defense response during stress conditions. Generally, ROS have been proposed to affect stress responses in two different ways: (1) ROS react with biological molecules and cause irreversible damage that can lead to cell death (Overmyer et al., 2003; Mittler et al., 2004; Karuppanapandian and Manoharan, 2008; Karuppanapandian et al., 2011); and (2) ROS influence the expression of several genes involved in various metabolic and signal transduction pathways (Overmyer et al., 2003; Mittler et al., 2004). Cells have evolved strategies to use ROS as environmental indicators and biological signals that activate and control various genetic stress response programs. Based on the literature, ROS may interact selectively with a target molecule that perceives an increase in ROS concentration and translates this information into signals that direct the plant responses to stress. ROS are ideally suited to act as signaling molecules because of their small size and ability to diffuse over short distances (Overmyer et al., 2003; Mittler et al., 2004). Since ROS act as signals that are triggered by various stress responses, their biological activities should exhibit a high degree of selectivity and specificity, so that the chemical identity and/or intracellular location of the stress signal are perceived. We have reviewed the latest body of literature relating to ROS generation, the role of ROS in signal transduction and cell death, and their scavenging mechanisms in plants. Given the intensive interest in this area of research, both regarding general aspects of ROS generation and the role of ROS during biotic and abiotic stress, we were unable to include all of the currently available information. Further characterization of the ROS genes identified as being involved in signal transduction and of their physiological

functions and response to environmental fluctuations will elucidate the detailed mechanisms underlying ROS activity in all aspects of plant growth and development.

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References

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Aravind P, Prasad MNV (2003) Zinc alleviates cadmium-induced oxidative stress in *Ceratophyllum demersum* L.: a free-floating freshwater macrophyte. Plant Physiol Biochem 41:391–397

Arora A, Byrem TM, Nair MG, Strasburg GM (2000) Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. Arch Biochem Biophys 373:102–109

Artetxe U, Hernández A, García-Plazaola JI, Becerril JM (2006) Do light acclimation mechanisms reduce the effects of light-dependent herbicides in duckweed (*Lemna minor*)? Weed Sci 54:230–236

Asada K (2000) The water-water cycle as alternative photon and electron sinks. Phil Trans R Soc Lond B Biol Sci 355:1419–1431

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Bai J, Gong C-M, Chen K, Kang H-M, Wang G (2009) Examination of antioxidative system's responses in the different phases of drought stress and during recovery in desert plant *Reaumuria soongorica* (Pall.) Maxim. J Plant Biol 52:417–425

Benderradji L, Brini F, Amar SB, Kellou K, Azaza J, Masmoudi K, Bouzerzour H, Hanin M (2011) Sodium transport in the seedlings of two bread wheat (*Triticum aestivum* L.) genotypes showing contrasting salt stress tolerance. Aust J Crop Sci 5:233–241

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 91:179–194

Cavalcanti FR, Lima JPMS, Ferreira-Silva SL, Viegas RS, Silveira JAG (2007) Roots and leaves display contrasting oxidative response during salt stress and recovery in cowpea. J Plant Physiol 164:591–600

Chalapathi Rao ASV, Reddy AR (2008) Glutathione reductase: a putative redox regulatory system in plant cells. In: Khan NA, Singh S, Umar S, Editors, Sulfur Assimilation and Abiotic Stresses in Plants, Springer, The Netherlands, pp 111–147

Chen Q, Zhang M, Shen S (2010) Effect of salt on malondialdehyde and antioxidant enzymes in seedling roots of Jerusalem artichoke (*Helianthus tuberosus* L.). Acta Physiol Plant 33:273–278

Chen S, Dickman MB (2004) Bcl-2 family members localize to tobacco chloroplasts and inhibit programmed cell death induced by chloroplast-targeted herbicides. J Exp Bot 55:2617–2623

Chen Z, Gallie DR (2006) Dehydroascorbate reductase affects leaf growth, development, and function. Plant Physiol 142:775–787

Collins A (2001) Carotenoids and genomic stability. Mutat Res 475:1–28

Contour-Ansel D, Torres-Franklin ML, Cruz H, de Carvalho M, D'arcy-Lameta A, Zuily-Fodil Y (2006) Glutathione reductase in leaves of cowpea: cloning of two cDNAs, expression and enzymatic activity under progressive drought stress, desiccation and abscisic acid treatment. Ann Bot 98:1279–1287

Corpas FJ, Barroso JB, del Río LA (2001) Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. Trends Plant Sci 6:145–150

Dangl, JL, Jones JDG (2001) Plant pathogens and integrated defence responses to infection. Nature 411:826–833

Dat JF, Pellinen R, Beeckman T, Van De Cotte B, Langebartels C, Kangasjarvi J, Inze D, Van Breusegem F (2003) Changes in hydrogen peroxide homeostasis trigger an active cell death process in tobacco. Plant J 33:621–632

del Rio LA, Corpas FJ, Sandalio LM, Palma JM, Gomez M, Barroso JB (2002) Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. J Exp Bot 53:1255–1272

del Rio LA, Palma JM, Sandalio LM, Corpas FJ, Pastori GM, Bueno P, Lopez-Huertas E (1996) Peroxisomes as a source of superoxide and hydrogen peroxide in stressed plants. Biochem Soc Trans 24:434–438

Dismukes GC, Klimov VV, Baranov SV, Kozlov YN, DasGupta J, Tyryshkin A (2001) The origin of atmospheric oxygen on Earth: The innovation of oxygenic photosynthesis. Proc Natl Acad Sci USA 98:2170–2175

Eltayeb AE, Kawano N, Badawi GH, Kaminaka H, Sanekata T, Shibahara T, Inanaga S, Tanaka K (2007) Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. Planta 225:1255–1264

Evans NH, McAinsh MR, Hetherington AM, Knight MR (2005) ROS perception in *Arabidopsis thaliana*: the ozone-induced calcium response. Plant J 41:615–626

Foyer CH, Noctor G (2005) Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ 29:1056–107

Foyer CH, Noctor G (2009) Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid Redox Signal 11:861–905

Frugoli JA, Zhong HH, Nuccio ML, McCourt P, McPeek MA, Thomas TL, McClung CR (1996) Catalase is encoded by a multigene family in *Arabidopsis thaliana* (L.) Heynh. Plant Physiol 112:327–336

Fryer MJ, Oxborough K, Mullineaux PM, Baker NR (2002) Imaging of photo-oxidative stress responses in leaves. J Exp Bot 53:1249–1254

Gadjev I, Stone JM, Gechev TS (2008) Programmed cell death in plants: new insights into redox regulation and the role of hydrogen peroxide. Int Rev Cell Mol Biol 270:87–144

Garcia-Plazaola JI, Hernandez A, Artetxe U, Becerril JM (2002) Regulation of the xanthophyll cycle pool size in duckweed (*Lemna minor*) plants. Physiol Plant 116:121–126

Giacomelli L, Masi A, Ripoll DR, Lee MJ, van Wijk KJ (2007) *Arabidopsis thaliana* deficient in two chloroplast ascorbate peroxidases shows accelerated light-induced

necrosis when levels of cellular ascorbate are low. Plant Mol Biol 65:627–644
Guan LM, Scandalios JG (2000) Hydrogen peroxide-mediated catalase gene expression in response to wounding. Free Rad Biol Med 28:1182–1190
Gunes A, Pilbeam D, Inal A, Coban S (2008) Influence of silicon on sunflower cultivars under drought stress, I: Growth, antioxidant mechanisms and lipid peroxidation. Commun Soil Sci Plant Anal 39:1885–1903
Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97:1634–1658
Halliwell B, Gutteridge JMC (2000) Free radicals in biology and medicine. Oxford University Press, Oxford, UK, Fourth Edition.
Haluskova L, Valentovicova K, Huttova J, Mistrik I, Tamas L (2009) Effect of abiotic stresses on glutathione peroxidase and glutathione *S*-transferase activity in barley root tips. Plant Physiol Biochem 47:1069–1074
Hao F, Wang X, Chen J (2006) Involvement of plasma-membrane NADPH oxidase in nickel-induced oxidative stress in roots of wheat seedlings. Plant Sci 170:151–158
Havaux M, Eymery F, Porfirova S, Rey P, Dörmann P (2005) Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana*. Plant Cell 17:3451–3469
Hernández JA, Ferrer MA, Jiménez A, Barceló AR, Sevilla F (2001) Antioxidant systems and $O_2^{\cdot-}/H_2O_2$ production in the apoplast of pea leaves. Its relation with salt-induced necrotic lesions in minor veins. Plant Physiol 127:817–831
Hess DF (2000) Light-dependent herbicides: an overview. Weed Sci 48:160–170
Higuchi T (2006) Look back over the studies of lignin biochemistry. J Wood Sci 52:2–8
Hojati M, Modarres-Sanavy SAM, Karimi M, Ghanati F (2010) Responses of growth and antioxidant systems in *Carthamus tinctorius* L. under water deficit stress. Acta Physiol Plant 33:105–112
Horemans N, Foyer CH, Potters G, Asard H (2000) Ascorbate function and associated transport systems in plants. Plant Physiol Biochem 38:531−540
Hossain Z, López-Climent MF, Arbona V, Pérez-Clemente RM, Gómez-Cadenas A (2009) Modulation of the antioxidant system in citrus under waterlogging and subsequent drainage. J Plant Physiol 166:1391–1404
Igamberdiev AU, Serege´lyes C, Manac'h N, Hill RD (2004) NADH-dependent metabolism of nitric oxide in alfalfa root cultures expressing barley haemoglobin. Planta 219:95–102
Jebara S, Jebara M, Limam F, Aouani ME (2005) Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. J Plant Physiol 162:929–936
Jezek P, Hlavata L (2005) Mitochondria is homeostasis of reactive oxygen species in cell, tissues, and organism. Int J Biochem Cell Biol 37:2478–2503
Jiang M, Zhang J (2001) Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol 42:1265–1273
Kang CH, Jung WY, Kang YH, Kim JY, Kim DG, Jeong JC, Baek DW, Jin JB, Lee JY, Kim MO, Chung WS, Mengiste T, Koiwa H, Kwak SS, Bahk JD, Lee SY, Nam JS, Yun DJ, Cho MJ (2006) AtBAG6, a novel calmodulin-binding protein, induces programmed cell death in yeast and plants. Cell Death Differ 13:84–95
Karuppanapandian T, Manoharan K (2008) Uptake and translocation of tri- and hexa-valent chromium and their effects on black gram (*Vigna mungo* L. Hepper cv. Co4) roots. J Plant Biol 51:192–201
Karuppanapandian T, Saranyadevi AR, Jeyalakshmi K, Manoharan K (2008) Mechanism, control and regulation of leaf senescence in plants. J Plant Biol 35:141–155
Karuppanapandian T, Sinha PB, Kamarul Haniya A, Manoharan K (2006a) Differential antioxidative responses of ascorbate-glutathione cycle enzymes and metabolites to chromium stress in green gram (*Vigna radiata* L. Wilczek) leaves. J Plant Biol 49:440–447
Karuppanapandian T, Sinha PB, Kamarul Haniya A, Manoharan K (2009) Chromium-induced accumulation of peroxide content, stimulation of antioxidative enzymes and lipid peroxidation in green gram (*Vigna radiata* L. cv. Wilczek) leaves. Afr J Biotechnol 8:475–479
Karuppanapandian T, Sinha PB, Kamarul Haniya A, Premkumar G, Manoharan K (2006b) Aluminium-induced changes in antioxidative enzyme activities, hydrogen peroxide content and cell wall peroxidase activity in green gram (*Vigna radiata* L. cv. Wilczek) roots. J Plant Biol 33:241–246
Karuppanapandian T, Sinha PB, Premkumar G, Manoharan K (2006c) Chromium toxicity: Correlated with increased in degradation of photosynthetic pigments and total soluble protein and increased peroxidase activity in green gram (*Vigna radiata* L.) seedlings. J Swamy Bot-Cl 23:117–122
Karuppanapandian T, Wang HW, Prabakaran N, Jeyalakshmi K, Kwon M, Manoharan K, Kim W (2011) 2,4-dichlorophenoxyacetic acid-induced leaf senescence in mung bean (*Vigna radiata* L. Wilczek) and senescence inhibition by co-treatment with silver nanoparticles. Plant Physiol Biochem 49:168–177
Kazzaz JA, Xu J, Palaia TA, Mantell L, Fein AM, Horowitz S (1996) Cellular oxygen toxicity: oxidant injury without apoptosis. J Biol Chem 271:15182–15186
Kiffin R, Bandyopadhyay U, Cuervo AM (2006) Oxidative stress and autophagy. Antioxid Redox Signal 8:152–162
Kim JH, Lee CH (2005) *In vivo* deleterious effects specific to reactive oxygen species on photosystem I and II after photo-oxidative treatments of rice (*Oryza sativa* L.) leaves. Plant Sci 168:1115–1125
Kim MJ, Ciani S, Schachtman DP (2010) A peroxidase contributes to ROS production during Arabidopsis root response to potassium deficiency. Mol Plant 3:420–427
Koji Y, Shiro M, Michio K, Mitsutaka T, Hiroshi M (2009) Antioxidant capacity and damages caused by salinity stress in apical and basal regions of rice leaf. Plant Prod Sci 12:319–326
Kwak JM, Mori IC, Pei Z-M, Leonhardt N, Torres MA, Dangl JL, Bloom RE, Bodde S, Jones JDG, Schroeder JI (2003) NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in *Arabidopsis*. EMBO J 22:2623–2633
Laloi C, Apel K, Danon A (2004) Reactive oxygen signalling: the latest news. Curr Opin Plant Biol 7:323–328
Langebartels C, Wohlgemuth H, Kschieschan S, Grün S, Sandermann H (2002) Oxidative burst and cell death in ozone-exposed plants. Plant Physiol Biochem 40:567–575
Ledford HK, Niyogi KK (2005) Singlet oxygen and photo-oxidative stress management in plants and algae. Plant Cell Environ 28:1037–1045
Lee SH, Ahsan N, Lee KW, Kim DH, Lee DG, Kwak SS, Kwon SY, Kim TH, Lee BH (2007) Simultaneous overexpression of both CuZn superoxide dismutase and ascorbate peroxidase in transgenic tall fescue plants confers increased tolerance to a wide range of abiotic stresses. J Plant Physiol 164:1626–1638

Li XM, He XY, Zhang LH, Chen W, Chen Q (2009) Influence of elevated CO_2 and O_3 on IAA, IAA oxidase and peroxidase in the leaves of ginkgo trees. Biol Plant 53:339–342

Liszkay A, van der Zalm E, Schopfer P (2004) Production of reactive oxygen intermediates ($O_2^{\cdot-}$, H_2O_2, and $OH^{\cdot}$) by maize roots and their role in wall loosening and elongation growth. Plant Physiol 136:3114–3123

Lopez-Huertas E, Charlton WL, Johnson B, Graham IA, Baker A (2000) Stress induces peroxisome biogenesis genes. EMBO J 19:6770–6777

Lopez-Huertas E, Corpas FJ, Sandalio LM, del Río LA (1999) Characterization of membrane polypeptides from pea leaf peroxisomes involved in superoxide radical generation. Biochem J 337:531–536

Lu Z, Liu D, Liu S (2007) Two rice cytosolic ascorbate peroxidases differentially improve salt tolerance in transgenic *Arabidopsis*. Plant Cell Rep 26:1909–1917

Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi Y (2010) Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Aust J Crop Sci 4:580–585

Manoharan K, Karuppanapandian T, Sinha PB, Prasad R (2005) Membrane degradation, accumulation of phosphatidic acid, stimulation of catalase activity and nuclear DNA fragmentation during 2,4-D-induced leaf senescence in mustard. J Plant Biol 48:394–403

Metwally A, Safronova VI, Belimov AA, Dietz KJ (2005) Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J Exp Bot 56:167–178

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. Trends Plant Sci 9:490–498

Mittova V, Guy M, Tal M, Volokita M (2004) Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. J Exp Bot 55:1105–1113

Moller IM (2001) Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol 52:561–591

Moller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. Annu Rev Plant Biol 58:459–481

Mortensen A, Skibsted LH, Truscott TG (2001) The interaction of dietary carotenoids with radical species. Arch Biochem Biophys 385:13–19

Moussa HR, Abdel-Aziz SM (2008) Comparative response of drought tolerant and drought sensitive maize genotypes to water stress. Aust J Crop Sci 1:31–36

Munne-Bosch S, Alegre L (2004) Die and let live: leaf senescence contributes to plant survival under drought stress. Funct Plant Biol 31:203–216

Nagesh Babu R, Devaraj VR (2008) High temperature and salt stress response in French bean (*Phaseolus vulgaris*). Aust J Crop Sci 2:40–48

Nakagami H, Kiegerl S, Hirt H (2004) OMTK1, a novel MAPKKK, channels oxidative stress signaling through direct MAPK interaction. J Biol Chem 279:26959–26966

Navrot N, Roubier N, Gelbaye E, Jacquot J-P (2007) Reactive oxygen species generation and antioxidant systems in plant mitochondria. Physiol Plant 129:185–195

Naya L, Ladrera R, Ramos J, González EM, Arrese-Igor C, Minchin FR, Becana M (2007) The response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to the subsequent recovery of plants. Plant Physiol 144:1104–1114

Neill S, Desikan R, Hancock J (2002) Hydrogen peroxide signalling. Curr Opin Plant Biol 5:388–395

Noctor G, Foyer CH (1998) Ascorbate and glutathione: keeping active oxygen under control. Annu Rev Plant Physiol Plant Mol Biol 49:249–279

Noctor G, Veljovic-Jovanovic S, Driscoll S, Novitskaya L, Foyer CH (2002) Drought and oxidative load in the leaves of C_3 plants: a predominant role for photorespiration? Ann Bot 89:841–850

Orozco-Cardenas ML, Narvaez-Vasquez J, Ryan CA (2001) Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. Plant Cell 13:179–191

Overmyer K, Brosche M, Kangasjarvi J (2003) Reactive oxygen species and hormonal control of cell death. Trends Plant Sci 8:325–342

Pan Y, Wu LJ, Yu ZL (2006) Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycorhiza uralensis* Fisch). J Plant Growth Regul 49:157–165

Panda SK (2001) Response of green gram seeds under salinity stress. Indian J Plant Physiol 6:438–440

Pei Z-M, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ, Grill E, Schroeder JI (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature 406:731–734

Pietrini F, Iannelli MA, Pasqualini S, Massacci A (2003) Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav.) Trin. Ex Steudel. Plant Physiol 133:829–837

Quartacci MF, Cosi E, Navari-Izzo F (2001) Lipids and NADPH-dependent superoxide production in plasma membrane vesicles from roots of wheat grown under copper deficiency or excess. J Exp Bot 52:77–84

Ramanjulu S, Bartels D (2002) Drought-and desiccation-induced modulation of gene expression in plants. Plant Cell Environ 24:141–151

Robson CA, Vanlerberghe GC (2002) Transgenic plant cells lacking mitochondrial alternative oxidase have increased susceptibility to mitochondria-dependent and -independent pathways of programmed cell death. Plant Physiol 129:1908–1920

Rodríguez AA, Córdoba AR, Ortega L, Taleisnik E (2004) Decreased reactive oxygen species concentration in the elongation zone contributes to the reduction in maize leaf growth under salinity. J Exp Bot 55:1383–1390

Rodríguez-Serrano M, Romero-Puertas MC, Sparkes I, Hawes C, del Rio LA, Sandalio LM (2009) Peroxisome dynamics in Arabidopsis plants under oxidative stress induced by cadmium. Free Radic Biol Med 47:1632–1639

Sagi M, Fluhr R (2001) Superoxide production by plant homologues of the $gp91^{phox}$ NADPH oxidase: modulation of activity by calcium and by tobacco mosaic virus infection. Plant Physiol 126:1281–1290

Sairam RK, Srivastava GC (2002) Changes in antioxidant activity in sub-cellular fraction of tolerant and susceptible wheat genotypes in response to long term salt stress. Plant Sci 162:897–904

Samuel MA, Ellis BE (2002) Double jeopardy: both overexpression and suppression of a redox-activated plant mitogen-activated protein kinase render tobacco plants ozone sensitive. Plant Cell 14:2059–2069

Samuel MA, Hall H, Krzymowska M, Drzewiecka K, Hennig J, Ellis BE (2005) SIPK signaling controls multiple components of harpin-induced cell death in tobacco. Plant J 42:406–416

Scandalios JG (2005) Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Braz J Med Biol Res 38:995–1014

Schopfer P (2001) Hydroxyl radical-induced cell-wall loosening *in vitro* and *in vivo*: implications for the control of elongation growth. Plant J 28:679–688

Schutzendübel A, Schwanz P, Teichmann T, Gross K, Langenfeld-Heyser R, Godbold DL, Polle A (2007) Cadmium-induced changes in antioxidative systems, H_2O_2 content and differentiation in pine (*Pinus sylvestris*) roots. Plant Physiol 127:887–898

Sharma P, Dubey RS (2005) Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. J Plant Physiol 162:854–864

Shen W, Nada K, Tachibana S (2000) Involvement of polyamines in the chilling tolerance of cucumber cultivars. Plant Physiol 124:431–439

Shi R, Sun Y-H, Li Q, Heber S, Sederoff R, Chiang VL (2010) Towards a systems approach for lignin biosynthesis in *Populus trichocarpa*: transcript abundance and specificity of the monolignol biosynthetic genes. Plant Cell Physiol 51:144–163

Smirnoff N (2000) Ascorbic acid: metabolism and functions of a multi-facetted molecule. Curr Opin Plant Biol 3:229–235

Spiteller G (2003) The relationship between changes in the cell wall, lipid peroxidation, proliferation, senescence and cell death. Physiol Plant 119:5–18

Stevens R, Page D, Gouble B, Garchery C, Zamir D, Causse M (2008) Tomato fruit ascorbic acid content is linked with monodehydroascorbate reductase activity and tolerance to chilling stress. Plant Cell Environ 31:1086–1096

Sweetlove LJ, Lytovchenko A, Morgan M, Nunes-Nesi A, Taylor NL, Baxter CJ, Eickmeier I, Fernie AR (2006) Mitochondrial uncoupling protein is required for efficient photosynthesis. Proc Natl Acad Sci USA 103:19587–19592

Torres MA, Dangl JL, Jones JDG (2002) Arabidopsis $gp91^{phox}$ homologues *AtrbohD* and *AtrbohF* are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci USA 99:517–522

Vandenabeele S, Vanderauwera S, Vuylsteke M, Rombauts S, Langebartels C, Seidilitz HK, Zabeau M, Van Montagu M, Inze D, Van Breusegem F (2004) Catalase deficiency drastically affects gene expression induced by high light in *Arabiodpsis thaliana*. Plant J 39:45–58

Vellosillo T, Vicente J, Kulasekaran S, Hamberg M, Castresana C (2010) Emerging complexity in reactive oxygen species production and signaling during the response of plants to pathogens. Plant Physiol 154:444–448

Vollenweider P, Ottiger M, Gunthardt-George MS (2003) Validation of leaf ozone symptoms in natural vegetation using microscopial methods. Environ Pollut 124:101–118

Vranova E, Inze D, Van Breusegem F (2002) Signal transduction during oxidative stress. J Exp Bot 53:1227–1236

Wang JP, Li YL, Zhang JG (2008) Effect of high-temperature and excessive-light stress on APX activity in apple peel. Acta Agri Boreali-Sin 23:144–147

Watanabe N, Lam E (2006) *Arabidopsis* Bax inhibitor-1 functions as an attenuator of biotic and abiotic type of cell death. Plant J 45:884–894

Winkel-Shirley B (2002) Biosynthesis of flavonoids and effects of stress. Curr Opin Plant Biol 5:218–223

Xiang C, Werner BL, Christensen EM, Oliver DJ (2001) The biological function of glutathione revisited in *Arabidopsis* transgenic plants with altered glutathione levels. Plant Physiol 126:564–574

Xiao X, Xu X, Yang F (2008) Adaptive responses to progressive drought stress in two *Populus cathayana* populations. Silva Fenn 42:705–719

Zhang Y, Yang J, Lu S, Cai J, Guo Z (2008) Overexpressing *SgNCED1* in tobacco increases ABA level, antioxidant enzyme activities, and stress tolerance. J Plant Growth Regul 27:151–158

Zhu BZ, Kitrossky N, Chevion M (2000) Evidence for production of hydroxyl radicals by pentachlorophenol metabolites and hydrogen peroxide: a metal-independent organic Fenton reaction. Biochem Biophys Res Commun 270:942–946

Zhu J-K (2001) Plant salt tolerance. Trends Plant Sci 6:66–71

Zolla L, Rinalducci S (2002) Involvement of active oxygen species in degradation of light-harvesting proteins under light stresses. Biochemistry 41:14391–14402