

PREFACE: PART OF A SPECIAL ISSUE ON REACTIVE OXYGEN AND NITROGEN SPECIES

Unravelling how plants benefit from ROS and NO reactions, while resisting oxidative stress

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- **Background and Aims** Reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as nitric oxide (NO), play crucial roles in the signal transduction pathways that regulate plant growth, development and defence responses, providing a nexus of reduction/oxidation (redox) control that impacts on nearly every aspect of plant biology. Here we summarize current knowledge and concepts that lay the foundations of a new vision for ROS/RNS functions – particularly through signalling hubs – for the next decade.
- **Scope** Plants have mastered the art of redox control using ROS and RNS as secondary messengers to regulate a diverse range of protein functions through redox-based, post-translational modifications that act as regulators of molecular master-switches. Much current focus concerns the impact of this regulation on local and systemic signalling pathways, as well as understanding how such reactive molecules can be effectively used in the control of plant growth and stress responses.
- **Conclusions** The spectre of oxidative stress still overshadows much of our current philosophy and understanding of ROS and RNS functions. While many questions remain to be addressed – for example regarding inter-organellar regulation and communication, the control of hypoxia and how ROS/RNS signalling is used in plant cells, not only to trigger acclimation responses but also to create molecular memories of stress – it is clear that ROS and RNS function as vital signals of living cells.

Key words: Reactive oxygen species, ROS, reactive nitrogen species, RNS, nitric oxide, NO, oxidative stress, plant signalling, redox.

Plants monitor their surroundings continuously and adjust their metabolic systems accordingly to optimize productivity. Plant cells display a high degree of plasticity, enabling them to withstand or acclimate to the swathe of environmental forces they are exposed to on a daily basis because of their sedentary habit. This plasticity is reflected in the dynamics of cellular reduction/oxidation (redox) processes, particularly the regulated accumulation of reactive oxygen species (ROS), which play important roles in signalling appropriate responses to changes in parameters such as light intensity or water availability. Stress-induced metabolic imbalances can lead to the activation of NADPH oxidases and increased production of ROS from other sources such as mitochondria, chloroplasts and peroxisomes. ROS accumulation leads to oxidative signalling. Similarly, the generation of nitric oxide (NO) in plants is important in the regulation of plant growth and defence responses. NO and its derivatives (reactive nitrogen species, RNS) are also produced in cells experiencing abiotic stress. Increasing evidence suggests that NO production in plants is linked, at least in part, to nitrogen assimilation through the conversion of nitrite to NO by the enzyme nitrate reductase, and that NO functions in a feedback loop that regulates plant nitrogen fluxes.

Considerable progress has been made in our understanding of the complexities of ROS and NO interactions over the last 10 years. Accumulating evidence in the literature demonstrates that ROS and RNS play key roles as biological messengers, functioning together to facilitate appropriate responses to biotic and abiotic stresses. ROS/RNS interactions are also important in the regulation of plant development and in the regulation of a wide range of physiological responses to the environment, from germination, root development and gravitropism to stomatal closure. The scope of this volume was influenced by a workshop held at the International University of Andalusia (UNIA) in Baeza, Spain to celebrate the achievements of Professor Luis del Rio (Fig. 1), who has dedicated a lifetime of research to this crucial topic. Our aim in this volume is to present recent advances in our understanding of how plant cells manage ROS and NO signalling, particularly the interactions between these redox signals, antioxidants and proteins that function as switches to regulate gene and pathway function in response to environmental and developmental triggers. The articles provide snapshots of key areas of current endeavour, together with overviews of key topics within this ever-growing field of intensive research interest. Taken together, the findings presented in this



Fig. 1. Professors Francisca Sevilla Valenzuela, Luisa Maria Sandalio, Luis Del Rio and Christine Helen Foyer at the workshop titled ‘Oxygen and nitrogen reactive species: generating a new vision for 2020’, that was held at the International University of Andalusia (UNIA) in Baeza, in October 2014.

volume demonstrate that ROS and NO impact on nearly every aspect of plant biology, making an important contribution to the cellular information exchange that governs cell function and cell fate. Several studies in this volume provide insights into the complexities of the redox regulation of cellular processes that govern plant stress responses. For example, Sandalio and Romero-Puertas (2015) provide a timely and useful overview of the central role played by peroxisomes in plant development and stress tolerance. They discuss how peroxisomes exert a strong influence on cellular redox processes through the generation of ROS and NO and associated signalling networks.

The crop varieties used in today’s agriculture have been selected largely on the basis of increased yields and not for stress-tolerance traits. It is therefore unlikely that current crop varieties will be able to sustain high yields under the increasingly stressful conditions that are predicated to occur in the future. An increase in global temperatures is considered to be one of the most important consequences of climate change. The consensus view held by climate change scientists is that the predicted increases in global temperatures that will occur over the next 50 years will have a strong negative impact on the productivity of major cereal crops such as wheat. Reductions in major crop yields of nearly 20 % have been predicted for each degree Centigrade increase in temperature during the growing season. It is therefore important to have a profound knowledge and understanding of thermotolerance mechanisms in crop plants, a topic that is reviewed by de Pinto *et al.* (2015), with a view to developing redox solutions with which to overcome the negative impacts of high temperatures. This article focuses on the role of ROS, redox metabolites and antioxidant enzymes in

signalling pathways that activate plant cell responses. High temperatures are often associated with drought stress under field conditions. It is perhaps not surprising therefore that many parts of the world will experience drought more frequently in coming decades. Marquez-Garcia *et al.* (2015) highlight the role of redox signals in the source–sink relationships underpinning the symbiotic union between soybean and *Bradyrhizobium* species exposed to drought. Drought can undermine symbiotic nitrogen fixation; however, as the authors state, defining this system is akin to a ‘chicken and egg’ situation. By monitoring primary and nitrogen metabolism, as well as markers of redox status in the leaf and crown nodules, they show that the leaves of lowest photosynthetic capacity are sacrificed before the symbiotic nodules. Similarly, Simancas and Munne-Bosch (2015) describe a trade-off between vitamin E and phosphorus nutrition in arabidopsis plants. Strikingly, the vitamin E-deficient *vte1* (tocopherol cyclase) plants display increased longevity under severe phosphorus limitation, implying a conceptual priority of vitamin E synthesis over plant longevity, i.e. a ‘vitamin E cost’ to sustained life. The authors suggest that the trade-off extends to a balance between photoprotection and jasmonate-mediated plant defence. Roach *et al.* (2015) investigate the relationships between the regulation of photosynthesis and ROS release into the surrounding water by several algal species. The role of the enigmatic enzyme polyphenol oxidase in photosynthesis is investigated by Boeckx *et al.* (2015), who provide preliminary evidence of a role for this thylakoid lumen-localized enzyme in oxidative stress tolerance.

There are clear differences in the ways that ROS and NO are produced and managed in animals and plants, not least because of the presence of chloroplasts and photosynthesis in plants but not in animals. The roles of ROS and NO in the pathways of anterograde and retrograde signalling between the organelles and the nucleus are hotbeds of current plant science research, as are the mechanisms of inter-organelle cross-talk. In this context, Ozgur *et al.* (2015) seek to define how the different organelles in plant cells influence the ROS-mediated unfolded protein response (UPR) in the endoplasmic reticulum (ER). While the UPR serves to facilitate protein-folding and degradation of misfolded proteins, prolonged stress exposures can lead to excessive accumulation of misfolded proteins. This triggers ER-associated degradation (ERAD), leading to the phenomenon called ER-stress. Ozgur *et al.* have studied the transcriptional responses of a number of UPR-hallmark genes to various concentrations of ROS-producing agents that are specific to different organelles. The findings of their study expand our understanding of ER responses to multiple oxidative signals from different organelles in plants. The alternative oxidase AOX1a has become a hallmark of oxidative stress and is known to be a key player in mitochondria–chloroplast cross-talk, as well as organelle to nucleus retrograde signalling pathways. The study described by Vishwakarma *et al.* (2015) suggests that lack of a functional AOX1a in arabidopsis has a negative impact on photosynthesis.

The regulation of genetically programmed cell death events and related mechanisms are another widely studied topic within the context of plant responses to the environment. Ortiz-Espín *et al.* (2015) examine the potential of the mitochondrial thioredoxin TRXO1 to mitigate ROS-induced programmed cell death in tobacco BY-2 cells. Over-expression of *TRXO1* was shown

to decrease markers of oxidative stress, and lower the cellular H₂O₂ and NO levels as well as delaying cell death. These features are linked to an increase in catalase activity and a greater capacity to maintain the cellular glutathione pool in a highly reduced state. Within this context, [Pu et al. \(2015\)](#) provide a useful and insightful description of the critical importance of the mitochondrial electron transport proteins such as AOX in the control of cellular redox homeostasis, and [Jozefczak et al. \(2015\)](#) examine the effects of low antioxidant status on plant responses to the heavy metal cadmium. Using a range of arabidopsis mutants that have low glutathione contents (*cad2-1*), low ascorbate (*vtc1-1*) or both (*cad2-1 vtc1-1*), [Jozefczak et al.](#) present data showing that the *cad2-1* mutants have an increased sensitivity to cadmium, but the *vtc1-1* mutants have a lower sensitivity. These differences are explained in terms of low phytochelatin production in the *cad2-1* and *cad2-1 vtc1-1* lines, while the *vtc2-1* lines have a higher capacity for phytochelatin synthesis. Returning to crop species, [Feigl et al. \(2015\)](#) examine markers of oxidative and nitrosative stress to evaluate the relative sensitivities of *Brassica napus* (oilseed rape) and *B. juncea* (Indian mustard) to zinc toxicity. Declines in the primary root length, total root fresh weight and root meristem cell viability of *B. napus* show it is substantially more sensitive to increasing zinc concentrations (50–300 μM) than *B. juncea*. This sensitivity of *B. napus* is underscored by considerable lipid peroxidation in the roots as well as accumulation of superoxide, which is not seen in *B. juncea*. By contrast, markers of nitrosative stress do not discriminate the sensitivities of the two species.

Jose Palma and colleagues ([Palma et al., 2015](#)) provide an overview of our current knowledge of the fruit ripening process using capsicum as a model system for climacteric fruit. They highlight the roles of ascorbate and pyridine nucleotides in developmental and environmental stress conditions in their review

of this important topic. Evidence of a role for protein tyrosine nitration in the control of pepper fruit ripening is presented by [Chaki et al. \(2015\)](#). [Tanou et al. \(2015\)](#) describe the respective functions of oxidative processes caused by ozone exposure and NO signalling imposed by application of SNP on kiwifruit ripening, with a particular focus on changes to the fruit proteome. Data are presented to show that ozone-induced inhibition of fruit ripening could be prevented by NO, highlighting the interactions between these signalling pathways.

Crop yield is dependent on seed quality. ROS are closely associated with different aspects of seed physiology from birth to death that regulate dormancy and play a role in combatting pathogen infection, among others. The dual role of ROS in seed germination and in cell death is discussed by [Jeevan Kumar et al. \(2015\)](#). New data concerning the role of the cellular redox environment in seed dormancy, ageing and deterioration are presented by [Morscher et al. \(2015\)](#), who show that high oxygen concentrations favour seed dormancy and that breaking dormancy and loss of viability are associated with oxidation of proteins in the cytosol rather than oxidation of membrane lipids. Following seed germination, [Airaki et al. \(2015\)](#) describe the spatial and temporal production of ROS and RNS in pepper, linking these changes to seedling establishment. Protein S-nitrosylation can regulate the activity, localization or conformation of target proteins. The role of protein denitrosylation in auxin-mediated root development is explored by [Correa-Aragunde et al. \(2015\)](#), who demonstrate that NADPH-dependent thioredoxin reductase is a key player in this process and suggest that a feedback mechanism exists to control unregulated protein S-nitrosylation.

Like seed germination, the transition from quiescence to a metabolically active state during bud burst requires the management of a transition from hypoxia to atmospheric oxygen levels,

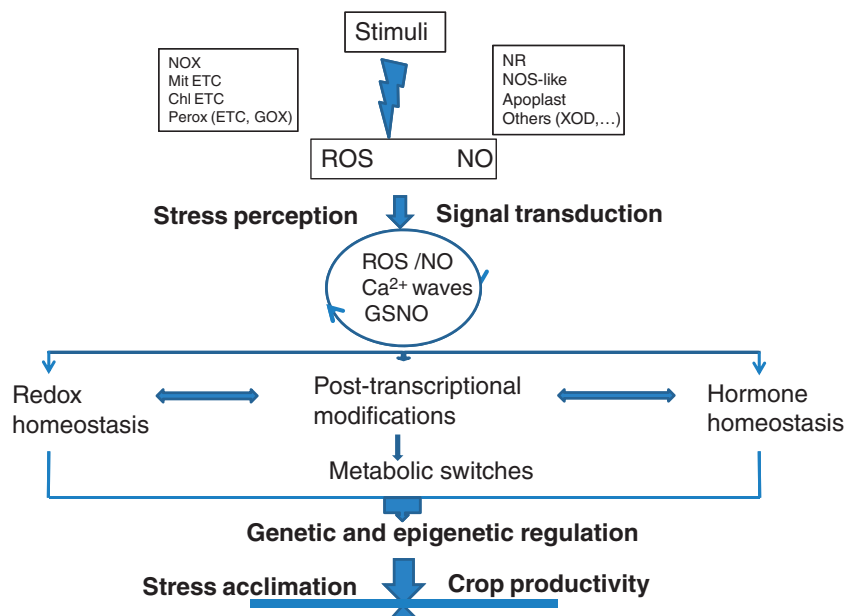


Fig. 2. A schematic diagram showing the central roles of reactive oxygen species (ROS) and nitric oxide (NO) in the signal transduction pathways that facilitate acclimation to stress allowing high crop productivity over a wide range of environmental conditions. ETC, respiratory electron transport chain; NOX, NADPH oxidases; Mit ETC, respiratory electron transport chain; Chl ETC, photosynthetic electron transport chain; Perox, peoxisomes; GOX, glycollate oxidase; NR, nitrate reductase; NOS-like, nitric oxide synthase-like enzymes; GSNO, S-Nitrosoglutathione.

together with the co-ordination of intercellular signalling networks including the cellular redox hub. Meitha *et al.* (2015) provide new information concerning the regulation of hypoxia release in ecodormant buds, showing that the transition to atmospheric oxygen levels upon budburst is accompanied by ROS accumulation in the developing vascular tissues of heterotrophic grapevine buds.

2-Cys peroxiredoxin A plays a dual role as both a peroxidase and a chaperone depending on its conformation and metabolic functions. The mechanisms involved in this functional switch are investigated by Lee *et al.* (2015), who provide evidence that substitution of Ser¹⁵⁰ with Cys¹⁵⁰ leads to a remarkable increase in chaperone and peroxidase activity. These changes are associated with structural alterations in the $\alpha 2$ helix, the fully folded $\alpha 2$ helix being associated with high peroxidase activity.

Research in the popular and ever-growing field of redox biology continues to be exciting and challenging. Slowly but surely we are gaining a better understanding of the roles of ROS and NO in the signalling networks that link cellular responses to changing environmental and developmental stimuli. In addition, NO readily reacts with glutathione to form S-nitrosoglutathione (GSNO), an important molecule that functions as a natural NO donor. Within this context ROS, NO and GSNO interact as control hubs that include master-switches regulating the genetic and epigenetic controls of plant growth and stress tolerance (Fig. 2). There are a large number of redox-based, post-translational modifications that act as regulators of the master-switches. This includes the addition of a glutathione to a protein cysteine thiol in a glutathionylation reaction or a NO moiety to form a S-nitrosothiol (SNO). The latter mechanism represents a key mechanism for the transfer of NO bioactivity. The size of the GSNO pool is regulated by GSNO reductase 1, which reduces GSNO to oxidized glutathione (GSSG) and ammonium. Despite this extensive and increasing knowledge, many questions remain unanswered. For example, further research is required to understand how plant cells use these mechanisms to transmit information in order to regulate gene expression. In addition, while local and systemic signalling is thought to involve waves of ROS, Ca²⁺ or NO production (Fig. 2), little is known about how plant cells perceive and distinguish between different stimuli. The characterization of new signalling molecules, such as oxidized or aggregated proteins is an important new field that is as yet relatively unexplored. Little is known about the functional dynamics of ROS/NO interactions or how ROS and NO regulate cross-talk between organelles. Deciphering the mechanisms by which ROS, NO and Ca²⁺ interact with phytohormones to create molecular memories of stress is an exciting new topic for future exploration. Answering these questions requires multidisciplinary approaches using physiological, biochemical, proteomic, molecular and cellular techniques. These, together with an armoury of more traditional whole-plant physiology, genetics, breeding and crop science approaches, and newer nanotechnological tools will enable us to dissect signalling networks at multiple levels of complexity from the cell, to the tissue to the whole plant. Such information is essential if we are to design improved biotechnology-driven strategies for sustained or enhanced productivity together with better plant resistance to biotic and abiotic stress conditions. Finally, it is worth remembering that in nature plants are often exposed to multiple stresses simultaneously. Regular exposures to changing mild

stresses results in acclimation of metabolic functions, together with molecular and physiological adjustments that include increased resistance to oxidative stress, which translate into plant growth and development that are optimized to prevailing environmental conditions. Without regular stress exposures that entrain genetic and epigenetic cross-tolerance responses, it is likely that sustainability and vigour in natural environments will be compromised because plants will be poorly adapted to stress, and hence less able to recover. At present, we understand relatively little about the mechanisms that create short- and long-lasting molecular memories of stress. Hence, there are many new avenues and developing topics for researchers in the field of plant redox biology to explore.

LITERATURE CITED

- Airaki M, Leterrier M, Valderrama R, Chaki M, Begara-Morales JC, Barroso JB, del Río LA, Palma JM, Corpas FJ. 2015. Spatial and temporal regulation of the metabolism of reactive oxygen and nitrogen species during the early development of pepper (*Capsicum annuum*) seedlings. *Annals of Botany* **116**: 679–693.
- Boeckx T, Webster R, Winters AL, Webb KJ, Gay A, Kingston-Smith AH. 2015. Polyphenol oxidase-mediated protection against oxidative stress is not associated with enhanced photosynthetic efficiency. *Annals of Botany* **116**: 529–540.
- Chaki M, Álvarez de Morales P, Ruiz C, Begara-Morales JC, Barroso JB, Corpas FJ, Palma JM. 2015. Ripening of pepper (*Capsicum annuum*) fruit is characterized by an enhancement of protein tyrosine nitration. *Annals of Botany* **116**: 637–647.
- Correa-Aragunde N, Cejudo FJ, Lamattina L. 2015. Nitric oxide is required for the auxin-induced activation of NADPH-dependent thioredoxin reductase and protein denitrosylation during root growth responses in arabidopsis. *Annals of Botany* **116**: 695–702.
- Feigl G, Lehotai N, Molnár A, Ördög A, Rodríguez-Ruiz M, Palma JM, Corpas FJ, Erdei L, Kolbert Z. 2015. Zinc induces distinct changes in the metabolism of reactive oxygen and nitrogen species (ROS and RNS) in the roots of two *Brassica* species with different sensitivity to zinc stress. *Annals of Botany* **116**: 613–625.
- Jeevan Kumar SP, Prasad SR, Banerjee R, Thammineni C. 2015. Seed birth to death: dual functions of reactive oxygen species in seed physiology. *Annals of Botany* **116**: 663–668.
- Jozefczak M, Bohler S, Schat H, Horemans N, Guisez Y, Remans T, Vangronsveld J, Cuypers A. 2015. Both the concentration and redox state of glutathione and ascorbate influence the sensitivity of arabidopsis to cadmium. *Annals of Botany* **116**: 601–612.
- Lee EM, Lee SS, Tripathi BN, Jung HS, Cao GP, Lee Y, Singh S, Hong SH, Lee KW, Lee SY, Cho J-Y, Chung BY. 2015. Site-directed mutagenesis substituting cysteine for serine in 2-Cys peroxiredoxin (2-Cys Prx A) of *Arabidopsis thaliana* effectively improves its peroxidase and chaperone functions. *Annals of Botany* **116**: 713–725.
- Marquez-Garcia B, Shaw D, Cooper JW, Karpinska B, Quain MD, Makgopa EM, Kunert K, Foyer CH. 2015. Redox markers for drought-induced nodule senescence, a process occurring after drought-induced senescence of the lowest leaves in soybean (*Glycine max*). *Annals of Botany* **116**: 497–510.
- Meitha K, Konnerup D, Colmer TD, Considine JA, Foyer CH, Considine MJ. 2015. Spatio-temporal relief from hypoxia and production of reactive oxygen species during bud burst in grapevine (*Vitis vinifera*). *Annals of Botany* **116**: 703–711.
- Morscher F, Kranner I, Arc E, Bailly C, Roach T. 2015. Glutathione redox state, tocopherols, fatty acids, antioxidant enzymes and protein carbonylation in sunflower seed embryos associated with after-ripening and ageing. *Annals of Botany* **116**: 669–678.
- Ortiz-Espín A, Locato V, Camejo D, Schiermeyer A, De Gara L, Sevilla F, Jiménez A. 2015. Over-expression of Trxo1 increases the viability of tobacco BY-2 cells under H₂O₂ treatment. *Annals of Botany* **116**: 571–582.
- Ozgur R, Uzilday B, Sekmen AH, Turkan I. 2015. The effects of induced production of reactive oxygen species in organelles on endoplasmic reticulum

- stress and on the unfolded protein response in arabidopsis. *Annals of Botany* **116**: 541–553.
- Palma JM, Sevilla F, Jiménez A, del Río LA, Corpas FJ, Álvarez de Morales P, Camejo DM. 2015.** Physiology of pepper fruit and the metabolism of antioxidants: chloroplasts, mitochondria and peroxisomes. *Annals of Botany* **116**: 627–636.
- de Pinto MC, Locato V, Paradiso A, De Gara L. 2015.** Role of redox homeostasis in thermo-tolerance under a climate change scenario. *Annals of Botany* **116**: 487–496.
- Pu X, Lv X, Tan T, Fu F, Qin G, Lin H. 2015.** Roles of mitochondrial energy dissipation systems in plant development and acclimation to stress. *Annals of Botany* **116**: 583–600.
- Roach T, Miller R, Aigner S, Kranner I. 2015.** Diurnal changes in the xanthophyll cycle pigments of freshwater algae correlate with the environmental hydrogen peroxide concentration rather than non-photochemical quenching. *Annals of Botany* **116**: 519–527.
- Sandalio LM, Romero-Puertas MC. 2015.** Peroxisomes sense and respond to environmental cues by regulating ROS and RNS signalling networks. *Annals of Botany* **116**: 475–485.
- Simancas B, Munné-Bosch S. 2015.** Interplay between vitamin E and phosphorus availability in the control of longevity in *Arabidopsis thaliana*. *Annals of Botany* **116**: 511–518.
- Tanou G, Minas IS, Karagiannis E, Tsikou D, Audebert S, Papadopoulou KK, Molassiotis A. 2015.** The impact of sodium nitroprusside and ozone in kiwifruit ripening physiology: a combined gene and protein expression profiling approach. *Annals of Botany* **116**: 649–662.
- Vishwakarma A, Tetali SD, Selinski J, Scheibe R, Padmasree K. 2015.** Importance of the alternative oxidase (AOX) pathway in regulating cellular redox and ROS homeostasis to optimize photosynthesis during restriction of the cytochrome oxidase pathway in *Arabidopsis thaliana*. *Annals of Botany* **116**: 555–569.