Dharmendra K. Gupta
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Preface

It is known that reactive oxygen species (ROS) are the by-products of aerobic breakdown and are inescapably formed by a number of metabolic pathways and electron transport chains. ROS are partially condensed form of molecular oxygen and normally result from the transfer of electrons to O_2 to form, in a succession of univalent reductions, superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$), respectively, or through an electron-independent energy transfer till an excited form of oxygen (singlet oxygen) (Gupta et al. 2016; Halliwell and Gutteridge 2015). Redox signal transduction is a complete feature of aerobic life enriched through evolution to balance evidence from metabolism and the environment. Like all other aerobic creatures, plants maintain most cytosolic thiols in the reduced (−SH) state because of the low thioldisulfide redox potential imposed by millimolar amount of the thiol buffer including glutathione.

Plants have developed cellular tactics where the endogenous content of antioxidant enzymes deliver them with amplified defense against harmful effects of oxidative stress encouraged by heavy metal and other stress sources (Palma et al. 2013). Stress-induced upsurges in ROS level can cause different degree of oxidation of cell components and a gross change in the redox status. Plant cells generally cope very well with high rates of generation of superoxide, H_2O_2, and even singlet oxygen. When the increment of ROS in plant cells quickly augments and the scavenging systems of ROS do not operate appropriately, a condition of oxidative stress and oxidative injury happens (Gupta et al. 2015). In plants, chloroplast is the most important among the organelles in respect of ROS generation as O_2 is constantly provided through the water autolysis and freely available inside the organelle (Gupta et al. 2015). In plant cells, compartmentalization of ROS production in the different organelles includes chloroplasts, mitochondria, or peroxisomes, and they also have a complex battery of antioxidant enzymes usually close to the site of ROS production (Corpas et al. 2015). Plant cells also contain a series of ROS-scavenging non-enzymatic antioxidants such as ascorbic acid, glutathione (GSH), and carotenoids, as well as a set of enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPX), peroxiredoxin (Prx), and the

ascorbate–glutathione cycle (Corpas et al. 2015). The total pool of redox-active complexes which are found in a cell in reduced and oxidized forms generates cellular redox buffers where NAD(P)H/NAD(P)$^+$, ascorbate/dehydroascorbate (AsA/DHA), glutathione/glutathione disulfide (GSH/GSSG), and reduced thioredoxin/oxidized thioredoxin (Trx_{red}/Trx_{ox}) are the main pairs. AsA and GSH are major constituents of the soluble redox shielding system, and they contribute pointedly to the redox environment of a cell. AsA cooperates tightly with GSH (γ-Glu-Cys-Gly) in the Foyer–Halliwell–Asada cycle (ascorbate–glutathione cycle), involving three codependent redox couples: AsA/DHA, GSH/GSSG, and NAD(P)H/NAD(P)$^+$. It undertakes subsequent reduction/oxidation reactions catalyzed by ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) that is universally responsible for H_2O_2 sifting and keeping AsA and GSH in the reduced state at the outflow of NADPH, this cycle being situated in all cellular partitions in which ROS detoxification is required.

One of the major consequences of stresses in plant cells is the enhanced generation of ROS which usually damage the cellular components such as membranes, nucleic acids, proteins, chloroplast pigments, and alteration in enzymatic and non-enzymatic antioxidants. The molecular mechanisms of signal transduction corridors in higher plant cells are vital for processes such as hormone and light sensitivity, growth, development, stress resistance, and nutrient uptake from soil and water (Gupta et al. 2013).

It is really great achievement for the plant biotechnologists who are working for years to know how redox state handled by plants. This edited volume will provide the recent advancements and overview to the plant scientists who are actively involved in redox signaling states and also a key player for cellular tolerance in plant cells under different stresses (biotic and abiotic). Other key features of this book are cellular redox homeostasis as central modulator, redox homeostasis and reactive oxygen species, redox balance in chloroplasts and in mitochondria, and oxidative stress and its role in peroxisome homeostasis. Some chapters are also focusing on glutathione-related enzyme system and metabolism under metal(ed) stress. Abiotic stress-induced redox changes and programmed cell death are also addressed in the edition. In summary, the information compiled in this volume will bring depth knowledge and current achievements in the field of redox state chemistry in plant cell.

Dr. Dharmendra K. Gupta, Prof. José M. Palma, and Dr. Francisco J. Corpas individually thank all authors for contributing their valuable time, knowledge, and enthusiasm to bring this book into in the current shape.

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References

Corpas FJ, Gupta DK, Palma JM (2015) Production sites of reactive oxygen species (ROS) in plants. In: Gupta DK, Palma JM, Corpas FJ (eds) Reactive oxygen species and oxidative damage in plants under stress. Springer Publication, Germany, p 1–22

Gupta DK, Corpas FJ, Palma JM (2013) Heavy metal stress in plants. Springer-Verlag, Germany

Gupta DK, Palma JM, Corpas FJ (2015) Reactive oxygen species and oxidative damage in plants under stress. Springer-Verlag, Germany

Gupta DK, Peña LB, Romero-Puertas MC, Hernández A, Inouhe M, Sandalio LM (2016) NADPH oxidases differently regulates ROS metabolism and nutrient uptake under cadmium toxicity. Plant Cell Environ doi:10.1111/pce.12711

Halliwell B, Gutteridge JMC (2015) Free radicals in biology and medicine. Oxford University Press, Oxford, UK

Palma JM, Gupta DK, Corpas FJ (2013) Metalloproteins involved in the metabolism of Reactive Oxygen Species (ROS) and heavy metal stress. In: Gupta DK, Corpas FJ, Palma JM (eds) Heavy metal stress in plants. Springer Publication, Germany, p 1–18

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Chapter 1
Cellular Redox Homeostasis as Central Modulator in Plant Stress Response

C. Paciolla, A. Paradiso and M.C. de Pinto

Abstract Plants are frequently exposed to different stressful factors, both of biotic or abiotic nature, which limit their growth and productivity. To survive under stress conditions, plants must activate stress-specific signalling pathways, which finally lead to morphological, physiological, and biochemical changes that allow to adapt to the adverse environment. Cellular redox homeostasis, determined by a complex interplay between pathways that produce and scavenge reactive oxygen species (ROS), plays a key role in the adaptive response. Each deviation in the cellular redox state, due to an imbalance of ROS production and/or scavenging, is indicative of environmental disturbance and works as a signal. Under stress conditions, different ROS are produced in many cell compartments. Plants have very proficient, versatile and flexible antioxidant machinery, which comprises enzymes and metabolites with distinct biochemical properties and distinct sub-cellular localization. The antioxidant systems play a key role in the control of redox homeostasis, determining either the extent or the specificity of ROS signals and the downstream redox-dependent responses. Redox signalling is responsive to a number of environmental cues, and the complex and dynamic pathways of redox regulation occur in different cell compartments. The redox-dependent modification of sensitive signalling proteins is proposed as a key mode of redox signal transmission. Each redox-dependent interaction is opportunely regulated by a restricted environment, whose change transfers the complex system of information and influences the plant response to external changes.

Keywords Ascorbate · Antioxidants · Glutathione · Peroxidases · Reactive oxygen species · Redox homeostasis · Redox signalling · Stress

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1.1 Introduction

Plants, as sessile organisms, are frequently exposed to various environmental cues, which can potentially limit their growth and development. To cope with their sessile life, plants possess different stress-specific signalling pathways that permit to perceive the external signals and trigger changes in the expression of numerous genes. Stress-responsive genes may encode both for functional proteins, that protect cells from damages, and regulatory proteins, such as transcription factors that control stress signalling and adaptation (Hirayama and Shinozaki 2010; Zhang et al. 2011). The activation of stress-specific signalling pathways causes morphological, physiological, and biochemical changes that allow plants to adapt to adverse environment. Many studies point out that changes in cellular redox environment play a key role in the integration of external stimuli and the complex network of stress-signalling pathways (Fujita et al. 2006; Spoel and Loake 2011; Suzuki et al. 2012; Scheibe and Dietz 2012).

The redox environment of a cell is determined by the global poise of its oxidation/reduction systems; in this view, the oxidative and reductive reactions have to be considered together as complementary processes. There is a complex link between redox state and metabolism: the redox state could be considered an integrator of cellular and apoplastic metabolism and at the same time is regulated by different metabolic processes (Geigenberger and Fernie 2014; Noctor et al. 2015). Thus, redox homeostasis plays a key role for appropriate plant responses to both developmental and environmental stimuli. Redox changes, due to endogenous or exogenous inputs, will be sensed, integrated and converted through different signalling pathways, which ultimately will lead to the redox-dependent reprogramming of gene expression.

Two regulated variables are dynamically implicated in maintaining the redox environment: on the one hand, the production of reactive oxygen species (ROS), and on the other hand the presence of different redox couples and antioxidant machinery. The redox homeostasis of the different cellular compartments is determined by a complex interplay between multiple ROS-producing pathways, and ROS-scavenging mechanisms. The processes that produce and balance oxidants and antioxidants are useful for the control of plant responses to the changing environment (Fig. 1.1).

1.2 ROS Production Pathways

ROS are natural byproducts of the aerobic metabolism, formed either by energy or by electron transfer to oxygen (Apel and Hirt 2004). Generation of singlet oxygen ($^1O_2$) is due to an energy transfer-dependent mechanism that rearranges the configuration of the unpaired electrons of oxygen, remarkably increasing its oxidising capability. $^1O_2$ has a half-life of 4 μs in aqueous solution and reacting with

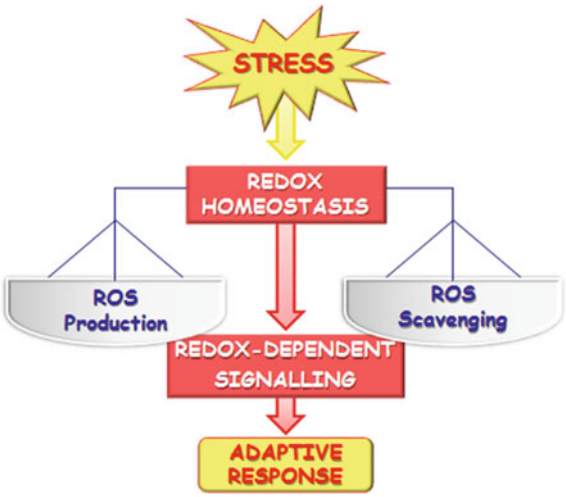

Fig. 1.1 Redox homeostasis, due to the balance among ROS production and scavenging, is altered in stress conditions and activates a redox-dependent signalling that trigger the adaptive response

biological molecules mainly forms endoperoxides and hydroperoxides (Halliwell 2006). The superoxide radical ($O_2^{\cdot -}$) is formed for the transfer of a single electron to O_2; this ROS can reduce quinones and transition metal as copper and iron, affecting the activity of metal-containing enzymes; however, $O_2^{\cdot -}$, being moderately reactive, and having a short half-life (2–4 μs), does not cause extensive damage by itself, but undergoes transformation into more reactive and toxic hydroxyl radical (OH) (Halliwell 2006). Because of its high instability at physiological pH, $O_2^{\cdot -}$ rapidly disproportionates to O_2 and hydrogen peroxide (H_2O_2), either spontaneously or by the action of superoxide dismutases (SODs, Alscher et al. 2002). H_2O_2 can cause inactivation of enzymes by oxidizing their thiol groups (Møller et al. 2007). However, H_2O_2, like $O_2^{\cdot -}$, is a relative poor oxidant. For this reason, the abundance of enzymes able to scavenge this ROS may be due to the requirement to reduce the production of OH, the most reactive and toxic ROS. OH can be formed at neutral pH through Haber-Weiss or Fenton reactions, catalysed by redox-active metal ions, especially iron and copper. This ROS is able to damage different cellular components and, due to the lack of enzymatic systems able to scavenge this toxic radical, its accumulation can lead to cell death (Møller et al. 2011). On the other hand, H_2O_2 has been proposed as the most valuable ROS functioning as second messenger (Petrov and Van Breusegem 2012). Indeed, due to its significantly longer half-life (1 ms) compared to other ROS members and its capability to cross cell membranes, being facilitated via aquaporins (Bienert et al. 2007), H_2O_2 can cover considerable long distances within the cell.

ROS are diffusely produced by a large number of physiological processes, occurring in both intracellular and extracellular locations. ROS production occurring in the photosynthetic and respiratory electron transport chains has a regulatory function in alleviating over-reduction, particularly during stress conditions (Noctor et al. 2014). Chloroplasts and mitochondria, together with peroxisomes, which

generate $O_2^{\cdot -}$ and H_2O_2 through multiple reactions, are the main producing sites of ROS in plant cells (Foyer and Noctor 2003). ROS overproduction in these organelles has been shown to participate in the responses to different kinds of stress, both of biotic or abiotic nature (del Río et al. 2006; Rhoads et al. 2006; Miller et al. 2010a, b; Nomura et al. 2012; Suzuki et al. 2012; Sandalio et al. 2013; Huang et al. 2016). The apoplast is another principal site of ROS generation. Cell wall peroxidases (PODs), catalyzing cell wall formation, have been proposed as a source of pathogen-induced oxidative burst (Daudi et al. 2012). Apoplastic ROS production during plant–pathogen interaction also occurs via respiratory burst oxidase homologs (RBOHs), localized at the plasma membrane. The pathogen recognition determines symplastic signals, including calcium influx and protein phosphorylation that activate the protein, which in turn transfers electrons from symplastic NADPH to apoplastic oxygen, generating $O_2^{\cdot -}$ at the apoplastic side of the plasma membrane (Torres et al. 2002; Suzuki et al. 2011). Apoplastic ROS production by RBOHs is not only involved in pathogen defence but also occurs in response to abiotic stresses (Zhang et al. 2001; Suzuki et al. 2012).

Other cell compartments have been proposed for ROS production in plant stress response. For instance, salt stress in Arabidopsis causes ROS production in endosomes targeted to the central vacuole. The inhibition of the fusion of H_2O_2-containing vesicles with the tonoplast leads to the formation of cytoplasmic H_2O_2-containing megavesicles and improves plant salt tolerance (Leshem et al. 2006). An example of nuclear ROS production has also been reported. Tobacco BY-2 cells treated with the elicitor cryptogein accumulate ROS firstly in the nucleus and later in other cell compartments, like endomembranes and cytoplasm. The isolated nuclei of these cells are able to produce H_2O_2 in a calcium-dependent manner, implying that nuclei could be an active source of ROS (Ashtamker et al. 2007).

Many stresses induce ROS production in specific sub-cellular compartments, which, in turn, results in ROS accumulation in other compartments. Alteration in ROS production or scavenging in one sub-cellular compartment influences the ROS level in other compartments (Davletova et al. 2005; Miller et al. 2007; Vanderauwera et al. 2011). Moreover, it should been considered that a continuous ROS flow through the cell can be necessary to transmit information between different sub-cellular compartments. The connections between different ROS locations in the plant cell make it very difficult to study the contribution of a single sub-cellular compartment in ROS production. These observations could explain why the mechanisms, by which stress conditions are sensed and integrated, and how ROS accumulation is interconnected with stress signalling, are not completely clear (Noctor and Foyer 2016). Further complexity is added by the interactions between ROS and hormone signalling (Overmyer et al. 2003; Blomster et al. 2011; Mittler and Blumwald 2015; Berkowitz et al. 2016).

The environmental cues causing ROS overproduction can lead to oxidative stress that has been generally categorized as a negative condition for the cells. Indeed, ROS are able to react readily with lipids, proteins, carbohydrates, and nucleic acids causing significant cell damage and negatively affecting metabolic activities and integrity of organelles (Foyer and Noctor 2003; Pfannschmidt et al.

2007). However, in the last two decades it has become more and more clear that transient oxidative imbalance can be needed to activate signalling pathways enabling cells to acclimate to adverse environment (Jaspers and Kangasjarvi 2010; Suzuki et al. 2012). Thus, ROS, although are involved in the generation of stress-induced oxidative damages, have an important role in cell signalling, being able to activate gene expression and to facilitate the development of plant tolerance to environmental stress.

1.3 ROS-Scavenging Mechanisms

The principal function of antioxidant defences is to control ROS accumulation; the homeostatic regulation, due to antioxidant redox buffering, determines the extent and the specificity of ROS signals and ultimately regulates the redox-dependent signalling pathways, deciding cell fate (de Pinto et al. 2006). However, also antioxidant systems are finely regulated to permit variations in ROS levels in order to make easy appropriate signalling functions (Munné-Bosch et al. 2013). Antioxidants are not inactive spectators, but key compounds that dynamically work at the cross-point between stress perception and physiological responses.

Plants have a very proficient, versatile and flexible antioxidant machinery comprising enzymatic and non-enzymatic components, with various biochemical properties and distinct sub-cellular localization (Foyer and Noctor 2003, 2005).

1.3.1 Non-enzymatic Antioxidants and Ascorbate-Glutathione Cycle

Tocopherols and carotenoids are key lipophilic antioxidants. Carotenoids, localized in the plastids, perform their antioxidant activity by protecting the photosynthetic machinery. For instance, an increase in the number of carotenoid molecules per chlorophyll unit provides protection from oxidative damages under drought stress (Munné-Bosch and Alegre 2000). Carotenoids interact with α-tocopherol in the protection of the $^1O_2$-dependent damages of the photosystem II in presence of herbicides (Trebst et al. 2002). Tocopherols, in particular α-tocopherol, are efficient scavengers of different ROS, including $^1O_2$ and lipid radicals, thus are indispensable for the protection of biological membranes. Tocopherol deficiency leads to an increase in lipid peroxidation (Abbasi et al. 2009). The Arabidopsis *vte1* and *vte4* mutants, deficient in α-tocopherol, are hypersensitive to salt stress (Ellouzi et al. 2013). On the other hand, tobacco plants over-expressing Arabidopsis VTE1 subjected to drought stress show decreased lipid peroxidation and H_2O_2 content when compared with wild-type plants (Liu et al. 2008). Under various adverse environmental conditions, tocopherols work in cooperation with other antioxidants, such as ascorbate (ASC) and glutathione

(GSH), contributing to the maintenance of a suitable redox state, particularly in chloroplasts (Munné-Bosch 2005; Szarka et al. 2012).

Among the non-enzymatic antioxidants, the hydrophilic redox couples ASC/dehydroascorbate (DHA) and GSH/glutathione disulphides (GSSG) have a key role in maintaining redox homeostasis and in participating to redox signalling (Foyer and Noctor 2005, 2011, 2013).

ASC is the most abundant hydrophilic antioxidant in plants and is widely distributed in all cell compartments. ASC takes part in the detoxification mechanisms of chloroplasts, such as the water–water cycle and the xanthophyll cycle, and is a major ROS scavenger in a wide range of abiotic and biotic stress (Smirnoff and Pallanca 1996; Asada 1999; Foyer and Noctor 2005; Yabuta et al. 2007; Gallie 2013).

The increase in the enzymes involved in ASC biosynthesis and in the reduction of its oxidized forms, monodehydroascorbate (MDHA) and DHA, in presence of adverse environmental cues highlights the important role of this antioxidant in the resistance to several stress (Urzica et al. 2012; Gallie 2013; Holler et al. 2015). Moreover, the exogenous treatment of different plant systems with galactone-γ-lactone, the biosynthetic precursor of ASC, increases the tolerance to various kinds of abiotic stress (Maddison et al. 2002; Paradiso et al. 2008; Sgobba et al. 2015). In Arabidopsis plants inoculated with different RNA viruses, the treatment with ASC is able to alleviate disease symptoms and inhibit virus replication (Wang et al. 2011).

GSH is a multifunctional tripeptide, containing a sulfhydryl group; it is an abundant metabolite in plants, and it has been considered a master regulator of intracellular redox homeostasis (Foyer and Noctor 2011, 2013; Gill et al. 2013). GSH participates in the reduction of DHA, but also plays a key role in the direct ROS scavenging and in the protection of the thiol groups of proteins (Zagorchev et al. 2013). The principal role of GSH in redox regulation occurring during the response to both abiotic and biotic stress has been recently reviewed extensively (Frendo et al. 2013; Gill et al. 2013; Zagorchev et al. 2013).

ASC and GSH are linked into a network of reactions, the so-called ASC-GSH cycle, whose components are essential for the control of redox homeostasis (Foyer and Noctor 2011, 2013).

ASC acts as specific electron donor for ascorbate peroxidase (APX) that catalyses the conversion of H_2O_2 to H_2O and O_2. APX belongs to class I peroxidase family, which possess a haem prosthetic group. Due to the high affinity for H_2O_2, APX is able to efficiently scavenge this ROS, even when it is present at low concentrations. In plants, cytosolic, mitochondrial, peroxisomal/glyoxysomal and chloroplastic APX have been identified (Shigeoka et al. 2002; Teixeira et al. 2006; Najami et al. 2008). The various APX enzymes are differently regulated by several abiotic stresses in different plant species. However, an enhancement of APX activity and expression is a good indicator for the acquisition of tolerance to different adverse environmental conditions (reviewed in Caverzan et al. 2012). Nevertheless, when programmed cell death has to be activated as a defence strategy a decrease in APX occurs at both transcriptional and post-translational level

(de Pinto et al. 2012, 2013). APX down-regulation has been also reported in the hypersensitive response activated by tobacco plants in response to tobacco mosaic virus (Mittler et al. 1998).

The scavenging of H_2O_2 by APX leads to the formation of MDHA that can spontaneously undergoes dismutation giving ASC and DHA. In chloroplast, particularly, near the thylakoid membranes, the main pathway of ASC regeneration from MDHA is at expense of ferredoxin. In other sub-cellular compartments, reduction of MDHA can occur via MDHA reductase (MDHAR), which utilizes NAD(P)H as electron donors. MDHAR activity has been detected in several cell compartments, such as chloroplasts, mitochondria, peroxisomes and cytosol (Jiménez et al. 1997; López-Huertas et al. 1999; Mittova et al. 2004; Kavitha et al. 2010). DHA can be regenerated by a reductase (DHAR) at the expense of GSH. If not reduced by DHAR, DHA can undergo irreversible hydrolysis; thus, DHAR has a significant role in maintaining the reduced ASC pool (Gallie 2013). DHARs have been identified in cytosol, chloroplasts, mitochondria and peroxisomes (Chew et al. 2003; Kataya and Reumann 2010). GSH is regenerated from GSSG by the NADPH-dependent glutathione reductase (GR). GR regenerating the reduced form of GSH maintains not only a high ratio of GSH/GSSG, but also the balance between reduced GSH and ASC pools (Ding et al. 2009). GR is mainly localized in the chloroplasts, although the enzyme is also present in cytosol, mitochondria and peroxisomes (Edwards et al. 1990; Jiménez et al. 1997; Romero-Puertas et al. 2006).

Various environmental stresses can differently affect the enzymes of the ASC-GSH pathway, depending on the plant species, the metabolic and developmental status, and the duration and intensity of the stress (Gill et al. 2013; de Pinto et al. 2015; Pandey et al. 2015). However, the use of mutants and transgenic plants over- or under-expressing enzymes of the ASC-GSH cycle has highlighted that a high correlation exists between the enhancement of the enzymes and metabolites of this pathway and the stress tolerance (Gill and Tuteja 2010; Gill et al. 2013; Pandey et al. 2015).

In response to biotic stress, the ASC-GSH cycle is also finely regulated according to the kind of plant–pathogen interaction, namely compatible or incompatible, the pathogen life style and the developmental stage of the plants (De Gara et al. 2003). The different susceptibility to the pathogens among cultivars of the same plant species correlates with a different activity/expression of the enzymes of the ASC-GSH cycle. For instance, maize genotypes resistant to the fungus *Fusarium* have higher levels of these defence-related enzymes than the susceptible ones (Lanubile et al. 2012, 2015).

1.3.2 ROS Removal Enzymes

In addition to ASC-GSH cycle, many other enzymatic proteins are involved in ROS removal (Fig. 1.2). SODs, being involved in the $O_2^{\cdot-}$ dismutation, avoid the possibility of $^{\cdot}OH$ formation and constitute the first line of defence against ROS. SODs,

based on their metal co-factor, are classified as Mn-SODs, Fe-SODs and Cu/Zn-SODs that show different cellular localization. Almost all cell compartments are equipped with this mandatory defence (Alscher et al. 2002). An increase in the various SOD enzymes occurs in response to different abiotic stress although, also in this case, this response can take place with different intensity depending on the plant species, plant developmental stage and stress intensity. Moreover, the improvement of stress tolerance in plants over-expressing SOD genes underlines the important role of these enzymes in counteracting the potential negative effects of ROS (Gill et al. 2015). Recently, it has been reported that the over-expression of a Cu/Zn-SOD gene in wheat and Arabidopsis enhances the tolerance to salt and oxidative stress. Interestingly, the improved stress tolerance in these transgenic lines seems to be due to the modulation of redox homeostasis obtained by the promotion of activity and expression of NADPH oxidase (Wang et al. 2016). The over-expression of cytosolic Cu/Zn-SOD is also able to increase disease tolerance against bacterial pathogens (Faize et al. 2012).

Numerous antioxidative enzymes are involved in the removal of H_2O_2 (Fig. 1.2). Catalases (CATs) are haem proteins able to dismutate H_2O_2, without the need for reducing cofactors. Since the CAT affinity for H_2O_2 is much lower than that of other H_2O_2 removal enzymes, it seems that CATs function only when this ROS is present at high levels. At sub-cellular level, CATs are undoubtedly localized in peroxisomes, even if their presence in other cell compartments, such as cytosol, chloroplasts and mitochondria, cannot be excluded (Mhamdi et al. 2010). Three different classes of CATs have been found in almost all plant species, and they are expressed in different tissues. In Arabidopsis, the knockout of CAT1 and CAT3 slightly reduces or has no effect, respectively, on total CAT activity. On the other hand, *cat2* mutants reduce the total CAT activity by 80 % and show defects not only in photorespiration but also in response to pathogens (Chaouch and Noctor 2010). CAT genes are highly expressed even under optimal conditions; thus, exposure to stress not always requires up-regulation and in many cases some stresses cause down-regulation of CATs expression and/or activity (Mhamdi et al. 2012).

All the other H_2O_2-removing enzymes are peroxidases, which require reducing cofactors. Peroxidases can be divided in haeme-based and thiol-based peroxidases.

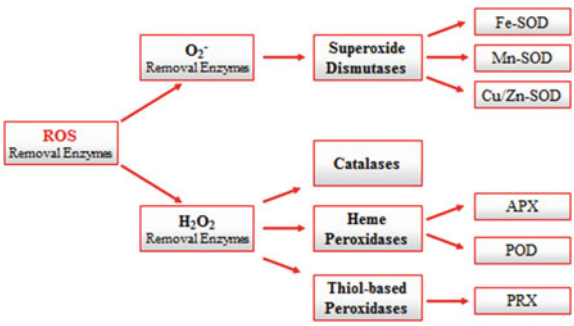

Fig. 1.2 Schematic representation of the enzymes involved in ROS removal. *APX* Ascorbate peroxidase; *PRX* Peroxiredoxins; *POD* Class III peroxidases; *SOD* Superoxide dismutase

The first group comprises APX (discussed above) and class III haeme peroxidases (PODs), which can be involved in both ROS removal and ROS generation. PODs use different compounds, mainly of phenolic nature, as electron donors, and their role seem to be correlated principally to the oxidation of the reducing substrate, rather than to the H_2O_2 removal (De Gara et al. 2003). PODs are involved in the stiffening and lignification of the cell wall, which represent an optimal mechanical barrier for the slowdown of pathogen penetration. Consistently, an increase in POD activity occurs during different plant–pathogen interactions (Ding et al. 2011; Lanubile et al. 2012, 2015; Mandal et al. 2014; Oliveira et al. 2014).

The second group of peroxidases, the thiol-based peroxidases, is constituted by peroxiredoxins (PRX, Tripathi et al. 2009). These proteins, not having a prosthetic group, remain in an inactive form at the end of their catalytic cycle; the regeneration of active PRX depends on external electron donors, such as thioredoxins (TRX), glutaredoxins (GRX), cyclophilins and NADPH-dependent TRX reductase (TR, Bhatt and Tripathi 2011). TRX and GRX, key proteins involved in the regulation of cysteine/protein redox state, are generally reduced by TR and GSH, respectively. Glutathione peroxidases (GPX) belong to the PRX superfamily; although initially defined as GSH-dependent peroxidases, GPX use only TRX for their regeneration and do not react with GSH or GRX (Navrot et al. 2006; Bela et al. 2015). Due to the thiol-dependent activities, GPX isoenzymes, besides detoxification, may be involved in the regulation of cellular redox homeostasis by maintaining the thiol/disulphide or NADPH/$NADP^+$ balance (Navrot et al. 2006). GPX proteins are involved in the response to both biotic and abiotic stress (Navrot et al. 2006; Bela et al. 2015).

The thiol-based peroxidases, changing the thiol status of TRX and/or GRX, can have repercussions on redox-sensitive target proteins, thus can be directly involved in redox-dependent signalling (Foyer and Noctor 2016).

1.4 Redox-Dependent Signalling

Redox homeostasis is a crucial requirement of plant cells: each variation in the redox state, due to an imbalance of ROS production and scavenging, could be indicative of environmental disturbance and function as a signal (Fig. 1.1; Potters et al. 2010). Moreover, any stimulus altering cellular redox homeostasis may function as an inducer for the same set of defence-related genes. For instance, it has been reported that both low levels of ascorbate or changes in glutathione pool are able to induce pathogenesis-related (PR) proteins, acting as elicitors of resistance response to pathogens (Pastori et al. 2003; Barth et al. 2004; Chaouch et al. 2010; Han et al. 2013). However, the two hydrophilic redox couples ASC/DHA and GSH/GSSG seem to function in a different way in the redox signalling (Munné-Bosch et al. 2013). ASC, that is the only reductant present at a significant level in the apoplast, can be oxidized in this compartment by ASC oxidase (Parsons and Fry 2012), which can contribute to create a redox gradient across the plasma membrane,

connecting intra- and extra-cellular environments. Thus, ASC/DHA redox pair would principally function in defining opportune thresholds for apoplastic and cytoplasmic signalling (de Pinto et al. 1999; Pignocchi et al. 2003; de Pinto and De Gara 2004). On the other hand, GSH, that has a principal role in defining intracellular redox potential, would be involved mainly in the redox-dependent signalling pathways occurring inside the cell (Foyer and Noctor 2005; Han et al. 2013). At this regard, the distribution of GSH among distinct intracellular compartments is crucial to define cellular redox environment in which both metabolism and signalling take place (Zechmann 2014). During oxidative stress, GSH is not only oxidized but also redistributed in intracellular compartments. In Arabidopsis *cat2* mutants producing more H_2O_2, GSH levels are higher and more oxidized than in the wild-type plants. Interestingly, the increase in GSH is higher in the vacuole and chloroplasts than in the cytosol (Queval et al. 2011). The glutathione compartmentation occurring during oxidative stress represents a significant aspect of redox homeostasis and signalling, since it is useful to avoid an excessive oxidising cytosolic redox environment and to allow the signalling termination (Noctor et al. 2013).

1.4.1 Redox Signalling in Different Cell Compartments

Redox signalling is reactive to innumerable environmental cues, which influence cellular metabolism and apoplastic environment (Foyer and Noctor 2012). It should be considered that the content and the redox state of redox-active compounds greatly vary among different cell compartments; therefore, redox regulation occurring in various cell compartments can influence differently plant response to external environment changes (Noctor and Foyer 2016).

The apoplast is as a crucial site for the plant redox-dependent response to external stimuli, both of biotic and abiotic nature. It has been suggested that in the apoplast oxidants are not only produced but also perceived. The redox buffering capability of the apoplast is weaker than that of intracellular compartments, since it is deficient in NAD(P)H and GSH while it is rich in enzymes that remove antioxidant compounds (Horemans et al. 2000; Pignocchi et al. 2003; Pignocchi and Foyer 2003; Ohkama-Ohtsu et al. 2007; Parsons and Fry 2012). For this reason, the ROS lifetime in the apoplast is longer than inside the cell. Different hypotheses have been issued to explain the transmission of the redox signal from the apoplast to inside the cell (Fig. 1.3). One emerging and interesting possibility is that the redox signal can be transmitted by redox-sensitive proteins on the plasma membrane, such as the K^+ channel SKOR or the cysteine-rich receptor-like kinases (García-Mata et al. 2010; Wrzaczek et al. 2010, 2013). On the other hand, the transmission of the redox signal could be due to the oxidation of the extracellular ASC pool (Foyer and Noctor 2012). Finally, it is possible that H_2O_2 produced in the apoplast migrates inside the cells through aquaporins and is transduced into the cytosol (Miller et al. 2010a, b). In this way, the apoplastic oxidative burst can be sensed and transduced also by neighbouring cells, leading to the formation of the so-called “ROS wave” (Mittler et al. 2011). ROS

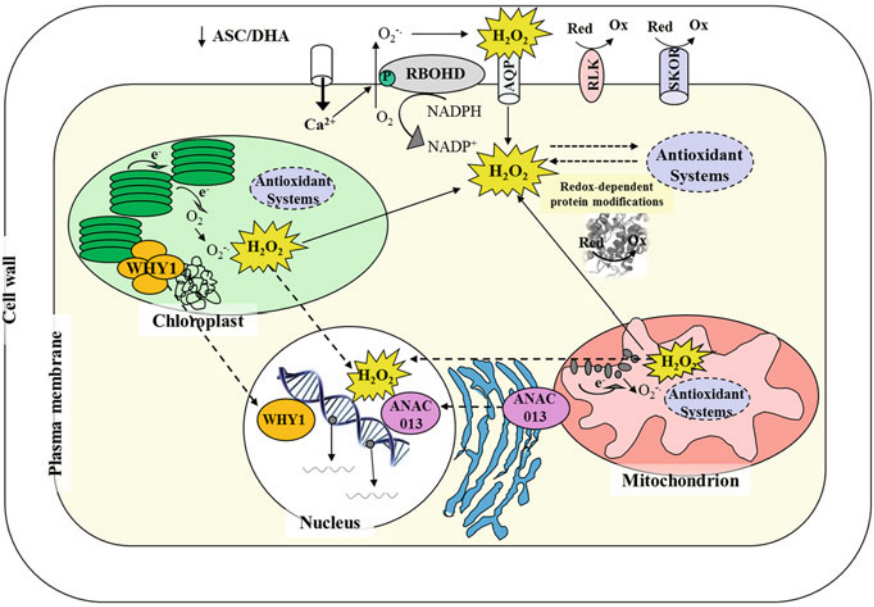

Fig. 1.3 Schematic model of redox signalling occurring in different cell compartments in response to stress. After stress, ROS can be generated in the apoplast by respiratory burst oxidase homologs (RBOHD), which is activated by a calcium influx, which in turn phosphorylates (P) the protein. Redox signalling in the apoplast can be sensed by changes in ASC oxidative state (↓ASC/DHA). In addition, redox signal can be transmitted by changes in the redox-sensitive proteins, such as the receptor-like kinases (RLK) and the K^+ channel (SKOR), localized on the plasma membrane. It is also possible that H_2O_2 migrates inside the cell through aquaporins (AQP) and is transduced into the cytosol. Redox imbalances occurring in the organelles could participate to retrograde signalling through the action of proteins with double localization. In particular Whirly1 (WHY1), changing its redox state and conformation, can move from chloroplasts to the nucleus where it stimulates gene transcription. The membrane-associated NAC protein, ANAC013, located in the endoplasmic reticulum, in response to ROS can undergo proteolytic activation and move into the nucleus where it induces the expression of genes conferring stress tolerance. Moreover, ROS from the organelles can pass in the cytosol. Changes in cytosolic redox homeostasis, due to interaction between ROS accumulation and antioxidant systems, can be transduced by redox-dependent modifications of redox-sensitive signalling proteins. More details are given in the text

generated in the apoplast, as part of the ROS wave, could enter a non-activated cell and trigger the release of calcium, which in turn phosphorylates and activates the RBOHD proteins; turning on ROS production, this new activated cell participates to the activated group of cells involved in the ROS wave (Suzuki et al. 2013; Gilroy et al. 2014). Thus, the ROS wave travels in the apoplast from the initiating tissue to the whole plant and, together with abscisic acid, is responsible for the activation of systemic acquired acclimation in response to local environmental stimuli (Suzuki et al. 2013; Mittler and Blumwald 2015).

Different environmental stresses cause redox imbalances principally in the organelles. Chloroplasts and mitochondria can respond either by rapidly fine tuning

the altered electron fluxes or by inducing changes in gene expression for long-term adaptation (Scheibe et al. 2005; Rhoads et al. 2006; Rhoads and Subbaiah 2007). In the latter case, the imbalance in the electron transport chains must initiate signalling processes that have to be sensed in the nucleus, with the results of changes in the expression of genes coding for proteins located in the organelles; such pathway has been identified as retrograde signalling (Nott et al. 2006; Rhoads and Subbaiah 2007; Leister 2012). Many actors have been proposed to function in retrograde signalling (reviewed in Kleine and Leister 2016) and among these ROS, and in particular H_2O_2, produced in the organelles could represent good intermediates, in particular for acclimation to stress (Rhoads et al. 2006; Petrov and Van Breusegem 2012; Galvez-Valdivieso and Mullineaux 2010). Another appealing hypothesis regarding the redox control of retrograde signalling is linked to the possibility that proteins with double localization move from the organelles to the nucleus, in order to directly mediate alteration in gene transcription (Fig. 1.3). An interesting example is the single-stranded DNA-binding protein Whirly1 (WHY1), which has been identified both in the nucleus and in the chloroplasts, where it is situated between thylakoids and nucleoids (Krause et al. 2005; Grabowski et al. 2008). WHY1 is able to move from the chloroplasts to the nucleus where it stimulates the transcription of PR genes (Isemer et al. 2012). The WHY proteins have a cysteine residue in a conserved region involved in the formation of disulphide bridges; they form tetramers and are also able to assemble into oligomers (Desveaux et al. 2002; Cappadocia et al. 2012); redox regulation has been demonstrated for the chloroplastic WHY3 protein (Stroher and Dietz 2008). Consequently, it has been speculated that stress-induced over-reduction of thylakoidal electron transport chain could destabilize the oligomeric WHY1, probably acting on cysteine residues, and release the monomer that will be then translocated to the nucleus. In this way, WHY1 might work as a redox-regulated element in chloroplastic retrograde signalling, involved in acclimation and immunity responses (Foyer et al. 2014). An example of this kind of regulation has been also proposed for mitochondrial retrograde signalling. The membrane-associated NAC protein, ANAC013, under non-stressed conditions is located in the endoplasmic reticulum. This compartment can be physically connected with mitochondria (Hayashi et al. 2009). Through these physical connections, mitochondrial ROS, implicated in retrograde signalling (Rhoads and Subbaiah 2007), could facilitate the proteolytic activation of ANAC013. In this way, ANAC013 migrates into the nucleus where it binds and activates the mitochondrial dysfunction motif(s), inducing gene expression that confers tolerance to oxidative stress (De Clercq et al. 2013).

In addition to the apoplast and the organelles, the cytosol, although not directly involved in ROS production, plays a key role in the integration of redox signals (Noctor and Foyer 2016). Indeed, it has been shown that different stresses are able to render the cytosolic environment more oxidized (Meyer et al. 2007; Jubany-Mari et al. 2010). Moreover, in *cat2* mutants the deficiency in catalase, which is primarily, localized in the peroxisomes, mainly impacts the transcription of cytosolic antioxidant enzymes (Rahantaniaina et al. 2013). The redox-dependent modification of proteins could be a principal way of redox signalling within this

compartment (Fig. 1.3). For instance, redox-dependent modifications of the translational apparatus in the cytosol permit a fast and efficient control of protein synthesis (Moore et al. 2016).

1.4.2 The Role of Redox-Sensitive Proteins in Signal Transduction

Since different stresses can induce a diverse locally restricted ROS production, it is possible that the specific redox signalling is determined by microenvironments (Terada 2006; Zachgo et al. 2013). Changes in the redox state of specific cell compartments or microenvironments have to be sensed and transduced. A potential way of perception and signalling engages the redox-dependent modification of proteins and in particular the modifications of cysteinyl residues, which can be oxidized to different degrees. The redox-dependent post-translational modifications of proteins comprise the formation of disulphide bridges, sulphenic, sulphinic and sulphonic acids as well as *S*-glutathionylation and *S*-nitrosylation, due to interaction of cysteine with GSH and nitric oxide (NO), respectively. These modifications can determine alteration in protein conformation and activity. Except for the formation of sulphonic acid, all other redox modifications are virtually reversible (Ghezzi et al. 2005). The reversible oxidation/reduction of the redox-sensitive proteins can be mediated directly by ROS or indirectly via the redox-sensitive molecules GSH, TRX and GRX, which, as discussed above, control the cellular redox environment (Foyer and Noctor 2005). In this perspective, a key role in the perception of cellular redox environment has been attributed to peroxiredoxins. For instance, chloroplastic peroxiredoxins, changing their aggregation state in function of their oxidative state, can act as sensors of oxidative stress and initiators of a signalling cascade that involves a multiplicity of protein–protein interactions that link redox changes with the necessary responses (Dietz 2008; Muthuramalingam et al. 2009).

Redox-sensitive proteins comprise metabolic enzymes that directly adjust cellular metabolism to the changing environment and redox-sensitive signalling proteins that perform their tasks through downstream components, such as kinases, phosphatases and transcription factors (Foyer and Noctor 2005, 2013; Dietz 2008).

Transcription factors (TFs) can also be direct targets of redox-dependent modulation of their activity (Dietz 2014). Among the directly redox-regulated TFs involved in the stress response, attention has been paid to the study of the heat shock factors (HSFs), since it was previously shown that these proteins are redox regulated in animals (Ahn and Thiele 2003; Miller and Mittler 2006). HSFs act by binding to the highly conserved heat shock element in the promoters of target genes. A great number of HSF genes are present in the plant genome, and the HSF network is extremely plastic and controls the response of plants to various stress conditions (Miller and Mittler 2006). Recently, it has been reported that heat stress and H_2O_2 treatment activate the Arabidopsis HSFA1, inducing its binding to the

promoters of two heat shock proteins; this binding can be reversed by reducing agents. Thus, it has been proposed that the activation of HSFA1 is redox-regulated, although the mechanisms of this activation have not been clearly explained (Liu et al. 2013). A redox-dependent regulation of another Arabidopsis HSF, the HSFA8, has been described with more details. H_2O_2 treatment of Arabidopsis protoplasts causes a translocation of HSFA8 to the nucleus. Interestingly, the site-directed mutagenesis of two conserved cysteine residues blocks this translocation. Therefore, a role for HSFA8 as a redox sensing TF in the stress-responsive transcriptional network has been suggested (Giesguth et al. 2015).

One of the best studied events of redox signalling regards the salicylic acid (SA)-mediated induction of PR proteins, occurring through the NPR1 (non-expressor of PR genes1) protein. NPR1 is a key regulator in the plant defence against pathogens (Pajerowska-Mukhtar et al. 2013); NPR1 is also involved in the response to some abiotic stress (Jayakannan et al. 2015). Nuclear localization of NPR1 is essential for the induction of PR genes (Kinkema et al. 2000). Indeed, NPR1 acts as a transcriptional co-activator that, interacting with the TFs of the TGA (TGACG motif binding factor) family, regulates their DNA-binding activity to the promoters of PR genes, thus inducing transcription (Despres et al. 2003). NPR1 can be found in the cytosol as oligomers, linked through intermolecular disulphide bridges, or in the nucleus as monomers that are the active form of the protein. Pathogen infection or SA treatment, modulating the cellular redox environment, can control the redox state of NPR1 (Mou et al. 2003). The reduction of the oligomeric form to the monomeric one is catalysed by TRX; this reduction permits the nuclear translocation of NPR1; on the other hand, the oligomerization of NPR1 is facilitated by *S*-nitrosylation (Tada et al. 2008). The importance of the redox-dependent changes in NPR1 status and localization has been amply reported. Indeed, in an *apx* mutant and in CAT antisense plants that accumulate H_2O_2, nuclear translocation of NPR1 and induction of PR genes are prevented (Peleg-Grossman et al. 2010). Similarly, treatment with reduced GSH or oxidizing conditions promotes or inhibits, respectively, the nuclear accumulation of NPR1 (Kovacs et al. 2015). The translocation of NPR1 into the nucleus is also promoted by the NO donor nitrosoglutathione (GSNO) (Lindermayr et al. 2010). However, GSNO-induced nuclear translocation is not due to the *S*-nitrosylation of NPR1, but rather due to the action of GSNO as a positive effector upstream of SA. An interesting crosstalk between NO and GSH for the control of NPR1 activity has been proposed (Kovacs et al. 2015). It is known that pathogen attack induces an NO burst that dynamically modulates the redox state of glutathione (Vanacker et al. 2000; Mou et al. 2003; Koornneef et al. 2008). Since NO fumigation transiently increases the oxidation of GSH and leads to an increase in the total GSH pool, it has been suggested that the initial oxidation of GSH promotes de novo GSH biosynthesis and is necessary for SA accumulation and the NPR1-dependent defence signalling pathway (Kovacs et al. 2015). In addition to the redox-controlled translocation into the nucleus, there is also evidence for the contribution of a redox control of TFs in the nucleus. For instance, a redox regulation of the TGAs activity has been proposed. Under oxidizing conditions, TGA1 is in an inactive conformation, due to the formation of intermolecular

disulphide bridges between cysteinyl residues (Despres et al. 2003; Lindermayr et al. 2010). However, TGA1 DNA-binding activity is considerably enhanced in presence of GSNO, probably for glutathionylation or *S*-nitrosylation of the Cys 260 and Cys 266; these redox modifications could be responsible of conformational changes of TGA that enhance its activity (Lindermayr et al. 2010). Interestingly, the redox-sensitive GRXs have been suggested as possible mediators of the redox-dependent changes of TGAs (Ndamukong et al. 2007; Li et al. 2011).

1.5 Conclusion and Perspectives

A large number of molecular components are involved in the control of cellular redox homeostasis. The various ROS, produced in different cell compartments and a plethora of antioxidants, finely regulate the cellular redox environment. Thus, the interactions between ROS and antioxidants constitute a strong network, able to give information on cellular environment. The imbalances in this network, occurring under stress conditions, act as redox signals that have to be transduced in order to induce specific adaptive responses. The redox-dependent signalling is not linear; on the contrary, it is defined by complex and dynamic pathways, which can be connected and sometimes overlapping. Each redox-dependent interaction is opportunely regulated by a restricted environment, whose changes are of primary importance to transfer the complex system of information. Different cellular compartments are involved in mediating redox-dependent signalling. For this reason, it is very important to shed light on the role of each compartment and the interaction among them in the transfer of redox-dependent signals in response to specific stresses. Undoubtedly, it will be necessary to take into account also the diversity of ROS and antioxidants that participate in the specific response.

Another important point that needs to be deepened regards the mode of transmission of the redox-dependent signals. Many data suggest that reversible oxidation/reduction of redox-sensitive proteins could have a key role in signal transduction. An important step is to understand if these modifications are induced directly by ROS or indirectly by changes in the oxidative state of the redox-sensitive molecules GSH, TRX and GRX. Moreover, the identification of new redox-sensitive signalling proteins involved in specific environmental conditions could help to identify common and divergent points of redox regulation in the response to different kinds of stress.

References

Abbasi AR, Saur A, Hennig P, Tschiersch H, Hajirezaei M, Hofius D, Sonnewald U, Voll LM (2009) Tocopherol deficiency in transgenic tobacco (*Nicotiana tabacum* L.) plants leads to accelerated senescence. Plant Cell Environ 32:144–157

Ahn SG, Thiele DJ (2003) Redox regulation of mammalian heat shock factor 1 is essential for Hsp gene activation and protection from stress. Gene Dev 17:516–528

Alscher RG, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53:1331–1341

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

Asada K (1999) The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. Annl Rev Plant Physiol Plant Mol Biol 50:601–639

Ashtamker C, Kiss V, Sagi M, Davydov O, Fluhr R (2007) Diverse subcellular locations of cryptogein-induced reactive oxygen species production in tobacco bright yellow-2 cells. Plant Physiol 143:1817–1826

Barth C, Moeder W, Klessig DF, Conklin PL (2004) The timing of senescence and response to pathogens is altered in the ascorbate-deficient Arabidopsis mutant vitamin c-1. Plant Physiol 134:1784–1792

Bela K, Horvath E, Galle A, Szabados L, Tari I, Csiszar J (2015) Plant glutathione peroxidases: emerging role of the antioxidant enzymes in plant development and stress responses. J Plant Physiol 176:192–201

Berkowitz O, De Clercq I, Van Breusegem F, Whelan J (2016) Interaction between hormonal and mitochondrial signalling during growth, development and in plant defence responses. Plant Cell Environ 39:1127–1139

Bhatt I, Tripathi BN (2011) Plant peroxiredoxins: catalytic mechanisms, functional significance and future perspectives. Biotechnol Adv 29:850–859

Bienert GP, Moller ALB, Kristiansen KA, Schulz A, Moller IM, Schjoerring JK, Jahn TP (2007) Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 282:1183–1192

Blomster T, Salojarvi J, Sipari N, Brosche M, Ahlfors R, Keinanen M, Overmyer K, Kangasjarvi J (2011) Apoplastic reactive oxygen species transiently decrease auxin signaling and cause stress-induced morphogenic response in Arabidopsis. Plant Physiol 157:1866–1883

Cappadocia L, Parent JS, Zampini E, Lepage E, Sygusch J, Brisson N (2012) A conserved lysine residue of plant Whirly proteins is necessary for higher order protein assembly and protection against DNA damage. Nucl Acid Res 40:258–269

Caverzan A, Passaia G, Rosa SB, Ribeiro CW, Lazzarotto F, Margis-Pinheiro M (2012) Plant responses to stresses: Role of ascorbate peroxidase in the antioxidant protection. Genet Mol Biol 35:1011–1019

Chaouch S, Noctor G (2010) Myo-inositol abolishes salicylic acid-dependent cell death and pathogen defence responses triggered by peroxisomal hydrogen peroxide. New Phytol 188:711–718

Chaouch S, Queval G, Vanderauwera S, Mhamdi A, Vandorpe M, Langlois-Meurinne M, Van Breusegem F, Saindrenan P, Noctor G (2010) Peroxisomal hydrogen peroxide is coupled to biotic defense responses by ISOCHORISMATE SYNTHASE1 in a daylength-related manner. Plant Physiol 153:1692–1705

Chew O, Whelan J, Millar AH (2003) Molecular definition of the ascorbate-glutathione cycle in Arabidopsis mitochondria reveals dual targeting of antioxidant defenses in plants. J Biol Chem 278:46869–46877

Daudi A, Cheng ZY, 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

Davletova S, Rizhsky L, Liang H, Shengqiang Z, Oliver DJ, Coutu J, Shulaev V, Schlauch K, Mittler R (2005) Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis. Plant Cell 17:268–281

De Clercq I, Vermeirssen V, Van Aken O, Vandepoele K, Murcha MW, Law SR, Inze A, Ng S, Ivanova A, Rombaut D, van de Cotte B, Jaspers P, Van de Peer Y, Kangasjarvi J, Whelan J, Van Breusegem F (2013) The membrane-bound NAC transcription factor ANAC013 functions

in mitochondrial retrograde regulation of the oxidative stress response in Arabidopsis. Plant Cell 25:3472–3490
De Gara L, de Pinto MC, Tommasi F (2003) The antioxidant systems vis-a-vis reactive oxygen species during plant-pathogen interaction. Plant Physiol Biochem 41:863–870
de Pinto MC, De Gara L (2004) Changes in the ascorbate metabolism of apoplastic and symplastic spaces are associated with cell differentiation. J Exp Bot 55:2559–2569
de Pinto MC, Francis D, De Gara L (1999) The redox state of the ascorbate-dehydroascorbate pair as a specific sensor of cell division in tobacco BY-2 cells. Protoplasma 209:90–97
de Pinto MC, Paradiso A, Leonetti P, De Gara L (2006) Hydrogen peroxide, nitric oxide and cytosolic ascorbate peroxidase at the crossroad between defence and cell death. Plant J 48:784–795
de Pinto MC, Locato V, De Gara L (2012) Redox regulation in plant programmed cell death. Plant Cell Environ 35:234–244
de Pinto MC, Locato V, Sgobba A, Romero-Puertas MD, Gadaleta C, Delledonne M, De Gara L (2013) S-Nitrosylation of ascorbate peroxidase is part of programmed cell death signaling in tobacco bright yellow-2 cells. Plant Physiol 163:1766–1775
de Pinto MC, Locato V, Paradiso A, De Gara L (2015) Role of redox homeostasis in thermo-tolerance under a climate change scenario. Ann Bot 116:487–496
del Río LA, Sandalio LM, Corpas FJ, Palma JM, Barroso JB (2006) Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. Plant Physiol 141:330–335
Despres C, Chubak C, Rochon A, Clark R, Bethune T, Desveaux D, Fobert PR (2003) The Arabidopsis NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. Plant Cell 15:2181–2191
Desveaux D, Allard J, Brisson N, Sygusch J (2002) A new family of plant transcription factors displays a novel ssDNA-binding surface. Nat Struct Biol 9:512–517
Dietz KJ (2008) Redox signal integration: from stimulus to networks and genes. Physiol Plant 133:459–468
Dietz KJ (2014) Redox regulation of transcription factors in plant stress acclimation and development. Antioxid Redox Sign 21:1356–1372
Ding L, Xu H, Yi H, Yang L, Kong Z, Zhang L, Xue S, Jia H, Ma Z (2011) Resistance to hemi-biotrophic *F. graminearum* infection is associated with coordinated and ordered expression of diverse defense signaling pathways. PLoS ONE 6:e19008
Ding SH, Lu QT, Zhang Y, Yang ZP, Wen XG, Zhang LX, Lu CM (2009) Enhanced sensitivity to oxidative stress in transgenic tobacco plants with decreased glutathione reductase activity leads to a decrease in ascorbate pool and ascorbate redox state. Plant Mol Biol 69:577–592
Edwards EA, Rawsthorne S, Mullineaux PM (1990) Subcellular distribution of multiple forms of glutathione reductase in leaves of pea (*Pisum sativum* L.). Planta 180:278–284
Ellouzi H, Hamed KB, Cela J, Muller M, Abdelly C, Munné-Bosch S (2013) Increased sensitivity to salt stress in tocopherol-deficient Arabidopsis mutants growing in a hydroponic system. Plant Signal Behav 8:e23136
Faize M, Burgos L, Faize L, Petri C, Barba-Espin G, Díaz-Vivancos P, Clemente-Moreno MJ, Alburquerque N, Hernandez JA (2012) Modulation of tobacco bacterial disease resistance using cytosolic ascorbate peroxidase and Cu, Zn-superoxide dismutase. Plant Pathol 61:858–866
Foyer CH, Noctor G (2003) Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119:355–364
Foyer CH, Noctor G (2005) Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ 28:1056–1071
Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155:2–18
Foyer CH, Noctor G (2012) Managing the cellular redox hub in photosynthetic organisms. Plant Cell Environ 35:199–201

Foyer CH, Noctor G (2013) Redox signaling in plants. Antioxid Redox Sign 18:2087–2090
Foyer CH, Noctor G (2016) Stress-triggered redox signalling: what's in pROSpect? Plant Cell Environ 39:951–964
Foyer CH, Karpinska B, Krupinska K (2014) The functions of Whirly1 and redox-responsive transcription factor 1 in cross tolerance responses in plants: a hypothesis. Philos Trans Roy Soc Lond B Biol Sci 369:20130226
Frendo P, Baldacci-Cresp F, Benyamina SM, Puppo A (2013) Glutathione and plant response to the biotic environment. Free Radic Biol Med 65:724–730
Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K (2006) Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9:436–442
Gallie DR (2013) The role of L-ascorbic acid recycling in responding to environmental stress and in promoting plant growth. J Exp Bot 64:433–443
Galvez-Valdivieso G, Mullineaux PM (2010) The role of reactive oxygen species in signalling from chloroplasts to the nucleus. Physiol Plant 138:430–439
García-Mata C, Wang J, Gajdanowicz P, Gonzalez W, Hills A, Donald N, Riedelsberger J, Amtmann A, Dreyer I, Blatt MR (2010) A minimal cysteine motif required to activate the SKOR K + channel of Arabidopsis by the reactive oxygen species H_2O_2. J Biol Chem 285:29286–29294
Geigenberger P, Fernie AR (2014) Metabolic control of redox and redox control of metabolism in plants. Antioxid Redox Sign 21:1389–1421
Ghezzi P, Bonetto V, Fratelli M (2005) Thiol-disulfide balance: from the concept of oxidative stress to that of redox regulation. Antioxid Redox Sign 7:964–972
Giesguth M, Sahm A, Simon S, Dietz KJ (2015) Redox-dependent translocation of the heat shock transcription factor AtHSFA8 from the cytosol to the nucleus in *Arabidopsis thaliana*. FEBS Lett 589:718–725
Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930
Gill SS, Anjum NA, Hasanuzzaman M, Gill R, Trivedi DK, Ahmad I, Pereira E, Tuteja N (2013) Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. Plant Physiol Biochem 70:204–212
Gill SS, Anjum NA, Gill R, Yadav S, Hasanuzzaman M, Fujita M, Mishra P, Sabat SC, Tuteja N (2015) Superoxide dismutase-mentor of abiotic stress tolerance in crop plants. Environ Sci Pollut Res Int 22:10375–10394
Gilroy S, Suzuki N, Miller G, Choi WG, Toyota M, Devireddy AR, Mittler R (2014) A tidal wave of signals: calcium and ROS at the forefront of rapid systemic signaling. Trend Plant Sci 19:623–630
Grabowski E, Miao Y, Mulisch M, Krupinska K (2008) Single-stranded DNA-binding protein Whirly1 in barley leaves is located in plastids and the nucleus of the same cell. Plant Physiol 147:1800–1804
Halliwell B (2006) Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiol 141:312–322
Han Y, Chaouch S, Mhamdi A, Queval G, Zechmann B, Noctor G (2013) Functional analysis of Arabidopsis mutants points to novel roles for glutathione in coupling H(2)O(2) to activation of salicylic acid accumulation and signaling. Antioxid Redox Sign 18:2106–2121
Hayashi T, Rizzuto R, Hajnoczky G, Su TP (2009) MAM: more than just a housekeeper. Trends Cell Biol 19:81–88
Hirayama T, Shinozaki K (2010) Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant J 61:1041–1052
Holler S, Ueda Y, Wu L, Wang Y, Hajirezaei MR, Ghaffari MR, von Wiren N, Frei M (2015) Ascorbate biosynthesis and its involvement in stress tolerance and plant development in rice (*Oryza sativa* L.). Plant Mol Biol 88:545–560
Horemans N, Foyer CH, Asard H (2000) Transport and action of ascorbate at the plant plasma membrane. Trend Plant Sci 5:263–267

Huang S, Van Aken O, Schwarzlander M, Belt K, Millar AH (2016) Roles of mitochondrial reactive oxygen species in cellular signalling and stress response in plants. Plant Physiol 171:1551–1559

Isemer R, Mulisch M, Schafer A, Kirchner S, Koop HU, Krupinska K (2012) Recombinant Whirly1 translocates from transplastomic chloroplasts to the nucleus. FEBS Lett 586:85–88

Jaspers P, Kangasjarvi J (2010) Reactive oxygen species in abiotic stress signaling. Physiol Plant 138:405–413

Jayakannan M, Bose J, Babourina O, Shabala S, Massart A, Poschenrieder C, Rengel Z (2015) The NPR1-dependent salicylic acid signalling pathway is pivotal for enhanced salt and oxidative stress tolerance in Arabidopsis. J Exp Bot 66:1865–1875

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

Jubany-Mari T, Alegre-Batlle L, Jiang K, Feldman LJ (2010) Use of a redox-sensing GFP (c-roGFP1) for real-time monitoring of cytosol redox status in *Arabidopsis thaliana* water-stressed plants. FEBS Lett 584:889–897

Kataya AR, Reumann S (2010) Arabidopsis glutathione reductase 1 is dually targeted to peroxisomes and the cytosol. Plant Signal Behav 5:171–175

Kavitha K, George S, Venkataraman G, Parida A (2010) A salt-inducible chloroplastic monodehydroascorbate reductase from halophyte *Avicennia marina* confers salt stress tolerance on transgenic plants. Biochimie 92:1321–1329

Kinkema M, Fan W, Dong X (2000) Nuclear localization of NPR1 is required for activation of PR gene expression. Plant Cell 12:2339–2350

Kleine T, Leister D (2016) Retrograde signaling: organelles go networking. Biochim Biophys Acta 1857:1313–1325

Koornneef A, Leon-Reyes A, Ritsema T, Verhage A, Den Otter FC, Van Loon LC, Pieterse CMJ (2008) Kinetics of salicylate-mediated suppression of jasmonate signaling reveal a role for redox modulation. Plant Physiol 147:1358–1368

Kovacs I, Durner J, Lindermayr C (2015) Crosstalk between nitric oxide and glutathione is required for NONEXPRESSOR OF PATHOGENESIS-RELATED GENES 1 (NPR1)-dependent defense signaling in *Arabidopsis thaliana*. New Phytol 208:860–872

Krause K, Kilbienski I, Mulisch M, Rodiger A, Schafer A, Krupinska K (2005) DNA-binding proteins of the Whirly family in *Arabidopsis thaliana* are targeted to the organelles. FEBS Lett 579:3707–3712

Lanubile A, Bernardi, Marocco A., Logrieco A. Paciolla C (2012) Differential activation of defense genes and enzymes in maize genotypes with contrasting levels of resistance to *Fusarium verticillioides*. Environ Exp Bot 78:39–46

Lanubile A, Maschietto V, De Leonardis S, Battilani P, Paciolla C, Marocco A (2015) Defense responses to mycotoxin-producing fungi *Fusarium proliferatum*, *F. subglutinans*, and *Aspergillus flavus* in kernels of susceptible and resistant maize genotypes. Mol Plant Microbe Inter 28:546–557

Leister D (2012) Retrograde signaling in plants: from simple to complex scenarios. Front Plant Sci 3:135

Leshem Y, Melamed-Book N, Cagnac O, Ronen G, Nishri Y, Solomon M, Cohen G, Levine A (2006) Suppression of Arabidopsis vesicle-SNARE expression inhibited fusion of H_2O_2-containing vesicles with tonoplast and increased salt tolerance. Proc Nat Acad Sci U S A 103:18008–18013

Li ST, Gutsche N, Zachgo S (2011) The ROXY1 C-Terminal L**LL motif is essential for the interaction with TGA transcription factors. Plant Physiol 157:2056–2068

Lindermayr C, Sell S, Muller B, Leister D, Durnera J (2010) Redox regulation of the NPR1-TGA1 system of *Arabidopsis thaliana* by nitric oxide. Plant Cell 22:2894–2907

Liu X, Hua X, Guo J, Qi D, Wang L, Liu Z, Jin Z, Chen S, Liu G (2008) Enhanced tolerance to drought stress in transgenic tobacco plants overexpressing VTE1 for increased tocopherol production from *Arabidopsis thaliana*. Biotechnol Lett 30:1275–1280

Liu YF, Zhang CX, Chen J, Guo LH, Li XL, Li WP, Yu ZF, Deng JS, Zhang PY, Zhang KQ, Zhang LM (2013) Arabidopsis heat shock factor HsfA1a directly senses heat stress, pH changes, and hydrogen peroxide via the engagement of redox state. Plant Physiol Biochem 64:92–98

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

Maddison J, Lyons T, Plochl M, Barnes J (2002) Hydroponically cultivated radish fed L-galactono-1,4-lactone exhibit increased tolerance to ozone. Planta 214:383–391

Mandal S, Acharya P, Kar I (2014) Reactive oxygen species signaling in eggplant in response to *Ralstonia solanacearum* infection. J Plant Pathol 96:525–534

Meyer AJ, Brach T, Marty L, Kreye S, Rouhier N, Jacquot JP, Hell R (2007) Redox-sensitive GFP in *Arabidopsis thaliana* is a quantitative biosensor for the redox potential of the cellular glutathione redox buffer. Plant J 52:973–986

Mhamdi A, Noctor G, Baker A (2012) Plant catalases: peroxisomal redox guardians. Arch Biochem Biophys 525:181–194

Mhamdi A, Queval G, Chaouch S, Vanderauwera S, Van Breusegem F, Noctor G (2010) Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. J Exp Bot 61:4197–4220

Miller EW, Dickinson BC, Chang CJ (2010a) Aquaporin-3 mediates hydrogen peroxide uptake to regulate downstream intracellular signaling. Proc Nat Acad Sci U S A 107:15681–15686

Miller G, Mittler R (2006) Could heat shock transcription factors function as hydrogen peroxide sensors in plants? Ann Bot 98:279–288

Miller G, Suzuki N, Rizhsky L, Hegie A, Koussevitzky S, Mittler R (2007) Double mutants deficient in cytosolic and thylakoid ascorbate peroxidase reveal a complex mode of interaction between reactive oxygen species, plant development, and response to abiotic stresses. Plant Physiol 144:1777–1785

Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R (2010b) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ 33:453–467

Mittler R, Blumwald E (2015) The roles of ROS and ABA in systemic acquired acclimation. Plant Cell 27:64–70

Mittler R, Feng X, Cohen M (1998) Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. Plant Cell 10:461–473

Mittler R, Vanderauwera S, Suzuki N, Miller G, Tognetti VB, Vandepoele K, Gollery M, Shulaev V, Van Breusegem F (2011) ROS signaling: the new wave? Trend Plant Sci 16:300–309

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

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

Møller IM, Rogowska-Wrzesinska A, Rao RSP (2011) Protein carbonylation and metal-catalyzed protein oxidation in a cellular perspective. J Proteom 74:2228–2242

Moore M, Gossmann N, Dietz KJ (2016) Redox regulation of cytosolic translation in plants. Trend Plant Sci 21:388–397

Mou Z, Fan WH, Dong XN (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell 113:935–944

Munné-Bosch S (2005) The role of alpha-tocopherol in plant stress tolerance. J Plant Physiol 162:743–748

Munné-Bosch S, Alegre L (2000) Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. Planta 210:925–931