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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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José M. Palma has more than 30 years experience in plant sciences and related fields. He also served as the deputy director and later director of the Estación Experimental del Zaidín (EEZ-CSIC), Granada, Spain. He has published more than 100 research papers/review articles in peer reviewed journals and edited five books.

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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

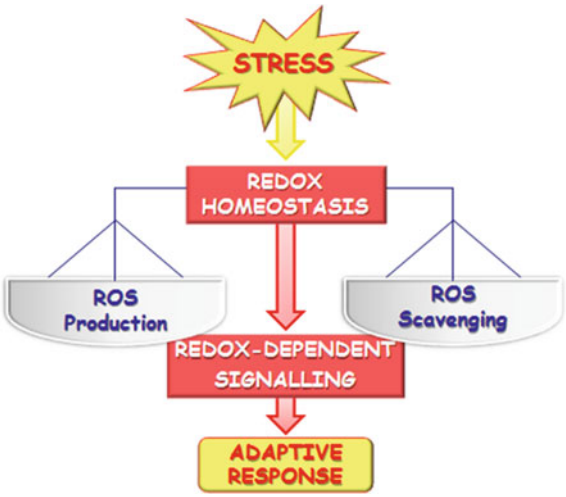

Fig. 1.1 Redox homeostasis, due to the balance among ROS production and scavenging, is altered in stress conditions and activates a redox-dependent signalling that trigger the adaptive response

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