Production Sites of Reactive Oxygen Species (ROS) in Organelles from Plant Cells

Francisco J. Corpas, Dharmendra K. Gupta, and José M. Palma

Contents

Abstract Reactive oxygen species (ROS) have been considered for a long time as undesirable by-product of the cellular metabolism, but recently the role of ROS in molecular signaling processes has been reported. Consequently, the cell must keep a fragile equilibrium between ROS production and the antioxidant defenses that protect cells in vivo against potential damages (oxidative stress) and, alternatively, allow the inter- and intra-cell communications. This equilibrium may become disturbed under different array of adverse conditions by an excessive generation of ROS or by an impaired antioxidant defenses. Plant cells have a compartmentalization of ROS production in the different organelles including chloroplasts,

F.J. Corpas (✉) • J.M. Palma (✉)
Group of Antioxidants, Free Radicals and Nitric Oxide in Biotechnology, Food and Agriculture, Department of Biochemistry, Cell and Molecular Biology of Plants, Estación Experimental del Zaidín, CSIC, Apartado 419, E-18008 Granada, Spain
e-mail: javier.corpas@eez.csic.es; josemanuel.palma@eez.csic.es

D.K. Gupta
Institut für Radioökologie und Strahlenschutz (IRS), Gottfried Wilhelm Leibniz Universität Hannover, Herrenhäuser Str. 2, Gebaüde 4113, 30419 Hannover, Germany

© Springer International Publishing Switzerland 2015

D.K. Gupta et al. (eds.), *Reactive Oxygen Species and Oxidative Damage in Plants Under Stress*, DOI 10.1007/978-3-319-20421-5_1

mitochondria, or peroxisomes, and they also have a complex battery of antioxidant enzymes usually close to the site of ROS production. Cell compartmentalization has been demonstrated to be an additional mechanism of cellular ROS modulation for signaling purposes. This chapter will provide a general overview of the main system of ROS production/regulation in plant cells.

Keywords Reactive oxygen species • Chloroplasts • Mitochondria • Peroxisomes

1 Introduction

Reactive oxygen species (ROS) is a term which includes radical and non-radical oxygen species formed by the partial reduction of oxygen. The main ROS mostly investigated are superoxide radical ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) as radicals molecules, and hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone (O_3), and hypochlorous acid (HClO) as non-radical. Under normal conditions, these molecules are produced in many metabolic pathways as normal by-product, being the respective electron transport chains present in chloroplasts and mitochondria the main sources of these ROS (Halliwell 2006; del Río 2015). However, the presence of free metals, such as iron, copper, and manganese, released from metalloprotein complexes can also contribute to ROS production. Plant cells enclose a wide range of enzymatic and nonenzymatic antioxidant systems which usually are nearby the ROS production site being an excellent mechanism to avoid the undesirable potential negative effects of ROS (oxidative stress) but also to modulate their signaling role.

In parallel, plant cells contain a series of ROS-scavenging nonenzymatic antioxidants such as ascorbic acid, glutathione (GSH), carotenoids, and others, as well as a wide battery of enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), peroxiredoxin (Prx), and the ascorbate–glutathione cycle. All these latter elements have multiple isozymes located in all cell compartments which provide a highly efficient system for detoxifying ROS. The main goal of this chapter is to offer a general overview of the main system of ROS production/scavenging in the principal plant organelles.

2 Chloroplasts

Due to their abundance and diversity of pigments, chloroplasts are the cell organelles more susceptible to be attacked by ROS. These photosynthetic compartments are also great sources of ROS production, including basically $O_2^{\bullet -}$ and singlet oxygen ($^1O_2$). Chloroplasts harbor in thylakoids the key elements to fully carry out

the photosynthesis, with the structures involved in the light-dependent phase being mainly responsible for the ROS generation (Tripathy and Oelmüller 2012). Complementarily, these organelles contain powerful antioxidant systems to counterbalance the ROS production under normal conditions.

2.1 Production of Reactive Oxygen Species

The major site of superoxide radical's production is linked to the photosystem I (PSI). Under illumination conditions, O_2 is continuously provided by the water autolysis performed in the PSII as indicated in reaction [1], so light would favor the superoxide radical formation reaction [2] at the PSI location. There, under excessive reduced ferredoxin and low NADP availability, the autoxidation of this iron–sulfur protein occurs with the formation of $O_2^{\bullet -}$, as depicted in reaction [3].

Reaction [1] $2H_2O \rightarrow 4e^- + O_2 + 4H^+$
Reaction [2] $2O_2 + 2e^- \rightarrow 2O_2^{\bullet -}$
Reaction [3] $Fdred + O_2 \rightarrow Fdox + O_2^{\bullet -}$

If the conditions persist, the reduced ferredoxin is able to react with superoxide radicals to form hydrogen peroxide, and this is what Mehler (1951) found when he performed his experiments with illuminated chloroplasts (reaction 4).

Reaction [4] $Fdred + O_2^{\bullet -} + 2H^+ \rightarrow Fdox + H_2O_2$

Asada and colleagues (1974) corroborated later that all the H_2O_2 formation attributable to chloroplasts was a consequence of the disproportionation of superoxide radicals previously formed. It has been found that the H_2O_2 photo produced via $O_2^{\bullet -}$ accumulates in thylakoids, whereas in intact chloroplasts this ROS does not accumulate (Asada 2006). The steady-state level of H_2O_2 in chloroplasts was determined to be about 0.5 μM, with increases under stress conditions up to 1–15 μM.

The direct production of $O_2^{\bullet -}$ to a lower extent at the level of the PSI was also reported, and it was postulated that, when the NADP availability lowers and the Calvin–Benson cycle does not operate properly, the ferredoxin autoxidation takes place initially and afterwards the direct formation of superoxide radicals from the PSI (Halliwell and Gutteridge 2007). Simultaneously, another source of superoxide radicals is also associated to PSII, for instance, through the autoxidation of PSII electronic acceptors and mostly at the level of the plastoquinone (Gupta and Igamberdiev 2015). The superoxide radical's production in chloroplasts is promoted above the normal conditions under certain circumstances, basically stress situations which proceed with stomata closure. Then, the CO_2 availability decreases and the photosynthetic carbon reductive pathway (Calvin–Benson cycle) is somehow impaired, with the concomitant lower provision of NADP for the thylakoid-linked ferredoxin-NADP reductase. Accordingly, reduced ferredoxin accumulates

and develops the scenario described above. Overall, the rate of $O_2^{\bullet -}$ production in isolated chloroplasts was initially reported to be about 30 μmol mg^{-1} Chl h^{-1} (Asada 1992). Later, it was probed to that the superoxide radical's generation increased from 240 to 720 μM s^{-1} under stress conditions (Polle 2001).

Singlet oxygen is produced at the PSII (P680) by excitation of oxygen of the ground (triplet) state $^3O_2$ till singlet state ($^1O_2$), as indicated in reaction [5].

Reaction [5] $^3O_2 + {}^3P680^*$ (excited P680) $\rightarrow {}^1O_2 + {}^3P680$

Under intense illumination conditions and/or low CO_2 assimilation rate undergone due to environmental stresses or certain physiological conditions, electrons from chlorophyll are excited to a higher energy layer, and this energy excess is transferred to oxygen, thus generating singlet oxygen responsible for photodynamic damages such as bleaching of leaves (Telfer et al. 1994; Hideg et al. 1998; Asada 2006). Additionally, it has been also found that biosynthetic and catabolic intermediates of chlorophyll are photosensitizers which generate singlet oxygen (Wagner et al. 2004; Pruzinska et al. 2005). Although $^1O_2$ is rapidly quenched by water, its lifetime and diffusion distance from the generation site are very short. So, the distance among the generation and the target sites of $^1O_2$ is a critical factor to evaluate the biological effect of this ROS (Asada 2006).

Many herbicides, including methyl viologen (paraquat), diquat, DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], atrazine, and others base their mechanism of action by promoting the generation of ROS. Thus, cationic herbicides such as methyl viologen trigger the formation of superoxide radicals at the level of PSI; other polar compounds like DCMU uncouple the electron fluxes at the PSII level with excitation of chlorophyll and the energy excess of excited chlorophyll being transferred toward the formation of $^1O_2$. It has been demonstrated that many plants (tobacco, tomato, potato, and alfalfa, among others) transfected with additional *SOD* genes showed reduced damage symptoms after being subjected to diverse herbicides.

2.2 ROS Scavenging Systems

Chloroplasts contain a battery of scavengers that not only protect chloroplasts from the direct effects of ROS but also relax the electron excess stress. Thus, a series of antioxidant enzymes and small molecules regulate the endogenous ROS levels, thus allowing a coordinated response under stress conditions (Foyer et al. 1991, 1994; Gill and Tuteia 2010). Chloroplastic membranes are rich of carotenoids (provitamin A) and α-tocopherol (vitamin E), two powerful $^1O_2$ scavengers, so this ROS with high ability to diffuse in hydrophobic environments can be promptly removed by these antioxidants, although ascorbate can also be an active scavenger of this species.

Carotenoids, mainly β-carotene, besides working as complementary light-absorbing pigments, can dissipate the photodynamic effect directly and indirectly. Hence, the energy excess accumulated in the triplet state of chlorophyll as consequence of intense illumination can be transferred to carotenoids which move up to their triplet state. These excited carotenoids go back to their ground state by dissipating their excess energy as heat. On the other hand carotenoids are able to counterbalance the production of $^1O_2$ promoted by the triplet-state chlorophyll. Again, excited carotenoids, as consequence of their interaction with $^1O_2$, dissipate their higher energy as heat rendering the ground-state pigments. Up to 11 molecule of β-carotene have been assigned to the PSII reaction center and antenna subunit complex (Asada 2006). Xanthophylls, a series of molecules framed within the carotenoids group, are also involved in the antioxidant metabolism in a stroma–lumen interaction. This mechanism implies to violaxanthin, antheraxanthin, and zeaxanthin which are interconverted one in another by epoxidation/de-epoxidation reactions, thus giving rise to the so-called xanthophylls cycle (Adams and Demmig-Adams 1992; Demmig-Adams and Adams 2006). The epoxidation pathway (zeaxanthin–antheraxanthin–violaxanthin), carried out at neutral pH under low light in the stroma, depends on the provision of NADPH, whereas the de-epoxidation is achieved in the lumen at acid pH (around 5, high light) with the participation of ascorbate which is converted into dehydroascorbate (Adams and Demmig-Adams 1992; Demmig-Adams and Adams 2006).

Alpha-tocopherol is another molecule which can quench $^1O_2$, although its effectiveness regarding β-carotene is much lower, about two orders of magnitude. After the reaction of α-tocopherol with $^1O_2$, α-tocopherylquinone is formed (Halliwell and Gutteridge 2007), and this can regenerate again α-tocopherol by the reaction with ascorbate. As a result of this reaction chain, monodehydroascorbate is formed, and this is integrated within the enzymatic pathways displayed below (Fig. 1). Tocopherols are also involved in suppressing the lipid peroxidation of thylakoids by trapping lipid radicals (Muller et al. 2006).

From all antioxidant molecules, ascorbate seems to be the most versatile since this compound not only scavenges all types of ROS by itself but also participates in the ascorbate–glutathione cycle (see below) and in the regeneration of other antioxidants as reported above for α-tocopherol. Thus, a very significant role in the chloroplast redox homeostasis is attributed to ascorbate. In fact, chloroplasts are the main cellular pool of ascorbate in spite that this antioxidant is synthesized in mitochondria (Foyer et al. 1991; Smirnoff 2001).

The presence of several superoxide dismutases (SOD; EC 1.15.1.1) has been reported in chloroplasts (Hayakawa et al. 1984; Grace 1990). SODs are a class of metalloenzymes with different nature depending on the heavy metal located in the active site of the protein which catalyze the reaction [6]:

Reaction [6]: $2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$

Three main SOD types have been described in plants: copper–zinc-, iron-, and manganese-containing superoxide dismutases (CuZn–SODs, Fe–SODs, and Mn–SODs, respectively; Rodríguez-Serrano et al. 2007). Chloroplasts commonly house

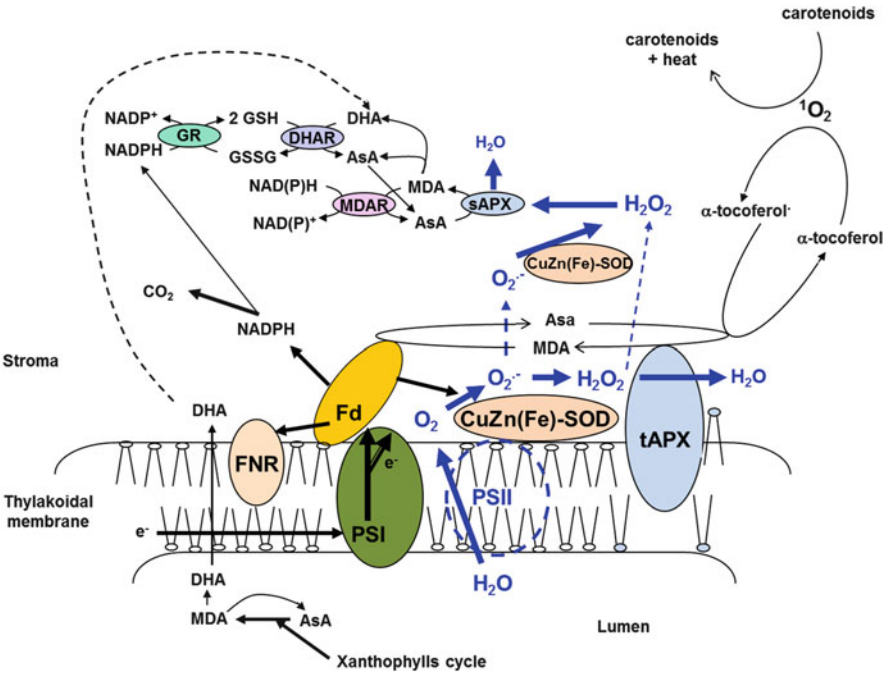

Fig. 1 Integrated model of production and scavenging of reactive oxygen species in chloroplasts. Electrons in PSI are usually "sailing" toward the PSI-linked ferredoxin (Fd) and by action of the NADP–ferredoxin reductase (FNR), NADPH is formed which can be used in the photosynthetic carbon fixation. Subsidiary, superoxide radicals ($O_2^{\bullet-}$) can be generated continuously in the presence of O_2 provided by PSII after H_2O photolysis. $O_2^{\bullet-}$ is then dismutated either by the thylakoid-linked superoxide dismutase (both CuZn–SOD and Fe–SOD) or the soluble forms of these isozymes. The H_2O_2 generated by the action of SOD is decomposed by either the ascorbate peroxidase bound to thylakoid membranes (tAPX) or the soluble isozyme (sAPX), using ascorbate (AsA) as reducing source. sAPX is integrated within the chloroplastic ascorbate–glutathione cycle (AGC) which implies the participation of the monodehydroascorbate reductase (MDAR), the dehydroascorbate reductase (DAR), and glutathione reductase (GR). This redox pathway is involved in the removal of H_2O_2 with expenses of NADPH. AsA could also be used to regenerate α-tocopherol from α-tocopherylquinone (α-tocopheryl radical), after this lipophilic antioxidant has been used as a singlet oxygen ($^1O_2$) quencher. $^1O_2$ can be also scavenged by carotenoids with excess energy being dissipated as heat. Throughout these processes, monodehydroascorbate (MDA) is formed, and this radical can be used to regenerate ascorbate in the stroma by either direct action of reduced Fd or through the AGC. MDA is also produced at the chloroplastic lumen in the xanthophylls cycle. MDA dismutates into ascorbate and dehydroascorbate which can migrate through the thylakoid membrane and be coupled to the stroma AGC. As depicted as blue arrows, a water–water cycle occurs, with consume of water in the lumen and production in the stroma side

CuZn–SOD and Fe–SOD isozymes, although the presence of one Mn–SOD has been reported in chromoplasts from pepper fruits (Martí et al. 2009). Both SOD isoenzyme types have been reported to be attached to the thylakoids near the PSI where $O_2^{\bullet-}$ is produced but also soluble in the stroma (Asada 2006; Mittova et al. 2015) (Fig. 1).

H_2O_2 is mainly removed by the action of the ascorbate peroxidase (reaction [7]; APX; EC 1.11.1.11) which, like SODs, is located either attached to the thylakoid membrane (tAPX) or soluble in the stroma (sAPX) (Yoshimura et al. 1999; Shigeoka et al. 2002; Maruta et al. 2010). In thylakoids, APX is in the vicinity of PSI so the flux of electrons through PSI, SODs, and tAPX forms a thylakoidal scavenging system which functions as the first defense against ROS, with the participation of reduced ferredoxin which directly provides electrons to monodehydroascorbate to regenerate ascorbate (reaction [8]; Fig. 1).

Reaction [7] $H_2O_2 + AsA \rightarrow 2H_2O + 2MDA$
Reaction [8] $2MDA + 2Fdred \rightarrow 2Asa + 2Fdox$

The sAPX is integrated within the ascorbate–glutathione cycle, also called Foyer–Halliwell–Asada cycle, where the enzymes monodehydroascorbate reductase (MDAR; EC 1.6.5.4), dehydroascorbate reductase (DAR; EC 1.8.5.1), and glutathione reductase (GR; EC 1.6.4.2) are involved in the H_2O_2 scavenging associated to the NADPH expense (Corpas and Barroso) (Fig. 1).

Overall, as the result of the series of reactions which involved the formation (reactions 1 and 2) and scavenging (reactions 6, 7 and 8) of ROS in chloroplasts renders the final stoichiometry given in reaction [9]:

Reaction [9] $2H_2O + O_2 \rightarrow O_2 + 2H_2O$

which allows introducing the concept water–water cycle proposed by Professor Kozi Asada (1999) as a unique pathway located in chloroplasts involving the dynamics of oxygen in these organelles and integrating a network of molecules which goes beyond the simple ROS-antioxidant pair.

Peroxiredoxins and thioredoxins are also systems involved in the detoxification of hydrogen peroxide in chloroplasts. Peroxiredoxins are thiol-based peroxidases which may utilize the reducing power provided through thioredoxins to scavenge H_2O_2(Puerto-Galán 2013). Thioredoxins are crucial for the chloroplast redox network, mediating environmental signals to the organelle proteins. Thus, chloroplast thioredoxins have been found to be very versatile and to control the structure and function of proteins by reducing disulfide bridges in the redox active site of a protein (Schürman and Jacquot 2000; Nikkanen, and Rintamaki 2014). A thioredoxin system which gains electrons from the PSI-linked ferredoxin and involves a ferredoxin–thioredoxin reductase has been found. Besides, a thioredoxin that uses NADPH as the reducing source through a NADPH–thioredoxin reductase has been reported (Nikkanen and Rintamaki 2014). Finally, a more complex system where the reducing power from NADPH is successively transferred following the sequence thioredoxin reductase, thioredoxin, and peroxiredoxin to reduce H_2O_2 up to water has been displayed (Dietz 2003). The possibility that this latter system may function as a water–water cycle under certain conditions was already proposed by Asada (2006).

3 Mitochondria

In mammalian cells, mitochondria are the major cell loci for ROS production. In plants, mitochondria constitute one of the main ROS production sites due to unavoidable impairments of the electron transport chain (ETC) responsible of the aerobic respiration which is located at the inner mitochondrial membrane. A short review of the ROS metabolism, both generation and scavenging involved systems, will be given in this chapter, although a wider view of this subject will be displayed in chapter "What Do the Mitochondrial Antioxidant and Redox Systems Have to Say Under Salinity, Drought and Extreme Temperature?" (F. Sevilla and colleagues).

Similarly to what happened in chloroplasts, the first reports on ROS in mitochondria in the mid-1960s revealed that these organelles were able to produce H_2O_2 (Hinkle et al. 1967). Years later, the demonstration of $O_2^{\bullet -}$ generation by submitochondrial particles bearing diverse ETC complexes (Loschen and Azzi 1975), along with the discovery of the presence of SOD activity in the organelle, led to conclude that the original ROS formed in mitochondria were superoxide radicals. About 2–5 % of the consumed O_2 in mitochondria is derived toward the formation of this species. By further research and thanks to the use of inhibitors of the ETC, namely, rotenone and antimycin, it was found that the $O_2^{\bullet -}$ production sites reside in complex I and complex III (Fig. 2a) (Møller 2001; Sweetlove and Foyer 2004;

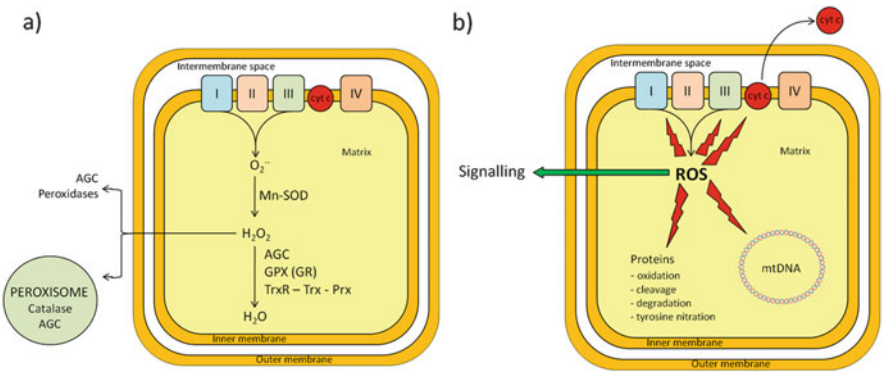

Fig. 2 Production and effects of ROS in mitochondria. (**a**) ROS production in mitochondria. Superoxide radicals ($O_2^{\bullet -}$) are generated at the complexes I and III from the electron transport chain located in the inner membrane. Mn–SOD disproportionates $O_2^{\bullet -}$ into H_2O_2 which, in turn, is removed by the ascorbate–glutathione (AGC) cycle enzymes in plants and in animal cells by a glutathione peroxidase (GPX) and a system involving thioredoxin (Trx), peroxiredoxin (Prx), and a thioredoxin reductase (TrxR). H_2O_2 can also come out of the mitochondria and be either scavenged in the cytosol by soluble peroxidases and the cytosolic AGC or driven to peroxisomes where catalase and AGC decompose it. (**b**) Effects of ROS on mitochondrial macromolecules. Under controlled conditions, ROS produced in mitochondria participates in signaling processes. However, when ROS generation exceeds the scavenging systems, ROS may attack mitochondrial DNA and trigger mutations, promote oxidation, cleavage and degradation or nitration of proteins, and favor the release of cytochrome *c* from the organelle membranes toward the cytosol, as it occurs in apoptosis

Gupta and Igamberdiev 2015). Rotenone inhibits the electron transfer from complex I (NADH–ubiquinone oxidoreductase) to ubiquinone, whereas antimycin binds to complex III (ubiquinol–cytochrome *c* oxidoreductase), thus avoiding this complex capturing electrons from the previous ETC components. A more precise study of the mitochondrial localization of $O_2^{\bullet -}$ production reported that this event develops in two ubiquinone pools: one associated to complex I and the other one linked to complex III (Raha and Robinson 2000; Popov 2015).

According to the mechanism of action of complexes I and III and the position of the respective ubiquinone pools in mammalian cells, it was postulated that $O_2^{\bullet -}$ generated in complex I was disposed of at the matrix of the organelle, whereas complex III dropped this ROS to the intermembrane space (Raha and Robinson 2000; Murphy 2009). In the matrix, $O_2^{\bullet -}$ dismutates by the action of a Mn–SOD (Fig. 2a), characteristic of mitochondria (del Río et al. 2002; Rodríguez-Serrano et al. 2007; Palma et al. 2013), and, in animal cells, the resulting H_2O_2 is detoxified by a selenium-dependent glutathione peroxidase (SeGPX) which, in turn, is coupled to a GR for the continuous provision of reduced glutathione (GSH). However, very few references have reported the presence of a CuZn–SOD in the intermembrane space, and this eventuality is far to be still consensed by the scientific community. H_2O_2 from the matrix can be pumped off to the cytosol through the mitochondrial membranes and then scavenged by diverse detoxifying systems such as peroxidases and the ascorbate–glutathione cycle or enters the peroxisomes, where catalase/ascorbate–glutathione cycle would decompose it. A thioredoxin–peroxiredoxin system located in the matrix could also remove H_2O_2 with the participation of a thioredoxin reductase which would utilize NADPH, provided by a NADP-dependent isocitrate dehydrogenase as electron donor (Murphy 2009). In plants, the presence of all enzyme components of the AGC in mitochondria has been demonstrated (Jiménez et al. 1997), and the participation of this pathway to remove H_2O_2 in this compartment is the most accepted issue for plant biologists (Fig. 2a) (Mittova et al. 2015). The necessary NADPH for the action of the GR is a common metabolite in plant mitochondria (Møller 2001). Alternative oxidase (AOX) has been reported to be activated when the reduction level of ubiquinone increases, so this is a dissipating mechanism which is also useful to prevent the overproduction of superoxide radicals (Maxwell et al. 1999; Rhoads et al. 2006; Gupta and Igamberdiev 2015).

Under certain stress conditions where H_2O_2 production overtakes the scavenging barriers and in the presence of transition metals, basically Fe^{3+} and Cu^{2+}, $^{\bullet}OH$ radicals can be formed in a Fenton-type reaction. Hydroxyl radicals could then be able to attack the mitochondrial genome provoking mutations in many of the ETC components which are encoded by the mitochondrial DNA (Fig. 2b) (Raha and Robinson 2000; Murphy 2009). ROS also damage proteins by diverse mechanisms which include oxidation, cleavage, and degradation of backbones and tyrosine nitration (Gupta and Igamberdiev 2015). Overall, ROS are important molecules to promote redox signaling events in mitochondria (Møller and Sweetlove 2010; Hebelstrup and Møller 2015), but under mitochondrial dysfunction, the overproduction of ROS under stress conditions and senescence ROS may lead to

apoptosis (programmed cell death, PCD) and necrosis. PCD is characterized by the release of cytochrome *c* from the inner mitochondrial membrane to the cytosol as a consequence of the damage (lipid peroxidation) undergone in membranes by ROS attack (Fig. 2b) (Murphy 2009).

3.1 Ascorbate Biosynthesis

A very important event in the antioxidant balance is the synthesis of ascorbate. This antioxidant molecule is synthesized by the great majority of phyla, excepting primates, rodents, and some others. Human cells lack the last enzyme of the ascorbate synthesis, the L-gulono-lactone oxidase, that makes human beings strictly dependent on an external ascorbate source, mainly fruits and vegetables. In plants, although several alternative pathways have been described, the main last step of the ascorbate biosynthesis is catalyzed by the L-galactono-lactone dehydrogenase (GalLDH), an enzyme which oxidizes L-galactono-lactone to ascorbic acid without the participation of any redox cofactor (Smirnoff 2001; Valpuesta and Botella 2004). GalLDH has been reported to be located in the inner mitochondrial membrane, neighbor to the ETC, and providing the electrons from the L-galactono-lactone to the terminal oxidase of complex IV (Bartoli et al. 2000). Thus, an interesting issue as a source of the investigation in plant antioxidant arises: ascorbate is synthesized in mitochondria but the major pool of this antioxidant is found in chloroplasts. The presence of ascorbate in other organelles suggest a very complex mechanism by which the ascorbate biosynthesis is triggered under certain stress conditions and how this important molecule is addressed to the diversity of organelles, mainly chloroplasts.

4 Plasma Membrane

Plant membrane-bound NADPH oxidase (NOX), also called respiratory burst oxidase homologue (RBOH), has the capacity to transfer electrons from intracellular NADPH across the plasma membrane to molecular oxygen in the apoplast site and generate $O_2^{\bullet -}$ which can then dismutate through different mechanisms to H_2O_2. *RBOH* genes belong to a multigenic family with 10 members in *Arabidopsis thaliana* (*RBOHA-RBOHJ*) and 9 in rice (*Oryza sativa*) but also with five groups of orthologous sequences (Torres et al. 2002; Sagi and Fluhr 2006; O'Brien et al. 2012; Skelly and Loake 2013).

The plant Rboh protein has two main components: (i) membrane-bound respiratory burst oxidase homologue (Rboh) with a molecular weight between 105 and 112 kDa (being homologue of $gp91^{phox}$ from mammalian phagocyte NAPDH oxidase) and (ii) its cytosolic regulator Rop (Rho-like protein) which is a Rac homologue of plants. Thus, the integral plasma membrane protein is composed of

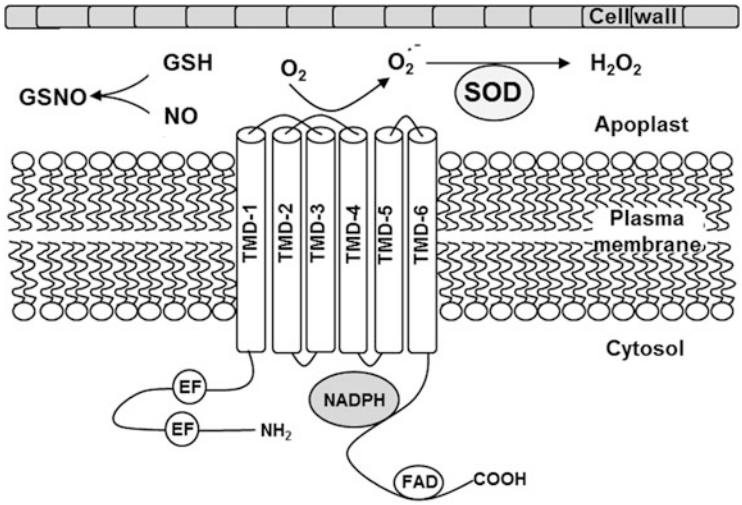

Fig. 3 Simple model of the structure and localization of the components of the plant membrane-bound respiratory burst oxidase homologues (RBOH) protein and other antioxidant elements. EF hand domains, FAD flavin adenine dinucleotide, GSH glutathione, GSNO S-nitrosoglutathione, NADPH reduced form of the nicotinamide adenine dinucleotide phosphate, NO nitric oxide, SOD superoxide dismutase, TMD-1 to TMD-6 transmembrane domains

six transmembrane domains (TMD-1 to TMD-6) connected by five loops (loops A–E) where TMD-3 and TMD-5 contain pairs of His residues required to bind two heme groups, C-terminal FAD and NADPH hydrophilic domains, and two N-terminal calcium-binding (EF-hand) motifs and some phosphorylation target sites (Yoshie et al. 2005; Marino et al. 2012) (Fig. 3). Besides this complex structure, there are also regulatory components involving phosphorylation and Ca^{2+} (Ogasawara et al. 2008) such as calcium-dependent protein kinases (CDPKs are Ser/Thr protein kinases that include a Ca^{2+}-binding calmodulin-like domain) (Kobayashi et al. 2007), Ca^{2+}/CaM-dependent protein kinase (CCaMK) (Shi et al. 2012), and Rop (Wong et al. 2007). Moreover, new mechanisms of regulation have been reported including phosphatidic acid binding (Zhang et al. 2009) and *S*-nitrosylation, which are posttranslational protein modifications mediated by nitric oxide-derived molecules (Corpas et al. 2015). Thus, in the *Arabidopsis* Rboh isoform D (AtRBOHD), the *S*-nitrosylation of Cys 890, thus abolishing the ability to generate $O_2^{\bullet-}$ (Yun et al. 2011), provides a clear interrelationship between reactive oxygen and nitrogen species.

Rboh is involved in many plant processes including cell growth (Foreman et al. 2003), plant development, stomatal closure (Shi et al. 2012), pollen tube growth (Kaya et al. 2014), symbiotic interactions (Marino et al. 2012; Kaur et al. 2014), abiotic stress, and pathogen response (Wojtaszek 1997; Torres et al. 2002; Daudi et al. 2012; Siddique et al. 2014). However, the number of Rboh isozymes which are differentially expressed suggests a certain grade of specialization for each one. For example, in *Arabidopsis thaliana* which has

10 genes, the focus has been pointed toward *AtRbohB*, *AtRbohC*, *AtRbohD*, and *AtRbohF*, especially *AtRbohD*, because it is constitutively and ubiquitously expressed (Kadota et al. 2014); however, the information about the other six *Rboh* genes is very scarce.

On the other hand, the apoplast space seems to be more complex than we could expect because it contains other elements such as SOD (Streller et al. 1997; Vanacker et al. 1998; Kukavica et al. 2005), the antioxidant glutathione (GSH) (Vanacker et al. 1999; Pignocchi and Foyer 2003), and nitric oxide (Stöhr and Ullrich 2002; Bethke et al. 2004). Thus, the SOD must regulate the H_2O_2 production during the dismutation of $O_2^{\bullet -}$ generated by Rboh being a mechanism of regulation of signaling between cells mediated by H_2O_2. Moreover, GSH and NO can interact to form *S*-nitrosoglutathione (GSNO), which is also recognized as a signaling molecule (Corpas et al. 2013), and can mediate the posttranslational modifications of proteins affecting their activities such as it occurs to ascorbate peroxidase (Begara-Morales et al. 2014).

Besides the mechanism of the local production of $O_2^{\bullet -}$ by Rboh, it has been proposed that after some stimuli (i.e., pathogens) and the generation of a local burst of ROS mediated by Rboh in an specific cells, there is a cascade of cell-to-cell communication events that carries a systemic signal over long distances throughout different tissues of the plants (see chapter "ROS as Key Players of Abiotic Stress Responses in Plants" of this book by Suzuki for deeper discussion) which opens a new perspective of the Rboh functions (Marino et al. 2012; Kaur et al. 2014).

5 Peroxisomes

Unlike other subcellular compartments, peroxisome is a single membrane-bounded compartment with a diverse range of specific metabolic functions depending on the tissue localization, the plant developmental step, and the environmental conditions (del Río et al. 2002; Mano and Nishimura 2005; Palma et al. 2009; Hu et al. 2012; Baker and Paudyal 2014). Among the principal functions of peroxisomes in plant cells, the fatty acid β-oxidation, the glyoxylate cycle, the photorespiration cycle, the metabolism of ureides, and the metabolism of reactive oxygen and nitrogen species (ROS and RNS) can be included, being the peroxisomal characteristic enzymes catalase and H_2O_2-generating flavin oxidases, which reflects a prominent oxidative metabolism. Table 1 summarizes the main peroxisomal ROS-producing systems and the involved enzymes.

5.1 H_2O_2-Producing System

Peroxisomal H_2O_2 generation is considered a side product of diverse pathways where peroxisomes are involved; however, the capacity to go through membranes

Table 1 Summary of the main ROS-producing systems and involved enzymes identified in peroxisomes from higher plants

Pathway	Peroxisomal enzyme	Reaction
H_2O_2-producing system		
β-oxidation	Acyl CoA oxidase (EC:1.3.3.6)	Acyl-CoA → *trans*-2-enoyl-CoA + H_2O_2
Photorespiration	Glycolate oxidase (EC 1.1.3.15)	Glycolate + O_2 → glyoxylate + H_2O_2
Sulphite detoxification	Sulfite oxidase (EC 1.8.3.1)	Sulfite + O_2+ H_2O → sulfate + H_2O_2
ROS metabolism	Superoxide dismutase (EC 1.15.11)	$O_2^{\bullet-}$ + $O_2^{\bullet-}$ + H+ → H_2O_2 + O_2
Purine metabolism	Urate oxidase (EC 1.7.3.3)	Uric acid + O_2 + H_2O → 5-hydroxyisourate+ H_2O_2 → allantoin + CO_2
Sarcosine metabolism	Sarcosine oxidase (EC 1.5.3.1)	Sarcosine + O_2 + H_2O → glycine + formaldehyde + H_2O_2 and L-pipecolate → Δ^1-piperideine-6-carboxylate + H_2O_2
Polyamine catabolism	Polyamine oxidase (EC 1.5.3.3)	Spermine + O_2 + H_2O → spermidine + 3-aminopropanal + H_2O_2
Superoxide-generating system		
Purine metabolism	Xanthine oxidase (EC 1.1.3.22)	Xanthine + O_2→ uric acid + $O_2^{\bullet-}$
Peroxisomal membrane polypeptides	PMP32 (membrane monodehydroascorbate reductase)	NADH + PMP32 → $O_2^{\bullet-}$

involves the capacity of this molecule to be used as a signal. Thus, peroxisomal fatty acid β-oxidation allows the breakdown of these molecules to acetyl-CoA and the subsequent conversion of acetyl-CoA to succinate via the glyoxylate cycle. In the β-oxidation pathway, the enzyme acyl-CoA oxidase catalyzes the conversion of acyl-CoA into trans-2-enyl-CoA with the concomitant generation of H_2O_2 (Arent et al. 2008). This pathway has a relevant physiological function because it allows the conversion of triacylglyceride pools in seedlings, the turnover of membrane lipids during senescence or starvation situation, as well the synthesis of fatty acid-derived hormones such as indole acetic acid (IAA), jasmonic acid (JA), and salicylic acid (SA) which consequently are involved in stress response and growth regulation (Poirier et al. 2006; Delker et al. 2007; Baker and Paudyal 2014). Photorespiration involves the light-dependent uptake of O_2 and release of CO_2 during the metabolism of phosphoglycolate, the two-carbon by-product by the oxygenase activity of Rubisco. This pathway involves several organelles (chloroplasts, mitochondria, and peroxisomes) with the peroxisomal glycolate oxidase generating H_2O_2.

There are other peroxisomal H_2O_2-producing enzymes but the available information on their function is still scarce. Thus, sulfite oxidase (SO) catalyzes the conversion of sulfite to sulfate with the concomitant generation of H_2O_2(Hänsch et al. 2006). It has been reported that low concentrations of sulfite inhibit catalase activity (Veljovic-Jovanovic et al. 1998), which could therefore be a means of regulating both enzymes. Sarcosine, also known as *N*-methylglycine, is an intermediate and by-product of glycine synthesis and degradation which also generates H_2O_2. The enzyme responsible is the sarcosine oxidase (SOX) which is a 46-kDa monomer that covalently attaches FAD molecule. Moreover, the SOX activity also catalyzes the conversion of L-pipecolate to Δ^1-piperideine-6-carboxylate plus H_2O_2 being a side branch of lysine catabolism (Goyer et al. 2004). In Arabidopsis, among the family of polyamine oxidases (PAO), it has been identified a peroxisomal isoform (AtPAO4) which is involved in polyamine catabolism especially in roots (Kamada-Nobusada et al. 2008; Planas-Portell et al. 2013).

5.2 Superoxide-Generating System

Xanthine oxidoreductase (XOR) is an FAD-, molybdenum-, iron-, and sulfur-containing hydroxylase enzyme that catalyzes the conversion of the purines hypoxanthine and xanthine into uric acid with the concomitant formation of either NADH or $O_2^{\bullet -}$ and plays an important role in nucleic acid degradation in all organisms (Harrison 2002). The enzyme is a homodimer, and each subunit contains one molybdenum atom, one FAD group, and two Fe_2S_2 centers. The molybdenum cofactor (Moco) present in XOR is also shared by other key enzymes that catalyze basic reactions in carbon, nitrogen, and sulfur metabolism, such as aldehyde oxidase, nitrate reductase, and sulfite oxidase (Schwarz and Mendel 2006). XOR exists in two interconvertible forms: an NAD-dependent dehydrogenase or xanthine dehydrogenase (XDH; EC 1.1.1.204), which can be converted into an oxygen-dependent oxidase or xanthine oxidase (XOD; EC 1.1.3.22). The presence of XOD activity in peroxisomes has been reported in different plant species (Sandalio et al. 1988; del Río et al. 1989; Mateos et al. 2003). More recently, additional biochemical and immunological results demonstrate the presence of XOR in leaf peroxisomes, showing that the XOD form, which generates superoxide radicals, is the predominant form in these oxidative organelles being differentially modulated under cadmium-induced oxidative stress (Corpas et al. 2008).

On the other hand, the peroxisomal membrane is another potential source of ROS, specifically $O_2^{\bullet -}$, through the existence of a small electron transport chain using NADH as electron donor. This is composed of a flavoprotein NADH:ferricyanide reductase of about 32 kDa and a cytochrome b (López-Huertas et al. 1999). The identity of the membrane protein of 32 kDa seems to be the enzyme monodehydroascorbate reductase (MDAR) since this enzyme has been described to be present in both matrix and membrane polypeptide of peroxisomes (Leterrier et al. 2005; Lisenbee et al. 2005). Additionally, using NADPH it was found a peroxisomal

membrane of 29 kDa that had the capacity to generate $O_2^{\bullet -}$ and to reduce cytochrome *c* (López-Huertas et al. 1999). The identity of this protein is not clear, but it could be related to the family of NADPH:cytochrome P450 reductase (López-Huertas et al. 1999).

5.3 *Peroxisomal Antioxidant Systems*

Besides the presence of catalase, a well-characterized peroxisomal antioxidant enzyme which keeps the H_2O_2 under control (Palma et al. 2013), there is another complementary antioxidant systems to regulate the content of $O_2^{\bullet -}$ and H_2O_2 in these organelles (Corpas et al. 2001).

In the case of the H_2O_2, plant peroxisomes enclose a particular ascorbate–glutathione cycle (Jiménez et al. 1997; Reumann and Corpas 2010) since its components have a special distribution with some membrane-bound enzymes such as the APX (Bunkelmann and Trelease 1996; Corpas and Trelease 1998) and the MDAR (Leterrier et al. 2005; Lisenbee et al. 2005) and others located in the matrix, such as the GR (Romero-Puertas et al. 2006) and also the DAR (Fig. 4). This peroxisomal system has been described to participate in the mechanism of response to different processes including growth (Narendra et al. 2006), leaf

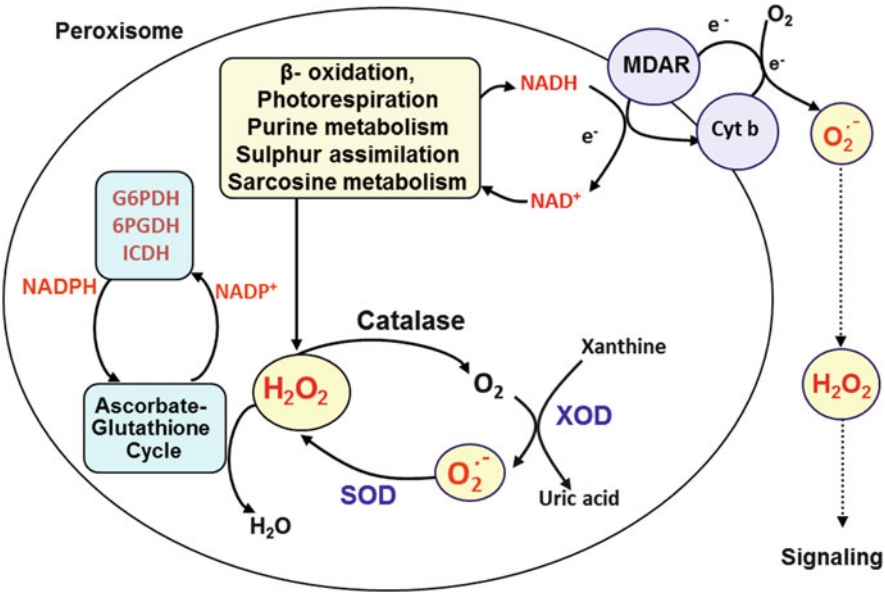

Fig. 4 Model of ROS production in plant peroxisomes. APX ascorbate peroxidase, G6PDH glucose-6-phosphate dehydrogenase, 6PGDH 6-phosphogluconate dehydrogenase, ICDH NADP–isocitrate dehydrogenase, MDAR monodehydroascorbate reductase, XOD xanthine oxidase

senescence (Jiménez et al. 1998; Palma et al. 2006), fruit ripening (Mateos et al. 2003), or heavy metal stress (Leterrier et al. 2005).

In animal cells, peroxisomes have been reported to contain exclusively a CuZn–SOD; however, in plant peroxisomes, it can be found, depending on the tissue and/or plant species and the three types of SOD isozymes, located in the matrix and/or in the membrane. Although the presence of either a CuZn–SOD or a Mn–SOD is the most common issue (del Río et al. 1983; Corpas and Trelease 1998; del Río et al. 2002), there are other cases where the presence of a Mn–SOD plus a CuZn–SOD (del Río et al. 2002) or a Fe–SOD has been demonstrated (Droillard and Paulin 1990).

Additionally, during the last decade, new components related with the peroxisomal metabolism of ROS have been discovered such as a closer family of molecules designated as reactive nitrogen species (RNS) (Corpas et al. 2013). All this indicates that peroxisomes enclose and complex nitro-oxidative apparatus characterized by a relevant flexibility which can adapt to fluctuating conditions.

6 Conclusions

In comparison to animal cells, higher plants have a most complex and active ROS metabolism under optimal environmental conditions which is in part consequence of the photosynthesis and photorespiration processes. ROS are obligated site products of many physiological pathways which are present in all cell compartments, including chloroplasts, mitochondria, plasma membrane, and peroxisomes. Although ROS have been considered as toxic molecules, this concept has changed because under a controlled production ROS are part of the mechanism of signaling or defense. This control is achieved by cellular complex of antioxidative systems which usually are close to the different sites of ROS production at subcellular level. However, under adverse environmental and/or certain physiological conditions, the cellular equilibrium between ROS production and scavenging could be broken and overcome the defense battery, which can provoke oxidative damage with fatal consequences for the normal cell functions. Future research is needed to get deeper knowledge and to decipher new mechanisms of regulation to keep under control the ROS production and their signaling implications in combination with RNS.

Acknowledgments Work in our laboratories is supported by ERDF grants co-financed by the Ministry of Economy and Competitiveness (projects AGL2011-26044, BIO2012-33904) and the Junta de Andalucía (group BIO192) in Spain.

References

Adams WW, Demmig-Adams B (1992) Operation of the xanthophyll cycle in higher plants in response to diurnal changes in incident sunlight. Planta 186:390–398

Arent S, Pye VE, Henriksen A (2008) Structure and function of plant acyl-CoA oxidases. Plant Physiol Biochem 46:292–301

Asada K, Kiso K, Yoshikawa K (1974) Univalent reduction of molecular oxygen by spinach chloroplasts on illumination. J Biol Chem 249:2175–2181

Asada K (1992) Production and scavenging of active oxygen in chloroplasts. In: Scandalios JG (ed) Molecular biology of free radical scavenging system. Cold Spring Harbor Laboratory Press, Plainview, NY, pp 173–192

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

Baker A, Paudyal R (2014) The life of the peroxisome: from birth to death. Curr Opin Plant Biol 22:39–47

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Valderrama R, Mata-Pérez C, López-Jaramillo J, Padilla MN, Carreras A, Corpas FJ, Barroso JB (2014) Dual regulation of cytosolic ascorbate peroxidase (APX) by tyrosine nitration and S-nitrosylation. J Exp Bot 65:527–538

Bethke PC, Badger MR, Jones RL (2004) Apoplastic synthesis of nitric oxide by plant tissues. Plant Cell 16:332–341

Bunkelmann JR, Trelease RN (1996) Ascorbate peroxidase. A prominent membrane protein in oilseed glyoxysomes. Plant Physiol 110:589–598

Corpas FJ, Barroso JB (2014) NADPH-generating dehydrogenases: their role in the mechanism of protection against nitro-oxidative stress induced by adverse environmental conditions. Front Environ Sci 2:55

Corpas FJ, Trelease RN (1998) Differential expression of ascorbate peroxidase and a putative molecular chaperone in the boundary membrane of differentiating cucumber seedling peroxisomes. J Plant Physiol 153:332–338

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 6:145–50

Corpas FJ, Palma JM, Sandalio LM, Valderrama R, Barroso JB, del Río LA (2008) peroxisomal xanthine oxidoreductase: characterization of the enzyme from pea (*Pisum sativum* L.) leaves. J Plant Physiol 165:1319–1330

Corpas FJ, Alché JD, Barroso JB (2013) Current overview of S-nitrosoglutathione (GSNO) in higher plants. Front Plant Sci 4:126

Corpas FJ, Begara-Morales JC, Sánchez-Calvo B, Chaki M, Barroso JB (2015) Nitration and S-nitrosylation: two post-translational modifications (PTMs) mediated by reactive nitrogen species (RNS) which participate in signaling processes of plant cells. In: Gupta KJ, Igamberdiev AU (eds) Reactive oxygen and nitrogen species signalling and communication in plants. Springer, Berlin

Daudi A, Cheng Z, O'Brien JA, Mammarella N, Khan S, Ausubel FM, Bolwell GP (2012) The apoplastic oxidative burst peroxidase in Arabidopsis is a major component of pattern-triggered immunity. Plant Cell 24:275–287

Demmig-Adams B, Adams W (2006) Photoprotection in an ecological context: the remarkable complexity of thermal energy dissipation. New Phytol 172:11–21

del Río LA (2011) Peroxisomes as a cellular source of reactive nitrogen species signal molecules. Arch Biochem Biophys 506:1–11

del Río LA (2015) ROS and RNS in plant physiology: an overview. J Exp Bot 66:2827–2837

del Río LA, Lyon DS, Olah I, Glick B, Salin ML (1983) Immunocytochemical evidence for a peroxisomal localization of manganese superoxide dismutase in leaf protoplasts from a higher plant. Planta 158:216–224

del Río LA, Fernández VM, Rupérez FL, Sandalio LM, Palma JM (1989) NADH induces the generation of superoxide radicals in leaf peroxisomes. Plant Physiol 89:728–31

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

Delker C, Zolman BK, Miersch O, Wasternack C (2007) Jasmonate biosynthesis in Arabidopsis thaliana requires peroxisomal β-oxidation enzymes-additional proof by properties of pex6 and aim1. Phytochemistry 68:1642–1650

Dietz KJ (2003) Plant peroxiredoxins. Annu Rev Plant Physiol Plant Mol Biol 54:93–107

Droillard MJ, Paulin A (1990) Isozymes of superoxide dismutase in mitochondria and peroxisomes isolated from petals of carnation (*Dianthus caryophyllus*) during senescence. Plant Physiol 94:1187–1192

Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD, Davies JM, Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature 422:442–446

Foyer CH, Lelandais M, Edwards EA, Mullineaux PM (1991) The role of ascorbate in plants, interaction with photosynthesis, and regulatory significance. In: Pell E, Steffen K (eds) Active oxygen/oxidative stress and plant metabolism. American Society of Plant Physiologists, Rockville, pp 131–143

Foyer CH, Lescure JC, Lefebvre C, Morot-Gaudry JF, Vincentz M, Vaucheret H (1994) Adaptations of photosynthetic electron transport, carbon assimilation, and carbon partitioning in transgenic *Nicotiana plumbaginifolia* plants to changes in nitrate reductase activity. Plant Physiol 104:171–178

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930

Goyer A, Johnson TL, Olsen LJ, Collakova E, Shachar-Hill Y, Rhodes D, Hanson AD (2004) Characterization and metabolic function of a peroxisomal sarcosine and pipecolate oxidase from Arabidopsis. J Biol Chem 279:16947

Grace SC (1990) Phylogenetic distribution of superoxide dismutase supports an endosymbiotic origin for chloroplasts and mitochondria. Life Sci 47:1875–86

Gupta KJ, Igamberdiev AU (2015) Compartmentalization of reactive oxygen species and nitric oxide production in plant cells: an overview. In: Gupta KJ, Igamberdiev AU (eds) Reactive oxygen and nitrogen species signaling and communications in plants. Springer International Publishing, Switzerland, pp 1–14

Hänsch R, Lang C, Riebeseel E, Lindigkeit R, Gessler A, Rennenberg H, Mendel RR (2006) Plant sulfite oxidase as novel producer of H_2O_2: combination of enzyme catalysis with a subsequent non-enzymatic reaction step. J Biol Chem 281:6884–6888

Halliwell B (2006) Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol 141:312–322

Halliwell B, Gutteridge JMC (2007) Free radicals in biology and medicine. Oxford University Press, Oxford, UK

Harrison R (2002) Structure and function of xanthine oxidoreductase: where are we now? Free Radic Biol Med 33:774–797

Hayakawa T, Kanematsu S, Asada K (1984) Occurrence of CuZn-superoxide dismutase in the intrathylakoid space of spinach chloroplasts. Plant Cell Physiol 25:883–889

Hebelstrup KH, Møller I (2015) Mitochondrial signaling in plants under hypoxia: use of reactive oxygen species (ROS) and reactive nitrogen species (RNS). In: Gupta KJ, Igamberdiev AU (eds) Reactive oxygen and nitrogen species signaling and communications in plants. Springer International Publishing, Switzerland, pp 63–77

Hideg E, Kalai T, Hideg K, Vass I (1998) Photoinhibition of photosynthesis in vivo results in singlet oxygen production detection via nitroxide-induced fluorescence quenching in broad bean leaves. Biochemistry 37:11405–11411

Hinkle PC, Butow RA, Rackers E (1967) Partial resolution of the enzymes catalyzing oxidative phosphorylation. XV Reverse electron transfer in the flavin-cytochrome b region of the respiratory chain of beef heart submitochondrial particles. J Biol Chem 242:5169–5173

Hu J, Baker A, Bartel B, Linka N, Mullen RT, Reumann S, Zolman BK (2012) Plant peroxisomes: biogenesis and function. Plant Cell 24:2279–2303

Jiménez A, Hernández JA, del Río LA, Sevilla F (1997) Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol 114:275–284

Jiménez A, Hernández JA, Pastori G, del Rio LA, Sevilla F (1998) Role of the ascorbate-glutathione cycle of mitochondria and peroxisomes in the senescence of pea leaves. Plant Physiol 118:1327–1335

Kadota Y, Sklenar J, Derbyshire P, Stransfeld L, Asai S, Ntoukakis V, Jones JD, Shirasu K, Menke F, Jones A, Zipfel C (2014) Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Mol Cell 54:43–55

Kamada-Nobusada T, Hayashi M, Fukazawa M, Sakakibara H, Nishimura M (2008) A putative peroxisomal polyamine oxidase, AtPAO4, is involved in polyamine catabolism in *Arabidopsis thaliana*. Plant Cell Physiol 49:1272–1282

Kaur G, Sharma A, Guruprasad K, Pati PK (2014) Versatile roles of plant NADPH oxidases and emerging concepts. Biotechnol Adv 32:551–563

Kaya H, Nakajima R, Iwano M, Kanaoka MM, Kimura S, Takeda S, Kawarazaki T, Senzaki E, Hamamura Y, Higashiyama T, Takayama S, Abe M, Kuchitsu K (2014) Ca2+-activated reactive oxygen species production by Arabidopsis RbohH and RbohJ is essential for proper pollen tube tip growth. Plant Cell 26:1069–1080

Kobayashi M, Ohura I, Kawakita K, Yokota N, Fujiwara M, Shimamoto K, Doke N, Yoshioka H (2007) Calcium-dependent protein kinases regulate the production of reactive oxygen species by potato NADPH oxidase. Plant Cell 19:1065–1080

Kukavica B, Vucinić Z, Vuletić M (2005) Superoxide dismutase, peroxidase, and germin-like protein activity in plasma membranes and apoplast of maize roots. Protoplasma 226:191–197

Leterrier M, Corpas FJ, Barroso JB, Sandalio LM, del Río LA (2005) Peroxisomal monodehydroascorbate reductase. Genomic clone characterization and functional analysis under environmental stress conditions. Plant Physiol 138:2111–2123

Lisenbee CS, Lingard MJ, Trelease RN (2005) Arabidopsis peroxisomes possess functionally redundant membrane and matrix isoforms of monodehydroascorbate reductase. Plant J 43:900–914

López-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

Loschen G, Azzi A (1975) On the formation of hydrogen peroxide and oxygen radicals in heart mitochondria. Recent Adv Stud Cardiac Struct Metab 7:3–12

Mano S, Nishimura M (2005) Plant peroxisomes. Vitam Horm 72:111–154

Marino D, Dunand C, Puppo A, Pauly N (2012) A burst of plant NADPH oxidases. Trends Plant Sci 17:9–15

Martí MC, Camejo D, Olmos E, Sandalio LM, Fernández-García N, Jiménez A, Sevilla F (2009) Characterisation and changes in the antioxidant system of chloroplasts and chromoplasts isolated from green and mature pepper fruits. Plant Biol 11:613–624

Maruta T, Tanouchi A, Tamoi M, Yabuta Y, Yoshimura K, Ishikawa T, Shigeoka S (2010) Arabidopsis chloroplastic ascorbate peroxidase isoenzymes play a dual role in photoprotection and gene regulation under photooxidative stress. Plant Cell Physiol 51:190–200

Mateos RM, León AM, Sandalio LM, Gómez M, del Río LA, Palma JM (2003) Peroxisomes from pepper fruits (*Capsicum annuum* L.): purification, characterisation and antioxidant activity. J Plant Physiol 160:1507–1516

Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci U S A 96:8271–8276

Mehler AH (1951) Studies on reactions of illuminated chloroplasts. II. Stimulation and inhibition of the reaction with molecular oxygen. Arch Biochem Biophys 34(2):339–351

Mittova V, Volokita M, Guy M (2015) Antioxidative systems and stress tolerance: insights from wild and cultivated tomato species. In: Gupta KJ, Igamberdiev AU (eds) Reactive oxygen and nitrogen species signaling and communications in plants. Springer International Publishing, Switzerland, pp 89–131

Møller 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

Muller M, Hernández I, Alegre L, Munné-Bosch S (2006) Enhanced alpha-tocopherol quinone levels and xanthophyll cycle de-epoxidation in rosemary plants exposed to water deficit during a Mediterranean winter. J Plant Physiol 163:601–606

Narendra S, Venkataramani S, Shen G, Wang J, Pasapula V, Lin Y, Kornyeyev D, Holaday AS, Zhang H (2006) The Arabidopsis ascorbate peroxidase 3 is a peroxisomal membrane-bound antioxidant enzyme and is dispensable for Arabidopsis growth and development. J Exp Bot 57:3033–3042

Nikkanen L, Rintamäki E (2014) Thioredoxin-dependent regulatory networks in chloroplasts under fluctuating light conditions. Phil Trans R Soc B 369:20130224

O'Brien JA, Daudi A, Butt VS, Bolwell GP (2012) Reactive oxygen species and their role in plant defence and cell wall metabolism. Planta 236:765–779

Palma JM, Jiménez A, Sandalio LM, Corpas FJ, Lundqvist M, Gómez M, Sevilla F, del Río LA (2006) Antioxidative enzymes from chloroplasts, mitochondria, and peroxisomes during leaf senescence of nodulated pea plants. J Exp Bot 57:1747–58

Palma JM, Corpas FJ, del Río LA (2009) Proteome of plant peroxisomes: new perspectives on the role of these organelles in cell biology. Proteomics 9:2301–2312

Palma JM, Gupta DK, Corpas FJ (2013) Metalloenzymes involved in the metabolism of reactive oxygen species and heavy metal stress. In: Gupta DK, Corpas FJ, Palma JM (eds) Heavy metal stress in plants. Springer, Berlin

Bartoli CG, Pastori GM, Foyer CH (2000) Ascorbate biosynthesis in mitochondria is linked to the electron transport chain between complexes III and IV. Plant Physiol 123:335–344

Pignocchi C, Foyer CH (2003) Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. Curr Opin Plant Biol 6:379–389

Planas-Portell J, Gallart M, Tiburcio AF, Altabella T (2013) Copper-containing amine oxidases contribute to terminal polyamine oxidation in peroxisomes and apoplast of *Arabidopsis thaliana*. BMC Plant Biol 13:109

Poirier Y, Antonenkov VD, Glumoff T, Hiltunen JK (2006) Peroxisomal β-oxidation-a metabolic pathway with multiple functions. Biochim Biophys Acta 1763:1413–1426

Polle A (2001) Dissecting the superoxide dismutase-ascorbate-glutathione-pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis. Plant Physiol 126:445–462

Popov VN (2015) Feedback loop of non-coupled respiration and reactive oxygen species production in plant mitochondria. In: Gupta KJ, Igamberdiev AU (eds) Reactive oxygen and nitrogen species signaling and communications in plants. Springer International Publishing, Switzerland, pp 79–88

Pruzinska A, Tanner G, Aubry S, Anders I, Moser S, Muller T, Ongania K-H, Krautler B, Youn J-Y, Liljegren SL et al (2005) Chlorophyll breakdown in senescent Arabidopsis leaves: characterization of chlorophyll catabolites and of chlorophyll catabolic enzymes involved in the degreening reaction. Plant Physiol 139:52–63

Puerto-Galán L, Pérez-Ruiz JM, Ferrández J, Cano B, Naranjo B, Nájera VA, González M, Lindahl AM, Cejudo FJ (2013) Overoxidation of chloroplast 2-Cys peroxiredoxins: balancing toxic and signaling activities of hydrogen peroxide. Front Plant Sci 4:310

Raha S, Robinson BH (2000) Mitochondria, oxygen free radicals, disease and ageing. Trends Biochem Sci 25:502–508

Reumann S, Corpas FJ (2010) The peroxisomal ascorbate-glutathione pathway: molecular identification and insights into its essential role under environmental stress conditions. In: Anjum

NA, Umar S, Chan MT (eds) Ascorbate-glutathione pathway and stress tolerance in plants. Springer, Berlin

Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol 141:357–366

Rodríguez-Serrano M, Romero-Puertas MC, Pastori GM, Corpas FJ, Sandalio LM, del Río LA, Palma JM (2007) Peroxisomal membrane manganese superoxide dismutase: characterization of the isozyme from watermelon cotyledons. J Exp Bot 58:2417–2427

Romero-Puertas MC, Corpas FJ, Sandalio LM, Leterrier M, Rodríguez-Serrano M, del Río LA, Palma JM (2006) Glutathione reductase from pea leaves: response to abiotic stress and characterization of the peroxisomal isozyme. New Phytol 170:43–52

Sagi M, Fluhr R (2006) Production of reactive oxygen species by plant NADPH oxidases. Plant Physiol 141:336–340

Sandalio LM, Fernández VM, Rupérez FL, del Río LA (1988) Superoxide free radicals are produced in glyoxysomes. Plant Physiol 87:1–4

Schürman P, Jacquot JP (2000) Plant thioredoxin systems revisited. Annu Rev Plant Physiol Plant Mol Biol 51:371–400

Schwarz G, Mendel RR (2006) Molybdenum cofactor biosynthesis and molybdenum enzymes. Annu Rev Plant Biol 57:623–647

Shi YC, Fu YP, Liu WQ (2012) NADPH oxidase in plasma membrane is involved in stomatal closure induced by dehydroascorbate. Plant Physiol Biochem 51:26–30

Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, Yoshimura K (2002) Regulation and function of ascorbate peroxidase isoenzymes. J Exp Bot 53:1305–1319

Siddique S, Matera C, Radakovic ZS, Hasan MS, Gutbrod P, Rozanska E, Sobczak M, Torres MA, Grundler FM (2014) Parasitic worms stimulate host NADPH oxidases to produce reactive oxygen species that limit plant cell death and promote infection. Sci Signal 7(320):ra33

Skelly MJ, Loake GJ (2013) Synthesis of redox-active molecules and their signaling functions during the expression of plant disease resistance. Antioxid Redox Signal 19:990–997

Smirnoff N (2001) L-ascorbic acid biosynthesis. Vitam Horm 61:241–266

Stöhr C, Ullrich WR (2002) Generation and possible roles of NO in plant roots and their apoplastic space. J Exp Bot 53:2293–2303

Streller S, Schinkel H, Wingsle G (1997) Apoplasmic CuZn-superoxide dismutase in *Pinus sylvestris*. Phyton Ann Rei Bot 37:271–276

Sweetlove LJ, Foyer CH (2004) Roles for reactive oxygen species and antioxidants in plant mitochondria. In: Day DA, Millar AH, Whelan J (eds) Plant mitochondria: from genome to function, vol 1, Advances in photosynthesis and respiration. Kluwer Academic, Dordrecht

Telfer A, Dhami S, Bishop SM, Phillips D, Barber J (1994) Beta-carotene quenches singlet oxygen formed by isolated Photosystem-II reaction centers. Biochemistry 33:14469–14474

Torres MA, Dangl JL, Jones JDG (2002) Arabidopsis gp91phox 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

Tripathy BC, Oelmüller R (2012) Reactive oxygen species generation and signaling in plants. Plant Signal Behav 7:1621–1633

Valpuesta V, Botella MA (2004) Biosynthesis of L-ascorbic acid in plants: new pathways for an old antioxidant. Trends Plant Sci 9:573–577

Vanacker H, Carver TL, Foyer CH (1998) Pathogen-induced changes in the antioxidant status of the apoplast in barley leaves. Plant Physiol 117:1103–1114

Vanacker H, Foyer CH, Carver TLW (1999) Changes in apoplastic antioxidants induced by powdery mildew attack in oat genotypes with race non-specific resistance. Planta 208:444–452

Veljovic-Jovanovic S, Oniki T, Takaham U (1998) Detection of Monodehydroascorbic acid radical in sulfite-treated leaves and mechanism of its formation. Plant Cell Physiol 39:1203–1208

Wagner DE, Przybyla D, op den Camp RG, Kim C, Landgraf F, Lee KP, Wursch M, Laloi C, Nater M, Hideg E, Apel K (2004) The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. Science 306:1183–1185

Wojtaszek P (1997) Oxidative burst: an early plant response to pathogen infection. Biochem J 322:681–692

Wong HL, Pinontoan R, Hayashi K, Tabata R, Yaeno T, Hasegawa K, Kojima C, Yoshioka H, Iba K, Kawasaki T, Shimamoto K (2007) Regulation of rice NADPH oxidase by binding of Rac GTPase to its N-terminal extension. Plant Cell 19:4022–4034

Yoshie Y, Goto K, Takai R, Iwano M, Takayama S, Isogai A, Che FS (2005) Function of the rice gp91phox homologs OsrbohA and OsrbohE genes in ROS-dependent plant immune responses. Plant Biotechnol 22:127–135

Yoshimura K, Yabuta Y, Tamoi M, Ishikawa T, Shigeoka S (1999) Alternatively spliced mRNA variants of chloroplast ascorbate peroxidase isoenzymes in spinach leaves. Biochem J 338(Pt 1):41–48

Yun BW, Feechan A, Yin M, Saidi NB, Le Bihan T, Yu M, Moore JW, Kang JG, Kwon E, Spoel SH, Pallas JA, Loake GJ (2011) S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. Nature 478:264–268

Zhang Y, Zhu H, Zhang Q, Li M, Yan M, Wang R, Wang L, Welti R, Zhang W, Wang X (2009) Phospholipase dalpha1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in Arabidopsis. Plant Cell 21:2357–2377