Dharmendra K. Gupta · José M. Palma
Francisco J. Corpas *Editors*

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Preface

Nitric oxide (NO) can be considered an ancient molecule in the history of life, which is correlated with the rise of atmospheric levels of O_2 (Feelish and Martin 1995). NO is a free radical molecule which also has lipophilic properties; consequently, it does not require a specific carrier to cross cell membranes and to reach a specific intracellular target, since it can also diffuse very rapidly due to its gaseous nature and moderate shelf life. However, the existence of canonical cellular receptors involved in the signaling process among cells cannot be discarded. NO and hydrogen peroxide (H_2O_2) have a family of derived molecules referred to as reactive oxygen and nitrogen species (ROS/RNS, respectively). However, both NO and H_2O_2 potentially cover much of intra- and inter-cell communications acting as signal molecules (Begara-Morales et al. 2018; Noctor et al. 2018).

ROS are derived forms of molecular oxygen (O_2) and normally result from the transfer of electrons to O_2 to generate, in a succession of univalent reductions, superoxide radical ($O_2^{\cdot -}$), H_2O_2, and hydroxyl radical ($^{\cdot}OH$), among others (Corpas et al. 2015; Gupta et al. 2018). Stress-induced increment in ROS level can trigger different degrees of oxidation in the different cell constituents (fatty acids, proteins, and nucleic acids) as well as relevant changes in the redox status. However, plant cells generally cope very well with high rates of ROS generation throughout the diversity of antioxidant enzymatic and non-enzymatic systems which modulate their levels in the different subcellular compartments (Gupta et al. 2016).

The enzymatic source of NO in plants is still under deep debate, but there are two main candidates: L-arginine-dependent NO synthase-like activity and NADH-dependent nitrate reductase (NR), a cytosolic enzyme related to nitrogen adjustment, whose prime purpose is reduction of nitrate to nitrite in cells (Corpas and Barroso 2017; Astier et al. 2018). Now, it is well recognized that NO is a key participant in the regulation of several developmental processes in plants, which comprise photomorphogenesis, plant defense, stomatal closure, leaf senescence, flowering, fertilization, and fruit ripening. In plants, there is some direct evidence showing that NO regulates ion channels, which comes mostly from electrophysiological studies in guard cells.

Biotic and abiotic stressors, such as pathogen attack, high CO_2 concentrations, drought, and ABA-associated stomata closure, induce NO synthesis.

Hydrogen peroxide (H_2O_2) plays a central role in both biotic/abiotic stress and is produced primarily in plant cells during photosynthesis and at photorespiration, and to a minor degree, in respiration processes. It is also called "master hormone" because it controls various ROS/hormonal homeostasis; therefore, it plays a vital role as signaling molecule in various physiological events in plants. H_2O_2 interrelates with thiol-rich proteins and stimulates diverse signaling corridors as well as transcription factors, which in turn regulate gene expression and cell-cycle practices. Recently, in plant systems, genetic regulatory cellular redox homeostasis and H_2O_2 signaling are discussed profoundly. Moreover, extracellular matrix (ECM) after photosynthetic and respiratory metabolism also plays an important role in the generation of H_2O_2, which controls plant growth, development, and acclamatory/defense responses. Among various environmental stresses the uppermost levels of H_2O_2 are detected in the leaf veins. In the recent past, our knowledge about how H_2O_2 is generated in C3 plants has experimental significant advances. However, in other plant systems such as C4 metabolism and CAM (Crassulacean acid metabolism) plants the potential role of H_2O_2 in photosynthetic mode of carbon assimilation is still discussed. Nevertheless, the increment of H_2O_2 generations is associated with adverse situations, which, at the same time, depends on the strength and duration of the imposed stress.

The most remarkable features of this book are related to how NO and H_2O_2 play important roles in signaling as well as in multidisciplinary functions in plants. Chapter 1 deals with an overview of how NO and H_2O_2 are generated and metabolized in plants. Chapters 2 and 3 focus on signaling network of NO and H_2O_2 and posttranslational modification in higher plants. Chapters 4–6 focus on transcriptional regulation of gene expression, NO and H_2O_2 metabolism, and relationship between ROS and RNS in mitochondria and in chloroplast. Chapters 7–9 put the emphasis in the participation of NO and H_2O_2 in physiological processes including seed germination, root organogenesis, and fruit ripening. Finally, the last three chapters (10–12) discuss the application and functions of NO and H_2O_2 in biotic/abiotic stresses and their biotechnological application in higher plants. Overall, the information gathered in this volume will bring in-depth knowledge of NO- and H_2O_2-related issues in higher plants, which is going to be helpful to so many in course and future plant bio/physiologists.

Dr. Dharmendra K. Gupta, Prof. José M. Palma, and Dr. Francisco J. Corpas deeply thank all the cooperative authors for contributing their valuable knowledge, time, and zeal to bring this book into its present form.

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References

Astier J, Gross I, Durner J (2018) Nitric oxide production in plants: an update. J Exp Bot 69:3401–3411

Begara-Morales JC, Chaki M, Valderrama R, Sánchez-Calvo B, Mata-Pérez C, Padilla MN, Corpas FJ, Barroso JB (2018) Nitric oxide buffering and conditional nitric oxide release in stress response. J Exp Bot 69:3425–3438

Corpas FJ, Barroso JB (2017) Nitric oxide synthase-like activity in higher plants. Nitric Oxide 68:5–6

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, Cham pp 1–22

Feelisch M, Martin JF (1995) The early role of nitric oxide in evolution. Trend Ecol Evol 10:496–499

Gupta DK, Palma JM, Corpas FJ (2016) Redox state as a central regulator of plant-cell stress response. Springer, Cham

Gupta DK, Palma JM, Corpas FJ (2018) Antioxidants and antioxidant enzymes in higher plants. Springer, Cham

Noctor G, Reichheld JP, Foyer CH (2018) ROS-related redox regulation and signaling in plants. Semin Cell Dev Biol 80:3–12

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About the Editors

Dharmendra K. Gupta is Sr. Scientist of environmental biotechnology/radioecology and has already published more than 90 referred research papers/review articles in peer-reviewed journals and has edited thirteen books. His field of research includes abiotic stress by radionuclides/heavy metals and xenobiotics in plants, antioxidative system in plants, and environmental pollution (radionuclides/heavy metals) remediation through plants (phytoremediation).

José Manuel Palma is Research Professor of antioxidants and free radicals in plant systems with more than 100 peer-reviewed research papers published and editor of five books from different publishers including four from Springer and several special issues of diverse international journals. At present, he is involved in the investigation of the interaction between nitric oxide and antioxid**a**nts during fruit ripening. He leads the research group "Antioxidants, Free Radicals and Nitric Oxide in Biotechnology, Food and Agriculture" at Estación Experimental del Zaidín, CSIC, Granada, Spain. He was also Deputy Director and Acting Director of the institute in the period 2007–2014.

Francisco J. Corpas is a staff member of the Spanish National Research Council (CSIC) who has more than 26 years of research experience in the metabolism of antioxidants and nitric oxide (NO) in higher plants under physiological and adverse environmental conditions. He was the Head of the Department of Biochemistry, Cell and Molecular Biology of Plants (2014–2018) at Estación Experimental del Zaidín, CSIC, Granada, Spain. He has already published more than 160 refereed research papers/review articles in peer-reviewed journals (according to Scopus database with *h*-index: 52) and edited seven books.

Hydrogen Peroxide and Nitric Oxide Generation in Plant Cells: Overview and Queries

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

Contents

Abstract Hydrogen peroxide (H_2O_2) and nitric oxide (NO) are two key molecules representative of two families of related compounds designated as reactive oxygen and nitrogen species (ROS and RNS, respectively). Our present knowledge about where, when, and how these molecules are produced in a specific plant tissue either under physiological or stress conditions and how they interact support the relevant crosstalk between these molecules which in many cases are autoregulated throughout posttranslational modifications. Thus, either *S*-nitrosation or nitration of different enzymes of the ROS metabolism including superoxide-generating NADPH oxidase (NOX) or antioxidant enzymes such as catalase and superoxide dismutase (SOD) and components of the ascorbate-glutathione cycle may take place under diverse situations. However, H_2O_2 and NO may react among them giving rise to a more powerful toxic species, the hydroxyl radical ($^{\cdot}OH$), which may react with most biomolecules (nucleic acids, proteins, and lipids), leading to irreversible damages within cells. This chapter will provide a comprehensive and easy overview about H_2O_2 and NO production, on how these molecules are generated within different cell compartments, and about their metabolic interaction. A proposed model on how such interaction between H_2O_2 and NO may influence the organelles' signaling

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network under normal physiological and stress conditions and/or developmental metabolic shifts is discussed.

Keywords Chloroplast · Hydrogen peroxide · Hydroxyl radical · Mitochondrion · Nitric oxide · Peroxisome · Reactive oxygen species · Reactive nitrogen species · Signaling · *S*-nitrosoglutathione · *S*-nitrosylation

Abbreviations

GSNO	*S*-nitrosoglutathione
GSH	Reduced glutathione
H_2O_2	Hydrogen peroxide
NR	Nitrate reductase
NO	Nitric oxide
$ONOO^-$	Peroxynitrite
PTM	Posttranslational modification
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SNOs	*S*-nitrosothiols

1 Introduction

Hydrogen peroxide (H_2O_2) and nitric oxide (NO) are two key molecules representative of two families of related compounds designated as reactive oxygen and nitrogen species (ROS and RNS, respectively). Both families of molecules participate in a myriad of plant processes (del Río 2015; Lindermayr 2017; Corpas and Barroso 2018a), and their relevance in plant cells is well recognized because they are involved in dual roles under both physiological events (seed and pollen germination, plant development and growth, stomatal movement, leaf senescence, and fruit ripening, among others) and in the mechanism of response against biotic and abiotic stresses. This dual face of both molecules implies their role in signaling processes during the initial phases of the plant response to diverse situations and as potential responsible of cellular damages when these molecules are overproduced without control. Figure 1 summarizes some of the plant processes where both H_2O_2 and NO participate at different level. Our present knowledge about where, when, and how these molecules are produced in a specific plant tissue either under physiological or stress conditions and how they interact supports the relevant interrelationship between these molecules which in many cases are autoregulated throughout post-translational modifications (PTMs). As a matter of fact, these families of molecules cannot be studied as separated areas because they are interconnected at metabolic level where many of the enzymes involved in their corresponding metabolisms are autoregulated by those PTMs. Thus, either *S*-nitrosation or nitration of different enzymes of the ROS metabolism including superoxide-generating NADPH oxidase (NOX) or antioxidant enzymes such as catalase and superoxide dismutase (SOD)

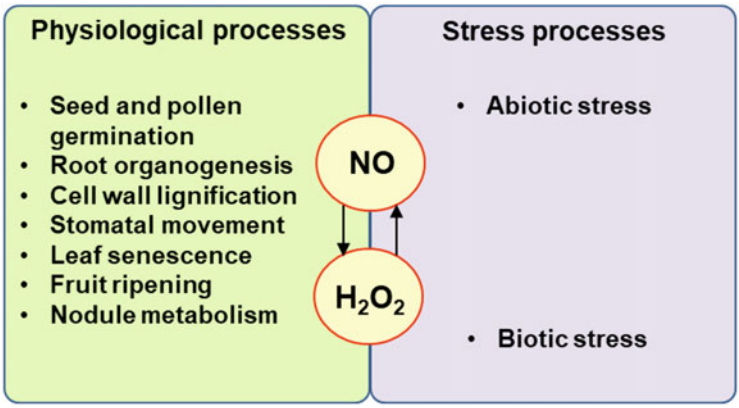

Fig. 1 Nitric oxide (NO) and hydrogen peroxide (H_2O_2) in plant cells participate in complex processes both under physiological conditions (seed and pollen germination, plant development and growth, stomatal movement, leaf senescence, fruit ripening, and others) and in the mechanism of response against biotic and abiotic stresses. They play their roles independently or interacting one with another under two perspectives, as signaling molecules in the initial phases of their respective response and as potential responsible of cellular damages when these molecules are overproduced without control

and components of the ascorbate-glutathione cycle may take place under diverse situations (Yun et al. 2011; Begara-Morales et al. 2015; Chaki et al. 2015).

Both reactive species (H_2O_2 and NO) are generated in multiple cell loci (Foyer and Noctor 2003; Corpas et al. 2015; Gupta et al. 2018a), but their respective diffusion rates within the cell spaces allow them to move from one organelle to another. Accordingly, the potential interaction between H_2O_2 and NO may have repercussions not only in situ where they are produced but also several dozens of micrometers away, thus exporting their effect to other cell compartments.

Consequently, the main goal of this chapter is to provide a wide and comprehensive overview of the metabolism of these two molecules indicating how they are produced and how they are interrelated in the plant metabolism. A prospective of the cell scenario that can be found under physiological and stress conditions will be also depicted.

2 Generation and Scavenging of H_2O_2 in Plant Cells

Hydrogen peroxide (H_2O_2) is one of the main reactive oxygen species (ROS) basically generated in living beings as a secondary metabolite of the aerobic metabolism. Early studies in cell organelles found out initially this molecule as the principal ROS directly generated in chloroplasts, mitochondria, and peroxisomes. Thus, in chloroplast, the first conclusive reports on the production of H_2O_2 were given by Mehler, who discovered that these organelles, besides producing oxygen

by the Hill reaction, also consume it through the so-called Mehler reaction (Mehler 1951). Years later, it was proved that this species was not directly generated in chloroplasts, but through the dismutation of superoxide radicals ($O_2^{\cdot-}$) spontaneously or achieved by the enzymatic system superoxide dismutase (SOD; EC 1.15.1.1) (Asada et al. 1974). Indeed, it was demonstrated that superoxide radicals are firstly produced, by the autoxidation of reduced ferredoxin at the photosystem PSI and the plastoquinone level in photosystem PSII, and then dismutated into H_2O_2 by the action of either chloroplastic Fe-SOD or CuZn-SODs (Asada 2006; Corpas et al. 2015). Diverse aspects complementing this scheme have been recently reviewed (Smirnoff and Arnaud 2018).

Once H_2O_2 is produced, it is decomposed basically by both stromal and thylakoidal ascorbate peroxidase (sAPX and tAPX, respectively; EC 1.11.1.11) (Yoshimura et al. 1999; Shigeoka et al. 2002; Maruta et al. 2016) which could work in cooperation with the other enzymes of the ascorbate-glutathione cycle, with consumption of reduced ascorbate and NADPH provided by the Calvin-Benson cycle. All these actors which play a role in the chloroplast scenario (PSI and PSII with their respective electron acceptors, ferredoxin, SOD, APX, and the ascorbate-glutathione cycle) are integrated within the water-water cycle which years ago postulated Professor Asada's works (Asada 1999, 2006; Corpas et al. 2015; Mano et al. 2016) and was accepted worldwide. Peroxiredoxins (Prxs) and thioredoxins (Trxs) are also systems with coordinated functions among them involved in the hydrogen peroxide scavenging in chloroplasts (Puerto-Galán et al. 2013). They can also interact with PSI through the ferredoxin site, thus sharing some connection points with the water-water cycle (Asada 2006; Nikkanen and Rintamäki 2014).

Globally, all these partners which participate in the H_2O_2 metabolism within chloroplasts are key points to modulate the concentration of this ROS for signaling purposes and confer to this organelle a relevant role in the signal transduction network within the plant cell (Smirnoff and Arnaud 2018) which will be dependent on the lighting conditions.

As in chloroplasts, the former reports in the 1960s on ROS in mitochondria demonstrated the generation of H_2O_2 in this cell compartment (Jensen 1966a, b), and still some years later, this issue was corroborated under different conditions (Boveris and Chance 1973). However, soon after the proposal of superoxide a radical as precursors of H_2O_2 in mitochondria was issued (Loschen et al. 1974). Later, a series of well-designed studies were developed that were focused on complexes I and III from the mitochondrial electron transport chain (ETC) as sources of $O_2^{\cdot-}$ (Boveris and Cadenas 1982; Turrens 1997; Raha and Robinson 2000; Murphy 2009; Huang et al. 2016). Further detection of Mn-SOD activity in these organelles (Weisiger and Fridovich 1973) supported this precursory of superoxide radicals as source of H_2O_2. A thorough review on this subject can be followed in Corpas et al. (2015).

In animals, it has been reported that the H_2O_2 formed at the mitochondrial ETC is scavenged by a selenium-dependent glutathione peroxidase (SeGPX), which uses reduced glutathione (GSH) provided by a glutathione reductase (GR) located at the matrix site (Ursini et al. 1995; Handy et al. 2009; Halliwell and Gutteridge 2015). In plants, a role of GPX in the mitochondrial H_2O_2 homeostasis has been also

referenced (Passaia et al. 2013), but the early report of all enzymatic components of the ascorbate-glutathione cycle (Jiménez et al. 1997) allowed proposing this pathway as the main H_2O_2 processing route in these cell loci (Corpas et al. 2015; Mittova et al. 2015). Besides, a thioredoxin-peroxiredoxin system has been also reported in the matrix which could remove H_2O_2 with the participation of a thioredoxin reductase that would utilize NADPH provided by a NADP-dependent isocitrate dehydrogenase as electron donor (Murphy 2009; Corpas et al. 2015; Sevilla et al. 2015). H_2O_2 can escape from the organelle and be pumped off to the cytosol where it can be either detoxified by diverse systems, including peroxisomes when it is released at high concentration, or driven to signaling processes (Foyer and Noctor 2003; Smirnoff and Arnaud 2018).

Regarding peroxisomes, this descriptive name was proposed in Professor de Duve's laboratories in which it was found that the "microbodies" reported in the mid-1950s (Rhodin 1954) displayed a very active H_2O_2 metabolism (de Duve and Baudhuin 1966). Peroxisomes are organelles with a highly oxidative metabolism whose main characteristics are the presence of flavin oxidases responsible for the H_2O_2 production and catalase (CAT; EC 1.11.1.6), the principal enzyme which removes H_2O_2 in the eukaryotic cell. Enzymes such as acyl-CoA oxidase, xanthine oxidase, urate oxidase, glycolate oxidase (mainly in plants), diamine oxidase, and polyamine oxidase, among others, have been reported in peroxisomes, all of them acting as sources of hydrogen peroxide (Corpas et al. 2015, 2019; Smirnoff and Arnaud 2018). Besides, the relevant presence in peroxisomes of superoxide dismutase (SOD) activity, either as Mn-SOD, Fe-SOD, or CuZn-SOD, can be also considered as a significant generator of H_2O_2 at the cell level (del Río 2011; Palma et al. 2015; del Río et al. 2018).

As indicated above, peroxisomes bear the H_2O_2-scavenging catalase as its archetypical enzyme. In fact, this protein is considered as the typical marker for peroxisomes in biochemistry and cell biology research. However, this plant organelle also contains the four enzymes of the ascorbate-glutathione cycle (Jiménez et al. 1997; del Río 2011) which finely tune the concentration of H_2O_2 within this compartment. Thus, the peroxisomal APX has been well studied in many species and under different situations (Corpas et al. 1994, 2015; Yamaguchi et al. 1995; Bunkelmann and Trelease 1996; Corpas and Trelease 1998; Narendra et al. 2006; Palma et al. 2006).

Some other cell sources of H_2O_2 have been reported in the cell, including the plasma membrane and the apoplast and the endoplasmic reticulum (Smirnoff and Arnaud 2018), but because the main organelles involved in the metabolism of this ROS (Gupta et al. 2018a) and its relationship with NO are better documented in chloroplasts, mitochondria, and peroxisomes, we will focus our attention in the potential signaling networks where these three cell organelles may be integrated.

3 Generation of NO in Plant Cells

Such as it has been mentioned, NO could be considered the most relevant component of a family of related molecules designated as RNS. However, one of the key points in the metabolism of NO in plant cells is the identification and subcellular localization of the endogenous NO sources. Plants can generate NO by nonenzymatic and enzymatic mechanisms, but the contribution of each NO source to a specific physiological process is still unclear (Astier et al. 2018; Corpas and Palma 2018).

Figure 2 shows a simple model of the main recognized NO sources in higher plants. It is known that the nonenzymatic reduction of nitrite (NO_2^-) can lead to the formation of NO, and this reaction is favored at acidic pH. Thus, NO_2^- can also be chemically reduced by ascorbic acid at pH 3–6 to yield NO. This reaction could occur at micro-localized pH conditions in barley aleurone layers, in the chloroplast, and in apoplastic space where ascorbic acid is known to be present (Stöhr et al. 2001; Stöhr and Stremlau 2006). Another nonenzymatic mechanism proposed for NO formation is the light-mediated reduction of NO_2^- by carotenoids (Bethke et al. 2004).

Related with the enzymatic source of NO, there are two main candidates in higher plant, nitrate reductase (NR) and L-arginine-dependent nitric oxide synthase (NOS)-like activity (Corpas and Barroso 2017; Astier et al. 2018). NR is a molybdoenzyme that reduces nitrate (NO_3^-) to nitrite (NO_2^-) using NADH as electron donor. Thus, it has been shown that purified maize NR can generate NO in vitro conditions using NADH (Yamasaki et al. 1999) and this NO production seems to be implicated in some physiological processes such as stomatal closure (Chen et al. 2016). However, there is little information on the direct involvement of NR-derived NO in plant stress situations. More recently, using the unicellular alga *Chlamydomonas reinhardtii* as model photosynthetic organism, it has been demonstrated that the interaction between the mitochondrial amidoxime reducing component (mARC) and NR can generate NO from NO_2^- where the ARC catalyzes the NO generation from NO_2^- using electrons from NR (Chamizo-Ampudia et al. 2016). On the other hand, the NOS-like activity in higher plants is characterized to have similar requirements (L-Arg, NADPH, FMN, FAD, calmodulin, and Ca^{2+}) to that of the mammalian NOSs (Barroso et al. 1999; Corpas et al. 2004). Nevertheless, in higher plants, no

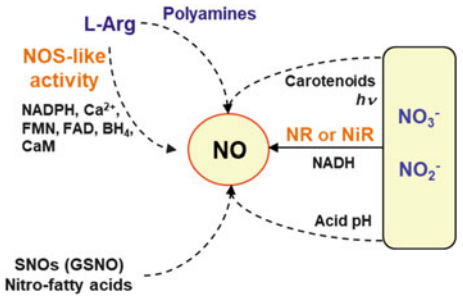

Fig. 2 Potential enzymatic and nonenzymatic sources of NO in higher plant cells. BH_4, tetrabiopterin; CaM, calmodulin; GSNO, *S*-nitrosoglutathione; L-Arg, arginine; NOS, nitric oxide synthase; NR, nitrate reductase; NiR, nitrite reductase; SNOs, nitrosothiols

ortholog genes have been found of any of the classic mammalian NO synthases (NOSs). In this sense, using also as a model the green alga *Ostreococcus tauri* it has been demonstrated the existence of an NOS-like protein (Foresi et al. 2010) whose occurrence has been extended to another 15 algal species (Jeandroz et al. 2016). On the other hand, there are evidences which correlated polyamines metabolism with NO generation (Tun et al. 2006; Wimalasekera et al. 2011; Agurla et al. 2018).

At subcellular level chloroplasts, mitochondria, and peroxisomes are the main organelles where NO generation has been mainly reported in higher plants. Although there is other potential place such as the apoplastic space, the available information is very limited (Stöhr and Ullrich 2002). The presence of NO into chloroplasts has been demonstrated by different experimental approaches such as electron spin resonance (ESR) and specific fluorescent probes (Jasid et al. 2006; Puntarulo et al. 2007; Galatro et al. 2013; Galatro and Puntarulo 2016), and the available data indicate that the main source of NO in this organelle is a NOS-like protein but not NR (Tewari et al. 2013; for more details see Chapter "Hydrogen Peroxide and Nitric Oxide Metabolism in Chloroplasts" of this book).

Plant mitochondria are the major producers of ATP via oxidative phosphorylation with the O_2 being the terminal electron acceptor of the mitochondrial electron transport chain (ETC). So far the mitochondrial NO generation is through the reduction of NO_2^- where the electron donors could be different depending of the mitochondrial oxygen tension, principally under hypoxia/anoxia conditions because the NO_2^- reduction to ammonium is inhibited and, consequently, NO_2^- is accumulated allowing the NO generation. In this situation the electron donors to generate NO is through the action of cytochrome *c* oxidase and other ETC components, such as complexes III and IV, by using NO_2^- (Wulff et al. 2009; Blokhina and Fagerstedt 2010; Igamberdiev et al. 2014; Gupta et al. 2018b). There are also some evidences indicating that plant mitochondria also generate NO even under normoxic conditions through alternative oxidase (AOX) (Alber et al. 2017; for more details see Chapter "Metabolism and Interplay of Reactive Oxygen and Nitrogen Species in Plant Mitochondria" of this book).

Plant peroxisomes are organelles where the presence of NO generation has been also shown by different technical approaches including spin-trapping electron paramagnetic resonance (EPR) spectroscopy and fluorescence-specific probes (Corpas et al. 2004, 2009). In this case, the experimental biochemical data support that the NO is generated by an L-arginine-dependent NOS-like activity that requires the same cofactors of animal NOSs including NADPH, FAD, FMN, calmodulin, and calcium (Barroso et al. 1999; Corpas et al. 2004). Additionally, different reports have also provided evidences that plant peroxisomes contain other NO-derived molecules including *S*-nitrosoglutathione (GSNO) and peroxynitrite ($ONOO^-$) (Barroso et al. 2013; Corpas and Barroso 2014a). Furthermore, additional analyses demonstrate that plant peroxisomes have the capacity to generate NADPH (for a review see Corpas and Barroso 2018b) and contain both calmodulin (Chigri et al. 2012) and Ca^{2+} (Costa et al. 2010; Corpas and Barroso 2018a). All these

experimental data are in good agreement with the presence of a NOS protein in animal peroxisomes (Stolz et al. 2002; Loughran et al. 2005).

4 Interplay Among Cell Organelles by NO and H_2O_2 Signaling: Overview and Queries

Both H_2O_2 and NO are able to exert their respective roles both as signal molecules but also as damaging species by themselves, as already probed and thoroughly reported (see diverse chapters in this book). But they can also react between them, thus generating a more powerful ROS, hydroxyl radicals ($^{\cdot}OH$) according to the following reaction (Gray et al. 1972; Nappi and Vass 1998):

$$H_2O_2 + NO \rightarrow {}^{\cdot}OH + HNO_2$$

This could be a feasible mechanism to generate hydroxyl radicals in biological systems in the absence of transition metals as it occurs in the superoxide-mediated Fenton reaction, thus providing a new focus to address tissue-specific damage caused by this ROS (Nappi and Vass 1998). But also, the way on how many moles of each species is consumed by this reaction opens novel concerns on the ways to conceive signaling processes leaded by either H_2O_2 or NO. Thus, in Fig. 3, as an exemplifying model, the specific interaction of these species generated in plant peroxisomes with other cell compartments is depicted. Under normal physiological conditions, catalase (CAT) and ascorbate peroxidase (APX) control the level of internally generated H_2O_2 by either a battery of oxidases, superoxide dismutases, or spontaneous dismutation from superoxide radicals, as well as the H_2O_2 imported from other cell loci (Corpas et al. 2015). Due to the presence of peroxisomal NOS-like activity, the formation of NO usually takes place within the organelle. Under those circumstances the small H_2O_2 amount which might escape from the action of the tandem CAT/APX could react with NO, thus giving rise to certain levels of $^{\cdot}OH$, and this radical may exert its damaging effect not only in the own peroxisome but also in neighboring organelles/loci (Fig. 3a) (Corpas et al. 2015). However, this potential mechanism seems to be finely regulated since no episodes promoted through these events have been neither observed nor reported under normal physiological conditions. This appears to mirror what hypothetically could take place in peroxisomes (and other organelles) where the simultaneous presence of diverse ROS and RNS could lead to the formation of singlet oxygen ($^1O_2$), hydroxyl radicals ($^{\cdot}OH$), peroxynitrite ($ONOO^-$), and other highly reacting molecules if their formation were not tuned with precision (Corpas et al. 2017).

However, this tight equilibrium could be disturbed under certain conditions such as those promoted by any kind of stress (biotic and abiotic) as well as by metabolic changes triggered by shifts in the developmental stages (seed germination, fruit ripening, etc.) (Corpas and Barroso 2014a; Corpas et al. 2017). Under those

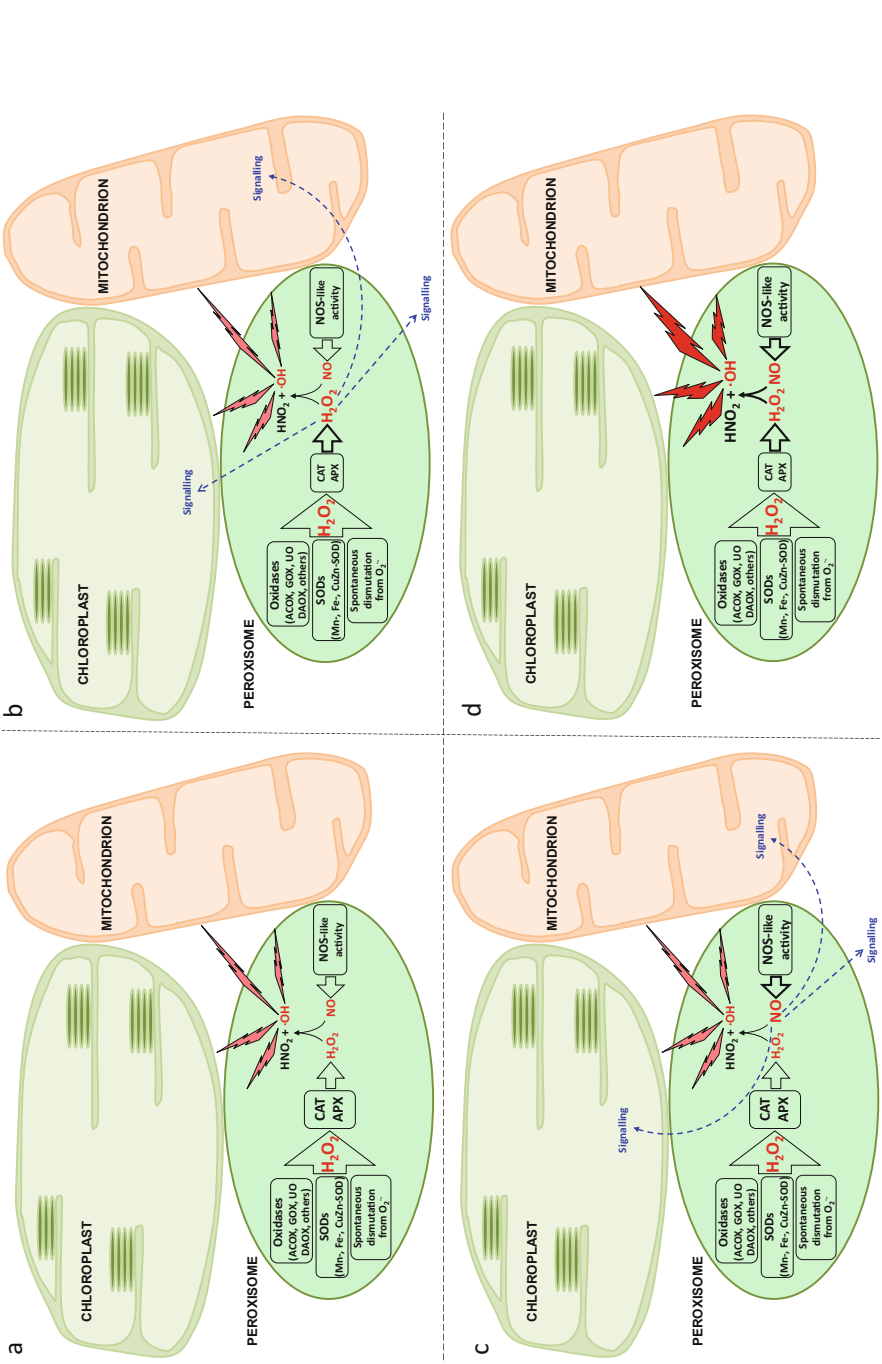

Fig. 3 Interaction of H_2O_2 and NO in peroxisomes from plant cells and their repercussions in the cell physiology. H_2O_2 can be generated by spontaneous dismutation and through the action of diverse enzymes. This species is basically removed by catalase and ascorbate peroxidase. NO is produced in the organelle

Fig. 3 (continued) by a NOS-like activity, and its reaction with H_2O_2 can give rise to hydroxyl radicals (˙OH) which is one of the most powerful damaging reactive oxygen species. H_2O_2 and NO can not only act as signaling molecules at the peroxisomal level but also interact with other cell organelles. (**a**) Normal physiological conditions in which H_2O_2 an NO reacts in a controlled form. (**b**) Situations where H_2O_2 is overproduced. In such cases this species may also act as signal molecule. (**c**) NO synthesis exceeds the H_2O_2 concentration with imbalanced stoichiometry being used for signaling purposes. (**d**) The overproduction of both, H_2O_2 and NO, leads to the generation of high ˙OH levels with strong damaging effects. ACOX, acyl-CoA oxidase; GOX, glycolate oxidase; UO, urate oxidase; DAOX, diamine oxidase; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase; NOS, nitric oxide synthase

circumstances, the ability of CAT and APX to scavenge all the generated H_2O_2 might be limited by the inhibition and/or repression of both enzymes, and the concentration of H_2O_2 could overtake that of the NO formation. The generation of $^{\cdot}OH$ could be maintained, but the H_2O_2 excess could be driven to diverse signaling processes which involve cell organelles such as chloroplasts, mitochondria, and others (Fig. 3b). It may also happen that the NO synthesis within peroxisomes overcomes the H_2O_2 levels, so, besides the formation of $^{\cdot}OH$ and all its negative effects, NO could also participate in signaling events (Fig. 3c) through posttranslational modifications facilitated by *S*-nitrosation and nitration events (Corpas and Barroso 2014b; Corpas et al. 2017). Finally, the levels of both species could be considerably enhanced due to activation of the NOS-like activity and lowered CAT and APX activities, thus rendering an environment where the formation of $^{\cdot}OH$ is potentiated. In those conditions, the damaging processes would prevail leading to degradation and disorganization of cell components (Fig. 3d).

It should be kept in mind that all these events may also be triggered by the interactions between the H_2O_2 and NO generated in other organelles (chloroplasts, mitochondria, cytosol, etc.), thus building a complex ROS/RNS network where a considerable number of actors participate and that can be altered by many factors and situations. In the following chapters of this book, we will learn more precisely on how all this metabolic labyrinth is depicted according to the latest contributing knowledge in this field.

5 Conclusions

Hydrogen peroxide and NO are common metabolites in the cell which are generated in most organelles. Due to their relative moderate life span and diffusion rates, they are good candidates to exert independently a role in the signaling network either directly or indirectly. Nevertheless, both molecules can react in the cell loci where they are produced (chloroplasts, mitochondria, peroxisomes, and others), thus generating the more powerful reactive species hydroxyl radical ($^{\cdot}OH$), which can trigger deleterious effects for life. Under normal physiological stages, this condition is somehow balanced by the own cell metabolism, and the action of $^{\cdot}OH$ is controlled. Under pathological situations and/or unfavorable conditions, this balance could be broken down displacing the molecular stoichiometry of these species to favor non-coordinated signaling processes or, even worse, to enhance the production of $^{\cdot}OH$ promoting a cell-wide damage. The ROS/RNS homeostasis within each cell organelle would be fundamental to avoid the expansive wave of this eventuality inside of the cell. Understanding the intimate regulation of the interaction between H_2O_2 and NO will be useful to know how important physiological processes such as fruit ripening, which is regulated by NO (Corpas et al. 2018), occur.

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References

Agurla S, Gayatri G, Raghavendra AS (2018) Polyamines increase nitric oxide and reactive oxygen species in guard cells of *Arabidopsis thaliana* during stomatal closure. Protoplasma 255: 153–162

Alber NA, Sivanesan H, Vanlerberghe GC (2017) The occurrence and control of nitric oxide generation by the plant mitochondrial electron transport chain. Plant Cell Environ 40: 1074–1085

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

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

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

Astier J, Gross I, Durner J (2018) Nitric oxide production in plants: an update. J Exp Bot 69: 3401–3411

Barroso JB, Corpas FJ, Carreras A, Sandalio LM, Valderrama R, Palma JM, Lupiáñez JA, del Río LA (1999) Localization of nitric-oxide synthase in plant peroxisomes. J Biol Chem 274: 36729–36733

Barroso JB, Valderrama R, Corpas FJ (2013) Immunolocalization of *S*-nitrosoglutathione, *S*-nitrosoglutathione reductase and tyrosine nitration in pea leaf organelles. Acta Physiol Plant 35:2635–2640

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Mata-Pérez C, Valderrama R, Padilla MN, López-Jaramillo J, Luque F, Corpas FJ, Barroso JB (2015) Differential molecular response of monodehydroascorbate reductase and glutathione reductase by nitration and *S*-nitrosylation. J Exp Bot 66:5983–5996

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

Blokhina O, Fagerstedt KV (2010) Reactive oxygen species and nitric oxide in plant mitochondria: origin and redundant regulatory systems. Physiol Plant 138:447–462

Boveris A, Cadenas E (1982) Production of superoxide radical and hydrogen peroxide in mitochondria. In: Oberley LW (ed) Superoxide dismutase, vol II. CRC Press, Boca Raton, FL, pp 15–30

Boveris A, Chance B (1973) The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem J 134:707–716

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

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. Ann Bot 116:637–647

Chamizo-Ampudia A, Sanz-Luque E, Llamas Á, Ocaña-Calahorro F, Mariscal V, Carreras A, Barroso JB, Galván A, Fernández E (2016) A dual system formed by the ARC and NR molybdoenzymes mediates nitrite-dependent NO production in *Chlamydomonas*. Plant Cell Environ 39:2097–2107

Chen ZH, Wang Y, Wang JW, Babla M, Zhao C, García-Mata C, Sani E, Differ C, Mak M, Hills A, Amtmann A, Blatt MR (2016) Nitrate reductase mutation alters potassium nutrition as well as

nitric oxide-mediated control of guard cell ion channels in *Arabidopsis*. New Phytol 209: 1456–1469

Chigri F, Flosdorff S, Pilz S, Kölle E, Dolze E, Gietl C, Vothknecht UC (2012) The *Arabidopsis* calmodulin-like proteins AtCML30 and AtCML3 are targeted to mitochondria and peroxisomes, respectively. Plant Mol Biol 78:211–222

Corpas FJ, Barroso JB (2014a) Peroxynitrite ($ONOO^-$) is endogenously produced in *Arabidopsis* peroxisomes and is overproduced under cadmium stress. Ann Bot 113:87–96

Corpas FJ, Barroso JB (2014b) Functional implications of peroxisomal nitric oxide (NO) in plants. Front Plant Sci 5:97

Corpas FJ, Barroso JB (2017) Nitric oxide synthase-like activity in higher plants. Nitric Oxide 68: 5–6

Corpas FJ, Barroso JB (2018a) Calmodulin antagonist affects peroxisomal functionality by disrupting both peroxisomal Ca^{2+} and protein import. J Cell Sci 131:jcs.201467.

Corpas FJ, Barroso JB (2018b) Peroxisomal plant metabolism – an update on nitric oxide, Ca^{2+} and the NADPH recycling network. J Cell Sci 131:jcs202978.

Corpas FJ, Palma JM (2018) Assessing nitric oxide (NO) in higher plants: an outline. Nitrogen 1: 12–20

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

Corpas FJ, Bunkelmann J, Trelease RN (1994) Identification and immunochemical characterization of a family of peroxisome membrane proteins (PMPs) in oilseed glyoxysomes. Eur J Cell Biol 65:280–290

Corpas FJ, Barroso JB, Carreras A, Quirós M, León AM, Romero-Puertas MC, Esteban FJ, Valderrama R, Palma JM, Sandalio LM, Gómez M, del Río LA (2004) Cellular and subcellular localization of endogenous nitric oxide in young and senescent pea plants. Plant Physiol 136: 2722–2733

Corpas FJ, Hayashi M, Mano S, Nishimura M, Barroso JB (2009) Peroxisomes are required for *in vivo* nitric oxide accumulation in the cytosol following salinity stress of *Arabidopsis* plants. Plant Physiol 151:2083–2094

Corpas FJ, Gupta DK, Palma JM (2015) Production sites of reactive oxygen species (ROS) in organelles from plant cells. In: Gupta DK, Palma JM, Corpas FJ (eds) Reactive oxygen species and oxidative damage in plants under stress. Springer, Cham, pp 1–22

Corpas FJ, Barroso JB, Palma JM, Rodríguez-Ruiz M (2017) Plant peroxisomes: a nitro-oxidative cocktail. Redox Biol 11:535–542

Corpas FJ, Freschi L, Marta Rodríguez-Ruiz M, Mioto PT, González-Gordo S, Palma JM (2018) Nitro-oxidative metabolism during fruit ripening. J Exp Bot 69:3449–3463

Corpas FJ, del Río LA, Palma JM (2019) Plant peroxisomes are in the crossroad of NO and H_2O_2 metabolism. J Integr Plant Biol. https://doi.org/10.1111/jipb.1772

Costa A, Drago I, Behera S, Zottini M, Pizzo P, Schroeder JI, Pozzan T, Lo Schiavo F (2010) H_2O_2 in plant peroxisomes: an *in vivo* analysis uncovers a Ca^{2+}-dependent scavenging system. Plant J 62:760–772

De Duve C, Baudhuin P (1966) Peroxisomes (microbodies and related particles). Physiol Rev 46: 323–357

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

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

del Río LA, Corpas FJ, López-Huertas E, Palma JM (2018) Plant superoxide dismutases: function under abiotic stress conditions. In: Gupta DK, Palma JM, Corpas FJ (eds) Antioxidants and antioxidant enzymes in higher plants. Springer, Cham, pp 1–26

Foresi N, Correa-Aragunde N, Parisi G, Caló G, Salerno G, Lamattina L (2010) Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga *Ostreococcus tauri* is light irradiance and growth phase dependent. Plant Cell 22:3816–3830

Foyer CH, Noctor G (2003) Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiol Plant 119:355–364

Galatro A, Puntarulo S (2016) Measurement of nitric Oxide (NO) generation rate by chloroplasts employing electron spin resonance (ESR). Methods Mol Biol 1424:103–112

Galatro A, Puntarulo S, Guiamet JJ, Simontacchi M (2013) Chloroplast functionality has a positive effect on nitric oxide level in soybean cotyledons. Plant Physiol Biochem 66:26–33

Gray D, Lissi E, Heicklen J (1972) The reaction of hydrogen peroxide with nitrogen dioxide and nitric oxide. J Phys Chem 76:1919–1924

Gupta DK, Palma JM, Corpas FJ (2018a) Generation and scavenging of reactive oxygen species (ROS) in plant cells: an overview. In: Gupta DK, Palma JM, Corpas FJ (eds) Antioxidants and antioxidant enzymes in higher plants. Springer, Cham

Gupta KJ, Kumari A, Florez-Sarasa I, Fernie AR, Igamberdiev AU (2018b) Interaction of nitric oxide with the components of the plant mitochondrial electron transport chain. J Exp Bot 69:3413–3424

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

Handy DE, Lubos E, Yang Y, Galbraith JD, Kelly N, Zhang YY, Leopold JA, Loscalzo J (2009) Glutathione peroxidase-1 regulates mitochondrial function to modulate redox-dependent cellular responses. J Biol Chem 284:11913–11921

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

Igamberdiev AU, Ratcliffe RG, Gupta KJ (2014) Plant mitochondria: source and target for nitric oxide. Mitochondrion 19(Pt B):329–333

Jasid S, Simontacchi M, Bartoli CG, Puntarulo S (2006) Chloroplasts as a nitric oxide cellular source. Effect of reactive nitrogen species on chloroplastic lipids and proteins. Plant Physiol 142: 1246–1255

Jeandroz S, Wipf D, Stuehr DJ, Lamattina L, Melkonian M, Tian Z, Zhu Y, Carpenter EJ, Wong GK, Wendehenne D (2016) Occurrence, structure, and evolution of nitric oxide synthase-like proteins in the plant kingdom. Sci Signal 9(417):re2

Jensen PK (1966a) Antimycin-insensitive oxidation of succinate and reduced nicotinamide-adenine dinucleotide in electron-transport particles. I. pH dependency and hydrogen peroxide formation. Biochim Biophys Acta 122:157–166

Jensen PK (1966b) Antimycin-insensitive oxidation of succinate and reduced nicotinamide-adenine dinucleotide in electron-transport particles. II. Steroid effects. Biochim Biophys Acta 122: 167–174

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

Lindermayr C (2017) Crosstalk between reactive oxygen species and nitric oxide in plants: key role of *S*-nitrosoglutathione reductase. Free Radic Biol Med 122:110–115

Loschen G, Azzi A, Richter C, Flohé L (1974) Superoxide radicals as precursors of mitochondrial hydrogen peroxide. FEBS Lett 42:68–72

Loughran PA, Stolz DB, Vodovotz Y, Watkins SC, Simmons RL, Billiar TR (2005) Monomeric inducible nitric oxide synthase localizes to peroxisomes in hepatocytes. Proc Natl Acad Sci U S A 102:13837–13842

Mano J, Endo T, Miyake C (2016) How do photosynthetic organisms manage light stress? A tribute to the late Professor Kozi Asada. Plant Cell Physiol 57:1351–1353

Maruta T, Sawa Y, Shigeoka S, Ishikawa T (2016) Diversity and evolution of ascorbate peroxidase functions in chloroplasts: more than just a classical antioxidant enzyme? Plant Cell Physiol 57: 1377–1386

Mehler A (1951) Studies on reactions of illuminated chloroplasts: I. Mechanism of the reduction of oxygen and other hill reagents. Arch Biochem Biophys 33:65–77

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

Murphy MP (2009) How mitochondria produce reactive oxygen species. Biochem J 417:1–13

Nappi AJ, Vass E (1998) Hydroxyl radical formation resulting from the interaction of nitric oxide and hydrogen peroxide. Biochim Biophys Acta 1380:55–63

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

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

Palma JM, Jiménez A, Sandalio LM, Corpas FJ, Lundqvist M, Gómez M, Sevilla F, del Río LA (2006) Antioxidative enzymes from chloroplasts, mitochondria, and peroxisomes during leaf senescence of nodulated pea plants. J Exp Bot 57:1747–1758

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. Ann Bot 116:627–636

Passaia G, Spagnolo-Fonini L, Caverzan A, Jardim-Messeder D, Christoff AP, Gaeta ML, de Araujo Mariath JE, Margis R, Margis-Pinheiro M (2013) The mitochondrial glutathione peroxidase GPX3 is essential for H_2O_2 homeostasis and root and shoot development in rice. Plant Sci 208:93–101

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

Puntarulo S, Jasid S, Simontacchi M (2007) Reactive nitrogen species-dependent effects on soybean chloroplasts. Plant Signal Behav 2:96–98

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

Rhodin J (1954) Correlation of ultrastructural organization and function in normal and experimentally changed proximal tubule cells of the mouse kidney. Karolinska Institutet, Doctoral Thesis. Stockholm, Sweden

Sevilla F, Jiménez A, Lázaro JJ (2015) What do the plant mitochondrial antioxidant and redox systems have to say under salinity, drought, and extreme temperature? In: Gupta DK, Palma JM, Corpas FJ (eds) Reactive oxygen species and oxidative damage in plants under stress. Springer, Cham, pp 23–55

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

Smirnoff N, Arnaud D (2018) Hydrogen peroxide metabolism and functions in plants. New Phytol 221:1197–1214

Stöhr C, Stremlau S (2006) Formation and possible roles of nitric oxide in plant roots. J Exp Bot 57: 463–470

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

Stöhr C, Strube F, Marx G, Ullrich WR, Rockel P (2001) A plasma membrane-bound enzyme of tobacco roots catalyses the formation of nitric oxide from nitrite. Planta 212:835–841

Stolz DB, Zamora R, Vodovotz Y, Loughran PA, Billiar TR, Kim YM, Simmons RL, Watkins SC (2002) Peroxisomal localization of inducible nitric oxide synthase in hepatocytes. Hepatology 36:81–93

Tewari RK, Prommer J, Watanabe M (2013) Endogenous nitric oxide generation in protoplast chloroplasts. Plant Cell Rep 32:31–44

Tun NN, Santa-Catarina C, Begum T, Silveira V, Handro W, Floh EI, Scherer GF (2006) Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. Plant Cell Physiol 47:346–354

Turrens JF (1997) Superoxide production by the mitochondrial respiratory chain. Biosci Rep 17: 3–8

Ursini F, Maiorino M, Brigelius-Flohe R, Aumann KD, Roveri A, Schomburg D, Flohé L (1995) Diversity of glutathione peroxidases. Method Enzymol 252:38–53

Weisiger RA, Fridovich I (1973) Mitochondrial superoxide simutase. Site of synthesis and intramitochondrial localization. J Biol Chem 248:4793–4796

Wimalasekera R, Villar C, Begum T, Scherer GF (2011) COPPER AMINE OXIDASE1 (CuAO1) of *Arabidopsis thaliana* contributes to abscisic acid- and polyamine-induced nitric oxide biosynthesis and abscisic acid signal transduction. Mol Plant 4:663–678

Wulff A, Oliveira HC, Saviani EE, Salgado I (2009) Nitrite reduction and superoxide-dependent nitric oxide degradation by *Arabidopsis* mitochondria: influence of external NAD(P)H dehydrogenases and alternative oxidase in the control of nitric oxide levels. Nitric Oxide 21:132–139

Yamaguchi K, Mori H, Nishimura M (1995) A novel isoenzyme of ascorbate peroxidase localized on glyoxysomal and leaf peroxisomal membranes in pumpkin. Plant Cell Physiol 36:1157–1162

Yamasaki H, Sakihama Y, Takahashi S (1999) An alternative pathway for nitric oxide production in plants: new features of an old enzyme. Trend Plant Sci 4:128–129

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

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

Hydrogen Peroxide and Nitric Oxide Signaling Network

Lijuan Niu, Jihua Yu, Weibiao Liao, and Jian Yu

Contents

Abstract Nitric oxide (NO), as a diatomic free radical gas, appears to be involved in development, growth, and biotic/abiotic responses in plants. Also, hydrogen peroxide (H_2O_2), a reactive oxygen species, has been considered as an important signaling molecule that regulates various physiological and biochemical processes in plants. A large of evidences implied that NO and H_2O_2 signaling could affect each other at different levels of regulation under common conditions. Meanwhile, NO might have similar kinetics with H_2O_2. The interaction between NO and H_2O_2 is complex which has essential role in mediating signaling transduction pathway in plants. This chapter aims to introduce these evidences in our understanding of the roles of NO and H_2O_2 signaling network in plants and their interaction.

Keywords Nitric oxide · Hydrogen peroxide · Signaling molecules · Interaction

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1 Introduction

Previous evidences indicated that nitric oxide (NO), as multifunctional molecule, is involved in the processes of plant growth and development such as seed germination (Li et al. 2018), root development (Liao et al. 2012; Cao et al. 2017), pollen tube growth (Wang et al. 2009), as well as stomatal closure (Shi et al. 2015; Lv et al. 2018). Moreover, NO plays an essential role in response to various abiotic stresses (Tripathi et al. 2017; Akram et al. 2018; Alamri et al. 2018).

Hydrogen peroxide (H_2O_2) signaling is a core regulator during plant physiological process and stress adaption including root development growth (Li et al. 2016; Mei et al. 2017), stomatal movement (Liu et al. 2015), and immune resistance (Tian et al. 2016). Also, H_2O_2 has been demonstrated to be involved in abiotic stress responses such as drought stress (Khan et al. 2017), salt stress (Wang et al. 2018), and many others. Until now, it has become accepted that NO and H_2O_2 signaling and their interaction have the ability to regulate the development and growth as well as abiotic stress resistance in plants. In this chapter, we pay more attention to elaborate the signaling network of NO and H_2O_2 and their interaction in plants.

2 NO Signaling Network in Plants

2.1 NO Synthesis

Free radical NO, which is widely found in bacteria (Gusarov et al. 2009), animals (Mishra et al. 2017; Fahey and Girotti 2017), and plants (Zhu et al. 2017; Hu et al. 2017), is considered to be an essential regulator in various biological and physiological processes. For example, NO as a multifunctional messenger plays various functions in mammals including dilation of blood vessels to neurotransmission, defense during immune response, and so on (Gow and Ischiropoulos 2001). In mammals, NO could be synthesized through three different isoforms of NO synthase (NOS) which mainly include endothelial NOS (eNOS), neuronal NOS (nNOS; Förstermann et al. 1994), and inducible NOS (iNOS; Nathan and Hibbs 1991). Generally, NO is synthesized via nonenzymatic and enzymatic pathways in plants. The nonenzymatic pathway of NO contains nitrification or denitrification processes (Skiba et al. 1993). The enzymatic pathway of NO generation mainly includes nitric oxide synthase (NOS)-like (Guo et al. 2003; del Río 2015; Corpas et al. 2017; Astier et al. 2018), nitrate reductase (NR; Rockel et al. 2002), nitrite-NO reductase (Ni-NOR; Stöhr et al. 2001), and xanthine oxidase (XO; Corpas et al. 2004) pathways.

Table 1 The physiological effects of NO in plants

Physiological effects	Species	Tissue	NO effect	References
Seed germination	*Oryza sativa* L.	Seed	+	He et al. (2014)
	Arabidopsis thaliana	Seed		Li et al. (2018)
Root development	*Arabidopsis thaliana*	Primary root	–	Wang et al. (2017)
	Lycopersicon esculentum Mill.	Lateral root	+	Cao et al. (2017), Pagnussat et al. (2004)
	Cucumis sativus	Adventitious root	+	Lanteri et al. (2006), Liao et al. (2012), Niu et al. (2017)
Ripening and senescence	*Prunus salicina* Lindell	Fruit	+	Singh et al. (2009)
	Solanum lycopersicum L.	Fruit		Eum et al. (2009)
	Rosa hybrida L.	Flower	–	Liao et al. (2013)
Stomatal closure	*Arabidopsis thaliana*	Leaf	+	Scuffi et al. (2014), Shi et al. (2015), Sun et al. (2018)
Pollen tube growth	*Lilium longiflorum*	Pollen	+	Prado et al. (2004)
	Pinus bungeana *Olea europaea* L.	Pollen Pollen		Wang et al. (2009) Jiménez-Quesada et al. (2017)
Disease resistance	*Prunus persica* L.	Fruit	+	Li et al. (2017a, b, c)
	Arabidopsis thaliana	Seedling	+	Zou et al. (2018)

2.2 *Response to NO in Plants*

NO is known as a versatile signaling molecule involved in the regulation of plant growth and developmental processes (Corpas et al. 2006).

2.2.1 Seed Germination

Generally, seed germination is the first developmental stage in the plant life cycle. Previous studies suggested that NO has a positive effect on promoting seed germination in plants (Table 1). For example, Li et al. (2018) found that NO might promote phytochrome B (PHYB)-dependent seed germination through suppressing the expression level of PHYTOCHROME-INTERACTION FACTOR1 (*PIF1*) and PIF1 activity. Also, the inhibition of seed germination under copper toxicity is ameliorated via the application of NO treatment. Moreover, He et al. (2014) indicated that application of NO attenuated the inhibition of rice seed germination and seedling growth through stimulating the activities of antioxidant enzymes including superoxide dismutases (SOD), ascorbate peroxidases (APX), guaiacol peroxidase

(POD), and catalases (CAT) under Cd stress. Thus, NO may initiate different mechanism, promoting seed germination in plants (Table 1).

2.2.2 Root Growth and Development

Root systems play an essential role in enhancing plant nutrient uptake (Bassiri Rad et al. 2001). Previous results implied that NO might be involved in regulating root formation in plants (Table 1). For example, NO might significantly inhibit primary root growth by *S*-nitrosylation of plastidial glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in *Arabidopsis thaliana* (Wang et al. 2017). However, NO has ability to promote development and elongation of later root in tomato (Cao et al. 2017). During the development of later roots of tomato, endogenous NO level significantly increased by $H_{2,}$ but the positive role of H_2 in inducing later root development was obviously suppressed via application of NO scavenger (2-4-carboxyphenyl-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, cPTIO) implying that NO was involved in H_2-induced later root development. In addition, NO might significantly increase the adventitious rooting under stress or stress-free condition (Pagnussat et al. 2004; Lanteri et al. 2006; Liao et al. 2012; Niu et al. 2017; Table 1).

2.2.3 Ripening and Senescence

NO fumigation might delay fruit ripening of Japanese plums (*Prunus salicina* L.) through increasing flesh firmness, decreasing respiration rate, and inhibiting ethylene production rate during fruit ripening in "Amber Jewel" plums (Singh et al. 2009). Moreover, Eum et al. (2009) indicated that NO might play a positive role in postharvest metabolism by decreasing and delaying the expression level of ethylene biosynthetic genes such as *LeACO1*, *LeACOH2*, and *LeACO4* in tomato fruit. Furthermore, NO might significantly delay senescence of cut flower through decreasing the activities of ethylene synthetic enzymes and thereafter inhibiting endogenous ethylene generation (Liao et al. 2013). Therefore, the process of ripening and senescence in plants may require the involvement of NO (Table 1).

2.2.4 Stomatal Closure

Stomatal movement is related to the plant growth and environmental stresses adaptation (Ding et al. 2014). NO was considered to be an essential regulator during stomatal closure which is a part of immunity response to pathogen in plants (Agurla et al. 2014). Also, Lv et al. (2018) indicated that NO production is required for the strigolactone (SLs)-triggered stomatal closure in *Arabidopsis thaliana*. Moreover, there are various evidences implied that NO plays an essential role in regulating stomatal closure in plants (Scuffi et al. 2014; Shi et al. 2015; Sun et al. 2018; Table 1).

2.2.5 Pollen Tube Growth

Previous studies suggested that the guidance of pollen tube plays an essential role in sexual plant reproduction. Some evidences have already demonstrated that NO plays a role in regulating the guidance of pollen tube and NO was mostly found in peroxisomes using DAF2-DA (Prado et al. 2004). Wang et al. (2009) indicated that NO donor SNAP might significantly enhance pollen tube growth through mediating extracellular Ca^{2+} influx and actin filament organization for cell wall construction in *Pinus bungeana* (Table 1).

2.2.6 Disease Resistance

Evidences indicated that NO might positively enhance disease resistance against *Monilinia fructicola* in peach fruit by regulating phenylpropanoid pathway (Li et al. 2016). Moreover, Zou et al. (2018) found that brassinosteroid (BR) might significantly reduce the accumulation of virus resistance in *Arabidopsis thaliana* through enhancing NIA1-mediated endogenous NO level, antioxidant system, and photosynthetic system (Table 1).

2.2.7 Abiotic Stress

As a multifunctional regulator, NO signaling also is involved in mediating responses to various abiotic stresses such as drought (Akram et al. 2018; Hasanuzzaman et al. 2018), salt (Ahmad et al. 2016; Arora and Bhatla 2017), cold (Lv et al. 2017; Dong et al. 2018), heat (Wang et al. 2014; Alamri et al. 2018), UV-B (Esringu et al. 2016), and heavy metal (Tripathi et al. 2017; He et al. 2017; Table 2). These studies have

Table 2 Responses of NO in plants under abiotic stress

Stress	Species	Tissue	NO effect	References
Drought	*Brassica napus* L.	Leaf	+	Akram et al. (2018)
	Triticum aestivum L.	Seedlings		Hasanuzzaman et al. (2018)
Salt	*Cicer arietinum* L.	Seedlings	+	Ahmad et al. (2016)
	Helianthus annuus	Cotyledons		Arora and Bhatla (2017)
Cold	*Solanum lycopersicum* L.	Seedlings	+	Lv et al. (2017)
	Juglans regia L.	Shoot		Dong et al. (2018)
Heat	*Arabidopsis thaliana*	Seedlings	+	Wang et al. (2014)
	Vicia faba L.	Seedlings		Alamri et al. (2018)
UV-B	*Lactuca sativa* L.	Seedlings	+	Esringu et al. (2016)
Heavy metal	*Pisum sativum*	Seedlings	+	Tripathi et al. (2017)
	Arachis hypogaea L.	Root	+	He et al. (2017)

demonstrated that the NO signaling has the potential to regulate cell metabolism, balance of cellular redox, and gene expression in plants. As a result, regulatory mechanism is activated by NO signaling to affect developmental and physiological processes and mitigate abiotic stresses in plants.

2.3 NO Signaling Transduction with Other Signaling Molecules

NO has been highly appreciated for its versatile properties as a signaling molecule regulating a diversity of physiological processes in animals and plants. Several researches have indicated that NO signaling transduction is really complicated for interaction between various signaling transductions. Therefore, we provide several evidences about the interaction between NO signaling molecule and other signaling molecules in plants (Asgher et al. 2017). For example, previous results implied that NO might crosstalk with phytohormones during plant growth and development (Freschi 2013; Sanz et al. 2015) as well as stress responses. Li et al. (2017a, b, c) found that there exists an interrelationship between NO and ABA in stomatal close of *Arabidopsis thaliana* through elevating endogenous NO level. However, Sivakumaran et al. (2016) showed that ABA might significantly inhibit *Botrytis cinerea*-stimulated NO generation, further affecting endogenous H_2O_2 generation and enhancing host susceptibility in tomato. Moreover, Zou et al. (2018) suggested that brassinosteroid (BR) treatment significantly increased endogenous NO level accompanying with an obvious reduction of virus accumulation in *Arabidopsis thaliana*. However, NO scavenger (cPTIO) or inhibitor remarkably reversed the promotive role of BR-induced virus resistance indicating that NO might be a downstream signaling molecule of BR in systematic resistance of *Arabidopsis*. Also, the interaction between NO and ethylene has been found during adventitious rooting (Jin et al. 2017) and senescence delay of cut flowers (Liao et al. 2013). Moreover, NO has the ability to interact with other gaseous signaling molecules such as hydrogen gas (H_2; Zhu et al. 2016), carbon oxide (CO; Wang and Liao 2016), H_2S (Shi et al. 2014; Scuffi et al. 2014), methane (CH_4; Qi et al. 2017), and so on. Therefore, relationship between NO and other signaling molecules in plants is extremely complex. The interplay of these signaling molecules and their mechanism needs to be elaborated clearly in the future.

3 H_2O_2 Signaling Network in Plants

3.1 H_2O_2 Generation

H_2O_2, a by-product produced by plants' aerobic metabolism (Mittler 2002), might be synthesized either enzymatically or nonenzymatically. There are various pathways of H_2O_2 production in plant cells such as photorespiration, electron transport chains (ETC), and redox reaction. Previous studies have showed that numerous enzymes are involved in H_2O_2 production including cell wall peroxidases (Francoz et al. 2015), oxalate (Hu et al. 2003), amine oxidases (Cona et al. 2006), nicotinamide adenine dinucleotide phosphate (NADPH; Brewer et al. 2015), and so on. In addition, some other oxidases such as sulfite oxidases (Brychkova et al. 2012) and glycolate oxidases (Chang and Tang 2014) may also produce H_2O_2 via oxidizing their own substrates. Besides, several nonenzymatic reactions, for instance, the processes of photosynthesis and respiration, are also known to be responsible for the generation of H_2O_2 (Mehler 1951; Dickinson and Chang 2011; Noctor and Foyer 2016).

3.2 Responses to H_2O_2 in Plants

3.2.1 Growth and Development

Numerous studies have investigated the importance of H_2O_2 signaling in regulating plant growth and development (Table 3). According to these researches, it has been confirmed that H_2O_2 as an essential molecule was involved in mediating the growth and development of plants by regulating endogenous H_2O_2 content even by affecting relative gene expression (Airaki et al. 2015). However, the mechanisms that H_2O_2 signaling exerts multifunction in plants are still unclear. More work needs to be done to further investigate.

3.2.2 Stress Response

It has been well known that H_2O_2 is a crucial signaling molecule participating in signaling transduction and affecting abiotic stress defense in plants. Several researches have shown that H_2O_2 is involved in various abiotic stress including drought (Khan et al. 2017; Shan et al. 2018), salt (Freitas et al. 2018; Wang et al. 2018), cold (Fedurayev et al. 2018; Lv et al. 2018), heat (Zhou et al. 2014), ozone (Oksanen et al. 2004), and heavy metal (Hasanuzzaman et al. 2017; Table 4). In abiotic stress conditions, H_2O_2 signaling might enhance cell structure stability and regulate the expression of resistance genes and proteins for strengthening antioxidant system and alleviating abiotic stress.

Table 3 The physiological effects of H_2O_2 in plants

Physiological effects	Species	Tissue	H_2O_2 effect	References
Seed germination	*M. sativa*	Seed	+	Wojtyla et al. (2016), Amooaghaie and Tabatabaie (2017)
	Zea may L.			Li et al. (2018)
Root development	*Arabidopsis thaliana*	Lateral root	+	Qu et al. (2017) Chen et al. (2018) Mei et al. (2017)
	Medicago sativa			
	Lycopersicon esculentum Mill.			
	Cucumis sativus L.	Adventitious root	+	Li et al. (2016)
Ripening	*Vitis vinifera*	Fruit	+	Xu et al. (2018)
	Capsicum annuum L.	Fruit		Corpas et al. (2018)
	Solanum lycopersicum L.			
Stomatal closure	*Solanum lycopersicum*	Leaf	+	Xia et al. (2014) Liu et al. (2015)
	Oryza sativa			
Pollen tube growth	*Cupressus arizonica*	Pollen	+	Pasqualini et al. (2015) Maksimov et al. (2018)
	Picea pungens			
Disease resistance	*Nicotiana tabacum*	Leaf	+	Tian et al. (2016)

Table 4 Responses of H_2O_2 in plants under abiotic stress

Stress	Species	Tissue	H_2O_2 effect	References
Drought	*Brassica napus* L.	Seedlings	+	Khan et al. (2017)
	Triticum aestivum L.	Seedlings		Shan et al. (2018)
Salt	*Aquilaria sinensis*	Calli	+	Wang et al. (2018)
	Zea mays L.	Leaf		Freitas et al. (2018)
Cold	*Synechocystis*	Cyanobacteria	+	Fedurayev et al. (2018)
	Solanum lycopersicum L.	Seedling		Lv et al. (2018)
Heat	*Solanum lycopersicum* L.	Seedlings	+	Zhou et al. (2014)
Ozone	*Betula papyrifera*	Leaf	+	Oksanen et al. (2004)
Heavy metal	*Brassica napus*	Seedlings	+	Hasanuzzaman et al. (2017)

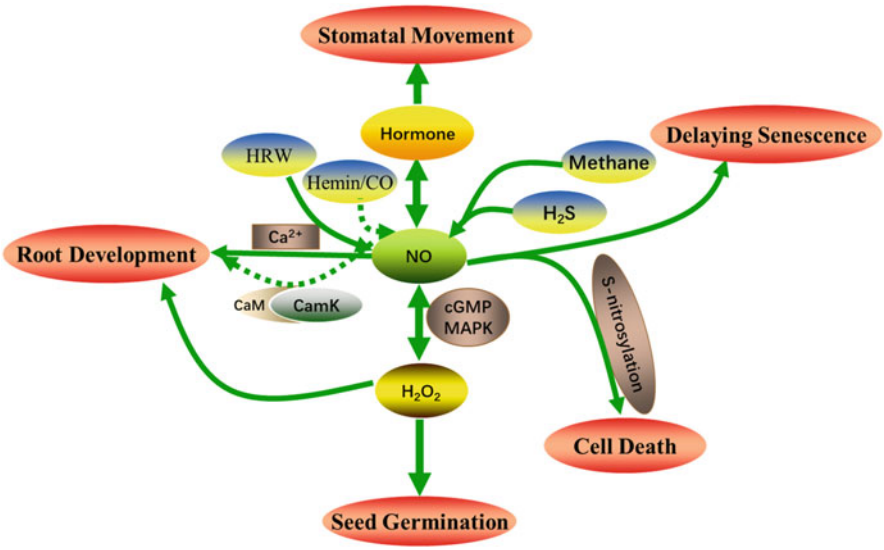

Fig. 1 Schematic model of interaction between NO and H_2O_2 during growth and development in plant

4 Crosstalk Between NO and H_2O_2 Signaling in Plants

4.1 Interaction in Growth and Development

NO and H_2O_2 signaling molecules have been highly appreciated for its versatile properties in regulating diverse physiological processes in plants (Lindermayr 2018; Fig. 1). Up to now, it has been clearly demonstrated that the interaction between NO and H_2O_2 might play a critical role in affecting plant developmental and physiological processes such as dormancy release (Liu et al. 2011; Ma et al. 2016), root growth (Duan et al. 2015; Yu et al. 2017; Recalde et al. 2018), cell death (Lin et al. 2012), delay senescence (Iakimova and Woltering 2015), and stomatal closure (Zhang et al. 2017; Sun et al. 2018; Lv et al. 2018; Table 5). As stated above, the signaling interaction between NO and H_2O_2 has a potential role at every stage of plant development and growth during cell extension and division, antioxidant enzyme activity, and transcriptional expression and protein expression. On the one side, NO may affect H_2O_2 level, and H_2O_2 may also regulate endogenous NO generation during plant growth and development.

Table 5 The physiological effects of interaction between NO and H_2O_2 in plants

Physiological effects	Species	Tissue	References
Dormancy	*Amaranthus retroflexus*	Seed	Liu et al. (2011)
	Hordeum vulgare L.	Seed	Ma et al. (2016)
Root growth	*Triticum aestivum*	Root	Duan et al. (2015)
	Lycopersicon esculentum Mill.	Root	Yu et al. (2017)
	Triticum aestivum	Root	Recalde et al. (2018)
Cell death	*Oryza sativa*	Leaf	Lin et al. (2012)
Senescence	*L. sativa* L.	Organ	Iakimova and Woltering (2015)
Stomatal closure	*Arabidopsis thaliana*	Leaf	Zhang et al. (2017)
Disease resistance	*Arabidopsis thaliana* Kidney bean Potato	Leaf Seedling Seedling	Sun et al. (2018) Keshavarz-Tohid et al. (2016) Floryszak-Wieczorek and Arasimowicz-Jelonek (2016)

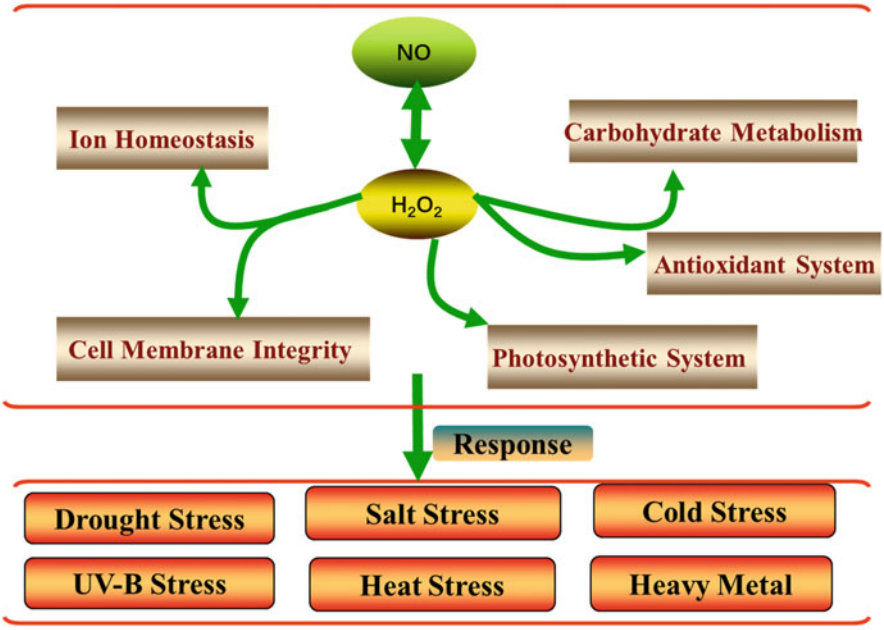

Fig. 2 Schematic model of interaction between NO and H_2O_2 in stress responses

4.2 Interaction in Stress Responses

Several studies have established the roles of NO and H_2O_2 signaling and their crosstalk in mediating plant response to abiotic stresses (Fig. 2; Table 6).

Table 6 Interaction between NO and H_2O_2-mediated effect under abiotic stresses in plants

Stress	Species	Tissue	References
Wounding Drought	*Cakile maritima* L. *Tagetes erecta* L.	Seedling Root	Houmani et al. (2018) Liao et al. (2012)
Salt	*Oryza sativa* L.	Seedlings	Mostofa et al. (2015)
	Populus euphratica	Calluses	Zhang et al. (2007)
Cold	*Solanum lycopersicum* Mill.	Seedlings	Diao et al. (2017)
UV-B	*Raphanus sativus*	Seedlings	Wu et al. (2016)
Heat	*Zea mays* L.	Seedlings	Li et al. (2015)
	Triticum aestivum L.	Seedlings	Karpets et al. (2015)
Heavy metal	*Triticum aestivum* L.	Seedlings	Sun et al. (2018)
	Triticum aestivum L.	Seedlings	Duan et al. (2015)

4.2.1 Drought

Drought stress is a critical environmental factor that affects plant growth and development. As reported by previous study, endogenous NO and H_2O_2 may participate in ABA-induced drought tolerance through enhancing activities of antioxidant enzyme in Bermuda grass (Lu et al. 2009). Moreover, H_2O_2 was also demonstrated as a downstream signaling of NO which induced the adventitious rooting through attenuating destruction of mesophyll cell ultrastructure and enhancing photosynthetic system as well as carbohydrate content in hypocotyl under drought stress (Liao et al. 2012). In addition, the interplay between NO and H_2O_2 signaling may increase the activity of myo-inositol phosphate synthase to alleviate drought stress (Tan et al. 2013). Therefore, the interaction between NO and H_2O_2 may regulate antioxidant defense system to balance ion homeostasis and strengthen cell membrane integrity under drought stress in plants (Table 6).

4.2.2 Salt

Salt stress is also one of the most serious factors restraining the plant development and growth. The crosstalk between NO and H_2O_2 was involved in plant tolerance to salt stress (Tan et al. 2013; Mostofa et al. 2015). For instance, Zhang et al. (2007) studied that involvement of NO and H_2O_2 in alleviating salt stress in *Populus euphratica*. Their results indicated that NO and H_2O_2 might enhance the salt resistance through increasing the K/Na ratio and the activity of plasma membrane (PM) H^+-ATPase. In addition, NO might significantly elevate the endogenous H_2O_2 level during salt resistance. Moreover, NO was investigated to participate in H_2O_2- or SA-induced salt tolerance via enhancing antioxidant system and methylglyoxal detoxification systems in rice seedlings. H_2O_2 or SA is responsible for increase of the endogenous NO content during this process (Mostofa et al. 2015; Table 6).

4.2.3 UV-B

UV-B, a key environmental signal, initiates diverse responses in plants (Jansen and Bornman 2012). Interaction among Ga protein, NO, and H_2O_2 was found to mediate UV-B-induced stomatal closure in *Arabidopsis* leaves through remarkably increasing NO or H_2O_2 levels in the wild type under UV-B stress (He et al. 2013), implying that the crosstalk between NO and H_2O_2 signaling might play a significant role during UV-B-induced stomatal closure in guard cells. Tossi et al. (2014) also suggested both NO and H_2O_2 generation in response to UV-B exposure. Previous research has indicated that application of exogenous H_2O_2 might significantly promote the accumulation of anthocyanin and *UVR8* expression under UV-B stress. However, NO scavenger (cPTIO) addition significantly reverses the promotive role of H_2O_2 in anthocyanin accumulation and *UVR8* transcription under UV-B stress (Wu et al. 2016; Table 6).

4.2.4 Cold

Cold stress can be divided into chilling and freezing stress which depends on whether the temperature is lower than 20°C or 0°C (Chinnusamy et al. 2007). Diao et al. (2017) indicated that application of polyamines (PAs) significantly increases the endogenous level of NO and H_2O_2; meanwhile, PAs-induced enhancement of endogenous NO level is dependent on H_2O_2 pathway in tomato seedlings under chilling stress. In addition, interaction of NO and H_2O_2 might increase cold tolerance through upregulating polyamine oxidation in alfalfa. Moreover, Lv et al. (2018) found that calcium-dependent protein kinases (CPKs) have a positive function for ABA generation through activating the production of NO and H_2O_2 in cold adaptation, which indicated that the crosstalk between NO and H_2O_2 might regulate the cold adaptation. As mentioned above, the interaction between NO and H_2O_2 may initiate different mechanisms to response to cold stresses (Table 6).

4.2.5 Heat

Several researches implied that the interaction between NO and H_2O_2 was involved in the response to heat stress in plants. For example, Wang et al. (2014) indicated that NO is a downstream factor of H_2O_2 in the tolerance to heat shock via stimulating DNA-binding activity of HS factors and heat shock protein accumulation. Similarly, H_2O_2-induced thermal tolerance was promoted by the application of NO or H_2S, but NO or H_2S inhibitors or scavengers significantly reversed this effect in maize seedlings (Li et al. 2015). Furthermore, Karpets et al. (2015) suggested that crosstalk between NO and H_2O_2 plays a functional role in heat resistance in wheat seedlings. These studies illustrated the existence of crosstalk between NO and H_2O_2 in thermal tolerance in plants (Table 6).

4.2.6 Heavy Metal

Aluminum (Al) toxicity is the primary limiting factor on plant growth and development. Sun et al. (2018) found that NO signaling might be a downstream of H_2O_2-induced antioxidant defense under Al stress. Meanwhile, exogenous H_2O_2 might elevate endogenous NO level in wheat seedling under Al stress (Sun et al. 2018). The interplay of NO and H_2O_2 might be involved in regulating root growth and increasing antioxidant system, protecting the integrity of cell membrane as well as regulating resistance gene expression in wheat seedlings under zinc stress (Duan et al. 2015). Also, NO and H_2O_2 signaling interaction was found to mediate activities of antioxidant enzyme and expression of relative gene in *Ulva compressa* in order to mitigating copper stress (González et al. 2012). Thus, the interaction between NO and H_2O_2 may trigger a serial of antioxidant responses in plants under heavy metal stress condition; meanwhile, the physiological effects of NO and H_2O_2 are similar and synergetic. In addition, it has been demonstrated that the interaction between NO and H_2O_2 also be involved in brassinosteroid (BR)-regulated systemic virus resistance in *Nicotiana benthamiana*; meanwhile, NO synthesis is responsible for the BR-activated H_2O_2 generation during virus resistance (Deng et al. 2016; Table 6).

5 Conclusion

As we all know, the interaction among signaling molecules in plant is extremely complicated. NO and H_2O_2 as two critical signaling molecules involved in various physiological and biological processes during plant growth and development. Different plant species and environmental stresses are responsible for the interaction form of NO and H_2O_2. This interaction might promote NO and H_2O_2 to affect each other level for synergistically regulating ability of antioxidant system, expression of relative gene, or modification of target proteins which are related to metabolism, signaling transduction, and defense resistance in plants. Therefore, the interaction between them has functional implications for signaling reception and transduction in plants. Future work will need to elucidate the complicated molecular mechanism of the crosstalk between NO and H_2O_2 during signaling transduction in plants.

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References

Agurla S, Gayatri G, Raghavendra AS (2014) Nitric oxide as a secondary messenger during stomatal closure as a part of plant immunity response against pathogens. Nitric Oxide 43:89–96

Ahmad P, Abdel Latef AA, Hashem A, Abd Allah EF, Gucel S, Tran LSP (2016) Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea. Front Plant Sci 7:347

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. Ann Bot 116:679–693

Akram NA, Iqbal M, Muhammad A, Ashraf M, Al-Qurainy F, Shafiq S (2018) Aminolevulinic acid and nitric oxide regulate oxidative defense and secondary metabolisms in canola (*Brassica napus* L.) under drought stress. Protoplasma 255:163–174

Alamri SA, Siddiqui MH, Al-Khaishany MY, Khan MN, Ali HM, Alakeel KA (2018) Nitric oxide-mediated cross-talk of proline and heat shock proteins induce thermotolerance in *Vicia faba* L. Environ Exp Bot. https://doi.org/10.1016/j.envexpbot.2018.06.012

Amooaghaie R, Tabatabaie F (2017) Osmopriming-induced salt tolerance during seed germination of alfalfa most likely mediates through H_2O_2 signaling and upregulation of heme oxygenase. Protoplasma 254:1791–1803

Arora D, Bhatla SC (2017) Melatonin and nitric oxide regulate sunflower seedling growth under salt stress accompanying differential expression of Cu/Zn SOD and Mn SOD. Free Radic Biol Med 106:315–328

Asgher M, Per TS, Masood A, Fatma M, Freschi L, Corpas FJ, Khan NA (2017) Nitric oxide signaling and its crosstalk with other plant growth regulators in plant responses to abiotic stress. Environ Sci Pollut Res Int 24:2273–2285

Astier J, Gross I, Durner J (2018) Nitric oxide production in plants: an update. J Exp Bot 69:3401–3411

Bassiri Rad H, Gutschick VP, Lussenhop J (2001) Root system adjustments: regulation of plant nutrient uptake and growth responses to elevated CO_2. Oecologia 126:305–320

Brewer TF, Garcia FJ, Onak CS, Carroll KS, Chang CJ (2015) Chemical approaches to discovery and study of sources and targets of hydrogen peroxide redox signaling through NADPH oxidase proteins. Annl Rev Biochem 84:765–790

Brychkova G, Yarmolinsky D, Fluhr R, Sagi M (2012) The determination of sulfite level sand its oxidation in plant leaves. Plant Sci 190:123–130

Cao Z, Duan X, Yao P, Cui W, Cheng D, Zhang J, Jin Q, Chen J, Dai T, Shen W (2017) Hydrogen gas is involved in auxin-induced lateral root formation by modulating nitric oxide synthesis. Int J Mol Sci 18:2084

Chang Q, Tang H (2014) Optical determination of glucose and hydrogen peroxide using an anocomposite prepared from glucose oxidase and magnetite nanoparticles immobilized on graphene oxide. Microchim Acta 181:527–534

Chen Z, Gu Q, Yu X, Huang L, Xu S, Wang R, Shen W, Shen W (2018) Hydrogen peroxide acts downstream of melatonin to induce lateral root formation. Ann Bot 121:1127–1136

Chinnusamy V, Zhu JH, Zhu JK (2007) Cold stress regulation of gene expression in plants. Trend Plant Sci 12:444–451

Cona A, Rea G, Botta M, Corelli F, Federico R, Angelini R (2006) Flavin-containing polyamine oxidase is a hydrogen peroxide source in the oxidative response to the protein phosphatase inhibitor cantharidin in *Zea mays* L. J Exp Bot 57:2277–2289

Corpas FJ, Barroso JB, del Río LA (2004) Enzymatic sources of nitric oxide in plant cells-beyond one protein–one function. New Phytol 162:246–248

Corpas FJ, Barroso JB, Carreras A, Valderrama R, Palma JM, León AM, Sandalio LM, del Río LA (2006) Constitutive arginine-dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. Planta 224:246–254

Corpas FJ, Barroso JB, Palma JM, Rodriguez-Ruiz M (2017) Plant peroxisomes: a nitro-oxidative cocktail. Redox Biol 11:535–542

Corpas FJ, Freschi L, Rodríguez-Ruiz M, Mioto PT, González-Gordo S, Palma JM (2018) Nitro-oxidative metabolism during fruit ripening. J Exp Bot 69:3449–3463

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

Deng XG, Zhu T, Zou LJ, Han XY, Zhou X, Xi DH, Zhang DW, Lin HH (2016) Orchestration of hydrogen peroxide and nitric oxide in brassinosteroid-mediated systemic virus resistance in *Nicotiana benthamiana*. Plant J 85:478–493

Diao QN, Song YJ, Shi DM, Qi HY (2017) Interaction of polyamines, abscisic acid, nitric oxide, and hydrogen peroxide under chilling stress in tomato (*Lycopersicon esculentum* Mill.) seedlings. Front Plant Sci 8:203

Dickinson BC, Chang CJ (2011) Chemistry and biology of reactive oxygen species in signaling or stress responses. Nat Chem Biol 7:504–511

Ding ZJ, Yan JY, Xu XY, Yu DQ, Li GX, Zhang SQ, Zheng SJ (2014) Transcription factor WRKY 46 regulates osmotic stress responses and stomatal movement independently in *Arabidopsis*. Plant J 79:13–27

Dong N, Li Y, Qi J, Chen Y, Hao Y (2018) Nitric oxide synthase-dependent nitric oxide production enhances chilling tolerance of walnut shoots *in vitro* via involvement chlorophyll fluorescence and other physiological parameter levels. Scient Horti 230:68–77

Duan X, Li X, Ding F, Zhao J, Guo A, Zhang L, Yao J, Yang YL (2015) Interaction of nitric oxide and reactive oxygen species and associated regulation of root growth in wheat seedlings under zinc stress. Ecotox Environ Safe 113:95–102

Esringu A, Aksakal O, Tabay D, Kara AA (2016) Effects of sodium nitroprusside (SNP) pretreatment on UV-B stress tolerance in lettuce (*Lactuca sativa* L.) seedlings. Environ Sci Pollut Res 23:589–597

Eum HL, Kim HB, Choi SB, Lee SK (2009) Regulation of ethylene biosynthesis by nitric oxide in tomato (*Solanum lycopersicum* L.) fruit harvested at different ripening stages. Eur Food Res Technol 228:331

Fahey JM, Girotti AW (2017) Nitric oxide-mediated resistance to photodynamic therapy in a human breast tumor xenograft model: improved outcome with NOS2 inhibitors. Nitric Oxide 62:52–61

Fedurayev PV, Mironov KS, Gabrielyan DA, Bedbenov VS, Zorina AA, Shumskaya M, Los DA (2018) Hydrogen peroxide participates in perception and transduction of cold stress signal in *Synechocystis*. Plant Cell Physiol 59:1255–1264

Floryszak-Wieczorek J, Arasimowicz-Jelonek M (2016) Contrasting regulation of NO and ROS in potato defense-associated metabolism in response to pathogens of different lifestyles. PLoS One 11:e0163546

Förstermann U, Closs EI, Pollock JS, Nakane M, Schwarz P, Gath I, Kleinert H (1994) Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension 23:1121–1131

Francoz E, Ranocha P, Nguyen-Kim H, Jamet E, Burlat V, Dunand C (2015) Roles of cell wall peroxidases in plant development. Phytochemistry 112:15–21

Freitas VS, de Souza Miranda R, Costa JH, de Oliveira DF, de Oliveira Paula S, de Castro Miguel E, Souza Freire R, Tarquinio Prisco J, Gomes Filho E (2018) Ethylene triggers salt tolerance in maize genotypes by modulating polyamine catabolism enzymes associated with H_2O_2 production. Environ Exp Bot 145:75–86

Freschi L (2013) Nitric oxide and phytohormone interactions: current status and perspectives. Front Plant Sci 4:398

González A, delos Ángeles Cabrera M, Henríquez MJ, Contreras RA, Morales B, Moenne A (2012) Crosstalk among calcium, hydrogen peroxide, and nitric oxide and activation of gene expression

involving calmodulins and calcium-dependent protein kinases in *Ulva compressa* exposed to copper excess. Plant Physiol 158:1451–1462

Gow AJ, Ischiropoulos H (2001) Nitric oxide chemistry and cellular signaling. J Cell Physiol 187:277–282

Guo F, Okamoto M, Crawford NM (2003) Identification of a plant nitric oxide synthase gene involved in hormonal signaling. Science 302:100–103

Gusarov I, Shatalin K, Starodubtseva M, Nudler E (2009) Endogenous nitric oxide protects bacteria against a wide spectrum of antibiotics. Science 325:1380–1384

Hasanuzzaman M, Nahar K, Gill SS, Alharby HF, Razafindrabe BH, Fujita M (2017) Hydrogen peroxide pretreatment mitigates cadmium-induced oxidative stress in *Brassica napus* L.: An intrinsic study on antioxidant defense and glyoxalase systems. Front Plant Sci 8:115

Hasanuzzaman M, Nahar K, Rahman A, Inafuku M, Oku H, Fujita M (2018) Exogenous nitric oxide donor and arginine provide protection against short-term drought stress in wheat seedlings. Physiol Mol Biol Plants 24:1–12

He J, Ren Y, Chen X, Chen H (2014) Protective roles of nitric oxide on seed germination and seedling growth of rice (*Oryza sativa* L.) under cadmium stress. Ecotoxicol Environ Saf 108:114–119

He JM, Ma XG, Zhang Y, Sun TF, Xu FF, Chen YP, Liu X, Yue M (2013) Role and interrelationship of Ga protein, hydrogen peroxide, and nitric oxide in ultra violet B-induced stomatal closure in *Arabidopsis* leaves. Plant Physiol 161:1570–1583

He H, Huang W, Oo TL, Gu M, He LF (2017) Nitric oxide inhibits aluminum-induced programmed cell death in peanut (*Arachis hypogaea* L.) root tips. J Hazard Mater 333:285–292

Houmani H, Rodríguez-Ruiz M, Palma JM, Corpas FJ (2018) Mechanical wounding promotes local and long distance response in the halophyte *Cakile maritima* through the involvement of the ROS and RNS metabolism. Nitric Oxide 74:93–101

Hu X, Bidney DL, Yalpani N, Duvick JP, Crasta O, Folkerts O, Lu G (2003) Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. Plant Physiol 133:170–181

Hu J, Yang H, Mu J, Lu T, Peng J, Deng X, Kong Z, Bao S, Cao X, Zuo J (2017) Nitric oxide regulates protein methylation during stress responses in plants. Mol Cell 67:702–710

Iakimova ET, Woltering EJ (2015) Nitric oxide prevents wound-induced browning and delays senescence through inhibition of hydrogen peroxide accumulation in fresh-cut lettuce. Innov Food Sci Emerg 30:157–169

Jansen MAK, Bornman JF (2012) UV-B radiation: from generic stressor to specific regulator. Physiol Plant 145:501–504

Jiménez-Quesada MJ, Carmona R, Lima-Cabello E, Traverso JÁ, Castro AJ, Claros MG, Alché JD (2017) Generation of nitric oxide by olive (*Olea europaea* L.) pollen during in vitro germination and assessment of the S-nitroso- and nitro-proteomes by computational predictive methods. Nitric Oxide 68:23–37

Jin X, Liao WB, Yu JH, Ren PJ, Dawuda MM, Wang M, Niu LJ, Li XP, Xu XT (2017) Nitric oxide is involved in ethylene-induced adventitious rooting in marigold (*Tagetes erecta* L.). Can J Plant Sci 97:620–631

Karpets YV, Kolupaev YE, Vayner AA (2015) Functional interaction between nitric oxide and hydrogen peroxide during formation of wheat seedling induced heat resistance. Russ J Plant Physl 62:65–70

Keshavarz-Tohid V, Taheri P, Taghavi SM, Tarighi S (2016) The role of nitric oxide in basal and induced resistance in relation with hydrogen peroxide and antioxidant enzymes. J Plant Physiol 199:29–38

Khan A, Anwar Y, Hasan MM, Iqbal A, Ali M, Alharby HF, Hakeem KR, Hasanuzzaman M (2017) Attenuation of drought stress in Brassica seedlings with exogenous application of Ca^{2+} and H_2O_2. Plants 6:20

Lanteri ML, Pagnussat GC, Lamattina L (2006) Calcium and calcium-dependent protein kinases are involved in nitric oxide-and auxin-induced adventitious root formation in cucumber. J Exp Bot 57:1341–1351

Li XP, Xu QQ, Liao WB, Ma ZJ, Xu XT, Wang M, Ren PJ, Niu LJ, Jin X, Zhu YC (2016) Hydrogen peroxide is involved in abscisic acid-induced adventitious rooting in cucumber (*Cucumis sativus* L.) under drought stress. J Plant Biol 59:536–548

Li G, Zhu S, Wu W, Zhang C, Peng Y, Wang Q, Shi J (2017a) Exogenous nitric oxide induces disease resistance against *Monilinia fructicola* through activating the phenylpropanoid pathway in peach fruit. J Sci Food Agric 97:3030–3038

Li Q, Wang YJ, Liu CK, Pei ZM, Shi WL (2017b) The crosstalk between ABA, nitric oxide, hydrogen peroxide, and calcium in stomatal closing of *Arabidopsis thaliana*. Biologia 72:1140–1146

Li Z, Xu J, Gao Y, Wang C, Guo G, Luo Y, Huang Y, Hu W, Sheteiwy MS, Guan Y, Hu J (2017c) The synergistic priming effect of exogenous salicylic acid and H_2O_2 on chilling tolerance enhancement during maize (*Zea mays* L.) seed germination. Front Plant Sci 8:1153

Li R, Jia Y, Yu L, Yang W, Chen Z, Chen H, Hu X (2018) Nitric oxide promotes light-initiated seed germination by repressing *PIF1* expression and stabilizing HFR1. Plant Physiol Biochem 123:204–212

Li ZG, Luo LJ, Sun YF (2015) Signal crosstalk between nitric oxide and hydrogen sulfide may be involved in hydrogen peroxide-induced thermotolerance in maize seedlings. Russ J Plant Physiol 62:507–514

Liao WB, Huang GB, Yu JH, Zhang ML (2012) Nitric oxide and hydrogen peroxide alleviate drought stress in marigold explants and promote its adventitious root development. Plant Physiol Biochem 58:6–15

Liao WB, Zhang ML, Yu JH (2013) Role of nitric oxide in delaying senescence of cut rose flowers and its interaction with ethylene. Sci Hortic 155:30–38

Lin AH, Wang YQ, Tang JY, Xue P, Li CL, Liu LC, Hu B, Yang FQ, Loake GJ, Chu CC (2012) Nitric oxide and protein *S*-nitrosylation are integral to hydrogen peroxide-induced leaf cell death in rice. Plant Physiol 158:451–464

Lindermayr C (2018) Crosstalk between reactive oxygen species and nitric oxide in plants: key role of *S*-nitrosoglutathione reductase. Free Radic Biol Med 122:110–115

Liu J, Zhang C, Wei C, Wang M, Liu X, Yu F, Xie Q, Tu J (2015) The RING finger ubiquitin E3 ligase OsHTAS enhances heat tolerance by promoting H_2O_2-induced stomatal closure in rice. Plant Physiol 170:429–443

Lu SY, Su W, Li HH, Guo ZF (2009) Abscisic acid improves drought tolerance of triploid bermudagrass and involves H_2O_2-and NO- induced antioxidant enzyme activities. Plant Physiol Biochem 47:132–138

Liu XY, Deng ZJ, Cheng HY, He XH, Song SQ (2011) Nitrite, sodium nitroprusside, potassium ferricyanide and hydrogen peroxide release dormancy of *Amaranthus retroflexus* seeds in a nitric oxide-dependent manner. Plant Growth Regul 64:155–161

Lv X, Ge S, Jalal Ahammed G, Xiang X, Guo Z, Yu J, Zhou Y (2017) Crosstalk between nitric oxide and MPK1/2 mediates cold acclimation-induced chilling tolerance in tomato. Plant Cell Physiol 58:1963–1975

Lv X, Li H, Chen X, Xiang X, Guo Z, Yu J, Zhou Y (2018) The role of calcium-dependent protein kinase in hydrogen peroxide, nitric oxide and ABA-dependent cold acclimation. J Exp Bot 69:4127–4139

Ma Z, Marsolais F, Bykova NV, Igamberdiev AU (2016) Nitric oxide and reactive oxygen species mediate metabolic changes in barley seed embryo during germination. Front Plant Sci 7:138

Maksimov N, Evmenyeva A, Breygina M, Yermakov I (2018) The role of reactive oxygen species in pollen germination in *Picea pungens* (blue spruce). Plant Reprod 31:357–365

Mehler AH (1951) Studies on reactions of illuminated chloroplasts II. Stimulation and inhibition of their action with molecular oxygen. Arch Biochem Biophys 33:339–351

Mei Y, Chen H, Shen W, Shen W, Huang L (2017) Hydrogen peroxide is involved in hydrogen sulfide-induced lateral root formation in tomato seedlings. BMC Plant Biol 17:162

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

Mishra BB, Lovewell RR, Olive AJ, Zhang G, Wang W, Eugenin E, Smith CM, Phuah JY, Long JE, Dubuke ML, Palace SG, Goguen JD, Baker RE, Nambi S, Mishra R, Booty MG, Baer CE, Shaffer SA, Dartois V, McCormick BA, Chen X, Sassetti CM (2017) Nitric oxide prevents a pathogen-permissive granulocytic inflammation during tuberculosis. Nat Microbiol 2:17072

Mostofa MG, Fujita M, Tran LSP (2015) Nitric oxide mediates hydrogen peroxide-and salicylic acid-induced salt tolerance in rice (*Oryza sativa* L.) seedlings. Plant Growth Regul 77:265–277

Nathan CF, Hibbs JB (1991) Role of nitric oxide synthesis in macrophage antimicrobial activity. Curr Opin Immunol 3:65–70

Niu L, Yu J, Liao W, Yu J, Zhang M, Dawuda MM (2017) Calcium and calmodulin are involved in Nitric Oxide-induced adventitious rooting of cucumber under simulated osmotic stress. Front Plant Sci 8:1684

Noctor G, Foyer CH (2016) Intracellular redox compartmentation and ROS-related communication in regulation and signaling. Plant Physiol 171:1581–1592

Oksanen E, Häikiö E, Sober J, Karnosky DF (2004) Ozone-induced H_2O_2 accumulation in field-grown aspen and birch is linked to foliar ultrastructure and peroxisomal activity. New Phytol 161:791–799

Pagnussat GC, Lanteri ML, Lombardo MC, Lamattina L (2004) Nitric oxide mediates the indole acetic acid induction activation of a mitogen-activated protein kinase cascade involved in adventitious root development. Plant Physiol 135:279–286

Pasqualini S, Cresti M, Del Casino C, Faleri C, Frenguelli G, Tedeschini E, Ederli L (2015) Roles for NO and ROS signalling in pollen germination and pollen-tube elongation in *Cupressus arizonica*. Biol Plant 59:735–744

Prado AM, Porterfield DM, Feijó JA (2004) Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. Development 131:2707–2714

Recalde L, Vázquez A, Groppa MD, Benavides MP (2018) Reactive oxygen species and nitric oxide are involved in polyamine-induced growth inhibition in wheat plants. Protoplasma 255:1295–1307

Qi F, Xiang Z, Kou N, Cui W, Xu D, Wang R, Zhu D, Shen W (2017) Nitric oxide is involved in methane-induced adventitious root formation in cucumber. Physiol Plant 159:366–377

Qu Y, Wang Q, Guo J, Wang P, Song P, Jia Q, Zhang X, Kudla J, Zhang W, Zhang Q (2017) Peroxisomal CuAOζ and its product H_2O_2 regulate the distribution of auxin and IBA-dependent lateral root development in *Arabidopsis*. J Exp Bot 68:4851–4867

Rockel P, Strube F, Rockel A, Wildt J, Kaiser WM (2002) Regulation of nitric oxide (NO) production by plant nitrate reductase *in vivo* and *in vitro*. J Exp Bot 53:103–110

Sanz L, Albertos P, Mateos I, Sánchez-Vicente I, Lechón T, Fernández-Marcos M, Lorenzo O (2015) Nitric oxide (NO) and phytohormones crosstalk during early plant development. J Exp Bot 66:2857–2868

Scuffi D, Núñez Á, Laspina N, Gotor C, Lamattina L, García-Mata C (2014) Hydrogen sulfide generated by L-cysteine desulfhydrase acts upstream of nitric oxide to modulate ABA-dependent stomatal closure. Plant Physiol 166:2065–2076

Shan C, Zhang S, Ou X (2018) The roles of H_2S and H_2O_2 in regulating AsA-GSH cycle in the leaves of wheat seedlings under drought stress. Protoplasma 255:1257–1262

Shi H, Ye T, Chan Z (2014) Nitric oxide-activated hydrogen sulfide is essential for cadmium stress response in bermuda grass (*Cynodon dactylon* L. Pers.). Plant Physiol Biochem 74:99–107

Shi C, Qi C, Ren H, Huang A, Hei S, She X (2015) Ethylene mediates brassinosteroid-induced stomatal closure via Gα protein-activated hydrogen peroxide and nitric oxide production in *Arabidopsis*. Plant J 82:280–301

Singh SP, Singh Z, Swinny EE (2009) Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina* Lindell). Postharvest Biol Technol 53:101–108

Sivakumaran A, Akinyemi A, Mandon J, Cristescu SM, Hall MA, Harren FJ, Mur LA (2016) ABA suppresses Botrytis cinerea elicited NO production in tomato to influence H_2O_2 generation and increase host susceptibility. Front Plant Sci 7:709

Skiba U, Smith KA, Fowler D (1993) Nitrification and denitrification as sources of nitric oxide and nitrous oxide in a sandy loam soil. Soil Biol Biochem 25:1527–1536

Stöhr C, Strube F, Marx G, Ullrich WR, Rockel P (2001) A plasma membrane-bound enzyme of tobacco roots catalyses the formation of nitric oxide from nitrite. Planta 212:835–841

Sun C, Liu L, Lu L, Jin C, Lin X (2018) Nitric oxide acts downstream of hydrogen peroxide in regulating aluminum-induced antioxidant defense that enhances aluminum resistance in wheat seedlings. Environ Exp Bot 145:95–103

Tan JL, Wang CY, Xiang B, Han RH, Guo ZF (2013) Hydrogen peroxide and nitric oxide mediated cold-and dehydration-induced myo-inositol phosphate synthase that confers multiple resistances to abiotic stresses. Plant Cell Environ 36:288–299

Tian S, Wang X, Li P, Wang H, Ji H, Xie J, Qui Q, Shen D, Dong H (2016) Plant aquaporin AtPIP1; 4 links apoplastic H_2O_2 induction to disease immunity pathways. Plant Physiol 171:1635–1650

Tossi V, Lamattina L, Jenkins GI, Cassia RO (2014) Ultraviolet-B-induced stomatal closure in *Arabidopsis* is regulated by the UV RESISTANCE LOCUS8 photoreceptor in a nitric oxide-dependent mechanism. Plant Physiol 164:2220–2230

Tripathi DK, Singh S, Singh S, Srivastava PK, Singh VP, Singh S, Prasad SM, Singh PK, Dubey NK, Pandey AC, Chauhan DK (2017) Nitric oxide alleviates silver nanoparticles (AgNps)-induced phytotoxicity in *Pisum sativum* seedlings. Plant Physiol Biochem 110:167–177

Wang Y, Chen T, Zhang C, Hao H, Liu P, Zheng M, Baluska F, Samai J, Lin J (2009) Nitric oxide modulates the influx of extracellular Ca^{2+} and actin filament organization during cell wall construction in *Pinus bungeana* pollen tubes. New Phytol 182:851–862

Wang L, Guo Y, Jia L, Chu H, Zhou S, Chen K, Zhao L (2014) Hydrogen peroxide acts upstream of nitric oxide in the heat shock pathway in *Arabidopsis* seedlings. Plant Physiol 164:2184–2196

Wang J, Wang Y, Lv Q, Wang L, Du J, Bao F, He YK (2017) Nitric oxide modifies root growth by S-nitrosylation of plastidial glyceraldehyde-3-phosphate dehydrogenase. Biochem Biophys Res Commun 488:88–94

Wang M, Liao WB (2016) Carbon monoxide as a signaling molecule in plants. Front Plant Sci 7:572

Wang X, Dong X, Feng Y, Liu X, Wang J, Zhang Z, Li J, Shi S, Tu P (2018) H_2O_2 and NADPH oxidases involve in regulation of 2-(2-phenylethyl) chromones accumulation during salt stress in *Aquilaria sinensis* calli. Plant Sci 269:1–11

Wojtyla Ł, Lechowska K, Kubala S, Garnczarska M (2016) Different modes of hydrogen peroxide action during seed germination. Front Plant Sci 7:66

Wu Q, Su NN, Zhang XY, Liu YY, Cui J, Liang YC (2016) Hydrogen peroxide, nitric oxide and UV RESISTANCE LOCUS8 interact to mediate UV-B-induced anthocyanin biosynthesis in radish sprouts. Sci Rep 6:29164

Xia XJ, Gao CJ, Song LX, Zhou YH, Shi K, Yu JQ (2014) Role of H_2O_2 dynamics in brassinosteroid-induced stomatal closure and opening in *Solanum lycopersicum*. Plant Cell Environ 37:2036–2050

Xu L, Yue Q, Xiang G, Bian FE, Yao Y (2018) Melatonin promotes ripening of grape berry via increasing the levels of ABA, H_2O_2, and particularly ethylene. Horticult Res 5:41

Yu QX, Ahammed GJ, Zhou YH, Shi K, Zhou J, Yu YL, Yu JQ, Xia XJ (2017) Nitric oxide is involved in the oxytetracycline-induced suppression of root growth through inhibiting hydrogen peroxide accumulation in the root meristem. Sci Rep 7:43096

Zhang F, Wang YP, Yang YL, Wu H, Wang D, Liu JQ (2007) Involvement of hydrogen peroxide and nitric oxide in salt resistance in the calluses from *Populus euphratica*. Plant Cell Environ 30:775–785

Zhang TY, Li FC, Fan CM, Li X, Zhang FF, He JM (2017) Role and interrelationship of MEK1-MPK6 cascade, hydrogen peroxide and nitric oxide in darkness-induced stomatal closure. Plant Sci 262:190–199

Zhou J, Wang J, Li X, Xia XJ, Zhou YH, Shi K, Chen ZX, Yu JQ (2014) H_2O_2 mediates the crosstalk of brassinosteroid and abscisic acid main tomato responses to heat and oxidative stresses. J Exp Bot 65:4371–4383

Zhu Y, Liao W, Niu L, Wang M, Ma Z (2016) Nitric oxide is involved in hydrogen gas-induced cell cycle activation during adventitious root formation in cucumber. BMC Plant Biol 16:146

Zhu XF, Zhu CQ, Wang C, Dong XY, Shen RF (2017) Nitric oxide acts upstream of ethylene in cell wall phosphorus reutilization in phosphorus-deficient rice. J Exp Bot 68:753–760

Zou LJ, Deng XG, Zhang LE, Zhu T, Tan WR, Muhammad A, Zhu LJ, Zhang C, Zhang DW, Lin HH (2018) Nitric oxide as a signaling molecule in brassinosteroid-mediated virus resistance to Cucumber mosaic virus in *Arabidopsis thaliana*. Physiol Plant 163:196–210

Hydrogen Peroxide (H_2O_2)- and Nitric Oxide (NO)-Derived Posttranslational Modifications

R. Valderrama, J. C. Begara-Morales, M. Chaki, C. Mata-Pérez, M. N. Padilla, and J. B. Barroso

Contents

Abstract Posttranslational modifications (PTMs) of proteins can be considered an additional step in protein metabolism that greatly expands the proteome of living organisms. In plant systems, PTMs affect protein structure and activity which are associated with concomitant changes in signaling and gene expression that make cellular systems more dynamic.

Redox reactions, which are a very old signaling phenomenon in evolutionarily terms, occur in prokaryotes and eukaryotes. The most important redox molecules are reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), hydroxyl radical ($^{\cdot}OH$), superoxide anion ($O_2^{\cdot -}$), and hydrogen peroxide (H_2O_2), as well as reactive nitrogen species (RNS) such as nitric oxide (NO) and derived compounds. H_2O_2 and NO are essential signaling molecules involved in many physiological processes and plant responses to unfavorable conditions. These diverse functions can be partly explained by the fact that ROS and NO rapidly interact to form a number of reactive nitrogen species, such as peroxynitrite ($ONOO^-$), nitrogen dioxide (NO_2),

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dinitrogen trioxide (N_2O_3), and other NO_x species. Moreover, as these molecules share signaling pathways, it is not surprising that cross talk enables pathways to control each other's functions.

In this chapter, we will explore up-to-date knowledge concerning the effects of H_2O_2- and NO-derived PTMs such as carbonylation, sulfhydryl oxidations, nitration, S-nitrosylation, and nitroalkylation while emphasizing in the importance of the interplay between their signaling pathways in plant systems.

Keywords Hydrogen peroxide (H_2O_2) · Nitric oxide (NO) · Posttranslational modifications (PTMs) · Carbonylation · Sulfhydryl oxidation · Nitration · *S*-nitrosylation · Nitroalkylation

1 Introduction

Plant genomes encode for the primary amino acid sequence of proteins, which, once synthetized in ribosomes, undergo different steps before reaching their mature and active version. This is often followed by posttranslational modifications (PTMs) of specific amino acid residues that can result in changes in oligomeric state, subcellular location, stabilization/degradation, and (de)activation (Huber and Hardin 2004). In addition, a PTM can be considered as an adaptation mechanism that facilitates rapid changes in plant proteins in order to maintain their functionality and fitness upon sudden alterations in environmental conditions, while taking into account that the modulation of gene expression results in relatively long-term changes (Prabakaran et al. 2012).

PTMs, which can be reversible or irreversible, are, in some cases, carried out by specific modifying enzymes. However, in other cases, they can occur spontaneously (nonenzymatically) depending on (a) the physical-chemical properties of reactive amino acids, (b) their exposure to the protein surface, (c) the characteristics of their neighboring residues, and (d) the cellular environment (e.g., pH, oxygen, and metabolites, among others) (Ryšlavá et al. 2013). PTMs can be found on both evolutionarily conserved and nonconserved residues, with Cys, Lys, and N-terminal residues being the protein targets of multiple PTMs (Friso and van Wijk 2015).

Multiple PTMs often occur simultaneously in a single protein. Moreover, depending on cellular status, residues can also alternate between PTMs, many of which are reversible; another level of complexity arises from the influence that one PTM can have on the generation of other PTMs. This greatly expands the proteome and cross talk between PTMs in the same protein, whose biological activity and/or interactions can also be affected. However, it is important to point out that not all PTMs have biological significance (Friso and van Wijk 2015).

With respect to subcellular distribution, it is important to note that most PTMs have been studied only in intracellular compartments. Nevertheless, recent studies

have indicated the plausibility of redox PTMs in extracellular proteins, including cell wall-associated proteins (Ruiz-May et al. 2018).

In recent years, the concept of oxidative and nitrosative PTMs has emerged. These modifications are nonenzymatically mediated by small oxygen and/or nitrogen metabolites termed reactive oxygen and nitrogen species or ROS and RNS, respectively. These molecules can display both beneficial (signaling) and deleterious (oxidative and nitrosative stress and damage) effects, depending on the concentration and exposure time (Lindermayr and Durner 2015). As to be expected, PTMs induced by ROS/RNS are typically of higher frequency where these species are produced.

The archetypal RNS second messenger is nitric oxide (NO). In addition to binding the heme of soluble guanylate cyclase (sGC), which affects downstream function (Hess et al. 2005), the molecular actions of NO as a redox modulator also include a wide range of PTMs on a variety of protein targets. Hydrogen peroxide (H_2O_2) is another important small signaling molecule, which, in recent years, has been viewed only as an oxidative stress marker (Miller and Chang 2007) in spite of, once produced, it can diffuse and reversibly oxidize cysteines (Cys) (Woo et al. 2003; Biteau et al. 2003; Wood et al. 2003), histidines (His) (Lee and Helmann 2006), and methionines (Met) (Stadtman et al. 2005) in protein targets which ultimately control cellular processes ranging from protein phosphorylation to gene expression (Miller and Chang 2007).

In this chapter, we will assess the role of both molecules in generating PTMs and will also focus on their effect on protein functionality.

2 H_2O_2-Derived Posttranslational Modifications

The term ROS encompasses molecules such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) which are widely used in plants as powerful subcellular and intercellular signaling molecules, despite the damaging and dangerous effects of their uncontrolled production. Hence, antioxidative enzymes may not only maintain ROS at low levels but also enable cells to sense and signal altered ROS availability and redox perturbations (Noctor et al. 2017).

H_2O_2 is widely recognized to play a crucial role in oxidative signaling (Foyer and Noctor 2016; Sies 2017; Foyer et al. 2017). This molecule can be synthesized in different ways, ranging from the reduction of molecular oxygen (O_2), the chemical reduction or dismutation of superoxide or H_2O_2, a reaction that is accelerated by superoxide dismutases (SODs) found in chloroplasts, peroxisomes, and mitochondria, as well as by a two-electron reduction of O_2 through various oxidases such as glycolate oxidase (GOX) located in peroxisomes (Foyer and Noctor 2016).

PTMs induced by ROS are typically of higher frequency where the ROS are produced. For instance, the major players involved in superoxide/H_2O_2 generation in plants are probably the photosynthetic electron transport chain (chloroplasts),

photorespiratory GOX (peroxisomes), the respiratory electron transport chain (mitochondria), and NADPH oxidases (plasmalemma). However, the contributions of these different players are highly dependent on plant species (Noctor et al. 2017).

The main H_2O_2-derived PTMs, protein carbonylation and sulfhydryl group's oxidations, will be discussed in Sects. 2.1 and 2.2.

2.1 Carbonylation

Carbonylation is an oxidative PTM that can affect macromolecules such as DNA, RNA, lipids, and proteins which are the preferential targets (Davies 2005). This irreversible PTM is considered to be a marker of protein oxidation which takes place during oxidative stress and aging both in prokaryotes and eukaryotes organisms. Carbonylation is associated with protein function loss, with protein inactivation, and ultimately with degradation processes (Nyström 2005; Polge et al. 2009; Friso and van Wijk 2015). Several carbonylated proteins, such as glycolytic enzymes, aldose reductase, methionine synthase (MS), and molecular chaperones, previously characterized in mammals, yeast, and bacteria (Tamarit 1998; Dalle-Donne et al. 2001; Shenton and Grant 2003), have been shown to be oxidized in plants. This supports the contention that protein carbonylation is not at all a random process, but rather a specific process which is capable of regulating common biological functions in living organisms (Lounifi et al. 2013).

Carbonylation is typically caused by metal-catalyzed ROS-induced oxidation of side chains from lysine (Lys), proline (Pro), arginine (Arg), and threonine (Thr) residues, with Lys residues being the most sensitive to carbonylation (Rao and Møller 2012). It can also be indirectly induced by ROS through lipid peroxides (for Cys, His, and Lys) and even by glycation/glycoxidation (for Lys).

Carbonylated proteins have been particularly detected in cytosol, mitochondria (Smakowska et al. 2014), and chloroplasts. However, carbonylation is not evenly distributed, and specific proteins appear to be preferentially targeted for this PTM (Friso and van Wijk 2015). With respect to chloroplasts, the pattern of protein carbonylation in *Arabidopsis* (and presumably other plants) is distinct from that in non-photosynthetic eukaryotes, with carbonylation initially increasing with age (similar to other species) but dropping abruptly prior to the vegetative-to-reproductive transition and also during senescence (Johansson et al. 2004). This suggests that old leaves rid themselves of oxidized proteins before falling (Lounifi et al. 2013).

Several seed biology studies have pointed out the involvement of protein carbonylation in physiological transitions including dormancy, germination, and aging (Job et al. 2005; Oracz et al. 2007; Rajjou et al. 2008; Arc et al. 2011). With regard to sunflower seeds, the increase in the amount of carbonyl groups was found to favor the transition from dormant to nondormant seeds (Oracz et al. 2007); this transition is accompanied by an increased ROS production and is in line with the finding that treatment of seeds with ROS donors is an efficient way of alleviating dormancy (Oracz et al. 2007; Leymarie et al. 2012).

During prolonged or inappropriate storage, seeds deteriorate and undergo aging processes (Rajjou and Debeaujon 2008), which increase carbonylated protein levels. Thus, in *Arabidopsis* and rice seeds, the accumulation of carbonylated proteins gradually increases with the loss of germination capacity, during both accelerated and natural aging (Tesnier et al. 2002; Rajjou et al. 2008).

Upon imbibition, seeds development is switched on by a metabolic and respiration activation (Rajjou et al. 2012). In this sense, *Arabidopsis* seed germination is accompanied by drastic changes in the profiles of carbonylated proteins (Job et al. 2005; El-Maarouf-Bouteau and Bailly 2008). Again in *Arabidopsis*, following seed germination and seedling establishment, the extent of protein carbonylation appears to increase as the rosette leaves become chronologically older until the end of the vegetative development (Johansson et al. 2004). Similarly, kiwifruit ripening is accompanied by an increase in protein carbonylation (Minas et al. 2012), which reinforces the involvement of this modification in physiological plant transitions (Lounifi et al. 2013).

This PTM is considered to be an important indicator of oxidative stress severity in plants, as oxidative burst in response to (biotic and abiotic) stress is commonly associated with a shift in protein carbonylation (Xiong et al. 2006; Oracz et al. 2007) with a direct correlation between the amount of carbonylated proteins, the exposure time, and the level of ROS generated under stress conditions (Nguyen and Donaldson 2005; Song et al. 2009). For example, heat stress leads to a sharp increase in the protein carbonyl content of *Arabidopsis* leaves (Sundaram and Rathinasabapathi 2010). On the other hand, far from being an isolated process, protein carbonylation in plants appears to be modified by several compounds, such us exogenous NO, associated with stress tolerance. This issue will be discussed in Sect. 4, where the interplay between H_2O_2- and NO-derived PTMs will be explored.

2.2 *Sulfhydryl Oxidations*

The wide variety of oxidation states from sulfur-containing amino acid (Cys and Met) facilitates the formation of a diverse range of oxidative PTMs including sulfenylation, intra-/intermolecular disulfide bridge formation, *S*-glutathionylation, sulfinylation, sulfonylation, and hydrogen sulfide-mediated S-sulfhydration. Interestingly, the rate of oxidation of Cys and Met residues inversely correlates with their dissociation constant (pKa), accessibility, and the local pH (Akter et al. 2015; Waszczak et al. 2015).

In contrast to Met, Cys oxidation and its important role in plant redox homeostasis have been extensively studied. The reactivity of Cys residues is strongly influenced by its pKa, which, when is lower than the surrounding pH, most thiols are present as thiolate ($R\text{-}S^-$) and prone to oxidation. In addition, while free Cys has a theoretical pKa of 8.3–8.5 (Tajc et al. 2004; Luo et al. 2005), most oxidation-sensitive Cys residues described thus far have pKa $\leq$ 3.5. The pKa of Cys residues is largely determined by the local electrostatic environment, as determined by

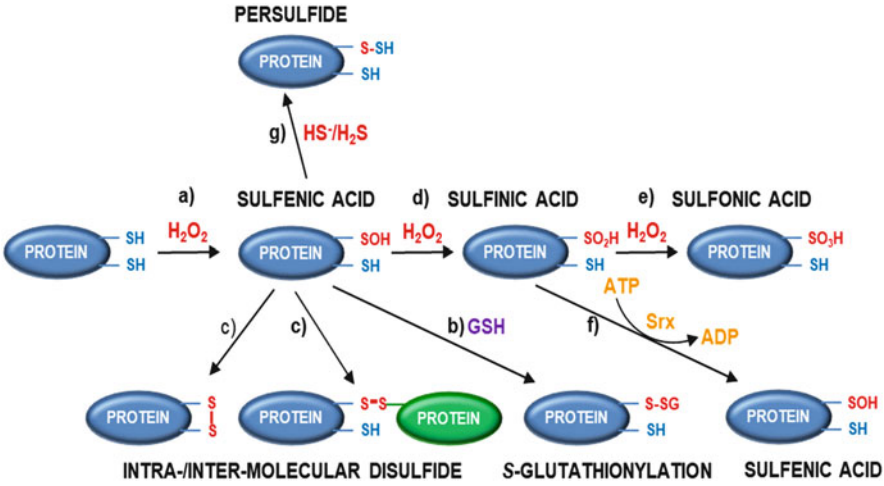

Fig. 1 Summary of the main Ox-PTMs of Cys residues: (**a**) sulfenylation, (**b**) *S*-glutathionylation, (**c**) intra-/intermolecular disulfide bridge formation, (**d**) sulfinylation, (**e**) sulfonylation, (**f**) sulfenic acid reduction by an ATP-dependent sulfiredoxin enzyme (Srx), and (**g**) the hydrogen sulfide-mediated S-sulfhydration

neighboring charged residues and dipoles and by hydrogen bonding between thiols/thiolates and neighboring residues (Harris and Turner 2002). In general, the more hydrogen bonds received by Cys-sulfur, the lower the p*K*a, and the more the thiolate form is stabilized (Roos et al. 2013). However, in some cases, stabilization of the thiolate in Cys residues needs to be lower in order to increase its nucleophilicity and reactivity.

Another important factor which controls the reactivity of Cys residues is their steric accessibility in the protein's three-dimensional structure (Marino and Gladyshev 2010).

Cys oxidation plays an important role in plant redox homeostasis. The initial oxidation of Cys by hydrogen peroxide (H_2O_2) typically results in sulfenic acid (R-SOH) (Fig. 1a), which can then form a mixed disulfide bond with glutathione (GSH; *S*-glutathionylation) (Fig. 1b) or intra-/intermolecular disulfide bonds with other thiols (R-S-S-R/R-S-S-R′) (Fig. 1c). Further sulfenic acid oxidation by H_2O_2 leads to the formation of sulfenic acid ($R\text{-}SO_2H$) (Fig. 1d) and irreversible sulfonic acid ($R\text{-}SO_3H$) (Fig. 1e). Although sulfenic acid is mostly irreversible, it can be reduced by an ATP-dependent sulfiredoxin enzyme (Srx) in the case of specific mitochondrial and chloroplastic peroxiredoxins (Rey et al. 2007) (Fig. 1f). Sulfenic acids can also be protected from over-oxidation by reacting with the backbone nitrogen of the adjacent residue to form sulfenylamides (R-SN), which have been well documented in relation to the human protein tyrosine phosphatase (PTP1B) (Salmeen et al. 2003; Haque et al. 2011). However, how plant sulfenylamide formation is used as a Cys-protection strategy remains unclear.

A relevant case of H_2O_2 Cys-oxidation PTM which mediates changes in cellular dynamics is the plasma membrane-bound transcription factor ERFVII (ethylene-responsive group factor VII), which crucially enables gene expression to be altered under hypoxic conditions. In the presence of oxygen, ERFVII cysteine residues are oxidized to sulfenic acid, conjugated with arginine, and directed to degradation. However, under low oxygen conditions, ERFVII is released from the plasma membrane and translocates to the nucleus in order to activate hypoxia-responsive gene expression (Dietz 2014). On the other hand, this Ox-PTM can be a positive regulator of gene transcription promoting the translocation of heat shock proteins (HSF) transcription factors from the cytosol to the nucleus following Cys oxidation by H_2O_2 (Habibi 2014).

With regard to *S*-glutathionylation, it is important to point out that it was initially regarded as a protective mechanism in active-site Cys residues to prevent over-oxidation and subsequent permanent protein damage. The role played by *S*-glutathionylation in redox signaling has only recently been recognized (Zaffagnini et al. 2012b) due to evidences of its central role in physiological and abiotic stress responses which modulate numerous cellular processes affecting proteins, transcription factors, and chromatin structure (Farnese et al. 2016). In this respect, the signal generated by Cys-PTM is necessarily transient, being glutaredoxin (Grx) enzymes the major deglutathionylating agents regulating protein *S*-glutathionylation (Begara-Morales et al. 2016a).

This Cys-PTM involves the formation of a mixed disulfide bridge between a reactive protein Cys and glutathione (GSH). Various mechanisms, involving thiol-disulfide exchange, sulfenic acids, and S-nitrosothiols, among others, leading to *S*-glutathionylation in vitro, have been reported (Gallogly and Mieyal 2007; Zaffagnini et al. 2012b). However, the precise mechanism involved and the physiological importance of in vivo protein *S*-glutathionylation in plants remain unclear (Zaffagnini et al. 2012b; Zagorchev et al. 2013).

An increased oxidative state inside the cell, leading to a decrease in the GSH:GSSG ratio, can promote protein *S*-glutathionylation (Dalle-Donne et al. 2007). Although hundreds of *S*-glutathionylated proteins have been identified, little information exits on the impact of this Cys-PTM on protein structure/function and its role under physiological conditions or in plant responses to abiotic stress (Zagorchev et al. 2013). One of the best-characterized *S*-glutathionylated proteins in plants is glyceraldehyde-3-phosphate dehydrogenase (GAPDH), with both chloroplastic and cytoplasmic isoforms being inhibited by this modification (Zaffagnini et al. 2007; Holtgrefe et al. 2008; Bedhomme et al. 2012), which can also protect against irreversible oxidation caused by H_2O_2 (Zaffagnini et al. 2007, 2013). Other identified targets of *S*-glutathionylation include peroxiredoxins, glutathione transferase, and heat shock proteins (Michelet et al. 2008; Gao et al. 2009; Zaffagnini et al. 2012a), as well as two components of the ascorbate-glutathione (Asa-GSH) cycle, the monodehydroascorbate reductase (MDAR) and the dehydroascorbate reductase (DHAR1) (Dixon et al. 2005). In this regard, a reversible inactivation of DHAR1 activity following *S*-glutathionylation has been reported (Dixon et al. 2002), which reflects the negative impact of this PTM on the functioning of this antioxidant system. It has also been reported that ascorbate peroxidase (APX) can be glutathionylated in vitro (Kitajima et al. 2008), although

no clear evidence exits on the impact of *S*-glutathionylation on APX activity. In addition, the NADP-malic enzyme (ME), one of the NADPH-generating systems, has also been identified as target of *S*-glutathionylation under induced oxidative stress (Dixon et al. 2005). However, the effect of *S*-glutathionylation on NADP-ME activity needs to be elucidated given the wide range of implications of NADPH on redox homeostasis.

Another type of Ox-PTM of protein Cys residues is the formation of an R-SSH persulfide group (Mustafa et al. 2009) by a process called S-sulfhydration, which is based on the chemical properties of the highly reactive and toxic hydrogen sulfide (H_2S) molecule (Fig. 1g). This compound displays important physiological signaling functions in both health and disease (Li et al. 2011; Kolluru et al. 2013). The possible role of H_2S as an endogenous neuromodulator was first described in 1996. It is now considered to be the third most prevalent gasotransmitter after nitric oxide (NO) and carbon monoxide (CO) in animal systems (Abe and Kimura 1996; Vandiver and Snyder 2012), in which its biosynthesis occurs through the action of three enzymes involved in the metabolism of sulfur-containing amino acids (Wang 2012). With regard to plant systems, emerging data in recent years also suggest that H_2S, like NO or H_2O_2, may function as an important signaling molecule (Aroca et al. 2015). Moreover, the nucleophilic properties of the H_2S molecule and its capacity to react with oxygen, H_2O_2, and peroxynitrite suggest that it could be involved in reducing cellular oxidative stress (Kabil and Banerjee 2010; Fukuto et al. 2012).

The biochemical processes underlying protein S-sulfhydration remain controversial; perhaps several chemical processes might be involved in modifying protein sulfhydryl groups in order to form persulfides. The Cys residue local environment determines its pKa to form a thiolate anion ($R\text{-}S^-$) and thus its susceptibility to oxidation by ROS to generate a sulfenic residue (R-SOH) (Gruhlke and Slusarenko 2012). This residue can also react with HS^- and H_2S to ultimately form a persulfide residue, as it has been described for the protein Tyr phosphatase 1B (PTP1B) (Krishnan et al. 2011).

S-sulfhydration is capable of either activating or inactivating enzymatic activities. In this regard, a *S*-sulfhydration-related activation has been reported in relation to glyceraldehyde-3-phosphate dehydrogenase, Parkin E3 ligase, and PTP1B (Mustafa et al. 2009; Krishnan et al. 2011; Vandiver and Snyder 2012).

In contrast to Cys oxidation, relatively little is known about of Met oxidation and its impact on plants. The oxidation of this amino acid residue leads to the formation of methionine sulfoxide (MetSO) (Boschi-Muller et al. 2008), which can alter both the activity and conformation of many proteins (Vieira Dos Santos et al. 2005; Rouhier et al. 2006; Li et al. 2012). An elegant study in *Arabidopsis* identified and quantified hundreds of Met oxidation sites induced by H_2O_2 generated in situ in peroxisomes through enhanced photorespiration by shifting from high to low [CO_2] and in a peroxisomal catalase mutant which detoxifies H_2O_2 (Jacques et al. 2015). Furthermore, Met oxidation can be reversed by a family of Met sulfoxide reductases (MSR) (Rouhier et al. 2006; Tarrago et al. 2009), thus repairing these oxidized (damaged) proteins (Friso and van Wijk 2015). Interestingly, *Arabidopsis* methionine sulfoxide reductase B1 (MSRB1) has also been reported to be glutathionylated

in vitro, and although the physiological role played by this modification in in vivo systems is unclear, these findings suggest that *S*-glutathionylation affects the regeneration of reduced methionine under oxidative stress conditions (Tarrago et al. 2009).

3 NO-Derived Posttranslational Modifications

Nitric oxide (NO) is a biological messenger that orchestrates a plethora of plant functions. However, unlike animals, which have a specific NO synthase, the source of NO in plants is under continuous debate. Although NO could be generated spontaneously in higher plants depending mainly on nitrite/nitrate levels, it can also be produced enzymatically by an L-Arg-dependent NO synthase (NOS)-like activity, with biochemical requirements similar to those of animal NOS (Barroso et al. 1999; Corpas and Borroso 2004; Corpas et al. 2004; Jasid et al. 2006; Chaki et al. 2011a), and a nitrate reductase (NR) activity (Yamasaki et al. 1999; Rockel et al. 2002; Wilson et al. 2008; Corpas et al. 2009; Corpas and Barroso 2015). With respect to the first one, the identification of a protein complex similar to that of animal NOS has not been reported thus far. In this context, it has been postulated that the L-Arg-dependent NO synthase-like activity found in higher plants could be the result of cooperation between discrete proteins which, together, can generate NO (Corpas and Barroso 2017).

The term reactive nitrogen species (RNS) refers to NO and NO-derived molecules such as dinitrogen trioxide (N_2O_3), nitrogen dioxide ($^{\cdot}NO_2$), peroxynitrite ($ONOO^-$), S-nitrosothiols (RSNOs), and *S*-nitrosoglutathione (GSNO). RNS react with reactive Cys thiols to form reversible *S*-nitrosothiols or with Tyr residues to form 3-nitrotyrosine, a mostly irreversible PTM in plants. These RNS-driven PTMs are involved in nearly every aspect of plant biology, thus highlighting the important role played by NO in plant-signaling mechanisms (Wang et al. 2013; Yu et al. 2014; Sanz et al. 2015; Trapet et al. 2015). Recent studies have also identified *S*-guanylation as a stable NO-related PTM in *Arabidopsis* caused by 8-nitro-cyclic GMP (cGMP), which is generated by the reaction of NO with cGMP (Sawa et al. 2013). 8-Nitro-cGMP has been proven to be essential for guard cell signaling in plants (Sawa et al. 2011; Joudoi et al. 2013).

In Sects. 3.1, 3.2, and 3.3, the main NO-derived PTMs, protein tyrosine nitration, *S*-nitrosylation, and nitroalkylation will be discussed.

3.1 Tyrosine Nitration

Protein tyrosine nitration is a selective process consisting of the addition of a nitro ($-NO_2$) group to one of the two equivalent ortho carbons in the aromatic ring of tyrosine residues, which leads to 3-nitrotyrosine (Gow et al. 2004; Radi 2004) (Fig. 2). This process involves the following two steps: oxidation of the phenolic

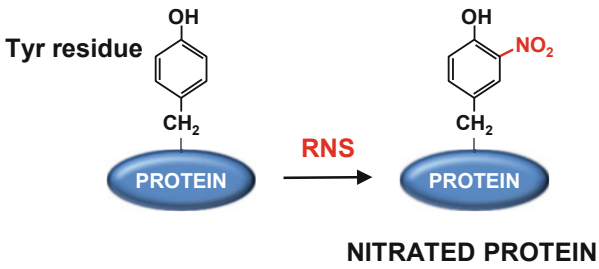

Fig. 2 Schematic representation of tyrosine nitration. RNS, reactive nitrogen species

ring of tyrosine to tyrosyl radical (Tyr·) and the addition of -NO_2 to the Tyr· by a nitrating agent (Mata-Pérez et al. 2016d). Although Tyr residues are preferentially nitrated and most studies in plants are concerned with tyrosine nitration (Tyr-NO_2), Trp, Cys, and Met can also be modified by this NO-PTM.

Nitration converts Tyr into a negatively charged hydrophilic residue and causes a marked shift in the local pKa of the hydroxyl group (Turko 2002; Abello et al. 2009) resulting in a gain, loss, or no change in the target protein's function (Souza et al. 2008; Radi 2013). Tyrosine nitration has traditionally been regarded as an irreversible mechanism and a nitrosative stress marker. Nevertheless, the existence of tyrosine denitrase activity that reduces 3-nitrotyrosine in mammalian cells (Görg et al. 2007; Deeb et al. 2013) points toward the role of tyrosine nitration in NO-mediated signaling processes in these cells. However, in plants, no specific denitrase protein has been identified and no information concerning these issues is currently available.

In recent years, a number of proteins involved in photosynthesis, as well as in antioxidant, ATP, carbohydrate, and nitrogen metabolism, have been shown to undergo tyrosine nitration (Chaki et al. 2009b). Since this well-established posttranslational modification is mediated by $ONOO^-$ and other oxidants including $O_2^{\cdot -}$ and H_2O_2 (Saito et al. 2006), it represents a candidate mechanism that could contribute to oxidative and nitrosative cross-talk signaling (Molassiotis and Fotopoulos 2011), which is further discussed in Sect. 4. These observations indicate that ROS- and RNS-based posttranslational modifications are key regulators of the oxidative and nitrosative signal transduction mechanisms (Molassiotis and Fotopoulos 2011).

During the last 10 years, research on higher plants has demonstrated that protein nitration must play an important regulatory role at the physiological level. Physiological protein nitration has been shown to be present in different plant organs as sunflower (*Helianthus annus* L.) seedlings grown under optimal conditions (Chaki et al. 2009b), in 2-week-old *Arabidopsis* (*Arabidopsis thaliana* L.) seedlings (Lozano-Juste et al. 2011) and in bitter orange (*Citrus aurantium* L.) roots (Tanou et al. 2009), among others. Interestingly, in green pepper (*Capsicum annuum* L.) fruits, the number of nitrated proteins increases during fruit ripening, thus suggesting that this PTM acts as a potential indicator of fruit ripening (Chaki et al. 2015). Moreover, the nitration analysis of different pea plants (*Pisum sativum* L.) organs during development and senescence indicates a pattern of protein nitration specific to each organ (Begara-Morales et al. 2013a, b). In this respect, nitroproteome analysis

of pea leaf peroxisomes has led to the detection of the endogenous nitration of hydroxypyruvate reductase (HPR1) involved in the photorespiration pathway, whose activity is inhibited by $ONOO^-$ in vitro (Corpas et al. 2013). Furthermore, site-directed mutagenesis has confirmed that Tyr-198 of *Arabidopsis* HPR1 is the primary nitration site responsible for the inhibition of its enzymatic activity by $ONOO^-$. Taken together, these results suggest that peroxisomal NO metabolism may contribute to the regulation of physiological processes under non-stress conditions (Mata-Pérez et al. 2016a, b, c).

On the other hand, it is important to note that protein nitration and NO signaling are clearly involved in plant hormone regulation. In this regard, NO may inhibit abscisic acid (ABA) signaling given the hypersensitivity of NO-deficient plants to ABA (Lozano-Juste and León 2010). Moreover, the in vitro nitration of several ABA receptors such as PYR1 and PYL1 by SIN-1 has recently been described (Castillo et al. 2015), thus demonstrating the inactivation of ABA signaling. Tyr nitration of PYR1 has been detected both in vivo and in planta, and the nitrated PYR1, which is polyubiquitinated, is subsequently marked for degradation by proteasomes.

As pointed out above, another key physiological process such as photosynthesis has been associated with tyrosine nitration processes. In this regard, the exposure of *Arabidopsis* plants to high concentrations of nitrogen dioxide (NO_2) generated a set of tyrosine-nitrated proteins (Takahashi et al. 2015), which include selectively nitrated of the photosystem II (PSII) proteins PsbO and PsbP.

Moreover, it is important to note that, with the aid of proteomic techniques, all Asa-GSH cycle enzymes have been identified as potential nitrated proteins (Chaki et al. 2009b; Lin et al. 2012; Tanou et al. 2012), thus showing that this NO-PTM could compromise the functioning of the cycle and, consequently, H_2O_2 detoxification (Begara-Morales et al. 2014, 2015). $ONOO^-$-mediated nitration causes inhibition of pea cytosolic APX and MDAR and orange plants DHAR activities, while chloroplastic and cytosolic pea glutathione reductase (GR) activity is unaffected by peroxynitrite-mediated tyrosine nitration (Begara-Morales et al. 2015, 2016b).

On the other hand, when plants are exposed to adverse environmental conditions, an increase in nitrated tyrosine levels could be considered a reliable marker of nitro-oxidative stress (Corpas and Barroso 2013). In this respect, an increase in free 3-nitrotyrosine was detected in *Taxus cuspidata* suspension cultures under shear stress conditions (Gong and Yuan 2006), in in vitro olive (*Olea europaea* L.) plantlets exposed to 200 mM salinity stress (Valderrama et al. 2007), in the hypocotyls of a susceptible sunflower (*Helianthus annuus*) cultivar infected by the *Plasmopara halstedii* pathogen (Chaki et al. 2009b) and subjected to mechanical wounding (Chaki et al. 2011a), in pepper (*Capsicum annuum*) leaves under low temperatures (Airaki et al. 2012), in 3-week-old pea seedlings and sunflower hypocotyls subjected to high temperatures (Corpas et al. 2008; Chaki et al. 2011b), in *Arabidopsis* seedlings under arsenic stress conditions (Leterrier et al. 2012), and in *Lotus japonicus* roots and leaves exposed to water stress (Signorelli et al. 2013), among others. Moreover, the sensitivity of different varieties of *Brassica* species (*B. napus* and *B. juncea*) to zinc has also recently been analyzed, with the greater tolerance of *B. napus* to Zn possibly related to the distinct pattern of nitration found

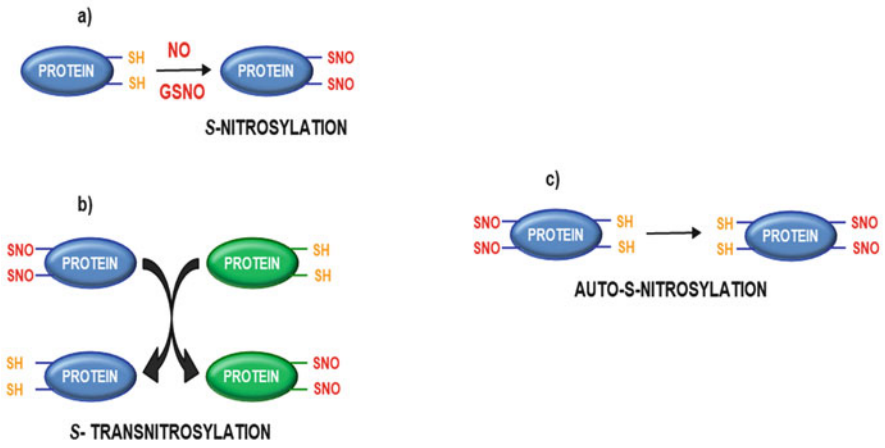

Fig. 3 Schematic representation of (**a**) *S*-nitrosylation, (**b**) *S*-transnitrosylation, and (**c**) auto-*S*-(trans)nitrosylation

in both species (Feigl et al. 2016). These results provide solid evidences suggesting that nitrosative responses are closely linked to tolerance mechanisms against (a) biotic stress conditions in plants.

3.2 S-*nitrosylation*

S-nitrosylation is a redox modification involving the addition of an NO group to a specific cysteine thiol group, which then gives rise to *S*-nitrosothiols (SNOs) (Fig. 3a) (Hess et al. 2005). *S*-nitrosylation is a transient/reversible NO modification which has emerged as the main redox signal through which NO transmits its bioactivity (Benhar et al. 2008; Kneeshaw et al. 2014). Nucleophilic Cys residues with low pKa values are preferential targets for nitrosylation (Seth and Stamler 2011). In addition to being directly nitrosylated, non-NO-reactive Cys residues can be enzymatically nitrosylated by so-called protein nitrosylases through the transfer of an NO moiety from a low-molecular-weight nitrosothiol, such as *S*-nitrosoglutathione (GSNO) (Fig. 3a) (Barroso et al. 2006), or from an already *S*-nitrosylated protein to the target protein involving a process called *S*-transnitrosylation (Fig. 3b) or auto-*S*-nitrosylation when the NO group is transferred intramolecularly (Anand and Stamler 2012) (Fig. 3c).

GSNO, considered to be the major low-molecular-weight *S*-nitrosothiol, plays an important role in *S*-nitrosylation due to its stability in solution which enables it to act as a NO reservoir and transporter over long distances in the phloem (Gaston et al. 1993; Durner et al. 1999; Leitner et al. 2009; Leterrier et al. 2011). GSNO can therefore act as a NO buffer, from which NO is released to orchestrate plant responses to basal and stress-related conditions (Begara-Morales et al. 2018). GSNO is formed by the *S*-nitrosylation of the antioxidant glutathione (GSH) by

NO. GSNO synthesis can also regulate NO concentrations in the cell by inhibiting nitrogen assimilation pathways (Frungillo et al. 2014). GSNO can also carry out transnitrosylation reactions, transferring its NO group to a protein cysteine thiol (Hess et al. 2005; Broniowska et al. 2013), thereby regulating target protein functions (Astier et al. 2011). In addition, the *S*-nitrosoglutathione reductase (GSNOR) enzyme breaks down GSNO by catalyzing its deamination into glutathione disulfide (GSSG) and NH_3. This enzyme indirectly controls the overall SNOs levels in cells (Feechan et al. 2005; Astier et al. 2011), thus suggesting that GSNO plays a crucial role in regulating the pool of total SNOs. In this context, GSNO can also be considered to have a central role in NO-dependent signal transduction by acting as a pivotal modulator of NO/SNOs metabolism in plant responses to stress. In this regard, different stress conditions can alter both GSNOR activity and GSNO levels, thus affecting NO-dependent signaling responses.

Of equal importance is denitrosylation, which regulates the extent and reversibility of protein nitrosylation and ensures the specificity of protein-SNO signaling networks (Malik et al. 2011; Kneeshaw and Spoel 2018). Although several proteins have been proposed as potential denitrosylases in animal tissues, only the GSNOR (Liu et al. 2001; Feechan et al. 2005) and thioredoxin/thioredoxin reductase (Trx/TR) (Benhar et al. 2008; Kneeshaw et al. 2014) plant systems have been well documented (Benhar et al. 2009; Malik et al. 2011). The nonenzymatic decomposition of *S*-nitrosothiols by antioxidants such as ascorbate and glutathione has also been described.

Given the important role of SNOs as fundamental players in the NO-signaling pathways of plants (Belenghi et al. 2007; Romero-Puertas et al. 2007, 2008; Lindermayr and Durner 2009; Astier et al. 2011; Hu et al. 2015), increased efforts have been made to identify the processes that might be regulated by *S*-nitrosylation; over the last 10 years, hundreds of proteins that undergo *S*-nitrosylation under physiological and adverse conditions have been identified in different plant species (Begara-Morales et al. 2016b). Many researchers have pointed out the important role of this NO-PTM in seed germination and seedling growth (Albertos et al. 2015), plant immunity (Feechan et al. 2005; Romero-Puertas et al. 2008; Yu et al. 2014), and plant responses to abiotic stress conditions (Rusterucci et al. 2007; Valderrama et al. 2007; Corpas et al. 2008; Chaki et al. 2009a, b, 2011a, b; Puyaubert et al. 2014). Recent findings highlight the important role played by *S*-nitrosylation in seed germination by promoting the degradation of the growth repressor transcription factor ABI5 in *Arabidopsis* plants (Albertos et al. 2015).

Salinity is probably the most studied abiotic stress in relation to *S*-nitrosylated proteins. An increase in SNOs levels as a consequence of NaCl stress has been detected in leaves of in vitro cultured olive seedlings (Valderrama et al. 2007), *Brassica juncea* plants (Abat and Deswal 2009) and pea leaves (Begara-Morales et al. 2014, 2015). Conversely, a decrease in total *S*-nitrosylated proteins under salt stress conditions has also been reported in different plant species (Tanou et al. 2009; Camejo et al. 2013; Ziogas et al. 2013). Various redox-related proteins have been identified as targets of *S*-nitrosylation under NaCl treatments such as APX, Fe-SOD, MDAR, and glutaredoxin in citrus plants (Tanou et al. 2009, 2012) and Mn-SOD in

pea mitocondria (Camejo et al. 2013). In addition, PRxIIF has been identified as a target of S-nitrosylation during salt stress, which had a negative impact on protein activity (Camejo et al. 2013, 2015). It is worth noting that *S*-nitrosylation may have different consequences on enzymatic activities, so while *S*-nitrosylation of pea leaf APX positively regulates its activity, an inhibition of MDAR was observed (Begara-Morales et al. 2014, 2015). In this respect, Yang et al. (2015) have also demonstrated a similar effect on APX activity in *Arabidopsis* plants.

In addition, as extreme temperatures can modify *S*-nitrosothiols levels in plants, heat stress has been reported to increase SNOs levels in sunflower hypocotyls (Chaki et al. 2011b). Although little information is available on low-temperature stress in relation to *S*-nitrosylated-mediated responses, certain proteins have been reported to be *S*-nitrosylated (Abat and Deswal 2009; Sehrawat et al. 2013; Puyaubert et al. 2014). In this regard, Fe-SOD has been identified as a *S*-nitrosylation target under cold stress conditions in *Brassica juncea*, whose enzymatic activity is positively regulated and therefore contributes to the detoxification of superoxide radicals (Sehrawat et al. 2013). However, *S*-nitrosylation has been reported to inhibit DHAR activity in potato and *Arabidopsis* plants under physiological (Fares et al. 2011; Kato et al. 2013; Puyaubert et al. 2014) but not cold stress conditions (Puyaubert et al. 2014). These results suggest that this type of stress could cause DHAR denitrosylation, thus enabling its activity to increase in response to low temperatures.

S-nitrosylation also appears to be critical for GSNO and $ONOO^-$ homeostasis, as this NO-PTM inhibits GSNOR and Prx II E activities (Romero-Puertas et al. 2007; Frungillo et al. 2014) which decompose GSNO and $ONOO^-$, respectively. This could favor the accumulation of these NO-derived molecules which, in turn, may enhance the effects of the generated nitro-oxidative stress (Begara-Morales et al. 2016b). On the other hand, some authors have suggested that *S*-nitrosylation protects against oxidative stress by avoiding the irreversible oxidation of critical Cys residues (Abat and Deswal 2009; Tanou et al. 2009; Begara-Morales et al. 2014). In this regard, a reduction in the protein carbonylation levels has been observed following GSNO treatment (Jasid et al. 2006).

3.3 Nitroalkylation

Although protein nitration and *S*-nitrosylation are the most studied processes, the interaction of NO with other important macromolecules such as lipids has been scarcely studied in plant systems (Sánchez-Calvo et al. 2013; Fazzari et al. 2014). Recently, a new class of NO-derived signaling mediators called nitro-fatty acids (NO_2-FA), also called nitrolipids or nitroalkenes, has emerged. Given their chemical stability and longer half-life, nitro-fatty acids increase NO-dependent signaling significantly (Geisler and Rudolph 2012).

In animal systems, nitrated fatty acids are formed endogenously under oxidative and nitrosative conditions. They are produced by the reaction of NO and NO-derived

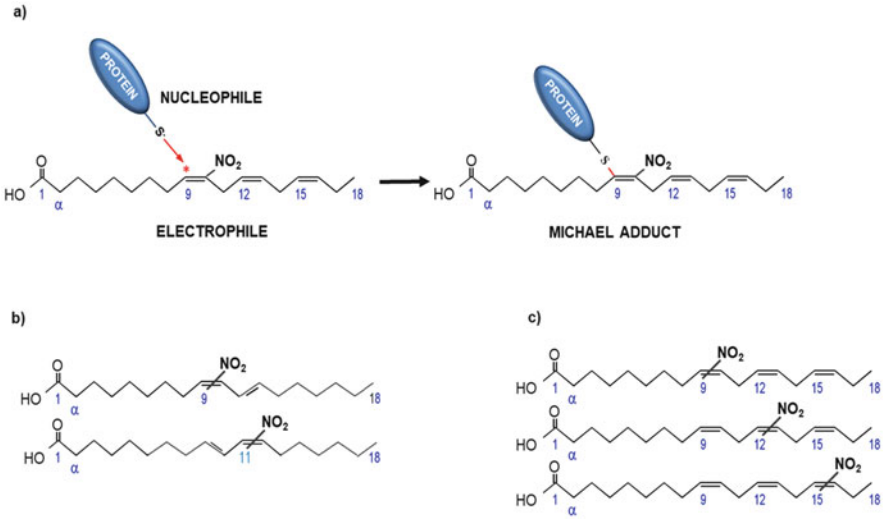

Fig. 4 (**a**) Schematic representation of nitroalkylation reaction and chemical structure of (**b**) isomers of nitro-conjugated linoleic acid (NO_2-cLA) and (**c**) isomers of nitro-linolenic acid (NO_2-Ln)

nitrogen oxides, such as nitrogen dioxide (NO_2) and peroxynitrite ($ONOO^-$), and oxygen-derived inflammatory mediators, including superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and lipid peroxyl radicals ($LOO^{\cdot}$), with mono- and polyunsaturated fatty acids (Baker et al. 2004; Schopfer et al. 2005a, b; Freeman et al. 2008; Jain et al. 2008).

Research in this field has demonstrated that NO_2-FAs can occur in vivo as free fatty acids, esterified in complex lipids in hydrophobic compartments and adducted with proteins, with the latter two being the most abundant reservoirs (Schopfer et al. 2005a, b; Trostchansky and Rubbo 2008; Rudolph et al. 2009). Protein adduction, which is the predominant via actuation of electrophilic NO_2-FAs (Schopfer et al. 2009; Geisler and Rudolph 2012), is a PTM that enables a covalent and reversible Michael addition reaction, so-called nitroalkylation (Fig. 4a), with nucleophiles such as protein His and Cys residues (Baker et al. 2007; Geisler and Rudolph 2012). This reversible reaction modifies the structure of proteins and amino acids and subsequently their biological function (Batthyany et al. 2006; Baker et al. 2007; Rudolph et al. 2010).

More recently, the presence of endogenous nitro-conjugated linoleic acid (NO_2-cLA) (Fig. 4b) has been reported in extra virgin olive oil (EVOO), and nitro-oleic acid (NO_2-OA) Cys adducts have been detected in fresh olives (Fazzari et al. 2014). Moreover, the endogenous presence of nitro-linolenic acid (NO_2-Ln), the major NO_2-FA in plants (Fig. 4c), has been detected in *Arabidopsis thaliana*, and the modulation of its content throughout the development of this plant has been described (Mata-Pérez et al. 2016c). NO_2-Ln has also been detected in other model plant species such as rice (*Oryza sativa*) and pea (*Pisum sativum*) (Mata-Pérez et al. 2017).

The abundance (between 0.07 and 3.8 pmol g^{-1} FW) of NO_2-Ln in plant species suggests its potential role as a signaling molecule (Mata-Pérez et al. 2017).

The biological properties of NO_2-FAs include their ability to release NO into aqueous environments, which reinforces their signaling capacity as potent NO donors (Schopfer et al. 2005a). In this respect, the ability of NO_2-Ln to release NO both in vivo and in vitro has recently been reported (Mata-Pérez et al. 2016a, b). Thus, the higher levels of this nitro-fatty acid detected at the beginning of the life of *Arabidopsis* plants (seeds and 14-day-old seedlings) suggest that, acting as a NO donor, it plays a key role at the early stages of plant life (Mata-Pérez et al. 2018). As newly detected by our research group (unpublished data), its NO-releasing capacity could be responsible for the shift in SNOs levels through the inhibitory effects of *S*-nitrosylation on GSNOR activity in *Arabidopsis* plants. The important finding that NO_2-FAs can promote the generation of NO in a similar way to that attributed to the biological NO donor, *S*-nitrosoglutathione (GSNO) (Mata-Pérez et al. 2016a), suggests that NO_2-Ln in plants could also constitute an important reserve of NO in conjunction with *S*-nitrosothiols (SNOs) (Mata-Pérez et al. 2018).

As previously reported in animal systems (Nadtochiy et al. 2009; Schopfer et al. 2009; Rudolph et al. 2010), a significant increase in NO_2-Ln levels under different abiotic stress conditions, including mechanical wounding, salinity, low temperature, and heavy-metal stress, has been detected in both cell suspension cultures and 14-day-old *Arabidopsis* seedlings (Mata-Pérez et al. 2016c). This increase could be due, on the one hand, to unsaturated fatty acid nitration caused by higher NO and other RNS content (Schopfer et al. 2005a, 2009) and, on the other, to releases from cellular reservoirs such as cell membranes and protein adducts (Schopfer et al. 2011; Begara-Morales et al. 2013a). In this latter regard, it has recently been demonstrated the in vitro release of NO_2-FAs from protein-adducted reservoirs after treatment with oxidizing agents, such as H_2O_2 and $ONOO^-$, commonly induced under stress conditions which can promote the oxidation of Michael adducts between NO_2-FAs and Cys residues (Padilla et al. 2017) (Fig. 5). This release of NO_2-FA may be partly responsible for the increase observed in their content in various stressful situations and may initiate multiple signaling activities such as the activation of antioxidant and defense responses (Mata-Pérez et al. 2018). In this respect, NO_2-Ln has recently been shown to be able to prompt the transcriptional expression of a large set of heat shock proteins (HSPs) and to induce antioxidant defense responses against abiotic stress processes such as those involving APX or MSR. Nevertheless, the mechanisms enabling NO_2-Ln to launch these responses remain unclear (Mata-Pérez et al. 2016a). Very recently, it has been hypothesized that NO_2-Ln may be involved in mediating nitroalkylation reactions with these systems, which might also affect protein activity (Mata-Pérez et al. 2018).

These data highlight the involvement of NO_2-Ln in plant physiology and abiotic stress responses, thus suggesting that NO_2-FAs also play an important role in plants.

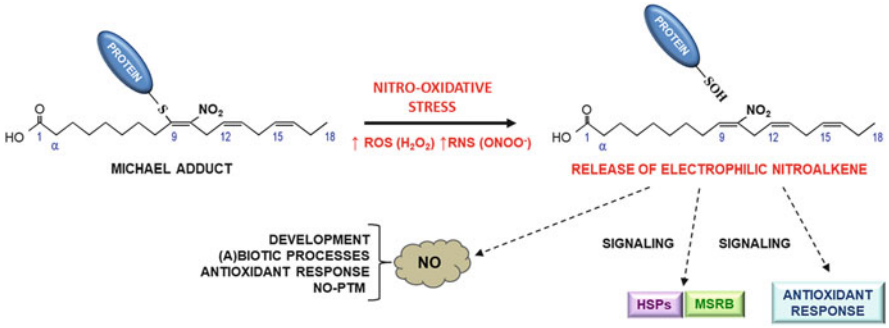

Fig. 5 Signaling mechanisms mediated by NO_2-FAs under nitro-oxidative stress conditions. When nitro-oxidative stress occurs, ROS and/or RNS production leads to the Michael adduct oxidation and nitroalkene release. This NO_2-FA release can display signaling actions by NO liberation and the induction of heat shock proteins (HSPs) and methionine sulfoxide reductase B (MSRB) or antioxidant enzymes (Modified from Padilla et al. 2017)

4 Interplay Between H_2O_2- and NO-Derived Posttranslational Modifications

There are many experimental data highlighting active interactions between ROS and RNS, which are especially evidenced by H_2O_2 and NO. Both signaling molecules, which are sometimes produced interdependently and can affect each other synthesis, are simultaneously present during various physiological processes. Moreover, the fact that they can react with each other reinforces their cross talk (Fig. 6). Therefore, the close interplay between these two signals under both physiological and stress conditions is very plausible in order to modulate cellular responses (Molassiotis and Fotopoulos 2011).

The first evidence of physiological interplay between NO and ROS was provided by Delledonne et al. (2001), who demonstrated that hypersensitive cell death is triggered by a balanced production of NO and ROS, in which the interaction of NO and H_2O_2 is required. In addition, as part of the innate immune responses in *Arabidopsis*, NO inhibits NADPH oxidase and regulates cell death (Yun et al. 2011). Another example of the involvement of RNS and ROS in plant cell death is provided by tobacco and *Arabidopsis* mutant plants with reduced leaf catalase activity (CAT), in which an H_2O_2-induced leaf cell death phenotype was observed (Dat et al. 2001; Vandenabeele et al. 2004; Queval et al. 2007). In the CAT-deficient rice mutant *noe1*, with excess of nitric oxide, the increased H_2O_2 levels induce the generation of NO and SNOs, suggesting that NO acts as an important endogenous mediator of H_2O_2-induced leaf cell death (Lin et al. 2012).

Evidence of cross talk between H_2O_2 and NO has also been observed in physiological processes such as pollen-stigma recognition in angiosperms. Furthermore, stigma epidermal cells have been found to accumulate relatively large amounts of ROS, mainly H_2O_2, in contrast to the production of NO by pollen (McInnis et al.

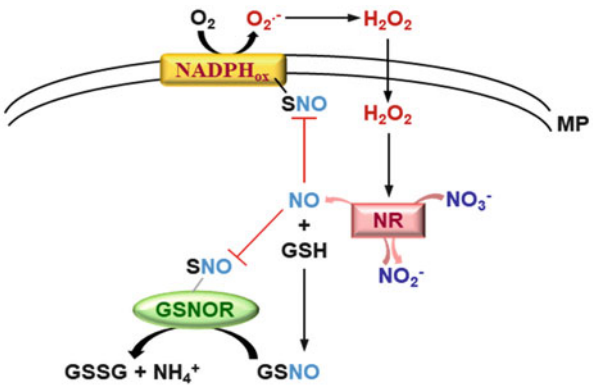

Fig. 6 Schematic model of cross-talk regulation between ROS and NO in plant cells. H_2O_2 produced by NADPH oxidase ($NADPH_{ox}$) leads to activation of nitrate reductase (NR) and concomitant NO production. NO accumulation, in turn, blunts $NADPH_{ox}$ activity by *S*-nitrosylation, thus preventing excess ROS accumulation. On the other hand, nitrate (NO_3^-) is reduced to nitrite (NO_2^-) by NR. NO_2^- can be reduced to NO by NR. NO reacts with reduced glutathione (GSH) in the presence of O_2 to form *S*-nitrosoglutathione (GSNO). GSNO is converted by the GSNO reductase enzyme (GSNOR) into oxidized glutathione (GSSG) and ammonium (NH_4^+). NO accumulation leads to GSNOR *S*-nitrosylation and inhibition and prevents GSNO degradation. MP, plasma membrane (Modified from Damiani et al. 2016)

2006; Hiscock et al. 2007). Moreover, Zafra et al. (2010) have shown that both ROS and NO are actively produced in the reproductive tissues of olive plants throughout flower development.

With respect to antioxidant enzymes, the metabolic interplay between NO and ROS metabolism is evidenced by the positive regulation by NO of the cytosolic ROS-degrading enzyme APX1 by *S*-nitrosylation, which enhances resistance to oxidative stress and improves immune responses (Yang et al. 2015). Interestingly, pea cytosolic APX 1 is regulated by NO in a dual fashion. While *S*-nitrosylation enhances APX 1 activity, Tyr nitration results in its inhibition (Begara-Morales et al. 2014). However, glutathione reductase (GR) is unaffected by these NO-PTMs, suggesting that GR attempts to maintain GSH regeneration and hence the cellular redox state in order to sustain the Asa-GSH cycle resistance to nitro-oxidative cell conditions (Begara-Morales et al. 2016b). With respect to the antioxidant enzymes peroxiredoxin IIE and superoxide dismutase, a connection between NO and ROS has also been demonstrated. In this context, *S*-nitrosylation inhibits the hydrogen peroxide-reducing and peroxynitrite-detoxifying activity of *Arabidopsis* peroxiredoxin IIE. In this way, NO regulates and fine-tunes the effects of its own radicals but also those of ROS (Romero-Puertas et al. 2007). Interestingly, *Arabidopsis* SODs are differentially inhibited by peroxynitrite, thus suggesting that it plays a regulatory role under stress conditions (Holzmeister et al. 2015).

With regard to stress, both H_2O_2 and NO clearly act in parallel with multiple phytohormones signaling pathways in response to abiotic stress (Zhang et al. 2011).

In addition, Tun et al. (2006) provided evidence that polyamines (PAs) induce NO production in *Arabidopsis* and that NO could be a link between polyamine-mediated stress responses and other stress mediators. Polyamines have also been proposed as important substrates for H_2O_2 production, as they are degraded by copper-containing amine oxidases (Yoda 2006). It is therefore possible that PAs participate in loops involving interaction with signal transduction pathways which may control oxidative and nitrosative events (Molassiotis and Fotopoulos 2011).

Moreover, as postulated in Sect. 2.1, several compounds associated with stress tolerance can promote or reduce protein carbonylation in plants. These include exogenous applications of NO either during or prior to abiotic stress conditions, which prevent stress-induced protein carbonylation. For instance, NO enhances the desiccation tolerance of recalcitrant *Antiaris toxicaria* seeds by activating the Asa-GSH pathway enzymes, thereby avoiding ROS accumulation and promoting *S*-nitrosylation rather than carbonylation of relevant proteins (Bai et al. 2011). Similarly, in salt-treated citrus leaves, the increase observed in carbonylated proteins is prevented by pretreatment with either NO donors or H_2O_2 (Tanou et al. 2009). However, in both cases, the reduction in carbonyl content does not appear to be directly mediated by NO, but is rather a consequence of the NO-mediated induction of the antioxidant machinery.

Recently, it has been suggested that the GSNOR enzyme is involved in cross talk between ROS and NO given its role in fine-tuning NO signaling and its inhibition by Ox-PTMs (Lindermayr 2018). In this regard, ROS has been shown to inhibit GSNOR in yeast (Men and Wang 2007), *Arabidopsis* (Kovacs et al. 2016), and *Baccaurea ramiflora* (Burmese grape) (Bai et al. 2012). Interestingly, ROS-targeted cysteine residues are different to the ones affected in the SNO-dependent inhibition of GSNOR. Hence, oxidative inhibition of GSNOR increases cellular SNOs and induces NO-dependent signaling mechanisms; this leads to GSH accumulation, enhances GSH-related enzymes activity, and ends in protection against oxidative stress. The importance of this cross talk resides in the constitutive expression and predominant presence of GSNOR in nearly all plant tissues/cells, thus demonstrating the important role played by this enzyme in plant growth and development, as well as in responses to environmental changes (Lindermayr 2018).

The in vitro release of NO_2-FAs from Michael protein adducts under nitro-oxidative stress conditions has recently been found to be an example of interplay between H_2O_2- and NO-PTMs (see Sect. 3.3). In these situations, ROS and RNS content increases significantly, then oxidize Cys-adducted NO_2-FAs, and break the nitroalkylation addition, which is followed by the release of free nitroalkenes. This release, attributed to these molecules, may be partly responsible for the increase observed in NO_2-FA content under different stress conditions in both animal and plant systems, as well as for activating antioxidant and anti-inflammatory signaling pathways (Padilla et al. 2017). Moreover, as described in Sect. 3.3, free NO_2-FAs are able to directly release NO into aqueous media acting as signaling molecules due to their capacity to induce different changes mediated by NO or NO-related molecules such as nitration and S-nitrosylation (Mata-Pérez et al. 2016a).

All these data demonstrate the importance of the interplay and cross talk between NO and ROS in both physiological and pathological processes. Moreover, their involvement in the regulation of redox homeostasis is clearly evidenced by NO-based modifications of antioxidant enzymes (Yang et al. 2015).

It is also important to note the fundamental role played by the cellular redox state in modulating the occurrence of H_2O_2-/NO-PTMs, which is ultimately caused by the physiological state and/or local environment in which the plant has to develop.

5 Conclusions and Future Perspectives

In this chapter, we have highlighted the importance of biologically active interplay between NO and H_2O_2 signaling which modulates cellular responses by showing the synergistic, antagonistic, and parallel mechanisms involved. In this context, given that ROS and RNS are well known to be key components in plant cell redox homeostasis, in the future, redox proteomics will enable us to identify physiological and molecular links between oxidative and nitrosative modifications. In this sense, studies focused on the functional characterization and quantification of H_2O_2- and NO-modified proteins under physiological and stress conditions are needed. Moreover, it is essential to elucidate the cellular compartmentalization of these posttranslational modified proteins. These approaches would increase our knowledge of the manipulation of plant stress tolerance in order to develop productive crops capable of dealing more efficiently with environmental insults. Furthermore, although the portfolio of proteins that potentially undergo PTMs is continuously growing, in many cases the number of proteins for which the occurrence of PTMs has been validated is scarce. Future studies therefore need to focus on the functional characterization and quantification of H_2O_2- and NO-modified proteins under physiological and stress conditions. Moreover, it is essential to elucidate the cellular compartmentalization of these posttranslational modified proteins. These approaches would increase our knowledge of the manipulation of plant stress tolerance in order to develop productive crops capable of dealing more efficiently with environmental insults.

In addition, little information is available on the initiation of the signaling mediated by ROS and NO, the mechanisms involved in the perception and the specificity of the generated signal, as well as the regulation of the delicate balance between production and scavenging of reactive species. Detailed studies of crosstalk regulation among ROS, NO, hormones, cyclic nucleotides, MAPKs, and other signaling molecules are therefore needed to clarify how these molecules interact with one another. It will also be necessary to evaluate in more detail how these signaling molecules alter gene expression by analyzing, for example, their possible involvement in epigenetic processes.

On the other hand, while fatty acid nitration reveals a new signaling pathway in plants, a growing effort is required to study the unexplored presence and physiological function of NO_2-FAs in higher plants as well the implications for stress situations in order to increase our knowledge in this field.

Finally, in order to gain better understanding of oxidative and nitrosative networks, it will be necessary to elucidate the mechanism of NO production in plant systems.

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References

Abat JK, Deswal R (2009) Differential modulation of S-nitrosoproteome of *Brassica juncea* by low temperature: change in S-nitrosylation of Rubisco is responsible for the inactivation of its carboxylase activity. Proteomics 9:4368–4380

Abe K, Kimura H (1996) The possible role of hydrogen sulfide as an endogenous neuromodulator. J Neurosci 16:1066–1071

Abello N, Kerstjens HAM, Postma DS, Bischoff R (2009) Protein tyrosine nitration: selectivity, physicochemical and biological consequences, denitration, and proteomics methods for the identification of tyrosine-nitrated proteins. J Proteome Res 8:3222–3238

Airaki M, Leterrier M, Mateos RM, Valderrama R, Chaki M, Barroso JB, del Río LA, Palma JM, Corpas FJ (2012) Metabolism of reactive oxygen species and reactive nitrogen species in pepper (*Capsicum annuum* L.) plants under low temperature stress. Plant Cell Environ 35:281–295

Akter S, Huang J, Waszczak C, Jacques S, Gevaert K, Van Breusegem F, Messens J (2015) Cysteines under ROS attack in plants: a proteomics view. J Exp Bot 66:2935–2944

Albertos P, Romero-Puertas MC, Tatematsu K, Mateos I, Sánchez-Vicente I, Nambara E, Lorenzo O (2015) S-nitrosylation triggers ABI5 degradation to promote seed germination and seedling growth. Nat Commun 6:8669

Anand P, Stamler JS (2012) Enzymatic mechanisms regulating protein s-nitrosylation: implications in health and disease. J Mol Med 90:233–244

Arc E, Galland M, Cueff G, Godin B, Lounifi I, Job D, Rajjou L (2011) Reboot the system thanks to protein post-translational modifications and proteome diversity: how quiescent seeds restart their metabolism to prepare seedling establishment. J Proteome 11:1606–1618

Aroca Á, Serna A, Gotor C, Romero LC (2015) *S*-Sulfhydration: a cysteine posttranslational modification in plant systems. Plant Physiol 168:334–342

Astier J, Rasul S, Koen E, Manzoor H, Besson-Bard A, Lamotte O, Jeandroz S, Durner J, Lindermayr C (2011) S-nitrosylation: an emerging post-translational protein modification in plants. Plant Sci 181:527–533

Bai X, Yang L, Tian M, Chen J, Shi J, Yang Y, Hu X (2011) Nitric Oxide enhances desiccation tolerance of recalcitrant antiaris toxicaria seeds via protein S-nitrosylation and carbonylation. PLoS One 6:e20714

Bai XG, Chen JH, Kong XX, Todd CD, Yang YP, Hu XY, Li DZ (2012) Carbon monoxide enhances the chilling tolerance of recalcitrant *Baccaurea ramiflora* seeds via nitric oxide-mediated glutathione homeostasis. Free Radic Biol Med 53:710–720

Baker PRS, Schopfer FJ, Sweeney S, Freeman B (2004) Red cell membrane and plasma linoleic acid nitration products: synthesis, clinical identification, and quantitation. Proc Natl Acad Sci U S A 101:11577–11582

Baker LMS, Baker PRS, Golin-Bisello F, Schopfer FJ, Fink M, Woodcock SR, Branchaud BP, Radi R, Freeman BA (2007) Nitro-fatty acid reaction with glutathione and cysteine: kinetic analysis of thiol alkylation by a Michael addition reaction. J Biol Chem 282: 31085–31093

Barroso JB, Corpas FJ, Carreras A, Sandalio LM, Valderrama R, Palma JM, Lupiánez JA, Del Río LA (1999) Localization of nitric-oxide synthase in plant peroxisomes. J Biol Chem 274: 36729–36733

Barroso JB, Corpas FJ, Carreras A, Rodríguez-Serrano M, Esteban FJ, Fernández-Ocaña A, Chaki M, Romero-Puertas MC, Valderrama R, Sandalio LM, del Río LA (2006) Localization of S-nitrosoglutathione and expression of S-nitrosoglutathione reductase in pea plants under cadmium stress. J Exp Bot 57:1785–1793

Batthyany C, Schopfer FJ, Baker PRS, Durán R, Baker LMS, Huang Y, Cerveñansky C, Branchaud BP, Freeman BA (2006) Reversible post-translational modification of proteins by nitrated fatty acids in vivo. J Biol Chem 281:20450–20463

Bedhomme M, Adamo M, Marchand CH, Couturier J, Rouhier N, Lemaire SD, Zaffagnini M, Trost P (2012) Glutathionylation of cytosolic glyceraldehyde-3-phosphate dehydrogenase from the model plant *Arabidopsis thaliana* is reversed by both glutaredoxins and thioredoxins in vitro. Biochem J 445:337–347

Begara-Morales JC, Chaki M, Sánchez-Calvo B, Mata-Pérez C, Leterrier M, Palma JM, Barroso JB, Corpas FJ (2013a) Protein tyrosine nitration in pea roots during development and senescence. J Exp Bot 64:1121–1134

Begara-Morales JC, López-Jaramillo FJ, Sánchez-Calvo B, Carreras A, Ortega-Muñoz M, Santoyo-González F, Corpas FJ, Barroso JB (2013b) Vinyl sulfone silica: application of an open preactivated support to the study of transnitrosylation of plant proteins by S-nitrosoglutathione. BMC Plant Biol 13:61

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

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Mata-Pérez C, Valderrama R, Padilla MN, López-Jaramillo J, Luque F, Corpas FJ, Barroso JB (2015) Differential molecular response of monodehydroascorbate reductase and glutathione reductase by nitration and S-nitrosylation. J Exp Bot 66:5983–5996

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Valderrama R, Mata-Pérez C, Corpas FJ, Barroso JB (2016a) Protein S-nitrosylation and S-glutathionylation as regulators of redox homeostasis during abiotic stress response. In: Gupta DK, Palma JM, Corpas FJ (eds) Redox state as a central regulator of plant-cell stress responses. Springer, Cham, pp 365–386

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Valderrama R, Mata-Pérez C, Padilla MN, Corpas FJ, Barroso JB (2016b) Antioxidant systems are regulated by nitric oxide-mediated post-translational modifications (NO-PTMs). Front Plant Sci 7:152

Begara-Morales JC, Chaki M, Valderrama R, Sánchez-Calvo B, Mata-Pérez C, Padilla MN, Corpas FJ, Barroso JB (2018) Nitric oxide buffering and conditional nitric oxide release in stress response. J Exp Bot 69:3425–3438

Belenghi B, Romero-Puertas MC, Vercammen D, Brackenier A, Inzé D, Delledonne M, Van Breusegem F (2007) Metacaspase activity of *Arabidopsis thaliana* is regulated by S-nitrosylation of a critical cysteine residue. J Biol Chem 282:1352–1358

Benhar M, Forrester MT, Hess DT, Stamler JS (2008) Regulated protein denitrosylation by cytosolic and mitochondrial thioredoxins. Science 320:1050–1054

Benhar M, Forrester MT, Stamler JS (2009) Protein denitrosylation: enzymatic mechanisms and cellular functions. Nat Rev Mol Cell Biol 10:721–732

Biteau B, Labarre J, Toledano MB (2003) ATP-dependent reduction of cysteine-sulphinic acid by *S. cerevisiae* sulphiredoxin. Nature 425:980–984

Boschi-Muller S, Gand A, Branlant G (2008) The methionine sulfoxide reductases: catalysis and substrate specificities. Arch Biochem Biophys 474:266–273

Broniowska KA, Diers AR, Hogg N (2013) S-Nitrosoglutathione. Biochim Biophys Acta 1830: 3173–3181

Camejo D, Romero-Puertas MC, Rodríguez-Serrano M, Sandalio LM, Lázaro JJ, Jiménez A, Sevilla F (2013) Salinity-induced changes in S-nitrosylation of pea mitochondrial proteins. J Proteome 79:87–99

Camejo D, Ortiz-Espín A, Lázaro JJ, Romero-Puertas MC, Lázaro-Payo A, Sevilla F, Jiménez A (2015) Functional and structural changes in plant mitochondrial PrxII F caused by NO. J Proteome 119:112–125

Castillo MC, Lozano-Juste J, González-Guzmán M, Rodriguez L, Rodriguez PL, León J (2015) Inactivation of PYR/PYL/RCAR ABA receptors by tyrosine nitration may enable rapid inhibition of ABA signaling by nitric oxide in plants. Sci Signal 8:ra89

Chaki M, Fernández-Ocaña AM, Valderrama R, Carreras A, Esteban FJ, Luque F, Gómez-Rodríguez MV, Begara-Morales JC, Corpas FJ, Barroso JB (2009a) Involvement of reactive nitrogen and oxygen species (RNS and ROS) in sunflower-mildew interaction. Plant Cell Physiol 50:265–279

Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, López-Jaramillo J, Luque F, Palma JM, Pedrajas JR, Begara-Morales JC, Sánchez-Calvo B, Gómez-Rodríguez MV, Corpas FJ, Barroso JB (2009b) Protein targets of tyrosine nitration in sunflower (*Helianthus annuus L.*) hypocotyls. J Exp Bot 60:4221–4234

Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, Gómez-Rodríguez MV, Pedrajas JR, Begara-Morales JC, Sánchez-Calvo B, Luque F, Leterrier M, Corpas FJ, Barroso JB (2011a) Mechanical wounding induces a nitrosative stress by down-regulation of GSNO reductase and an increase in S-nitrosothiols in sunflower (*Helianthus annuus*) seedlings. J Exp Bot 62: 1803–1813

Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, Gómez-Rodríguez MV, López-Jaramillo J, Begara-Morales JC, Sánchez-Calvo B, Luque F, Leterrier M, Corpas FJ, Barroso JB (2011b) High temperature triggers the metabolism of S-nitrosothiols in sunflower mediating a process of nitrosative stress which provokes the inhibition of ferredoxin-NADP reductase by tyrosine nitration. Plant Cell Environ 34:1803–1818

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. Ann Bot 116:637–647

Corpas FJ, Barroso J (2004) Cellular and subcellular localization of endogenous nitric oxide in young and senescent pea plants. Plant Physiol 136:2722–2733

Corpas FJ, Barroso JB (2013) Nitro-oxidative stress vs oxidative or nitrosative stress in higher plants. New Phytol 199:633–635

Corpas FJ, Barroso JB (2015) Nitric oxide from a "green" perspective. Nitric Oxide Biol Chem 45: 15–19

Corpas FJ, Barroso JB (2017) Nitric oxide synthase-like activity in higher plants. Nitric Oxide 68: 5–6

Corpas FJ, Barroso JB, del Río LA (2004) Enzymatic sources of nitric oxide in plant cells – beyond one protein-one function. New Phytol 162:243–251

Corpas FJ, Chaki M, Fernández-Ocaña A, Valderrama R, Palma JM, Carreras A, Begara-Morales JC, Airaki M, del Río LA, Barroso JB (2008) Metabolism of reactive nitrogen species in pea plants under abiotic stress conditions. Plant Cell Physiol 49:1711–1722

Corpas FJ, Palma JM, Del Rio L, Barroso JB (2009) Evidence supporting the existence of l-arginine-dependent nitric oxide synthase activity in plants. New Phytol 184:9–14

Corpas FJ, Leterrier M, Begara-Morales JC, Valderrama R, Chaki M, López-Jaramillo J, Luque F, Palma JM, Padilla MN, Sánchez-Calvo B, Mata-Pérez C, Barroso JB (2013) Inhibition of peroxisomal hydroxypyruvate reductase (HPR1) by tyrosine nitration. Biochim Biophys Acta 1830:4981–4989

Dalle-Donne I, Rossi R, Giustarini D, Gagliano N, Lusini L, Milzani A, Di Simplicio P, Colombo R (2001) Actin carbonylation: from a simple marker of protein oxidation to relevant signs of severe functional impairment. Free Radic Biol Med 31:1075–1083

Dalle-Donne I, Rossi R, Giustarini D, Colombo R, Milzani A (2007) S-glutathionylation in protein redox regulation. Free Radic Biol Med 43:883–898

Damiani I, Pauly N, Puppo A, Brouquisse R, Boscari A (2016) Reactive oxygen species and nitric oxide control early steps of the legume – rhizobium symbiotic interaction. Front Plant Sci 7:454

Dat JF, Inze D, van Breusegem F (2001) Catalase-deficient tobacco plants: tools for in planta studies on the role of hydrogen peroxide. Redox Rep 6:37–42

Davies MJ (2005) The oxidative environment and protein damage. Biochim Biophys Acta 1703: 93–109

Deeb RS, Nuriel T, Cheung C, Summers B, Lamon BD, Gross SS, Hajjar DP (2013) Characterization of a cellular denitrase activity that reverses nitration of cyclooxygenase. AJP Hear Circ Physiol 305:H687–H698

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci U S A 98:13454–13459

Dietz KJ (2014) Redox regulation of transcription factors in plant stress acclimation and development. Antioxid Redox Signal 21:1356–1372

Dixon DP, Davis BG, Edwards R (2002) Functional divergence in the glutathione transferase superfamily in plants: Identification of two classes with putative functions in redox homeostasis in *Arabidopsis thaliana*. J Biol Chem 277:30859–30869

Dixon DP, Skipsey M, Grundy NM, Edwards R (2005) Stress-induced protein S-glutathionylation in arabidopsis. Plant Physiol 138:2233–2244

Durner J, Gow AJ, Stamler JS, Glazebrook J (1999) Ancient origins of nitric oxide signaling in biological systems. Proc Natl Acad Sci U S A 96:14206–14207

El-Maarouf-Bouteau H, Bailly C (2008) Oxidative signaling in seed germination and dormancy. Plant Signal Behav 3:175–182

Fares A, Rossignol M, Peltier JB (2011) Proteomics investigation of endogenous S-nitrosylation in Arabidopsis. Biochem Biophys Res Commun 416:331–336

Farnese FS, Menezes-Silva PE, Gusman GS, Oliveira JA (2016) When bad guys become good ones: the key role of reactive oxygen species and nitric oxide in the plant responses to abiotic stress. Front Plant Sci 7:471

Fazzari M, Trostchansky A, Schopfer FJ, Salvatore SR, Sánchez-Calvo B, Vitturi D, Valderrama R, Barroso JB, Radi R, Freeman BA, Rubbo H (2014) Olives and olive oil are sources of electrophilic fatty acid nitroalkenes. PLoS One 9(1):e84884

Feechan A, Kwon E, Yun BW, Wang Y, Pallas JA, Loake GJ (2005) A central role for S-nitrosothiols in plant disease resistance. Proc Natl Acad Sci U S A 102:8054–8059

Feigl G, Kolbert Z, Lehotai N, Molnár Á, Ördög A, Bordé Á, Laskay G, Erdei L (2016) Different zinc sensitivity of Brassica organs is accompanied by distinct responses in protein nitration level and pattern. Ecotoxicol Environ Saf 125:141–152

Foyer CH, Noctor G (2016) Stress-triggered redox signalling: what's in pROSpect? Plant Cell Environ 39:951–964

Foyer CH, Ruban AV, Noctor G (2017) Viewing oxidative stress through the lens of oxidative signalling rather than damage. Biochem J 474:877–883

Freeman BA, Baker PRS, Schopfer FJ, Woodcock SR, Napolitano A, D'Ischia M (2008) Nitro-fatty acid formation and signaling. J Biol Chem 283:15515–15519

Friso G, van Wijk KJ (2015) Update: post-translational protein modifications in plant metabolism. Plant Physiol 169:1469–1487

Frungillo L, Skelly MJ, Loake GJ, Spoel SH, Salgado I (2014) S-nitrosothiols regulate nitric oxide production and storage in plants through the nitrogen assimilation pathway. Nat Commun 5:1–10

Fukuto JM, Carrington SJ, Tantillo DJ, Harrison JG, Ignarro LJ, Freeman BA, Chen A, Wink DA (2012) Small molecule signaling agents: the integrated chemistry and biochemistry of nitrogen oxides, oxides of carbon, dioxygen, hydrogen sulfide, and their derived species. Chem Res Toxicol 25:769–793

Gallogly MM, Mieyal JJ (2007) Mechanisms of reversible protein glutathionylation in redox signaling and oxidative stress. Curr Opin Pharmacol 7:381–391

Gao XH, Bedhomme M, Veyel D, Zaffagnini M, Lemaire SD (2009) Methods for analysis of protein glutathionylation and their application to photosynthetic organisms. Mol Plant 2: 218–235

Gaston B, Reilly J, Drazen JM, Fackler J, Ramdev P, Arnelle D, Mullins ME, Sugarbaker DJ, Chee C, Singel DJ (1993) Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. Proc Natl Acad Sci U S A 90:10957–10961

Geisler AC, Rudolph TK (2012) Nitroalkylation – a redox sensitive signaling pathway. Biochim Biophys Acta 1820:777–784

Gong YW, Yuan YJ (2006) Nitric oxide mediates inactivation of glutathione S-transferase in suspension culture of *Taxus cuspidata* during shear stress. J Biotechnol 123:185–192

Görg B, Qvartskhava N, Voss P, Grune T, Häussinger D, Schliess F (2007) Reversible inhibition of mammalian glutamine synthetase by tyrosine nitration. FEBS Lett 581:84–90

Gow AJ, Farkouh CR, Munson DA, Posencheg MA, Ischiropoulos H (2004) Biological significance of nitric oxide-mediated protein modifications. Am J Phys Lung Cell Mol Phys 287: L262–L268

Gruhlke MCH, Slusarenko AJ (2012) The biology of reactive sulfur species (RSS). Plant Physiol Biochem 59:98–107

Habibi G (2014) Hydrogen peroxide (H_2O_2) generation, scavenging and signaling in plants. In: Ahmad P (ed) Oxidative damage to plants: antioxidant networks and signaling. Academic Press, Amsterdam, pp 557–584

Haque A, Andersen JN, Salmeen A, Barford D, Tonks NK (2011) Conformation-sensing antibodies stabilize the oxidized form of PTP1B and inhibit its phosphatase activity. Cell 147:185–198

Harris TK, Turner GJ (2002) Structural basis of perturbed pK a values of catalytic groups in enzyme active sites. IUBMB Life 53:85–98

Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS (2005) Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol 6:150–166

Hiscock SJ, Bright J, McInnis SM, Desikan R, Hancock JT (2007) Signaling on the stigma: potential new roles for ROS and NO in plant cell signaling. Plant Signal Behav 2:23–24

Holtgrefe S, Gohlke J, Starmann J, Druce S, Klocke S, Altmann B, Wojtera J, Lindermayr C, Scheibe R (2008) Regulation of plant cytosolic glyceraldehyde 3-phosphate dehydrogenase isoforms by thiol modifications. Physiol Plant 133:211–228

Holzmeister C, Gaupels F, Geerlof A, Sarioglu H, Sattler M, Durner J, Lindermayr C (2015) Differential inhibition of Arabidopsis superoxide dismutases by peroxynitrite-mediated tyrosine nitration. J Exp Bot 66:989–999

Hu J, Huang X, Chen L, Sun X, Lu C, Zhang L, Wang Y, Zuo J (2015) Site-specific nitrosoproteomic identification of endogenously *S*-Nitrosylated proteins in Arabidopsis. Plant Physiol 167:1731–1746

Huber SC, Hardin SC (2004) Numerous posttranslational modifications provide opportunities for the intricate regulation of metabolic enzymes at multiple levels. Curr Opin Plant Biol 7:318–322

Jacques S, Ghesquière B, De Bock PJ, Demol H, Wahni K, Willems P, Messens J, Van Breusegem F, Gevaert K (2015) Protein methionine sulfoxide dynamics in *Arabidopsis thaliana* under oxidative stress. Mol Cell Proteomics 14:1217–1229

Jain K, Siddam A, Marathi A, Roy U, Falck JR, Balazy M (2008) The mechanism of oleic acid nitration by $^{\cdot}NO_2$. Free Radic Biol Med 45:269–283

Jasid S, Simontacchi M, Bartoli CG, Puntarulo S (2006) Chloroplasts as a nitric oxide cellular source. Effect of reactive nitrogen species on chloroplastic lipids and proteins. Plant Physiol 142: 1246–1255

Job C, Rajjou L, Lovigny Y, Belghazi M, Job D (2005) Patterns of protein oxidation in *Arabidopsis* seeds and during germination. Plant Physiol 138:790–802

Johansson E, Olsson O, Nyström T (2004) Progression and specificity of protein oxidation in the life cycle of *Arabidopsis thaliana*. J Biol Chem 279:22204–22208

Joudoi T, Shichiri Y, Kamizono N, Akaike T, Sawa T, Yoshitake J, Yamada N, Iwai S (2013) Nitrated cyclic GMP modulates guard cell signaling in *Arabidopsis*. Plant Cell 25:558–571

Kabil O, Banerjee R (2010) Redox biochemistry of hydrogen sulfide. J Biol Chem 285: 21903–21907

Kato H, Takemoto D, Kawakita K (2013) Proteomic analysis of S-nitrosylated proteins in potato plant. Physiol Plant 148:371–386

Kitajima S, Kurioka M, Yoshimoto T, Shindo M, Kanaori K, Tajima K, Oda K (2008) A cysteine residue near the propionate side chain of heme is the radical site in ascorbate peroxidase. FEBS J 275:470–480

Kneeshaw S, Spoel SH (2018) Thioredoxin-dependent decomposition of protein S-nitrosothiols. Method Mol Biol 1747:281–297

Kneeshaw S, Gelineau S, Tada Y, Loake GJ, Spoel SH (2014) Selective protein denitrosylation activity of thioredoxin-h5 modulates plant immunity. Mol Cell 56:153–162

Kolluru GK, Shen X, Bir SC, Kevil CG (2013) Hydrogen sulfide chemical biology: pathophysiological roles and detection. Nitric Oxide 35:5–20

Kovacs I, Holzmeister C, Wirtz M, Geerlof A, Fröhlich T, Römling G, Kuruthukulangarakoola GT, Linster E, Hell R, Arnold GJ, Durner J, Lindermayr C (2016) ROS-mediated inhibition of S-nitrosoglutathione reductase contributes to the activation of anti-oxidative mechanisms. Front Plant Sci 7:1669

Krishnan N, Fu C, Pappin DJ, Tonks NK (2011) H_2S-induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. Sci Signal 4:ra86–ra86

Lee JW, Helmann JD (2006) The PerR transcription factor senses H_2O_2 by metal-catalysed histidine oxidation. Nature 440:363–367

Leitner M, Vandelle E, Gaupels F, Bellin D, Delledonne M (2009) NO signals in the haze: nitric oxide signalling in plant defence. Curr Opin Plant Biol 12:451–458

Leterrier M, Chaki M, Airaki M, Valderrama R, Palma JM, Barroso JB, Corpas FJ (2011) Function of S-nitrosoglutathione reductase (GSNOR) in plant development and under biotic/abiotic stress. Plant Signal Behav 6:789–793

Leterrier M, Airaki M, Palma JM, Chaki M, Barroso JB, Corpas FJ (2012) Arsenic triggers the nitric oxide (NO) and S-nitrosoglutathione (GSNO) metabolism in *Arabidopsis*. Environ Pollut 166:136–143

Leymarie J, Vitkauskaité G, Hoang HH, Gendreau E, Chazoule V, Meimoun P, Corbineau F, El-Maarouf-Bouteau H, Bailly C (2012) Role of reactive oxygen species in the regulation of *Arabidopsis* seed dormancy. Plant Cell Physiol 53:96–106

Li L, Rose P, Moore PK (2011) Hydrogen sulfide and cell signaling. Annu Rev Pharmacol Toxicol 51:169–187

Li CW, Lee SH, Chieh PS, Lin CS, Wang YC, Chan MT (2012) Arabidopsis root-abundant cytosolic methionine sulfoxide reductase B genes MsrB7 and MsrB8 are involved in tolerance to oxidative stress. Plant Cell Physiol 53:1707–1719

Lin A, Wang Y, Tang J, Xue P, Li C, Liu L, Hu B, Yang F, Loake GJ, Chu C (2012) Nitric oxide and protein S-nitrosylation are integral to hydrogen peroxide-induced leaf cell death in rice. Plant Physiol 158:451–464

Lindermayr C (2018) Crosstalk between reactive oxygen species and nitric oxide in plants: key role of S-nitrosoglutathione reductase. Free Radic Biol Med 122:110–115

Lindermayr C, Durner J (2009) S-Nitrosylation in plants: pattern and function. J Proteome 73:1–9

Lindermayr C, Durner J (2015) Interplay of reactive oxygen species and nitric oxide: nitric oxide coordinates reactive oxygen species homeostasis. Plant Physiol 167:1209–1210

Liu L, Hausladen A, Zeng M, Que L, Heitman J, Stamler JS (2001) A metabolic enzyme for *S*-nitrosothiol conserved from bacteria to humans. Nature 410:490–494

Lounifi I, Arc E, Molassiotis A, Job D, Rajjou L, Tanou G (2013) Interplay between protein carbonylation and nitrosylation in plants. J Proteome 13:568–578

Lozano-Juste J, León J (2010) Enhanced abscisic acid-mediated responses in nia1nia2noa1-2 triple mutant impaired in NIA/NR- and AtNOA1-dependent nitric oxide biosynthesis in *Arabidopsis*. Plant Physiol 152:891–903

Lozano-Juste J, Colom-Moreno R, León J (2011) In vivo protein tyrosine nitration in *Arabidopsis thaliana*. J Exp Bot 62:3501–3517

Luo D, Smith SW, Anderson BD (2005) Kinetics and mechanism of the reaction of cysteine and hydrogen peroxide in aqueous solution. J Pharm Sci 94:304–316
Malik SI, Hussain A, Yun BW, Spoel SH, Loake GJ (2011) GSNOR-mediated de-nitrosylation in the plant defence response. Plant Sci 181:540–544
Marino SM, Gladyshev VN (2010) Cysteine function governs its conservation and degeneration and restricts its utilization on protein surfaces. J Mol Biol 404:902–916
Mata-Pérez C, Sánchez-Calvo B, Begara-Morales JC, Carreras A, Padilla MN, Melguizo M, Valderrama R, Corpas FJ, Barroso JB (2016a) Nitro-linolenic acid is a nitric oxide donor. Nitric Oxide 57:57–63
Mata-Pérez C, Sánchez-Calvo B, Begara-Morales JC, Padilla MN, Valderrama R, Corpas FJ, Barroso JB (2016b) Nitric oxide release from nitro-fatty acids in Arabidopsis roots. Plant Signal Behav 11(3):e1154255
Mata-Pérez C, Sánchez-Calvo B, Padilla MN, Begara-Morales JC, Luque F, Melguizo M, Jiménez-Ruiz J, Fierro-Risco J, Peñas-Sanjuán A, Valderrama R, Corpas FJ, Barroso JB (2016c) Nitro-fatty acids in plant signaling: nitro-linolenic acid induces the molecular chaperone network in *Arabidopsis*. Plant Physiol 170:686–701
Mata-Perez C, Begara-Morales JC, Chaki M, Sánchez-Calvo B, Valderrama R, Padilla MN, Corpas FJ, Barroso JB (2016d) Protein tyrosine nitration during development and abiotic stress response in plants. Front Plant Sci 7:1699
Mata-Pérez C, Sánchez-Calvo B, Padilla MN, Begara-Morales JC, Valderrama R, Corpas FJ, Barroso JB (2017) Nitro-fatty acids in plant signaling: new key mediators of nitric oxide metabolism. Redox Biol 11:554–561
Mata-Pérez C, Padilla MN, Sánchez-Calvo B, Begara-Morales JC, Valderrama R, Chaki M, Barroso JB (2018) Biological properties of nitro-fatty acids in plants. Nitric Oxide 78:176–179
McInnis SM, Desikan R, Hancock JT, Hiscock SJ (2006) Production of reactive oxygen species and reactive nitrogen species by angiosperm stigmas and pollen: potential signalling crosstalk? New Phytol 172:221–228
Men L, Wang Y (2007) The oxidation of yeast alcohol dehydrogenase-1 by hydrogen peroxide in vitro. J Proteome Res 6:216–225
Michelet L, Zaffagnini M, Vanacker H, Le Maréchal P, Marchand C, Schroda M, Lemaire SD, Decottignies P (2008) In vivo targets of S-thiolation in *Chlamydomonas reinhardtii*. J Biol Chem 283:21571–21578
Miller EW, Chang CJ (2007) Fluorescent probes for nitric oxide and hydrogen peroxide in cell signaling. Curr Opin Chem Biol 11:620–625
Minas IS, Tanou G, Belghazi M, Job D, Manganaris GA, Molassiotis A, Vasilakakis M (2012) Physiological and proteomic approaches to address the active role of ozone in kiwifruit post-harvest ripening. J Exp Bot 63:2449–2464
Molassiotis A, Fotopoulos V (2011) Oxidative and nitrosative signaling in plants: two branches in the same tree? Plant Signal Behav 6:210–214
Mustafa AK, Gadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, Snyder SH (2009) H_2S signals through protein S-Sulfhydration. Sci Signal 2:ra72
Nadtochiy SM, Burwell LS, Ingraham CA, Spencer CM, Friedman AE, Pinkert CA, Brookes PS (2009) In vivo cardioprotection by S-nitroso-2-mercaptopropionyl glycine. J Mol Cell Cardiol 46:960–968
Nguyen AT, Donaldson RP (2005) Metal-catalyzed oxidation induces carbonylation of peroxisomal proteins and loss of enzymatic activities. Arch Biochem Biophys 439:25–31
Noctor G, Reichheld JP, Foyer CH (2017) ROS-related redox regulation and signaling in plants. Semin Cell Dev Biol 80:3–12
Nyström T (2005) Role of oxidative carbonylation in protein quality control and senescence. EMBO J 24:1311–1317
Oracz K, Bouteau HEM, Farrant JM, Cooper K, Belghazi M, Job C, Job D, Corbineau F, Bailly C (2007) ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. Plant J 50:452–465

Padilla MN, Mata-Pérez C, Melguizo M, Barroso JB (2017) In vitro nitro-fatty acid release from Cys-NO_2-fatty acid adducts under nitro-oxidative conditions. Nitric Oxide 68:14–22

Polge C, Jaquinod M, Holzer F, Bourguignon J, Walling L, Brouquisse R (2009) Evidence for the existence in *Arabidopsis thaliana* of the proteasome proteolytic pathway: activation in response to cadmium. J Biol Chem 284:35412–35424

Prabakaran S, Lippens G, Steen H, Gunawardena J (2012) Post-translational modification: nature's escape from genetic imprisonment and the basis for dynamic information encoding. Rev Syst Biol Med 4:565–583

Puyaubert J, Fares A, Rézé N, Peltier JB, Baudouin E (2014) Identification of endogenously S-nitrosylated proteins in *Arabidopsis* plantlets: effect of cold stress on cysteine nitrosylation level. Plant Sci 215–216:150–156

Queval G, Issakidis-Bourguet E, Hoeberichts FA, Vandorpe M, Gakière B, Vanacker H, Miginiac-Maslow M, Van Breusegem F, Noctor G (2007) Conditional oxidative stress responses in the *Arabidopsis* photorespiratory mutant cat2 demonstrate that redox state is a key modulator of daylength-dependent gene expression, and define photoperiod as a crucial factor in the regulation of H_2O_2-induced cell. Plant J 52:640–657

Radi RCNC (2004) Nitric oxide, oxidants, and protein tyrosine nitration. Proc Natl Acad Sci U S A 101:4003–4008

Radi R (2013) Protein tyrosine nitration: biochemical mechanisms and structural basis of functional effects. Acc Chem Res 46:550–559

Rajjou L, Debeaujon I (2008) Seed longevity: survival and maintenance of high germination ability of dry seeds. C R Biol 331:796–805

Rajjou L, Lovigny Y, Groot SPC, Belghazi M, Job C, Job D (2008) Proteome-wide characterization of seed aging in *Arabidopsis*: a comparison between artificial and natural aging protocols. Plant Physiol 148:620–641

Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C, Job D (2012) Seed germination and vigor. Annu Rev Plant Biol 63:507–533

Rao SR, Møller IM (2012) Pattern of occurrence and occupancy of carbonylation sites in proteins. J Proteome 11:4166–4173

Rey P, Bécuwe N, Barrault MB, Rumeau D, Havaux M, Biteau B, Toledano MB (2007) The *Arabidopsis thaliana* sulfiredoxin is a plastidic cysteine-sulfinic acid reductase involved in the photooxidative stress response. Plant J 49:505–514

Rockel P, Strube F, Rockel A, Wildt J, Kaiser WM (2002) Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro. J Exp Bot 53:103–110

Romero-Puertas MC, Laxa M, Matte A, Zaninotto F, Finkemeier I, Jones AME, Perazzolli M, Vandelle E, Dietz KJ, Delledonne M (2007) S-Nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. Plant Cell Online 19:4120–4130

Romero-Puertas MC, Campostrini N, Mattè A, Righetti PG, Perazzolli M, Zolla L, Roepstorff P, Delledonne M (2008) Proteomic analysis of S-nitrosylated proteins in *Arabidopsis thaliana* undergoing hypersensitive response. J Proteome 8:1459–1469

Roos G, Foloppe N, Messens J (2013) Understanding the pK (a) of redox cysteines: the key role of hydrogen bonding. Antioxid Redox Signal 18:94–127

Rouhier N, Dos SCV, Tarrago L, Rey P (2006) Plant methionine sulfoxide reductase A and B multigenic families. Photosynth Res 89:247–262

Rudolph V, Schopfer FJ, Khoo NKH, Rudolph TK, Cole MP, Woodcock SR, Bonacci G, Groeger AL, Golin-Bisello F, Chen CS, Baker PRS, Freeman BA (2009) Nitro-fatty acid metabolome: saturation, desaturation, β-oxidation, and protein adduction. J Biol Chem 284:1461–1473

Rudolph V, Rudolph TK, Schopfer FJ, Bonacci G, Woodcock SR, Cole MP, Baker PRS, Ramani R, Freeman BA (2010) Endogenous generation and protective effects of nitro-fatty acids in a murine model of focal cardiac ischaemia and reperfusion. Cardiovasc Res 85:155–166

Ruiz-May E, Segura-Cabrera A, Elizalde-Contreras JM, Shannon LM, Loyola-Vargas VM (2018) A recent advance in the intracellular and extracellular redox post-translational modification of proteins in plants. J Mol Recognit. https://doi.org/10.1002/jmr.2754

Rusterucci C, Espunya MC, Diaz M, Chabannes M, Martinez MC (2007) S-Nitrosoglutathione reductase affords protection against pathogens in Arabidopsis, both locally and systemically. Plant Physiol 143:1282–1292

Ryšlavá H, Doubnerová V, Kavan D, Vaněk O (2013) Effect of posttranslational modifications on enzyme function and assembly. J Proteome 92:80–109

Saito S, Yamamoto-Katou A, Yoshioka H, Doke N, Kawakita K (2006) Peroxynitrite generation and tyrosine nitration in defense responses in tobacco BY-2 cells. Plant Cell Physiol 47: 689–697

Salmeen A, Andersen JN, Myers MP, Meng TC, Hinks JA, Tonks NK, Barford D (2003) Redox regulation of protein tyrosine phosphatase 1B involves a sulphenyl-amide intermediate. Nature 423:769–773

Sánchez-Calvo B, Barroso JB, Corpas FJ (2013) Hypothesis: nitro-fatty acids play a role in plant metabolism. Plant Sci 199–200:1–6

Sanz L, Albertos P, Mateos I, Sánchez-Vicente I, Lechón T, Fernández-Marcos M, Lorenzo O (2015) Nitric oxide (NO) and phytohormones crosstalk during early plant development. J Exp Bot 66:2857–2868

Sawa T, Ihara H, Akaike T (2011) Antioxidant effect of a nitrated cyclic nucleotide functioning as an endogenous electrophile. Curr Top Med Chem 11:1854–1860

Sawa T, Ihara H, Ida T, Fujii S, Nishida M, Akaike T (2013) Formation, signaling functions, and metabolisms of nitrated cyclic nucleotide. Nitric Oxide 34:10–18

Schopfer FJ, Baker PRS, Giles G, Chumley P, Batthyany C, Crawford J, Patel RP, Hogg N, Branchaud BP, Lancaster JR, Freeman BA (2005a) Fatty acid transduction of nitric oxide signaling: nitrolinoleic acid is a hydrophobically stabilized nitric oxide donor. J Biol Chem 280:19289–19297

Schopfer FJ, Lin Y, Baker PRS, Cui T, Garcia-Barrio M, Zhang J, Chen K, Chen YE, Freeman BA (2005b) Nitrolinoleic acid: an endogenous peroxisome proliferator-activated receptor ligand. Proc Natl Acad Sci U S A 102:2340–2345

Schopfer FJ, Batthyany C, Baker PRS, Bonacci G, Cole MP, Rudolph V, Groeger AL, Rudolph TK, Nadtochiy S, Brookes PS, Freeman BA (2009) Detection and quantification of protein adduction by electrophilic fatty acids: mitochondrial generation of fatty acid nitroalkene derivatives. Free Radic Biol Med 46:1250–1259

Schopfer FJ, Cipollina C, Freeman BA (2011) Formation and signaling actions of electrophilic lipids. Chem Rev 111:5997–6021

Sehrawat A, Abat JK, Deswal R (2013) RuBisCO depletion improved proteome coverage of cold responsive S-nitrosylated targets in *Brassica juncea*. Front Plant Sci 4:342

Seth D, Stamler JS (2011) The SNO-proteome: causation and classifications. Curr Opin Chem Biol 15:129–136

Shenton D, Grant CM (2003) Protein S-thiolation targets glycolysis and protein synthesis in response to oxidative stress in the yeast *Saccharomyces cerevisiae*. Biochem J 374:513–519

Sies H (2017) Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: oxidative eustress. Redox Biol 11:613–619

Signorelli S, Corpas FJ, Borsani O, Barroso JB, Monza J (2013) Water stress induces a differential and spatially distributed nitro-oxidative stress response in roots and leaves of *Lotus japonicus*. Plant Sci 201–202:137–146

Smakowska E, Czarna M, Janska H (2014) Mitochondrial ATP-dependent proteases in protection against accumulation of carbonylated proteins. Mitochondrion 19:245–251

Song H, Fan P, Li Y (2009) Overexpression of organellar and cytosolic at HSP90 in arabidopsis thaliana impairs plant tolerance to oxidative stress. Plant Mol Biol Report 27:342–349

Souza JM, Peluffo G, Radi R (2008) Protein tyrosine nitration—functional alteration or just a biomarker? Free Radic Biol Med 45:357–366

Stadtman ER, Van Remmen H, Richardson A, Wehr NB, Levine RL (2005) Methionine oxidation and aging. Biochim Biophys Acta 1703:135–140

Sundaram S, Rathinasabapathi B (2010) Transgenic expression of fern *Pteris vittata* glutaredoxin PvGrx5 in *Arabidopsis thaliana* increases plant tolerance to high temperature stress and reduces oxidative damage to proteins. Planta 231:361–369

Tajc SG, Tolbert BS, Basavappa R, Miller BL (2004) Direct determination of thiol pK (a) by isothermal titration microcalorimetry. J Am Chem Soc 126:10508–10509

Takahashi M, Shigeto J, Sakamoto A, Izumi S, Asada K, Morikawa H (2015) Dual selective nitration in *Arabidopsis*: almost exclusive nitration of PsbO and PsbP, and highly susceptible nitration of four non-PSII proteins, including peroxiredoxin II E. Electrophoresis 36:2569–2578

Tamarit J (1998) Identification of the major oxidatively damaged proteins in *Escherichia coli* cells exposed to oxidative stress. J Biol Chem 273:3027–3032

Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Molassiotis A, Job D (2009) Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. Plant J 60:795–804

Tanou G, Filippou P, Belghazi M, Job D, Diamantidis G, Fotopoulos V, Molassiotis A (2012) Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. Plant J 72:585–599

Tarrago L, Laugier E, Zaffagnini M, Marchand C, Le Marechal P, Rouchier N, Lemaire SD, Rey P (2009) Regeneration mechanisms of *Arabidopsis thaliana* methionine sulfoxide reductases B by glutaredoxins and thioredoxins. J Biol Chem 284:18963–18971

Tesnier K, Strookman-Donkers HM, van Pijlen JG, van der Geest AHM, Bino RJ, Groot SPC (2002) A controlled deterioration test for *Arabidopsis thaliana* reveals genetic variation in seed quality. Seed Sci Technol 30:149–165

Trapet P, Kulik A, Lamotte O, Jeandroz S, Bourque S, Nicolas-Francès V, Rosnoblet C, Besson-Bard A, Wendehenne D (2015) NO signaling in plant immunity: a tale of messengers. Phytochemistry 112:72–79

Trostchansky A, Rubbo H (2008) Nitrated fatty acids: mechanisms of formation, chemical characterization, and biological properties. Free Radic Biol Med 44:1887–1896

Tun NN, Santa-Catarina C, Begum T, Silveira V, Handro W, Segal Floh EI, Scherer GFE (2006) Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. Plant Cell Physiol 47:346–354

Turko IV (2002) Protein nitration in cardiovascular diseases. Pharmacol Rev 54:619–634

Valderrama R, Corpas FJ, Carreras A, Fernandez-Ocana A, Chaki M, Luque F, Gomez-Rodriguez MV, Colmenero-Varea P, del Rio LA, Barroso JB (2007) Nitrosative stress in plants. FEBS Lett 581:453–461

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

Vandiver MS, Snyder SH (2012) Hydrogen sulfide: a gasotransmitter of clinical relevance. J Mol Med 90:255–263

Vieira Dos Santos C, Cuiné S, Rouhier N, Rey P (2005) The *Arabidopsis* plastidic methionine sulfoxide reductase B proteins. Sequence and activity characteristics, comparison of the expression with plastidic methionine sulfoxide reductase A, and induction by photooxidative stress. Plant Physiol 138:909–922

Wang R (2012) Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. Physiol Rev 92:791–896

Wang Y, Loake GJ, Chu C (2013) Cross-talk of nitric oxide and reactive oxygen species in plant programed cell death. Front Plant Sci 4:314

Waszczak C, Akter S, Jacques S, Huang J, Messens J, Van Breusegem F (2015) Oxidative post-translational modifications of cysteine residues in plant signal transduction. J Exp Bot 66: 2923–2934

Wilson ID, Neill SJ, Hancock JT (2008) Nitric oxide synthesis and signalling in plants. Plant Cell Environ 31:622–631

Woo HA, Chae HZ, Hwang SC, Yang KS, Kang SW, Kim K, Rhee SG (2003) Reversing the inactivation of peroxiredoxins caused by cysteine sulfinic acid formation. Science 300:653–656

Wood ZA, Poole LB, Karplus PA (2003) Peroxiredoxin evolution and the signaling. Science 300: 6506–6553

Xiong Y, Contento AL, Nguyen PQ, Bassham DC (2006) Degradation of oxidized proteins by autophagy during oxidative stress in *Arabidopsis*. Plant Physiol 143:291–299

Yamasaki H, Sakihama Y, Takahashi S (1999) An alternative pathway for nitric oxide production in plants: new features of an old enzyme. Trend Plant Sci 4:128–129

Yang H, Mu J, Chen L, Feng J, Hu J, Li L, Zhou JM, Zuo J (2015) *S* -Nitrosylation positively regulates ascorbate peroxidase activity during plant stress responses. Plant Physiol 167: 1604–1615

Yoda H (2006) Polyamine oxidase is one of the key elements for oxidative burst to induce programmed cell death in tobacco cultured cells. Plant Physiol 142:193–206

Yu M, Lamattina L, Spoel SH, Loake GJ (2014) Nitric oxide function in plant biology: a redox cue in deconvolution. New Phytol 202:1142–1156

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

Zaffagnini M, Michelet L, Marchand C, Sparla F, Decottignies P, Le Maréchal P, Miginiac-Maslow M, Noctor G, Trost P, Lemaire SD (2007) The thioredoxin-independent isoform of chloroplastic glyceraldehyde-3- phosphate dehydrogenase is selectively regulated by glutathionylation. FEBS J 274:212–226

Zaffagnini M, Bedhomme M, Groni H, Marchand CH, Puppo C, Gontero B, Cassier-Chauvat C, Decottignies P, Lemaire SD (2012a) Glutathionylation in the photosynthetic model organism *Chlamydomonas reinhardtii*: a proteomic survey. Mol Cell Proteomics 11:M111.014142

Zaffagnini M, Bedhomme M, Marchand CH, Morisse S, Trost P, Lemaire SD (2012b) Redox regulation in photosynthetic organisms: focus on glutathionylation. Antioxid Redox Signal 16: 567–586

Zaffagnini M, Morisse S, Bedhomme M, Marchand CH, Festa M, Rouhier N, Lemaire SD, Trost P (2013) Mechanisms of nitrosylation and denitrosylation of cytoplasmic glyceraldehyde-3-phosphate dehydrogenase from *Arabidopsis thaliana*. J Biol Chem 288:22777–22789

Zafra A, Rodríguez-García MI, Alché JDD (2010) Cellular localization of ROS and NO in olive reproductive tissues during flower development. BMC Plant Biol 10:36

Zagorchev L, Seal CE, Kranner I, Odjakova M (2013) A central role for thiols in plant tolerance to abiotic stress. Int J Mol Sci 14:7405–7432

Zhang A, Zhang J, Zhang J, Ye N, Zhang H, Tan M, Jiang M (2011) Nitric oxide mediates brassinosteroid-induced ABA biosynthesis involved in oxidative stress tolerance in maize leaves. Plant Cell Physiol 52:181–192

Ziogas V, Tanou G, Filippou P, Diamantidis G, Vasilakakis M, Fotopoulos V, Molassiotis A (2013) Nitrosative responses in citrus plants exposed to six abiotic stress conditions. Plant Physiol Biochem 68:118–126

Transcriptional Regulation of Gene Expression Related to Hydrogen Peroxide (H_2O_2) and Nitric Oxide (NO)

Juan C. Begara-Morales, Mounira Chaki, Raquel Valderrama, Capilla Mata-Pérez, María Padilla, and Juan B. Barroso

Contents

Abstract Hydrogen peroxide (H_2O_2) and nitric oxide (NO) are biological messengers that control a plethora of physiological functions integral to plant biology such as seed germination, growth, development, flowering, or plant response to stress. Furthermore, the interplay between the signaling pathways governed by these redox molecules has emerged as crucial during plant response to different stress situations. In recent years, to gain in the knowledge of the mode of action of these signaling molecules at molecular levels, different NO donors and H_2O_2 have been used in medium- and large-scale transcriptomic analyses including microarray, cDNA-amplification fragment length polymorphism (AFLP), and high-throughput sequencing (RNA-seq technology). Following this strategy, a high transcriptional reprogramming induced by both NO and H_2O_2 has been proposed. In this regard, thousands of NO- and H_2O_2-cell targets have been identified in different plant species and organs and predicted to be related to a wide diversity of biological processes. However, some authors have identified by comparing different transcriptomic analysis that there is a low overlap in the transcriptomic data available

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under different treatment conditions as well as different organ analyzed. In this sense, more transcriptomic data comparisons will help in the identification of the NO- and H_2O_2-specific targets and even the common genes involved in both H_2O_2- and NO-dependent signaling events.

In this book chapter, we will offer an update about the recent knowledge concerning the transcriptional regulation induced by NO and H_2O_2. With this purpose, the recent data from the different medium- and large-scale transcriptomic analyses have been discussed. In addition, it is also provided an overview about the interplay between H_2O_2- and NO-dependent signaling mechanism and the need to further identification of common targets during the coordinated response to different stress situations.

Keywords Nitric oxide · Hydrogen peroxide · Signaling · Transcriptomic analysis · RNA-seq · Microarray · cDNA-AFLP

1 Introduction

Redox molecules are essential components of the signaling mechanisms integral to plant biology. Among the most important redox molecules are reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and reactive nitrogen species (RNS) as nitric oxide (NO) and its derived molecules. ROS and RNS are a double-edge sword because at low concentrations they act as essential molecules involved in plant signaling processes, and at high concentrations they can alter cellular redox homeostasis and therefore being toxic and inducing cellular damages (Cerny et al. 2018). It is well established that during plant response to different stress situations, a burst of ROS and RNS takes place and therefore a nitro-oxidative stress is induced (Corpas and Barroso 2013). Under these conditions, ROS and RNS could act independently or coordinately in the regulation of signaling events leading to face the adverse situations. In this context, the interplay between both signaling pathways has emerged as crucial to regulate plant response to stress (Lindermayr and Durner 2015; Begara-Morales et al. 2016; Niu and Liao 2016; Lindermayr 2018). To transmit their bioactivity, these redox molecules act via posttranslational modifications that can regulate protein function and therefore being an essential part of signaling mechanisms (Astier and Lindermayr 2012; Cerny et al. 2018). In addition, as part of the signaling events governed by these redox molecules, a transcriptional regulation under these adverse conditions takes place to coordinate the plant response to stress. In the last decade, the development of the transcriptomic approaches, which use a part of a whole-genome analysis of different plant species, such as cDNA-amplification fragment length polymorphism (AFLP), microarray, and especially the high-throughput sequencing (RNA-seq technology), has facilitated the identification of thousands of H_2O_2- and NO-specific target genes under different physiological and stress situations (Ferrarini et al. 2008; Boscari et al. 2013; Begara-Morales et al. 2014b; Blaby et al. 2015; Li et al. 2017). Therefore, significant efforts have been made to unravel the transcriptional regulation mediated by these

ROS and RNS that leads to a strong transcriptional reprogramming. However, most of these studies have been performed via the exogenous administration of H_2O_2 and different NO donors (Ferrarini et al. 2008; Boscari et al. 2013; Begara-Morales et al. 2014b; Blaby et al. 2015; Li et al. 2017). In this regard, further analyses to analyze the in vivo implication of these molecules during plant response to different stress conditions are required.

In this book chapter, we will explore the recent state-of-the-art knowledge concerning recent results obtained by a wide range of transcriptomic approaches to offer an overview of the understanding of H_2O_2- and NO-induced gene expression profile changes under different physiological and stress situations in plants. Due to the low overlap in the results obtained under different treatment conditions, plant species, or even NO donors (Ferrarini et al. 2008; Besson-Bard et al. 2009b; Blaby et al. 2015), the need to perform more comparisons of the transcriptomic data available to improve our knowledge in the H_2O_2- and NO-signaling events is also discussed.

2 Nitric Oxide Induces a High Transcriptional Reprogramming Under Physiological and Stress Conditions

Nitric oxide (NO) is a key biological messenger that governs a multitude of functions integral to plant biology (Mur et al. 2013). For instance, NO has been involved in seed germination (Albertos et al. 2015), flowering (He et al. 2014; Kumar et al. 2016), stomata closure (Wang et al. 2015), or plant response to stress (Yu et al. 2014; Fancy et al. 2016). It usually transmits its bioactivity through interaction with essential biomolecules such as proteins, nucleic acids, and fatty acids. In this regard, NO research has been traditionally focused on the regulation of protein function via posttranslational modifications (NO-PTMs) such as S-nitrosylation and tyrosine nitration (Astier and Lindermayr 2012) (Fig. 1). S-nitrosylation consists of the reversible addition of a NO group to a specific thiol group in a cysteine (Cys) residue leading to S-nitrosothiols (SNOs) formation (Hess et al. 2005). On the other hand, tyrosine nitration, which is usually mediated by peroxynitrite, is produced by the irreversible attachment of a NO_2 radical to the aromatic ring of the tyrosine residue yielding 3-nitrotyrosine (Radi 2004) that is considered a nitrosative stress marker rather than a signaling process (Corpas et al. 2013). In last years, the development of new proteomic approaches has allow the identification of hundreds of proteins that are NO-PTM targets under different physiological and stress situations (Tanou et al. 2009; Hu et al. 2015). Consequently a wide range of cellular processes have been proposed to be regulated by these NO-PTMs.

Otherwise, gene expression changes are also part of NO-dependent signaling events (Fig. 1). One of the first evidence showing the NO-dependent induction of defense gene was carried out in tobacco plants and tobacco suspension cells. In this

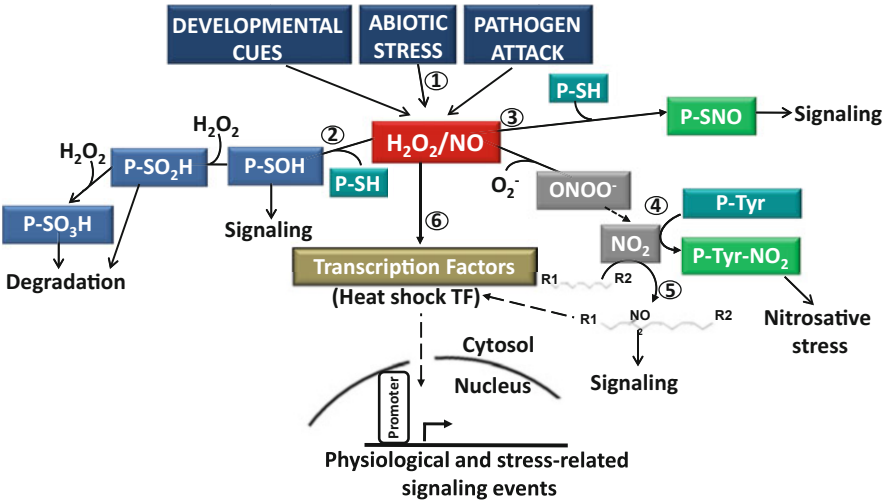

Fig. 1 Hydrogen peroxide and nitric oxide-dependent signaling events. Different molecular cues derived from developmental processes or different stress situations, among others, can induce the production of hydrogen peroxide (H_2O_2) and nitric oxide (NO) (1). These signaling molecules can transmit their bioactivity through different posttranslational modifications. In this regard, H_2O_2 can oxidized the cysteine (Cys) residue in a target protein (P-SH) generating sulfenic acid (P-SOH) that can be involved in signaling processes (2). High H_2O_2 could produce the irreversible oxidation of the Cys residue to sulfinic (P-SO_2H) and sulfonic (P-SO_3H) acids that usually act as markers for protein degradation. On the other hand, NO can interact with the thiol group of a Cys residue leading to S-nitrosothiols (P-SNO) formations that are key players in signaling processes (3). Moreover, NO can interact with the superoxide anion ($O_2^{\cdot-}$) generating peroxynitrite ($ONOO^-$) that ultimately can mediate tyrosine nitration (4) or nitration of fatty acids (5) via the formation of the intermediate NO_2 radical. Due to its irreversibility, the 3-nitrotyrosine (P-Tyr-NO_2) is considered a marker of the nitrosative stress rather than signaling molecules, whereas nitro-fatty acids are involved in signaling mechanisms via nitroalkylation processes or regulating gene expression profile in plant response to stress via control of heat shock transcription factors. Finally, both H_2O_2 and NO are able to modulate the expression of a wide range of transcription factors that ultimately mediate the specific gene expression related to different physiological or stress response processes

study, recombinant nitric oxide synthase (NOS) from mammals and NO donors as *S*-nitrosoglutathione (GSNO), and *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP), induced the expression of defense-related genes such as pathogenesis-related 1 protein (PR1) and phenylalanine ammonia lyase (PAL) (Durner et al. 1998). From this initial work, the development of the omic technologies has allowed to analyze the effect of NO on transcriptional regulation using a large part or the whole-genome (Badri et al. 2008; Ferrarini et al. 2008; Palmieri et al. 2008; Ahlfors et al. 2009; Begara-Morales et al. 2014b). Therefore, these technologies permit to have a general perspective of the NO-dependent signaling in a single experiment. In the following sections, we will analyze the most recent and relevant data concerning the

NO-dependent transcriptional reprogramming obtained by different technology approaches.

2.1 Nitric Oxide-Responsive Genes Identified by cDNA-Amplification Fragment Length Polymorphism (cDNA-AFLP) and Microarray Analysis

The transcriptomic technologies have allowed significant advances in understanding the molecular basis of NO action. Most of these studies have been carried out under plant response to pathogen attack and/or using different NO donors (Badri et al. 2008; Ferrarini et al. 2008; Ahlfors et al. 2009; Begara-Morales et al. 2014b). First studies were conducted using a microarray analysis in which a cDNA microarray containing 200 defense-related genes and 50 genes related to primary metabolism was employed to determine gene expression changes in *Arabidopsis* suspension cells treated with the NO donor NOR-3 ((E)-ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexeneamide) (Huang et al. 2002). Following this approach, pathogenesis-related (PR) and antioxidant genes such as peroxidases and glutathione S-transferases were proposed to be modulated by NO. Interestingly, cytochrome c respiration was inhibited by NO, whereas one of the NO-induced genes was alternative oxidase 1a (AOX1a) that resulted in an increased respiration via the alternative pathway. Furthermore, NO-induced AOX was independent of salicylic acid (SA), suggesting that it may participate to counteract the toxicity of NO and therefore having an essential function for cellular homeostasis under NO stress (Huang et al. 2002). Moreover, *Arabidopsis* gene expression profile was analyzed by cDNA-amplification fragment length polymorphism (AFLP) transcript profiling after leaves infiltration with the NO donor sodium nitroprusside (SNP) (Polverari et al. 2003). Following this approach, it was observed the modulation of 120 genes among the 2500 cDNA examined. The NO-responsive genes were mainly related to signal transduction, disease resistance and cell death, ROS-related and stress response, photosynthesis, cellular transport, and basic metabolism (Polverari et al. 2003). Interestingly, most of NO-modulated genes identified were previously proposed to be regulated in other stress-related experiments, especially in disease-related conditions according to public microarray analysis data (Polverari et al. 2003). These results, pointing toward the NO-responsive genes have a crucial role during plant response to stress. Subsequently, it was shown that lipopolysaccharides (LPS) induced a rapid burst of NO with a concomitant regulation of gene expression, suggesting a functional link between the production of NO and gene induction by LPS (Zeidler et al. 2004). Moreover, a custom-designed cDNA microarray containing about 700 defense-related genes, which encodes PR proteins or protein induced by pathogens and abiotic stresses, was used to analyze gene expression changes caused by LPS and linked to a functional regulation of these genes by NO (Zeidler et al. 2004). In this context, the cDNA microarray analysis identified different defense or stress-associated genes

including glutathione *S*-transferases, cytochrome P450, and different PR proteins, altogether contributing to the activation of plant defense responses (Zeidler et al. 2004). Besides these custom-designed arrays containing mainly genes related to defense, different genome-scale analyses have been performed too deep in the knowledge of the transcriptional reprogramming after perception of the NO signal by the cell. In this sense, stress-related genes were also proposed to be modulated by NO in *Arabidopsis thaliana* using SNP as NO donor (Parani et al. 2004). The advantage of this study was to use the whole-genome ATH1 microarray, allowing analyzing over 24,000 genes. After *Arabidopsis* irrigation with SNP and 2-[4-carboxyphenyl]-4,4,5,5-tetramethylimidazoline-1-oxy-3-oxide (cPTIO) as NO scavenger, 342 upregulated and 80 downregulated genes in response to NO were identified. Most of these genes were proposed to be involved in plant defense response such as some genes containing leucine-rich repeats (NBS-LRRs), non-race-specific disease resistance gene (NDR1), and different proteins related to disease resistance. Among these defense-related genes highlight some transcription factors with important functions during plant response to disease such as some members of the WRKY and ethylene-responsive element-binding (ERBP) transcription factor families. In this regard, about 10% of the NO-responsive genes were identified as transcription factors, suggesting the strong effect of NO on modulating gene expression profile. Moreover, genes related to protection against oxidative stress, iron homeostasis, or signal transduction including different kinases were identified as regulated by NO (Parani et al. 2004). In addition, NO treatment triggered the expression of transcripts encoding for dehydration-responsive element-binding proteins (DREB1 and DREB2) and late embryogenesis abundant (LEA) proteins that could confer drought, cold, and salinity tolerance in plants (Parani et al. 2004). Moreover, another microarray containing 26,090 genes and therefore covering almost the whole-genome in *Arabidopsis* was employed to identify NO targets in *Arabidopsis* roots (Badri et al. 2008). With this purpose, *Arabidopsis* plants were treated with 250 μM of SNP, and roots and root exudates were analyzed 3 and 6 h posttreatment. As a result, 87 genes differentially expressed with a fold change more than twofold were identified after NO treatment. It is interesting to note that following NO treatment there are a high number of repressed genes and few induces genes. In this analysis, different transport systems probably related to root exudation such as MATE, ABC, or MFS transporters appear to be modulated by NO treatment. In addition, different genes involved in defense signaling, antimicrobial activities, and regulation of cellular redox state were also identified to be modulated by NO in roots (Badri et al. 2008). Interestingly, in parallel to the identification of NO-responsive genes, in this work the effect of other signaling compounds such as methyl jasmonate (MetJA) and salicylic acid (SA) on gene expression changes was also analyzed. The processes affected by these elicitors are similar to those identified in NO treatment, but the set of genes regulated by each compound were different, suggesting that these signaling molecules act through different signaling mechanisms. In this regard, the results of this study only identified the NAC3, a member of NAC transcription factor family, as regulated by the three signaling compounds analyzed (Badri et al. 2008).

Another dedicated microarray was constructed to analyze the behavior of NO-responsive genes during pathogenic and symbiotic interactions in *Medicago truncatula* plants (Ferrarini et al. 2008). First, roots from 4-week-old plants were treated with SNP or GSNO as NO donors, and a cDNA-AFLP analysis with an estimated coverage of 55% of the *Medicago truncatula* transcriptome was performed. In this way, 1023 cDNA fragments were differentially regulated by these NO donors. These fragments that corresponded to 999 NO-responsive genes were then employed to construct the dedicated MtNO array (Ferrarini et al. 2008). This MtNO was first used to analyze GSNO and SNP-responsive genes to identify if these NO donors modulate a common set of genes. Surprisingly, GSNO and SNP exerted the same regulatory effects on only 11% of genes in leaves and 1.6% in roots, and there was a coincident between roots and leaves of only 1.6% of the differentially expressed genes. These results suggest that the NO-dependent modulation of gene expression could be dependent on the NO donor and it also could be organ-specific (Ferrarini et al. 2008), as lately confirmed (Begara-Morales et al. 2014b). Subsequently, this MtNO array was employed to monitor the modulation of these NO-responsive genes during the incompatible and symbiotic interactions. During the incompatible infection of *M. truncatula* with *C. trifolii*, there was a modulation of 275 NO-responsive genes mainly related to defense signaling, ROS metabolism, lipid signaling pathways, proteasome degradation, and different genes related to signal transduction such as kinases, phosphatases, and different transcription factors. On the other hand, the symbiotic interaction regulated the expression of 290 out of the 999 NO-responsive genes in the MtNO array. These genes were proposed to be mainly related to flavonoid biosynthesis, redox signaling, and primary metabolism. After transcriptomic data analysis, the authors suggest that NO could have an essential role during symbiotic nodule development and functioning (Ferrarini et al. 2008) as it was subsequently demonstrated (Puppo et al. 2013; Damiani et al. 2016). SNP was also used as NO donor in another microarray assay leading to identify NO targets and its relation with ozone-induced cell death in *Arabidopsis thaliana* (Ahlfors et al. 2009). Using a full genome array in leaves sprayed with 0.5 mM SNP for 3 h, 614 NO-responsive genes (579 upregulated and 35 downregulated) were identified, which were mostly related to various abiotic and biotic stresses or hormone metabolism. In addition, the treatment of *Arabidopsis* leaves with ozone (O_3) showed about an 80% of similarity in the expression profile to NO, with only three genes that are modulated in an opposite manner by these compounds. Interestingly, the combination of NO and O_3 had a significant effect on hormone concentrations, occurring a large increase in JA hormone. In addition, the authors showed that the combined treatments of O_3 and SNP or SNAP produced the attenuation of SA and ethylene-related genes and therefore NO could be involved in the induction and regulation of the O_3-induced cell death (Ahlfors et al. 2009).

In an elegant work, cadmium (Cd^{2+}) was proposed to induce NOS-like-mediated NO production in *Arabidopsis* roots and leaves, with NO having a role in Cd^{2+}-induced root growth inhibition (Besson-Bard et al. 2009a). To identify the root genes regulated by NO during Cd^{2+} treatment, a genome-scale array covering 22,089 nuclear genes was used. Thus, plants were exposed to 30 μM Cd^{2+} and/or

L-NAME, a NOS animal inhibitor, and those genes whose modulation by Cd^{2+} was modified or completely abolished by L-NAME were selected as NO targets. In this way, 783 genes were identified as Cd^{2+}-responsive genes and 43 of them were also NO-dependent genes. These NO-modulated genes appear to be related to iron homeostasis, proteolysis, nitrogen assimilation and metabolism and root growth. Interestingly, the authors conclude that NO contributes to Cd^{2+} toxicity by favoring Cd^{2+} versus Ca^{2+} uptake and by initiating a cellular pathway resembling those activated upon iron deprivation (Besson-Bard et al. 2009a). In the same work, plants treated with 4 mM of L-NAME for 24 h provide a valuable information on genes whose expression could be modulated by a NOS-like activity sensitive to L-NAME (Besson-Bard et al. 2009a, b). In this regard, L-NAME produced expression changes in 2656 genes, among which the L-NAME-repressed genes are mainly related to primary and secondary metabolism, abiotic and biotic stress, and transport of micronutrients ions, ions, and carbohydrates. Interestingly, L-NAME produced the repression of genes related to auxin metabolism and transport (Besson-Bard et al. 2009b).

A microarray analysis was also performed to analyze the effect of NO on root architecture in sunflower plants (Monzón et al. 2014). In this context, NO donors produced a reduction in the primary root length without effect on the lateral root development, whereas cPTIO induced a general change in the root architecture mainly affecting the number of the lateral roots. Therefore, a Sunflower Custom Oligo Microarray containing 41,013 genes was used to characterize those genes specifically affected by cPTIO. Following this approach, 330 genes regulated by cPTIO were identified in sunflower roots. These genes are peroxidases, cytochrome P450, glutathione reductases, and different transmembrane transports among other genes differentially expressed. Interestingly, NO depletion caused the regulation of an important set of genes related to secondary metabolism, especially phenylpropanoid biosynthesis and particularly lignin biosynthesis. These results suggest that NO is crucial for regulating root architecture and lignin composition and therefore for plant growth and development (Monzón et al. 2014).

Using a cDNA-AFLP analysis, senescence-related genes were identified in *Medicago truncatula* plants (De Michele et al. 2009). Interestingly, this data collection was compared to previously reported NO-responsive genes in the same plant species (Ferrarini et al. 2008) obtaining about 50 genes being modulated in both senescence and NO treatments (De Michele et al. 2009). Interestingly, taking into account the high proportion of regulative genes expressed in both conditions, the authors suggested that there are conserved mechanisms in the onset and execution of the plant cell death events (De Michele et al. 2009) as previously pointed by other study (Quirino et al. 1999).

2.2 *Nitric Oxide-Induced Transcriptional Regulation Determined by RNA-seq Analysis*

Apart from the cDNA-AFLP and microarray analysis, in the last decade, new massively parallel sequencing or RNA-seq has emerged as a useful tool that could replace and improve existing methods because of their advantages over array-based methods (Wilhelm and Landry 2009), highlighting it is not necessary to have previous knowledge of the transcribed regions and allow gene expression quantification in a single experiment (Wilhelm and Landry 2009). Consequently, this RNA-seq technology has been used to deep in the knowledge of the NO-signaling events under physiological and stress situations. In this regard, Illumina RNA-seq technology was employed to analyze the dynamic changes in gene expression at two different stages of the symbiotic interaction of *Medicago truncatula* with *S. meliloti*: early organogenesis of the nodules and fully differentiated and functioning nodules (Boscari et al. 2013). This approach allowed identifying 1670 new genes and 7595 new transcribed regions in *M. truncatula.* In addition, about 35% of the genes identified by RNA-seq to have differential regulation during nodulation were not present on the Affymetrix *Medicago* GeneChip. These results confirm that RNA-seq appears to have a higher sensitivity and ability to discover new genes than microarrays approaches (Boscari et al. 2013). An important point in this work was the analysis of NO function in the nodulation transcriptome. With this purpose, a RNA-seq analysis was performed on inoculated roots treated with cPTIO as a NO scavenger (Boscari et al. 2013). Removing NO caused differential expression of 2030 genes, and surprisingly, NO produced a downregulation in the number of reads identified, suggesting a potential role of NO in decreasing transcriptome complexity during nodulation. Interestingly, under NO depletion there are a high number of downregulated transcriptional regulators related to defense reactions. Consequently, it is suggested that NO plays a crucial role repressing the defense system during early nodulation and therefore favoring the plant-microbe interaction that takes place during the establishment of the symbiotic interaction. By contrast, during a pathogenic interaction, NO usually induces the expression of defense genes (Boscari et al. 2013). Another Illumina RNA-seq analysis was performed too deep in the knowledge of the resistance of the wheat variant Wangshuibai to the *Fusarium* head blight (FHB) disease in comparison to the susceptible mutant NAUH117 (Xiao et al. 2013). Among all the gene expression changes, some ROS and NO producing and removing systems were induced in the susceptible NAUH11 while were repressed or remained stable in the resistant variant Wangshuibai. Among these ROS and NO-related genes are NADPH oxidases, ascorbate peroxidase, glutathione peroxidase, superoxide dismutase, catalase, or peroxiredoxin. Consequently, authors suggested that ROS/NO could contribute to the necrotrophic phase during the infection and therefore the downregulation of these reactive species would allow to enhance the resistance to FHB in wheat plants (Xiao et al. 2013).

Besides these transcriptional reprogramming analyses upon pathogen infection, most of the transcriptional analyses using RNA-seq have been performed using NO

donors. For instance, dynamic changes in gene expression profile have been analyzed in birch cells (*Betula platyphylla*) after treatment with SNP for 12 h using Solexa sequencing (Zeng et al. 2014). In this way, 403 upregulated and 971 downregulated genes were identified after application of the exogenous SNP. An important set of these NO-responsive genes are involved in protection against ROS, probably as a consequence of the induction of $O_2^{\cdot -}$ production after SNP treatment. Consequently, it is not surprising that within the NO-regulated genes in birch cells, there were 30 upregulated genes encoding proteins with antioxidant functions such as glutathione S-transferases, thioredoxin peroxidase, superoxide dismutases, or ascorbate peroxidase. In addition, different processes integral to plant biology were identified as target of NO as carbohydrate metabolism and cell wall biosynthesis, terpenoid biosynthesis, or growth regulation (Zeng et al. 2014).

GSNO has been also used to analyze the transcriptional changes in *Arabidopsis* plants under nonstress conditions (Begara-Morales et al. 2014b). GSNO was exogenously applied by roots for 3 h, and an Illumina RNA-seq was carried out in leaves and roots of *Arabidopsis* plants given as a result the identification of 3263 GSNO-responsive genes in the whole plant. Overall, GSNO provokes expression changes of an important set of stress-related genes suggesting that GSNO is perceived as a molecular cue to trigger NO-downstream signaling events leading to protect against a stress situation (Begara-Morales et al. 2018). In this context, different PR genes, defense-related transcription factors, or genes involved in wounding response have been proposed to be modulated by GSNO. In addition, in leaves of *Arabidopsis*, there was an important induction of a member of methionine sulfoxide reductase B (MSRB) family, concretely MSRB7 which is related to the protection against oxidative damages via regeneration of methionine from oxidized methionine. Interestingly, GSNO also induced proteins related to methionine degradation, suggesting a potential role of NO in methionine metabolism as previously reported in *E. coli* (Flatley et al. 2005). However, the most interesting analysis using this transcriptomic data was to determine those genes differentially expressed in leaves and roots and especially those genes with an organ-specific modulation. In this regard, leaves-specific NO-responsive genes were related to plant response to stress processes, whereas root-specific genes were related to developmental processes. These results confirm that NO can regulate a different set of genes depending on the tissue analyzed and therefore conferring to NO the capacity to modulate gene expression in an organ-specific manner (Begara-Morales et al. 2014b) as previously suggested by other transcriptomic analyses (Ferrarini et al. 2008).

The involvement of NO in the flowering process in *Oncidium* plants has been recently analyzed by Solexa transcriptomic analysis (Kumar et al. 2016). After transcriptomic data analysis, differentially expressed genes related to NO metabolism such as nitrate and nitrite reductases were identified in flowering. In addition, a significant reduction of nitrate reductase activity and NO level was observed during this process, suggesting a key role of NO in the transition phase and flowering process. Furthermore, exogenously applied SNP on ascorbate *Arabidopsis*-deficient mutants induced a downregulation of flowering-associated genes and the concomitant delay in

flowering, suggesting an essential role of NO signaling in flowering repression (Kumar et al. 2016).

NO has been previously proposed to govern a multitude of physiological and stress response in plants as we can deduce for the transcriptomic analysis aforementioned. Following with this line, the infiltration of *Arabidopsis* leaves with 1 mM *S*-nitrosocysteine (Cys-NO), a potent NO donor, for 6 h and the subsequent RNA-seq analysis allowed to identify 1165 differentially expressed genes, with 463 upregulated and 702 downregulated. These Cys-NO-responsive genes were involved in a wide range of plant processes such as biotic and abiotic stress, hormone metabolism, or secondary metabolism among others (Hussain et al. 2016). It is interesting to note that 604 out of 1165 of these CysNO-responsive genes encode transcription factors (TFs), that in turn regulate a wide range of processes integral to plant biology (Hussain et al. 2016). The experimental analysis of the role of NO regulating these TFs could open new lines of research in the field by exploring the role of NO as a regulator of the processes in which these TFs are involved. Indeed, a new RNA-seq analysis following *Arabidopsis* leaves infiltration with 1 mM Cys-NO was focused on those genes with transcriptional activity (Imran et al. 2018). A total of 673 differentially expressed TFs with important function on a wide range of biological processes were identified. Three of these TFs, DDF1, RAP2.6, and AtMYB48, were randomly selected, and their functional implications were analyzed. In this regard, it was demonstrated that these genes act as key regulators of plant growth and immunity. Defective mutants of DDF1 and RAP2.6 compromised basal and effector triggered immunity, suggesting a vital role of these TFs in regulating these plant defense systems (Imran et al. 2018).

The involvement of NO as modulator of plant response to arsenic stress was also analyzed by a massive sequencing RNA analysis (Singh et al. 2017). With this purpose, rice plants were subjected to arsenic stress alone or combined with SNP at different time points, and an Illumina RNA-seq transcriptomic analysis was performed. Following the bioinformatic analysis, NO was proposed to regulate different metal transporters, stress-related genes, hormones, and secondary metabolism genes that together could be involved in the arsenic detoxification processes. Therefore, authors conclude that NO reduces arsenic toxicity by modulating regulatory networks involved in arsenic detoxification (Singh et al. 2017).

Similar biological processes to those described in the aforementioned transcriptomic analysis have been identified to be modulated by NO following RNA-seq analysis in upland cotton (*Gossypium hirsutum*) treated with SNP for 3 h (Huang et al. 2018). Interestingly, the authors performed a comparison of different NO-mediated transcriptomic reprogramming in different plant species and different NO donors. This comparison highlights that there is a low overlap between different NO donors and tissue analyzed, confirming that the NO effect could depend on the source of NO and also have an organ-specific mode of action. Furthermore, the results could depend on the transcriptomic sequencing strategy perform (Huang et al. 2018), with RNA-seq being a more sensitive approach.

Very recently, the occurrence of nitro-fatty acids (NO_2-FAs) has been described in plants for the first time (Mata-Pérez et al. 2016b). Interestingly, the nitro-linolenic

acid (NO_2-Ln) has been proposed to have an essential role during development and stress response in *Arabidopsis* plants (Mata-Pérez et al. 2016b). In this context, NO_2-Ln appears to have an essential role in the early stages of development as the greater levels have been detected in seeds with the subsequent decrease throughout the plant development. Furthermore, different abiotic stress situations such as salinity, cadmium exposure, or wounding are able to increase NO_2-Ln levels suggesting a role of this signal molecule in plant response to abiotic stress. In addition, NO_2-Ln has the capacity to act as a NO donor and therefore regulating the NO-mediated signaling (Mata-Pérez et al. 2016a). Too deep in the signaling mechanisms of this NO_2-Ln and to analyze the transcriptional reprogramming that it could induce, an Illumina RNA-seq analysis was performed in *Arabidopsis* suspension cells treated with 10 and 100 μM NO_2-Ln (Mata-Pérez et al. 2016b). The transcriptomic analysis revealed that NO_2-Ln induced expression changes in 1308 genes, with 129 upregulated and 187 downregulated at least twofold. These NO_2-Ln-responsive genes have been proposed to be mainly involved in plant response to abiotic and oxidative stress, mainly by activating heat shock proteins and supporting a conserved signaling mechanism in both animals and plants during the defense response (Kansanen et al. 2009; Mata-Pérez et al. 2016b).

3 Transcriptional Regulation Mediated by Hydrogen Peroxide

Reactive oxygen species (ROS) are a family of reactive molecules composed of singlet oxygen (O_2^1), hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\cdot-}$), or hydroxyl radical (OH). It is widely recognized that H_2O_2 plays crucial roles in oxidative signaling (Foyer and Noctor 2016). This ROS can be synthesized from the reduction of molecular oxygen (O_2), the chemical reduction or dismutation of superoxide or H_2O_2, a reaction that is accelerated by superoxide dismutases (SODs), as well as by a two-electron reduction of O_2 through various oxidases such as glycolate oxidase (GOX) located in peroxisomes (Foyer and Noctor 2016; Cerny et al. 2018). H_2O_2 is involved in different developmental and physiological processes such as seed germination, programmed cell death, senescence, flowering, or stomatal closure (Niu and Liao 2016). In addition, it is also a signaling molecule involved in plant response to different stresses such as drought, salt, extreme temperatures, or heavy metal (Niu and Liao 2016). H_2O_2 transmits its action through different PTMs that are produced by oxidation of different amino acid such as cysteine and methionine (Niu and Liao 2016; Cerny et al. 2018). Cysteine residue is very sensitive to oxidation, showing different oxidation states depending on the cellular redox state that induce different PTMs related to signaling or degradation processes (Fig. 1). These PTMs have been proposed to have important functions in the regulation of different physiological and stress response in plants (Cerny et al. 2018). Besides PTMs, H_2O_2 is also able to modulate gene expression changes as part

of its function as a signaling molecule (Fig. 1). In this regard, an interplay between H_2O_2 and SA has been proposed to control the expression of defense genes (Herrera-Vásquez et al. 2015). In addition, H_2O_2 was proposed to act as a second messenger after wounding stress in tomato plants (Orozco-Cárdenas et al. 2001). In this context, H_2O_2 appears to be involved in a cascade of intracellular events in which jasmonate activates the signaling genes (early genes), whereas H_2O_2 could act as a second messenger activating the defense genes (late genes) in response to wounding stress. Due to its low stability and the presence of H_2O_2 scavengers, it has been questioned its function as a long-distance molecule (Cerny et al. 2018). Keeping this in mind, the detection and use of H_2O_2 are not always straightforward tasks, so that catalase-deficient mutants, impaired in one of the major H_2O_2 scavenger, have been employed to analyze the effect of the sustained H_2O_2 stress over time. These mutants appear to produce a disruption of the redox state and peroxisome function, and therefore they are more sensitive to different abiotic stresses (Vandenabeele et al. 2004; Su et al. 2018). To analyze the H_2O_2_signaling events, different cDNA-AFLP and microarray analysis have been performed. In this regard, using a catalase-deficient tobacco plant exposed to high light, the effect of H_2O_2 accumulation on gene expression profile was analyzed by cDNA-AFLP (Vandenabeele et al. 2003). 713 differentially expressed genes were detected and predicted to be mainly involved in plant response to stress, proteolysis, mitochondrial metabolism, or cell death among other biological processes (Vandenabeele et al. 2003). In the same line, a microarray composed of 6008 cDNA from *Arabidopsis* was employed to analyze the effect of the accumulated H_2O_2 in catalase-deficient mutants exposed to high light stress for 3, 8, and 23 h (Vandenabeele et al. 2004). The main functional categories regulated by H_2O_2 were related to development, interaction with the environment, defense, cellular communication, and signal transduction or cellular transports, among others. Catalase-deficient mutants also induced the expression of nuclear genes under nonstress conditions, and these effects were amplified by high light stress (Vanderauwera et al. 2005). In this case, it was shown that H_2O_2 plays an essential role during high light stress through the transcriptional activation of the small heat shock proteins. Interestingly, the heat shock transcription factors were proposed to act as sensor of hydrogen peroxide under different stresses that induce an oxidative stress (Miller and Mittler 2006), with a significant H_2O_2-mediated upregulation of the heat shock proteins taking place in peroxisomes (Sewelam et al. 2016). In addition, an impairment in the anthocyanin biosynthesis pathway was identified and proposed to be responsible for the more sensitive phenotype of catalase-deficient mutants to abiotic stress (Vanderauwera et al. 2005). Taking together, these results show a high reprogramming of the transcriptome by H_2O_2. Moreover, the establishment of *Medicago truncatula-Sinorhizobium meliloti* symbiosis is also regulated by H_2O_2. The use of DPI as a NADPH oxidase inhibitor, which reduces ROS and specially H_2O_2 production, was used to determine the function of ROS on this symbiotic interaction (Andrio et al. 2013). By microarray analysis, 447 differentially expressed genes were identified after bacterial inoculation. Of these genes, 301 were also affected by DPI treatment in inoculated roots, suggesting a crucial role of ROS in the transcriptomic regulation during this

symbiotic condition. Within this group of genes, potentially regulated by ROS after bacterial inoculation, is the gene MtSpk1 that encodes a putative protein kinase whose deficiency impairs the establishment of the symbiotic interaction (Andrio et al. 2013). The role of H_2O_2 during plant-pathogen interaction was also demonstrated in grapevines plants infected with Flavescence dorée (FD), one of the most severe phytoplasma diseases affecting these plants (Gambino et al. 2013). In this work, the phenomenon of recovery after pathogen infection was investigated. In this line, infected plants exhibited a decrease in H_2O_2 levels probably due to an upregulation of the H_2O_2 scavenger systems compared to healthy and recovery plants. Interestingly, recovery plants showed an upregulation of genes involved in H_2O_2 production, whereas most of the scavenger systems were not affected, leading to an accumulation of H_2O_2. Following expression analysis of different defense genes, the recovery phenomenon was proposed to be mediated by the activation of ethylene biosynthesis and defense genes probably in a SA-independent manner (Gambino et al. 2013).

In an interesting work, glycolate oxidase overexpressing mutants associated with increased levels of H_2O_2 in chloroplasts and catalase-deficient mutants that accumulate H_2O_2 in peroxisomes were used to test if H_2O_2-mediated transcriptional changes have a subcellular localization specificity (Sewelam et al. 2016). These mutants were grown under normal conditions and then transferred to photorespiratory conditions to increase H_2O_2 production in both organelles. Following a microarray analysis, a set of differentially expressed genes were identified as peroxisome or chloroplast specific as well as genes with a response independent from the subcellular localization. Interestingly, the results showed that H_2O_2 from chloroplast modulate the expression of transcription factors, protein/receptor kinases, or defense genes, whereas H_2O_2 from peroxisomes are mainly involved in cell repair responses (Sewelam et al. 2016).

Besides microarray approaches, a few RNA-seq analyses focused on the unrevealing H_2O_2-induced signaling events have been performed. However, less transcriptomic analysis based on the RNA-seq technology is available in comparison to NO. In this context, a RNA-seq analysis showed that *cat1/2/3*-deficient triple mutant induced the differential expression of 2852 genes compared to wild-type, including 241 transcription factors that appear to be crucial in the perception and signaling events related to H_2O_2 in eukaryotes (Marinho et al. 2014; Su et al. 2018). Interestingly, 1972 differentially expressed genes were also identified in comparison to cat1/2 double mutant (Su et al. 2018). As a general conclusion, authors propose that the differentially expressed genes are mainly involved in plant growth regulation and stress response processes. Furthermore, they suggest that the H_2O_2 produced in peroxisomes could serve as a peroxisomal retrograde signal with important functions in plant development (Su et al. 2018). The exogenous application of 1 mM H_2O_2 for 0.5 and 1 h to cell cultures of *Chlamydomonas reinhardtii* induced expression changes in 1278 genes, with the upregulation of transcripts mainly related to protein degradation, ROS detoxification, and stress response, whereas downregulated genes are involved in photosynthesis and central carbon metabolism, suggesting a crucial role of H_2O_2 in the regulation of these physiological processes (Blaby et al. 2015).

After comparison of the transcriptomic results with previous public data, a significant overlap in the number of transcripts upregulated by H_2O_2 and singlet oxygen was detected. Interestingly, it was determined that the response of the heat shock proteins was specific to H_2O_2, supporting the idea that these proteins can act as sensors of H_2O_2 as mentioned above (Miller and Mittler 2006; Blaby et al. 2015). H_2O_2 is also involved in the adventitious roots formation in mung bean (Li et al. 2017). The treatment of mung bean plants after removing the hypocotyl basis with 10 mM H_2O_2 for 6 h induced a high transcriptional activity, whereas 24 h posttreatment a reduction in gene expression levels was observed. In this regard, after 6 h of treatment, the number of differentially expressed genes was 29.9% higher than in 24 h posttreatment. Overall, the most responsive genes were related to stress response, cell redox homeostasis, oxidative responses, cell modification, or hormone metabolism among others. Interestingly, after comparison of 6 h, 6–24 h, and 24 h posttreatment, two differentiate phases were identified in the adventitious roots formation, the induction, and the initiation stages. In this regard, protein degradation, cellular respiration, lipid transport, photosynthesis, flavonoid synthesis, and phenylalanine metabolism are among the biological processes proposed to be modulated by H_2O_2 during the induction stage. However, amino acids synthesis, transmembrane transporters, cell wall organization, protein synthesis, lipid metabolism, cytokinin-related pathway, and phenylpropanoid biosynthesis were more represented in the initiation stage. Interestingly, once again the upregulation of the heat shock proteins and heat shock transcription factors suggests their function as sensors of H_2O_2 (Li et al. 2017).

4 Interplay Between Hydrogen Peroxide and Nitric Oxide Signaling Events

Hydrogen peroxide (H_2O_2) and nitric oxide (NO) are considered secondary messengers involved in a plethora of processes integral to plant biology, ranging from seed germination and plant development to plant response to different stress situations (Niu and Liao 2016). It is well established that under different stress situations, a rapid burst of ROS and RNS is produced to mediate signaling events leading to cope these adverse situations. In this context, a growing number of evidences show that NO and H_2O_2 are produced under similar stress conditions and with similar kinetics, suggesting that the interplay between these two signaling molecules could have important functions in the modulation of the signaling events that ultimately coordinate plant defense (Niu and Liao 2016). Niu and Liao (2016) have reviewed the interaction of these signaling pathways during plant development and stress response. In this sense, the interplay between H_2O_2 and NO appears to be crucial in physiological processes such as cell death, senescence, root growth, pollen tube growth, and stomatal movement. In addition, this cross talk is also produced during

plant response to different abiotic stresses such as drought, salt, extreme temperatures, or heavy metals (Niu and Liao 2016).

The first evidence of H_2O_2 and NO cross talk was evidenced during the hypersensitive cell death response in soybean cell cultures (Delledonne et al. 1998). It was shown that not only ROS but also NO is necessary for the hypersensitive cell death response, concluding that NO and ROS are complementary and could trigger a synergistic induction of this process (Delledonne et al. 1998). Subsequently, it was demonstrated that a balanced production of NO and ROS is necessary to trigger this response, where the interaction of NO with H_2O_2 plays a crucial role (Delledonne et al. 2001). In addition, a connection between NO and ROS pathways under different physiological and stress conditions has been widely reviewed (Corpas et al. 2011; Gross et al. 2013; Procházková et al. 2014). This interplay can be produced by regulation of specific enzymes involved in ROS metabolism by NO or by a connection in the transcriptional regulation induced by both signaling pathways. Regarding NO-PTMs, *S*-nitrosylation can modulate ROS metabolism through the regulation of the ROS producers and scavengers systems such as NADPH oxidase (Yun et al. 2011), catalase (Ortega-Galisteo et al. 2012), peroxiredoxin IIE (Romero-Puertas et al. 2007), and peroxiredoxin IIF (Camejo et al. 2015). In addition, the function of the main antioxidant systems has been proposed to be modulated by NO-PTMs, highlighting the regulation of the ascorbate-glutathione (Asa-GSH) cycle by NO (Begara-Morales et al. 2016). Interestingly, the modulation of ascorbate peroxidase (APX) by NO has emerged as a crucial point during plant response to abiotic stress (Lindermayr and Durner 2015). In fact, APX activity exhibits a dual regulation by NO-PTMs, being enhanced by *S*-nitrosylation and inhibited by tyrosine nitration (Begara-Morales et al. 2014a). In this regard, the Cys-32 in APX has been identified as a crucial amino acid regulated by *S*-nitrosylation during plant response to abiotic and oxidative stresses (Begara-Morales et al. 2014a; Yang et al. 2015). *S*-nitrosoglutathione reductase (GSNOR) enzyme degrades GSNO and indirectly controls total SNO levels in cells (Liu et al. 2001; Feechan et al. 2005). This enzyme has been proposed to play a crucial role in the regulation of SNO levels during plant development and response to a wide range of stresses (Begara-Morales and Loake 2016). Very recently, it has been proposed that GSNOR also plays a crucial role during cross talk between ROS and NO in plants (Lindermayr 2018). In this sense, in vitro H_2O_2 treatment and in vivo paraquat-induced oxidative stress inhibit GSNOR activity with the concomitant increase in SNO and GSH levels (Kovacs et al. 2016). In addition, it was shown that in GSNOR-deficient mutants, there is an increase in the transcriptional activation of redox-regulated genes and antioxidant enzymes, suggesting that oxidation of GSNOR could be crucial in the antioxidant response to cope the oxidative damage during abiotic stress (Kovacs et al. 2016).

The interplay at transcriptional level of these signaling pathways has been also suggested. In this regard, the cross talk between NO and ROS appears to be also crucial during the legume-*Rhizobium* symbiotic interaction (Puppo et al. 2013; Damiani et al. 2016). H_2O_2 has been proposed to regulate the infection process and bacterial differentiation into the symbiotic form and NO as important for the

establishment of the symbiosis (Puppo et al. 2013). The transcriptomic data available also point toward a crucial regulation of *Medicago truncatula-Sinorhizobium meliloti* symbiosis by NO and H_2O_2 (Andrio et al. 2013; Boscari et al. 2013; Ferrarini et al. 2008). The comparison of these differentially expressed genes during symbiosis process as consequence of NO and H_2O_2 will allow identifying the common genes regulated by both signaling molecules. On the other hand, the transcriptomic data available highlight a high response of heat shock proteins and heat shock transcription factors to H_2O_2. In this regard, these transcription factors were proposed to act as sensor of hydrogen peroxide signal during plant response to stress (Miller and Mittler 2006). These transcription factors have been also recently shown to be transcriptionally activated by nitro-linolenic acid, which can act as a NO donor (Mata-Pérez et al. 2016a, b). These results suggest another point of interaction between H_2O_2 and NO that needs further investigations.

5 Conclusions and Future Perspectives

ROS and RNS encompass a set of redox molecules with an essential role in the cellular redox homeostasis, acting as crucial regulators of signaling events that coordinate fundamental processes integral to plant biology. In this regard, the interplay between ROS and RNS signaling pathways, especially H_2O_2 and NO, appears to be essential during plant response to a wide range of stress conditions. These signal molecules usually transmit their function via posttranslational modifications and regulation of the transcriptional activity in cells. Due to the wide range of processes and diverse functions that can be regulated by these signal molecules, it is more informative to analyze their function using different "omics" approaches such as large-scale proteomics or gene expression studies to better understand the signaling network in which they are involved. In this book chapter, we have analyzed recent data concerning the transcriptional reprogramming mediated by H_2O_2 and NO. These approaches have provided a huge availability of data concerning the gene expression profile changes induced by H_2O_2 and NO that sometimes overwhelm researchers during its analysis. In addition, these studies have been performed using different biological systems (*Arabidopsis thaliana*, *Medicago truncatula*, *Glycine max*, etc.) at different developmental stages and organs (cell cultures, seedlings, leaves, roots, hypocotyls, etc.) and even using different NO and ROS donors (GSNO, SNP, NOR-3, H_2O_2, O_2^{1}, etc.). Consequently, it is not a straightforward task to make effective comparisons of these studies to establish a real perspective of the molecular basis of H_2O_2 and NO mode of action. For example, in *Medicago truncatula* plants exposed to SNP and GSNO, it was shown that only 11% of genes in leaves and 1.6% in roots are common targets of these NO donors. This result suggests a specific modulation of the genes depending on the NO source (Ferrarini et al. 2008). In the same line, a recent comparison of the transcriptomic data available has also emphasized that even the same NO donor induces different responses depending on the time of exposure and concentration (Huang et al.

2018). In this regard, the results could also depend on the application mode of the donor and the technology used in the sequencing analysis. Something similar takes place when different concentration of H_2O_2 is used to induce the transcriptional response. In addition, an organ-specific regulation of NO target genes has been proposed in *Arabidopsis* and *Medicago* plants, highlighting the low percentage of coincidence in the common genes regulated by NO in these organs (Begara-Morales et al. 2014b; Ferrarini et al. 2008). In this regard, Besson-Bard et al. (2009b) performed a comparison of different medium- and large-scale transcriptional analyses available until 2009 to identify common NO-responsive genes. Although they showed that there was not a high overlap of NO targets identified in the different analysis, a high percentage of the NO-responsive genes are related to oxidative stress generated in response to different stress conditions, supporting the idea that NO acts as a signal molecule involved in the adaptive response to various plant stress situations (Besson-Bard et al. 2009b). All these results highlight the necessity of performing more transcriptional data comparisons to identify a reliable set of genes that are targets of NO or H_2O_2. In addition, due to the interplay between these two signaling molecules, a comparison between the transcriptomic data available on NO-responsive genes and H_2O_2-responsive genes would be a good starting point to identify those genes that are common to both signaling pathways and their potential effect on plant response to stress. Additionally, in spite of the high number of NO and H_2O_2 target genes identified, little is known concerning the molecular characterization of the effect of this regulation on gene or protein function. In this respect, the transcriptomic data could be complement with proteomic and protein structural analysis to definitively unravel the effect of NO and H_2O_2 on the whole-cell response to different stress situations. Finally, more information regarding promoter sequences having a crucial role during H_2O_2- and NO-mediated signaling events is required.

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References

Ahlfors R, Brosché M, Kollist H, Kangasjarvi J (2009) Nitric oxide modulates ozone-induced cell death, hormone biosynthesis and gene expression in *Arabidopsis thaliana*. Plant J 58:1–12

Albertos P, Romero-Puertas MC, Tatematsu K, Mateos I, Sánchez-Vicente I, Nambara E, Lorenzo O (2015) S-nitrosylation triggers ABI5 degradation to promote seed germination and seedling growth. Nat Commun 6:8669

Andrio E, Marino D, Marmeys A, de Segonzac MD, Damiani I, Genre A, Huguet S, Frendo P, Puppo A, Pauly N (2013) Hydrogen peroxide-regulated genes in the *Medicago truncatula-Sinorhizobium meliloti* symbiosis. New Phytol 198:179–189

Astier J, Lindermayr C (2012) Nitric oxide-dependent posttranslational modification in plants: an update. Int J Mol Sci 13:15193–15208

Badri DV, Loyola-Vargas VM, Du J, Stermitz FR, Broeckling CD, Iglesias-Andreu L, Vivanco JM (2008) Transcriptome analysis of *Arabidopsis* roots treated with signaling compounds: a focus on signal transduction, metabolic regulation and secretion. New Phytol 179:209–223

Begara-Morales JC, Loake GJ (2016) Protein denitrosylation in plant biology. In: Lamattina L, García-Mata C (eds) Gasotransmitters in plants, signaling and communication in plants. Springer, Cham, pp 201–215

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

Begara-Morales JC, Sánchez-Calvo B, Luque F, Leyva-Pérez MO, Leterrier M, Corpas FJ, Barroso JB (2014b) Differential transcriptomic analysis by RNA-seq of GSNO-responsive genes between Arabidopsis roots and leaves. Plant Cell Physiol 55:1080–1095

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Valderrama R, Mata-Pérez C, Padilla MN, Corpas FJ, Barroso JB (2016) Antioxidant systems are regulated by nitric oxide-mediated post-translational modifications (NO-PTMs). Front Plant Sci 7:152

Begara-Morales JC, Chaki M, Valderrama R, Sanchez-Calvo B, Mata-Perez C, Padilla MN, Corpas FJ, Barroso JB (2018) Nitric oxide buffering and conditional nitric oxide release in stress response. J Exp Bot 69:3425–3438

Besson-Bard A, Gravot A, Richaud P, Auroy P, Duc C, Gaymard F, Taconnat L, Renou JP, Pugin A, Wendehenne D (2009a) Nitric oxide contributes to cadmium toxicity in *Arabidopsis* by promoting cadmium accumulation in roots and by up-regulating genes related to iron uptake. Plant Physiol 149:1302–1315

Besson-Bard AL, Astier JR, Rasul S, Wawer I, Dubreuil-Maurizi C, Jeandroz S, Wendehenne D (2009b) Current view of nitric oxide-responsive genes in plants. Plant Sci 177:302–309

Blaby IK, Blaby-Haas CE, Pérez-Pérez ME, Schmollinger S, Fitz-Gibbon S, Lemaire SD, Merchant SS (2015) Genome-wide analysis on *Chlamydomonas reinhardtii* reveals the impact of hydrogen peroxide on protein stress responses and overlap with other stress transcriptomes. Plant J 84:974–988

Boscari A, del Giudice J, Ferrarini A, Venturini L, Zaffini A-L, Delledonne M, Puppo A (2013) Expression dynamics of the *Medicago truncatula* transcriptome during the symbiotic interaction with *Sinorhizobium meliloti*: which role for nitric oxide? Plant Physiol 161:425–439

Camejo D, Ortiz-Espín A, Lázaro JJ, Romero-Puertas MC, Lázaro-Payo A, Sevilla F, Jiménez A (2015) Functional and structural changes in plant mitochondrial PrxII F caused by NO. J Proteome 119:112–125

Cerny M, Habánová H, Berka M, Luklová M, Brzobohatý B (2018) Hydrogen peroxide: its role in plant biology and crosstalk with signalling networks. Int J Mol Sci 19:2812

Corpas FJ, Barroso JB (2013) Nitro-oxidative stress vs oxidative or nitrosative stress in higher plants. New Phytol 199:633–635

Corpas FJ, Leterrier M, Valderrama R, Airaki M, Chaki M, Palma JM, Barroso JB (2011) Nitric oxide imbalance provokes a nitrosative response in plants under abiotic stress. Plant Sci 181:604–611

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

Damiani I, Pauly N, Puppo A, Brouquisse R, Boscari A (2016) Reactive oxygen species and nitric oxide control early steps of the legume-*Rhizobium* symbiotic interaction. Front Plant Sci 7:454

De Michele R, Formentin E, Todesco M, Toppo S, Carimi F, Zottini M, Barizza E, Ferrarini A, Delledonne M, Fontana P (2009) Transcriptome analysis of *Medicago truncatula* leaf senescence: similarities and differences in metabolic and transcriptional regulations as compared with Arabidopsis, nodule senescence and nitric oxide signalling. New Phytol 181:563–575

Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. Nature 394:585–588

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci USA 98:13454–13459

Durner J, Wendehenne D, Klessig DF (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci USA 95:10328–10333

Fancy NN, Bahlmann AK, Loake GJ (2016) Nitric oxide function in plant abiotic stress. Plant Cell Environ 40:462–472

Feechan A, Kwon E, Yun BW, Wang Y, Pallas JA, Loake GJ (2005) A central role for *S*-nitrosothiols in plant disease resistance. Proc Natl Acad Sci USA 102:8054–8059

Ferrarini A, De Stefano M, Baudouin E, Pucciariello C, Polverari A, Puppo A, Delledonne M (2008) Expression of *Medicago truncatula* genes responsive to nitric oxide in pathogenic and symbiotic conditions. Mol Plant-Microbe Interact 21:781–790

Flatley J, Barrett J, Pullan ST, Hughes MN, Green J, Poole RK (2005) Transcriptional responses of *Escherichia coli* to S-nitrosoglutathione under defined chemostat conditions reveal major changes in methionine biosynthesis. J Biol Chem 280:10065–10072

Foyer CH, Noctor G (2016) Stress-triggered redox signalling: what's in pROSpect? Plant Cell Environ 39:951–964

Gambino G, Boccacci P, Margaria P, Palmano S, Gribaudo I (2013) Hydrogen peroxide accumulation and transcriptional changes in grapevines recovered from flavescence doree disease. Phytopathology 103:776–784

Gross F, Durner J, Gaupels F (2013) Nitric oxide, antioxidants and prooxidants in plant defence responses. Front Plant Sci 4:419

He H, He L, Gu M (2014) The diversity of nitric oxide function in plant responses to metal stress. Biometals 27:219–228

Herrera-Vásquez A, Salinas P, Holuigue L (2015) Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression. Front Plant Sci 6:171

Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS (2005) Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol 6:150–166

Hu J, Huang X, Chen L, Sun X, Lu C, Zhang L, Wang Y, Zuo J (2015) Site-specific nitrosoproteomic identification of endogenously *S*-nitrosylated proteins in *Arabidopsis*. Plant Physiol 167:1731–1746

Huang X, von Rad U, Durner J (2002) Nitric oxide induces transcriptional activation of the nitric oxide-tolerant alternative oxidase in *Arabidopsis* suspension cells. Planta 215:914–923

Huang J, Wei H, Li L, Yu S (2018) Transcriptome analysis of nitric oxide-responsive genes in upland cotton (*Gossypium hirsutum*). PLoS One 13:e0192367

Hussain A, Mun BG, Imran QM, Lee SU, Adamu TA, Shahid M, Kim KM, Yun BW (2016) Nitric oxide mediated transcriptome profiling reveals activation of multiple regulatory pathways in *Arabidopsis thaliana*. Front Plant Sci 7:975

Imran QM, Hussain A, Lee SU, Mun BG, Falak N, Loake GJ, Yun BW (2018) Transcriptome profile of NO-induced Arabidopsis transcription factor genes suggests their putative regulatory role in multiple biological processes. Sci Rep 8:771

Kansanen E, Jyrkkanen H-K, Volger OL, Leinonen H, Kivela AM, Hakkinen S-K, Woodcock SR, Schopfer FJ, Horrevoets AJ, Yla-Herttala S (2009) Nrf2-dependent and-independent responses to nitro-fatty acids in human endothelial cells: identification of heat shock response as the major pathway activated by nitro-oleic acid. J Biol Chem 284:33233–33241

Kovacs I, Holzmeister C, Wirtz M, Geerlof A, Fröhlich T, Römling G, Kuruthukulangarakoola GT, Linster E, Hell R, Arnold GJ, Durner J, Lindermayr C (2016) ROS-Mediated inhibition of S-nitrosoglutathione reductase contributes to the activation of anti-oxidative mechanisms. Front Plant Sci 7:1669

Kumar RS, Shen CH, Wu PY, Kumar SS, Hua MS, Yeh KW (2016) Nitric oxide participates in plant flowering repression by ascorbate. Sci Rep 6:35246

Li SW, Leng Y, Shi RF (2017) Transcriptomic profiling provides molecular insights into hydrogen peroxide-induced adventitious rooting in mung bean seedlings. BMC Genomics 18:188

Lindermayr C (2018) Crosstalk between reactive oxygen species and nitric oxide in plants: key role of *S*-nitrosoglutathione reductase. Free Radic Biol Med 122:110–115

Lindermayr C, Durner J (2015) Interplay of reactive oxygen species and nitric oxide: nitric oxide coordinates reactive oxygen species homeostasis. Plant Physiol 167:1209–1210

Liu L, Hausladen A, Zeng M, Que L, Heitman J, Stamler JS (2001) A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. Nature 410:490–494

Marinho HS, Real C, Cyrne L, Soares H, Antunes F (2014) Hydrogen peroxide sensing, signaling and regulation of transcription factors. Redox Biol 2:535–562

Mata-Pérez C, Sánchez-Calvo B, Begara-Morales JC, Carreras A, Padilla MN, Melguizo M, Valderrama R, Corpas FJ, Barroso JB (2016a) Nitro-linolenic acid is a nitric oxide donor. Nitric Oxide 57:57–63

Mata-Pérez C, Sánchez-Calvo B, de las Padilla-Serrano MN, Begara-Morales JC, Luque F, Melguizo M, Jiménez-Ruiz J, Fierro-Risco J, Peñas-Sanjuan A, Valderrama R (2016b) Nitro-fatty acides in plant signaling: nitro-linolenic acid induces the molecular chaperone network in *Arabidopsis*. Plant Physiol 170:686–701

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

Monzón GC, Pinedo M, Di Rienzo J, Novo-Uzal E, Pomar F, Lamattina L, de la Canal L (2014) Nitric oxide is required for determining root architecture and lignin composition in sunflower. Supporting evidence from microarray analyses. Nitric Oxide 39:20–28

Mur LAJ, Mandon J, Persijn S, Cristescu SM, Moshkov IE, Novikova GV, Hall MA, Harren FJM, Hebelstrup KH, Gupta KJ (2013) Nitric oxide in plants: an assessment of the current state of knowledge. AoB Plant 5:pls052

Niu L, Liao W (2016) Hydrogen peroxide signaling in plant development and abiotic responses: crosstalk with nitric oxide and calcium. Front Plant Sci 7:230

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

Ortega-Galisteo AP, Rodriguez-Serrano M, Pazmiño DM, Gupta DK, Sandalio LM, Romero-Puertas MC (2012) S-Nitrosylated proteins in pea (*Pisum sativum* L.) leaf peroxisomes: changes under abiotic stress. J Exp Bot 63:2089–2103

Palmieri MC, Sell S, Huang X, Scherf M, Werner T, Durner JR, Lindermayr C (2008) Nitric oxide-responsive genes and promoters in *Arabidopsis thaliana*: a bioinformatics approach. J Exp Bot 59:177–186

Parani M, Rudrabhatla S, Myers R, Weirich H, Smith B, Leaman DW, Goldman SL (2004) Microarray analysis of nitric oxide responsive transcripts in *Arabidopsis*. Plant Biotechnol J 2:359–366

Polverari A, Molesini B, Pezzotti M, Buonaurio R, Marte M, Delledonne M (2003) Nitric oxide-mediated transcriptional changes in *Arabidopsis thaliana*. Mol Plant-Microbe Interact 16:1094–1105

Procházková D, Sumaira J, Wilhelmová NA, Pavlíková D, Száková J (2014) Reactive nitrogen species and the role of NO in abiotic stress. In: Ahmad P (ed) Emerging technologies and managment of crops stress tolerance. Elsevier, London

Puppo A, Pauly N, Boscari A, Mandon K, Brouquisse R (2013) Hydrogen peroxide and nitric oxide: key regulators of the legume-rhizobium and mycorrhizal symbioses. Antioxid Redox Signal 18:2202–2219

Quirino BF, Normanly J, Amasino RM (1999) Diverse range of gene activity during *Arabidopsis thaliana* leaf senescence includes pathogen-independent induction of defense-related genes. Plant Mol Biol 40:267–278

Radi R (2004) Nitric oxide, oxidants, and protein tyrosine nitration. Proc Natl Acad Sci USA 101:4003–4008

Romero-Puertas MC, Laxa M, Matté A, Zaninotto F, Finkemeier I, Jones AME, Perazzolli M, Vandelle E, Dietz KJ, Delledonne M (2007) S-nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration. Plant Cell 19:4120–4130

Sewelam N, Jaspert N, Van Der Kelen K, Tognetti VB, Schmitz J, Frerigmann H, Stahl E, Zeier J, Van Breusegem F, Maurino VG (2016) Spatial H_2O_2 signaling specificity: H2O2 from chloroplasts and peroxisomes modulates the plant transcriptome differentially. Mol Plant 7:1191–1210

Singh PK, Indoliya Y, Chauhan AS, Singh SP, Singh AP, Dwivedi S, Tripathi RD, Chakrabarty D (2017) Nitric oxide mediated transcriptional modulation enhances plant adaptive responses to arsenic stress. Sci Rep 7:3592

Su T, Wang P, Li H, Zhao Y, Lu Y, Dai P, Ren T, Wang X, Li X, Shao Q (2018) The *Arabidopsis* catalase triple mutant reveals important roles of catalases and peroxisome derived signaling in plant development. J Integr Plant Biol 60:591–607

Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Molassiotis A, Job D (2009) Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. Plant J 60:795–804

Vandenabeele S, Van Der Kelen K, Dat J, Gadjev I, Boonefaes T, Morsa S, Rottiers P, Slooten L, Van Montagu M, Zabeau M (2003) A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. Proc Natl Acad Sci USA 100:16113–16118

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

Vanderauwera S, Zimmermann P, Rombauts S, Vandenabeele S, Langebartels C, Gruissem W, Inzé D, Van Breusegem F (2005) Genome-wide analysis of hydrogen peroxide-regulated gene expression in Arabidopsis reveals a high light-induced transcriptional cluster involved in anthocyanin biosynthesis. Plant Physiol 139:806–821

Wang P, Du Y, Hou YJ, Zhao Y, Hsu CC, Yuan F, Zhu X, Tao WA, Song CP, Zhu JK (2015) Nitric oxide negatively regulates abscisic acid signaling in guard cells by S-nitrosylation of OST1. Proc Natl Acad Sci USA 112:613–618

Wilhelm BT, Landry JR (2009) RNA-Seq-quantitative measurement of expression through massively parallel RNA-sequencing. Methods 48:249–257

Xiao J, Jin X, Jia X, Wang H, Cao A, Zhao W, Pei H, Xue Z, He L, Chen Q (2013) Transcriptome-based discovery of pathways and genes related to resistance against Fusarium head blight in wheat landrace Wangshuibai. BMC Genomics 14:197

Yang H, Mu J, Chen L, Feng J, Hu J, Li L, Zhou JM, Zuo J (2015) S-nitrosylation positively regulates ascorbate peroxidase activity during plant stress responses. Plant Physiol 167:1604–1615

Yu M, Lamattina L, Spoel SH, Loake GJ (2014) Nitric oxide function in plant biology: a redox cue in deconvolution. New Phytol 202:1142–1156

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

Zeidler D, Zähringer U, Gerber I, Dubery I, Hartung T, Bors W, Hutzler P, Durner J (2004) Innate immunity in *Arabidopsis thaliana*: lipopolysaccharides activate nitric oxide synthase (NOS) and induce defense genes. Proc Natl Acad Sci USA 101:15811–15816

Zeng F, Sun F, Li L, Liu K, Zhan Y (2014) Genome-scale transcriptome analysis in response to nitric oxide in birch cells: implications of the triterpene biosynthetic pathway. PLoS One 9: e116157

Metabolism and Interplay of Reactive Oxygen and Nitrogen Species in Plant Mitochondria

Abir U. Igamberdiev and Natalia V. Bykova

Contents

Abstract In the conditions when oxygen is available and redox level is moderate, the electron transport chain (ETC) of plant mitochondria reduces oxygen to water. However, when the redox level is increased, one-electron transfer to oxygen becomes more plausible and superoxide anion is formed, which is further metabolized to hydrogen peroxide, both representing reactive oxygen species (ROS). The alternative rotenone-insensitive NADH and NADPH dehydrogenases prevent the increase in redox level of NAD and NADP, while the alternative cyanide-resistant oxidase prevents the increase of redox level of ubiquinone. When oxygen is depleted, nitrite can substitute oxygen as the terminal acceptor of electrons in the mitochondrial ETC resulting in the formation of nitric oxide (NO). The interplay between NO and superoxide results in generation of peroxynitrite and other reactive nitrogen species (RNS). The reactions of peroxynitrite metabolism include participation of

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thioredoxin. The complex interaction between ROS and RNS in mitochondria results in the involvement of several regulatory mechanisms which include *S*-nitrosylation and tyrosine nitration of proteins and formation of *S*-nitrosoglutathione and its further conversion by *S*-nitrosoglutathione reductase and other reactions that aim to maintain the stable non-equilibrium state of mitochondrial metabolism. The balancing of ROS and RNS formation and scavenging represents an important function of plant mitochondria regulating cellular metabolism and initiating signal transduction events.

Keywords Nitric oxide · Reactive oxygen species · Reactive nitrogen species · *S*-Nitrosoglutathione · Alternative oxidase · Rotenone-insensitive dehydrogenases

1 Introduction

The steady and efficient operation of metabolism is possible when the fluxes of load and consumption of major metabolites are equilibrated (Stucki 1980; Igamberdiev and Kleczkowski 2009). This condition is called "the stable non-equilibrium state" (Bauer 1935). Mitochondria play an important role in providing the stable non-equilibrium state by oxidizing reducing equivalents and producing ATP. In plants, where in photosynthetic tissues chloroplasts produce NADPH and ATP, mitochondria rather equilibrate the fluxes of catabolism and anabolism and fulfill important regulatory role in metabolism. This explains the fact that plant mitochondria contain both the coupled and non-coupled ATP synthesis pathways of electron transport and that the non-coupled pathways are finely regulated depending on the redox level and concentration of major metabolites. The side products of the increased redox level and of the variation of concentrations of oxygen are the reactive oxygen and nitrogen species (ROS and RNS) (Igamberdiev et al. 2014; Gupta et al. 2018). Being the toxic side products, they also participate in signaling events as well as fulfill important metabolic functions. The interplay between ROS and RNS depending on the intensity of metabolism, redox level, and saturation of electron transport by oxygen plays an important role in the regulation of metabolic events in plant cells and in cellular communication. During this interplay, new reactive species are formed such as peroxynitrite that are also important in metabolic reactions and signaling. As a consequence of RNS formation, the reactions of *S*-nitrosylation of proteins and glutathione as well as nitration of tyrosine take place contributing to many aspects of regulation of cellular metabolism. In this chapter we will discuss the formation, scavenging, and functional role of ROS and RNS in mitochondria and the role of electron transport components, coupled and non-coupled, to the generation of proton gradient and ATP synthesis, in the balancing of ROS and RNS formation and supporting metabolic homeostasis.

2 Redox Level and Production of ROS and RNS in Mitochondria

ROS and RNS are produced in different cell compartments (Feelisch et al. 2008), and mitochondria represent an important source of these compounds due to active electron transport. The leakage of electrons from the main path of electron transport via one-electron transfer either to oxygen with the formation of superoxide anion or to nitrate with the formation of nitric oxide (NO) is an unavoidable consequence of active electron flux which increases in the conditions of high redox level. The balance between superoxide and NO formation depends on redox level, concentration of oxygen, involvement of non-coupled pathways of electron transport, and inhibition of electron transport by the formed ROS and RNS. Further reactions result in the formation of hydrogen peroxide (H_2O_2) via dismutation of superoxide by superoxide dismutase (SOD), peroxynitrite ($ONOO^-$) via interaction of NO with superoxide at near the diffusion controlled rates, nitrogen dioxide (NO_2) via the reaction of NO with peroxynitrite, and dinitrogen trioxide (N_2O_3) via the reaction of NO_2 with NO (Espey et al. 2002). In addition to these low molecular compounds, many other compounds such as *S*-nitrosoglutathione as well as *S*-nitrosylated and tyrosine-nitrated peptides and proteins are formed as a consequence of formation of RNS and ROS (Fig. 1). In addition to the reductive pathways of RNS formation, NO can be formed in the oxidative pathways, the most known of which is the nitric oxide

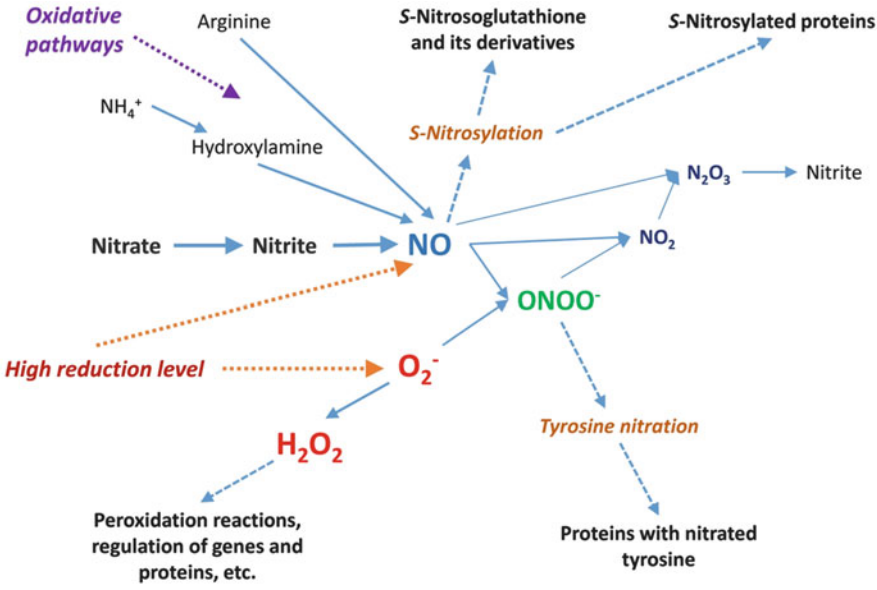

Fig. 1 Main reactions of the formation and conversion of reactive oxygen and nitrogen species (ROS and RNS). The reactions between different ROS and RNS result in the production of other species followed by *S*-nitrosylation and tyrosine nitration of proteins

synthase (NOS) reaction. This reaction was intensively studied in animals, and although biochemically demonstrated in plants, the plant enzyme responsible for this reaction and its gene have not been identified yet. Among other oxidative reactions of NO formation, hydroxylamine oxidation can take place; however, its physiological significance is not known (Rümer et al. 2009). The association of the oxidative reactions of NO formation with plant mitochondria has not been shown yet, and it is unlikely that these reactions of NO production significantly contribute to the balance of RNS in plant cells. It has been demonstrated that L-arginine-dependent NOS-like activity is associated with peroxisomes (Corpas and Barroso 2014; Corpas et al. 2017); however, further studies are needed to reveal its molecular mechanism in plants.

While NO directly reacts with superoxide, it does not react with H_2O_2; however, NO can keep H_2O_2 formation under control (Małolepsza and Rózalska 2005), in particular, via activation of the key H_2O_2 scavenging enzyme ascorbate peroxidase through its *S*-nitrosylation (Correa-Aragunde et al. 2013; Begara-Morales et al. 2014; Yang et al. 2015). Other mitochondrial enzymes that are *S*-nitrosylated are the subunits of glycine decarboxylase, serine hydroxymethyltransferase, lipoamide dehydrogenase (Palmieri et al. 2011), the mitochondrial isoform of NAD-malate dehydrogenase (Romero-Puertas et al. 2008), and subunits of Complex I (Burwell et al. 2006). It is not always known how the activity is modified by *S*-nitrosylation in each concrete case; however, it becomes evident that, by affecting these enzymes, *S*-nitrosylation modulates the redox level in mitochondria and can influence H_2O_2 production.

Tyrosine nitration has been demonstrated for NADP-isocitrate dehydrogenase and malate dehydrogenase causing their strong inhibition (Begara-Morales et al. 2014), while superoxide dismutase was activated albeit it may be not the mitochondrial isoform (Sehrawat et al. 2013). Tyrosine nitration was reported for serine hydroxymethyltransferase, formate dehydrogenase, cysteine synthase, and the subunit T of the glycine decarboxylase complex (GDC) (Lozano-Juste et al. 2011) with no information on modulation of activity of these enzymes. The effect of RNS and ROS on Fe-S clusters is a well-known phenomenon affecting aconitase and several complexes of the mitochondrial electron transport chain (ETC). The diverse effects of ROS and RNS on plant cell proteins reveal a complex phenomenon of action of ROS and RNS which includes their interaction and leads to the inhibition of several enzymes and activation of others. This complex picture of ROS and RNS action is getting clarification with the development of proteomic and metabolomic studies which reveal new targets of ROS and RNS and the dependence of their effects on environmental factors and stress conditions.

3 Regulation of ROS and RNS Production and Scavenging at the Level of Electron Transport from NADH/NADPH and Succinate to Ubiquinone

3.1 Complexes I and II

Complex I is an important site of superoxide formation (Møller 2001). It can be involved in the programmed cell death via its participation in the opening of the mitochondrial permeability pore (Chauvin et al. 2001). By producing superoxide, it also affects RNS levels due to the interaction between superoxide and NO. NO may also stimulate the activity of Complex I via binding to soluble guanylate cyclase that causes its phosphorylation (Durner et al. 1998). On the other hand, the excess of NO via the formation of peroxynitrite in the reaction with superoxide causes the inhibition of Complex I via binding to Fe-S clusters (Gupta et al. 2018). The role of Complex I in NO metabolism was studied using the cytoplasmic male-sterile (CMS) mutant of *Nicotiana sylvestris* lacking NAD7, one of the subunits of the respiratory Complex I (Shah et al. 2013). This mutant exhibited the tenfold reduction in NO production, which can be explained by the decreased flux of electrons to reduce nitrite and by the increased expression of the class I plant hemoglobin (phytoglobin) scavenging NO. This study shows the importance of Complex I in NO production by supplying electrons which under low oxygen can be used to reduce nitrite. The presence of nitrite under hypoxia facilitates the supercomplex I + III_2 formation (Gupta et al. 2017) which appears to be important for the maintenance of the mitochondrial function and integrity and NO production from nitrite. The supercomplex activity in potato mitochondria is increased under hypoxic conditions in the presence of nitrite (Ramírez-Aguilar et al. 2011) which may be explained by configuring the folding of the mitochondrial cristae (Cogliati et al. 2016).

The activity of Complex II (succinate-ubiquinone reductase) which represents the tricarboxylic acid (TCA) cycle enzyme succinate dehydrogenase associated with the subunits transferring electrons to ubiquinone is strongly inhibited by NO. This was demonstrated in animal mitochondria (Simonin and Galina 2013) where it was shown that NO significantly decreases the affinity to succinate and results in 70% reduction in the state 3 oxygen consumption rates. The inhibition takes place at Fe-S centers and the ubiquinone site. The accumulated NO by inhibiting Complex II inhibits also ROS production at this site. While the Complex II-dependent ROS production impairs the expression of genes of the cell cycle (Jardim-Messeder et al. 2015), the inhibition of Complex II by NO represents a major site of the interplay between ROS and RNS resulting in regulation of gene expression and initiating signaling events related to cell cycle regulation and programmed cell death.

3.2 Alternative NADH/NADPH Dehydrogenases

While the Complex I common to all types of mitochondria catalyzes the transfer of electrons from NADH to ubiquinone coupled to pumping of protons, plant mitochondria contain several alternative NADH and NADPH dehydrogenases which are resistant to the inhibitor of Complex I rotenone and do not pump protons (Møller et al. 1993; Møller 1997). The dehydrogenases located on the internal side of the inner mitochondrial membrane and oxidizing NADH are defined as NDA, the dehydrogenases located on the external side and oxidizing NADH or NADPH belong to the NDB group, and the dehydrogenase located on the internal side and oxidizing NADPH is designated as NDC. The dehydrogenases of the NDA-type are encoded in *Arabidopsis* by two genes and possess one order of magnitude lower affinity to NADH than Complex I and thus oxidize the excess of NADH formed inside the mitochondria. The NDC-type dehydrogenase is encoded in *Arabidopsis* by one gene, has Ca^{2+}-binding motif, and represents the only ETC component oxidizing NADPH internally (Michalecka et al. 2003; Escobar et al. 2004).

The dehydrogenases of the NDB-type are divided into two clusters having affinity either to NADH with low sensitivity to diphenyleneiodonium or to NADPH with high sensitivity to diphenyleneiodonium (Roberts et al. 1995) and oxidize reduced pyridine nucleotides from the external side of the inner mitochondrial membrane. Since the external mitochondrial membrane is penetrable to NADH and NADPH, these dehydrogenases can oxidize the cytosolic NADH and NADPH. They are specific for the protonated forms of NADH or NADPH (Edman et al. 1985) being activated in the conditions of cytosol acidification and contain an EF-hand domain for Ca^{2+} binding (Geisler et al. 2007; Hao et al. 2015). Four genes in *Arabidopsis* encode the dehydrogenases of the NDB-type, not all being expressed in all tissues or developmental stages (Rasmusson et al. 2008). There are some indications that the NDB-type dehydrogenases play an important role during hypoxia and in seed germination (Logan et al. 2001; Igamberdiev et al. 2004; Stoimenova et al. 2007). While NO stimulates Ca^{2+} release from mitochondria (Richter 1997; Igamberdiev and Hill 2018), the role of the NDB-type dehydrogenases in NO metabolism has been proposed. The insensitivity to rotenone of the turnover of nitrate, nitrite, and NO via the phytoglobin-NO cycle (Igamberdiev and Hill 2009) assumes the primary role of NDB-type dehydrogenases in this process.

It has been demonstrated that the NDB-type dehydrogenases can generate superoxide anion in the flavin-dependent mechanism which can further interact with NO forming peroxynitrite ($ONOO^-$) (de Oliveira et al. 2008). The process is stimulated by Ca^{2+} and inhibited by superoxide dismutase. This, the dehydrogenases of NDB-type participate in the interplay between ROS and RNS, and the formed peroxynitrite participates in tyrosine nitration (Poyton et al. 2009). The excess of peroxynitrite is readily scavenged in plants in the reactions involving thioredoxin and peroxiredoxin (Wulff et al. 2009; Selles et al. 2012). The NDB-type dehydrogenases are induced in the *Arabidopsis* plants knockdown in *S*-nitrosoglutathione reductase (Frungillo et al. 2013) which also indicates their role in RNS homeostasis.

The cross talk between calcium, H_2O_2, and NO can be induced by heavy metals (González et al. 2012) which may indicate possible role of the NDB-type dehydrogenases in this response.

It is possible that the dehydrogenases of the NDA and NDC type are also involved in the maintenance of ROS and RNS levels by oxidizing correspondingly the excess of intra-mitochondrial NADH and the formed intra-mitochondrial NADPH. This prevents over-reduction of the ubiquinone pool and thus superoxide and NO formation. Further studies are needed to clarify this possibility. Contrary to the NDB-type dehydrogenases that respond to various stress-related processes but not activated by light, the expression of NDA- and NDC-type dehydrogenases is related to photosynthesis (Michalecka et al. 2003; Escobar et al. 2004). Their role in balancing ROS and RNS levels during active photosynthesis needs further investigation, but theoretically this function of NDA- and NDC-type dehydrogenases seems very plausible.

4 Regulation of ROS and RNS Production and Scavenging at the Electron Transport Level from Ubiquinol to the Terminal Electron Acceptor

4.1 Alternative Oxidase in the Regulation of ROS and RNS Levels in Plants

The flux of electrons from ubiquinol to the terminal electron acceptor (mostly oxygen but in certain cases nitrite as we discuss below) is finely regulated, and the most important player in this regulation is the alternative oxidase (AOX) which uncouples electron transport from proton translocation and hence from ATP synthesis. This pathway is tightly regulated, and different isoforms of AOX are regulated in a different manner (Selinski et al. 2018a, b) to support stable non-equilibrium flux via the mitochondrial ETC and keep the level of ROS and RNS under control (Scheibe 2018). When the alternative cyanide-resistant path of respiration was initially detected (Bendall and Bonner 1971) and AOX was first identified in plants (Elthon and McIntosh 1987), its function remained enigmatic due to the common belief that dissipation of energy is not beneficial for living organisms. At that time Lambers (1982) introduced the "overflow" hypothesis claiming that AOX supports homeostasis by oxidation of the excess of reducing power generated during active metabolism, in particular, in active photosynthesis. Being generally correct, this hypothesis at that time did not take into consideration the mechanisms of fine regulation of this enzyme and the presence of its multiple isoforms that are differentially expressed in plants. Later it was shown that the oxidation of excess of reducing power by AOX prevents the increased formation of reactive oxygen species, namely, superoxide anion, which is formed at the high reduction level of ubiquinone (Maxwell et al. 1999; Umbach et al. 2005). This became the first

indication that the level of ROS is controlled via AOX expression in plant mitochondria. By limiting superoxide production, AOX also limits the production of H_2O_2 formed from superoxide by SOD (Møller 2001).

While the role of AOX in preventing the increased formation of ROS became evident, the accumulating data on the role of plant mitochondria in nitrogen metabolism and on the use of nitrite as alternative electron acceptor (Stoimenova et al. 2007; Gupta and Igamberdiev 2011) opened a possibility of the role of AOX in regulating the mitochondrial formation of NO and RNS. Despite its low affinity to oxygen as compared to cytochrome *c* oxidase (COX), during the intensive respiration, AOX dampens the leak of single electrons from ETC not only to O_2 but also to nitrite thus keeping NO levels under control, in particular, during various stress conditions (Cvetkovska and Vanlerberghe 2012, 2013; Alber et al. 2017). The knockdown of AOX causes "the stress state" of the signaling molecule pools resulting in elevation of ROS and RNS. One of the consequences of AOX suppression is the alteration of stomatal function via accumulation of NO in stomatal guard cells leading to alterations in their size and making them less responsive to the signals for stomatal closure (Cvetkovska et al. 2014). Thus the uncoupling of mitochondrial respiration at the level of AOX from proton gradient formation and ATP synthesis leads not only to the maintenance of the level of ROS such as superoxide and hydrogen peroxide but also of the levels of NO and thus of *S*-nitrosylation of glutathione and proteins in plant cells (Vanlerberghe 2013). The expression of AOX is in turn induced by ROS such as ozone and by NO (Ederli et al. 2006; Royo et al. 2015) which generates a feedback to keep low ROS and RNS levels.

An important question is whether AOX can itself participate in production of NO from nitrite. In the first experiments that showed that nitrite can be reduced to NO by plant mitochondria (Tischner et al. 2004; Planchet et al. 2005), not only cyanide, antimycin A, and myxothiazol inhibited NO production which indicates the involvement of the cytochrome pathway but also salicylhydroxamic acid which is the inhibitor of AOX. This was interpreted as a possibility that AOX directly participates in nitrite reduction to NO similarly as this was proposed for Complexes III and IV. While the inhibitors of AOX such as salicylhydroxamate and pyrogallol are not very specific for AOX and can have other targets, e.g., inhibit peroxidases, it was also proposed that the observed inhibition cannot be considered as the evidence of direct AOX involvement in NO production from nitrite. AOX is a non-heme protein – it belongs to the di-iron carboxylate family of proteins and contains a hydroxo-bridged binuclear iron center, analogous to that found in the enzyme methane monooxygenase (Siedow et al. 1995). It is known that nitrite reduction to NO can be catalyzed by hemeproteins and molybdocofactors in their deoxygenated state (Gladwin and Kim-Shapiro 2008); however, it is still not known whether di-iron carboxylate proteins can perform this reaction. On the other hand, the reaction of nitrite reduction to NO may take place even nonenzymatically at low pH, with reducing agents such as ascorbate and phenolics (Bethke et al. 2004). Recently Vishwakarma et al. (2018) demonstrated in *Arabidopsis* AOX transgenic plants that while in normoxia downregulation of AOX stimulated NO, superoxide,

peroxynitrite production, and tyrosine nitration, under hypoxia NO production was enhanced when AOX was overexpressed and was suppressed when AOX was downregulated. Although these data cannot provide the direct evidence in AOX involvement in NO formation when oxygen drops down, they clearly indicate such possibility.

Regulation of AOX activity and expression is achieved by different fine-tuned mechanisms, and this regulation aims to control ROS and RNS levels in plant cells. It was shown that the AOX protein is regulated by redox level when the inactive dimer linked by the S–S inter subunit bond is reduced forming active monomers with SH groups (Vanlerberghe et al. 1995). The activity of the reduced AOX is stimulated by oxo-acids among which pyruvate was considered most important (Day and Wiskich 1995) increasing AOX affinity to ubiquinol. Recently it was shown that different AOX isoforms respond differently to various oxo-acids (Selinski et al. 2017, 2018a, b). Both CysI and CysII residues of the isoforms AOX1A, AOX1C, and AOX1D in *Arabidopsis* are involved in 2-oxo-acid activation but with different strength: AOX1A activity was stimulated more by oxo-acids than AOX1C and AOX1D. While AOX1C was least sensitive to activation by organic acids, AOX1A and AOX1D were both activated by 2-oxoglutarate, and AOX1A was additionally activated by oxaloacetate (Selinski et al. 2018a). This all means that different oxo-acids act differently on AOX which may represent fine mechanism of regulation by the products of different metabolic pathways, like pyruvate from glycolysis, glyoxylate from photorespiration, 2-oxoglutarate from the TCA cycle and amino acid metabolism, and oxaloacetate from the malate dehydrogenase equilibrium (Selinski et al. 2018b). This may play a role in the regulation of levels of ROS and RNS in different conditions characterized by accumulation of different oxo-acids (Igamberdiev and Eprintsev 2016; Igamberdiev and Bykova 2018).

While at the level of protein AOX is regulated by redox level and oxo-acids, its expression is stimulated by the accumulation of citrate (Vanlerberghe et al. 1995). The efflux of citrate from mitochondria occurs at the increased redox level due to the reversal of NADP-isocitrate dehydrogenase reaction (Igamberdiev and Gardeström 2003), and it can be stimulated by the release of Mg^{2+} at low ATP displacing the citrate/isocitrate ratio toward citrate (Igamberdiev and Kleczkowski 2001). Another important feedback mechanism is the inhibition of aconitase by ROS (Morgan et al. 2008) and RNS (Navarre et al. 2000) which leads to citrate accumulation and thus to the induction of AOX expression. As it was shown on nitrate reductase mutants of *Arabidopsis*, the production of NO results in aconitase inhibition and activation of AOX expression due to citrate accumulation (Gupta et al. 2012), which indicates the important role of AOX in keeping NO levels under control. The absence of AOX inhibition by NO contrary to COX inhibition (Millar and Day 1996) makes AOX an important player in the regulation of NO levels and balancing ROS and RNS production in plants under stress or changing environmental conditions and representing a defense mechanism against metabolic fluctuations (Rasmusson et al. 2009).

4.2 Cytochrome Pathway in ROS/RNS Production and Scavenging

The cytochrome pathway of electron transport is common to mitochondria of all organisms and includes Complex III (ubiquinol:cytochrome *c* oxidoreductase), cytochrome *c*, and Complex IV (cytochrome *c* oxidase). Both complexes generate proton potential and thus participate in ATP synthesis. Complex III is the site of superoxide production due to the mechanism of one electron transfer to oxygen (Sun and Trumpower 2003). When the oxygen concentration drops down, electrons can reduce nitrite forming NO. This mechanism was shown for mammalian mitochondria (Kozlov et al. 1999) and later established for green algae (Tischner et al. 2004) and plants (Gupta et al. 2005; Planchet et al. 2005). Alber et al. (2017) by using the inhibitors of Complex III antimycin A and myxothiazol acting at different sites of the complex have found that nitrite reduction to NO takes place on the site of ubiquinol oxidation center (Q_o site). Similarly to the formation of superoxide, NO release can take place from both sides of the inner mitochondrial membrane (Muller et al. 2004; Xu and Arriaga 2009). These findings put Complex III at the place of key signal generator of ROS and NO production in plants, and the interaction between NO and superoxide can generate peroxynitrite from both sides of the inner mitochondrial membrane. This means that a part of NO does not need to penetrate from inside the mitochondria but is generated outside where it can easily move to the cytosol to be scavenged by nonsymbiotic hemoglobins (phytoglobins) (Abbruzzetti et al. 2011).

Cytochrome *c* itself can perform nitrite reduction to NO in the conditions of limitation of the electron transfer to Complex IV (Basu et al. 2008; Feelisch et al. 2008). This reaction takes place when cytochrome *c* turns to the pentacoordinate state from hexacoordinate, which is facilitated by nitration of tyrosines with peroxynitrite (Poyton et al. 2009). There were no studies of cytochrome *c* in plants in relation to NO production, but its location on the outer side of the inner mitochondrial membrane and release to the cytosol during apoptosis (Virolainen et al. 2002) may indicate that NO production by cytochrome *c* takes place at the outer side of the mitochondria and even in the cytosol.

Complex IV is the terminal oxidase transferring electrons to oxygen. In the absence of oxygen, nitrite can be used as a terminal electron acceptor forming NO. The sensitivity of NO production to KCN was the first indication of COX involvement in this process (Planchet et al. 2005; Stoimenova et al. 2007). The mechanism is associated with the oxidation of iron by nitrite after its binding at the fully reduced $Fe_{a3}Cu_B$ center and facilitated at lower pH (Castello et al. 2006). On the other hand, NO competitively inhibits the electron transport to O_2 (Millar and Day 1996) by elevating the apparent *K*m of respiration for oxygen (Brown 1999) thus regulating respiration in response to the decrease in oxygen levels and preventing oxygen depletion (Gupta et al. 2009; Zabalza et al. 2009). This means that the NO formed by nitrite reduction exerts regulatory functions on COX which results in keeping oxygen concentration and ROS homeostasis in plant cells under control upon fluctuation of oxygen in the environment.

5 Conclusions

The components of the mitochondrial ETC can participate in the formation and scavenging of ROS and RNS putting plant mitochondria in the center of regulation of ROS and RNS levels and retrograde signaling. The generalized scheme of formation of NO, superoxide anion, and peroxynitrite in the mitochondrial ETC is shown on Fig. 2. More research is needed to understand the mitochondrial pathways of ROS and RNS metabolism and their role in stress tolerance. In particular, the role of non-coupled electron transport at the levels of rotenone-resistant dehydrogenases and cyanide-resistant alternative oxidase in balancing the stable non-equilibrium metabolic flux and electron transport and making ROS and RNS important players in the system of signal transduction acting at the transcriptional, translational, and posttranslational levels should be further investigated. Interaction between ROS and RNS at different levels of oxygen is another important aspect that needs to be further explored, in particular, in the relation to modification of proteins and other cell components resulting in *S*-nitrosylation, tyrosine nitration, and peroxidation of essential residues. This will clarify the whole flexible metabolic structure of the plant cell and the central role of mitochondria in the maintenance of the homeostatic balance of biosynthetic and catabolic pathways in the adaptation to changing environment.

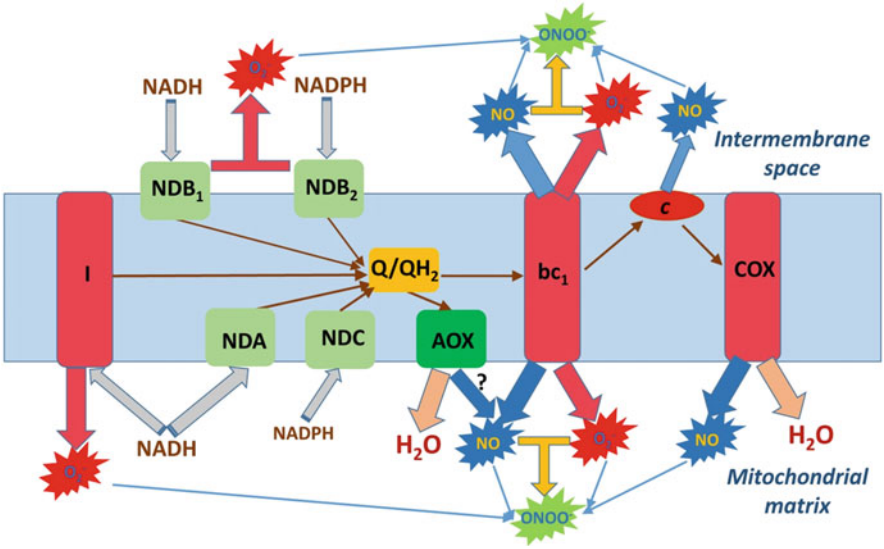

Fig. 2 Electron transport chain of mitochondria and the production of nitric oxide (NO), superoxide anion (O_2^-), and peroxynitrite ($ONOO^-$). Abbreviations: I, Complex I; NDA, NDB_1, NDB_2, NDC, rotenone-resistant NADH/NADPH dehydrogenases; bc_1, Complex III; COX, cytochrome *c* oxidase (Complex IV); AOX, alternative oxidase; *c*, cytochrome *c*; Q/QH_2, ubiquinone/ubiquinol pool. Complex II is not shown for simplification

References

Abbruzzetti S, Faggiano S, Spyrakis F, Bruno S, Mozzarelli A, Astegno A, Dominici P, Viappiani C (2011) Oxygen and nitric oxide rebinding kinetics in nonsymbiotic hemoglobin AHb1 from *Arabidopsis thaliana*. IUBMB Life 63:1094–1100

Alber NA, Sivanesan H, Vanlerberghe GC (2017) The occurrence and control of nitric oxide generation by the plant mitochondrial electron transport chain. Plant Cell Environ 40: 1074–1085

Basu S, Azarova NA, Font MD, King SB, Hogg N, Gladwin MT, Shiva S, Kim-Shapiro DB (2008) Nitrite reductase activity of cytochrome *c*. J Biol Chem 283:32590–32597

Bauer ES (1982) Theoretical biology. Akadémiai Kiadó, Budapest (Originally published 1935)

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

Bendall DS, Bonner WD (1971) Cyanide-insensitive respiration in plant mitochondria. Plant Physiol 47:236–245

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

Brown GC (1999) Nitric oxide and mitochondrial respiration. Biochim Biophys Acta 1411: 351–369

Burwell LS, Nadtochiy SM, Tompkins AJ, Young S, Brookes PS (2006) Direct evidence for *S*-nitrosation of mitochondrial complex I. Biochem J 394:627–634

Castello PR, David PS, McClure T, Crook Z, Poyton RO (2006) Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes. Cell Metab 3:277–287

Chauvin C, De Oliveira F, Ronot X, Mousseau M, Leverve X, Fontain E (2001) Rotenone inhibits the mitochondrial permeability transition- induced cell death in U937 and KB cells. J Biol Chem 276:41394–41398

Cogliati S, Enriquez JA, Scorrano L (2016) Mitochondrial cristae: where beauty meets functionality. Trend Biochem Sci 41:261–273

Corpas FJ, Barroso JB (2014) Peroxisomal plant nitric oxide synthase (NOS) protein is imported by peroxisomal targeting signal type 2 (PTS2) in a process that depends on the cytosolic receptor PEX7 and calmodulin. FEBS Lett 588:2049–2054

Corpas FJ, Barroso JB, Palma JM, Rodríguez-Ruiz M (2017) Plant peroxisomes: a nitro-oxidative cocktail. Redox Biol 11:535–542

Correa-Aragunde N, Foresi N, Delledonne M, Lamattina L (2013) Auxin induces redox regulation of ascorbate peroxidase1 activity by *S*-nitrosylation/denitrosylation balance resulting in changes of root growth pattern in Arabidopsis. J Exp Bot 64:3339–3349

Cvetkovska M, Vanlerberghe GC (2012) Alternative oxidase modulates leaf mitochondrial concentrations of superoxide and nitric oxide. New Phytol 195:32–39

Cvetkovska M, Vanlerberghe GC (2013) Alternative oxidase impacts the plant response to biotic stress by influencing the mitochondrial generation of reactive oxygen species. Plant Cell Environ 36:721–732

Cvetkovska M, Dahal K, Alber NA, Jin C, Cheung M, Vanlerberghe GC (2014) Knockdown of mitochondrial alternative oxidase induces the 'stress state' of signaling molecule pools in *Nicotiana tabacum*, with implications for stomatal function. New Phytol 203:449–461

Day DA, Wiskich JT (1995) Regulation of alternative oxidase activity in higher plants. J Bioenerg Biomembr 27:379–385

de Oliveira HC, Wulff A, Saviani EE, Salgado I (2008) Nitric oxide degradation by potato tuber mitochondria: evidence for the involvement of external NAD(P)H dehydrogenases. Biochim Biophys Acta 1777:470–476

Durner J, Wendehenne D, Klessig DF (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci U S A 95:10328–10333

Ederli L, Morettini R, Borgogni A, Wasternack C, Miersch O, Reale L, Ferranti F, Tosti N, Pasqualini S (2006) Interaction between nitric oxide and ethylene in the induction of alternative oxidase in ozone-treated tobacco plants. Plant Physiol 142:595–608

Edman K, Ericson I, Møller IM (1985) The regulation of exogenous NAD(P)H oxidation in spinach (*Spinacia oleracea*) leaf mitochondria by pH and cations. Biochem J 232:471–477

Elthon TE, McIntosh L (1987) Identification of the alternative terminal oxidase of higher plant mitochondria. Proc Natl Acad Sci U S A 84:8399–8403

Escobar MA, Franklin KA, Svensson AS, Salter MG, Whitelam GC, Rasmusson AG (2004) Light regulation of the *Arabidopsis* respiratory chain. Multiple discrete photoreceptor responses contribute to induction of type II NAD(P)H dehydrogenase genes. Plant Physiol 136:2710–2721

Espey MG, Thomas DD, Miranda KM, Wink DA (2002) Focusing of nitric oxide mediated nitrosation and oxidative nitrosylation as a consequence of reaction with superoxide. Proc Natl Acad Sci U S A 99:11127–11132

Feelisch M, Fernandez BO, Bryan NS, Garcia-Saura MF, Bauer S, Whitlock DR, Ford PC, Janero DR, Rodriguez J, Ashrafian H (2008) Tissue processing of nitrite in hypoxia: an intricate interplay of nitric oxide-generating and scavenging systems. J Biol Chem 283:33927–33934

Frungillo L, de Oliveira JF, Saviani EE, Oliveira HC, Martínez MC, Salgado I (2013) Modulation of mitochondrial activity by *S*-nitrosoglutathione reductase in *Arabidopsis thaliana* transgenic cell lines. Biochim Biophys Acta 1827:239–247

Geisler DA, Broselid C, Hederstedt L, Rasmusson AG (2007) Ca^{2+}-binding and Ca^{2+}-independent respiratory NADH and NADPH dehydrogenases of *Arabidopsis thaliana*. J Biol Chem 282: 28455–28464

Gladwin MT, Kim-Shapiro DB (2008) The functional nitrite reductase activity of the heme-globins. Blood 112:2636–2647

González A, Cabrera M de L, Henríquez MJ, Contreras RA, Morales B, Moenne A (2012) Cross talk among calcium, hydrogen peroxide, and nitric oxide and activation of gene expression involving calmodulins and calcium-dependent protein kinases in *Ulva compressa* exposed to copper excess. Plant Physiol 158:1451–1462

Gupta KJ, Igamberdiev AU (2011) The anoxic plant mitochondrion as a nitrite: NO reductase. Mitochondrion 11:537–543

Gupta KJ, Stoimenova M, Kaiser WM (2005) In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, in vitro and in situ. J Exp Bot 56:2601–2609

Gupta KJ, Zabalza A, van Dongen JT (2009) Regulation of respiration when the oxygen availability changes. Physiol Plant 137:383–391

Gupta KJ, Shah JK, Brotman Y, Jahnke K, Willmitzer L, Kaiser WM, Bauwe H, Igamberdiev AU (2012) Inhibition of aconitase by nitric oxide leads to induction of the alternative oxidase and to a shift of metabolism towards biosynthesis of amino acids. J Exp Bot 63:1773–1784

Gupta KJ, Lee CP, Ratcliffe RG (2017) Nitrite protects mitochondrial structure and function under hypoxia. Plant Cell Physiol 58:175–183

Gupta KJ, Kumari A, Florez-Sarasa I, Fernie AR, Igamberdiev AU (2018) Interaction of nitric oxide with the components of the plant mitochondrial electron transport chain. J Exp Bot 69:3413–3424

Hao MS, Jensen AM, Boquist AS, Liu YJ, Rasmusson AG (2015) The Ca^{2+}-regulation of the mitochondrial external NADPH dehydrogenase in plants is controlled by cytosolic pH. PLoS One 10:e0139224.

Igamberdiev AU, Bykova NV (2018) Role of organic acids in the integration of cellular redox metabolism and mediation of redox signalling in photosynthetic tissues of higher plants. Free Radic Biol Med 122:74–85

Igamberdiev AU, Eprintsev AT (2016) Organic acids: the pools of fixed carbon involved in redox regulation and energy balance in higher plants. Front Plant Sci 7:1042

Igamberdiev AU, Gardeström P (2003) Regulation of NAD- and NADP-dependent isocitrate dehydrogenases by reduction levels of pyridine nucleotides in mitochondria and cytosol of pea leaves. Biochim Biophys Acta 1606:117–125

Igamberdiev AU, Hill RD (2009) Plant mitochondrial function during anaerobiosis. Ann Bot 103: 259–268

Igamberdiev AU, Hill RD (2018) Elevation of cytosolic Ca^{2+} in response to energy deficiency in plants: the general mechanism of adaptation to low oxygen stress. Biochem J 475:1411–1425

Igamberdiev AU, Kleczkowski LA (2001) Implications of adenylate kinase-governed equilibrium of adenylates on contents of free magnesium in plant cells and compartments. Biochem J 360: 225–231

Igamberdiev AU, Kleczkowski LA (2009) Metabolic systems maintain stable non-equilibrium via thermodynamic buffering. BioEssays 31:1091–1099

Igamberdiev AU, Seregélyes C, Manac'h N, Hill RD (2004) NADH-dependent metabolism of nitric oxide in alfalfa root cultures expressing barley hemoglobin. Planta 219:95–102

Igamberdiev AU, Ratcliffe RG, Gupta KJ (2014) Plant mitochondria: source and target for nitric oxide. Mitochondrion 19:329–333

Jardim-Messeder D, Caverzan A, Rauber R, de Souza Ferreira E, Margis-Pinheiro M, Galina A (2015) Succinate dehydrogenase (mitochondrial complex II) is a source of reactive oxygen species in plants and regulates development and stress responses. New Phytol 208:776–789

Kozlov AV, Staniek K, Nohl H (1999) Nitrite reductase activity is a novel function of mammalian mitochondria. FEBS Lett 454:127–130

Lambers H (1982) Cyanide-resistant respiration–a non-phosphorylating electron-transport pathway acting as an energy overflow. Physiol Plant 55:478–485

Logan DC, Millar AH, Sweetlove LJ, Hill SA, Leaver CJ (2001) Mitochondrial biogenesis during germination in maize embryos. Plant Physiol 125:662–672

Lozano-Juste J, Colom-Moreno R, León J (2011) In vivo protein tyrosine nitration in *Arabidopsis thaliana*. J Exp Bot 62:3501–3517

Małolepsza U, Rózalska S (2005) Nitric oxide and hydrogen peroxide in tomato resistance. Nitric oxide modulates hydrogen peroxide level in o-hydroxyethylorutin-induced resistance to *Botrytis cinerea* in tomato. Plant Physiol Biochem 43:623–635

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

Michalecka AM, Svensson AS, Johansson FI, Agius SC, Johanson U, Brennicke A, Binder S, Rasmusson AG (2003) Arabidopsis genes encoding mitochondrial type II NAD(P)H dehydrogenases have different evolutionary origin and show distinct responses to light. Plant Physiol 133:642–652

Millar AH, Day DA (1996) Nitric oxide inhibits the cytochrome oxidase but not the alternative oxidase of plant mitochondria. FEBS Lett 398:155–158

Møller IM (1997) The oxidation of cytosolic NAD(P)H by external NAD(P)H dehydrogenases in the respiratory chain of plant mitochondria. Physiol Plant 100:85–90

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

Møller IM, Rasmusson AG, Fredlund KM (1993) NAD(P)H-ubiquinone oxidoreductases in plant mitochondria. J Bioenerg Biomembr 25:377–384

Morgan MJ, Lehmann M, Schwarzländer M, Baxter CJ, Sienkiewicz-Porzucek A, Williams TC, Schauer N, Fernie AR, Fricker MD, Ratcliffe RG, Sweetlove LJ, Finkemeier I (2008) Decrease in manganese superoxide dismutase leads to reduced root growth and affects tricarboxylic acid cycle flux and mitochondrial redox homeostasis. Plant Physiol 147:101–114

Muller FL, Liu Y, Van Remmen H (2004) Complex III releases superoxide to both sides of the inner mitochondrial membrane. J Biol Chem 279:49064–49073

Navarre DA, Wendehenne D, Durner J, Noad R, Klessig DF (2000) Nitric oxide modulates the activity of tobacco aconitase. Plant Physiol 122:573–582

Palmieri F, Pierri CL, De Grassi A, Nunes-Nesi A, Fernie AR (2011) Evolution, structure and function of mitochondrial carriers: a review with new insights. Plant J 66:161–181

Planchet E, Gupta KJ, Sonoda M, Kaiser WM (2005) Nitric oxide emission from tobacco leaves and cell suspensions: rate limiting factors and evidence for the involvement of mitochondrial electron transport. Plant J 41:732–743

Poyton RO, Ball KA, Castello PR (2009) Mitochondrial generation of free radicals and hypoxic signaling. Trend Endocrinol Metabol 20:332–340

Ramírez-Aguilar SJ, Keuthe M, Rocha M, Fedyaev VV, Kramp K, Gupta KJ, Rasmusson AG, Schulze WX, van Dongen JT (2011) The composition of plant mitochondrial supercomplexes changes with oxygen availability. J Biol Chem 286:43045–43053

Rasmusson AG, Geisler DA, Møller IM (2008) The multiplicity of dehydrogenases in the electron transport chain of plant mitochondria. Mitochondrion 8:47–60

Rasmusson AG, Fernie AR, van Dongen JT (2009) Alternative oxidase: a defence against metabolic fluctuations? Physiol Plant 137:371–382

Richter C (1997) Reactive oxygen and nitrogen species regulate mitochondrial Ca^{2+} homeostasis and respiration. Biosci Rep 17:53–66

Roberts TH, Fredlund KM, Møller IM (1995) Direct evidence for the presence of two external NAD(P)H dehydrogenases coupled to the electron transport chain in plant mitochondria. FEBS Lett 373:307–309

Romero-Puertas MC, Campostrini N, Mattè A, Righetti PG, Perazzolli M, Zolla L, Roepstorff P, Delledonne M (2008) Proteomic analysis of *S*-nitrosylated proteins in *Arabidopsis thaliana* undergoing hypersensitive response. Proteomics 8:1459–1469

Royo B, Moran JF, Ratcliffe RG, Gupta KJ (2015) Nitric oxide induces the alternative oxidase pathway in Arabidopsis seedlings deprived of inorganic phosphate. J Exp Bot 66:6273–6280

Rümer S, Gupta KJ, Kaiser WM (2009) Plant cells oxidize hydroxylamines to NO. J Exp Bot 60: 2065–2072

Scheibe R (2018) Maintaining homeostasis by controlled alternatives for energy distribution in plant cells under changing conditions of supply and demand. Photosynth Res https://doi.org/10.1007/s11120-018-0583-z

Sehrawat A, Abat JK, Deswal R (2013) RuBisCO depletion improved proteome coverage of cold responsive S-nitrosylated targets in *Brassica juncea*. Front Plant Sci 4:342

Selinski J, Hartmann A, Kordes A, Deckers-Hebestreit G, Whelan J, Scheibe R (2017) Analysis of posttranslational activation of alternative oxidase isoforms. Plant Physiol 174:2113–2127

Selinski J, Hartmann A, Deckers-Hebestreit G, Day DA, Whelan J, Scheibe R (2018a) Alternative oxidase isoforms are differentially activated by tricarboxylic acid cycle intermediates. Plant Physiol 176:1423–1432

Selinski J, Scheibe R, Day DA, Whelan J (2018b) Alternative oxidase is positive for plant performance. Trend Plant Sci 23:588–597

Selles B, Hugo M, Trujillo M, Srivastava V, Wingsle G, Jacquot JP, Radi R, Rouhier N (2012) Hydroperoxide and peroxynitrite reductase activity of poplar thioredoxin-dependent glutathione peroxidase 5: kinetics, catalytic mechanism and oxidative inactivation. Biochem J 442:369–380

Shah JK, Cochrane DW, De Paepe R, Igamberdiev AU (2013) Respiratory complex I deficiency results in low nitric oxide levels, induction of hemoglobin and upregulation of fermentation pathways. Plant Physiol Biochem 63:185–190

Siedow JN, Umbach AL, Moore AL (1995) The active site of the cyanide-resistant oxidase from plant mitochondria contains a binuclear iron center. FEBS Lett 362:10–14

Simonin V, Galina A (2013) Nitric oxide inhibits succinate dehydrogenase-driven oxygen consumption in potato tuber mitochondria in an oxygen tension-independent manner. Biochem J 449:263–273

Stoimenova M, Igamberdiev AU, Gupta KJ, Hill RD (2007) Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. Planta 226:465–474

Stucki JW (1980) The thermodynamic-buffer enzymes. Eur J Biochem 109:257–267

Sun J, Trumpower BL (2003) Superoxide anion generation by the cytochrome bc1 complex. Arch Biochem Biophys 419:198–206

Tischner R, Planchet E, Kaiser WM (2004) Mitochondrial electron transport as a source for nitric oxide in the unicellular green alga *Chlorella sorokiniana*. FEBS Lett 576:151–155

Umbach AL, Fiorani F, Siedow JN (2005) Characterization of transformed Arabidopsis with altered alternative oxidase levels and analysis of effects on reactive oxygen species in tissue. Plant Physiol 139:1806–1820

Vanlerberghe GC (2013) Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. Int J Mol Sci 14: 6805–6847

Vanlerberghe GC, Day DA, Wiskich JT, Vanlerberghe AE, McIntosh L (1995) Alternative oxidase activity in tobacco leaf mitochondria. Dependence on tricarboxylic acid cycle-mediated redox regulation and pyruvate activation. Plant Physiol 109:353–361

Virolainen E, Blokhina O, Fagerstedt K (2002) Ca^{2+}-induced high amplitude swelling and cytochrome c release from wheat (*Triticum aestivum* L.) mitochondria under anoxic stress. Ann Bot 90:509–516

Vishwakarma A, Kumari A, Mur LAJ, Gupta KJ (2018) A discrete role for alternative oxidase under hypoxia to increase nitric oxide and drive energy production. Free Radic Biol Med 122: 40–51

Wulff A, Oliveira HC, Saviani EE, Salgado I (2009) Nitrite reduction and superoxide-dependent nitric oxide degradation by *Arabidopsis* mitochondria: influence of external NAD(P)H dehydrogenases and alternative oxidase in the control of nitric oxide levels. Nitric Oxide 21:132–139

Xu X, Arriaga EA (2009) Qualitative determination of superoxide release at both sides of the mitochondrial inner membrane by capillary electrophoretic analysis of the oxidation products of triphenylphosphonium hydroethidine. Free Radic Biol Med 46:905–913

Yang H, Mu J, Chen L, Feng J, Hu J, Li L, Zhou JM, Zuo J (2015) S-nitrosylation positively regulates ascorbate peroxidase activity during plant stress responses. Plant Physiol 167: 1604–1615

Zabalza A, van Dongen JT, Froehlich A, Oliver SN, Faix B, Gupta KJ, Schmälzlin E, Igal M, Orcaray L, Royuela M, Geigenberger P (2009) Regulation of respiration and fermentation to control the plant internal oxygen concentration. Plant Physiol 149:1087–1098

Hydrogen Peroxide and Nitric Oxide Metabolism in Chloroplasts

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Contents

Abstract Chloroplasts are ubiquitous organelles with a notable oxidative metabolism that are involved in free energy transduction through photophosphorylation. In addition, thioredoxin-mediated redox regulation of Calvin cycle enzymes has been shown to determine the efficiency of carbon assimilation. These provide significant evidence that chloroplasts are key cellular sites of reactive oxygen species (ROS) production, including molecules like superoxide ($O_2^{\bullet-}$) and H_2O_2. In addition to ROS, there are compelling indications that nitric oxide (NO) can be generated in chloroplasts by both reductive and oxidative pathways. NO is involved in many physiological and biochemical processes in plants, including photosynthesis. However, many unanswered questions remain concerning how, when, and where NO is generated (enzymatically or nonenzymatically) in higher plants. ROS and reactive

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nitrogen species (RNS) play a key role in signaling, but they can also be deleterious via oxidation of cell components when overproduced, as a consequence of redox imbalance and/or adverse conditions. In chloroplasts, besides the redox state and regulation of thiol groups of cysteinyl residues in proteins, identification of other types of posttranslational protein modifications (PTMs) such as Tyr nitration, *S*-nitrosylation, and glutathionylation has been reported. Therefore, this chapter is focused on photosynthetic light/dark reactions, antioxidant defense and thiol-based redox regulation, ROS production, NO synthesis, and its implication together with that of ROS, in PTM-mediated regulation, with the aim of drawing a picture of the importance of all of them in the regulation of the chloroplast function.

Keywords Plant stress · Chloroplast · Hydrogen peroxide · Nitric oxide · Posttranslational modification · Reactive nitrogen species · Reactive oxygen species

1 Introduction

In plant cells, generation of ROS (reactive oxygen species) and RNS (reactive nitrogen species) is characteristic of a number of metabolic reactions that take place in different cell compartments. The best known ROS—superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), single oxygen ($^1O_2$), and hydroxyl radical ($^{\bullet}OH$)—are all strong oxidizing compounds and therefore potentially harmful for cell integrity by causing loss of redox homeostasis. Among them, H_2O_2 is thermostable and can be produced in all compartments by the dismutation of $O_2^{\bullet-}$, with the electron transport in mitochondria, but mainly that in chloroplast during photosynthesis and in peroxisomes during photorespiration, being the most important ROS sources (Foyer and Shigeoka 2011). Besides their toxicity, ROS are also considered to be powerful signaling molecules that regulate plant growth and development as well as biotic and abiotic stress responses (Mittler et al. 2004; Baxter et al. 2014; del Río 2015).

H_2O_2 in chloroplasts, even at low levels, is a potent inhibitor of photosynthesis due to its capacity to oxidize the thiol-modulated enzymes of the Calvin cycle. Therefore, chloroplasts are equipped with a robust ROS detoxification system including nonenzymatic and enzymatic components, able to maintain a strict control of ROS content produced during the normal metabolism, and increased under unfavorable stress conditions. The ascorbate-glutathione (ASC-GSH) cycle and the water-water cycle are in charge of metabolizing H_2O_2 and to dissipate excess of excitation energy in chloroplasts (Foyer and Shigeoka 2011), whereas superoxide dismutases (SODs) metabolize $O_2^{\bullet-}$. Other enzymes involved in H_2O_2 and hydroperoxides detoxification are glutathione peroxidase (GPX) and peroxiredoxin (Prx), which function together with sulfiredoxin (Srx) and thioredoxin (Trx) in chloroplasts ROS detoxification and redox homeostasis (Bernier-Villamor et al. 2004; Iglesias-Baena et al. 2010, 2011; Mock and Dietz 2016).

Thioredoxins (Trxs) are an important class of regulatory small proteins presenting two cysteines in their active site that modulate the activity of target thiol proteins by

reducing specific disulfide bounds (Montrichard et al. 2009). In chloroplasts, in the photosynthetic electron transport (PET), reducing equivalents are transferred to specific proteins containing redox-active cysteine residues mediated by the thioredoxin system (Yoshida et al. 2015). This Trx system, composed of ferredoxin (Fd)-Trx reductase (FTR) and Trx, ensures light-responsive control of multiple key functions (Crawford et al. 1989; Barajas-López et al. 2012) as well as the thiol-based H_2O_2 reduction through the activity of Prxs. Thus, these proteins participate in the cell signaling network, where redox signals, comprising ROS and redox balance sensors, are perceived and transmitted (Sevilla et al. 2015a; Calderón et al. 2018).

Nitric oxide (NO) is a gaseous small reactive molecule which exists in three different forms, i.e., NO^+ (nitrosonium cation), NO^- (nitroxyl anion), and $^{\bullet}NO$ (nitric oxide radical). As a free radical, NO and its derived molecules are designated as reactive nitrogen species (RNS) including peroxynitrite ($ONOO^-$), *S*-nitrosothiols (SNOs), and *S*-nitrosoglutathione (GSNO) (Yu et al. 2014). There is evidence of the NO production in plants due to a variety of sources, including an L-arginine-dependent nitric oxide synthase (NOS)-like enzymes (Corpas et al. 2017), nitrite and nitrate reductase (NR), or nonenzymatic sources, occurring in different cell compartments (see below). Additionally, an excess of NO can act synergistically with ROS and result in nitro-oxidative stress eliciting undesirable damaging effects in plant cells. In chloroplasts, photosynthesis is affected not only by ROS but also by reaction with NO. Thus under conditions of high NO content in chloroplast, the generation of RNS may provoke an impairment of the photosynthetic machinery (Jasid et al. 2006). On the other hand, as an endogenous metabolite, NO has various signaling functions. NO and RNS can interact at different levels, as part of a complex network of signaling processes as well as a response mechanism against environmental stress conditions (Turkan 2017; Umbreen et al. 2018). Interaction of RNS with ROS occurs during plant growth and development in processes such as seed germination and flowering (Zafra et al. 2016) and in defense (Asai et al. 2008; Romero-Puertas et al. 2008; Lin et al. 2012) and abiotic stress responses (Wang et al. 2006; del Río 2015). Prxs are important proteins at the crossroads of the ROS and NO signaling pathways, because they can reduce the H_2O_2, peroxynitrite, and organic peroxides generated in the cell, through their catalytic cysteine residues by using the thiol-containing Trx system (Mock and Dietz 2016). Moreover, transduction and specificity of ROS and RNS bioactivity under physiological processes as well as under stress conditions involve posttranslational modification of proteins, such as sulfenylation, *S*-glutathionylation, nitration, and *S*-nitrosylation (Mittler et al. 2004; Corpas et al. 2009; Lozano-Juste et al. 2011; Ortega-Galisteo et al. 2012; Lázaro et al. 2013; Camejo et al. 2015).

In this chapter, we will focus on the role of antioxidant and redox-modulated enzymes in chloroplasts and the effects played by ROS, mainly H_2O_2 and NO on the photosynthesis functionality through specific targets. The sources of NO in plants cells together with some examples of posttranslational modification driven by ROS and RNS in chloroplasts proteins are also presented.

2 ROS Metabolism

Oxygen (O_2) represents approximately 21% of the volume of the composition of the terrestrial atmosphere. The reduction of the oxygen to water provides the energy that allows the impressive complexity of the superior organisms, but when its reduction is not complete, the generated species are called ROS.

2.1 ROS Generation in Plants

These reactive species are generated as products of the aerobic metabolism in the different cellular compartments as a by-product of a wide variety of processes, among them those which imply transport of electrons such as respiration in mitochondria and photosynthesis in chloroplasts (Asada 2006; Sevilla et al. 2015b) or during photorespiration and oxidation of fatty acids in peroxisomes and glyoxysomes (del Río and López-Huertas 2016). Also certain enzymatic activities generate ROS, such as NADPH-oxidoreductases and peroxidases of the plasmatic membrane and cell wall, respectively (Mittler et al. 2004) (Table 1).

A common feature of ROS is the generation of oxidative damage to proteins inactivating enzymes by oxidizing thiol groups or producing redox changes in the metals of the active centers. They also cause breaks and mutations in the DNA oxidizing the aromatic compounds of this molecule, as well as altering the functionality of the lipids in the membranes forming hydroperoxides.

Light energy distribution in the photosynthetic process regulates ROS production in leaf tissues (Tikkanen et al. 2014). In chloroplasts, ROS are generated within the electron transport chains (ETC) of PSII and PSI, and this production is increased as consequence of stressful conditions when CO_2 is limited and ATP synthesis is impaired (Nishiyama and Murata 2014; Noctor et al. 2014). The oxygen-evolving complex (OEC) in PSII liberates O_2 from H_2O, and electrons are then transferred to PSI via ETC from plastocyanin (PC) to ferredoxin (Fd) (Tikkanen and Aro 2014). FNR can then reduce NADP+ to NADPH from the reduced Fd (Fig. 1) (Rochaix

Table 1 ROS generation in different plant cell compartments

Localization	Process	ROS
Chloroplasts	Photosynthesis PSI, PSII	$O_2^{\bullet-}$
	Excited chlorophyll	$^1O_2$
Mitochondria	Respiration CI and CIII	$O_2^{\bullet-}$
Peroxisomes	Xantine oxidase	$O_2^{\bullet-}$
	Glycolate oxidase	H_2O_2
	Fatty acids β-oxidation	H_2O_2
Plasma membrane	NADPH oxidase	$O_2^{\bullet-}$
Cell wall	Peroxidases, Mn^{2+} and NADH	$H_2O_2/O_2^{\bullet-}$
Apoplast	Oxalate and amine oxidase	H_2O_2

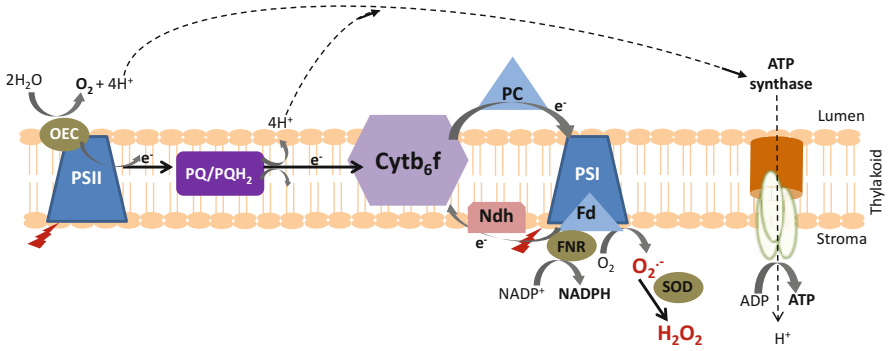

Fig. 1 Generation of ROS in the photosynthetic electron transport chain. Linear electron flow (continuous arrows) is catalyzed by PSII from water oxidation through PQ pool to Cytb6f and plastocyanin to PSI and ferredoxin. Ultimately, FNR reduces $NADP^+$ to NADPH. Electrons can be derived to O_2 to generate $O_2^{\bullet-}$ which dismutates to H_2O_2 by SOD. Cyclic electron flow occurs through PSI and Cytb6f using Fd, NADPH, and Ndh complex or directly from Fd to Cytc in the Cytb6 complex. *Cytb6f* cytochrome b6f complex, *Fd* ferredoxin, *FNR* Fd reductase, *Ndh* NADH dehydrogenase, *OEC* oxygen-evolving complex, *PSI* photosystem I, *PSII* photosystem II, *PC* plastocyanin, *PQ* plastoquinone, *SOD* superoxide dismutase (modified from Rochaix 2013)

2013). The cyclic electron flow (CEF) occurs through PSI and Cytb6f mainly in the stroma by two pathways, one involving Fd, NADPH, and the NADH dehydrogenase (Ndh) complex and the other involving Fd and Cytc in the Cytb6f complex. Electron transport coupled to proton translocation generates a pH gradient that drives ATP synthase to produce ATP (Rochaix 2014). These reactions produce ROS, which may cause oxidative damage and disruption of OEC, mainly under continuous exposure to high light (HL), so provoking photoinhibition (Murata et al. 2007; Gururani et al. 2015). Additionally, the pseudo-CEF comprises the so-called Mehler reaction, which is very important in the dark-light induction phase or during CO_2-limited photosynthesis (Asada 2006; Michelet and Krieger-Liszkay 2012). This reaction consists of the direct reduction of dioxygen at the acceptor side of PSI with consequent production of $O_2^{\bullet-}$. This ROS can be generated under certain stress conditions, such as high light intensity and a low concentration of CO_2, diminishing NADP availability in the chloroplast and thus increasing the electronic flow to $O_2^{\bullet-}$. In turn, the $O_2^{\bullet-}$ can protonate to $HO^{\bullet}_2$ in the internal surface of thylakoid membrane (lumen) or dismutate to H_2O_2 spontaneously or by the Fe and Cu,Zn superoxide dismutase enzymes (SODs), in the external surface of the membrane of the thylakoid or in the stroma (Gómez et al. 2004; Asada 2006; Pospíšil 2012) (Fig. 2). Also, chloroplastic NADPH oxidase-like activity has been shown to mediate H_2O_2 generation in *Brassica napus* (Tewari et al. 2012). This H_2O_2 is mainly detoxified by the activity of ascorbate peroxidase (APX) present in thylakoids and stroma (Gómez et al. 2004) in collaboration with the other components of the so-called ascorbate-glutathione (ASC-GSH) cycle, first described in chloroplasts, and/or by the activity of the different peroxiredoxins (Prxs) (Dietz 2016) in accordance with thioredoxins

(Trxs) and sulfiredoxins (Srxs). All these systems collaborate in the so-called water-water cycle, which, in fact, supposes the reduction of $O_2^{\bullet-}$ into H_2O. It has two quite important main functions under redox-stress conditions: photoprotection and ATP/NADPH ratio balancing (Asada 2006; Eberhard et al. 2008; Foyer and Shigeoka 2011). Another interesting photo-protective strategy involves a class of flavodiiron proteins (FLVs), located in the proximity of PSII and/or PSI. These proteins present several domains, including a flavodoxin-like and an NAD(P)H-flavin reductase-like domain. They are present in *Chlamydomonas*, bryophyte, and gymnosperms although they have been reported in Archaea and anaerobic bacteria and are able to reduce O_2 to water using NADPH as electron donor. They are believed to protect PSI from light stress, under changing light conditions (Gerotto et al. 2016), while another form of the protein FLV2/FLV4 seems to be active in photoprotection of PSII (Allahverdiyeva et al. 2015).

ROS can also be generated in the Fe-S centers in which the Fe^{2+} is involved in Fenton's reaction, where H_2O_2 produces $^{\bullet}OH$, one of the most powerful oxidants (Halliwell and Gutteridge 2015). Another reactive species generated in these organelles is $^1O_2$ as product of the activity lipoxygenase (Triantaphylides and Havaux 2009), and it is also linked to the excitation of chlorophylls associated with the

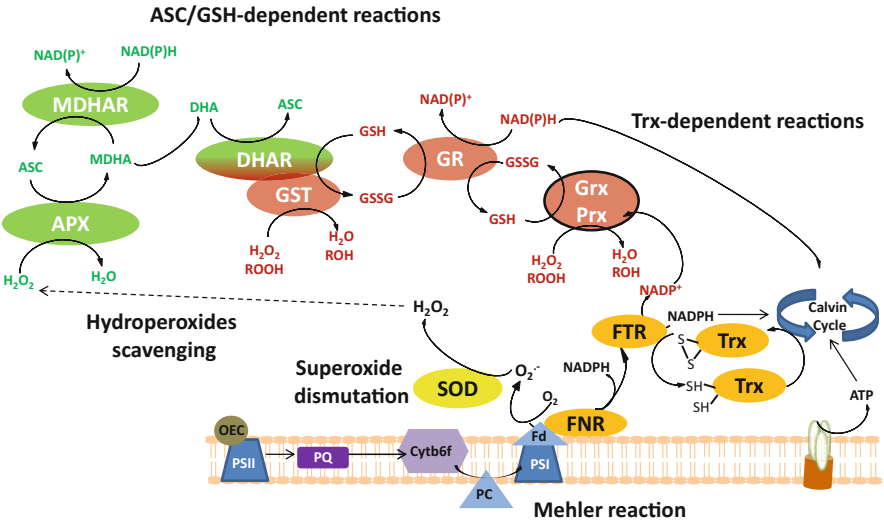

Fig. 2 The water-water cycle and the antioxidant system involved in ROS scavenging in chloroplasts. Pseudo-cyclic electron flow occurs in the PSI (Mehler reaction) to generate $O_2^{\bullet-}$ which dismutates to H_2O_2. ASC-GSH cycle in coordination with the Trx/Prx/Grx system is in charge of the control of hydroperoxide (ROOH, H_2O_2) levels. *APX* ascorbate peroxidase, *Cytb6f* cytochrome b6f complex, *DHAR* dehydroascorbate reductase, *Fd* ferredoxin, *FNR* Fd reductase, *GST* glutathione S-transferase, *GR* glutathione reductase, *GRX* glutaredoxin, *MDHAR* monodehydroascorbate reductase; PSI, photosystem I, *PSII* photosystem II, *Prx* peroxiredoxin, *Trx* thioredoxin, *TR* thioredoxin reductase, *PC* plastocyanin, *SOD* superoxide dismutase (modified from Foyer and Noctor 2011)

electron transport. Specifically it is produced in the PSII and its light-harvesting antennae (Shapiguzov et al. 2012) and is able to promote cell death and to regulate gene expression (Telfer 2014; Laloi and Havaux 2015). Carotenoids are the main $^1O_2$ scavengers in the chloroplast, and its oxidized form has been shown to induce gene expression of singlet oxygen signaling pathway but with scarce effect on H_2O_2-responsive gene expression (Ramel et al. 2012).

Several alternative electron pathways have been proposed to increase ATP in the chloroplast, and among them, cyclic electron flow around photosystem I (CEF) is the most important. In this cycle, electrons flow from PSI to PQ, producing ATP without generating of NADPH. This cycle has been reported to be activated by H_2O_2 produced by the imbalance of chloroplast redox state in vivo (Strand et al. 2015), probably through the regulation of proteins involved in the antimycin A-insensitive pathway of CEF or by inactivation of Calvin cycle enzymes.

2.2 ROS Scavenging

2.2.1 ASC-GSH Cycle and SOD

Related to the scavenging and redox control of H_2O_2, as mentioned above, the ASC-GSH cycle has been described in chloroplast and later on in other cellular compartments as mitochondria, peroxisomes, apoplast, and cytosol (Jiménez et al. 1997; del Río and López-Huertas 2016), involving the antioxidants ascorbate and glutathione and the enzymes ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR). In this cycle, the H_2O_2 is eliminated by the APX using ASC as electron donor (Fig. 2). The MDHA formed, and from this, the DHA resulting spontaneous dismutation is used for recycling the ASC by the action of NADH-dependent MDHAR and by DHAR, which uses reduced GSH as electron donors, respectively. DHA can also suffer an irreversible hydrolysis, so DHAR may have a significant role in the maintenance of reduced ASC pool at cellular level (Gallie 2013).The role of this cycle in chloroplast has been reported under many different stress situations (Noctor and Foyer 1998; Gómez et al. 1999; Sharma and Dubey 2005; Lázaro et al. 2013; Bartoli et al. 2017; Ortiz-Espín et al. 2017a, b). As an example of the beneficial role of one of the components APX, overexpression of this enzyme in chloroplast, cytosol, and peroxisome was reported to enhance tolerance to salinity, cold, and heat stresses, diminishing the oxidative damage induced (Badawi et al. 2004; Lu et al. 2007; Wei-Feng et al. 2008). On the other hand, mutants lacking stromal and thylakoidal APX are more susceptible to photooxidative damage induced by high light (Kangasjärvi et al. 2008), and increase in ASC and GSH is a response to high irradiation (Bartoli et al. 2009). An antagonist effect of H_2O_2 and $^1O_2$ has been reported in chloroplast when overexpression of tAPX decreased H_2O_2 levels but increased $^1O_2$-mediated response in an excess mutant *Arabidopsis flu* (*Arabidopsis* fluorescent, Laloi et al. 2007). An increase in chloroplast H_2O_2 was

reported in pea plants under salinity together with augmented Fe-SOD, Cu, Zn-SOD II and APX, DHAR, and GR activities (Gómez et al. 1999). These last two enzymes seem to be responsible for the regeneration of the oxidized antioxidants ascorbate and glutathione in this condition and, all together, collaborate to cope with the stress situation. Later on, an increase in H_2O_2 by a decrease in tAPX parallel to increased thylakoid- and stroma-located Fe-SOD and Cu,Zn-SOD activities was shown in these pea plants under salinity (Gómez et al. 2004), with a proposed role for these isoenzymes in the signaling events leading to plant acclimation. All these examples demonstrate the fine tuning of the different organelles through the regulation of the SOD isoenzymes and ASC-GSH components in the protection and acclimation to changing environment.

Alternatively, the regeneration of ASC may also take place due to other redox compounds such as glutaredoxin (Grx) and Trx system (Meyer et al. 2012; Potters et al. 2002), as described below. Oxidized glutathione (GSSG) can also diminish due to the NADPH-dependent thioredoxin reductase (NTR) (Barranco-Medina et al. 2007; Martí et al. 2009; Cejudo et al. 2014). In consequence, the cellular redox status will depend partly on the thiol and disulfide groups of the antioxidants, among others, the antioxidant GSH, and the proteins Trx, Prx, Srx, and Grx. On the other hand, it is considered that the redox state in the different cellular compartments depends to a great extent on the relative rate between reduced and oxidized groups of GSH and Trx (Go and Jones 2010; Foyer and Noctor 2013; Yoshida et al. 2018).

The accepted role of ROS/RNS as part of signaling events is changing the traditional concept of antioxidant systems in charge of their control, to that of "ROS processes systems," as recently suggested by Foyer (2018). In fact, cellular components that interact with ROS are able to transmit oxidative signals from the different cell compartments, including chloroplasts, as described for phytohormones involved in plant growth and stress response (Bartoli et al. 2013; Xia et al. 2015; Foyer et al. 2017).

2.2.2 Thioredoxins

Thioredoxins (Trxs) are small oxidoreductase proteins of around 12–14 kDa involved in the reduction of the disulfide bonds of other proteins through a dithiol-disulfide exchange, in this way modulating the stability and function of their target proteins (Sevilla et al. 2015a, b). These enzymes present an active preserved site formed by a few amino acids and two cysteine residues (-WC(G/P)PC-). The maintenance in the cell of the reduced state of Trx depends on an external electron donor in every cellular compartment. In this way, mitochondrial, cytoplasmic, and nuclear Trxs are reduced by NADPH-dependent TR (NTR), which uses NADPH as a donor of electrons via FAD, whereas the chloroplastic Trxs use the electrons proceeding from the ET via ferredoxin (Fd) to be reduced by Fd/TR (FTR) (Balsera et al. 2014; Cejudo et al. 2014). A different NADPH NTR (NTRC) has been described in chloroplasts and nonphotosynthetic plastids presenting two sites, one Trx and another NTR, in the same polypeptide chain (Serrato et al. 2004;

Kirchsteiger et al. 2012), allowing to function also as Trx on the target chloroplastic 2-Cys Prx (Spinola et al. 2008; Pérez-Ruiz et al. 2009). This is an alternative system to the ferredoxin-dependent one, using NADPH produced by ET in the photosynthetic process during the day or in the first reactions of the oxidative pentose phosphate pathway during the night (Pérez-Ruiz et al. 2006; Alkhalfioui et al. 2007).

Location of Trxs is mainly cytoplasmic and chloroplastic, although there are members of the family in mitochondria, nucleus, apoplast, and endoplasmic reticulum (Gelhaye et al. 2004; Martí et al. 2009; Pulido et al. 2009; Meng et al. 2010; Calderón et al. 2017a). Among the chloroplastic Trxs, several types are described in *Arabidopsis*: Trxs *f* and *m* with important functions in the regulation of photosynthesis, specifically in the Calvin cycle (Jacquot et al. 1978; Wolosiuk et al. 1979), and also in the light-driven synthesis of ATP, catalyzed by ATP synthase. Recently, it has been described that this Trx-dependent redox modulation of ATP synthase may be involved in regulating the dark stability of the photosynthetic apparatus, most likely by controlling thylakoid membrane transport of proteins and ions (Kohzuma et al. 2017).

Other chloroplasts Trxs include Trx *x* involved in the oxidative stress by activation of 2-Cys Prxs (Mestres-Ortega and Meyer 1999; Pulido et al. 2010), Trx *y* with the capacity to reduce different plastidial Prxs (Collin et al. 2004; Lemaire et al. 2003), and Trx *z* (Arsova et al. 2010), presenting an important function in the development of the chloroplast by controlling the transcription of specific genes (Rivas et al. 2004; Pfalz et al. 2006). Trx *z* is required for the PEP polymerase and the PEP-dependent transcription of plastid genes like psbA (D1 protein of PSII) (Dietz and Pfannschmidt 2011). Interestingly, this Trx *z* has a unique function, due to its property of being reduced by other Trxs (Chibani et al. 2011). A role for Trx *x*, Trx *y* together with the atypical CDSP32 has also been described in the response to oxidative stress in chloroplasts (Broin 2002; Collin et al. 2003, 2004). CDSP32 protein has an important role during drought (Broin 2002), and the APR (*a*denosine 5′-*p*hosphosulfate *r*eductase) proteins, similar to PDI, participate in the sulfur metabolism through the reduction of sulfate to sulfite, among other functions (Martin et al. 2005). As an interesting example, another chloroplast stroma-localized atypical Trx from *Arabidopsis*, designated as Trx-like2 (TrxL2), has been characterized as presenting redox-active properties with an unusually lower negative redox potential (Yoshida et al. 2018). This Trx interacts with a range of chloroplast redox-regulated proteins with the special characteristic that it is able to efficiently oxidize, but not reduce, its target proteins Rubisco activase (RCA), fructose-1,6-bisphosphatase (FBPase), and sedoheptulose-1,7-bisphosphatase (SBPase) efficiently. In fact, Trx *f* could do it as well but much less efficiently. TrxL2 is then a redox-mediator protein specialized in oxidizing redox-regulated proteins and is strongly dependent on 2-Cys Prx and H_2O_2. Interestingly, it seems that TrxL2/2CP allows chloroplasts to turn off the photosynthetic metabolism at night as an acquired mechanism of land plants.

Taking into account the identification of target proteins and the phenotype of transformed plants, we can assume that the main function of Trxs is their action as redox proteins and antioxidants. A multifunction has been described for Trxs in several processes including plant development via regulation of the embryo

formation, control of intercellular communication, reserve mobilization, chloroplast structure and biogenesis, carbon and phosphate metabolism, plant cell death, photorespiration, plant respiration, ATP generation, stress response, and redox regulation of cellular homeostasis in the different compartments including chloroplasts (Reichheld et al. 2007; Martí et al. 2009, 2011; Arsova et al. 2010; Lázaro et al. 2013; Calderón et al. 2017a, 2018; Ying et al. 2017). The participation of Trx in the control of the redox state of Cys residues in the cellular proteome is a key function because it allows the modulation of the protein function in various cellular cycles such as the Calvin cycle, cellular cycle, or tricarboxylic acid cycle (Daloso et al. 2015; Calderón et al. 2017a). As an antioxidant it is able to transfer electrons in a cascade of oxido-reduction to eliminate ROS. An example in chloroplast is the activation by reduction of PrxIIE, an enzyme able to eliminate H_2O_2, among other hydroperoxides (Gama et al. 2008).

One interesting concept is the regulation by reductants of the ROS production and antioxidant regeneration, described by Foyer and Noctor (2016). In chloroplast, a "push-pull model" was presented by these authors for the dependence of ROS generation on reductants in light, when Fd-NTR/Trx pools become more reduced. In this situation, an increase in ROS is favored by an excess of reductants, which has been described as "reductive stress."

2.2.3 Peroxiredoxins and Sulfiredoxins

Peroxiredoxins (Prxs) are a family of peroxidases dependent on thiol groups that catalyze the scavenging of peroxides (ROOH) from H_2O_2, alkyl hydroperoxides, and peroxynitrite to water and the corresponding alcohol (ROH) (Bryk et al. 2000; Rhee et al. 2005; Pedrajas and Bárcena 2018). In plants, the Prxs are classified into four subclasses depending on the number and position of the conserved Cys residues: 1-Cys-Prxs, 2-Cys Prxs, Prx associated to bacterioferritin (Prx Q), and Prx type II (associated to the YLR109 protein). In this last group, there are several members in *Arabidopsis*, poplar, pea, and rice with cytoplasmic, PrxIIC; plastidial, PrxIIE; and mitochondrial location, PrxIIF (Horling et al. 2002; Dietz et al. 2006; Barranco-Medina et al. 2007; Martí et al. 2009). It has been demonstrated that Prxs participate in several processes as 1-Cys-Prx does in seed germination (Haslekås et al. 2003) and in photooxidative stress in chloroplasts (Prx Q) (Rey et al. 2005).

During the catalytic cycle, Prxs are oxidized by H_2O_2 to sulfenic form (Cys-SOH) in the peroxidatic cysteine. The disulfure form and/or sulfenic form is then specifically reduced by cellular thiol groups as Trx or Grx (Barranco-Medina et al. 2007, 2009; Meyer et al. 2012; Couturier et al. 2013) (Fig. 3, lower part). Moreover, the sulfenic form can be over-oxidized by H_2O_2 to the more stable sulfinic form ($Cys\text{-}SO_2H$) with concomitant inactivation of the peroxidase activity, allowing a transient H_2O_2 accumulation which could act as signaling event in response to the oxidative situation (D'Autreaux and Toledano 2007). The action mechanism of Prx is not limited only to its peroxidase function but also to the chaperone modulation

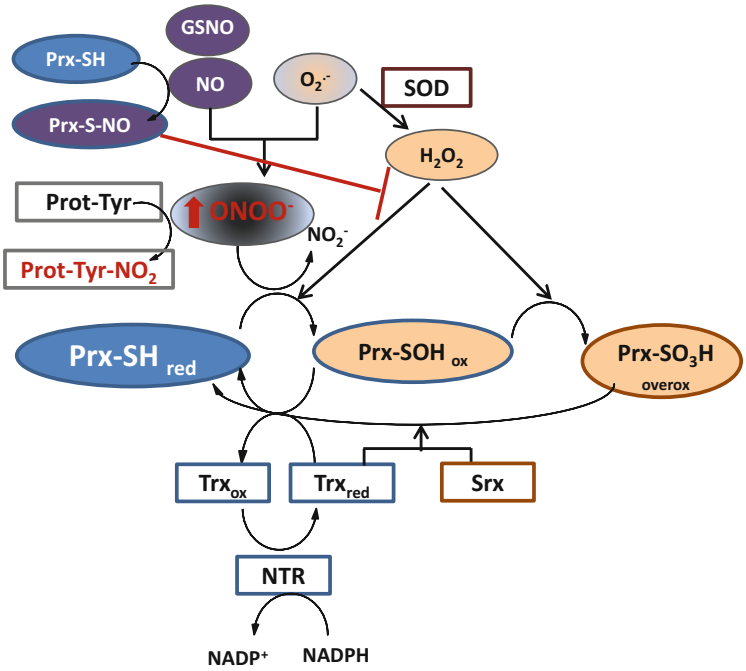

Fig. 3 Proposed mechanism of action of reactive oxygen and nitrogen species on PrxIIE. Dismutation of $O_2^{\bullet-}$ by superoxide dismutase (SOD) generates H_2O_2 which oxidizes and inactivates the peroxidase PrxIIE, which is regenerated from its oxidized (ox) forms by the thioredoxin (Trx)/thioredoxin reductase (NTR)/sulfiredoxin (Srx) system at expense of NADPH. $NO/O_2^{\bullet-}$ reaction leads to the formation of $ONOO^-$ which could be metabolized by reduced (red) PrxIIE. The *S*-nitrosylation of PrxIIE by NO or nitrosoglutathione (GSNO) inhibits its peroxynitrite reductase activity (in red) provoking an increase in Tyr nitration of target proteins (modified from Romero-Puertas et al. 2007; Iglesias-Baena et al. 2010)

protecting cellular structures from over-oxidation (Finkemeier et al. 2005; Barranco-Medina et al. 2008, 2009). On the other hand, changes in oligomerization and redox states lead to postulate that Prxs could translate and inform about cellular H_2O_2. In this sense, these proteins could act as very sensitive H_2O_2 sensors linking oxidative metabolism to a variety of processes dependent on redox state in the different compartments.

The over-oxidized form of Prx can be reverted by sulfiredoxin (Srx) in an ATP-dependent manner in the 2-Cys Prx (Iglesias-Baena et al. 2010). This mechanism operates for plant Srx, which presents a double localization: mitochondrial and chloroplastic (Iglesias-Baena et al. 2010, 2011). However, the hyper-oxidation to a sulfonic form is considered to be an irreversible mechanism. The function of the Srx relates to the maintenance of the redox balance. As an example, it has been shown that under H_2O_2 treatment *AtSrx* gene expression increased and that KO *AtSrx* mutants present higher oxidative stress than wild-type plants. Also, paraquat

treatment provokes an increase in ROS levels in chloroplast of KO mutants, whereas this hypersensitivity is reversed on having restored AtSrx's expression by genetic transformation. This information indicates that the expression and AtSrx's function in chloroplasts are essential for a suitable response of the plant to the conditions of stress (Liu et al. 2006). This role in the balance redox, accompanied by its specificity for Prx and the need for a reductant, such as Trx, to carry out Srx activity, as well as the union of Trx-Prx, has led to the description of the existence in the cell of a system of redox regulation formed by Trx, Prx, and Srx—the so-called Trx/Prx/Srx system. These proteins participate in the signaling network to perceive and transmit mainly ROS and redox sensors (Sevilla et al. 2015a, b).

3 NO Metabolism

3.1 NO Synthesis in Plants

Biosynthesis of NO in plants is still a matter of debate, and several sources have been proposed as possible pathways for NO generation (Gupta et al. 2011; Mur et al. 2013), which depend upon either reductive or oxidative routes taking place in different cell compartments, including cytosol, mitochondria, chloroplasts and plastids, peroxisomes, and apoplast, where RNS and ROS accumulate during stress (Jasid et al. 2006; Corpas et al. 2009; Igamberdiev et al. 2014; del Río 2015; Astier et al. 2018). In mammals, NO is synthesized via an oxidative mechanism by NO synthase (NOS: EC 1.14.13.39), which consists of three well-characterized isoforms, endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) (Alderton et al. 2001), and which was originally isolated from macrophages. NOS proteins use L-arginine as substrate, generating a hydroxyl-arginine intermediate, to produce citrulline and NO. The reaction is NADPH dependent, utilizes O_2 as a co-substrate, and needs the presence of tetrahydrobiopterin (BH_4). Recently, a related protein has been discovered in *Ostreococcus tauri*, a single-cell green algae that shares a common ancestor with higher plants, bacteria, and human. The NOS sequence possesses 45% similarity to human NOS and exhibited NOS activity in vitro, showing similar properties to animal NOS proteins (Foresi et al. 2010). Genes encoding a structurally related NOS enzyme have not been identified in higher plants, even though over 1000 transcriptome sequences of land plants have been analyzed for the presence of canonical NOS sequences (Jeandroz et al. 2016). These results indicate that the NOS activity reported in plants comes from a different type of enzyme (Gas et al. 2009). Taken together, the emerging evidence supports that NO generation could occur from multiple reductive and oxidative sources in land plants and also in different cellular localization.

3.2 Sources of NO in Plants

Reductive routes are dependent upon nitrite as the primary substrate and include reduction via nitrate reductase (NR EC 1.7.1.1), a plasma membrane-bound nitrite-NO reductase (NiNOR) and mitochondrial nitrite reduction. Nitrate reductase (NR) is a key enzyme for NO production in plants and facilitates NO homeostasis (Chamizo-Ampudia et al. 2011). NR in addition to the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) can also catalyze the reduction of nitrite to NO. However, the efficiency of this reaction is low, and it requires small oxygen tensions and light and high nitrite concentrations (Rockel et al. 2002; Igamberdiev et al. 2014). NO production via NR was demonstrated either in vivo as in vitro (Rockel et al. 2002; Seligman et al. 2008). It has been shown that under in vitro conditions, commercial purified maize NR generated NO under ambient air conditions (Yamasaki et al. 1999; Ischiropoulos 2003). In *Arabidopsis*, NR enzyme is encoded through two homologues genes (*Nia1*and *Nia2*) (Chaki et al. 2015). Double *nia1nia2* mutants were used to demonstrate the involvement of NR in some physiological processes. Thus, NR was shown to be involved in the NO generation during the control of stomatal closure induced by ABA and other physiological processes as flower development (Seligman et al. 2008) and plant immunity (Thalineau et al. 2016).

The presence of a PM-bound NiNOR enzyme has been suggested in tobacco roots plasma membrane (Stöhr et al. 2001). The nitrite as substrate for NiNOR is probably provided by plasma membrane-bound NR in a coupled reaction. Unfortunately, the identity of NiNOR remains to be determined. Moreover the nonenzymatic reduction of nitrite leading to the formation of NO and nitrate is known to be favored at acidic pH values, such as those in apoplasts and plastids (Cooney et al. 1994; Bethke et al. 2004).

Mitochondria also support the reduction of nitrite to NO (Tischner et al. 2004; Modolo et al. 2005; Igamberdiev et al. 2014) in the mitochondrial inner membrane, probably via cytochrome c oxidase and/or reductase (Planchet and Kaiser 2006). This process contributes to ATP production under hypoxic conditions (Stoimenova et al. 2007). Pharmacological evidence also suggests that complex III and AOX are both involved in nitrite to NO reduction, although a clear mechanism is established only for cytochrome oxidase under hypoxia (Gupta and Igamberdiev 2016).

In peroxisomes, as other source, NO can also be generated by reversible regulation of *S*-nitrosoglutathione (GSNO) by the action of GSNO reductase (GSNOR) (Corpas et al. 2013a).

Regarding the oxidative NO sources, evidence for the production of NO through an L-Arg-dependent NOS-like activity and via polyamine-/hydroxylamine-mediated synthesis has been described (Delledonne et al. 2001; Corpas et al. 2006, 2017; Durner et al. 1998). In this regard, Guo and Crawford (2005) showed that the *Arabidopsis* protein named NO synthase 1 (AtNOS1) is targeted to the mitochondria. This protein was further characterized as a functional small GTPase and therefore renamed AtNOA1 (nitric oxide associated 1) (Moreau et al. 2008) which might be implied in mitochondrial biogenesis. To date, in plant mitochondria, in

contrast with mammalian tissue, the production of NO by a NOS-like enzyme remains elusive (Astier et al. 2018).

The localization of a NOS-like activity has been also reported in the subcellular compartments of peroxisomes and chloroplasts, both organelles taking part in NO production in environmental conditions (Jasid et al. 2006; Corpas and Barroso 2014).

On the other hand, increases in the concentrations of the polyamines spermine and spermidine induce NO release, but the actual reaction mechanism has not yet been determined. Recently it has been reported that in *Arabidopsis thaliana*, copper-containing amino oxidase 1 (CuAO1) mediates the generation of NO in response to both polyamine and ABA (Tun et al. 2006).

3.3 NO Generation in Chloroplasts

Early evidence obtained by fluorescence microscopy (Foissner et al. 2000; Gould et al. 2003) and immunogold electron microscopy (Barroso et al. 1999) suggested that chloroplasts participate in NO synthesis in plants. Moreover, it was reported that chloroplasts were the first organelle where NO increased after elicitation of rape (*B. napus*) cells with cryptogein (Foissner et al. 2000). Further evidence obtained in soybean showed that at least two pathways for NO production are operative in chloroplasts: one of them was dependent on L-Arg-dependent NOS-like enzyme activity and the other on nitrite, as suggested by in vitro exposure assays (Jasid et al. 2006). In this regard, based on loss of function of *Atnoa1* mutants, it has been also sustained that chloroplasts are involved in NO generation (Gas et al. 2009), and it is known that AtNOA1, as a cyclic GTPase, is involved in protein translation in the chloroplast (Gas et al. 2009; Chen et al. 2010). The decrease in accumulation of NO in *Atnoa1* mutants has been indirectly linked to the inability of that mutant to fix carbon, resulting in a decrease in fumarate stores, which is inherently leading to a decrease in L-arginine accumulation and chloroplast dysfunction (Van Ree et al. 2011). Phenotype of maize NOA1 knockdown is lethal. Recently, the study of generation of NO in *B. napus* without any external supply of arginine or NO donors suggests the involvement of a NOS-like protein, but not nitrate reductase, in NO generation in the leaf chloroplasts and protoplasts (Tewari et al. 2013). Additionally, the reduction of nitrite to NO by ascorbic acid under microlocalized pH conditions has also been proposed in chloroplasts (Beligni et al. 2002). The light-mediated reduction of NO_2 by carotenoids is also a source of NO generation (Cooney et al. 1994).

3.4 NO Targets in Chloroplasts

There are interesting reviews on the effects of NO in chloroplasts mainly in the photosynthetic process (Misra et al. 2014; Simontacchi et al. 2015; Turkan 2018). In this chapter, we present some examples using mutant plants with modified endogenous NO content as well as results obtained after exogenous treatments with NO donors. Early studies reported the reduction in CO_2 assimilation by NO (Hill and Bennett 1970). Furthermore, both photosynthesis and photorespiration have been found to be affected by NO in different plants at concentrations below those required to result in visible injuries (Yamasaki 2000; Takahashi and Yamasaki 2002). Elsewhere, it has been reported that NO is involved in light-mediated greening of barley seedlings (Zhang et al. 2006) and it stimulates chlorophyll biosynthesis and chloroplast differentiation by increasing iron availability (Graziano et al. 2002; Kumar et al. 2010).

The analysis of *NOA1*-suppressed mutants which presented low contents of NO has revealed a lower PSII efficiency in comparison with WT plants, suggesting a decrease in the energy transfer efficiency (Liu et al. 2016). NO is able to bind reversibly to several sites in PSII and inhibit electron transport, so decreasing photosynthetic activity in isolated thylakoids of spinach (Sanakis et al. 1997). In this regard, by applying exogenous NO or different NO donors on leaves and/or isolated chloroplasts, several electron transport chain components of PSII have been identified as target sites of NO, including the non-heme iron between Q_A and Q_B quinone binding sites, which results in an important decrease of the electron transport rate between both Q_A and Q_B (Diner and Petrouleas 1990). Also, NO binds at the catalytic Mn cluster of the oxygen-evolving complex (OEC) (Schansker et al. 2002) and interacts with the tyrosine residue (Y_D) of the D2 protein (Sanakis et al. 1997). The action of NO as a reductant in destabilizing the excited states of the Mn cluster complex is well demonstrated, as is the decrease of phothosynthetic oxygen evolution in a NO-dependent manner (Schansker et al. 2002). Regarding the Yp-NO couple, NO binding seems to result in a decreased redox potential that becomes a more efficient electron donor in isolated thylakoids than the immediate redox-active tyrosine residue Yz in D1 protein (Vladkova et al. 2011). As another example, the interaction of NO with cytochrome b_6f complex—the electron transport mediator between PSII and PSI complex—has also been reported. The possible involvement of this NO cytb_6f interaction in signaling pathways, by modulation of cyclic electron flow around PSI, and so regulating redox potential in chloroplasts, has been suggested (Twigg et al. 2009). Interestingly, it has been observed that mutants that exhibit constitutively high rates of cyclic electron flow (CEF) also present increased production of H_2O_2. Further evidence appeared that H_2O_2 resulting from imbalances on plant chloroplasts redox state acts as signaling agent to activate CEF in vivo (Strand et al. 2015).

On the other hand, most of the studies employing exogenous application of NO and/or application of NO chemical donors find that NO effects in photosynthesis are dependent on the nature and concentration of the donor used and also on

environmental conditions. Contradictory results may be obtained if they are analyzed under nonstress or under stressful conditions (Misra et al. 2014). As an example, leaves treated with sodium nitroprusside (SNP) and GSNO showed decreased maximum quantum efficiency (Fv/Fm). However, SNP-treated leaves under different abiotic stresses (including drought, salinity, heat, UVB, metal toxicity) had higher Fv/Fm when compared with the nonstressed SNP-treated leaves, pointing to the stimulating effect of the NO donor treatment on PSII photochemistry, in contrast to that described under nonstress conditions. This positive effect of SNP on PSII has been correlated with an enhancement in the proportion of the open PSII reaction centers (Gupta et al. 2012; Manjunatha et al. 2012). Other protective effects have also been reported by NO under stressful conditions. As an example, and related to that commented on above, NO has been shown to prevent chlorophyll loss. This was shown in sunflower plants subjected to Cd-induced chlorophyll decay (Laspina et al. 2005), where the results were similar to those reported in mesophyll cells from Fe-deficient maize plants, in which SNP prevented leaf chlorosis by significantly increasing chlorophyll content and chloroplasts development (Graziano et al. 2002). A protective effect of NO was also reported in relation to carbonyl and lipid peroxidation contents in chloroplasts (Jasid et al. 2006; Galatro et al. 2013).

Additionally, the effects of NO on metabolism related to photosynthesis have been reported to depend on its concentrations. As an example, Abat et al. (2008) described that NO slows photosynthesis through inhibiting Rubisco by *S*-nitrosylation in a dose-dependent manner (see below). In this context, an enhanced carbonic anhydrase activity was induced by micromolar levels of SNP treatment in tomato, which indirectly resulted in a constant supply of CO_2 to ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Opposite effects were observed at high NO concentrations, which decreased carbonic anhydrase as well as Rubisco activities (Ferreira et al. 2008; Hayat et al. 2011). This may also be related to the fact that elevated NO content induces the closure of stomata (García-Mata and Lamattina 2001).

4 ROS/RNS and Stress

It has been well established that ROS/RNS generation is a key feature of stress. Thus, the control of ROS/RNS levels by modulation of their production and scavenging can change the role or these species as toxic molecules to signaling ones, so enabling a good response and adaptation to the changing stressful conditions. Interestingly, chloroplasts as described above, together with other cell compartments, are ROS/NO producers and use oxygen/nitrogen derived undesired metabolites to send signals to other compartments (retrograde signaling), so provoking metabolic responses to avoid severe damages (Locato et al. 2018). In this way, redox and antioxidant systems are coordinated to counteract the oxidative stress situation imposed depending on the stress type, duration, and sensibility. As an example, it has been shown that H_2O_2 increase after downregulation of sAPX and

tAPX in defective *Arabidopsis* mutants provokes a negative regulation of a cold stress response but a positive regulation under biotic stress. In these situations, a different gene expression was observed, pointing to the specificity of the response (Maruta et al. 2012).

Another interesting concept related to retrograde regulation is the fact that not only ROS generated in the chloroplasts are involved as intermediates in signaling between this organelle and the nucleus, as reported during acclimation of photosynthesis (Galvez-Valdivieso and Mullineaux 2010), but that photosynthetic functions are also regulated by cues perceived in the apoplast (Shapiguzov et al. 2012), pointing to a larger network collaborating for the stress adaptation. Other plastidial components like glutathione and the Trx system are not only involved in dynamic regulation of photosynthesis but also seem to be able to communicate this redox state in the organelle to the cytoplasm (Bashandy et al. 2010; Noctor et al. 2012).

Several chloroplastic redox hubs, including the plastoquinone and the glutathione pools and the thioredoxin system, provide not only dynamic local regulation of photosynthesis but may also communicate the chloroplast redox status to the cytosol (Marty et al. 2009; Bashandy et al. 2010; Foyer and Noctor 2011; Noctor et al. 2012; Rochaix 2012). For example, the redox state of plastoquinone, a component of the photosynthetic electron transfer chain, is monitored through the thylakoid-associated protein kinase State Transition 7 (STN7). STN7-dependent phosphorylation of chloroplast proteins leads, on the one hand, to optimization of photosynthesis in response to changing light conditions (via the reversible reallocation of light-harvesting antennae called state transitions) and, on the other, to a retrograde signal (Bonardi et al. 2005; Rochaix 2012). Another redox sensor in chloroplast is SAL1 phosphatase, which is inhibited by oxidation (provoking the dimerization of the protein) and glutathionylation. SAL1 dephosphorylates PAP (phosphonucleotide 30-phosphoadenosine 50-phosphate) into AMP (adenosine monophosphate) (Chan et al. 2016). PAP has been shown to accumulate under drought and high light stress, and the translocation to the cytosol provokes RNA cleavage and transcription termination (Chan et al. 2016). In this way, SAL1 is considered as a sensor of changing redox status in the organelle, and ROS-induced accumulation of this and other metabolites may serve as retrograde signals to provoke an adequate stress response.

Another emerging concept is that all types of oxidative modifications may be involved in signaling (Foyer et al. 2017) and ROS can act as death or life signals. In fact, PTMs induced by ROS/RNS are being considered as part of the redox signaling network inducing (or not) the repair, regulation or even desirable cell death, as part of the acclimation or fight against external invasion (biotic stress) or changing abiotic stressful environments.

5 ROS-/RNS-Mediated Protein Modifications

The specificity of redox signaling is achieved by reactive oxygen and nitrogen species generated in the vicinity of certain susceptible proteins in the different cell compartments in which an appropriate environment may support redox-based post-translational modifications (PTMs) (Umbreen et al. 2018). Interestingly, the reversibility of certain modifications is also part of the specificity as well as of the interactions among the different ROS/RNS players and the proteins involved in this reversibility.

5.1 Sulfenylation

ROS have a strong impact in the chemistry of thiol groups in proteins. Cysteine sulfenylation (–SOH) is a posttranslational modification related to the ROS-mediated oxidative product of a thiol (–SH) (Akter et al. 2017). An efficient photosynthetic carbon metabolism is necessary, and plants have evolved to adjust their physiology toward light fluctuations. One important mechanism is the reversible activation-inactivation during light/dark cycles due to reduction-oxidation switches of Cys residues, termed as redox regulation (Yoshida et al. 2018).

A lot of work is going on to discover the sensor proteins of ROS as a means of transduction of stimuli able to provoke a response to cope with the stress situations. Indeed, plant protein sulfenylation (sulfenome) under oxidative stress is considered a validated method in which reactive cysteine thiols are identified. Several chloroplastic proteins have thus been identified such as some specific Calvin cycle enzymes (Michelet et al. 2013), ribulose bisphosphate carboxylase, ATP synthase, NADP-dependent malate dehydrogenase, adenosine kinase 1, and glutamine synthetase 2 (Akter et al. 2017). Another experimental approach is the identification of chloroplastic thioredoxin redox-regulated protein targets. In this sense, a lot of research has been carried out with many different proteins identified that cover the main processes in the organelle, including gene expression, photosynthesis, Calvin cycle, biogenesis of plastids, translation, biosynthetic and antioxidant metabolisms, and stress response (review by Montrichard et al. 2009; Nikkanen et al. 2017). Usually oxidation of the proteins provokes inactivation of the enzymatic activity pointing thioredoxins as key components for protein functionality in both normal metabolism and stress responses. As an example, oxidation of Rubisco induces conformational changes and the PSII efficiency decreases, correlated with the sulfenylation state of the enzyme (Akter et al. 2017). Other redox-regulated proteins in the thylakoid lumen are immunophilin, FKBP13, polyphenol oxidase, and violaxanthin de-epoxidase, which are oxidatively activated by the oxygen release at the PSII site (Buchanan and Luan 2005). Also oxidation of peroxiredoxins provokes oligomerization, leading to a loss in the peroxidase function but gaining a role as chaperone (Barranco-Medina et al. 2009), although Cerveau et al. (2016) suggested

that chaperone function of chloroplastic 2-Cys Prx may not be essential in plants, due to the absence of abundant high-molecular-weight complexes under water deficit or photooxidative conditions. All these are examples that point to ROS-induced posttranslational modification sulfenylation as a key event in the regulation of the structure and function of essential processes in the chloroplasts with a high impact in the metabolism of living cells.

5.2 S-*Nitrosylation and Tyr Nitration*

NO mediates several posttranslational modifications (PTMs) in plants, such as protein tyrosine nitration and *S*-nitrosylation, which are processes that have a high impact in the activity of several enzymes collaborating with other defense mechanisms in the adaptation or tolerance to stress situations (Romero-Puertas et al. 2013; Chaki et al. 2011; Camejo et al. 2013, 2015). In the protein nitration, a nitro group ($-NO_2$) from nitric oxide is added to Tyr, Trp, Cys, or Met residues of a target protein (Corpas et al. 2009). *S*-nitrosylation, in turn, is the result of the covalent binding of an NO group to a Cys residue (Seth and Stamler 2011). Since both PTMs are involved in signaling, they are presented in more detail below.

Peroxynitrite results from the reaction between NO and $O_2^{\bullet-}$ and together with GSNO, acts as a natural reservoir and contributes to the control of NO in plant cells. $ONOO^-$ presents a very strong nitrating capacity to mediate Tyr nitration. Various studies on the nitro-Tyr proteome have revealed 127 proteins, with several targets located in different organelles including chloroplasts and mitochondria in *Arabidopsis* (Galetskiy et al. 2011a,b; Lozano-Juste et al. 2011; Tanou et al. 2012). In chloroplasts, nitrated enzymes are related to several processes, such as Calvin cycle, photosynthesis, ROS metabolism, and protein synthesis (Table 2). This PTM can irreversibly modify protein conformation, also affecting the catalytic activity and susceptibility to proteolysis (Corpas et al. 2009). As examples of its effect, Tyr nitration inhibits the activity of FNR and chloroplast SOD 3 (Chaki et al. 2011; Holzmeister et al. 2014). Other inactivated enzymes are G3PDH, carbonic anhydrase (Lozano-Juste et al. 2011; Chaki et al. 2013), or the destabilization of the electron transport from the PSI to Fd after Tyr nitration of the D1 protein (Galetskiy et al. 2011a), pointing to nitration as being related to photodamage and disassembly of complexes leading to protein degradation (Galetskiy et al. 2011a). NO treatment of thylakoid membranes isolated from pea leaves provoked the inhibition of the electron transport by binding of NO to several PSII sites as described above (Wodala et al. 2008), and Tyr nitration of D2 protein induced a decrease in the redox potential enough to become a more efficient electron donor than D1 protein (Vladkova et al. 2011). The presence of nitrotyrosine has been considered as a marker for strong nitrosative stress, due to the increase in $ONOO^-$ generation as a consequence of environmental and/or biotic stress conditions, although physiological protein nitration also occurs. Moreover, Tyr nitration is quite specific because only certain Tyr residues in target proteins are preferentially nitrated (Corpas et al. 2013b). As an

Table 2 Some Tyr-nitrated proteins in chloroplasts of photosynthetic organisms. For abbreviated protein name see text

Protein	Reference	Organism and treatment
Calvin cycle		
Rubisco	Cecconi et al. (2009)	Hypersensitive response *Arabidopsis*
	Tanou et al. (2012)	Citrus leaf
Rubisco	Lozano-Juste et al. (2011)	*Arabidopsis*
Rubisco activase	Cecconi et al. (2009)	Hypersensitive response *Arabidopsis* Sunflower hypocotyls
	Chaki et al. (2009) Tanou et al. (2012)	Citrus leaf
Glyceraldehyde 3-P dehydrogenase	Lozano-Juste et al. (2011)	*Arabidopsis*
	Tanou et al. (2012)	Citrus leaf
Phosphoglycerate kinase	Lozano-Juste et al. (2011)	*Arabidopsis*
	Tanou et al. (2012)	Citrus leaf and root
Phosphoribulokinase	Lozano-Juste et al. (2011)	*Arabidopsis*
	Tanou et al. (2012)	Citrus leaf
Sedoheptulose-1,7-bisphosphatase	Tanou et al. (2012)	Citrus leaf
Carbonic anhydrase	Chaki et al. (2009, 2013)	Sunflower hypocotyls
	Lozano-Juste et al. (2011)	*Arabidopsis*
Fructose-1,6-bisphosphate aldolase	Tanou et al. (2012)	Citrus leaf
Photosynthetic proteins		
Lhcb1, Lhcb2, Lhcb4, Lhcb5, Lhcb6 (PSII)	Galetskiy et al. (2011b)	*Arabidopsis* light stress
Lhcb6 (PSII)	Galetskiy et al. (2011b)	*Arabidopsis* light stress
PsbA protein of D1 subunit (PSII)	Galetskiy et al. (2011b)	*Arabidopsis* light stress
PsbD protein of D2 subunit (PSII)	Galetskiy et al. (2011b)	*Arabidopsis* light stress
PsbC (CP43)(PSII)	Galetskiy et al. (2011b)	*Arabidopsis* light stress
	Tanou et al. (2012)	Citrus leaf
HCF136 factor (PSII)	Lozano-Juste et al. (2011)	*Arabidopsis*
PbsP: Oxygen-evolving enhancer protein 2-1 (PSII)	Lozano-Juste et al. (2011)	*Arabidopsis*
Cyt b_6f	Galetskiy et al. (2011a, b)	*Arabidopsis* light stress

(continued)

Table 2 (continued)

Protein	Reference	Organism and treatment
ATP synthase gamma chain 1,	Lozano-Juste et al. (2011)	*Arabidopsis*
Lhca3 (PSI)	Galetskiy et al. (2011b)	*Arabidopsis* light stress
Lhca4 (PSI)	Galetskiy et al. (2011b)	*Arabidopsis* light stress
PsaF (PSI)	Galetskiy et al. (2011b)	*Arabidopsis* light stress
P700 apoprotein A (PSI)	Tanou et al. (2012)	Citrus leaf
Ferredoxin-NADP reductase	Chaki et al. (2011)	Heat stress sunflower hypocotyl
Ferredoxin-nitrite reductase	Chaki et al. (2011)	Heat stress sunflower hypocotyl
Others		
Elongation factor Tu	Cecconi et al. (2009)	Hypersensitive response *Arabidopsis*
Glutamine synthetase	Lozano-Juste et al. (2011)	*Arabidopsis*
	Chaki et al. (2009)	Sunflower hypocotyls
	Cecconi et al. (2009)	Hypersensitive response *Arabidopsis*
	Tanou et al. (2012)	Citrus leaf
Ketol-acid reductoisomerase	Lozano-Juste et al. (2011)	*Arabidopsis*
Phosphoglucomutase	Lozano-Juste et al. (2011)	*Arabidopsis*
Aspartate aminotransferase	Lozano-Juste et al. (2011)	*Arabidopsis*
	Tanou et al. (2012)	Citrus root
Malate dehydrogenase	Lozano-Juste et al. (2011)	*Arabidopsis*
Signal recognition particle 54 kDa protein,	Lozano-Juste et al. (2011)	*Arabidopsis*
3-Oxoacyl-[acyl-carrier-protein] synthase I	Lozano-Juste et al. (2011)	*Arabidopsis*
Thiazole biosynthetic enzyme	Lozano-Juste et al. (2011)	*Arabidopsis*
3-Oxoacyl-[acyl-carrier-protein] reductase	Lozano-Juste et al. (2011)	*Arabidopsis*
Magnesium-chelatase subunit chlD	Tanou et al. (2012)	Citrus leaf
Diaminopimelate decarboxylase 1	Tanou et al. (2012)	Citrus leaf
Antigen peptide transporter-like 1	Tanou et al. (2012)	Citrus leaf
Glutamate synthase	Tanou et al. (2012)	Citrus leaf
Pyruvate orthophosphate dikinase		
Sulfite reductase	Tanou et al. (2012)	Citrus leaf

(continued)

Table 2 (continued)

Protein	Reference	Organism and treatment
NAD-dependent epimerase	Tanou et al. (2012)	Citrus leaf
Lipoxygenase	Tanou et al. (2012)	Citrus root
Serine-type peptidase	Tanou et al. (2012)	Citrus root
Acylaminoacyl-peptidase1	Tanou et al. (2012)	Citrus root
Phosphoserine aminotransferase	Tanou et al. (2012)	Citrus root
Hsp70	Tanou et al. (2012)	Citrus root
6-phosphogluconolactonase	Tanou et al. (2012)	Citrus root
Glyoxalase	Tanou et al. (2012)	Citrus root
Carboxymethylenebutenolidase	Tanou et al. (2012)	Citrus root
Carbonic anhydrase	Chaki et al. (2011)	Heat stress sunflower hypocotyl

example, in control growth conditions, *Arabidopsis* PSII-LHCII complexes present low levels of phosphorylation and high nitration, while under high light conditions, the opposite occurred (Galetskiy et al. 2011b).

S-nitrosylation is mediated by NO and GSNO, with the latter also provoking *S*-glutathionylation (Camejo et al. 2015; Calderón et al. 2017b). *S*-nitrosylation is reversible, and thus it appears to be suitable for a signaling function. It has been reported that it affects protein activities, structure, localization, and interaction between proteins in animal and plant systems (Hess et al. 2005; Camejo et al. 2015; Zaffagnini et al. 2016). Several targets of *S*-nitrosylation have been identified in different cell compartments including chloroplasts (some are listed in Table 3). Several targets are involved in photosynthesis (in both, light-harvesting and carbon fixation), redox and antioxidant homeostasis, and amino acid biosynthesis, among others. Although this PTM may increase enzyme activity (Astier et al. 2012), the chloroplastic targets are mainly inhibited by *S*-nitrosylation (Lindermayr et al. 2005).

Related to GSNO metabolism, it is interesting to point out that GSNOR is the only enzyme capable of metabolizing GSNO, in a NADH-dependent manner, transforming GSNO into GSSG and ammonia. Regulation of GSNO cellular homeostasis allows GSNO to control the level of protein nitrosothiols. In fact, KO GSNO *Arabidopsis* plants presented higher levels of *S*-nitrosylated proteins than control wild-type plants (Feechan et al. 2005; Chaki et al. 2011), while the overexpression provoked decreased levels (Lin et al. 2012). This change in the nitrosylated pattern may affect the functioning of photosynthesis among other processes. Moreover, it has been proposed that a high cross talk exists among the different PTMs and that the influence of *S*-nitrosylation may be comparable with that of ubiquitination and phosphorylation (Zaffagnini et al. 2016).

Table 3 Some *S*-nitrosylated proteins in chloroplasts of photosynthetic organisms. For abbreviated protein name see text

Protein	Reference	Organism and treatment
Calvin cycle		
Rubisco	Abat and Deswal (2009) Abat et al. (2008)	GSNO treatment Low temperature NO treatment poplar
	Vanzo et al. (2016)	*Brassica juncea*, *Kalanchoe pinnata*
Rubisco	Romero-Puertas et al. (2008)	Hypersensitive response *Arabidopsis* and *Pseudomonas*
Rubisco	Lindermayr et al. (2005)	*Arabidopsis*, GSNO and NO treatment
Rubisco	Kato et al. (2013)	Potato GSNO treatment
Rubisco	Tanou et al. (2012)	Citrus leaf
Rubisco	Camejo et al. (2013)	Salinity, pea leaf
Rubisco activase	Vanzo et al. (2014, 2016)	NO treatment poplar
	Tanou et al. 2012	Citrus leaf
Ribose 5-P isomerase	Vanzo et al. (2014, 2016)	NO treatment poplar
Phosphoribulokinase	Vanzo et al. (2014, 2016)	NO treatment poplar
	Romero-Puertas et al. (2008)	Hypersensitive response
	Kato et al. (2013)	*Arabidopsis* and *Pseudomonas*
	Tanou et al. (2012)	Potato GSNO treatment Citrus leaf
Sedoheptulose-1,7-bisphosphatase	Vanzo et al. (2014, 2016)	NO treatment poplar
	Tanou et al. (2012)	Citrus leaf
Triose phosphate isomerase	Vanzo et al. (2014, 2016)	NO treatment poplar
	Lindermayr et al. (2005)	Salinity *Arabidopsis*
	Tanou et al. (2012)	*Arabidopsis*, GSNO and NO treatment Citrus root
Glyceraldehyde 3-P dehydrogenase	Vanzo et al. (2014, 2016)	NO treatment poplar
	Romero-Puertas et al. (2008)	Hypersensitive response
	Lindermayr et al. (2005)	*Arabidopsis* and *Pseudomonas* *Arabidopsis*, GSNO and NO treatment
	Tanou et al. (2012)	Citrus leaf
Phosphoglycerate kinase	Vanzo et al. (2014, 2016)	NO treatment poplar
	Lindermayr et al. (2005)	*Arabidopsis*, GSNO and NO treatment
	Tanou et al. (2012)	Citrus leaf

(continued)

Table 3 (continued)

Protein	Reference	Organism and treatment
Fru-1,6-bisphosphate aldolase	Lindermayr et al. (2005)	*Arabidopsis*, GSNO and NO treatment
	Tanou et al. (2012)	Citrus leaf
Fructose-1,6-bisphosphatase	Serrato et al. (2018)	In vitro GSNO and SNAP treatment, in vivo GSNO
Ribulose-5-phosphate-3-epimerase	Tanou et al. (2012)	Citrus leaf
Photosynthetic proteins		
PsbQ protein (PSII)	Romero-Puertas et al. (2008)	Hypersensitive response *Arabidopsis* and *Pseudomonas*
	Tanou et al. (2012)	Citrus leaf
PsbO protein (PSII)	Lindermayr et al. (2005)	*Arabidopsis*, GSNO and NO treatment
	Tanou et al. (2012)	Citrus leaf
PsbC proten (PSII)	Tanou et al. (2012)	Citrus leaf
PsbS protein (PSII)	Tanou et al. (2012)	Citrus leaf
PsbP protein (PSII)	Tanou et al. (2012)	Citrus leaf
PsbE(PSII)	Tanou et al. (2012)	Citrus leaf
P680 (PSII)	Lindermayr et al. (2005)	*Arabidopsis*, GSNO and NO treatment
D2 protein (PSII)	Tanou et al. (2012)	Citrus leaf
Lhcb protein (PSII)	Tanou et al. (2012)	Citrus leaf
Cyt b_6f	Lindermayr et al. (2005)	*Arabidopsis*, GSNO and NO treatment
	Tanou et al. (2012)	Citrus leaf
Plastocyanin	Kato et al. (2013)	Potato GSNO treatment
P700 apoprotein A (PSI)	Tanou et al. (2012)	Citrus leaf
PsaC (PSI)	Tanou et al. (2012)	Citrus leaf
PsbaN (PSI)	Tanou et al. (2012)	Citrus leaf
ATP synthase F1 (subunits α and β)	Tanou et al. (2012)	Citrus leaf
Antioxidants		
Prx IIE	Romero-Puertas et al. (2008); Lindermayr et al. (2005)	Hypersensitive response *Arabidopsis* and *Pseudomonas*
Peroxiredoxin	Tanou et al. (2012)	Citrus root
Fe-SOD	Holzmeister et al. (2014)	In vitro *Arabidopsis* peroxynitrite treatment
	Tanou et al. (2012)	Citrus leaf
Dehydroascorbate reductase	Lindermayr et al. (2005)	*Arabidopsis*, GSNO adn NO treatment
Monodehydroascorbate reductase	Tanou et al. (2012)	Citrus root

(continued)

Table 3 (continued)

Protein	Reference	Organism and treatment
Others		
Putative translation elongation factor EF-Tu precursor, chloroplast	Romero-Puertas et al. (2008)	Hypersensitive response *Arabidopsis* and *Pseudomonas*
Aldolase	Kato et al. (2013)	Potato GSNO treatment
Cysteine synthase 1	Kato et al. (2013)	Potato GSNO treatment
Transketolase	Tanou et al. (2012)	Citrus leaf
HSP 70	Tanou et al. (2012)	Citrus leaf
4-Hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	Tanou et al. (2012)	Citrus leaf
Chaperonin 60 (subunits α and β)	Tanou et al. (2012)	Citrus leaf
Glutamine synthase	Tanou et al. (2012)	Citrus leaf
NAD dependent epimerase	Tanou et al. (2012)	Citrus root
Carbonic anhydrase	Tanou et al. (2012)	Citrus root
Major latex protein	Tanou et al. (2012)	Citrus root

6 ROS/RNS Cross Talk

NO and H_2O_2 have been reported to act synergically in a dose-dependent manner during plant pathogen interaction (Delledonne et al. 2001; Zaninotto et al. 2006). Moreover, it has been reported that ROS activate NO biosynthesis and vice versa and that these reactive species collaborate to induce PCD (de Pinto et al. 2012). In fact, an increase in *S*-nitrosylating agents like GSNO and NO has been shown during cell death in tobacco TBY-2 cells after oxidative H_2O_2 treatments (de Pinto et al. 2013, Ortiz-Espín et al. 2015). In this sense, as referred above, protein PTMs are emerging as a key component of the signaling cross talk in cellular metabolism, and some examples are described below to illustrate this.

The *S*-nitrosylation of several phosphatases and kinases provokes changes in phosphorylation status (Hess and Stamler 2012), and the oxidative stress also influences protein succinylation and acetylation (Zhou et al. 2017) although the functional significance of these PTMs remains unclear. Another example is the regulation of the H_2O_2 scavenger 2-Cys Prx by NTRC/Trx system in chloroplasts (Kirchsteiger et al. 2012). Also PrxIIE has been described as able to detoxify $ONOO^-$, and in this way it can interfere with Tyr kinase signaling (Romero-Puertas et al. 2007), while *S*-nitrosylation inhibited the peroxidase function of this PrxIIE, interfering in ROS detoxification and signaling (Fig. 3, upper part). In fact chloroplast PrxIIE has been reported among the proteins specifically *S*-nitrosylated during the plant immune response (Lindermayr et al. 2005; Romero-Puertas et al. 2007) and in induced leaf death by H_2O_2 treatment in wild rice and *noe1* rice mutant (Lin et al. 2012). Elsewhere, Tyr nitration has also been reported to prevent Tyr phosphorylation, affecting this important regulatory mechanism (Galetskiy et al. 2011b). Thus,

PTMs mediated by NO are crucial components in ROS/RNS cross talk that regulate the transduction of these species during stress response. In animal systems, TR/Trxs are involved in protein *S*-denitrosylation, e.g., caspase-3 (Sengupta and Holmgren 2013), where Trx is found as an *S*-nitrosylated intermediate. However, in plants, little is known about this capacity. In fact, denitrosylation/*trans*-nitrosylation is an additional function for Trx that is important in regulating apoptotic processes in animal systems (Sun et al. 2013). *S*-nitrosylation of chloroplastic Trxs in plants has scarcely been reported: Trx*m*5 was found to be modified in a *nitric oxide excess1* (*noe*) mutants and not in WT plants and also in *Brassica juncea* seedlings under cold stress (Lindermayr et al. 2005; Sehrawat and Deswal 2014). However cytosolic Trx and GSNO have been reported as being involved in NPR1 (non-expressor of pathogenesis-related gene 1) translocation to the nucleus in *Arabidopsis* (Tada et al. 2008; Lindermayr et al. 2010), although the mechanism is not completely elucidated. Also the involvement of cytosolic Trx*h*5 as a denitrosylase of different proteins including TGA1 (GACG motif binding factor 1, a basic leucine zipper (bZIP) protein) has been described by Kneeshaw et al. (2014) in the immune response. All these examples may represent a link between redox changes and gene regulation provoked by pathogens (Maldonado-Alconada et al. 2011).

Cross talk between oxidative and nitrosative stress can also be shown through the carbonylation and *S*-nitrosylation of several proteins including Prx under salinity (Tanou et al. 2009) in which *S*-nitrosylation can prevent the irreversible protein oxidation. Also mitochondrial Prx IIF was found as *S*-nitrosylated in a time-dependent manner, probably associated with increased NO contents in the organelle under salt stress (Camejo et al. 2013). Later on, we described the functional switch of the *S*-nitrosylated protein from peroxidase to trans-nitrosylase (Camejo et al. 2015), as a new function of Prx involved in the cross talk of the ROS/RNS in plants (Calderón et al. 2017b).

7 Future Perspectives

In chloroplasts, the signaling function of ROS is related to the thiol redox regulatory network in which redox proteins peroxiredoxin and thioredoxin act as redox sensors and transmitters. It is now accepted that NO also has a key role to play in signaling in plant cells. However, there is a substantial lack of information on the intricacies of ROS and RNS signaling, which hinders determining its regulatory role in physiological processes and during the abiotic stress response. In this sense, the advances in cellular imaging techniques and real-time detection tools to measure localized ROS and NO production would substantially enhance our understanding of ROS/RNS signaling in chloroplasts. The sources of NO in plants have been difficult to determine, and there is an important debate about how to exactly identify NO produced in plant cells. Thus, at present, nitric oxide research basically centers on the characterization of a NOS enzyme and on deciphering the importance of each of the enzymes and sources involved in the physiological production of NO. These challenges are important and complex because some previous evidence has indicated that the importance of each of

these sources to the physiological production of NO will, likely, depend on the species, the cells/tissues, the environment under which the plants are grown, and of course, the signaling pathways active under those specific conditions.

The recently developed experimental tools have enabled the identification of a range of PTMs in chloroplast proteins. Our knowledge about protein redox regulation on photosynthetic reactions and antioxidant defense has considerably increased in the last decade, and there has been important progress in the signaling role of the redox state of thiols in Trxs and Prxs through PTMs, although the regulation of most of the metabolic pathways in the chloroplast is poorly understood. Because a specific amino acid residue can be targeted by different PTM types (e.g., Cys sulfenylation or *S*-glutathionylation, *S*-nitrosylation/denitrosylation), many challenges remain regarding thiol specificity. Moreover, ROS/NO interaction can result in Tyr nitration. These PTMs may have either antagonistic or cooperative effects; thus, understanding when and how they are coordinated to allow specific proteins to respond and their repercussion in the regulation of various metabolic pathways in chloroplasts during normal physiological processes, as well as under stressful environmental conditions, is a challenging task.

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References

Abat JK, Mattoo AK, Deswal R (2008) *S*-nitrosylated proteins of a medicinal CAM plant *Kalanchoe pinnata*—ribulose-1,5-bisphosphate carboxylase/oxygenase activity targeted for inhibition. FEBS J 275:2862–2872

Abat JK, Deswal R (2009) Differential modulation of S-nitrosoproteome of Brassica juncea by low temperature: change in S-nitrosylation of Rubisco is responsible for the inactivation of its carboxylase activity. Proteomics 9:4368–4380

Akter S, Carpentier S, Van Breusegem F, Messens J (2017) Identification of dimedone-trapped sulfenylated proteins in plants under stress. Biochem Biophys Rep 9:106–113

Alderton WK, Cooper CE, Knowles RG (2001) Nitric oxide synthases: structure, function and inhibition. Biochem J 357:593–615

Alkhalfioui F, Renard M, Montrichard F (2007) Unique properties of NADP-thioredoxin reductase C in legumes. J Exp Bot 58:969–978

Allahverdiyeva Y, Isojärvi J, Zhang P, Aro EM (2015) Cyanobacterial oxygenic photosynthesis is protected by flavodiiron proteins. Life (Basel) 5:716–743

Arsova B, Hoja U, Wimmelbacher M, Greiner E, Ustun S, Melzer M, Petersen K, Lein W, Bornke F (2010) Plastidial thioredoxin z interacts with two fructokinase-like proteins in a thiol-dependent manner: evidence for an essential role in chloroplast development in *Arabidopsis* and *Nicotiana benthamiana*. Plant Cell 22:1498–1515

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 41:391–396

Asai S, Ohta K, Yoshioka H (2008) MAPK signaling regulates nitric oxide and NADPH oxidase-dependent oxidative bursts in *Nicotiana benthamiana*. Plant Cell 20:1390–1406

Astier J, Besson-Bard A, Lamotte O, Bertoldo J, Bourque S, Terenzi H, Wendehenne D (2012) Nitric oxide inhibits the ATPase activity of the chaperone-like AAA^+ ATPase CDC48, a target for *S*-nitrosylation in cryptogein signalling in tobacco cells. Biochem J 447:249–260

Astier J, Jeandroz S, Wendehenne D (2018) Nitric oxide synthase in plants: the surprise from algae. Plant Sci 268:64–66

Badawi GH, Kawano N, Yamauchi Y, Shimada E, Sasaki R, Kubo A, Tanaka K (2004) Over-expression of ascorbate peroxidase in tobacco chloroplasts enhances the tolerance to salt stress and water deficit. Physiol Plant 121:231–238

Balsera M, Uberegui E, Schürmann P, Buchanan BB (2014) Evolutionary development of redox regulation in chloroplasts. Antiox Red Signal 21:1327–1355

Barajas-López JD, Tezycka J, Travaglia CN, Serrato AJ, Chueca A, Geigenberger TP, Sahrawy M (2012) Expression of the chloroplast thioredoxins *f* and *m* is linked to short-term changes in the sugar and thiol status in leaves of *Pisum sativum*. J Exp Bot 63:4887–4900

Barranco-Medina S, Krell T, Finkemeier I, Sevilla F, Lazaro JJ, Dietz KJ (2007) Biochemical and molecular characterization of the mitochondrial peroxiredoxin PsPrxIIF from *Pisum sativum*. Plant Physiol Biochem 45:729–739

Barranco-Medina S, Krell T, Bernier-Villamor L, Sevilla F, Lázaro JJ, Dietz KJ (2008) Hexameric oligomerization of mitochondrial peroxiredoxin PrxIIF and formation of an ultrahigh affinity complex with its electron donor thioredoxin Trx-*o*. J Exp Bot 59:3259–3269

Barranco-Medina S, Lázaro JJ, Dietz KJ (2009) The oligomeric conformation of peroxiredoxins links redox state to function. FEBS Lett 583:1809–1816

Barroso JB, Corpas FJ, Carreras A, Sandalio LM, Valderrama R, Palma JM, Lupianez JA, del Rio LA (1999) Localization of nitric-oxide synthase in plant peroxisomes. J Biol Chem 274:36729–36733

Bartoli CG, Tambussi EA, Fanello DD, Foyer CH (2009) Control of ascorbic acid synthesis and accumulation by the incident light red/far red ratio in *Phaseolus vulgaris* leaves. FEBS Lett 583:118–122

Bartoli CG, Casalongué CA, Simontacchi M, Marquez-Garcia B, Foyer CH (2013) Interactions between hormone and redox signalling pathways in the control of growth and cross tolerance to stress. Environ Exp Bot 94:73–88

Bartoli CG, Buet A, Grozeff GG, Galatro A, Simontacchi M (2017) Ascorbate-glutathione cycle and abiotic stress tolerance in plants. In: Hossain MA, Munné-Bosch S, Burritt D, Diaz-Vivancos P, Fujita M, Lorence A (eds) Ascorbic acid in plant growth, development and stress tolerance. Springer, Cham, pp 177–200

Bashandy T, Guilleminot J, Vernoux T, Caparros-Ruiz D, Ljung K, Meyer Y, Reichheld JP (2010) Interplay between the NADP-linked thioredoxin and glutathione systems in *Arabidopsis* auxin signaling. Plant Cell 22:376–391

Baxter A, Mittler R, Suzuki N (2014) ROS as key players in plant stress signalling. J Exp Bot 65:1229–1240

Beligni MV, Fath A, Bethke PC, Lamattina L, Jones RL (2002) Nitric oxide acts as an antioxidant and delays programmed cell death in barley aleurone layers. Plant Physiol 129:1642–1650

Bernier-Villamor L, Navarro E, Sevilla F, Lázaro JJ (2004) Cloning and characterization of a 2-Cysperoxiredoxin from *Pisum sativum*. J Exp Bot 55:2191–2199

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

Bonardi V, Pesaresi P, Becker T, Schleiff E, Wagner R, Pfannschmidt T, Jahns P, Leister D (2005) Photosystem II core phosphorylation and photosynthetic acclimation require two different protein kinases. Nature 437:1179–1182

Broin M (2002) The plastidic 2-Cysteine peroxiredoxin is a target for a thioredoxin involved in the protection of the photosynthetic apparatus against oxidative damage. Plant Cell 14:1417–1432

Bryk R, Griffin P, Nathan C (2000) Peroxynitrite reductase activity of bacterial peroxiredoxins. Nature 407:211–215

Buchanan BB, Luan S (2005) Redox regulation in the chloroplast thylakoid lumen: a new frontier in photosynthesis research. J Exp Bot 56:1439–1447

Calderón A, Ortiz-Espín A, Iglesias-Fernández R, Carbonero P, Pallardó FV, Sevilla F, Jiménez A (2017a) Thioredoxin (Trxo1) interacts with proliferating cell nuclear antigen (PCNA) and its overexpression affects the growth of tobacco cell culture. Redox Biol 11:688–700

Calderón A, Lázaro-Payo A, Iglesias-Baena I, Camejo D, Lázaro JJ, Sevilla F, Jiménez A (2017b) Glutathionylation of pea chloroplast 2-Cys Prx and mitochondrial PrxIIF affects their structure and peroxidase activity and sulfiredoxin deglutathionylates only the 2-Cys Prx. Front Plant Sci 8:118

Calderón A, Ortiz-Espín A, Iglesias-Fernández R, Carbonero P, Pallardó FV, Sevilla F, Jiménez A (2018) Redox protein thioredoxins: function under salinity, drought and extreme temperature conditions. In: Gupta DK, Palma JM, Corpas FJ (eds) Antioxidants and antioxidant enzymes in higher plants. Springer, Cham, pp 132–162

Camejo D, Romero-Puertas MDC, Rodríguez-Serrano M, Sandalio LM, Lázaro JJ, Jiménez A, Sevilla F (2013) Salinity-induced changes in S-nitrosylation of pea mitochondrial proteins. J Proteomics 79:87–99

Camejo D, Ortiz-Espín A, Lázaro JJ, Romero-Puertas MC, Lázaro-Payo A, Sevilla F, Jiménez A (2015) Functional and structural changes in plant mitochondrial PrxIIF caused by NO. J Proteomics 119:112–125

Cecconi D, Orzetti S, Vandelle E, Rinalducci S, Zolla L, Delledonne M (2009) Protein nitration during defense response in *Arabidopsis thaliana*. Electrophoresis 30:2460–2468

Cejudo FJ, Meyer AJ, Reichheld JP, Rouhier N, Traverso JA, Huber SC (2014) Thiol-based redox homeostasis and signaling. Front Plant Sci 5:266

Cerveau D, Ouahrani D, Marok MA, Blanchard L, Rey P (2016) Physiological relevance of plant 2-Cys peroxiredoxin overoxidation level and oligomerization status. Plant Cell Environ 39:103–119

Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, López-Jaramillo J, Luque F, Palma JM, Pedrajas JR, Begara-Morales JC, Sánchez-Calvo B, Gómez-Rodríguez MV, Corpas FJ, Barroso JB (2009) Protein targets of tyrosine nitration in sunflower (*Helianthus annuus* L.) hypocotyls. J Exp Bot 60:4221–4234

Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, Gómez- Rodríguez MV, Pedrajas JR, Begara-Morales JC, Sánchez-Calvo B, Luque F, Leterrier M, Corpas FJ, Barroso JB (2011) Mechanical wounding induces a nitrosative stress by down-regulation of GSNO reductase and an increase in S-nitrosothiols in sunflower (*Helianthus annuus*) seedlings. J Exp Bot 62:1803–1813

Chaki M, Carreras A, López-Jaramillo J, Begara-Morales JC, Sánchez-Calvo B, Valderrama R, Corpas FJ, Barroso JB (2013) Tyrosine nitration provokes inhibition of sunflower carbonic anhydrase (β-CA) activity under high temperature stress. Nitric Oxide 29:30–33

Chaki M, de Morales PÁ, 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. Ann Bot 116:637–647

Chamizo-Ampudia A, Galvan A, Fernandez E, Llamas A (2011) The Chlamydomonas reinhardtii molybdenum cofactor enzyme crARC has a Zn-dependent activity and protein partners similar to those of its human homologue. Eukaryot Cell 10:1270–1282

Chan KX, Mabbitt PD, Phua SY, Mueller JW, Nisar N, Gigolashvili T, Stroeher E, Grassl J, Arlt W, Estavillo GM, Jackson CJ, Pogson BJ (2016) Sensing and signaling of oxidative stress in chloroplasts by inactivation of the SAL1 phosphoadenosine phosphatase. Proc Natl Acad Sci U S A 113:E4567–E4576

Chen WW, Yang JL, Qin C, Jin CW, Mo JH, Ye T, Zheng SJ (2010) Nitric oxide acts downstream of auxin to trigger root ferric-chelate reductase activity in response to iron deficiency in *Arabidopsis*. Plant Physiol 154:810–819

Chibani K, Tarrago L, Schürmann P, Jacquot JP, Rouhier N (2011) Biochemical properties of poplar thioredoxin z. FEBS Lett 585:1077–1081

Collin V, Issakidis-Bourguet E, Marchand C, Hirasawa M, Lancelin J, Knaff D, Miginiac-Maslow M (2003) The arabidopsis plastidial thioredoxins—new functions. Environ Exp Bot 154:134–142

Collin V, Lamkemeyer P, Miginiac-Maslow M, Hirasawa M, Knaff DB, Dietz KJ, Issakidis-Bourguet E (2004) Characterization of plastidial thioredoxins from *Arabidopsis* belonging to the new y-type. Plant Phys 136:4088–4095

Cooney RV, Harwood PJ, Custer LJ, Franke AA (1994) Light-mediated conversion of nitrogen dioxide to nitric oxide by carotenoids. Environ Health Perspect 102:460–462

Corpas FJ, Barroso JB (2014) Functional implications of peroxisomal nitric oxide (NO) in plants. Front Plant Sci 5:97

Corpas FJ, Barroso JB, Carreras A, Valderrama R, Palma JM, León AM, Sandalio LM, del Río LA (2006) Constitutive arginine-dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. Planta 224:246–254

Corpas FJ, Chaki M, Leterrier M, Barroso JB (2009) Protein tyrosine nitration: a new challenge in plants. Plant Signal Behav 4:920–923

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

Corpas FJ, Palma JM, del Río LA, Barroso JB (2013b) Protein tyrosine nitration in higher plants grown under natural and stress conditions. Front Plant Sci 4:1–4

Corpas FJ, Barroso JB, Palma JM, Rodríguez-Ruiz M (2017) Plant peroxisomes: a nitro-oxidative cocktail. Redox Biol 11:535–542

Couturier J, Jacquot JP, Rouhier N (2013) Toward a refined classification of class I dithiol glutaredoxins from poplar: biochemical basis for the definition of two subclasses. Front Plant Sci 4:518

Crawford NA, Droux M, Kosower NS, Buchanan BB (1989) Evidence for function of the ferredoxin/thioredoxin system in the reductive activation of target enzymes of isolated intact chloroplasts. Arch Biochem Biophys 271:223–239

Daloso DM, Müller K, Obata T, Florian A, Tohge T, Bottcher A, Riondet C, Bariat L, Carrari F, Nunes-Nesi A, Buchanan BB, Reichheld JP, Araújo WL, Fernie AR (2015) Thioredoxin, a master regulator of the tricarboxylic acid cycle in plant mitochondria. Proc Natl Acad Sci U S A 12:1392–1400

D'Autreaux B, Toledano MB (2007) ROS as signaling molecules: mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol 8:813–824

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci U S A 98:13454–13459

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

del Río LA, López-Huertas E (2016) ROS generation in peroxisomes and its role in cell signalling. Plant Cell Physiol 57:1364–1376

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 MC, 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 Cell. Plant Physiol 163:1766–1775

Dietz KJ (2016) Thiol-based peroxidases and ascorbate peroxidases: why plants rely on multiple peroxidase systems in the photosynthesizing chloroplast? Mol Cells 39:20–25

Dietz KJ, Pfannschmidt T (2011) Novel regulators in photosynthetic redox control of plant metabolism and gene expression. Plant Physiol 155:1477–1485

Dietz KJ, Jacob S, Oelze ML, Laxa M, Tognetti V, de Miranda SMN, Baier M, Finkemeier I (2006) The function of peroxiredoxins in plant organelle redox metabolism. J Exp Bot 57:1697–1709

Diner BA, Petrouleas V (1990) Formation by NO of nitrosyl adducts of redox components of the photosystem II reaction center. II: evidence that HCO3/CO2 binds to the acceptor-side non-heme iron. Biochim Biophys Acta 1015:141–149

Durner J, Wendehenne D, Klessig DF (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci U S A 95:10328–10333

Eberhard S, Finazzi G, Wollman FA (2008) The dynamics of photosynthesis. Annu Rev Genet 42:463–515

Feechan A, Kwon E, Yun BW, Wang Y, Pallas JA, Loake GJ (2005) A central role for S-nitrosothiols in plant disease resistance. Proc Natl Acad Sci U S A 102:8054–8059

Ferreira FJ, Guo C, Coleman JR (2008) Reduction of plastid-localized carbonic anhydrase activity results in reduced *Arabidopsis* seedling survivorship. Plant Physiol 147:585–594

Finkemeier I, Goodman M, Lankemeyer P, Kandlbinder A, Sweetlove LJ, Dietz KJ (2005) The mitochondrial type II peroxiredoxin F is essential for redox homeostasis and root growth of *Arabidopsis thaliana* under stress. J Biol Chem 280:12168–12180

Foissner I, Wendehenne D, Langebartels C, Durner J (2000) In vivo imaging of an elicitor-induced nitric oxide burst in tobacco. Plant J 23:817–824

Foresi N, Correa-Aragunde N, Parisi Caló G, Salerno G, Lamattina L (2010) Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga *Ostreococcus tauri* is light irradiance and growth phase dependent. Plant Cell 22:3816–3830

Foyer CH (2018) Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. Environ Exp Bot 154:134–142

Foyer CH, Noctor G (2011) Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 155:2–18

Foyer CH, Noctor G (2013) Redox signaling in plants. Antiox Red Signal 18:2087–2090

Foyer CH, Noctor G (2016) Stress-triggered redox signaling: what's in pROSpect? Plant Cell Environ 39:951–964

Foyer CH, Shigeoka S (2011) Understanding oxidative stress and antioxidant functions to enhance photosynthesis. Plant Physiol 155:93–100

Foyer CH, Ruban AV, Noctor G (2017) Viewing oxidative stress through the lens of oxidative signaling rather than damage. Biochem J 474:877–883

Galatro A, González PM, Malanga G, Robello E, Piloni NE, Puntarulo S (2013) Nitric oxide and membrane lipid peroxidation in photosynthetic and non-photosynthetic organisms under several stress conditions. Front Physiol 4:276

Galetskiy D, Lohscheider JN, Kononikhin AS, Popov IA, Nikolaev EN, Adamska I (2011a) Mass spectrometric characterization of photooxidative protein modifications in *Arabidopsis thaliana* thylakoid membranes. Rapid Commun Mass Spectrom 25:184–190

Galetskiy D, Lohscheide JN, Kononikhin AS, Popov IA, Nikolaev EN, Adamska I (2011b) Phosphorylation and nitration levels of photosynthetic proteins are conversely regulated by light stress. Plant Mol Biol 77:461–473

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

Gama F, Bréhélin C, Gelhaye E, Meyer Y, Jacquot JP, Rey P, Rouhier N (2008) Functional analysis and expression characteristics of chloroplastic PrxIIE. Physiol Planta 133:599–610

García-Mata C, Lamattina L (2001) Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. Plant Physiol 126:1196–1204

Gas E, Flores-Pérez Ú, Saurez-Gúeto S, Rodriguez-Concepción M (2009) Hunting for plant nitric oxide synthase provides new evidence of the central role for plastids in nitric oxide metabolism. Plant Cell 21:18–23

Gelhaye E, Rouhier N, Gerard J, Jolivet Y, Gualberto J, Navrot N, Ohlsson PI, Wingsle G, Hirasawa M, Knaff DB, Wang H, Dizengremel P, Meyer Y, Jacquot JP (2004) A specific form of thioredoxin h occurs in plant mitochondria and regulates the alternative oxidase. Proc Natl Acad Sci U S A 101:14545–14550

Gerotto C, Alboresi A, Meneghesso A, Jokel M, Suorsa M, Aro EM, Morosinotto T (2016) Flavodiiron proteins act as safety valve for electrons in *Physcomitrella patens*. Proc Natl Acad Sci U S A 113:12322–12327

Go YM, Jones DP (2010) Redox control systems in the nucleus: mechanisms and functions. Antioxid Redox Signal 13:489–509

Gómez JM, Hernández JA, Jiménez A, del Río LA, Sevilla F (1999) Differential response of antioxidative enzymes of chloroplasts and mitochondria to long-term NaCl stress of pea plants. Free Radic Res 31:S11–S18

Gómez JM, Jiménez A, Olmos E, Sevilla F (2004) Location and effects of long-term NaCl stress on superoxide dismutase and ascorbate peroxidase isoenzymes of pea (*Pisum sativum* cv. Puget) chloroplasts. J Exp Bot 55:119–130

Gould KS, Lamotte O, Klinguer A, Pugin A, Wendehenne D (2003) Nitric oxide production in tobacco leaf cells: a generalized stress response? Plant Cell Environ 26:1851–1862

Graziano M, Beligni MV, Lamattina L (2002) Nitric oxide improves internal iron availability in plants. Plant Physiol 130:1852–1859

Guo FQ, Crawford NM (2005) Arabidopsis nitric oxide synthase1 is targeted to mitochondria and protects against oxidative damage and dark-induced senescence. Plant Cell 17:3436–3450

Gupta KJ, Igamberdiev AU (2016) Reactive nitrogen species in mitochondria and their implications in plant energy status and hypoxic stress tolerance. Front Plant Sci 7:369

Gupta KJ, Fernie AR, Kaiser WM, van Dongen JT (2011) On the origins of nitric oxide. Trend Plant Sci 6:160–168

Gupta KJ, Shah JK, Brotman Y, Jahnke K, Willmitzer L, Kaiser WM, Bauwe H, Igamberdiev AU (2012) Inhibition of aconitase by nitric oxide leads to induction of the alternative oxidase and to a shift of metabolism towards biosynthesis of amino acids. J Exp Bot 63:1773–1784

Gururani MA, Venkatesh J, Tran LSP (2015) Regulation of photosynthesis during abiotic stress-induced photoinhibition. Mol Plant 8:1304–1320

Halliwell B, Gutteridge JM (2015) Free radicals in biology and medicine. Oxford University Press, New York

Haslekås C, Viken MK, Grini PE, Nygaard V, Nordgard SH, Meza TJ, Aalen RB (2003) Seed 1-cysteine peroxiredoxin antioxidants are not involved in dormancy, but contribute to inhibition of germination during stress. Plant Physiol 133:1148–1157

Hayat S, Yadav S, Wani AS, Irfan M, Ahmad A (2011) Nitric oxide effects on photosynthetic rate growth, and antioxidant activity in tomato. Int J Veg Sci 17:333–348

Hess DT, Stamler JS (2012) Regulation by S-nitrosylation of protein posttranslational modification. J Biol Chem 287:4411–4418

Hess DT, Matsumoto A, Kim SO, Marshall HE, Stamler JS (2005) Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol 6:150–166

Hill AC, Bennett JH (1970) Inhibition of apparent photosynthesis by nitrogen oxides. Atmos Environ 4:341–348

Holzmeister C, Gaupels F, Geerlof A, Sarioglu H, Sattler M, Durner J, Lindermayr C (2014) Differential inhibition of Arabidopsis superoxide dismutases by peroxynitrite-mediated tyrosine nitration. J Exp Bot 66:989–999

Horling F, König J, Dietz KJ (2002) Type II peroxiredoxin C, a member of the peroxiredoxin family of *Arabidopsis thaliana*: its expression and activity in comparison with other peroxiredoxins. Plant Physiol Biochem 40:491–499

Igamberdiev AU, Ratcliffe RG, Gupta KJ (2014) Plant mitochondria: source and target for nitric oxide. Mitochondrion B 19 Pt B:329–333

Iglesias-Baena I, Barranco-Medina S, Lazaro-Payo A, Lopez-Jaramillo FJ, Sevilla F, Lazaro JJ (2010) Characterization of plant sulfiredoxin and role of sulphinic form of 2-Cys peroxiredoxin. J Exp Bot 61:1509–1521

Iglesias-Baena I, Barranco-Medina S, Sevilla F, Lazaro JJ (2011) The dual-targeted plant sulfiredoxin retroreduces the sulfinic form of atypical mitochondrial peroxiredoxin. Plant Physiol 155:944–955

Ischiropoulos H (2003) Biological selectivity and functional aspects of protein tyrosine nitration. Biochem Biophys Res Commun 305:776–783

Jacquot JP, Vidal J, Gadal P, Schürmann P (1978) Evidence for the existence of several enzyme-specific thioredoxins in plants. FEBS Lett 96:243–246

Jasid S, Simontacchi M, Bartolic G, Puntarulo S (2006) Chloroplasts as a nitric oxide cellular source. Effect of reactive nitrogen species on chloroplastic lipids and proteins. Plant Physiol 142:1246–1255

Jeandroz S, Wipf D, Stuehr DJ, Lamattina L, Melkonian M, Tian Z, Zhu Y, Carpenter EJ, Wong GK, Wendehenne D (2016) Occurrence, structure, and evolution of nitric oxide synthase-like proteins in the plant kingdom. Sci Signal 9:re2

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

Kangasjärvi S, Lepistö A, Hännikäinen K, Piippo M, Luomala E-M, Aro EM, Rintamäki E (2008) Diverse roles for chloroplast stromal and thylakoid-bound ascorbate peroxidases in plant stress responses. Biochem J 412:275–285

Kato H, Takemoto D, Kawakita K (2013) Proteomic analysis of S-nitrosylated proteins in potato plant. Physiol Plant 148:371–386

Kirchsteiger K, Ferrández J, Pascual MB, González M, Cejudo FJ (2012) NADPH thioredoxin reductase C is localized in plastids of photosynthetic and nonphotosynthetic tissues and is involved in lateral root formation in *Arabidopsis*. Plant Cell 24:1534–1548

Kneeshaw S, Gelineau S, Tada Y, Loake GJ, Spoel SH (2014) Selective protein denitrosylation activity of thioredoxin-h5 modulates plant immunity. Mol Cell 56:153–162

Kohzuma K, Froehlich JE, Davis GA, Temple JA, Minhas D, Dhingra A, Crz JA, Kramer DM (2017) The role of light–dark regulation of the chloroplast ATP synthase. Front Plant Sci 8:1248

Kumar P, Tewari RK, Sharma PN (2010) Sodium nitroprusside mediated alleviation of iron deficiency and modulation of antioxidant responses in maize plants. AoB Plants 2010:plq002

Laloi C, Havaux M (2015) Key players of singlet oxygen-induced cell death in plants. Front Plant Sci 6:1–9

Laloi C, Stachowiak M, Pers-Kamczyc E, Warzych E, Murgia I, Apel K (2007) Cross-talk between singlet oxygen- and hydrogen peroxide-dependent signaling of stress responses in *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 104:672–677

Laspina NV, Groppa MD, Tomaro ML, Benavides MP (2005) Nitric oxide protects sunflower leaves against Cd-induced oxidative stress. Plant Sci 169:323–330

Lázaro JJ, Jiménez A, Camejo D, Iglesias-Baena I, Martí M del C, Lázaro-Payo A, Barranco-Medina S, Sevilla F (2013) Dissecting the integrative antioxidant and redox systems in plant mitochondria. Effect of stress and S-nitrosylation. Front Plant Sci 4:460

Lemaire SD, Collin V, Keryer E, Quesada A, Miginiac-Maslow M (2003) Characterization of thioredoxin y, a new type of thioredoxin identified in the genome of *Chlamydomonas reinhardtii*. FEBS Lett 543:87–92

Lin A, Wang Y, Tang J, Xue P, Li C, Liu L, Hu B, Yang F, Loake GJ, Chu CH (2012) Nitric oxide and protein S-nitrosylation are integral to hydrogen peroxide-induced leaf cell death in rice. Plant Physiol 158:451–464

Lindermayr C, Saalbach G, Durner J (2005) Proteomic identification of S-nitrosylated proteins in *Arabidopsis*. Plant Physiol 137:921–930

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

Liu XP, Liu XY, Zhang J, Xia ZL, Liu X, Qin HJ, Wang DW (2006) Molecular and functional characterization of sulfiredoxin homologs from higher plants. Cell Res 16:287–296

Liu X, Liu B, Xue S, Cai Y, Qi W, Ch J, Xu S, Wang T, Ren H (2016) Cucumber (*Cucumis sativus* L.) nitric oxide synthase associated gene1 (CsNOA1) plays a role in chilling stress. Front Plant Sci 7:1652

Locato V, Cimini S, De Gara L (2018) ROS and redox balance as multifaceted players of cross-tolerance: epigenetic and retrograde control of gene expression. J Exp Bot 69:3373–3391

Lozano-Juste J, Colom-Moreno R, León J (2011) In vivo protein tyrosine nitration in *Arabidopsis thaliana*. J Exp Bot 62:3501–3517

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

Maldonado-Alconada AM, Echevarría-Zomeno S, Lindermayr C, Redondo-López I, Durner J, Jorrín-Novo JV (2011) Proteomic analysis of *Arabidopsis* protein S-nitrosylation in response to inoculation with *Pseudomonas syringae*. Acta Physiol Plant 33:1493–1514

Manjunatha G, Gupta KJ, Lokesh V, Mur LA, Neelwarne B (2012) Nitric oxide counters ethylene effects on ripening fruits. Plant Signal Behav 7:476–483

Martí MC, Olmos E, Calvete JJ, Díaz I, Barranco-Medina S, Whelan J, Lázaro JJ, Sevilla F, Jiménez A (2009) Mitochondrial and nuclear localization of a novel pea thioredoxin: identification of its mitochondrial target proteins. Plant Physiol 150:646–657

Martí M, Florez-Sarasa I, Camejo D, Ribas-Carbó M, Lázaro J, Sevilla F, Jiménez A (2011) Response of mitochondrial thioredoxin PsTrxo1, antioxidant enzymes, and respiration to salinity in pea (*Pisum sativum* L.) leaves. J Exp Bot 62:3863–3874

Martin MN, Tarczynski MC, Shen B, Leustek T (2005) The role of 5′-adenylylsulfate reductase in controlling sulfate reduction in plants. Photosynth Res 86:309–323

Marty L, Siala W, Schwarzländer M, Fricker MD, Wirtz M, Sweetlove LJ, Meyer Y, Meyer AJ, Reichheld JP, Hell R (2009) The NADPH-dependent thioredoxin system constitutes a functional backup for cytosolic glutathione reductase in *Arabidopsis*. Proc Natl Acad Sci U S A 106:9109–9114

Maruta T, Noshi M, Tanouchi A, Tamoi M, Yabuta Y, Yoshimura K, Ishikawa T, Shigeoka S (2012) H_2O_2-triggered retrograde signaling from chloroplasts to nucleus plays specific role in response to stress. J Biol Chem 287:11717–11729

Meng L, Wong JH, Feldman LJ, Lemaux PG, Buchanan BB (2010) A membrane-associated thioredoxin required for plant growth moves from cell to cell, suggestive of a role in intercellular communication. Proc Natl Acad Sci U S A 107:3900–3905

Mestres-Ortega D, Meyer Y (1999) The *Arabidopsis thaliana* genome encodes at least four thioredoxins m and a new prokaryotic-like thioredoxin. Gene 240:307–316

Meyer Y, Belin C, Delorme-Hinoux V, Reichheld JP, Riondet C, Meyer Y (2012) Thioredoxin and glutaredoxin systems in plants: molecular mechanisms, crosstalks, and functional significance. Antiox Redox Sign 17:1124–1160

Michelet L, Krieger-Liszkay A (2012) Reactive oxygen intermediates produced by photosynthetic electron transport are enhanced in short-day grown plants. Biochim Biophys Acta 1817:1306–1313

Michelet L, Zaffagnini M, Morisse S, Sparla F, Pérez-Pérez ME, Francia F, Danon A, Marchand CH, Fermani S, Trost P, Lemaire SD (2013) Redox regulation of the Calvin-Benson cycle: something old, something new. Front Plant Sci 4:470

Misra AN, Vladkova R, Singh R, Misra M, Dobrikova AG, Apostolova EL (2014) Action and target sites of nitric oxide in chloroplasts. Nitric Oxide 39:35–45

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

Mock HP, Dietz KJ (2016) Redox proteomics for the assessment of redox-related posttranslational regulation in plants. Biochim Biophys Acta 1864:967–973

Modolo LV, Augusto O, Almeida IMG, Magalhaes JR, Oliveira HC, Salgado I (2005) Nitrite as the major source of nitric oxide production by *Arabidopsis thaliana* in response to *Pseudomonas syringae*. FEBS Lett 579:3814–3820

Montrichard F, Alkhalfioui F, Yano H, Vensel WH, Hurkman WJ, Buchanan BB (2009) Thioredoxin targets in plants: the first 30 years. J Proteom 72:452–474

Moreau M, Lee GI, Wang Y, Crane BR, Klessig DF (2008) AtNOS/A1 is a functional *Arabidopsis thaliana* cGTPase and not a nitric oxide synthase. J Biol Chem 283:32957–32967

Mur LAJ, Mandon J, Persijn S, Cristescu SM, Moshkov IE, Novikova GV, Hall MA, Harren FJM, Hebelstrup KM, Gupta KJ (2013) Nitric oxide in plants: an assessment of the current state of knowledge. AoB Plants 5:pls052

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI (2007) Photoinhibition of photosystem II under environmental stress. Biochim Biophys Acta 1767:414–421

Nikkanen L, Toivola J, Guinea Diaz M, Rintamäki E (2017) Chloroplast thioredoxin systems: prospects for improving photosynthesis. Philos Trans R Soc Lond B Biol Sci 372:20160474

Nishiyama Y, Murata N (2014) Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. Appl Microbiol Biotechnol 98:8777–8796

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

Noctor G, Mhamdi A, Chaouch S, Han Y, Neukermans J, Marquez Garcia B, Queval G, Foyer CH (2012) Glutathione in plants: an integrated overview. Plant Cell Environ 35:454–484

Noctor G, Mhamdi A, Foyer CH (2014) The roles of reactive oxygen metabolism in drought: not so cut and dried. Plant Physiol 164:1636–1648

Ortega-Galisteo AP, Rodríguez-Serrano M, Pazmiño DM, Gupta DK, Sandalio LM, Romero-Puertas MC (2012) S-Nitrosylated proteins in pea (*Pisum sativum* L.) leaf peroxisomes: changes under abiotic stress. J Exp Bot 63:2089–2103

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 H_2O_2 treatment. Ann Bot 116:571–582

Ortiz-Espín A, Sevilla F, Jiménez A (2017a) The role of ascorbate in plant growth and development. In: Hossain M, Munné-Bosch S, Burritt D, Diaz-Vivancos P, Fujita M, Lorence A (eds) Ascorbic acid in plant growth, development and stress tolerance. Springer, Cham, pp 25–45

Ortiz-Espín A, Iglesias-Fernández R, Calderón A, Carbonero P, Sevilla F, Jiménez A (2017b) Mitochondrial AtTrxo1 is transcriptionally regulated by AtbZIP9 and AtAZF2 and affects seed germination under saline conditions. J Exp Bot 68:1025–1038

Pedrajas JR, Bárcena JA (2018) Peroxiredoxins: types, characteristics and functions in higher plants. In: Gupta DK, Palma JM, Corpas FJ (eds) Antioxidants and antioxidant enzymes in higher plants. Springer, Cham, pp 95–121

Pérez-Ruiz JM, Spinola MC, Kirchsteiger K, Moreno J, Sahrawy M, Cejudo FJ (2006) Rice NTRC is a high-efficiency redox system for chloroplast protection against oxidative damage. Plant Cell 18(9):2356–2368

Pérez-Ruiz JM, González M, Spinola MC, Sandalio LM, Cejudo FJ (2009) The quaternary structure of NADPH thioredoxin reductase C is redox-sensitive. Mol Plant 2:457–467

Pfalz J, Liere K, Kandlbinder A, Dietz KJ, Oelmuller R (2006) pTAC2, -6, and -12 are components of the transcriptionally active plastid chromosome that are required for plastid gene expression. Plant Cell 18:176–197

Planchet E, Kaiser WM (2006) Nitric oxide production in plants: facts and fictions. Plant Signal Behav 1:46

Pospíšil P (2012) Molecular mechanisms of production and scavenging of reactive oxygen species by photosystem II. Biochim Biophys Acta 1817:218–231

Potters G, DeGara L, Asard H, Horemans N (2002) Ascorbate and glutathione: guardians of the cell cycle, partners in crime? Plant Physiol Biochem 40:537–548

Pulido P, Cazalis R, Cejudo FJ (2009) An antioxidant redox system in the nucleus of wheat seed cells suffering oxidative stress. Plant J 57:132–145

Pulido P, Spinola MC, Kirchsteiger K, Guinea M, Pascual MB, Sahrawy M, Sandalio LM, Dietz KJ, Gonzalez M, Cejudo FJ (2010) Functional analysis of the pathways for 2-Cys peroxiredoxin reduction in *Arabidopsis thaliana* chloroplasts. J Exp Bot 61:4043–4054

Ramel F, Birtic S, Ginies C, Soubigou-Taconnat L, Triantaphylides C, Havaux M (2012) Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. Proc Natl Acad Sci U S A 109:5535–5540

Reichheld JP, Khafif M, Riondet C, Droux M, Bonnard G, Meyer Y (2007) Inactivation of thioredoxin reductases reveals a complex interplay between thioredoxin and glutathione pathways in *Arabidopsis* development. Plant Cell 19:1851–1865

Rey P, Cuiné S, Eymery F, Garin J, Court M, Jacquot JP, Rouhier N, Broin M (2005) Analysis of the proteins targeted by CDSP32, a plastidic thioredoxin participating in oxidative stress responses. Plant J 41:31–42

Rhee SG, Yang KS, Kang SW, Woo HA, Chang TS (2005) Controlled elimination of intracellular H_2O_2: regulation of peroxiredoxin, catalase, and glutathione peroxidase via post-translational modification. Antiox Redox Sign 7:619–626

Rivas S, Rougon-Cardoso A, Smoker M, Schauser L, Yoshioka H, Jones JD (2004) CITRX thioredoxin interacts with the tomato Cf-9 resistance protein and negatively regulates defence. EMBO J 23:2156–2165

Rochaix JD (2012) Regulation of photosynthetic electron transport. Biochim Biophys Acta 180:375–383

Rochaix JD (2013) Fine-tuning photosynthesis. Science 342:50–51

Rochaix JD (2014) Regulation and dynamics of the light-harvesting system. Annu Rev Plant Biol 65:287–309

Rockel P, Strube F, Rockel A, Wildt J, Kaiser WM (2002) Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro. J Exp Bot 53:103–110

Romero-Puertas MC, Laxa M, Delledonne M (2007) S-Nitrosylation of peroxiredoxin IIE promotes peroxynitrite-mediated tyrosine nitration. Plant Cell 19:4120–4130

Romero-Puertas MC, Campostrini N, Matte A, Righetti PG, Perazzolli M, Zolla L, Roepstorff P, Delledonne M (2008) Proteomic analysis of S-nitrosylated proteins in *Arabidopsis thaliana* undergoing hypersensitive response. Proteomics 8:1459–1469

Romero-Puertas MC, Rodríguez-Serrano M, Sandalio LM (2013) Protein S-nitrosylation in plants under abiotic stress: an overview. Front Plant Sci 4:373

Sanakis Y, Goussias C, Mason RP, Petrouleas V (1997) NO interacts with the tyrosine radical YD of photosystem II to form an iminoxyl radical. Biochemistry 36:1411–1417

Schansker G, Goussias C, Petrouleas V, Rutherford AW (2002) Reduction of the Mn cluster of the water-oxidizing enzyme by nitric oxide: formation of an S2 state. Biochem 41:3057–3064

Sehrawat A, Deswal R (2014) Sub-proteome S-nitrosylation analysis in Brassica juncea hints at the regulation of Brassicaceae specific as well as other vital metabolic pathway(s) by nitric oxide and suggests posttranslational modifications cross-talk. Nitric Oxide 43:97–111

Seligman K, Saviani EE, Oliveira HC, Pinto-Maglio CA, Salgado I (2008) Floral transition and nitric oxide emission during flower development in *Arabidopsis thaliana* is affected in nitrate reductase-deficient plants. Plant Cell Physiol 49:1112–1121

Sengupta R, Holmgren A (2013) Thioredoxin and thioredoxin reductase in relation to reversible S-nitrosylation. Antioxid Redox Signal 18:259–269

Serrato AJ, Pérez-Ruiz JM, Spínola MC, Cejudo FJ (2004) A novel NADPH thioredoxin reductase, localized in the chloroplast, which deficiency causes hypersensitivity to abiotic stress in *Arabidopsis thaliana*. J Biol Chem 279:43821–43827

Serrato AJ, Romero-Puertas MC, Lázaro-Payo A, Sahrawy M (2018) Regulation by S-nitrosylation of the Calvin-Benson cycle fructose-1, 6-bisphosphatase in Pisum sativum. Redox Biol 14:409–416

Seth D, Stamler JS (2011) The SNO-proteome: causation and classifications. Curr Opin Chem Biol 15:129–136

Sevilla F, Camejo D, Ortiz-Espín A, Calderón A, Lázaro JJ, Jiménez A (2015a) Thioredoxin/Peroxiredoxin/Sulfiredoxin system: current overview on its redox function in plants and regulation by ROS and RNS. J Exp Bot 66:2945–2955

Sevilla F, Jiménez A, Lázaro JJ (2015b) What do the plant mitochondrial antioxidant and redox systems have to say in salinity, drought and extreme temperature abiotic stress situations? In: Gupta DK, Palma JM, Corpas FJ (eds) Reactive oxygen species and oxidative damage in plants under stress. Springer, Cham, pp 23–55

Shapiguzov A, Vainonen JP, Wrzaczek M, Kangasjärvi J (2012) ROS-talk-how the apoplast, the chloroplast, and the nucleus get the message through. Front Plant Sci 3:292

Sharma P, Dubey RS (2005) Drought induces oxidative stress and enhances the activities of antioxidant enzymes in growing rice seedlings. Plant Growth Regul 46:209–221

Simontacchi M, Galatro A, Ramos-Artuso F, Santa-María GE (2015) Plant survival in a changing environment: the role of nitric oxide in plant responses to abiotic stress. Front Plant Sci 6:977

Spinola MC, Perez-Ruiz JM, Pulido P, Kirchsteiger K, Guinea M, Gonzalez M, Cejudo FJ (2008) NTRC new ways of using NADPH in the chloroplast. Physiol Planta 133:516–524

Stöhr C, Strube F, Marx G, Ullrich WR, Rockel P (2001) A plasma membrane-bound enzyme of tobacco roots catalyzes the formation of nitric oxide from nitrite. Planta 212:835–841

Stoimenova M, Igamberdiev AU, Gupta KJ, Hill RD (2007) Nitrite-driven anaerobic ATP synthesis in barley and rice root mitochondria. Planta 226:465–474

Strand DD, Livingston AK, Satoh-Cruz M, Froehlich JE, Maurino VG, Kramer DM (2015) Activation of cyclic electron flow by hydrogen peroxide in vivo. Proc Natl Acad Sci U S A 112:5539–5544

Sun N, Hao JR, Li XY, Yin XH, Zong YY, Zhang GY, Gao C (2013) GluR6-FasL-Trx2 mediates denitrosylation and activation of procaspase-3 in cerebral ischemia/reperfusion in rats. Cell Death Dis 4:771

Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zou J, Dong X (2008) Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. Science 321:952–956

Takahashi S, Yamasaki H (2002) Reversible inhibition of photophosphorylation in chloroplasts by nitric oxide. FEBS Lett 512:145–148

Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Job D (2009) Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. Plant J 60:795–804

Tanou G, Filippou P, Belghazi M, Job D, Diamantidis G, Fotopoulos V, Molassiotis A (2012) Oxidative and nitrosative-based signaling and associated post- translational modifications orchestrate the acclimation of citrus plants to salinity stress. Plant J 72:585–599

Telfer A (2014) Singlet oxygen production by PSII under light stress: mechanism, detection and the protective role of β-carotene. Plant Cell Physiol 55:1216–1223

Tewari RK, Watanabe D, Watanabe M (2012) Chloroplastic NADPH oxidase-like activity-mediated perpetual hydrogen peroxide generation in the chloroplast induces apoptotic-like death of *Brassica napus* leaf protoplasts. Planta 235:99–110

Tewari RK, Prommer J, Watanabe M (2013) Endogenous nitric oxide generation in protoplast chloroplasts. Plant Cell Rep 32:31–44

Thalineau E, Truong HN, Berger A, Fournier C, Boscari A, Wendehenne D, Jeandroz S (2016) Cross-regulation between N metabolism and nitric oxide (NO) signaling during plant immunity. Front Plant Sci 8:472

Tikkanen M, Aro EM (2014) Integrative regulatory network of plant thylakoid energy transduction. Trend Plant Sci 19:10–17

Tikkanen M, Gollan PJ, Mekala NR, Isojarvi J, Aro EM (2014) Light-harvesting mutants show differential gene expression upon shift to high light as a consequence of photosynthetic redox and reactive oxygen species metabolism. Phil Trans R Soc Lond B Biol Sci 369:20130229

Tischner R, Planchet E, Kaiser WM (2004) Mitochondrial electron transport as a source for nitric oxide in the unicellular green alga *Chlorella sorokiniana*. FEBS Lett 576:151–155

Triantaphylides C, Havaux M (2009) Singlet oxygen in plants: production, detoxification and signaling. Trend Plant Sci 14:219–228

Tun NN, Santa-Catarina C, Begum T, Silveira V, Handrow I, Flohe I, Scherer GF (2006) Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. Plant Cell Physiol 47:346–354

Turkan I (2017) Emerging roles for ROS and RNS-versatile molecules in plants. J Exp Bot 68:4413–4416

Turkan I (2018) ROS and RNS: key signalling molecules in plants. J Exp Bot 69:3313–3315

Twigg AI, Baniulis D, Cramer WA, Hendrich MP (2009) EPR detection of an O_2 surrogate bound to heme cn of the cytochrome b6f complex. J Amer Chem Soc 131:12536–12537

Umbreen S, Lubega J, Cui B, Pan Q, Jiang J, Loake G (2018) Specificity in nitric oxide signaling. J Exp Bot 69:3439–3448

Van Ree K, Gehl B, Wassim Chehab E, Tsai YC, Braam J (2011) Nitric oxide accumulation in *Arabidopsis* is independent of NOA1 in the presence of sucrose. Plant J 68:225–233

Vanzo E, Ghirardo A, Merl-Pham J, Lindermayr C, Heller W, Hauck SM, Durner J, Schnitzler JP (2014) S-nitrosoproteome in poplar leaves in response to acute ozone stress. PLoS One 9: e106886

Vanzo E, Merl-Pham J, Velikova V, Ghirardo A, Lindermayr C, Hauck SM, Bernhardt J, Riedel K, Durner J, Schnitzler JP (2016) Modulation of protein S-nitrosylation by isoprene emission in poplar. Plant Physiol 170:1945–1961

Vladkova R, Dobrikova AG, Singh R, Misra AN, Apostolova E (2011) Photoelectron transport ability of chloroplast thylakoid membranes treated with NO donor SNP: changes in flash oxygen evolution and chlorophyll fluorescence. Nitric Oxide 24:84–90

Wang Y, Feng H, Qu Y, Cheng J, Zhao Z, Zhang M, Wang X (2006) The relationship between reactive oxygen species and nitric oxide in ultraviolet-B-induced ethylene production in leaves of maize seedlings. Environ Exp Bot 57:51–61

Wei-Feng X, Wei-Ming S, Ueda A, Takabe T (2008) Mechanisms of salt tolerance in transgenic *Arabidopsis thaliana* carrying a peroxisomal ascorbate peroxidase gene from barley. Pedosphere 18:486–495

Wodala B, Deák Z, Vass I, Erdei L, Altorjay A, Horváth F (2008) In vivo target sites of nitric oxide in photosynthetic electron transport as studied by chlorophyll fluorescence in pea leaves. Plant Physiol 146:1920–1927

Wolosiuk RA, Crawford NA, Yee BC, Buchanan BB (1979) Isolation of three thioredoxins from spinach leaves. J Biol Chem 254:1627–1632

Xia XJ, Zhou YH, Shi K, Zhou J, Foyer CH, Yu JQ (2015) Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. J Exp Bot 66:2839–2856

Yamasaki H (2000) Nitrite-dependent nitric oxide production pathway: implications for involvement of active nitrogen species in photoinhibition in vivo. Philos Trans R Soc Lond B Biol Sci 355:1477–1488

Yamasaki H, Sakihama Y, Takahashi S (1999) An alternative pathway for nitric oxide production in plants: new features of an old enzyme. Trend Plant Sci 4:128–129

Ying Y, Yue W, Wang S, Li S, Wang M, Zhao Y, Wang CH, Mao C, Whelan J, Shou H (2017) Two h-type thioredoxins interact with the E2 ubiquitin conjugase PHO2 to fine-tune phosphate homeostasis in rice. Plant Physiol 173:812–824

Yoshida K, Hara S, Hisabori T (2015) Thioredoxin selectivity for thiol-based redox regulation of target proteins in chloroplasts. J Biol Chem 290:14278–14288

Yoshida K, Hara A, Sugiura K, Fukaya Y, Hisabori T (2018) Thioredoxin-like2/2-Cys peroxiredoxin redox cascade supports oxidative thiol modulation in chloroplasts. Proc Natl Acad Sci U S A 115:E8296–E8304

Yu M, Lamattina L, Spoel SH, Loake GJ (2014) Nitric oxide function in plant biology: a redox cue in deconvolution. New Phytol 202:1142–1156

Zaffagnini M, DeMi M, Morisse S, Di Giacinto N, Marchand CH, Maes A, Lemaire SD, Trost P (2016) Protein S-nitrosylation in photosynthetic organisms: a comprehensive overview with future perspectives. Biochim Biophys Acta 1864:952–966

Zafra A, Rejón JD, Hiscock SJ, Alché J de D (2016) Patterns of ROS accumulation in the stigmas of angiosperms and visions into their multi-functionality in plant reproduction. Front Plant Sci 7:1112

Zaninotto F, La Camera S, Polverari A, Delledonne M (2006) Cross talk between reactive nitrogen and oxygen species during the hypersensitive disease resistance response. Plant Physiol 141:379–383

Zhang L, Wang Y, Zhao L, Shi S, Zhang L (2006) Involvement of nitric oxide in light-mediated greening of barley seedlings. J Plant Physiol 163:818–826

Zhou H, Finkemeier I, Guan W, Tossounian M, Wei B, Young D, Jingjing J, Messens J, Yang X, Zhu J, Wilson M, Shen WB, Xie Y, Foyer CH (2017) Oxidative stress-triggered interactions between the succinyl- and acetyl-proteomes of rice leaves. Plant Cell Environ 41:1139–1153

Participation of Hydrogen Peroxide and Nitric Oxide in Improvement of Seed Germination Performance Under Unfavourable Conditions

David W. M. Leung

Contents

Abstract Seed germination is a complex process. Upon imbibition, many factors including phytohormones (gibberellin and abscisic acid) and reactive oxygen and nitrogen species [hydrogen peroxide (H_2O_2) and nitric oxide (NO), respectively] are involved in a complicated web of interactions. While there are some impressive recent progresses made in our understanding of these interactions, it is also of great interest to investigate treatments that help seeds with difficulties to germinate under unfavourable conditions including abiotic stress factors such as chilling and heavy metals. In this chapter, an update and critical interpretations of some recent investigations into the relationships among H_2O_2, NO, catalase activity and gene expression in cold stratification, light signal and abiotic stress are provided.

Keywords Abscisic acid (ABA) · Catalase · Chilling stress · Cold stratification · Gibberellic acid · Heme oxygenase · NADPH oxidase · NO donor · Phytochrome

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1 Introduction

Emergence of the radicle through the seed coat and other seed structures that may be enclosing or associated with the embryo is the first visible sign that a seed has completed germination (Bewley et al. 2013). This is a critical step towards the successful natural regeneration of plants in the wild as well as crop production. Seeds from some plant species may exhibit a low germination rate under otherwise favourable germination conditions for many other species. This problem has long been the subject of many studies. The possible reasons for a low rate of seed germination can be varied (Finch-Savage and Leubner-Metzger 2006; Bewley et al. 2013). For example, there may be a need for tuning in with certain environmental factors including light and cold temperature before the germination-associated processes in the hydrated seeds can be initiated and eventually culminated in radicle protrusion through the surrounding seed structures. Seeds may be sown under unfavourable growth conditions or stress such as chilling, salinity or heavy metals, and therefore their germination performance may be impaired. The storage conditions of seeds after harvest may not be appropriate and could lead to impairment in seed germination performance. In the literature, there is a classical view about the antagonism of two phytohormones, gibberellins (GA) and abscisic acid (ABA), in the decision for a seed to germinate or not (Nelson and Steber 2017). In the seeds with low germination performance for whatever reasons, the metabolism and signalling of ABA may predominate and ensure the no germination state (Finkelstein and Lynch 2000).

The focus on a few seeds as model lab systems, including *Arabidopsis thaliana*, has recently led to some rapid and impressive advances in our understanding of the interplay between reactive nitrogen species, particularly nitric oxide (NO) signalling and crosstalk with GA and ABA, occurring prior to the completion of seed germination (Nonogaki 2014, 2017). There has also been a great deal of investigations into the involvement of hydrogen peroxide (H_2O_2) in the regulation of seed germination. There may be a universal germination mechanism in different seeds. It is of great practical interest to be able to apply this knowledge to improve germination of seeds that is adversely affected by abiotic stress or less than favourable germination conditions. In this chapter, the objective is to provide an update of insights gained from the most recent papers (mainly published in between 2017 and 2018) on the participation of NO and H_2O_2 in seed germination under these conditions.

2 Cold Stratification

Freshly harvested *Arabidopsis thaliana* seeds (from wild-type plants of ecotype Col-0) were dormant (1% germinable seeds), but a majority of the seeds germinated after cold stratification at 4 °C for a few days (Bethke et al. 2004). However, supplementation of 4 or 5 mM hydrogen peroxide (H_2O_2) to a plant tissue culture

medium for seed germination led to a reduction in *Arabidopsis* seed germination by about 20 and 50%, respectively (Bi et al. 2017). Similar to the exogenous application of H_2O_2, supplementation of 3–10 mM of an inhibitor of catalase activity, 3-amino-1,2,4 triazole (3-AT), led to inhibition of the germination of the wild-type *Arabidopsis* seeds by about 30% (Bi et al. 2017). Catalase activity and H_2O_2 content in wild-type seeds treated with 5 mM 3-AT were only one-third and 80%, respectively, of those in the seeds germinated in the absence of the catalase activity inhibitor. This suggests that high catalase activity seems to be associated with a positive role in *Arabidopsis* seed germination. However, it is more complex to interpret the possible role of H_2O_2 content in cold-stratified *Arabidopsis* seed germination based on this result. Firstly, it is not readily clear why inhibiting catalase activity by 3-AT could lead to a reduction in endogenous H_2O_2 content in cold-stratified *Arabidopsis* seeds as the opposite would be expected to happen.

Since abscisic acid (ABA) is well-known to be associated with germination inhibition, it is of interest to probe the relationship of catalase activity and endogenous H_2O_2 content with ABA signalling in germinating *Arabidopsis* seeds after cold stratification (Bi et al. 2017). Abscisic acid (ABA)-insensitive 5 (ABI5) is a basic leucine zipper transcription factor. It is the core component of the ABA signalling pathway and has been shown to affect the expression of several ABA-responsive genes (Skubacz et al. 2016). In some earlier studies, for example, ABI5 was found to interact with the promoters of some ABA responsive genes including several late-embryogenesis-abundant genes during seed germination (Finkelstein and Lynch 2000). Recently, it was revealed that ABI5 could bind directly to the promoter of a catalase gene, *CAT1* but not *CAT2*, in the *Arabidopsis* genome. Furthermore, the expression of *CAT1*was also increased by ABI5 (Bi et al. 2017).

The possible roles of catalase activity and H_2O_2 content in cold-stratified *Arabidopsis* seeds were evaluated further using *Arabidopsis* plant lines with perturbation in sensitivity to ABA and the ABA signalling pathway. The plant lines that were investigated included mutant plants called *abi5-1* and *abi7*, which harbour mutations in the *ABI5* gene loci, and *ABI5*-overexpression lines. Based on the results obtained, it seems that the sensitivity of cold-stratified *Arabidopsis* seeds to exogenous H_2O_2 was dependent on ABA signalling (Bi et al. 2017). The cold-stratified seeds of *abi5-1* and *abi7* exhibited significantly lower germination percentages than wild type in response to exogenous application of 3–5 mM of H_2O_2. Conversely, overexpression of *ABI5* seemed to be associated with the insensitivity to the germination inhibitory effect of exogenous application of H_2O_2. These results would seem to be consistent with the possibility that catalase activity played a positive role in the germination of the cold-stratified *Arabidopsis* seed. Based on the link between *ABI5* and *catalase* gene expression, there would be an elevated level of catalase activity in *ABI5*-overexpression lines. Therefore any excess H_2O_2 from exogenous application could be decomposed so that germination process in the cold-stratified seeds was insensitive to the excess H_2O_2 (Bi et al. 2017).

Consistent with this, the seeds of the ABI5-overexpression lines and the *abi5-1* mutant plants exhibited a higher and lower catalase activity, respectively, than the

wild type (Bi et al. 2017). This finding was correlated with that of exogenous application of H_2O_2 having little or more germination inhibitory effect on the overexpression lines or the mutant plants, respectively, than the wild type. This result was in agreement with the results on the germination responses to 3 to 10 mM 3-AT of the cold-stratified seeds of *abi5-1* and *abi7* and ABI5-overexpression lines compared to wild type. Their germination responses mirrored those of the plant lines exposed to exogenous application of H_2O_2 (Bi et al. 2017). Therefore, it seemed that inhibition of catalase activity by 3-AT, like the seed germination response to exogenous application of H_2O_2, was also dependent on ABI5 expression levels (Bi et al. 2017). Catalase in cold-stratified *Arabidopsis* seeds would be working downstream of ABI5 in germinating seeds.

There is, however, evidence that calls for some caution in accepting a positive role for catalase in *Arabidopsis* seed germination. *ABI5* expression levels were linked to gene expression of *CAT1* which was higher in the cold-stratified seeds of the ABI5-overexpression lines than wild type. The cold-stratified *abi5-1* seeds exhibited a lower level of *CAT1* expression than wild type. As expected from the lack of interaction between *ABI5* and the promoter of *CAT2* in a yeast-one hybrid system, there was no difference in the expression levels of *CAT2* in the wild type and the *abi5-1* and *ABI5*-overexpression lines. Interestingly, the remaining member of the *CAT* gene family, *CAT3*, exhibited the lowest and highest expression levels in the seeds of the wild type and *abi5-1*, respectively. As far as germination percentage was concerned, there was, however, no difference exhibited by the seeds of wild type, *abi5-1* and *ABI5*-overexpression lines when the cold-stratified seeds were sown in a plant tissue culture medium in the absence of exogenous H_2O_2. Therefore, it is not clear how the cold stratification treatment would enable seeds to germinate independently of their different ABI5 expression levels. The relative significance of the varying catalase gene expression levels in the germination process must also await further elucidation.

Many seeds require a prior exposure to cold stratification conditions before they can germinate at a higher temperature. It would be interesting to investigate the changes in the catalase activity and endogenous H_2O_2 content in other cold stratification-requiring seeds to ascertain whether the finding from the studies on the *Arabidopsis* model system is translational to other non-model seeds. However, a survey of the recent literature shows a gap in our knowledge in this direction. For example, about 30% of *Zanthoxylum nitidum* seeds (of the Rutaceae family, a common Chinese medicinal plant) germinated at 15 °C after cold stratification at 4 °C for 3 months, while less than 3% of the non-cold-stratified seeds germinated (Lu et al. 2018). As expected from the classical literature about the involvement of gibberellins in the control of seed germination, the seeds without cold stratification but treated with gibberellic acid (300 mg L^{-1}) exhibited about 22% germination which was significantly higher than control. Based on the proteomic analytical approach iTRAQ-coupled LC-MS/MS, 484 proteins in the cold-stratified seeds were more abundant than the non-cold-stratified seeds (Lu et al. 2018). Only about eight proteins in the category of detoxification function, which could potentially include catalase gene, were found to be either up- or downregulated in association

with dormancy release. This is highly speculative but it is worthwhile to validate this further through direct measurement of catalase activity and determination of H_2O_2 content in the cold-stratified, GA-treated seeds and the control.

3 Abiotic Stress-Related Suppression of Seed Germination

3.1 Chilling Stress

At 10 °C for 10 days, about 20% of *Hedysarum scoparium* seeds germinated compared to 80% germination at 25 °C (Su et al. 2016). Interestingly, about 80% of the seeds could also germinate after 10 days at 10 °C provided the seeds were prior treated with 10 days of cold stratification (seeds mixed with sand at 4 °C). Therefore, the prior cold stratification aided the seeds to overcome chilling stress on seed germination. Exogenous application of 50 mM H_2O_2 promoted maximum germination (80%) of the non-cold stratified seeds at 10 °C. Exogenous application of an antioxidant, 50 mM N-acetyl cysteine (NAC), effectively prevented the stimulation of germination by 50 mM H_2O_2. This result is contrary to that implicating the need for preventing excess H_2O_2 content in cold-stratified, germinating *Arabidopsis* seeds (Bi et al. 2017).

In situ detection using confocal microscopy of H_2O_2 production at the tip of the embryonic axes of *H. scoparium* after 24 h of imbibition showed that there was only slightly detectable H_2O_2 content in the non-cold-treated seeds, but there was elevated accumulation of H_2O_2 in cold-stratified seeds imbibed in water and non-cold-stratified seeds treated with 50 mM exogenous H_2O_2 (Su et al. 2016). The treatment of the cold-stratified seeds with 50 mM NAC effectively nullified the endogenous H_2O_2 production. Hence, ROS generation following cold stratification of the seeds plays a positive role in seed germination. It would be worthwhile in future studies to evaluate the status of the catalase activity and gene expression in this system.

The cold-stratified *H. scoparium* seeds had lower ABA content than non-cold-stratified seeds that germinated poorly at 10 °C. There was an inverse correlation between H_2O_2 and ABA contents in the embryonic axes of *H. scoparium* (Su et al. 2016). Exogenous application of ABA prevented cold-stratified seeds from germinating and lowered H_2O_2 accumulation. This result suggests that ABA could influence upstream of ROS metabolism which was in turn linked to the completion of the germination process. However, exogenous application of H_2O_2 that enabled non-cold-stratified seeds to germinate led to a 20% reduction in ABA contents in the seeds within 24 h of imbibition. Conversely, NAC treatment of cold-stratified seeds led to an increase in ABA correlating with inhibition of seed germination. Hence, the precise relationship between changes in ABA and H_2O_2 contents in relation to the decision to germinate or not is still not completely clear (Su et al. 2016).

Seeds that normally germinate at a higher temperature can be prevented from germination at low temperatures. For example, 96% maize seeds germinated after 3–4 days at 25 °C, but after 3 and 4 days at 13 °C, only 15% germinated, respectively

(Li et al. 2017b). The seeds at 25 °C were found to have a higher endogenous H_2O_2 content as well as activities of catalase and other antioxidative enzymes than those at 13 °C after 48 h from sowing. Priming maize seeds with 50 mM H_2O_2 for 24 h enabled 50 and 90% of the seeds germinated at 13 °C after 3 and 4 days, respectively (Li et al. 2017b). Seed priming with H_2O_2 also led to a higher catalase activity at 48 h from sowing the primed seeds under chilling stress than non-primed seeds. The activities of other antioxidative enzymes assayed including ascorbate peroxidase, another enzyme beside catalase that can decompose H_2O_2, were also increased following seed priming with 50 mM H_2O_2. These results suggest that the net accumulation of H_2O_2 and probably other ROS was an important force for maize seed germination to occur.

There was no difference in the expression of *ZmNCED1* (a key gene for ABA biosynthesis in maize) and *ZmCYPT707A2* (a key gene for ABA catabolism) between non-primed and H_2O_2-primed maize seeds after 48 h of imbibition. However, the H_2O_2-primed maize seeds exhibited higher levels in the expression of the two genes after 72 h at 13 °C (Li et al. 2017b). Based on these results, it is difficult to interpret the relative contribution of ABA biosynthesis and ABA catabolism in H_2O_2-primed maize seeds without determination of ABA contents in the seeds. In contrast, the positive involvement of increased GA biosynthesis with concomitant reduction in GA catabolism in germination of H_2O_2-primed maize seeds was clearly evident early (6–24 h) during imbibition. Nevertheless, since higher levels of H_2O_2 and catalase activity were found in H_2O_2-primed than in non-primed maize seeds after 48 h of imbibition, this suggests that ROS homeostasis-related to seed germination might work upstream of ABA metabolism but downstream of GA metabolism (Li et al. 2017b).

3.2 Salinity and Heavy Metal Stress

Germination of alfalfa seeds in water and 150 mM NaCl (salinity stress) was about 98 and 45%, respectively (Amooaghaie and Tabatabaie 2017). Pretreatment of the seeds with 2 mM H_2O_2 or 300 mg L^{-1} polyethylene glycol (PEG 6000, osmopriming) for 6 h enabled more seeds (about 70%) to germinate in the presence of the salinity stress. Both priming with exogenous H_2O_2 and PEG 6000 led to a higher level of endogenous H_2O_2 than non-primed alfalfa seeds after 6 h from exposure to 150 mM NaCl. Application of *N,N*-dimethylthiourea (DMTU, a chemical absorbent of H_2O_2 and a ROS scavenger) prevented the stimulation of germination under salinity stress by priming with H_2O_2 or PEG 6000. In addition, application of DMTU suppressed an increase in endogenous H_2O_2 by priming the alfalfa seeds with H_2O_2 or PEG 6000. Interestingly, there was an increase in heme oxygenase activity and heme-oxygenase gene (*HO-1*) expression. These results suggest a sequence of signalling events involving endogenous H_2O_2 and heme oxygenase for osmopriming to overcome salinity stress on alfalfa seed germination (Fig. 1). Heme oxygenase coupled with carbon monoxide signalling has been

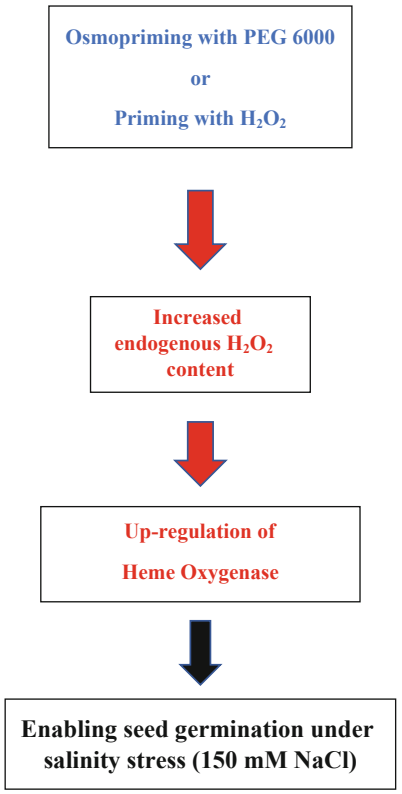

Fig. 1 An emerging signalling role involving H_2O_2 in alfalfa seed germination under abiotic stress. (See the results presented in Amooaghaie and Tabatabaie 2017.) The box in blue: experimental manipulation of seeds; red arrows and the boxes in red: the sequence of key internal signal changes in seeds; the black arrow and black box: germination events leading to improved % of seed germination under salinity stress

thought to be part of the response of plant cells under different environmental stress and ABA and may play a cytoprotective role (Shekhawat and Verma 2010).

Germination of maize seeds in water and 5 mM $PbCl_2$ (under Pb stress) was about 98 and 25% after 72 h from sowing (Zhang et al. 2018). The Pb stress-induced inhibition of seed germination could be reversed by an inhibitor (imidazole or diphenyliodonium) of NADPH oxidase (NOX) activity which is responsible for the production of superoxide free radical ($O_2^{\cdot -}$). Superoxide may be scavenged by superoxide dismutase to generate H_2O_2. Interestingly, EDTA (a metal chelator) or DMTU (a trap for H_2O_2) was not able to counteract the Pb stress-induced inhibition of seed germination. This suggests that although endogenous H_2O_2 content in maize seeds during imbibition was also increased under Pb stress, the Pb stress-triggered production of $O_2^{\cdot -}$ is a key suppressor of seed germination. Similar results were obtained in rice seeds germinated in the absence of abiotic stress (Li et al. 2017a).

3.3 Seed Storage Conditions

Two batches of barley seeds which were from the same harvest but were stored under two different conditions resulted in different germination performances under the same imbibition conditions: the seeds of higher germination performance were stored at 23 °C for 6 months, and those of lower germination performance were stored at −28 °C (Ishibashi et al. 2017). The level of H_2O_2 as a marker of reactive oxygen species (ROS) production in the batch of barley seeds exhibiting higher germination performance, both in relation to germination speed and higher germination percentage, was higher than in the other batches of barley seeds of lower germination performance. The significance of this correlation between hydrogen peroxide level and germination performance was supported with exogenous application of hydrogen peroxide and ascorbate (an antioxidant that resulted in a lower level of hydrogen peroxide in the seeds) during seed imbibition. After 48 h of imbibition in water, the germination percentage of high germination performing seeds was about 80%, but it was reduced significantly to about 20% in the presence of 20 mM sodium ascorbate. Conversely, the seeds of low germination performance exhibited a higher germination percentage (45%) when imbibed in 100 mM hydrogen peroxide than that of those imbibed in water (30%) (Ishibashi et al. (2017)).

Higher H_2O_2 and lower ABA contents were found in the barley seeds of higher germination performance compared to the batch of seeds of lower germination performance after 48 h of imbibition (Ishibashi et al. 2017). While there was little change in the expression of *HvNECD1* (a key gene for ABA biosynthesis in barley), that of *HvABA8′-OH1* (encoding ABA-8′-hydroxylase for ABA catabolism) was significantly higher in the barley seeds of higher germination performance than those of lower germination performance.

Higher H_2O_2 content in the barley seeds of higher germination performance was correlated with higher NADPH oxidase activity. On the other hand, of the two H_2O_2 scavenger enzymes, ascorbate peroxidase activity was higher in the barley seeds of high germination performance than those of low germination performance, while catalase activity showed the opposite relationship with the two batches of seeds during imbibition. It seems difficult to decipher the relative contributions of these two antioxidative enzymes on H_2O_2 signalling in seed germination. Further studies might include simultaneous investigations into the effect of exogenous ABA on the status of both ascorbate peroxidase and catalase at the levels of gene expression and enzyme activity in relation to the germination of the two batches of barley seeds.

4 The Scientific Basis for Improving Seed Germination by Exogenous Nitric Oxide

In many studies, application of exogenous NO has been found to improve seed germination performance due to diverse circumstances, although only some studies validated the effect with a NO-specific scavenger and evaluate the physiological significance of endogenous NO content (Table 1). For example, isolated embryos of mountain ash (*Sorbus pohuashanesis*) placed on filter paper wetted with distilled water for 8 days exhibited 42% germination. The promoter effect of pretreatment with 2 mM sodium nitroprusside (SNP, a nitric oxide donor) for 3 h, compared to pretreatment with water only, on germination of isolated embryos of mountain ash (*S. pohuashanesis*) on filter paper wetted with distilled water has been demonstrated to be 80 and 42%, respectively (Yang et al. 2018). The embryos treated with a combination of 2 mM SNP and 0.3 mM cPTIO (a NO-scavenger) did not exhibit dormancy release as only 42% of the embryos germinated. Furthermore, germination of embryos treated with 0.3 mM cPITO alone was slightly lower than imbibition in water only.

Upon seed imbibition, ABA in seeds could stimulate the expression of *ABI5* which is a key repressor of seed germination. There are four ways that exogenous application of NO or endogenous NO produced after start of seed imbibition can interfere with regulation of ABA in seed germination. For example, NO or other RNS (reactive nitrogen species) could inactivate the protein signalling intermediates between ABA and ABI5 by tyrosine nitration in these proteins (Signorelli and Considine 2018). As a result, the *ABI5* expression level would be reduced and hence has a less stronghold on suppression of seed germination.

In lettuce seeds, it was shown that light could stimulate NO production (An and Zhou 2017). Then NO was thought to in turn stimulate the activity of phospholipase D activity which is responsible for the hydrolysis of phospholipids to phosphatidic acid. Presumably, the phosphatidic acid formed could interact with phytohormones such as GA and ABA to allow lettuce seed germination in the light. The light-stimulated NO production in *Arabidopsis thaliana* seeds seems to be able to interact with the well-known phytochrome signalling in light-sensitive seed germination (Li et al. 2018). Further investigations are warranted to investigate between phosphatidic acid formation and phytochrome signalling in light-regulated seed germination.

NO could also interact with GA, a well-established germination promoter, and other phytohormones to release seed dormancy. For example, less than 10% of *Amaranthus retroflexus* seeds germinated at 25 °C in light. However, exogenous application of potassium nitrite (KNO_2, a NO donor) for 5 h or 10^{-3} M GA_3 led to 60% seed germination (Kepczynski et al. 2017). Likewise, exogenous application of ethylene or an ethylene biosynthesis precursor stimulated seed germination. Ethylene production seemed to be associated with the action of GA or NO in promoting germination. It was validated further that NO worked with GA or ethylene in germination stimulation because cPTIO blocked the effect of GA or ethylene.

Table 1 Some recent examples of seed germination stimulation following NO pretreatment

Plants	Nature of germination difficulty	Stimulation of seed germination by NO (compared to pretreatment without NO)	Effect of NO scavenger (cPTIO)	Endogenous NO production	References
Mountain Ash (*Sorbus pohuashanensis*)	Low natural seed germination: 60% after 8 days	Pretreatment with 2 mM SNP (NO donor): 90% seed germination	Reversed the promotive effect of SNP on seed germination	n.d	Yang et al. (2018)
Oat seeds	Artificial ageing of seeds (seed stored at 45 °C for 26 days) associated with increased H_2O_2 accumulation: 68% compared to 99% seed germination prior to storage	Pretreatment with 0.05 mM SNP: about 80% seeds germinated	n.d.	n.d.	Mao et al. (2018)
Lettuce (*Lactuca sativa* L., cv. Jianye Xianfeng)	Light (14 h at 50 μmol m^{-2} s^{-1} and 10 h darkness): over 80% seed germination compared to control (36 h in the dark): less than 10%	0.1 mM SNP: over 80% seed germination	0.2 mM cPTIO nullified the promotive effect of SNP on lettuce seed germination	NO content at 6 h from the start of imbibition under light was about fivefold higher than the seeds in the dark	An and Zhou (2017)
Coriander	Only 40% seed germination in water	80% seed germination	–	–	Panngom et al. (2018)
Carrot	60% seed germination in water	No effect	–	–	
Amaranthus retroflexus	10% seed germination in light at 25 °C	Pretreatment with KNO_2 (NO donor): 60% seed germination	Reversed the positive effect of NO	n.d.	Kepczynski et al. (2017)
Arabidopsis thaliana seeds (wild-type Col)	20% seed germination (1 h white light followed by 5 min of far-red light, then 96 h of darkness)	80% seed germination following a treatment with 10 μM SNAP or red light	Reversed the positive effect of NO	Red light stimulated NO accumulation but far-red light inhibited this	Li et al. (2018)

n.d. = not determined
SNP = sodium nitroprusside
SNAP = S-nitroso-N-acetylpenicillamine

The relative effectiveness of exogenous application of H_2O_2 and NO (SNP as NO donor) on promoting coriander and carrot seed germination was studied (Panngom et al. 2018). About 40% seeds germinated in water, but pretreatment with 25 mM H_2O_2 or 12.5 μM SNP led to 90 and 80% seed germination, respectively. Pretreatment of carrot seeds with 25–200 mM H_2O_2 resulted in about 70% seed germination compared to 60% germination in water. Pretreatment of carrot seeds with 0–100 μM SNP did not have any effect seed germination. This suggests that the sensitivity of different seeds to NO and H_2O_2 signalling may vary as far as seed germination is concerned.

5 Conclusion

Seed germination research is justified given its agricultural importance and implication for natural plant regeneration to maintain survival and propagation of wild plants. During imbibition, the current seed germination research communities seem to have fully embraced the concept that H_2O_2 and NO are important signal partners in the complex web of molecules including the well-known germination promoter and inhibitor played by GA and ABA, respectively. The details as to how either H_2O_2 or NO interacts with these key germination regulators are being revealed in different studies including the improvement in seed germination under unfavourable germination conditions or abiotic stress. There is yet no comprehensive study to investigate in detail the interrelationships of both H_2O_2 and NO and how they would interact with GA and ABA metabolism and signalling in the same seed during imbibition.

References

Amooaghaie R, Tabatabaie F (2017) Osmopriming-induced salt tolerance during seed germination of alfalfa most likely mediates through H_2O_2 signaling and upregulation of heme oxygenase. Protoplasma 254:1791–1803

An CF, Zhou CJ (2017) Light induces lettuce seed germination through promoting nitric oxide production and phospholipase D-derived phosphatidic acid formation. South Afr J Bot 108:416–422

Bethke PC, Gubler F, Jacobsen JV, Jones RL (2004) Dormancy of *Arabidopsis* seeds and barley grains can be broken by nitric oxide. Planta 219:847–855

Bewley DJ, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds: physiology of development, germination, and dormancy. Springer, New York

Bi C, Ma Y, Yu YT, Liang S, Lu K, Wang XF (2017) *Arabidopsis* ABI5 plays a role in regulating ROS homeostasis by activating *CATALASE 1* transcription in seed germination. Plant Mol Biol 94:197–213

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and control of seed germination. New Phytol 171:501–523

Finkelstein RR, Lynch TJ (2000) The *Arabidopsis* abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. Plant Cell 12:599–609

Ishibashi Y, Aoki N, Kasa S, Sakamoto M, Kai K, Tomokiyo R, Watabe G, Yuasa T, Iwaya-Inoue MI (2017) The interrelationship between abscisic acid and reactive oxygen species plays a key role in barley seed dormancy and germination. Front Plant Sci 8:275

Kepczynski J, Cembrowska-Lech D, Sznigir P (2017) Interplay between nitric oxide, ethylene, and gibberellic acid regulating the release of *Amaranthus retroflexus* seed dormancy. Acta Physiol Plant 39:254

Li WY, Chen BX, Chen ZJ, Gao YT, Chen Z, Liu J (2017a) Reactive oxygen species generated by NADPH oxidases promote radicle protrusion and elongation during rice seed germination. Int J Mol Sci 18:110

Li Z, Xu J, Gao Y, Wang C, Guo G, Luo Y, Huang Y, Hu W, Sheteiwy MS, Guan Y, Hu J (2017b) The synergistic priming effect of exogenous salicylic acid and H_2O_2 on chilling tolerance enhancement during maize (*Zea mays* L.) seed germination. Front Plant Sci 8:1153

Li R, Jia Y, Yu L, Yang W, Chen Z, Chen H, Hu X (2018) Nitric oxide promotes light-initiated seed germination by PIF1 expression and stabilizing HFR1. Plant Physiol Biochem 123:204–212

Lu Q, Zhang ZS, Zhan RT, He R (2018) Proteomic analysis of *Zanthoxylum nitidum* seeds dormancy release: influence of stratification and gibberellin. Ind Crop Prod 122:7–15

Mao C, Zhu Y, Chen H, Yan H, Zhao L, Tang J, Ma X, Mao P (2018) Nitric acid oxide regulates seedling growth and mitochondrial responses in aged oat seeds. Int J Mol Sci 19:1052

Nelson SK, Steber CM (2017) Transcriptional mechanisms associated with seed dormancy and dormancy loss in gibberellin-insensitive sly1-2 mutant of *Arabidopsis thaliana*. PLoS One 12: e0179143

Nonogaki H (2014) Seed dormancy and germination-emerging mechanisms and new hypotheses. Front Plant Sci 5:233

Nonogaki H (2017) Seed biology updates-highlights and new discoveries in seed dormancy and germination research. Front Plant Sci 8:524

Panngom K, Chuesaard T, Tamchan N, Jiwchan T, Srikongsritong K, Park G (2018) Comparative assessment for the effects of reactive species on seed germination, growth and metabolisms of vegetables. Scient Hort 227:85–91

Shekhawat GS, Verma K (2010) Haem oxygenase (HO): an overlooked enzyme of plant metabolism and defence. J Exp Bot 61:2255–2270

Signorelli S, Considine MJ (2018) Nitric oxide enables germination by a four-pronged attack on ABA-induced seed dormancy. Front Plant Sci 9:296

Skubacz A, Daszkowska-Golec A, Szarejko I (2016) The role and regulation of ABI5 (ABA-Insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. Front Plant Sci 7:1884

Su L, Lan Q, Pritchard HW, Xue H, Wang X (2016) Reactive oxygen species induced by cold stratification promote germination of *Hedysarum scoparium* seeds. Plant Physiol Biochem 109:406–415

Yang L, Zhang D, Liu H, Wei C, Wang J, Shen H (2018) Effects of nitric oxide donor and nitric oxide scavengers on *Sorbus pohuashanensis*. J For Res 29:631–638

Zhang Y, Deng B, Li Z (2018) Inhibition of NADPH oxidase increases defense enzyme activities and improves maize seed germination under Pb stress. Ecotoxicol Environ Saf 158:187–192

Nitric Oxide and Hydrogen Peroxide in Root Organogenesis

Javier Raya-González, Jesús Salvador López-Bucio, and José López-Bucio

Contents

Abstract Nitric oxide (NO) and reactive oxygen species (ROS) are central messengers in the way plants respond to environmental and hormonal stimuli and for the configuration of root architecture. ROS determine the boundaries between the meristem and cell elongation zone of the primary root and act in concert with NO to promote lateral root primordia maturation and epidermal cell differentiation. Overall, the capacity of roots to acquire nutrients such as phosphate, nitrate, and sulfate is determined by NO and ROS via their effects on root hair development and expression of genes for improving nutritional responses or orchestrating the activities of proteins of all major hormonal pathways, including auxin, ethylene, jasmonic acid, brassinosteroids, and abscisic acid. Specifically, ROS target phosphatases and transcription factors of two main families, MYB and BHLH, these later being probably recruited by the *mediator* complex to the promoters of genes for transcription. Here, we review the information about the functions and mechanisms of NO and ROS modulated-root organogenesis, including growth, patterning, and differentiation.

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1 Root System Architecture and Patterning

The root system provides support to photosynthetic and reproductive organs of plants and explores the soil to acquire water and nutrients. Its tridimensional configuration commonly referred to as root architecture depends upon cell division, elongation and differentiation in the primary root, and branching patterns that develop postembrionically, all these aspects being fine-tuned regulated by the growth conditions.

The model plant *Arabidopsis thaliana* develops a taproot system typical of dicots, in which the primary root sustains indeterminate growth and forms a dominant axis. In contrast, multiple embryonic primary and seminal roots are formed in cereals such as maize, sorghum, and wheat (Martínez-de la Cruz et al. 2015; Rogers and Benfey 2015). Adventitious and/or lateral roots increase the overall root absorptive capacity since every branch can develop specialized types of epidermal cells termed root hairs, which are directly involved in water and nutrient uptake (Salazar-Henao et al. 2016; Du and Scheres 2018; Stoeckle et al. 2018).

Roots are very sensitive and respond to light, gravity, acidity or alkalinity, temperature, and salinity (Mo et al. 2015; Ruiz-Herrera et al. 2015; Ha et al. 2018). In actively growing root tips, three main regions can be distinguished: the root apical meristem, an elongation zone, and a differentiation region. In the meristem, cells proliferate at a high rate through mitosis, which is sustained by the root stem cell niche (RSCN), composed by the quiescent center (QC) and peripheral initials or stem cells that divide asymmetrically for self-renewing (Cederholm et al. 2012). Later on, the daughter cells enter into the elongation zone where mitosis ceases, increase in size, and begin to differentiate (Tsukagoshi 2016). The differentiation region is typified by the formation of root hairs from epidermal cells termed trichoblasts, vascular tissue formation occurs, and lateral roots initiate from pericycle cells (Salazar-Henao et al. 2016; Du and Scheres 2018). All major root developmental transitions are related to specific NO and ROS signatures. This chapter updates the knowledge on the functions and mechanisms of NO and ROS in the signaling network that orchestrates root system development.

NO plays critical roles in plant growth and in all major developmental transitions, including embryogenesis, seed germination, root system configuration, flowering, fruit maturation, and leaf senescence (Domingos et al. 2015). Biochemical and cellular analyses show the presence of NO and NO-derived molecules in roots of several species, for instance, using fluorescent indicators, like 4,5-diaminofluorescein diacetate (DAF-2DA), which is a cell-permeable compound that when nitrosylated by NO emits fluorescence (Airaki et al. 2015; Corpas and Barroso 2015; Yamasaki et al. 2016), has revealed that in *Arabidopsis*, NO accumulates in the primary root tip, trichoblast cells, and lateral root cap, and its levels

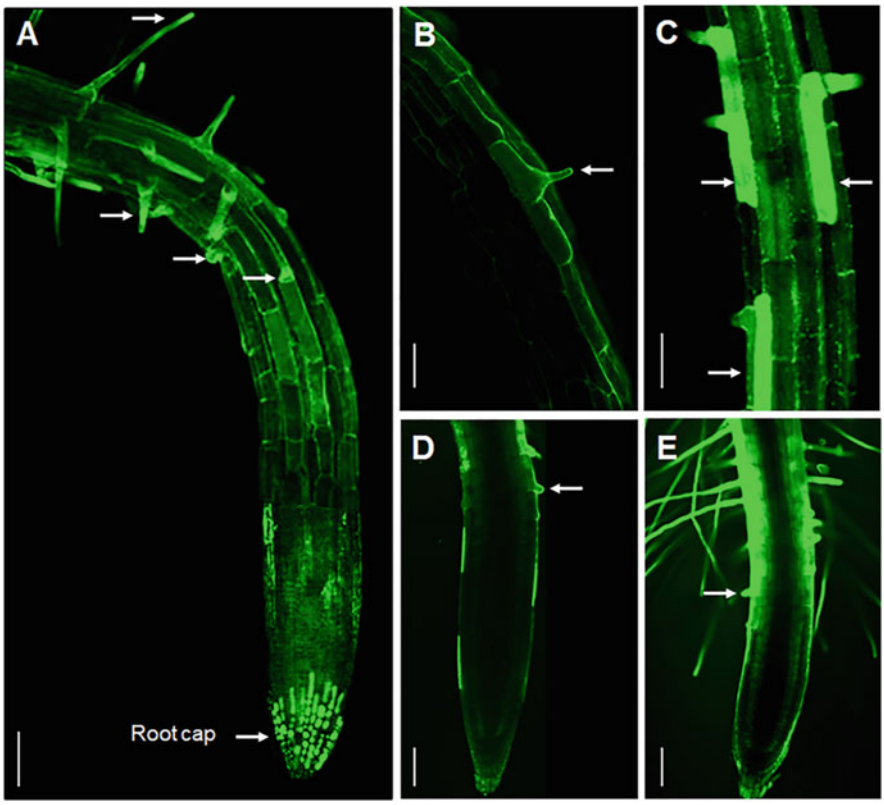

Fig. 1 NO detection in distinct cell types and zones of the *Arabidopsis* primary root. Representative confocal images of primary roots from 7-day-old *Arabidopsis* (Columbia-0 ecotype) seedlings that were stained with the specific NO indicator DAF-2DA for NO detection. (**a**) Primary root. (**b**) Root hair initiation in differentiation zone. (**c**) NO accumulation in trichoblast cells. (**d**, **e**) Root tips under standard growth conditions or supplemented with NO donor SNP, respectively. Arrows in (**a**) show NO accumulation in several stages of root hair development and in root cap cells, in (**b**) mark a trichoblast that just started to elongate, and in (**d**) and (**e**) show the first root hair developed. Fluorescence signal was detected using a confocal laser scanning microscope and monitored with an argon laser with an excitation line from 488 to 568 nm and an emission window from 585 to 610 nm. Scale bar = 70 μm in (**a**, **d**, **e**) and 50 μm in (**b**, **c**). The green color corresponds to the detection of NO. Note the correlation between root hair size and NO fluorescence in SNP-treated seedlings

increase upon treatment with NO donors (Fig. 1). Recent advancements further support the importance of NO cross talking with hormonal pathways for modulation of growth and patterning.

ROS comprise superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and hydroxyl radical ($^{\cdot}OH$). These reactive molecules are generated at organelles, including mitochondria, chloroplasts, and peroxisomes, but also in the plasma membrane by ROS-generating enzymes, such as the plant homologs of

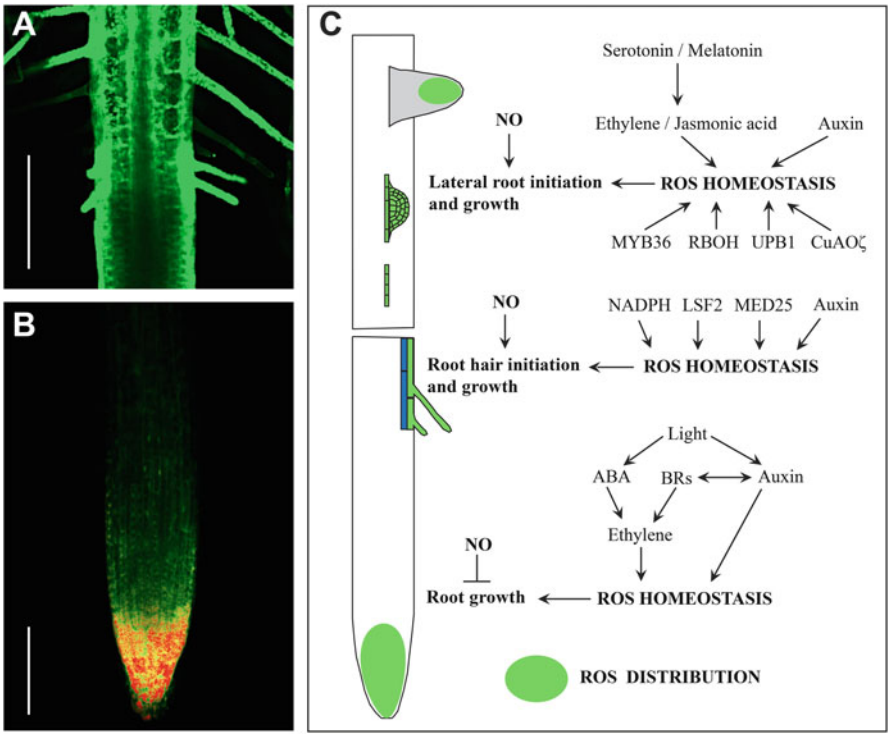

Fig. 2 Roles of NO and ROS in root organogenesis. ROS detection by confocal microscopy using: (**a**) fluorescent dye staining H2DCF-DA (2′,7′-dichlorofluorescein) in the differentiation zone and (**b**) the H_2O_2-specific sensor 35*s:Hyper:YFP* in the meristem. The image in (**b**) was acquired at 405 nm wave lengths for Hyper-H_2O_2-independent excitation and 485 nm for Hyper-H_2O_2-dependent excitation and an emission line of 530 nm. (**c**) NO and ROS signaling modulate primary root growth, root branching, and root hair production. Red/yellow color shows the greater H_2O_2 concentration within the primary root tip. Scale bars in **a** and **b** = 100 μM

respiratory-burst NADPH oxidases and class III peroxidases (Apel and Hirt 2004; Marino et al. 2012; Corpas et al. 2015; Tsukagoshi 2016). Plants accumulate ROS in specific root domains and/or in response to physiological or environmental stimuli (Fig. 2; Xia et al. 2015; Mittler 2017). ROS target DNA, proteins, lipids, and carbohydrates, causing conformational changes (Stadtman and Levine 2003; Nowicka et al. 2013; Tian et al. 2018), and their effects on cellular processes are balanced by the rate of production and cell detoxification via antioxidants or scavenging enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). ROS are not inherently toxic, such that the oxidation of regulatory proteins may improve their function and all types of oxidative modifications are important for cell, tissue, and organ specification (Foyer et al. 2017; Tian et al. 2018).

2 Primary Root Growth

Application of sodium nitroprusside (SNP), a NO donor to tomato, cucumber, and *Arabidopsis* plants, reduces primary root growth, whereas the NO scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO) blocks the action of NO donors and increases root elongation. Characterization of the *Arabidopsis* NO-hypersensitive mutant, *no overproducer/chlorophyll a/b binding protein underexpressed* 1 (*nox1*/*cue1*), showed the correlation between SNP hypersensitivity and formation of a shorter primary root (Correa-Aragunde et al. 2004; Fernández-Marcos et al. 2011).

In *Arabidopsis*, low NO levels cause programmed cell death (PCD), whereas NO accumulation induces DNA damage and cell cycle arrest at G1 and G2/M phases, restraining root growth (Bai et al. 2012). Shen et al. (2013) showed that the expression of CYCD3;1 is modulated by NO levels, NO production mutant *Atnos/noa1* defective at *Arabidopsis thaliana nitric oxide synthase 1/nitric 101 oxide associated 1* have repressed the *CYCD3;1* gene, whereas its constitutive expression complements the mutant phenotype and restores normal root development. The undifferentiated status of root stem cells requires expression of *WUSCHEL-related homeobox 5* (*WOX5*) in the quiescent center. Sanz et al. (2014) showed that application of NO synthesis inhibitors reduces WOX5 expression and this effect could be reverted by SNP supplementation. In addition, the development of the *Arabidopsis* triple mutant *nia1 nia2 noa1* defective on the nitrate reductases 1 and 2 confirmed that decreased NO production and signaling lead to small root meristems with abnormal divisions. The mentioned evidences point to NO as a key player in regulating stem cell activity and root meristem maintenance.

ROS determine the balance among cell proliferation and differentiation in the *Arabidopsis* primary root. ROS are distributed within the root tip, particularly in the meristem and elongation zone (Fig. 2; Tsukagoshi et al. 2010; Hernández-Barrera et al. 2015). Considerable advances have been made toward identifying the proteins orchestrating ROS homeostasis in roots. An *Arabidopsis* P-loop NTPase (APP1) protein located in mitochondria of root meristems displays ATPase activity and hydrolyzes nucleoside triphosphates. Mutation of *APP1* gene causes a reduction in ROS levels and increases cell division rate in the QC, which leads to stem cell differentiation (Yu et al. 2016). Treatments with methyl viologen (MV) and H_2O_2 normalized root patterning, implying that both an increased rate of cell division in the QC and stem cell differentiation can be attributed to a low level of ROS. APP1 acts upstream of the key transcription factors *scarecrow* (*SCR*) and *short root* (*SHR*) to control the undifferentiated status of the meristem and to fine-tune root stem cell niche activity. Impairment of root meristem proliferation could also be observed in *Arabidopsis* mutants lacking the mitochondrial protease AtFTSH4, for which exposure to high temperatures caused the precocious cessation of root growth, which correlates with oxidative stress and progressive mitochondria dysfunction (Dolzblasz et al. 2018).

Kong et al. (2018) characterized the *Arabidopsis* prohibitin PHB3, which belongs into a highly conserved family of proteins that restrict cell proliferation in all three domains of life. *phb3 Arabidopsis* mutants developed a short root with higher rate of QC division and meristem differentiation that correlates with both $O_2^{\cdot-}$ and H_2O_2 accumulation. RNA-seq analysis was performed to compare the root transcriptomes of WT and *phb3* mutants, in which the expression of ethylene response factors (ERF) 115, 114, and 109 was highly increased in *phb3* roots and was antagonized by diphenyleneiodonium (DPI), an inhibitor of NADPH oxidases. Tissue-specific gene expression analysis further indicated that PHB acts through phytosulfokine (PSK) peptide hormones and independently of the *plethora* (PLT) transcription factors. Taken together, these data indicate that ERF115, ERF114, and ERF109 mediate ROS signaling downstream of PHB3 to control root stem cell niche maintenance.

ROS mediates root meristem function during sensing of pathogens or molecules derived from microbes. Mabuchi et al. (2018) identified genes regulated by H_2O_2 in the meristem and elongation zone, including the transcription factor with basic helix-loop-helix (BHLH) domains termed *upbeat1* (UPB1), which controls the expression of a set of peroxidases that establish the ROS gradient correlated with root tip zonation. ROS-induced expression of MYB30, a transcription factor responsive to pathogens and targets genes involved in the transport of very-long-chain fatty acids (VLCFAs) in the epidermis and cortex cell layers of the root tip. Comparison of root growth of wild-type and *myb30-2* seedlings to treatment with Flg22, a microbe-associated molecular pattern (MAMP) that represses growth revealed the resistance of the mutants, suggesting that a MYB30-dependent regulatory network links root growth and immunity. This indicates that growth and defense trade-offs are orchestrated via a single regulatory node, and to this respect, roots colonized with the plant pathogen *Pseudomonas aeruginosa* or treatment with the bacterial virulence factor pyocyanin caused a strong reduction of cell division and elongation (Ortiz-Castro et al. 2014). In this case, however, pyocyanin modulation of the primary root growth required the gaseous hormone ethylene for signaling, since the *Arabidopsis etr1-1*, *ein2-1*, and *ein3-1* ethylene-related mutants were less sensitive to pyocyanin-induced root stoppage. Not only bacterial toxins modulate ROS levels in roots, Pelagio-Flores et al. (2016) while characterizing the role of the neurotransmitter serotonin as a plant signaling molecule found that the ethylene inhibitor $AgNO_3$ antagonized its growth-repressing effects on *Arabidopsis* primary roots, whereas *ethylene overproducer 3* mutants were oversensitive to this compound. Thus, ethylene and ROS are mediators in transducing serotonin and pyocyanin bioactivity.

3 Root Branching

Root branching occurs via the formation of lateral roots, structures formed by de novo organogenesis from pericycle, an inner tissue within mature roots. Overall, lateral root formation comprises two main programs: (1) initiation, through which a lateral root primordium is formed from lateral root founder cells, and (2) emergence,

which allows the passage of the primordium through at least three overlying cell layers, the endodermis, cortex, and epidermis, in order to emerge (Du and Scheres 2018; Stoeckle et al. 2018).

NO promotes lateral and adventitious root maturation (Liao et al. 2012; Ma et al. 2014). Early studies demonstrated that application of NO donors, SNP and SNAP, to hypocotyl explants of cucumber activated de novo root organogenesis. The cellular components sensitive to NO included Ca^{2+}-dependent protein kinases (CDPKs), phosphatidic acid (PA), and mitogen-activated protein kinases (MAPKs), which involve cGMP and Ca^{2+} as second messengers (Lanteri et al. 2006). Environmental stimuli, such as CO_2, promote lateral root initiation by increasing the production of NO, which subsequently increases cytosolic Ca^{2+} concentration-activating plasma membrane and/or intracellular Ca^{2+}-permeable channels. Besides, NO acts downstream of plant hormones to control root organogenesis, including auxin, jasmonic acid, and ethylene (Fig. 2c). In cucumber, *Arabidopsis*, and rice, NO donors mimic the effect of auxin in adventitious root formation, and NO accumulation in lateral root primordia promoted its maturation (Kolbert et al. 2008; Chen and Kao 2012). NO is indispensable for crown root primordia formation, whereas a reduction of intracellular levels blocks this process in rice seedlings (Xiong et al. 2009).

A few *Arabidopsis* mutants have been identified, which represent valuable tools for investigating NO biosynthesis and/or signaling, including *argh1/2* (*arginine amidohydrolase 1/2*), which is defective in an enzyme involved in arginine (Arg) biosynthesis (Flores et al. 2008). Mutations of either *Arabidopsis ARGH1*, two genes result in increased formation of lateral and adventitious roots, accompanied with NO accumulation. *argh1/2* double mutants show higher sensitivity on lateral root formation in response to auxin and increased expression of the auxin-responsive reporter *DR5:GUS* in root tips, suggesting that arginine or its derivatives are potential NO sources to control root morphogenesis (Flores et al. 2008).

The cell wall remodeling of pericycle overlying tissues correlates with ROS deposition in the apoplast, which overlaps with the expression domains of extracellular ROS donors of the *Respiratory burst oxidase homologs* (RBOH), and disrupting or enhancing expression of *RBOH* promotes or antagonizes lateral root emergence (Orman-Ligeza et al. 2016). Thus, RBOH-mediated ROS production establishes the lateral root primordium boundary that facilitates lateral root outgrowth, and it was recently found to depend on the activity of the MYB36 transcription factor (Fernández-Marcos et al. 2017). MYB36 is expressed in the endodermis of primary roots and in developing lateral root primordia, and in *myb36-1* mutants, these structures manifest defective progress after stages IV–V leading to a flat appearance in contrast to the dome-shaped form of the wild type. MYB36 controls a set of peroxidase genes, which maintain the ROS balance in cell tissues overlying the primordium and in this manner fine-tunes its emergence through the parent layers.

ROS are specifically involved in lateral root outgrowth, since H_2O_2 supplementation accelerates lateral root development from newly formed primordia. The effects of ROS is independent of auxin signaling, because H_2O_2 could restore lateral root formation in auxin-related mutants that are defective in cell wall softening and

remodeling (Orman-Ligeza et al. 2016). ROS may also act downstream of auxins as reported for the function of the *Arabidopsis* peroxisome-localized copper amine oxidase ζ (CuAOζ), which controls the ROS production essential for lateral root development. Mutation of CuAOζ results in deficient auxin-induced ROS generation and *pinformed2* (PIN2)-mediated auxin transport (Qu et al. 2017). The UPB1 transcription factor responsible of ROS distribution within the primary root tip is critical in the branching process as *upb1-1* mutants develop a higher number of emerged lateral roots and, conversely, UPB1 overexpressing roots accumulate more primordia, which nowadays failed to emerge (Manzano et al. 2014). In addition, hormonal stimuli that trigger the root branching program rely on ROS to break lateral root primordium quiescence. Treatment of *Arabidopsis* roots with neurotransmitters serotonin and melatonin increased lateral root development, which correlated with higher H_2O_2 levels (Pelagio-Flores et al. 2011, 2012; Chen et al. 2018). The compounds modulated expression of genes responsible for G2-M cell cycle transition, including CDKB1;1, CDKB2;1, CDKB1;1, and CDKB2;1, in a process that involves ethylene and jasmonic acid signaling (Pelagio-Flores et al. 2016; Chen et al. 2018).

4 Root Hair Development

Root hairs are tubular outgrowths from epidermal cells termed trichoblasts that are specialized in form and function to take up water and nutrients and represent an important niche for bacteria inhabiting the rhizosphere. Root hairs develop from a bulge in the trichoblast, which elongates via tip growth and reaches its maximum size (up to 1 mm in *Arabidopsis*) depending upon the plant hormonal status and nutrient availability in the soil and proceeds through the generation of a high Ca^{2+} gradient (Shin et al. 2005; Bhosale et al. 2018; Dindas et al. 2018). For instance, phosphate starvation increases up to threefold the overall root surface through inducing extra cell files of root hairs and increasing the growth of these structures in a NO- and ROS-mediated genetic program (López-Bucio et al. 2003; Ruiz-Herrera et al. 2015; Gutiérrez-Alanís et al. 2018).

NO promotes epidermal cell differentiation during root hair development of lettuce (*Lactuca sativa*) and *Arabidopsis* (Fig. 1a, d, and e; Lombardo et al. 2006). SNP application to lettuce plants resulted in almost all rhizodermal cells to be differentiated into root hairs. Treatment with the synthetic auxin 1-naphthyl acetic acid (NAA) increased root hair formation that was prevented by the NO scavenger, cPTIO (Lombardo et al. 2006). Two *Arabidopsis* mutants associated to NO production, namely, *Atnos1/noa1* and *nia1 nia2* single and double mutants, respectively, are affected in root hair growth, which could be phenocopied in wild-type plants by cPTIO (Lombardo et al. 2006). Interestingly, NO was detected inside the vacuole of root hairs and acts as a critical component for endocytosis, vesicle formation, and trafficking, nucleus migration, and vacuolar development during root hair growth, and NO application restored vesicle formation and trafficking in *nia1 nia2* mutants.

Root hair development is increased in *Atgsnor1-1* and reduced in *Atgsnor1-3* plants defective on *S-nitrosoglutathione (GSNO) reductase* (*AtGSNOR*), which catalyzes the posttranslational modifications of proteins via the addition of an NO moiety to a reactive cysteine thiol, to form an S-nitrosothiol (Kwon et al. 2012). Thus, nitrosylation can be regarded as an instructive signal for tip growth of root epidermal cells.

Different molecules have been reported to affect ROS homeostasis principally during root hair development. For instance, treatments with vanadate increases root hair density and length, and this correlated with induced ROS production. Lin et al. (2015) investigated the pathways involved in vanadate-induced root hair formation in *Arabidopsis* by supplying diphenylene iodonium (DPI), an inhibitor of NADPH oxidase, and using the NADPH oxidase mutant *root hair-defective* mutant 2 (*rhd2*) that encodes a NADPH oxidase (AtrbohC). Vanadate changed the levels of transcripts related to cell wall formation and ROS signaling and required the NADPH oxidase. Taken together, these studies support the important role of ROS homeostasis in regulating root hair growth in response to environmental stress.

Several screens identified *Arabidopsis* mutants lacking root hairs or producing short-root hairs that helped clarifying the roles of ROS in polar growth. The RHD (*root hair defective*) /RSL (*root hair defective like*) transcription factors control both the initiation and elongation phases during root hair development. Foreman et al. (2003) showed that *rhd2* regulates root hair growth through the activation of Ca^{2+} channels (Foreman et al. 2003). On the other hand, RHD6 activates the RSL4/RSL2 transcription factors, which act downstream of auxin to release *auxin response factors* (ARFs) ARF5, ARF7, ARF8, and ARF19 from Aux/IAA proteins. Auxin activation of RSL4 expression was related to changes in ROS homeostasis through the RBOHC, H, and J proteins and four type III-secreted peroxidases (Mangano et al. 2017, 2018).

Auxin response involves several components of the *mediator* (MED) transcriptional complex, which acts as a bridge between ARFs and the RNA polymerase II. *Phytochrome and flowering time1* (PFT1) corresponds to the MED25 subunit and its loss of function renders plants oversensitive to auxin (Raya-González et al. 2014). Global gene expression analysis revealed the activation of class III peroxidases by PFT1, while the corresponding *Arabidopsis* mutants had an altered $O_2^{\cdot -}$ and H_2O_2 distribution, indicating that PFT1 is critical to maintain redox homeostasis. Normalization of ROS levels rescued the *pft1* mutant phenotype, suggesting its essential prerequisite for root hair patterning through cell wall remodeling genes (Sundaravelpandian et al. 2013). These results link the MED complex via PFT1/MED25 to the transcriptional machinery orchestrating ROS distribution.

In plants, protein phosphatases regulate a myriad of cellular processes via dephosphorylation reactions that affect ROS homeostasis. The *starch excess4* (SEX4) and *like sex four2* (LSF2) are two glucan phosphatases controlled by the redox status. LSF2 is located in the chloroplast and cytoplasm and is related to starch metabolism. Zhao et al. (2016) characterized the *lsf2-1* mutant, which shows reduced rates of $O_2^{\cdot -}$ generation and higher levels of H_2O_2 in response to oxidative stress, which correlates with root hair growth. LSF2 interacts with mitogen-activated

protein kinase 8 (MPK8), a known component of ROS homeostasis pathways in the cytoplasm. Thus, a MAPK cascade may integrate LSF2 function, ROS homeostasis, and root hair development.

5 Shoot-to-Root Long-Distance Signaling

Roots respond to local soil conditions as well as to systemic signal cues, and this is important for adaptation and survival to the dynamic environment (Raya-González et al. 2017). Light is required for photosynthesis and reconfigures plant architecture, such that different wave lengths are perceived in stems and leaves through red/far-red photoreceptor phytochromes, or cryptochromes, which mediate primary root elongation, gravitropism, and hormone responses (Lee et al. 2016). Abscisic acid (ABA) accumulates in shoots following light exposure and is transported long distance to roots where it triggers developmental plasticity linked to ROS, antioxidants, and ROS-detoxifying enzymes, specifically during mitosis (de Tullio et al. 2010).

Two reports have clarified the mechanisms by which ABA, imported from shoots or locally produced in roots, influence meristematic activity, and both involved ROS as second messengers. Yang et al. (2014) identified a recessive, ABA-oversensitive *Arabidopsis* mutant with retarded growth named *abo8-1*, which is defective in a pentatricopeptide repeat (PPR) protein responsible for the correct functioning of the mitochondrial complex I. Interestingly, *abo8-1* mutants accumulated more ROS in root tips than the wild type, and this effect was exacerbated by ABA treatment. High ROS levels reduced root meristem activity through affecting the expression of genes that determine stem cell niche identity, whereas the normal growth could be reversibly recovered by treatment with the reducing agent GSH. In the other works, Ha et al. (2018) showed that in *Arabidopsis* plants exposed to light, the phyB photoreceptor stimulates ABA synthesis in shoots and then the hormone moves to roots and triggers a peroxidase-mediated ROS detoxification.

UV light may cause damage to DNA and the residing mutations often result in cell death. Genetic screens aimed at identifying ABA-related genes found the MED18 subunit of the transcriptional *mediator* complex, because the *med18* loss-of-function mutant is oversensitive to root growth inhibition by ABA (Zhu et al. 2017). Noteworthy, the *med18* mutants show delayed root growth, related to cell death in the root meristem, which exacerbates with age and/or exposition to DNA damaging agents (Raya-González et al. unpublished). Cell death was reduced in *med18* seedlings grown in darkness but remained when only the shoot is exposed to light, suggesting that MED18 acts to protect root meristem cells from local cell death, and/or in response to root-acting signal (s) such as ABA and/or ROS emitted by the shoot in response to light stimuli. *med18* mutants overexpress the cell regeneration factor ERF115, which triggers cell division and replenishes the stem cell pool during root tip regeneration in a similar manner to animal limb recovery, and in such case the lost part of the body could be replaced through conversion of

normal cells into stem cells that act as progenitors of the missing tissues (Efroni et al. 2016; Heyman et al. 2016). As mentioned above ERF115 overexpression is found in *phb3* mutants that displayed a short-root phenotype dependent of ROS deregulation. These data evidences the break point between root growth and adaptation to stress, which is integrated by a complex but fine-tuned pathway in which ABA, MED18/ERF115 and ROS are key components.

6 Hormone Cross Talk

NO and ROS interact with most signaling pathways underlying hormonal and nutritional responses in plants, which influence, at least to some degree, the endogenous levels of these reactive molecules (Freschi 2013; Sanz et al. 2015; Liu et al. 2018; Sun et al. 2018). Brassinosteroids-auxin-ethylene cross talk activates NO- and ROS-dependent mechanisms for growth modulation, which occur in a concentration and tissue-dependent manner. Brassinosteroid (BR) synthesis and signaling enable root growth and development, but their alteration by either pharmacological or genetic means induces a short-root phenotype through decreased cell division and elongation (Wei and Li 2016; Lv et al. 2018). The response of roots to BRs application correlated with enhanced NO levels and was blocked by cPTIO, suggesting that NO is required for BR-induced changes in root system architecture (Tossi et al. 2013). Indeed, the promoting effect of ethylene on adventitious rooting in cucumber explants could be reverted by cPTIO and NO synthesis inhibitors (Xu et al. 2017).

Tian et al. (2018) showed that BRs binding to its receptor kinase BRI1 promoted dephosphorylation of the transcription factor *brassinazole-resistant1* (BZR1), and increased intracellular levels of H_2O_2, which in turn caused oxidation of BZR1 at a conserved cysteine residue. This modification promoted the interaction with *auxin response factor6* (ARF6) and *phytochrome interacting factor4* (PIF4), which act as regulators in the auxin and light-signaling pathways, respectively. A genetic screen of *Arabidopsis* mutants producing short primary roots identified the *det2-9* mutant defective in a steroid 5α-reductase from the BR synthesis pathway. The *det2-9* root phenotype correlated with reduced cell number in meristem and decreased cell size at the maturation zone, which was caused by an enhanced rate of ethylene biosynthesis and was recovered in the *det2-9/acs9* double mutant and *det2-9/ein3/eil1-1* triple mutant, which have defects either in ethylene synthesis or ethylene signaling, respectively. These data indicate that ethylene signaling acts downstream of BRs for the modulation of cell processes that determine primary root growth. Interestingly, the *det2-9* mutant produced more $O_2^{\cdot -}$ than wild type plants through the peroxidase pathway (Lv et al. 2018).

The alkamides comprise a group of fatty acid amides, which have emerged as modulators of root development (López-Bucio et al. 2006). *Arabidopsis* root explants treated with *N*-isobutyl decanamide showed higher adventitious root number and an increase in NO accumulation in zones of adventitious root formation

(Campos-Cuevas et al. 2008). Later on, Méndez-Bravo et al. (2010, 2011) found that morphogenetic effects of alkamides decreased by cPTIO application. Interestingly, *Arabidopsis* mutants defective at the *DRR1* (*decanamide resistant root 1*) locus were less sensitive in both primary root reduction and lateral root promotion to NO treatments and bacterial quorum-sensing perception and had decreased senescence (Morquecho-Contreras et al. 2010), suggesting its role as a modulator in small lipid amide and NO sensing.

7 Conclusions

NO and ROS production by plants has been traditionally related with adaptation to stress and defense against pathogens. The emerging view is that accumulation and/or distribution of these reactive molecules support the basic cellular programs defining tissue and organ shape (Fig. 2c). Their fundamental role underlies root growth and development and goes beyond the polarized tip growth of the primary root, lateral roots, and root hairs.

Major roles of NO have been defined during lateral root formation, and its alteration causes root apical meristem defects and growth inhibition while reducing auxin transport. It also orchestrates root architecture configuration in response to bioactive metabolites such as alkamides, bacterial quorum-sensing signals, and cross talks with most phytohormone signaling pathways including auxin, ethylene, and jasmonic acid. The recent characterization of NO-related mutant *drr1* of *Arabidopsis*, unraveled its critical function in plant senescence, whereas NO production through nitrate reductases, NOS, and NOS-related enzymes supports a direct link among nutrition and metabolism that should influence all major plant phase transitions.

The environmental and hormonal long-distance communication between shoots and roots are orchestrated by ROS acting in the meristems. The ongoing characterization of *Arabidopsis* mutants has proven to be useful toward identifying the signaling players in ROS accumulation/detoxification, for which phosphatases and MYB and BHLH transcription factors orchestrate gene expression, probably being recruited by the *mediator* complex to the promoters of genes for transcription.

A very interesting perspective is that an ABA-ROS signaling could inform the root of the light quality in leaves to fine-tune cell division and elongation and, even more, to support regeneration of damaged tissues. Global gene expression analysis demonstrated that the regulatory network orchestrated by ROS is dynamic and specific and that the phytohormones auxin, ethylene, jasmonic acid, and brassinosteroids influence positively or negatively ROS levels in the meristem and elongation zones and determine the rate of growth of primary and lateral roots. Characterization of the activities of the proteins and other macromolecular targets of ROS may confirm that nitrosylation and oxidation have fundamental roles in organogenesis and in the way plants react to the provision of mineral nutrients, such as nitrate, phosphate, and sulfate, which are required in high amounts to support agriculture.

References

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. Ann Bot 116:679–693

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

Bai S, Li M, Yao T, Wang H, Zhang Y, Xiao L, Wang J, Zhang Z, Hu Y, Liu W, He Y (2012) Nitric oxide restrain root growth by DNA damage induced cell cycle arrest in *Arabidopsis thaliana*. Nitric Oxide 26:54–60

Bhosale R, Giri J, Pandey BK, Giehl RFH, Hartmann A, Traini R, Truskina J, Leftley N, Hanlon M, Swarup K, Rashed A, Voß U, Alonso J, Stepanova A, Yun J, Ljung K, Brown KM, Lynch JP, Dolan L, Vernoux T, Bishopp A, Wells D, von Wirén N, Bennett MJ, Swarup R (2018) A mechanistic framework for auxin dependent *Arabidopsis* root hair elongation to low external phosphate. Nat Commun 9:1409

Campos-Cuevas JC, Pelagio-Flores R, Raya-González J, Méndez-Bravo A, Ortiz-Castro R, López-Bucio J (2008) Tissue culture of *Arabidopsis thaliana* explants reveals a stimulatory effect of alkamides on adventitious root formation and nitric accumulation. Plant Sci 174:165–173

Cederholm H, Lyer-Pascuzzi AS, Benfey P (2012) Patterning the primary root in Arabidopsis. WIREs Dev Biol 1:675–691

Chen YH, Kao CH (2012) Calcium is involved in nitric oxide- and auxin-induced lateral root formation in rice. Protoplasma 249:187–195

Chen Z, Gu Q, Yu X, Huang L, Xu S, Wang R, Shen W, Shen W (2018) Hydrogen peroxide acts downstream of melatonin to induce lateral root formation. Ann Bot 121:1127–1136

Corpas FJ, Barroso JB (2015) Functions of nitric oxide (NO) in roots during development and under adverse stress conditions. Plants (Basel) 22:240–252

Corpas FJ, Gupta DK, Palma JM (2015) Production sites of reactive oxygen species (ROS) in organelles from plant cells. In: Gupta DK, Palma JM, Corpas FJ (eds) Reactive oxygen species and oxidative damage in plants under stress. Springer, Basel, pp 1–19

Correa-Aragunde N, Graziano M, Lamattina L (2004) Nitric oxide plays a central role in determining lateral root development in tomato. Planta 218:900–905

De Tullio MC, Jiang K, Feldman LJ (2010) Redox regulation of root apical meristem organization: connecting root development to its environment. Plant Physiol Biochem 45:328–336

Dindas J, Scherzer S, Roelfsema MRG, von Meyer K, Müller HM, Al-Rasheid KAS, Palme K, Dietrich P, Becker D, Bennett MJ, Hedrich R (2018) AUX1-mediated root hair auxin influx governs SCF(TIR1/AFB)-type Ca(2+) signaling. Nat Commun 9:1174

Dolzblasz A, Gola EM, Sokołowska K, Smakowska-Luzan E, Twardawska A, Janska H (2018) Impairment of meristem proliferation in plants lacking the mitochondrial protease AtFTSH4. Int J Mol Sci 19:853

Domingos P, Prado AM, Wong A, Gehring C, Feijo JA (2015) Nitric oxide: a multitasked signaling gas in plants. Mol Plant 8:506–520

Du Y, Scheres B (2018) Lateral root formation and the multiple roles of auxin. J Exp Bot 69:155–167

Efroni I, Mello A, Nawy T, Ip PL Rahni R, DelRose N, Powers A, Satija R, Birnbaum KD (2016) Root regeneration triggers an embryo-like sequence guided by hormonal interactions. Cell 165:1721–1733

Fernández-Marcos M, Sanz L, Lewis DR, Muday GK, Lorenzo O (2011) Nitric oxide causes root apical mersitem defects and growth inhibition while reducing PIN-FORMED 1 (PIN1)-dependent acropetal auxin transport. Proc Natl Acad Sci U S A 108:18506–18511

Fernández-Marcos M, Desvoyes B, Manzano C, Liberman LM, Benfey PN, del Pozo JC, Gutierrez C (2017) Control of *Arabidopsis* lateral root primordium boundaries by MYB36. New Phytol 213:105–112

Flores T, Todd CD, Tovar-Mendez A, Dhanoa PK, Correa-Aragunde N, Hoyos ME, Brownfield DM, Mullen RT, Lamattina L, Polacco JC (2008) Arginase-negative mutants of *Arabidopsis* exhibit increased nitric oxide signaling in root development. Plant Physiol 147:1936–1946

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

Foyer CH, Ruban AV, Noctor G (2017) Viewing oxidative stress through the lens of oxidative signalling rather than damage. Biochem J 474:877–883

Freschi L (2013) Nitric oxide and phytohormone interactions: current status and perspectives. Front Plant Sci 4:398

Gutiérrez-Alanís D, Ojeda-Rivera JO, Yong-Villalobos L, Cardenas-Torres L, Herrera-Estrella L (2018) Adaptation to phosphate scarcity: tips from *Arabidopsis* roots. Trend Plant Sci 23:721–730

Ha JH, Kim JH, Kim SG, Sim HS, Lee G, Halitschke R, Baldwin IT, Kim JI, Park CM (2018) Shoot phytochrome B modulates reactive oxygen species homeostasis in roots via abscisic acid signaling in *Arabidopsis*. Plant J 94:790–798

Hernández-Barrera A, Velarde-Buendía A, Zepeda I, Sánchez F, Quinto C, Sánchez-López R, Cheung AY, Wu HM, Cardenas L (2015) Hyper, a hydrogen peroxide sensor, indicates the sensitivity of the *Arabidopsis* root elongation zone to aluminum treatment. Sensors 15:855–867

Heyman J, Cools T, Canher B, Shavialenka S, Traas J, Vercauteren I, Van den Daele H, Persiau G, De Jaeger G, Sugimoto G, De Veylder L (2016) The heterodimeric transcription factor complex ERF115-PAT1 grants regeneration competence. Nat Plant 2:16165

Kolbert Z, Bartha B, Erdei L (2008) Exogenous auxin-induced NO synthesis in nitrate reductase-associated in *Arabidopsis thaliana* root primordia. J Plant Physiol 165:967–975

Kong X, Tian H, Yu Q, Zhang F, Wang R, Gao S, Xu W, Liu J, Shani E, Fu C, Zhou G, Zhang L, Zhang X, Ding Z (2018) PHB3 maintains root stem cell niche identity through ROS-responsive AP2/ERF transcription factors in *Arabidopsis*. Cell Rep 22:1350–1363

Kwon E, Feechan A, Yun BW, Hwang BH, Pallas JA, Kang JG, Loake GJ (2012) AtGSNOR1 function is required for multiple developmental programs in *Arabidopsis*. Planta 236:887–900

Lanteri ML, Pagnussat GC, Lamattina L (2006) Calcium and calcium-dependent protein kinases are involved in nitric oxide- and auxin-induced adventitious root formation in cucumber. J Exp Bot 57:1341–1351

Lee HJ, Ha JH, Kim SG, Choi HK, Kim ZH, Han YJ, Kim JI, Oh Y, Fragoso V, Shin K, Hyeon T, Choi HG, Oh KH, Baldwin IT, Park CM (2016) Stem-piped light activates phytochrome B to trigger light responses in *Arabidopsis thaliana* roots. Sci Signal 9:ra106

Liao WB, Zhang ML, Huang GB, Yu JH (2012) Ca^{2+} and CaM are involved in NO- and H_2O_2-induced adventitious root development in marigold. J Plant Growth Regul 31:253–264

Lin CY, Huang LY, Chi WC, Huang TL, Kakimoto T, Tsai CR, Huang HJ (2015) Pathways involved in vanadate-induced root hair formation in *Arabidopsis*. Physiol Plant 153:137–148

Liu M, Zhang H, Fang X, Zhang Y, Jin C (2018) Auxin acts downstream of ethylene and nitric oxide to regulate magnesium deficiency-induced root hair development in *Arabidopsis thaliana*. Plant Cell Physiol 59:1452–1465

Lombardo MC, Graziano M, Polacco JC, Lamattina L (2006) Nitric oxide functions as a positive regulator of root hair development. Plant Signal Behav 1:28–33

López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol 6:280–287

López-Bucio J, Acevedo-Hernández G, Molina-Torres J, Herrera-Estrella L (2006) Novel signals for plant development. Curr Opin Plant Biol 9:523–529

Lv B, Tian H, Zhang F, Liu J, Lu S, Bai M, Li C, Ding Z (2018) Brassinosteroids regulate root growth by controlling reactive oxygen species homeostasis and dual effect on ethylene synthesis in *Arabidopsis*. PLoS Genet 14:e1007144

Ma F, Wang L, Li J, Samma MK, Xie Y, Wang R, Wang J, Zhang J, Shen W (2014) Interaction between HY1 and H_2O_2 in auxin-induced lateral root formation in *Arabidopsis*. Plant Mol Biol 85:49–61

Mabuchi K, Maki H, Itaya T, Suzuki T, Nomoto M, Sakaoka S, Morikami A, Higashiyama T, Tada Y, Busch W, Tsukagoshi H (2018) MYB30 links ROS signaling, root cell elongation, and plant immune responses. Proc Natl Acad Sci U S A 115:E4710–E4719

Mangano S, Denita-Juarez SP, Choi HS, Marzol E, Hwang Y, Ranocha P, Velasquez SM, Borassi C, Barberini ML, Aptekmann AA, Muschietti JP, Nadra AD, Dunand C, Cho HT, Estevez JM (2017) The molecular link between auxin and ROS-controlled root hair growth. Proc Natl Acad Sci U S A 114:5289–5294

Mangano S, SP D-J, Marzol E, Borassi C, Estevez JM (2018) High auxin and high phosphate impact on RSL2 expression and ROS-homeostasis linked to root hair growth in *Arabidopsis thaliana*. Front Plant Sci 9:1164

Manzano C, Pallero-Baena M, Casimiro I, De Rybel B, Orman-Ligeza B, Van Isterdael G, Beeckman T, Draye X, Casero P, Del Pozo JC (2014) The emerging role of reactive oxygen species signaling during lateral root development. Plant Physiol 165:1105–1119

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

Martínez-de la Cruz E, García-Ramírez E, Vázquez-Ramos JM, Reyes de la Cruz H, López-Bucio J (2015) Auxins differentially regulate root system architecture and cell cycle protein levels in maize seedlings. J Plant Physiol 176:147–156

Méndez-Bravo A, Raya-González J, Herrera-Estrella L, López-Bucio J (2010) Nitric oxide is involved in alkamide-induced lateral root development in *Arabidopsis*. Plant Cell Physiol 51:1612–1626

Méndez-Bravo A, Calderón-Vázquez C, Ibarra-Laclette E, Raya-González J, Ramírez-Chavez E, Molina-Torres J, Guevara-García A, López-Bucio J, Herrera-Estrella L (2011) Alkamides actívate jasmonic acid biosynthesis and signaling pathways and confer resistance to *Botrytis cinerea* in *Arabidopsis thaliana*. PLoS One 6(11):e27251

Mittler R (2017) ROS are good. Trend Plant Sci 22:11–19

Mo M, Yokawa K, Wan Y, Baluska F (2015) How and why do root apices sense light under the soil surface? Front Plant Sci 6:775

Morquecho-Contreras A, Mendez-Bravo A, Pelagio-Flores R, Raya-Gonzalez J, Ortiz-Castro R, López-Bucio J (2010) Characterization of *drr1*, an alkamide resistant mutant of *Arabidopsis* reveals an important role for small lipid amides in lateral root development and plant senescence. Plant Physiol 152:1659–1673

Nowicka AM, Kowalczyk A, Sek S, Stojek Z (2013) Oxidation of DNA followed by conformational change after OH radical attack. Anal Chem 85:355–361

Orman-Ligeza B, Parizot B, de Rycke R, Fernandez A, Himschoot E, Van Breusegem F, Bennett MJ, Périlleux C, Beeckman T, Draye X (2016) RBOH-mediated ROS production facilitates lateral root emergence in *Arabidopsis*. Development 143:3328–3339

Ortiz-Castro R, Pelagio-Flores R, Méndez Bravo A, Ruíz Herrera LF, Campos-García J, López-Bucio J (2014) Pyocyanin, a virulence factor produced by *Pseudomonas aeruginosa*, alters root development through reactive oxygen species and ethylene signaling in *Arabidopsis*. Mol Plant Microbe Interact 27:364–378

Pelagio-Flores R, Ortíz-Castro R, Méndez-Bravo A, Macías-Rodríguez L, López-Bucio J (2011) Serotonin, a tryptophan-derived signal conserved in plants and animals, regulates root system architecture probably acting as a natural auxin inhibitor in *Arabidopsis thaliana*. Plant Cell Physiol 52:490–508

Pelagio-Flores R, Muñoz-Parra E, Ortiz-Castro R, López-Bucio J (2012) Melatonin regulates Arabidopsis root system architecture likely acting independently of auxin signaling. J Pineal Res 53:279–288

Pelagio-Flores R, Ruiz-Herrera LF, López-Bucio J (2016) Serotonin modulates *Arabidopsis* root growth via changes in reactive oxygen species and jasmonic acid-ethylene signaling. Physiol Plant 158:92–105

Qu Y, Wang Q, Guo J, Wang P, Song P, Jia Q, Zhang X, Kudla J, Zhang W, Zhang Q (2017) Peroxisomal CuAOζ and its product H_2O_2 regulate the distribution of auxin and IBA-dependent lateral root development in *Arabidopsis*. J Exp Bot 68:4851–4867

Raya-González J, Ortiz-Castro R, Ruíz-Herrera LF, Kazan K, López-Bucio J (2014) PHYTOCHROME AND FLOWERING TIME1/MEDIATOR25 regulates lateral root formation via auxin signaling in *Arabidopsis*. Plant Physiol 165:880–894

Raya-González J, López-Bucio JS, Prado-Rodríguez JC, Ruiz-Herrera LF, Guevara-García AA, López-Bucio J (2017) The MEDIATOR genes *MED12* and *MED13* control *Arabidopsis* root system configuration influencing sugar and auxin responses. Plant Mol Biol 95:141–156

Rogers ED, Benfey PN (2015) Regulation of plant root system architecture: implications for crop advancement. Curr Opin Biotechnol 32:93–98

Ruiz-Herrera LF, Shane MW, López-Bucio J (2015) Nutritional regulation of root development. WIREs Dev Biol 4:431–443

Salazar-Henao JE, Vélez-Bermúdez IC, Schmidt W (2016) The regulation and plasticity of root hair patterning and morphogenesis. Development 143:1848–1858

Sanz L, Fernández-Marcos M, Modrego A, Lewis DR, Muday GK, Pollmann S, Dueñas M, Santos-Buelga C, Lorenzo O (2014) Nitric oxide plays a role in stem cell niche homeostasis through its interaction with auxin. Plant Physiol 166:1972–1984

Sanz L, Albertos P, Mateos I, Sánchez-Vicente I, Lechón T, Fernández-Marcos M, Lorenzo O (2015) Nitric oxide (NO) and phytohormones crosstalk during early plant development. J Exp Bot 66:2857–2868

Shen Q, Wang YT, Tian H, Guo FQ (2013) Nitric oxide mediates cytokinin functions in cell proliferation and meristem maintenance in *Arabidopsis*. Mol Plant 6:1214–1225

Shin R, Berg RH, Schachtman DP (2005) Reactive oxygen species and root hairs in *Arabidopsis* root response to nitrogen, phosphorus and potassium deficiency. Plant Cell Physiol 46:1350–1357

Stadtman ER, Levine RL (2003) Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acid 25:207–218

Stoeckle D, Thellmann M, Vermeer JE (2018) Breakout-lateral root emergence in *Arabidopsis thaliana*. Curr Opin Plant Biol 41:67–72

Sun H, Feng F, Liu J, Zhao Q (2018) Nitric oxide affects Rice root growth by regulating auxin transport under nitrate supply. Front Plant Sci 9:659

Sundaravelpandian K, Chandrika NNP, Schmidt W (2013) PFT1, a transcriptional Mediator complex subunit, controls root hair differentiation through reactive oxygen species (ROS) distribution in *Arabidopsis*. New Phytol 197:151–161

Tian Y, Fan M, Qin Z, Lv H, Wang M, Zhang Z, Zhou W, Zhao N, Li X, Han C, Ding Z, Wang W, Wang ZY, Bai MB (2018) Hydrogen peroxide positively regulates brassinosteroid signaling through oxidation of the BRASSINAZOLE-RESISTANT1 transcription factor. Nat Commun 9:1063

Tossi V, Lamattina J, Cassia R (2013) Pharmacological and genetical evidence supporting nitric oxide requirement for 2,4-epibrassinolide regulation of root architecture in *Arabidopsis thaliana*. Plant Signal Behav 8:e24712

Tsukagoshi H (2016) Control of root growth and development by reactive oxygen species. Curr Opin Plant Biol 29:57–63

Tsukagoshi H, Busch W, Benfey PN (2010) Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. Cell 143:606–616

Wei Z, Li J (2016) Brassinosteroids regulate root growth, development, and symbiosis. Mol Plant 9:86–100

Xia XJ, Zhou YH, Shi K, Zhou J, Foyer CH, Yu JQ (2015) Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. J Exp Bot 66:2839–2856

Xiong J, Tao L, Zhu C (2009) Does nitric oxide play a pivotal role downstream of auxin in promoting crown root primordia initiation in monocots? Plant Sig Behav 4:999–1001

Xu XT, Jin X, Liao WB, Dawuda MM, Li XP, Wang M, Niu LJ, Ren PJ, Zhu YC (2017) Nitric oxide is involved in ethylene-induced adventitious root development in cucumber (*Cucumis sativus* L.) explants. Sci Hort 215:65–71

Yamasaki H, Watanabe NS, Sakihama Y, Cohen MF (2016) An overview of methods in plant nitric oxide (NO) research: why do we always need to use multiple methods? Method Mol Biol 1424:1–14

Yang L, Zhang J, He J, Qin Y, Hua D, Duan Y, Chen Z, Gong Z (2014) ABA-mediated ROS in mitochondria regulate root meristem activity by controlling PLETHORA expression in *Arabidopsis*. PLoS Genet 10:e1004791

Yu Q, Tian H, Yue K, Liu J, Zhang B, Li X, Ding Z (2016) A P-loop NTPase regulates quiescent center cell division and distal stem cell identity through the regulation of ROS homeostasis in *Arabidopsis* root. PLoS Genet 12:e1006175

Zhao P, Sokolov LN, Ye J, Tang CY, Shi J, Zhen Y, Lan W, Hong Z, Qi J, Lu GH, Pandey GK, Yang YH (2016) The LIKE SEX FOUR2 regulates root development by modulating reactive oxygen species homeostasis in *Arabidopsis*. Sci Rep 6:28683

Zhu Y, Wang B, Tang K, Hsu CC, Xie S, Du H, Yang Y, Tao WA, Zhu JK (2017) An *Arabidopsis* nucleoporin NUP85 modulates plant responses to ABA and salt stress. PLoS Genet 13: e1007124

Nitric Oxide and Hydrogen Peroxide: Signals in Fruit Ripening

Charlotte Steelheart, Andrea Galatro, Carlos Guillermo Bartoli, and Gustavo Esteban Gergoff Grozeff

Contents

Abstract Nitric oxide (NO) and hydrogen peroxide (H_2O_2) have profound effects in growth and development processes in plant physiology. They act as signal molecules under normal conditions or biotic and abiotic stress situations. Fruit ripening is a highly coordinated process that needs the control of several biosynthetic pathways, including signal molecules such as NO and H_2O_2. On the other hand, enzymatic and nonenzymatic antioxidants act to maintain the equilibrium and to prevent oxidative stress. This forward look reviews the relationship between these two active species and their interplay with ethylene in climacteric and non-climacteric fruit ripening. Throughout we emphasize the influence of NO and H_2O_2 crosstalk coupled to the hormone control, chloroplast to chromoplast transition, and their implications in postharvest technology.

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1 Introduction

Angiosperm and floral diversification appeared 140 million years ago in the early Cretaceous concomitant with the coevolution of insects to ensure cross-pollination and gene flow (Wing and Boucher 1998). About 70 million years ago, fruit (especially drupes and berries) appeared as a seed dispersal mechanism together with the rise of mammals and birds (Eriksson et al. 2000). The fruit-disperser interaction is sometimes specific, and this relationship depends on the nutritional quality and secondary metabolism compounds of the fruit (especially fleshy fruits) (Knapp 2002).

Fruit ripening physiology is complex (Giovanoni 2004) and depends on different metabolic pathways (Palma et al. 2011). This highly coordinated developmental process is connected with different biochemical events such as color change (Ronen et al. 2000), starch/sugar metabolism (Prabha and Bhagyalakshmi 1998), sugar/acid balance (Jayasena and Cameron 2008), fruit softening (Brummell 2006), texture changes (Goulao and Oliveira 2008), volatiles release (Goff and Klee 2006), and secondary metabolite synthesis such as many antioxidants, vitamins, flavonoids, and different pigments (Tohge et al. 2014). All these aspects and quality characteristics of the edible fleshy fruit have much importance from the agronomical point of view, coupled to the postharvest physiology, shelf life, and human nutrition (Kitijona et al. 2011). Different sensory and nutritional features that are developed during fruit ripening are highly regulated, and the discovery of genetic and molecular triggers that govern the process may provide new tools to understand and design the appropriate technologies to maintain and/or even improve the desired characteristics (Carrari and Fernie 2006).

Fruit ripening is characterized by two main physiologies: climacteric and non-climacteric. The first studies were reported by Kidd and West in 1925, when they found a peak of the respiratory rate during apple ripening process. This discovery leaded the classification of fruit ripening physiology. The fruits that displayed this peak were named "climacteric," and the rest that did not show this behavior were classified as "non-climacteric." Years later, Gane (1934) discovered that fruits can even release detectable amounts of ethylene and that this gaseous hormone was responsible for the ripening process of certain fruits (Burg and Thimann 1959; Burg and Burg 1965).

The plant gaseous hormone, ethylene, is produced from the *S*-adenosylmethionine, which is converted into l-aminocyclopropane-l-carboxylic acid (ACC) by the ACC synthase (ACS), and the ACC is finally oxidized by the ACC oxidase (ACO) into ethylene. To maintain high levels of ethylene production, the methionine is recycled in a circular pathway (Yang and Hoffman 1984).

Two regulatory systems that coordinate the ethylene biosynthesis were discovered. In plants, System 1 produces low amounts of ethylene in all tissues, including climacteric and non-climacteric fruits, and it has an auto-inhibitory control over this hormone synthesis, whereas System 2 has an autocatalytic control which produces a massive ethylene peak in climacteric fruits, while it is absent in non-climacteric ones (Bouzayen et al. 2009). The bursts in the respiratory rate and ethylene synthesis are important events to start the ripening process in climacteric fruits (Lelievre et al. 1997).

2 Exogenous Applications of Ethylene Has Differential Responses in Climacteric and Non-climacteric Fruits

In climacteric fruits the role of ethylene has been more amply studied (Liu et al. 2015), but the transition from System 1 to System 2 is still not completely understood; however, there are some clues that can explain this process (Cara and Giovannoni 2008). In unripe fruits, System 1 delays the ethylene catalysis up to a certain level till the exposure to the hormone reaches a threshold and switches the autocatalytic System 2 (Klee 2004). At a molecular level, the difference between these two syndromes, either climacteric or non-climacteric, is the induction of *ACS* transcription, which is limited in System 1 and stimulated in System 2. That is the reason why immature climacteric fruits respond to exogenous ethylene at a reception level but do not initiate the ripening process, whereas in mature climacteric fruits, there is an induction of genes associated with ethylene that triggers the chain of responses (Yang 1987). The use of a potent ethylene signaling inhibitor, 1-methylcyclopropene (1-MCP) (Sisler and Serek 1997), confirmed this hypothesis about the stimulation of the ethylene production during tomato ripening (Yokotani et al. 2009).

In non-climacteric fruits, the endogenous ethylene function in ripening is still not well understood. It triggers the color change in grapes (Chervin et al. 2004; Tesniere et al. 2004) and in citrus fruits (Shimokawa et al. 1978; Chaudhary et al. 2012). It was also found that ethylene can trigger the carotenoid biosynthetic pathway in orange flavedo (Rodrigo and Zacarias 2007), the synthesis of volatile organic compounds in citrus (Herrera et al. 2007), and it has a specific organ manner of action in strawberries related to fruit softening (Merchante et al. 2013). Ethylene has no action in internal fruit attributes such as total phenol, flavonoids, vitamin C content, or antioxidant activity in the citrus flesh (Mayouni et al. 2011), but it reduces respiration rate and loss of consuming quality in tangerines (Li et al. 2018). Exogenous application of ethylene has different effects in fruit physiology. In non-climacteric fruits, the increase in the ethylene concentration results in a rise in the respiration rate, being proportional to the amount of ethylene applied. On the other hand, in climacteric fruits there is a shortening in the time to reach the ethylene burst (Tucker and Grierson 1987). The most relevant ripening differences between climacteric and non-climacteric fruits are summarized in Table 1.

Table 1 Summary of the main differences between climacteric and non-climacteric physiologies during fruit ripening

Climacteric	Non-climacteric
A burst in the respiration rate during the ripening process	A drop in the respiration rate during fruit ripening
Ethylene burst during fruit ripening—high ethylene rates	No ethylene burst—low ethylene rates
High and differential sensitivity to exogenous ethylene exposure (firmness, color, starch degradation, etc.)	Low sensitivity to exogenous ethylene (color change and de-greening)
System 1 (auto-inhibition) and System 2 (autocatalytic)	Only System 1 (auto-inhibition)
Sudden and drastic changes during ripening	Gradual and slow changes during ripening
Coincidence in the peak of ethylene and ABA	A rise in ABA during ripening
Exogenous application of ethylene advances the ethylene peak	Exogenous application of ethylene does not advance the ethylene peak
A rise in the application of ethylene does not produce an increment in the respiration rate	A rise in the application of ethylene produces an increment in the respiration rate

3 ROS–Hormone Interaction in Fruit Ripening

Reactive oxygen species (ROS) are uncompleted reduced forms of molecular oxygen (O_2). The excitation of the oxygen or the transference of electrons produces different partially reduced forms that are very reactive with other molecules (Mittler 2002; Noctor et al. 2018). In photosynthetic organs the main source of ROS is the chloroplasts (Galvez-Valdivieso and Mullineaux 2010), while in non-photosynthetic organs, mitochondria are the main ROS source (Navrot et al. 2007). Other sources of ROS are peroxisomes, coupled, among others, to the photorespiration process (Foyer and Noctor 2009), and the NADPH oxidases in plasmalemma (Foreman et al. 2003).

ROS are controlled by ROS-scavenging mechanisms that include enzymatic (e.g., catalase, superoxide dismutase, and ascorbate peroxidase, among others) (Mittler 2002) and nonenzymatic systems (e.g., ascorbic acid, glutathione, tocopherols, anthocyanins, etc.) (Noctor and Foyer 1998).

Fruit set and development is controlled by the interaction of several hormones. While higher concentrations of auxin, gibberellins, and cytokinins are needed for fruit set and growth, increased levels of abscisic acid (ABA) and ethylene have a central role in the initiation and progress of ripening (Kumar et al. 2014). In particular, ethylene participation in several metabolic processes involved in fruit ripening has been largely studied. However, a peak in ABA concentration has been demonstrated to be crucial for the initiation of ripening, triggering ethylene production (Leng et al. 2014) (Fig. 1).

H_2O_2 integrates within redox metabolism with several plant signaling processes (Petrov and van Breusegem 2012). Plants have evolved mechanisms sensing H_2O_2

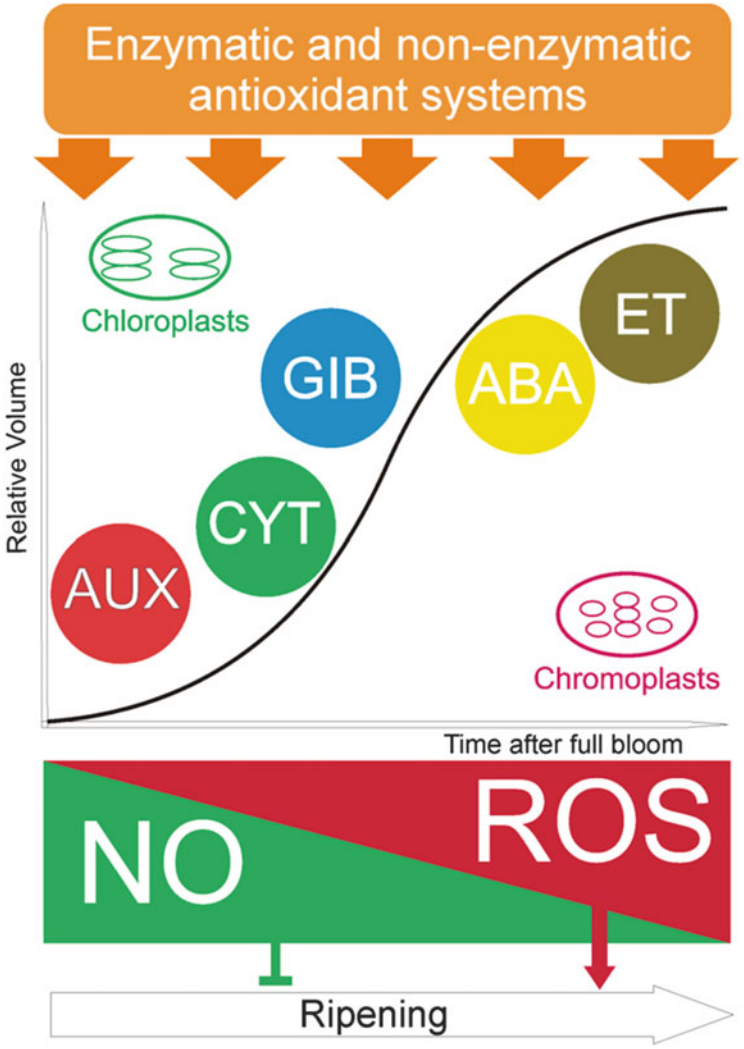

Fig. 1 Hormone influence during fruit growth/ripening and the interplay with NO, H_2O_2, and antioxidants. The black line represents the simple sigmoid pattern of fruit growth. During the first stage of fruit growth, auxins (AUX), gibberellins (GIB), and cytokinins (CYT) have a prevalent role during cell division and expansion, while abscisic acid (ABA) and ethylene (ET) play a central role in fruit ripening and color change in climacteric and non-climacteric fruit. NO content is higher in early stages and decreases with the ripening process, whereas reactive oxygen species (ROS) production rises at the ripening stage. The enzymatic [e.g., catalase, superoxide dismutase] and nonenzymatic [ascorbic acid (AA), glutathione (GSH), and lycopene] antioxidant systems act as a counterpart for the cellular redox homeostasis. The oxidant/antioxidant balance triggers the transition from chloroplasts to chromoplasts and the accumulation of pigments that produce the fruit color change. H_2O_2 acts as a promoter of fruit ripening, whereas NO possibly acts as an anti-senescence molecule

increments and triggering adaptive responses under abiotic stress conditions. Similarly, H_2O_2-dependent cell death was established in several physiological processes such as hypersensitive response or senescence (Gechev et al. 2006).

A peak in ROS production was registered during the initiation of ripening in fruits of several species. Natural or artificially induced increments in H_2O_2 levels correlate with the color change during pear ripening (Brennan and Frenkel 1977). The rise of catalase (CAT) and superoxide dismutase (SOD) activities during fruit ripening in tomato can be enhanced due to abiotic external stress, such as NaCl (Murshed et al. 2014). Similar effects were found when H_2O_2 was used in another climacteric fruit, such as pear, connecting also the effect of H_2O_2 to ethylene release and fruit ripening (Brennan et al. 1979). In pepper, a non-climacteric fruit, Martí et al. (2008) found an increase in the ascorbate/glutathione content during fruit ripening, coupled to the enhancement of Cu,Zn-SOD II activity (but not other SOD isoenzymes), suggesting a possible role in the control of H_2O_2 and $O_2^{\bullet-}$, and acting as modulators during fruit maturation. On the opposite side, Jiménez et al. (2002) found a decrease in some antioxidant enzymes in tomato, such as CAT and ascorbate peroxidase (APX). According to these results, the antioxidant enzymatic systems need to be taken into account to elucidate the function and the activity of these specific enzymes in different climacteric and non-climacteric fleshy fruits during ripening. The differences between experiments could be attributed to different environmental conditions or plant cultivars used in the layout.

The tomato fruit displays an increase of H_2O_2 production at breaker stage with simultaneous increments in oxidative damage to lipid and proteins (Jiménez et al. 2002). Tomato mutants with impaired ethylene signaling showed delay or absence of fruit ripening-associated processes. The concentration of H_2O_2 in these mutants was kept lower than in wild type fruits at the breaker stage (Kumar et al. 2015). Another work demonstrates that synthesis of ROS is crucial for the normal development of tomato plants since mutants with reduced NADPH oxidase (the enzyme that produces superoxide anion in the apoplast) activity showed severe alterations in some organs including impairment in seed and fruit formation (Sagi et al. 2004). Sweet pepper fruit also shows an increase in the level of H_2O_2 at the onset of ripening when fruits start to accumulate carotenoids (Imahori et al. 2000). The melatonin treatment delays maturation of peach fruit keeping lower ROS concentration and higher antioxidant levels than non-treated fruits (Gao et al. 2016). The papaya fruit also shows an oxidative burst during the progress of ripening (Pandey et al. 2013). All these evidences suggest that ROS participate actively in the fruit ripening; however, their specific physiological roles have not been elucidated yet. It has been proposed that an oxidative damage to proteins may be implicated in the mitochondria dysfunction and, as a consequence, in the acceleration of fruit senescence (Tian et al. 2013). Furthermore, fruits with increased antioxidant concentration undergo an extended postharvest life (Klee 2013; Cocaliadis et al. 2014 and references therein).

The interaction between hormones and ROS has been established in several processes in plants (Bartoli et al. 2013), but this relationship and its relevance have been scarcely reported for fruit ripening. Experimental evidences show a ROS–ethylene interaction in plant tissues. Treatments enhancing H_2O_2 are

accompanied by ethylene increase, and furthermore, ethylene treatment stimulates H_2O_2 increments in pears (Brennan and Frenkel 1977). Similarly, treatments with ethylene, or its inhibitor 1-MCP, on mango fruit either increase or decrease the concentration of H_2O_2, respectively (Wang et al. 2009). Ozone treatment provokes increments in ROS production in tomato leaves. This was followed by the increases in the concentration of the ethylene precursor and the expression of its biosynthetic enzymes, leading to an enhanced production of the hormone (Moeder et al. 2002). The treatment of tomato seedlings with H_2O_2 triggers the expression of genes related with ethylene synthesis and ripening/senescence (Kumar et al. 2015). However, it has not been established yet whether the change observed in ROS accumulation is directly connected with the hormone synthesis, or it is a consequence of an acceleration (or delay) of the ripening process in the fruit.

4 NO Levels During Fruit Development and Ripening: Where Does NO Come from?

Nitric oxide (NO) participates in morphogenesis and development of plants through the interaction with hormones, ROS, calcium, and protein posttranslational modifications (Simontacchi et al. 2015). NO has been strongly associated with plant stress (Moreau et al. 2010), and many advances on its effects were obtained by employing a variety of NO donors and treatments (Manjunatha et al. 2010). However, the sources of NO in plants are not fully known. In mammals, NO generation relies on the activity of nitric oxide synthases (NOS), a family of enzymes that employ L-arginine, O_2, and NADPH to produce NO (Stuehr 1999). In plants, NO may be produced from a variety of enzymatic and nonenzymatic sources that have been reviewed by several authors (Moreau et al. 2010; Fröhlich and Durner 2011; Gupta et al. 2011; Mur et al. 2013; del Río 2015; Astier et al. 2018; and references in chapter "Hydrogen Peroxide and Nitric Oxide Generation in Plant Cells: Overview and Queries"). Briefly, they can be classified as either oxidative or reductive (Table 2).

Nitrate reductase (NR)-dependent NO generation has emerged as the main enzymatic source of NO in plants. They have developed efficient mechanisms to assimilate and reduce nitrate and may have optimized the use of nitrite as a main source for NO (Jeandroz et al. 2016; Santolini et al. 2017). On the other hand, in photosynthetic organisms, only a few algal species contain NOS-like protein, and no typical NOS sequences were identified in 1087 sequenced transcriptomes of land plants (Jeandroz et al. 2016). NO production in the presence of arginine (NOS-like activity) and its inhibition through known arginine analogs are still an enigma, but it is observed in different species, organs, and cell compartments. Thus, the presence of NOS enzymes assembled from multipolypeptides and the existence of a NOS enzyme different to known NOS cannot be discarded in higher plants (Jeandroz et al. 2016; Corpas and Barroso 2017).

Table 2 Proposed sources of NO in plants. Oxidative and reductive pathways

Oxidative pathways	References
Arginine dependent	Simontacchi et al. (2004); Corpas et al. (2006); Jasid et al. (2006)
Polyamine-mediated	Tun et al. (2006); Yamasaki and Cohen (2006)
Hydroxylamine-mediated	Rümer et al. (2009)
Reductive pathways (nitrite dependent)	
Nitrate reductase	Yamasaki and Sakihama (2000); Rockel et al. (2002); Chamizo-Ampudia et al. (2017)
Membrane-bound nitrite NO reductase (NiNOR), root-specific	Stöhr et al. (2001)
Thylakoids supplemented with nitrite	Jasid et al. (2006)
Mitochondrial electron transfer chain[a]	Gupta et al. (2005); Gupta and Kaiser (2010)
Peroxisomal xanthine oxidoreductase (XOR)	Godber et al. (2000); Wang et al. (2010)
Nonenzymatic reduction of nitrite	Bethke et al. (2004)[b]

Taken and modified from Benavides et al. (2016)
[a]Under low oxygen conditions and determined by the availability in nitrite
[b]In the apoplast

The question about how and where NO is produced particularly in the fruit is another enigma. NO emission from fruits was reported by pioneering woks in the 1990s. Leshem et al. (1998) and Leshem and Haramaty (1996) supported a role of endogenously generated NO on plant growth regulatory mechanisms in higher plants, acting in the regulation of stress, fruit maturation, and senescence. Endogenously generated NO was measured employing a NO sensor inserted into fruit flesh or monitoring the emissions from intact fruits, vegetables, sprouts, and cut flowers. They provided evidences indicating that the NO content in unripe fruits or fresh flowers was higher than in ripe fruits or senescent flowers and that ethylene and NO levels were inversely correlated, as NO decrease paralleled with ethylene increase (Leshem et al. 1998). Hence, the idea that the exogenous NO application could delay the fruit ripening process in climacteric and non-climacteric fruit, and also could inhibit the onset of senescence, opened the possibility of an horticultural NO application to extend the shelf life of fruits and vegetables (Leshem et al. 1998; Leshem and Wills 1998).

Zhang et al. (2011) studied NO metabolism in apple fruit by monitoring endogenous NO levels, and measuring the activities of some enzymatic activities, described previously, probably linked to NO generation (such as NR and arginine dependent), under natural physiological conditions. The content of endogenous NO in the young fruit period was significantly higher than that in mature period and well correlated with a NOS-like activity (dependent of arginine and NADPH and inhibited by a known animal NOS inhibitor) but not with NR activity.

Zheng et al. (2011) investigated the role of different proposed sources of NO production in the NO-mediated disease resistance in tomato fruit (*Solanum lycopersicum* cv. No.4 Zhongshu). The treatment with an elicitor from *Botrytis*

cinerea induced a burst of NO fluorescence (within 10 min) in tomato fruit pericarp tissue cells (harvested at a mature green ripening stage) that was blocked by the treatment with a known mammalian NOS inhibitor. Thus, a NOS-like pathway may play a regulatory role in elicitor-induced disease resistance in tomato.

However, Bodanapu et al. (2016) could not detect endogenous levels of NO at mature green and red ripe stages of tomato fruit (*Solanum lycopersicum* cv. Ailsa Craig) employing electron paramagnetic resonance (EPR) spectroscopy and a fluorescent probe (DAF-2DA). Despite this, the influence of NO produced in the plant on central carbon metabolism and endogenous phytohormone levels affected fruit growth and ripening.

Parra-Lobato and Gómez-Jiménez (2011) described the presence and distribution of NO in the abscission zone (AZ) tissue from olive cultivars during activation and abscission. Higher NO production was detected in few layers of AZ cells (at the proximal side of the separation line and adjacent cells) at the early stages of olive fruit development and then declined. Also, it was found that endogenous NO and ACC maintained an inverse correlation in olive AZ, suggesting an antagonistic action of NO and ethylene not only during fruit ripening but also in abscission signaling.

NO levels seem to depend on the age, species, tissue, and environmental conditions and may also diverge depending on the techniques employed to measure it. In fact, few works detect NO concentration in situ in the fresh fruits, and some of the measurements are made by indirect detections of nitrite generation, or employing hemoglobin assay in fruits homogenates, and also in frozen samples. Nitrite and nitrate are used as indirect measurement of NO levels, but this correlation is not as straightforward, as nitrite and nitrate not solely arise from NO metabolism (Zhang et al. 2011).

Overall, despite the specific site of NO generation, and the source involved, an undoubted role for NO during fruit development, ripening, and postharvest storage can be proposed. In addition, the increasing concern in consuming fruit and vegetables due to the importance for human nutrition and health needs continue advances to improve postharvest strategies in order to extend shelf life of fruits and vegetables to satisfy demand. Hence, advances on the knowledge about the site and sources of NO generation in situ—in the fruit or in the plant—the factors that can influence them, and the possibility of exogenous treatments will be useful to develop adequate strategies to its application on postharvest industry. Nevertheless, the selection and use of NO-generating compounds need the assessment of data and consequences in each fruit commodity, to obtain information about the desired NO levels in ripening fruit and the adequate storage conditions. Also the possibility of inducing the endogenous NO levels employing an array of elicitors from abiotic and biotic origin may also be considered (Manjunatha et al. 2010).

Finally, NO also plays a critical role in suppressing ROS (del Río et al. 2002). It was observed that NO treatment reduced the increases in membrane permeability and lipid peroxidation, delayed the increases in both the rate of $O_2^{\bullet -}$ production and H_2O_2 content, and increased the activities of SOD, CAT, and ascorbate peroxidase (APX), indicating that NO positively maintains the balance between formation and detoxification of ROS in fruit postharvest (Wu et al. 2012).

5 From Chloroplasts to Chromoplasts: The Role of NO and H_2O_2 in Fruit Color Change

Plastid differentiation during ripening is a highly coordinated process involving programmed multiphase events that are provoked by a variety of stimuli. The activation of numerous morphological and biochemical changes ultimately affect plastid compartmentalization. This is particularly evident in chloroplasts undergoing the transformation to chromoplasts, an event characterized by the synthesis and accumulation of natural pigments—carotenoids—into unique plastid substructures (Camara et al. 1995). These changes are accompanied by the color shift in flowers, fruits, vegetables, and roots of certain plants and photosynthetic bacteria (Liedvogel et al. 1976; Namitha and Negi 2010). Carotenoids can be classified into two groups on the basis of a functional classification: xanthophylls, containing oxygen as functional group in side rings, including lutein and zeaxanthin, and carotenes, which only contain parent hydrocarbon chain without any functional group, such as α-carotene, β-carotene, and lycopene (Britton 2008).

Since the early 1980s, tomato has been recognized as a model system for studying the molecular basis of fleshy fruit development revealing the role of ethylene in controlling the ripening of climacteric fruits (Pirrello et al. 2009). Meanwhile, much of the available information related to antioxidants in fruit ripening was studied in pepper fruit, which has a non-climacteric syndrome (Yanishlieva et al. 2006; Song et al. 2010; Mateos et al. 2013).

From the beginning of pepper ripening, typical chloroplast pigments, such as lutein and neoxanthin, are gradually replaced by chromoplast pigments such as zeaxanthin and cryptoxanthin (Gómez-García and Ochoa-Alejo 2013). In a similar way, it has been reported that during tomato ripening, the activity of cyclases decreases and eventually disappears, resulting in the accumulation of lycopene and β-carotene, concomitant with the decrease in the chlorophyll content during this transition (Fraser et al. 1994).

Bouvier et al. (1998) showed that ROS molecular probes represent promising tools to study the biosynthesis of the chromoplast-specific carotenoids, such as capsanthin in pepper fruit. ROS are potent inducers of carotenogenic gene mRNA expression during chromoplast development. Other works revealed that tocopherols (especially α-tocopherol) apparently control the proliferation of peroxyl radical formation more effectively than carotenoids in this redox balance (Fryer 1992).

Recent data show that ROS and reactive nitrogen species (RNS) are involved in fruit ripening, during which molecules such as H_2O_2, NADPH, NO, peroxynitrite ($ONOO^-$), and *S*-nitrosothiols (SNOs) interact to regulate protein functions through posttranslational modifications (Corpas and Palma 2018). As part of these adjustments, it is remarkable that the different enzymatic components of the antioxidant systems (SOD, CAT, peroxidases (POD), and the ascorbate–glutathione cycle, among others) play a key role during fruit ripening (Jiménez et al. 2002; Camejo et al. 2010; Martí et al. 2011; Mateos et al. 2013; Racchi 2013; Palma et al. 2015; Huan et al. 2016) (Fig. 1).

According to Jiménez et al. (2002), during tomato breaker stage, there is a H_2O_2 accumulation coincidently with a decrease in enzymatic activities of CAT, APX, and monodehydroascorbate reductase (MDAR) that might be associated with the increase in lipid peroxidation and protein oxidation. These authors also observed that when the tomato fruit turned to the red stage, many components of the antioxidant system (mRNA levels, enzymatic activities, and antioxidant content) were enhanced. NO also participates during the transition from the immature green fruit stage to the red ripe stage. According to Chaki et al. (2015), the full transition in pepper fruit was accompanied with a sharp reduction in NO content, determined spectrofluorometrically, and by an increase in both nitrated and nitrosylated proteins (e.g., CAT, cytochrome c oxidase, peroxiredoxin II E, alcohol dehydrogenase) (Chaki et al. 2015; Rodríguez-Ruiz et al. 2017a), while treatments with NO delayed the ripening of pepper fruit, without changes in the nitrated and nitrosylated protein profile (Palma et al. 2015).

These data demonstrate that oxidative stress could be a potent driving force for the expression of carotenoid biosynthetic genes during the chloroplast to chromoplast transition in fruit. In terms of applying these findings, all the data described above indicate that it should be possible to increase the fruit's own supply of healthy nutrients by enhancing the transition from chloroplasts to chromoplasts, while keeping oxidative stress under control. Understanding the mechanisms for achieving redox control and manipulation of photo-/thermoprotection mechanisms in the plastid under real growth conditions will require further research to take full advantage of chloroplast fortification (Corpas et al. 2018) (Fig. 1).

6 NO and H_2O_2 in Fruit Postharvest: New Insights

NO is an important signal for many physiological functions in plants, including stress conditions (Hung et al. 2002; Kopyra and Gwóźdź 2003; Simontacchi et al. 2015), reacting with many targets such as metal centers, thiols, oxygen molecule, and free radicals to modulate plant responses (Simontacchi et al. 2015). In plants, NO has different effects over the activity of antioxidant enzymes such as CAT, SOD (Flores et al. 2008), and peroxidases (Kopyra and Gwóźdź 2003). Other authors suggest that NO acts as an antisenescent molecule (Jasid et al. 2009; Manjunatha et al. 2010; Procházková and Wilhelmová 2011). In climacteric fruit, NO and ethylene have antagonistic effects. In NO-treated fruits, there is a decrease in the ethylene synthesis rate and a delay in fruit ripening and senescence (Liu et al. 2007; Chaki et al. 2015). The interaction has not been clarified yet, but the effect of NO could be associated at the ethylene receptor level. As many other binding process, ethylene needs a cooper molecule to bind to the specific receptors (Rodríguez et al. 1999). There are some clues suggesting that the effect of NO is interfering with this binding process.

Recent research has focused on the effect of NO in postharvest technology. NO has been successfully used to extend the shelf life in strawberries (Wills et al. 2000)

and preventing chilling injury in mangos (Zaharah and Zora 2011) and plums (Singh et al. 2009). Manjunatha et al. (2010) reviewed some works done with different NO donors, including NO gas, DETANO (diethylenetriamine-nitric oxide), and sodium nitroprusside (SNP) used as different tools in postharvest technology, showing that a delicate balance between ROS and NO production is crucial for orchestrating large networks of genes and that the mechanisms of NO involvement between both climacteric and non-climacteric have to be interpreted appropriately for exploiting any commercial benefits.

In climacteric and non-climacteric fruits, NO has different targets of action. In strawberries, a non-climacteric fruit, there is a decrease in the ethylene synthesis, due to the reduction of the ACC synthase content (Zhu and Zhou 2007), whereas in peach, a climacteric fruit, the inhibition was at the ACC oxidase level, producing a ACC oxidase-NO complex leading also to a decrease in the ethylene synthetic pathway activity (Zhu et al. 2006). This ethylene synthesis suppression was confirmed in tomato, another climacteric fruit (Eum et al. 2009). Recent studies in climacteric fruit have focused the attention in the ethylene/NO interaction. In blueberries, the combination of a NO donor, *S*-nitrosoglutathione (GSNO), with an ethylene signal inhibitor, 1-MCP, has proved that the loss of firmness is delayed and the antioxidant content (ascorbate and glutathione) is increased, even after 14 days of cold storage (Gergoff et al. 2017). These authors hypothesize that there could be a synergic effect of these two molecules in climacteric fruits. Other works reported that NO acts in the cell wall disabling process, at the level of the enzymes polygalacturonase and pectin methylesterase in banana (Cheng et al. 2009), and has a strong influence in the last step of the ascorbate synthesis in mitochondria during fruit ripening (Rodríguez-Ruiz et al. 2017b).

ROS generation (including H_2O_2) is the first signal of oxidative burst in the apoplast cells, due to the action of different fungi (Vera-Estrella et al. 1992), bacteria (Baker et al. 1991), or some elicitors (Apostol et al. 1989). Therefore, the rise in the H_2O_2 content in fruit has been associated with an early resistance response to the pathogen attack, as reported in apple (Torres et al. 2003). On the other hand, NO has been also reported as an antifungal gas that inhibits the mycelium growth, the sporulation process, and the spore germination of different postharvest pathogens (Lazar et al. 2008) and induces pathogen resistance in tomato fruit coupled to H_2O_2 (Fan et al. 2008). In postharvest pathogen control, there are many reports that describe H_2O_2 as a highly effective fruit disinfectant (Forney et al. 1991).

In this context, NO and H_2O_2 are employed as postharvest tools to improve shelf life of several climacteric and non-climacteric fruits and horticultural crops. Tables 3 and 4 summarize some information about the use of NO donors and H_2O_2 in postharvest technology of climacteric and non-climacteric fruits, showing the interactions with hormones, the biochemical changes, and the postharvest effects on the physiology and fruit attributes.

Table 3 Effect of NO and H_2O_2 in climacteric fruit postharvest

Climacteric fruit							
Species	NO donor	Treatments	ROS interaction	Hormone interaction	Biochemical effects	Postharvest effects	References
Apple (*Malus* × *domestica* Borkh. cv. "Granny Smith")	DETANO[a]	Dipped in 10 mg L^{-1} DETANO	–	–	↓ Browning in apple slices	↑ Postharvest life	Pristijono et al. (2008)
Papaya (*Carica papaya* L. cv. "Sui you 2")	NO gas	Fumigated for 3 h with 60 μL L^{-1} NO	–	↓ Ethylene production ↓ Auxins ↓ Abscisic acid ↓ Zeatin riboside	↓ Cell wall softening enzymes ↓ Polygalacturonase (PG), pectin methylesterase (PME), and pectate lyase (PL)	↓ Softening Delays ripening	Guo et al. (2014)
Peach (*Prunus persica* L. cv. "Xiahui NO.5")	NO gas	Fumigated with 15 μL L^{-1} of NO for 3 h	Scavenging of H_2O_2	↑ ACO-NO-ACC complex ↓ Ethylene	↑ SOD ↑ Ascorbate–glutathione cycle enzymes ↑ Phenolics, flavonoids	↑ Resistance to disease and environmental stress	Kang et al. (2016); Li et al. (2017)
Tomato (*Solanum lycopersicum* L.)	SNP[b] and	Dipped from 0.2 to 1 mM SNP aqueous solution plus 0.1 mM DMTU[c]	H_2O_2 and NO are involved in disease response	↓ Ethylene production ↑ Total phenols content	↑ CAT, POD, and SOD ↑ The activity of phenylalanine ammonia-lyase (PAL), chitinase, glutathione S-transferase, and polyphenol oxidase	↓ Pericarp reddening in tomato fruit ↑ Resistance to fungal pathogens ↓ Total soluble solids (TSS) ↑ Firmness	Fan et al. (2008); Lai et al. (2011); Zheng et al. (2011)
	Elicitor from *Botrytis cinerea*	Injected with 20 μL of fungal elicitor					
Kiwi (*Actinidia chinensis* L. Planch cv. Xuxiang)	NO gas	20 μL L^{-1} of NO	Protection against lipid peroxidation by	↓ Ethylene production	↑ Antioxidant content ↓ Malondialdehyde	↓ TSS/TTA (total titratable acidity) ratio	Zhu et al. (2010)
	NO solution	1.0 μM NO aqueous solution					

(continued)

Table 3 (continued)

Climacteric fruit							
Species	NO donor	Treatments	ROS interaction	Hormone interaction	Biochemical effects	Postharvest effects	References
			increasing nonenzymatic antioxidants		content Delays chlorophyll degradation	↑ Ascorbate and tocopherol content ↑ Postharvest life	
Banana (*Musa* spp., AAA group cv. "Brazil")	SNP	5 mM	↓ $O_2^{\bullet-}$ production rate and H_2O_2 content	↓ Ethylene production ↓ Electrolyte leakage and malondialdehyde content	↓ Activities of PG, PME, and endo-β-1,4-glucanase ↑ SOD, CAT, POD, and APX activities ↑ Acid-soluble pectin and starch content	↓ Chilling injury	Cheng et al. (2009); Wu et al. (2014)
Blueberries (*Vaccinium corymbosum* cvs. "Blue Cuinex" and "Blue Chip" and *V. corymbosum* × *darrowii* cv. "Misty")	GSNO[d]	1 mM GSNO solution and 1-MCP (1-methylcyclopropene)		1-MCP (ethylene response inhibitor)	↑ Ascorbate and glutathione content	↑ Firmness ↓ Weight loss ↑ Postharvest life	Gergoff et al. (2017)
Mango (*Mangifera indica* L.)	SNP	Immersed in 0.1 mM SNP solution	–	↓ Ethylene production	↑ Activities of defense-related enzymes including PAL, cinnamate-4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL), POD, 1,3-glucanase (GLU), and chitinase (CHT)	↓ Softening ↓ Yellowing Changes in TSS and TTA ↓ Respiration peak ↑ Disease resistance	Hu et al. (2014)

Species	H_2O_2 donor	Treatments	NO interaction	Hormone interaction	Biochemical effects	Postharvest effects	References
Melon (*Cucumis melo* cv. reticulatus)	Sanosil-25[e]	Sprayed with 1000–10,000 μL L^{-1} of Sanosil-25	–	–	–	↓ Decay and preserves the quality during storage and shelf life	Aharoni et al. (1994); Sapers and Sites (2003)
	H_2O_2 solution	Sprayed with 0.1% v/v H_2O_2 solution					
Apple (*Malus* × *domestica*)	H_2O_2 solution and HPPA[f]	Dipped in 80/60–360/480 ppm of H_2O_2/HPPA solution for 7 or 15 min Immersed in 1% H_2O_2 solution for 15 or 30 min	–	–	–	↓ Colony number of sooty blotch and flyspeck	Batzer et al. (2002)

[a]Diethylenetriamine-nitric oxide
[b]Sodium nitroprusside
[c]H_2O_2 scavenger 1,3-dimethyl-2-thiourea
[d]*S*-Nitrosoglutathione
[e]Disinfectant containing 48% v/v H_2O_2 solution and 0.05% silver ion (Ag^+) as a stabilizing agent
[f]Peroxyacetic acid

Table 4 Effect of NO and H_2O_2 in non-climacteric fruit postharvest

Non-climacteric fruit							
Species	NO donor	Treatment	ROS interaction	Hormone interaction	Biochemical effects	Postharvest effects	References
Strawberry (*Fragaria* × *ananassa* Duch.)	NO gas	10 μL L^{-1} of NO for 3 h	–	↓ Ethylene production	↓ Respiration rate	↑ Postharvest life	Wills et al. (2007); Zhu and Zhou (2007)
	DETANO[a]	1 mg L^{-1} of DETANO in citric acid solution					
	SNP[b]	5.0 μmol L^{-1} SNP aqueous solution					
Sweet pepper (*Capsicum annuum* L. cv. Melchor)	NO gas	Fumigated with 160 μL L^{-1} of NO for 1 h	–	–	↑ Nitrosothiols ↑ Nitroproteins	Delays fruit ripening	Chaki et al. (2015)
Orange (*Citrus sinensis* L. Osbeck cv. Valencia)	SNP	50 μM SNP aqueous solution	↓ H_2O_2 content ↑ CAT activity	↑ Phenolic compounds	↓ PAL, POD, polyphenol oxidase enzymes activity ↑ Ascorbate ↑ Ascorbate–glutathione cycle activity	↓ Weight loss ↑ TSS ↓ TSS/TTA ratio ↑ Fungal disease resistance	Mohamed et al. (2016); Zhou et al. (2016)
	SNP + H_2O_2	1 mM SNP 2% v/v H_2O_2					
Bayberry (*Myrica rubra* Sieb. & Zucc. cv. Dongkui)	NO gas	Fumigated with 20 μL L^{-1} NO for 2 h	↓ Lipid peroxidation ↓ H_2O_2 content ↑ CAT, SOD, and APX activities	↓ Ethylene synthesis	↑ Phenolic content ↑ 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity	Preserves quality ↑ Disease resistance ↑ Firmness	Wu et al. (2012)
Cucumber (*Cucumis sativus* L. cv. Deltastar)	NO gas	Fumigated with 25 μL L^{-1} of NO for 12h	↓ Lipid peroxidation ↓ $O_2^{\bullet -}$ and H_2O_2 synthesis		↑ SOD, CAT, APX, and POD activity ↑ DPPH-radical scavenging ↓ Membrane permeability	↑ Chilling tolerance ↑ Antioxidant defense	Yang et al. (2011)

Species	H_2O_2 donor	Treatment	NO interaction	Hormone interaction	Biochemical effects	Postharvest effects	References
Sweet pepper (*Capsicum annuum* L.)	Sanosil-25[c]	0.1–1% Sanosil-25 solution for 60 s	–	–	↑ APX and dehydroascorbate reductase activity ↓ Nitrate content ↑ Ascorbate and total phenolic contents	↓ Weight loss Inhibits fungal spore germination and mycelial growth ↑ Disease resistance against fungus	Fallik et al. (1994); Bayoumi (2008); Hafez (2010)
	H_2O_2 solution	1–15 mM H_2O_2 solution for 30 min Sprayed with a 20 or 50 mM H_2O_2 solution					
Lemon (*Citrus limon* L. cv. Burm)	H_2O_2 solution	15–20 g L^{-1} of H_2O_2 solutions for 1 min	–	–	–	Control of postharvest diseases	Cerioni et al. (2013)
Eggplant (*Solanum melongena* L. cv. Classic)	Sanosil-25[c]	0.1, 0.5, and 1.0% Sanosil-25 solution for 60 s	–	–	–	Inhibits fungal spore germination and mycelial growth ↓ Decay	Fallik et al. (1994)
Grapes (*V. labrusca* L. cv Isabella) (*Vitis vinifera* L.)	H_2O_2 solution	2% v/v vapor phase H_2O_2 and flushed in 30% v/v H_2O_2 solution for 10 min	–	–	–	↓ Number of spores ↓ Decay caused by *Botrytis cinerea*	Forney et al. (1991); Fava et al. (2011)
Longan (*Dimocarpus longan* Lour.)	H_2O_2 solution	1.96 M H_2O_2 solution for 20 min	–	–	↑ Malondialdehyde content ↓ SOD, CAT, and APX activity ↓ Ascorbate, glutathione, and carotenoid content	↑ Pericarp browning	Lin et al. (2014)

[a]Diethylenetriamine-nitric oxide
[b]Sodium nitroprusside
[c]Disinfectant containing 48% v/v H_2O_2 solutions and 0.05% silver ion (Ag^+) as a stabilizing agent

7 Conclusions

Fruit ripening involves several interconnected pathways leading to many changes in edibles fleshy fruits, such as flavor and color. The coordination of this process depends on the synthesis of hormones coupled to signal molecules such as ROS and NO. The specific ripening control is also achieved by the equilibrium between prooxidants and antioxidants. ROS and NO seem to act in concomitance with ethylene and have a strong influence with different behaviors in climacteric and non-climacteric fruits. In this context, H_2O_2 and NO also provide new insights for improving postharvest technology and controlling fruit decay, antioxidant content, cell wall disabling, pathogen attack, and color change, among other effects that deeply affect shelf life and nutritional quality of fruit.

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References

Aharoni Y, Copel A, Fallik E (1994) The use of hydrogen peroxide to control postharvest decay on 'Galia' melons. Ann Appl Biol 125:189–193

Apostol I, Heinstein PE, Low PS (1989) Rapid stimulation of an oxidative burst during elicitation of cultured plant cells: role in defense and signal transduction. Plant Physiol 90:109–116

Astier J, Gross I, Durner J (2018) Nitric oxide production in plants: an update. J Exp Bot 69:3401–3411

Baker CJ, O'Neill NR, Keppler LD, Orlandi EW (1991) Early responses during plant-bacteria interactions in tobacco suspensions. Phytopathology 81:1504–1507

Bartoli CG, Casalongué CA, Simontacchi M, Marquez-Garcia B, Foyer CH (2013) Interactions between hormone and redox signalling pathways in the control of growth and cross tolerance to stress. Environ Exp Bot 94:73–88

Batzer JC, Gleason ML, Weldon B, Dixon PM, Nutter FWJ (2002) Evaluation of postharvest removal of sooty blotch and flyspeck on apples using sodium hypochlorite, hydrogen peroxide with peroxyacetic acid, and soap. Plant Dis 86:1325–1332

Bayoumi YA (2008) Improvement of postharvest keeping quality of white pepper fruits (*Capsicum annuum* L.) by hydrogen peroxide treatment under storage conditions. Acta Biol Szeged 52:7–15

Benavides MP, Gallego SM, Artuso FR, Checovich M, Galatro A (2016) The complexity of nitric oxide generation and function in plants. Biocell 40:1–5

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

Bodanapu R, Gupta SK, Basha PO, Sakthivel K, Yellamaraju Sreelakshmi S, Sharma R (2016) Nitric oxide overproduction in Tomato shr mutant shifts metabolic profiles and suppresses fruit growth and ripening. Front Plant Sci 7:1714

Bouvier F, Backhaus RA, Camara B (1998) Induction and control of chromoplast-specific carotenoid genes by oxidative stress. J Biol Chem 273:30651–30659

Bouzayen M, Latché A, Nath P, Pech JC (2009) Mechanisms of fruit ripening. In: Pua EC, Davey MR (eds) Plant developmental biology—biotechnological perspectives, vol 1. Springer, Berlin
Brennan T, Frenkel C (1977) Involvement of hydrogen peroxide in the regulation of senescence in pear. Plant Physiol 59:411–416
Brennan T, Rychter A, Frenkel C (1979) Activity of enzymes involved in the turnover of hydrogen peroxide during fruit senescence. Bot Gaz 140:384–388
Britton G (2008) Functions of intact carotenoids. In: Britton G, Liaaen-Jensen S, Pfander H (eds) Carotenoids. Birkhäuser, Basel, pp 189–212. Retrieved from http://link.springer.com/chapter/10.1007/978-3-7643-7499-0_10
Brummell DA (2006) Cell wall disassembly in ripening fruit. Funct Plant Biol 33:103–119
Burg SP, Burg EA (1965) Relationship between ethylene production and ripening in bananas. Bot Gaz 126:200–204
Burg SP, Thimann KV (1959) The physiology of ethylene formation in apples. Proc Natl Acad Sci U S A 45:335–344
Camara B, Hugueney P, Bouvier F, Kuntz M, Monéger R (1995) Biochemistry and molecular biology of chromoplast development. Int Rev Cytol 163:175–247
Camejo D, Martí MC, Román P, Ortíz A, Jiménez A (2010) Antioxidant system and protein pattern in peach fruits at two maturation stages. J Agric Food Chem 58:11140–11147
Cara B, Giovannoni JJ (2008) Molecular biology of ethylene during tomato fruit development and maturation. Plant Sci 175:106–113
Carrari F, Fernie AR (2006) Metabolic regulation underlying tomato fruit development. J Exp Bot 57:1883–1897
Cerioni L, Sepulveda M, Rubio-Ames Z, Volentini SI, Rodríguez-Montelongo L, Smilanick JL, Ramallo J, Rapisardaa VA (2013) Control of lemon postharvest diseases by low-toxicity salts combined with hydrogen peroxide and heat. Postharvest Biol Technol 33:72–80
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. Ann Bot 116:637–647
Chamizo-Ampudia A, Sanz-Luque E, Llamas A, Galván A, Fernández E (2017) Nitrate reductase regulates plant nitric oxide homeostasis. Trend Plant Sci 22:163–174
Chaudhary P, Jayaprakasha GK, Porat R, Bhimanagouda SP (2012) Degreening and postharvest storage influences 'Star Ruby' grapefruit (*Citrus paradisi* Macf.) bioactive compounds. Food Chem 135:1667–1675
Cheng G, Yang E, Lu W, Jia Y, Jinag Y, Duan X (2009) Effect of nitric oxide on ethylene synthesis and softening of banana fruit slice during ripening. J Agric Food Chem 57:5799–5804
Chervin C, El-Kereamy A, Roustan J, Latché A, Lamon J, Bouzayen M (2004) Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. Plant Sci 167:1301–1305
Cocaliadis MF, Fernández-Muñoz R, Pons C, Orzaez D, Granell A (2014) Increasing tomato fruit quality by enhancing fruit chloroplast function. A double-edged sword? J Exp Bot 65:4589–4598
Corpas FJ, Barroso JB (2017) Nitric oxide synthase-like activity in higher plants. Nitric Oxide 68:5–6
Corpas FJ, Barroso JB, Carreras A, Valderrama R, Palma JM, León AM, Sandalio LM, del Río LA (2006) Constitutive arginine-dependent nitric oxide synthase activity in different organs of pea seedlings during plant development. Planta 224:246–254
Corpas FJ, Freschi L, Rodríguez-Ruiz M, Mioto PT, González-Gordo S, Palma JM (2018) Nitro-oxidative metabolism during fruit ripening. J Exp Bot 69:3449–3463
Corpas FJ, Palma JM (2018) Nitric oxide on/off in fruit ripening. Plant Biol 20:805–807
del Río LA (2015) ROS and RNS in plant physiology: an overview. J Exp Bot 66:2827–2837
del Río LA, Corpas FJ, Sandalio LM, Palma JM, Gómez M, Barroso JB (2002) Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes. J Exp Bot 53:1255–1272

Eriksson O, Friis EM, LoÈfgren P (2000) Seed size, fruit size, and dispersal systems in angiosperms from the early cretaceous to the late tertiary. Am Nat 156:47–58

Eum HL, Kim HB, Choi SB, Lee SK (2009) Regulation of ethylene biosynthesis by nitric oxide in tomato (*Solanum lycopersicum* L.) fruit harvested at different ripening stages. Eur Food Res Technol 228:331–338

Fallik E, Aharoni Y, Grinberg S, Copel A, Klein JD (1994) Postharvest hydrogen peroxide treatment inhibits decay in eggplant and sweet red pepper. Crop Prot 13:451–454

Fan B, Shen L, Liu K, Zhao D, Yu M, Sheng J (2008) Interaction between nitric oxide and hydrogen peroxide in postharvest tomato resistance response to *Rhizopus nigricans*. J Sci Food Agric 88:1238–1244

Fava J, Hodara K, Nieto A, Guerrero S, Alzamora SM, Castro MA (2011) Structure (micro, ultra, nano), color and mechanical properties of *Vitis labrusca* L. (grape berry) fruits treated by hydrogen peroxide, UV–C irradiation and ultrasound. Food Res Int 44:2938–2948

Flores FB, Sanchez-Bel P, Valdenegro M, Romojaro F, Martinez-Madrid MC, Egea MI (2008) Effects of a pretreatment with nitric oxide on peach (*Prunus persica* L.) storage at room temperature. Eur Food Res Technol 227:1599–1611

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

Forney CF, Rij RE, Denis-Arrue R, Smilanick JL (1991) Vapor phase hydrogen peroxide inhibits postharvest decay of table grapes. Hort Sci 26:1512–1514

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

Fraser PD, Truesdale MR, Bird CR, Schuch W, Bramley PM (1994) Carotenoid biosynthesis during tomato fruit development (evidence for tissue-specific gene expression). Plant Physiol 105:405–413

Fröhlich A, Durner J (2011) The hunt for plant nitric oxide synthase (NOS): is one really needed? Plant Sci 181:401–404

Fryer MJ (1992) The antioxidant effects of thylakoid vitamin E (α-tocopherol). Plant Cell Environ 15:381–392

Galvez-Valdivieso G, Mullineaux PM (2010) The role of reactive oxygen species in signalling from chloroplasts to the nucleus. Physiol Plant 138:430–439

Gane R (1934) Production of ethylene by some ripening fruits. Nature 134:1008

Gao H, Zhang ZK, Chai HK, Cheng N, Yang Y, Wang DN, Yang T, Cao W (2016) Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. Postharvest Biol Technol 118:103–110

Gechev TS, Van Breusegem F, Stone JM, Denev I, Laloi C (2006) Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. Bioessays 28:1091–1101

Gergoff GEG, Alegre ML, Senn ME, Chaves AR, Simontacchi M, Bartoli CG (2017) Combination of nitric oxide and 1-MCP on postharvest life of the blueberry (*Vaccinium spp*.) fruit. Postharvest Biol Technol 33:72–80

Giovanoni JJ (2004) Genetic regulation of fruit development and ripening. Plant Cell 16:S170–S180

Godber BLJ, Doel JJ, Sapkota GP, Blake DR, Stevens CR, Eisenthal R, Harrison R (2000) Reduction of nitrite to nitric oxide catalyzed by xanthine oxide reductase. J Biol Chem 275:7757–7763

Goff SA, Klee HJ (2006) Plant volatile compounds: sensory cues for health and nutritional value? Science 311:815–819

Gómez-García M, Ochoa-Alejo N (2013) Biochemistry and molecular biology of carotenoid biosynthesis in Chili Peppers (*Capsicum spp*.). Int J Mol Sci 14:19025–19053

Goulao LF, Oliveira CM (2008) Cell wall modifications during fruit ripening: when a fruit is not the fruit. Trend Food Sci Technol 19:4–25

Guo Q, Wu B, Chen W, Zhang Y, Wang J, Li X (2014) Effects of nitric oxide treatment on the cell wall softening related enzymes and several hormones of papaya fruit during storage. Food Sci Technol Int 20:309–317

Gupta KJ, Kaiser WM (2010) Production and scavenging of nitric oxide by barley root mitochondria. Plant Cell Physiol 51:576–584

Gupta KJ, Stoimenova M, Kaiser WM (2005) In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, in vitro and in situ. J Exp Bot 56:2601–2609

Gupta KJ, Fernie AR, Kaiser WM, van Dongen JT (2011) On the origins of nitric oxide. Trend Plant Sci 16:160–168

Hafez YM (2010) Control of *Botrytis cinerea* by the resistance inducers Benzothiadiazole (BTH) and Hydrogen peroxide on white Pepper fruits under postharvest storage. Acta Phytopathol Entomol Hung 45:13–29

Herrera A, Rodrigo MJ, Gil J, Zacarías L (2007) Ethylene stimulates emission of terpenoids and aliphatic esters in citrus fruits. In: Ramina A, Chang C, Giovannoni J, Klee H, Perata P, Woltering E (eds) Advances in plant ethylene research. Springer, Dordrecht

Hu M, Yang D, Huber DJ, Jiang Y, Li M, Gao Z, Zhang Z (2014) Reduction of postharvest anthracnose and enhancement of disease resistance in ripening mango fruit by nitric oxide treatment. Postharvest Biol Technol 97:115–122

Huan C, Jiang L, An X, Yu M, Xu Y, Ma R, Yu Z (2016) Potential role of reactive oxygen species and antioxidant genes in the regulation of peach fruit development and ripening. Plant Physiol Biochem 104:294–303

Hung KT, Chang CJ, Kao CH (2002) Paraquat toxicity is reduced by nitric oxide in rice leaves. J Plant Physiol 159:159–166

Imahori Y, Kanetsune Y, Ueda Y, Chachin K (2000) Changes in hydrogen peroxide content and antioxidative enzyme activities during the maturation of sweet pepper (*Capsicum annuum* L) fruit. J Jpn Soc Hortic Sci 69:690–695

Jasid S, Simontacchi M, Bartoli CG, Puntarulo S (2006) Chloroplasts as a nitric oxide cellular source. Effect of reactive nitrogen species on chloroplastic lipids and proteins. Plant Physiol 142:1246–1255

Jasid S, Galatro A, Villordo JJ, Puntarulo S, Simontacchi M (2009) Role of nitric oxide in soybean cotyledon senescence. Plant Sci 176:662–668

Jayasena V, Cameron I (2008) °Brix/acid as a predictor of consumer acceptability of Crimson Seedless table grapes. J Food Qual 31:736–750

Jeandroz S, Wipf D, Stuehr DJ, Lamattina L, Melkonian M, Tian Z, Zhu Y, Carpenter EJ, Wong GKS, Wendehenne D (2016) Occurrence, structure, and evolution of nitric oxide synthase-like proteins in the plant kingdom. Sci Signal 9:re2

Jiménez A, Creissen G, Kular B, Firmin J, Robinson S, Verhoeyen M, Mullineaux PM (2002) Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. Planta 214:751–758

Kang R, Zhang L, Jiang L, Yu M, Ma R, Yu Z (2016) Effect of postharvest nitric oxide treatment on the proteome of peach fruit during ripening. Postharvest Biol Technol 112:277–289

Kidd F, West C (1925) Respiratory activity and duration of life of apples gathered at different stages of development and subsequently maintained at a constant temperature. Plant Physiol 20:467–504

Kitijona L, Saran S, Roy SK, Kader AA (2011) Postharvest technology for developing countries: challenges and opportunities in research, outreach and advocacy. J Sci Food Agric 91:597–603

Klee HJ (2004) Ethylene signal transduction. Moving beyond Arabidopsis. Plant Physiol 135:660–667

Klee HJ (2013) Purple tomatoes: longer lasting, less disease, and better for you. Curr Biol 23:R520–R521

Knapp S (2002) Tobacco to tomatoes: a phylogenetic perspective on fruit diversity in the Solanaceae. J Exp Bot 53:2001–2022

Kopyra M, Gwóźdź EA (2003) Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. Plant Physiol Biochem 41:1011–1017

Kumar R, Khurana A, Sharma AK (2014) Role of plant hormones and their interplay in development and ripening of fleshy fruits. J Exp Bot 65:4561–4575

Kumar V, Irfan M, Ghosh S, Chakraborty N, Chakraborty S, Datta A (2015) Fruit ripening mutants reveal cell metabolism and redox state during ripening. Protoplasma 253:581–594

Lai T, Wang Y, Li B, Qin G, Tian S (2011) Defense responses of tomato fruit to exogenous nitric oxide during postharvest storage. Postharvest Biol Technol 62:127–132

Lazar EE, Wills RBH, Ho BT, Harris AM, Spohr LJ (2008) Antifungal effect of gaseous nitric oxide on mycelium growth, sporulation and spore germination of the postharvest horticulture pathogens, *Aspergillus niger*, *Monilinia fructicola* and *Penicillium italicum*. Lett Appl Microbiol 246:688–692

Lelievre JM, Latche A, Jones B, Bouzayen M, Pech JC (1997) Ethylene and fruit ripening. Physiol Planta 101:727–739

Leng P, Yuan B, Guo Y (2014) The role of abscisic acid in fruit ripening and responses to abiotic stress. J Exp Bot 65:4577–4588

Leshem YY, Haramaty E (1996) The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn. foliage. J Plant Physiol 148:258–263

Leshem YY, Wills R (1998) Harnessing senescence delaying gases nitric oxide and nitrous oxide: a novel approach to postharvest control of fresh horticultural produce. Biol Plant 41:1–10

Leshem YY, Wills RRH, Ku VVV (1998) Evidence for the function of the free radical gas—nitric oxide ($NO^{\bullet}$) as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiol Biochem 36:825–833

Li G, Zhu S, Wu W, Zhang C, Peng Y, Wang Q, Shi J (2017) Exogenous nitric oxide induces disease resistance against *Monilinia fructicola* through activating the phenylpropanoid pathway in peach fruit. J Sci Food Agric 97:3030–3038

Li Y, Golding JB, Arcot J, Wills RBH (2018) Continuous exposure to ethylene in the storage environment adversely affects 'Afourer' mandarin fruit quality. Food Chem 242:585–590

Liedvogel B, Sitte P, Falk H (1976) Chromoplasts in the daffodil: fine structure and chemistry. Cytobiologie 12:155–174

Lin Y, Lin H, Zhang S, Chen Y, Chen M, Lin Y (2014) The role of active oxygen metabolism in hydrogen peroxide-induced pericarp browning of harvested longan fruit. Postharvest Biol Technol 96:42–48

Liu MC, Song WH, Zhu SH, Zhou J (2007) Effects of nitric oxide and exogenous ethylene treatments on ethylene biosynthesis in Feicheng Peach. Agric Sci China 6:290–295

Liu M, Pirrello J, Chervin C, Roustan JP, Bouzayen M (2015) Ethylene control in fruit ripening: revisiting the complex network of transcriptional regulation. Plant Physiol 169:2380–2390

Manjunatha G, Lokesh V, Neelwarne B (2010) Nitric oxide in fruit ripening: trends and opportunities. Biotechnol Adv 28:489–499

Martí MC, Camejo D, Olmos E, Sandalio LM, Fernández-García N, Jiménez A, Sevilla F (2008) Characterization and changes in the antioxidant system of chloroplasts and chromoplasts isolated from green and mature pepper fruits. Plant Biol 11:613–624

Martí MC, Camejo D, Vallejo F, Romojaro F, Bacarizo S, Palma JM, Sevilla F, Jiménez A (2011) Influence of fruit ripening stage and harvest period on the antioxidant content of sweet pepper cultivars. Plant Foods Hum Nutr 66:416–423

Mateos RM, Jiménez A, Román P, Romojaro F, Bacarizo S, Leterrier M, Gómez M, Sevilla F, del Río LA, Corpas FJ, Palma JM (2013) Antioxidant systems from pepper (*Capsicum annuum* L.): involvement in the response to temperature changes in ripe fruits. Int J Mol Sci 14:9556–9580

Mayouni L, Tietel Z, Patil BS, Porat R (2011) Does ethylene degreening affect internal quality of citrus fruit? Postharvest Biol Technol 62:50–58

Merchante C, Vallarino JG, Osorio S, Aragüez I, Villarreal N, Ariza MT, Martínez GA, Medina-Escobar N, Civello MP, Fernie AR, Botella MA, Valpuesta V (2013) Ethylene is involved in strawberry fruit ripening in an organ-specific manner. J Exp Bot 64:4421–4439

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

Moeder W, Barry CS, Tauriainen AA, Betz C, Tuomainen J, Utriainen M, Grierson D, Sandermann H, Langebartels C, Kangasjärvi J (2002) Ethylene synthesis regulated by biphasic induction of 1-aminocyclopropane-1-carboxylic acid synthase and 1-aminocyclopropane-1-carboxylic acid oxidase genes is required for hydrogen peroxide accumulation and cell death in ozone-exposed tomato. Plant Physiol 130:1918–1926

Mohamed MAAT, Abd El-khalek AF, Elmehrat HG, Mahmoud GA (2016) Nitric oxide, oxalic acid and hydrogen peroxide treatments to reduce decay and maintain postharvest quality of 'Valencia' orange fruits during cold storage. Egypt J Hortic 43:137–161

Moreau M, Lindermayr C, Durner J, Klessig DF (2010) NO synthesis and signaling in plants—where do we stand? Physiol Plant 138:372–383

Mur LAJ, Mandon J, Persijn S, Cristescu SM, Moshkov IE, Novikova GV, Hall MA, Harren FJM, Hebelstrup KH, Gupta KJ (2013) Nitric oxide in plants: an assessment of the current state of knowledge. AoB Plant 5:pls052

Murshed R, Lopez-Lauri F, Sallanon H (2014) Effect of salt stress on tomato fruit antioxidant systems depends on fruit development stage. Physiol Mol Biol Plant 20:15–29

Namitha KK, Negi PS (2010) Chemistry and biotechnology of carotenoids. Crit Rev Food Sci Nutr 50:728–760

Navrot N, Rouhier N, Gelhaye E, Jacquot JP (2007) Reactive oxygen species generation and antioxidant systems in plant mitochondria. Physiol Plant 129:185–195

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

Noctor G, Reichheld JP, Foyer CH (2018) ROS-related redox regulation and signaling in plants. Semin Cell Dev Biol 80:3–12

Palma JM, Corpas FJ, del Río LA (2011) Proteomics as an approach to the understanding of the molecular physiology of fruit development and ripening. J Proteom 74:1230–1243

Palma JM, Sevilla F, Jiménez A, del Río LA, Corpas FJ, Álvarez de Morales P, Camejo DM (2015) Physiology of pepper fruits and the metabolism of antioxidants: chloroplasts, mitochondria and peroxisomes. Ann Bot 116:627–636

Pandey VP, Singh S, Jaiswal N, Awasthi M, Pandey B, Dwivedi UN (2013) Papaya fruit ripening: ROS metabolism, gene cloning, characterization and molecular docking of peroxidase. J Mol Catal B Enzym 98:98–105

Parra-Lobato MC, Gómez-Jiménez MC (2011) Polyamine-induced modulation of genes involved in ethylene biosynthesis and signalling pathways and nitric oxide production during olive mature fruit abscission. J Exp Bot 62:4447–4465

Petrov VD, Van Breusegem F (2012) Hydrogen peroxide—a central hub for information flowing plant cells. AoB Plants pls:014

Pirrello J, Regad F, Latché A, Pech JC, Bouzayen M (2009) Regulation of tomato fruit ripening. CAB Rev 4:1–14

Prabha TN, Bhagyalakshmi N (1998) Carbohydrate metabolism in ripening banana fruit. Phytochemistry 48:915–919

Pristijono P, Wills RBH, Golding JB (2008) Use of the nitric oxide donor compound, diethylenetriamine-nitric oxide (DETANO), as an inhibitor of browning in apple slices. J Hortic Sci Biotechnol 83:555–558

Procházková D, Wilhelmová N (2011) Nitric oxide, reactive nitrogen species and associated enzymes during plant senescence. Nitric Oxide 24:61–65

Racchi M (2013) Antioxidant defenses in plants with attention to *Prunus* and *Citrus spp*. Antioxidants 2:340–369

Rockel P, Strube F, Rockel A, Wildt J, Kaiser WM (2002) Regulation of nitric oxide (NO) production by plant nitrate reductase in vivo and in vitro. J Exp Bot 53:103–110

Rodrigo MJ, Zacarias L (2007) Effect of postharvest ethylene treatment on carotenoid accumulation and the expression of carotenoid biosynthetic genes in the flavedo of orange (*Citrus sinensis* L. Osbeck) fruit. Postharvest Biol Technol 43:14–22

Rodríguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB (1999) A cooper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. Science 283:996–998

Rodríguez-Ruiz M, Mioto P, Palma JM, Corpas FJ (2017a) S-nitrosoglutathione reductase (GSNOR) activity is down-regulated during pepper (*Capsicum annuum* L.) fruit ripening. Nitric Oxide 68:51–55

Rodríguez-Ruiz M, Mateos RM, Codesido V, Corpas FJ, Palma JM (2017b) Characterization of the galactono-1,4-lactone dehydrogenase from pepper fruits and its modulation in the ascorbate biosynthesis. Role of nitric oxide. Redox Biol 12:171–181

Ronen G, Carmel-Goren L, Zamir D, Hirscheberg J (2000) An alternative pathway to β-carotene formation in plant chromoplasts discovered by map-based cloning of *Beta* and *old-gold* color mutations in tomato. Proc Natl Acad Sci U S A 97:11102–11107

Rümer S, Gupta KJ, Kaiser WM (2009) Plant cells oxidize hydroxylamines to NO. J Exp Bot 60:2065–2072

Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratmann JW, Fluhr R (2004) Plant respiratory burst oxidase homologs impinge on would responsiveness and development in *Lycopersicon esculentum*. Plant Cell 16:616–628

Santolini J, André F, Jeandroz S, Wendehenne D (2017) Nitric oxide synthase in plants: where do we stand? Nitric Oxide 63:30–38

Sapers GM, Sites JE (2003) Efficacy of 1% hydrogen peroxide wash in decontaminating apples and Cantaloupe melons. J Food Sci 68:1793–1797

Shimokawa K, Shimada S, Yaeo K (1978) Ethylene-enhanced chlorophyllase activity during degreening of *Citrus unshiu* Marc. Sci Hortic 8:129–135

Simontacchi M, Jasid S, Puntarulo S (2004) Nitric oxide generation during early germination of sorghum seeds. Plant Sci 167:839–847

Simontacchi M, Galatro A, Ramos-Artuso F, Santa-María GE (2015) Plant survival in a changing environment: the role of nitric oxide in plant responses to abiotic stress. Front Plant Sci 6:977

Singh SP, Singh Z, Swinny EE (2009) Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina* Lindell). Postharvest Biol Technol 53:101–108

Sisler EC, Serek M (1997) Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiol Plant 100:577–582

Song W, Derito CM, Liu MK, He X, Dong M, Liu RH (2010) Cellular antioxidant activity of common vegetables. J Agric Food Chem 58:6621–6629

Stöhr C, Strube F, Marx G, Ullrich WR, Rockel P (2001) A plasma membrane-bound enzyme of tobacco roots catalyses the formation of nitric oxide from nitrite. Planta 212:835–841

Stuehr DJ (1999) Mammalian nitric oxide synthases. Biochim Biophys Acta 1411:217–230

Tesniere C, Pradal M, El-Kereamy A, Torregrosa L, Chatelet P, Roustan JP, Chervin C (2004) Involvement of ethylene signalling in a non-climacteric fruit: new elements regarding the regulation of ADH expression in grapevine. J Exp Bot 55:2235–2240

Tian S, Qin S, Li B (2013) Reactive oxygen species involved in regulating fruit senescence and fungal pathogenicity. Plant Mol Biol 82:593–602

Tohge T, Alseekh S, Fernie AR (2014) On the regulation and function of secondary metabolism during fruit development and ripening. J Exp Bot 65:4599–4611

Torres R, Valentines MC, Usall J, Vinas I, Larrigaudiere C (2003) Possible involvement of hydrogen peroxide in the development of resistance mechanisms in 'Golden Delicious' apple fruit. Postharvest Biol Technol 27:235–242

Tucker GA, Grierson D (1987) Fruit ripening. In: Davies DD (ed) Biochemistry of plants: a comprehensive treatise, vol 12. Academic, London, pp 265–318

Tun NN, Santa-Catarina C, Begum T, Silveira V, Handro W, Segal Floh EI, Scherer GFE (2006) Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. Plant Cell Physiol 47:346–354

Vera-Estrella R, Blumwald E, Higgins VG (1992) Effect of specific elicitors of *Cladosporium fulvum* on tomato suspension cells. Plant Physiol 99:1208–1215

Wang B, Wang J, Feng X, Lin L, Zhao Y, Jiang W (2009) Effects of 1-MCP and exogenous ethylene on fruit ripening and antioxidants in stored mango. Plant Growth Regul 57:185–192

Wang BL, Tang XY, Cheng LY, Zhang AZ, Zhang WH, Zhang FS, Liu JQ, Cao Y, Allan DL, Vance CP, Shen JB (2010) Nitric oxide is involved in phosphorus deficiency-induced cluster-root development and citrate exudation in white lupin. New Phytol 187:1112–1123

Wills RBH, Ku VVV, Leshem YY (2000) Fumigation with nitric oxide to extend the postharvest life of strawberries. Postharvest Biol Technol 18:75–79

Wills RB, Soegiarto L, Bowyer MC (2007) Use of a solid mixture containing diethylenetriamine/nitric oxide (DETANO) to liberate nitric oxide gas in the presence of horticultural produce to extend postharvest life. Nitric Oxide 17:44–49

Wing SL, Boucher LD (1998) Ecological aspects of the Cretaceous flowering plant radiation. Annu Rev Earth Planet Sci 26:379–421

Wu F, Yang H, Chang Y, Cheng J, Bai S, Yin J (2012) Effects of nitric oxide on reactive oxygen species and antioxidant capacity in Chinese Bayberry during storage. Sci Hortic 135:106–111

Wu B, Guo Q, Li Q, Ha Y, Li X, Chena W (2014) Impact of postharvest nitric oxide treatment on antioxidant enzymes and related genes in banana fruit in response to chilling tolerance. Postharvest Biol Technol 92:157–163

Yamasaki H, Cohen MF (2006) NO signal at the crossroads: polyamine-induced nitric oxide synthesis in plants? Trend Plant Sci 11:522–524

Yamasaki H, Sakihama Y (2000) Simultaneous production of nitric oxide and peroxynitrite by plant nitrate reductase: in vitro evidence for the NR-dependent formation of active nitrogen species. FEBS Lett 468:89–92

Yang SF (1987) The role of ethylene and ethylene synthesis in fruit ripening. In: Thompson W, Nothnagel E, Huffaker R (eds) Plant senescence: its biochemistry and physiology. The American Society of Plant Physiologists, Rockville, MD, pp 156–165

Yang SF, Hoffman NE (1984) Ethylene biosynthesis and its regulation in higher plants. Ann Rev Plant Physiol 35:155–189

Yang H, Wu F, Cheng J (2011) Reduced chilling injury in cucumber by nitric oxide and the antioxidant response. Food Chem 127:1237–1242

Yanishlieva NV, Marinova E, Pokorny J (2006) Natural antioxidants from herbs and spices. Eur J Lipid Sci Technol 108:776–793

Yokotani N, Nakano R, Imanishi S, Nagata M, Inaba A, Kubo Y (2009) Ripening-associated ethylene biosynthesis in tomato fruit is auto catalytically and developmentally regulated. J Exp Bot 60:3433–3442

Zaharah SS, Zora S (2011) Postharvest nitric oxide fumigation alleviates chilling injury, delays fruit ripening and maintains fruit quality in cold-storage 'Kensington Pride' mango. Postharvest Biol Technol 60:202–210

Zhang LL, Zhu SH, Chen CB, Zhou J (2011) Metabolism of endogenous nitric oxide during growth and development of apple fruit. Sci Hortic 127:500–506

Zheng Y, Shen L, Yu M, Fan B, Zhao D, Liu L, Sheng J (2011) Nitric oxide synthase as a postharvest response in pathogen resistance of tomato fruit. Postharvest Biol Technol 60:38–46

Zhou Y, Li S, Zeng K (2016) Exogenous nitric oxide-induced postharvest disease resistance in citrus fruit to *Colletotrichum gloeosporioides*. J Sci Food Agric 96:505–512

Zhu S, Zhou J (2007) Effect of nitric oxide on ethylene production in strawberry fruit during storage. Food Chem 100:1517–1522

Zhu S, Liu M, Zhou J (2006) Inhibition by nitric oxide of ethylene biosynthesis and lipoxygenase activity in peach fruit during storage. Postharvest Biol Technol 42:41–48

Zhu S, Sun L, Zhou J (2010) Effects of different nitric oxide application on quality of kiwifruit during 20°C storage. Int J Food Sci Technol 45:245–251

Plant Abiotic Stress: Function of Nitric Oxide and Hydrogen Peroxide

Małgorzata Janicka, Małgorzata Reda, Natalia Napieraj, and Katarzyna Kabała

Contents

Abstract The negative effect of various environmental stresses is partially due to the generation of reactive oxygen species (ROS). ROS were originally considered to be detrimental to cells. However, it is now recognized that hydrogen peroxide functions as a trigger for induction of many genes encoding enzymes involved in cellular protection under stress. In a number of abiotic responses, NO generation occurs in parallel with H_2O_2, and both molecules can act synergistically and/or independently. Studies have shown that NO and H_2O_2 function as stress signals in plants, mediating a range of resistance mechanisms. The main place of the signal perception of worsening environmental conditions is the plasma membrane. On the other hand, one of the major proteins of the plant cell membrane is the plasma membrane H^+-ATPase (PM H^+-ATPase), a key enzyme in adaptation of plants to abiotic stresses. In plants exposed to different abiotic stresses, e.g., salinity, heavy metals, and low or high temperature, an increase in permeability related to membrane damage is observed. Maintaining ionic balance and replenishing the loss of essential substances are important issues. Support of active transport of ions through the plasma membrane requires increased generation of an electrochemical proton gradient by PM H^+-ATPase, which results in a proton-motive force used by active transporters for assimilation of various nutrients, as well as for releasing toxic ions from cells. NO and H_2O_2 are important elements for understanding the mechanisms

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of PM H^+-ATPase modification at both genetic and posttranslational level. Nowadays the role of NO and H_2O_2 as well as the signal cascades by which signaling molecules participate in plant responses to changing environmental conditions is under intensive study.

Keywords Abiotic stress · NO · H_2O_2 · Plasma membrane H^+-ATPase

1 Introduction

Plants are continuously subjected to numerous environmental stresses, such as heavy metals, salinity, drought, and low or high temperature. These adverse external factors limit plant growth and productivity. Understanding plant responses to abiotic stresses is essential for reasonable production of more resistant crop plants. The process by which plant cells sense the stress signals and transmit them to cellular machinery for activation of adaptive responses is referred as signal transduction. In plants, the signal transduction pathways are still elucidated. Despite many physiological and molecular studies, the knowledge of how plants sense and transduce extreme temperature, drought, salinity, or heavy metals needs to be explained. The negative effect of various environmental stresses is partially due to the generation of reactive oxygen species (ROS). Accumulation of ROS is a general feature of abiotic stresses and together with changes in cell antioxidant capacity can lead not only to oxidative damage but also to ROS signaling (Tanou et al. 2009; Foyer and Noctor 2009; Groβ et al. 2013; Arora et al. 2016). In a number of abiotic responses, H_2O_2 generation occurs in parallel with NO (nitric oxide), and these molecules can act both synergistically and independently (Clarke et al. 2000; Neill et al. 2002; Zhang et al. 2007; Qiao et al. 2014). NO is a key signaling molecule that mediates a variety of physiological functions and defense mechanisms against abiotic stresses in plants (Qiao and Faan 2008).

In general, extreme temperature mainly results in mechanical embarrassment, whereas heavy metals, salinity, and drought exert their scathing effects mainly by disrupting the ionic and osmotic equilibrium of the cell. In plants exposed to abiotic stresses, an increase in permeability related to membrane damage is observed (Guy 1990; Breckle and Kahle 1991; Devi and Prasad 1999). Moreover, under heavy metals or salt stress, accumulation of toxic ions in plant cells occurs. Ion homeostasis is the physiological fundament for living cells. Maintaining ionic balance and replenishing the loss of essential substances in repair processes are important issues under such conditions (Janicka-Russak 2011). Support of active transport of ions and organic compounds through the plasma membrane requires increased generation of a proton gradient by plasma membrane H^+-ATPase (Serrano 1989). The generation of an electrochemical proton gradient across the membrane results in a protonmotive force that is used by active transporters for uptake of various nutrients as well as for releasing toxic ions and substances from cells (Janicka-Russak 2011). It has been proven that the activity of the plasma membrane H^+-ATPase (PM H^+-ATPase)

is a key index of plant adaptation to abiotic stresses (Kłobus and Janicka-Russak 2004; Janicka-Russak et al. 2008, 2012a, b, 2013; Sahu and Shaw 2009; Lopez-Pérez et al. 2009). Moreover, the reported studies show that signaling molecules are important elements for understanding the mechanisms of modification of the plasma membrane proton pump activity. Both NO and H_2O_2 play an essential role in regulation of PM H^+-ATPase activity under stress conditions (Zhang et al. 2007; Siddiqui et al. 2010; Janicka-Russak and Kabała 2012; Janicka-Russak et al. 2012a; Janicka et al. 2018). The pathways by which signaling molecules participate in plant responses to changes in environmental conditions are under intensive study. The purpose of this chapter is to explain the role of NO and H_2O_2 in plant adaptation to abiotic stresses.

2 Abiotic Stress in Plants

Stress is a physical definition. In biological terms, it is problematic to define stress. A physiological condition, stressful for one plant, may be optimal for another plant. The most applied definition of biological stress is an adverse force or a condition which limits the normal functioning of biological systems such as plants (Jones and Jones 1989). It is well known that NO and H_2O_2 play key functions in the regulation of plant tolerance to environmental stress factors such as drought, salinity, low or high temperature, heavy metals, and wounding (Neil et al. 2002, 2008; Vandenabeele et al. 2003; Shapiro 2005; Suzuki and Mittler 2006; Volkov et al. 2006; Qiao and Faan 2008; Janicka et al. 2018).

Exposure to high salinity can affect all the major plant processes, including protein synthesis, photosynthesis, and lipid metabolism (Parida and Das 2005). A high concentration of salt in the soil is one of the stressogenic stimuli. Excessive salinity imposes two stress factors on plants: an osmotic component that results from the reduced water availability caused by an increase in osmotic pressure in the soil and an ionic stress resulting from a solute imbalance, causing changes in the K^+/Na^+ ratio and increasing the concentration of Na^+ and Cl^- in the cytosol (Alvarez et al. 2003). Sodium toxicity results from the similarity of the Na^+ and K^+ ions that compete for plant transporters and binding sites in enzymes (Silva and Geros 2009). Osmolytes like proline, glycine-betaine, trehalose, mannitol, and sorbitol that are abundantly produced and accumulated in salt-treated plants represent an important component of salt stress responses. These compounds lower the osmotic potential of cells or protect various cellular structures and proteins against stress-induced damage (Sahi et al. 2006). It is well established that the efficient exclusion of Na^+ excess from cytoplasm is the most important step toward the maintenance of ion homeostasis inside the cell (Zhu 2003; Silva and Geros 2009). Majority of plants adapted to salinity maintain a relatively low concentration of Na^+ in the cytosol achieved through the active exclusion of sodium ions in the apoplast and vacuole. Removal of sodium ions out of the cell, catalyzed by the specific plasma membrane and tonoplast Na^+/H^+ antiporters, depends on the membrane electrochemical proton gradient (Chinnusamy

et al. 2005; Silva and Geros 2009; Kabała and Janicka-Russak 2012). The only pump which generates proton gradient across the plasma membrane is H^+-ATPase. For this reason, it is believed that plant plasma membrane H^+-ATPase plays a major role in salt stress tolerance. The importance of the proton pump in plant adaptation to salinity was indicated by the observations carried out on salt-tolerant plants, showing increased activity of this enzyme in both normal and salt conditions (Vera-Estrella et al. 1994; Chen et al. 2007; Sahu and Shaw 2009). Moreover, the salinity induces the activities of the plasma membrane proton pumps both in halophytes and glycophytes (Kłobus and Janicka-Russak 2004; Sahu and Shaw 2009; Lopez-Pérez et al. 2009; Janicka-Russak et al. 2013). NaCl stress induces the expression of PM H^+-ATPase genes (Niu et al. 1993; Janicka-Russak and Kłobus 2007; Janicka-Russak et al. 2013). The positive correlation between accumulation of mRNAs of H^+-ATPase and salt tolerance is well documented (Niu et al. 1993; Perez-Prat et al. 1994; Janicka-Russak and Kłobus 2007; Sahu and Shaw 2009). It seems that both NO and H_2O_2 are generated under salt stress to activate resistance mechanisms in plants. These signal molecules maintain adequate K nutrition and a favorable K^+ to Na^+ ratio in cytosol (Zhu 2003). It was shown that NO produced under salt stress can serve as a second messenger to induce H_2O_2 generation mediated by the induction of plasma membrane NADPH oxidase expression (Zhang et al. 2007). Subsequently, the H_2O_2 contributes to induce the expression of PM H^+-ATPase that creates an electrochemical proton gradient for the establishment of ionic homeostasis to confer salt resistance (Zhang et al. 2007; Janicka et al. 2018).

Unpredictable falls in temperature are a common phenomenon in a temperate climate. Membranes are most susceptible to damage resulting from low temperature. An increase in membrane permeability and a change in its viscosity and fluidity result in a decrease of cell turgor due to low temperature (Guy 1990). It is well known that lipids containing saturated fatty acids solidify at temperatures higher than those containing unsaturated fatty acids. Therefore, the relative proportion of unsaturated fatty acids in the membrane strongly influences its fluidity (Mahajan and Tuteja 2005). Membrane changes at cold concern positional redistribution of saturated and unsaturated fatty acids. Membrane reconstruction has been observed in response of plants to low temperature. Chilling-sensitive plants usually have a higher proportion of saturated fatty acids. On the other hand, chilling-resistant species are marked by higher proportion of unsaturated fatty acids (Mahajan and Tuteja 2005). Probably, the change in membrane fluidity in sensitive species initiates a signal chain reaction that leads to acclimation to cold stress. Furthermore, the increase in H_2O_2 accumulation at low temperatures occurs (Kocsy et al. 2001). Glutathione is an important component of the ascorbate-glutathione cycle, which is indirectly involved in the regulation of H_2O_2 concentration in plants (Foyer et al. 1997). At low temperature, high GSH content and NADPH-dependent glutathione reductase (GR) activity were detected in plants indicating their possible contribution to chilling tolerance and cold acclimation. Changes in H_2O_2 concentration and GSH/GSSG ratio alter the redox state of the cells and activate specific defense mechanisms through a redox signaling chain (Kocsy et al. 2001). ROS produced in response to low temperature contributes to membrane damage. The integrity of intracellular

organelles is also disrupted, leading to a loss of compartmentalization and impairing of photosynthesis, protein synthesis, and general metabolic processes. Low temperatures induce alteration in cellular components, including unsaturated fatty acids, glycerolipids, proteins, and carbohydrate composition as well as the activation of ion channels (Knight et al. 1996). Cold acclimation results in protection and stabilization of the integrity of cellular membranes, enhancement of the antioxidative mechanisms, increased intracellular sugar level, as well as accumulation of cryoprotectants like polyamines (Krishna et al. 1995; Polisensky and Braam 1996; Xiong and Zhu 2001; Mahajan and Tuteja 2005; Chinnusamy et al. 2006). Cold acclimation involves precise signaling and regulation of the transcriptome. Cold-induced ROS (such as H_2O_2) may activate a mitogen-activated protein kinase cascade that regulates tolerance to freezing (Chinnusamy et al. 2006). Altered transcript levels of PM H^+-ATPase genes were also observed (Ahn et al. 1999; Janicka-Russak et al. 2012a) due to exposure of plants to low temperature. Published data also indicate that activity of PM H^+-ATPase is modulated under low temperature by changes in plasma membrane lipids (Martz et al. 2006). The activity of plasma membrane H^+-ATPase may increase more than twofold following cold acclimation (Martz et al. 2006). Moreover, fatty acid-regulated PM proton pump activity is involved in the cellular response underlying cold acclimation and de-acclimation (Martz et al. 2006).

Temperature is a major factor in determining the natural distribution of plants. High temperatures can damage several plant metabolic and physiological processes (Larkindale and Huang 2004; Johnston et al. 2007). Plants are able to sense, respond to, and acclimate to mild high temperatures. This property allows them to survive extreme temperature shocks. High temperature causes protein dysfunction. Maintaining proteins in their functional conformations and preventing their aggregation are important for cell survival under high temperature stress (Wang et al. 2004; Timperio et al. 2008). The treatment of plants with temperatures near the upper limit for survival induces a specific set of proteins, the heat shock proteins (HSPs). This response is common to all living organisms. Königshofer et al. (2008) observed that as the final indicator of the heat signaling cascade, the synthesis of HSPs occurs. Their results indicated that elevated production of hydrogen peroxide, in the early stages of the heat signaling pathways, which responds rapidly to changes in membrane fluidity, is required for the activation of HSP synthesis. Similar observations were made by Miller and Mittler (2006) and Volkov et al. (2006). It was demonstrated that increased H_2O_2 production in apoplast under abiotic stresses occurs as a result of elevated levels of ABA (Hu et al. 2005, 2006). There is some evidence that ABA may be involved in heat stress responses in plants (Robertson et al. 1994; Gong et al. 2002). It was shown that ABA added to plants protected them from heat-induced oxidative damage (Larkindale and Knight 2002; Larkindale and Huang 2004).

Bioaccumulation of heavy metals in the environment has become a problem for all living organisms including plants. Heavy metals have the competence to interact with several cellular biomolecules such as proteins and DNA. Elevated levels of heavy metals lead to the increased generation of reactive oxygen species and induction of oxidative stress (Howlett and Avery 1997; Pál et al. 2005; Hossain et al. 2012). In response to heavy metals, plants are equipped with a range of mechanisms to counteract

their toxicity. These mechanisms include chelation of metals by forming complexes with phytochelatins or metallothioneins, which is followed by the removal of heavy metals ions from cytoplasm. The plant plasma membrane may be regarded as the first structure that is a target for heavy metal toxicity. An increase in permeability related to membrane damage is observed in plants that have been subjected to heavy metal stress (Demidchik et al. 1997; Murphy and Taiz 1997; Murphy et al. 1999). It is well known that metal ions are easily bound to both the sulfhydryl groups of proteins and hydroxyl part of phospholipids (Devi and Prasad 1999). They can also replace the calcium ions at essential sites of cell membranes (Breckle and Kahle 1991). All these events result in disruption of membrane integrity and ionic homeostasis of cells. A factor that may be important in the maintenance of plasma membrane integrity in the presence of heavy metals could be enhanced membrane repair after damage (Salt et al. 1998). This could involve heat shock proteins. Moreover maintaining ionic balance and replenishing the loss of essential substances are important under heavy metal stress. A few observations have indicated that H^+-ATPase activity was changed under heavy metal stresses (Demidchik et al. 1997; Astolfi et al. 2003, 2005; Janicka-Russak et al. 2008, 2012b; Kabała et al. 2008). Moreover, the relative expression of PM-H^+-ATPase genes in cucumber roots was affected due to Cd treatment (Janicka-Russak et al. 2012b).

Many studies indicate that NO and H_2O_2 could be a common and mutual elements of different signaling pathways activated by plants in response to adverse environmental factors. Hydrogen peroxide and nitric oxide are involved in both salt and heat stress tolerance. Pretreatment of seedlings with H_2O_2 or SNP (NO donor) before salt or heat treatment caused decreases in deleterious effects of stress in plants (Uchida et al. 2002). Cross-resistance factors are able to reduce the effects of other stresses. For instance drought-hardened maize plants become more chilling-tolerant as well (Pérez de Juan et al. 1997). These phenomena are common in wide range of stresses (Uchida et al. 2002). This suggests that environmental factors activate similar signal transduction pathways. Stress conditions often lead to similar disturbances in plants, including dehydration and membrane damage. Control of ion movement across plasma membrane is an essential element of cellular adaptation to abiotic stresses. Because active transport of ions across the plasma membrane requires increased generation of a proton gradient by PM H^+-ATPase, it is suspected that this enzyme could play a key role in abiotic stress tolerance.

3 Plasma Membrane H^+-ATPase

The main place of the signal perception of worsening environmental conditions is the outer cell membrane, i.e., the plasma membrane. One of the most important proteins of the plant cell membrane is PM H^+-ATPase. This protein belongs to a large superfamily of pumps termed P-type ATPases. Plasma membrane H^+-ATPase is a tightly bound and integral transmembrane protein. The enzyme is a single polypeptide of ~100 kDa. By the use of the chemical energy of ATP hydrolysis, PM ATPases extrude protons from cells to apoplast to generate an electrochemical

proton gradient (Serrano 1989). PM H^+-ATPase as a proton pump plays a central role in many physiological functions such as nutrient uptake, intracellular pH regulation, stomatal opening, and cell growth. Besides regulation of physiological processes, this proton pump also participates in the adaptation of plants to changing environmental conditions, especially stress conditions (Janicka-Russak 2011). Generation of an electrochemical proton gradient across the membrane is necessary for active transporters involved in accumulation of nutrients as well as removal of ions and other toxic substances from cells. Thus, the plasma membrane H^+-ATPase can be a key element for resistance mechanisms activated under various stress conditions. Therefore, the regulation of enzyme activity may represent an important cellular mechanism for abiotic stress resistance. Modification of the plasma membrane H^+-ATPase activity could take place at the genetic and posttranslational level.

The molecular study of plant H^+-ATPase has shown that this enzyme is encoded by a multigene family. In addition to tissue-specific expression, the plasma membrane H^+-ATPase genes are differentially expressed by environmental factors. Several studies have indicated that the H^+-ATPase genes might be activated by various abiotic stresses. With such a phenomenon, the amount of H^+-ATPase might be increased under conditions requiring greater transport activity. The external signals resulting in changes in plant plasma membrane H^+-ATPase gene expression include salinity (Niu et al. 1993; Janicka-Russak and Kłobus 2007), low temperature (Ahn et al. 1999; Janicka-Russak et al. 2012a), heavy metals (Janicka-Russak et al. 2008, 2012b), and mechanical stress (Oufattole et al. 2000). It was shown that signaling molecules NO and H_2O_2 contribute to increased activity of PM H^+-ATPase by stimulation of expression of gene encoding this enzyme (Zhang et al. 2007; Janicka et al. 2018).

As to posttranslational regulation, the best known mechanism described to date involves the auto inhibitory action of the C-terminal domain (approximately 100 amino acids) of the enzyme protein. The activity of the enzyme is well known to be regulated by 14-3-3 proteins, the association of which requires phosphorylation of the penultimate H^+-ATPase residues of Thr 947 (Svennelid et al. 1999; Fuglsang et al. 2006). The binding of 14-3-3 regulatory protein displaces the inhibitory domain, activating the enzyme. One 14-3-3 protein dimer binds two C-terminal polypeptides simultaneously, so a high activity state of H^+-ATPase could involve formation of dimers or multimeric complexes (Kanczewska et al. 2005). Recent studies have shown that NO participates in posttranslational modifications of plasma membrane proton pump because it leads to an increased level of enzyme phosphorylation and to an increased H^+/ATP coupling ratio (Janicka et al. 2018).

It is well known that the plant plasma membrane H^+-ATPase requires lipids for activity. This lipid dependency suggests a possible mode of regulation via modification of lipid environment (Kasamo 2003). Abiotic stresses lead to changes in the plasma membrane lipid composition altering the fluidity of the membrane. The modulation of the phospholipid environment of the plasma membrane regulates the activity of H^+-ATPase (Kasamo 2003). The activation of H^+-ATPase is dependent on the degree of saturation or unsaturation of the fatty acyl chain and its length. The activity decreased with an increase in the length of the chain and in the degree of unsaturation (Kasamo 2003; Martz et al. 2006).

4 Function of H_2O_2 in Abiotic Stress in Plants

It is known that the negative effect of the various environmental stresses, e.g., salinity, heavy metals, and extreme temperature, is partially due to the generation of reactive oxygen species and the inhibition of the systems which defend against them (Alscher et al. 1997; Quan et al. 2008). The reactive oxygen species H_2O_2 is a harmful cellular metabolite. To some extent, excess H_2O_2 accumulation can lead to oxidative stress in plants, which then triggers cell death (Quan et al. 2008). Although ROS were originally considered to be detrimental to cells, they are now widely recognized as key factors modulating cellular activities (Suzuki and Mittler 2006; Quan et al. 2008). There are several possible sources of H_2O_2 in plants which can be activated during abiotic stress, e.g., electron transport chains in chloroplast and mitochondria, photorespiration in peroxisomes, or enzymatic sources including plasma membrane NADPH oxidase and cell wall-bound peroxidases/amine oxidase. However, under stress conditions, much attention is focused on plasma membrane and apoplast compartments, in which increases in H_2O_2 could be associated with the activities of NADPH oxidases and peroxidases (Bolwell et al. 2002). Plants tolerate much higher concentration of endogenous H_2O_2 than other organisms (Queval et al. 2008). This increased tolerance could be attributed to the fact that accumulation preferentially occurs in the apoplast and that intracellular concentrations are much lower. ROS originating from the plasma membrane NADPH oxidase have been focus in ROS signaling (Jiang and Zhang 2002). It has been shown that NADPH oxidase is involved in plant growth and development and in plant responses to pathogens, elicitors, and wounding (Sagi and Fluhr 2006), chilling (Piotrovskii et al. 2011), drought (Jiang and Zhang 2002), and salt stress (Yang et al. 2007). This enzyme oxidizes cytoplasmic NADPH and transfers an electron to molecular O_2 to form O_2^-; the latter is then converted to H_2O_2 by superoxide dismutase (SOD) (van Gestelen et al. 1997). NADPH is mainly generated by the pentose phosphate pathway (Yu et al. 2014). Glucose-6-phosphate dehydrogenase (G6PDH) is the rate-limiting enzyme of this pathway. Recent studies demonstrated that G6PDH plays important role under salt stress and heavy metals (Wang et al. 2008; Jakubowska et al. 2015). However, studies that have been carried to elucidate the physiological role of this protein in plant tolerance to abiotic stresses are still limited.

In addition, apoplastic ROS generation can be mediated by two amine oxidases, the diamine oxidase (DAO) and polyamine oxidase (PAO). DAO and PAO are localized in peroxisomes and the apoplast (Moschou et al. 2008). DAO oxidizes putrescine, whereas PAO oxidizes spermidine and spermine and yields Δ^1-pyrroline and 1,5-diazbicyclononane, respectively, along with 1,3-diaminopropane and H_2O_2. Increased DAO activity resulted in increased tolerance to salinity (Waie and Rajam 2003). It was also shown that upon salt stress spermidine is secreted into apoplast and catabolized by PAO to produce H_2O_2 (Moschou et al. 2008). Polyamines (PAs) are low molecular weight polycationic compounds present ubiquitously in all living cells. Data suggest that PAs are able to affect the activity of H_2O_2-scavening enzymes, moderating this signal at molecular level (Kubiś 2003). Some evidence

has shown that PA oxidation is directly involved in plant adaptation to abiotic stresses (Moschou et al. 2008). H_2O_2 derived from PA catabolism could also exert signaling effects. Toumi et al. (2010) have shown that the intrinsic ABA signal upregulates PA metabolism, which in turn increases endogenous H_2O_2 load through the apoplastic PAs exodus/catabolism pathway. Also Paschalidis et al. (2010) have indicated that ABA is partly responsible for the induction of the polyamine exodus pathway in plants. They demonstrated that ABA is an upstream signal for the induction of the polyamine catabolic pathway.

Increasing evidence indicates that hydrogen peroxide functions as a signaling molecule responsible for induction of many genes encoding enzymes involved in cellular protection under stress conditions (Vandenabeele et al. 2003; Volkov et al. 2006; Quan et al. 2008). It was observed that treatment of plants with H_2O_2 contributes to increased expression of PM H^+-ATPase genes in cucumber roots (Janicka-Russak et al. 2012a, 2018). Furthermore, it was shown that abscisic acid causes enhanced generation of ROS (Pei et al. 2000; Laloi et al. 2004). ABA is known as a stress hormone, which mediates responses to a variety of stress factors. Some studies demonstrated that increased H_2O_2 production in apoplast under abiotic stresses occurs as a result of elevated levels of ABA (Hu et al. 2005, 2006). Furthermore, ABA stimulates H_2O_2 production by NADPH oxidases (Hu et al. 2005). So, it seems that under abiotic stress, the elevated level of H_2O_2 could partly result from increased amounts of ABA.

5 Function of NO in Abiotic Stress in Plants

Nitric oxide is a small, highly diffusible, gaseous molecule with ubiquitous bioactivity. It is synthesized in plant cells either during physiological or stress conditions. Endogenously generated NO and NO-derived compounds referred as reactive nitrogen species (RNS) are involved in many primary physiological processes including seed germination, root growth and development, control of stomatal movements, flowering, regulation of pollen tube growth, ripening of fruits, and senescence (Domingos et al. 2015; Sami et al. 2018). NO and RNS play also an extensive role in triggering the plant defense mechanisms in plant response to abiotic stresses such as salinity, drought, high and low temperature, and heavy metals (Corpas et al. 2011; Sahay and Gupta 2017; Sami et al. 2018).

Biosynthesis of NO occurs in certain cellular compartment including cytoplasm, chloroplasts, mitochondria, peroxisomes, and apoplast space (Tischner et al. 2004; Jasid et al. 2006; del Río et al. 2003; Stöhr and Stremlau 2006). Several oxidative and reductive and enzymatic and nonenzymatic pathways for NO biosynthesis have been discovered in plants. Reductive route of NO production involves reduction of nitrite ions and is dominantly associated with catalytic activity of cytoplasmic nitrate reductase (cNR). To date, the numerous studies confirmed the role of cNR in NO production in plants during physiological processes and under abiotic stresses (Kolbert et al. 2010; Hancock 2012; Sun et al. 2014). Further, during anoxia, mitochondrial inner membrane

can also participate in reductive pathways of NO biosynthesis (Tischner et al. 2004; Gupta and Igamberdiev 2011). Xanthine oxidoreductase (XOR), a molybdenum-containing enzyme is responsible for NO production occurring in peroxisomes (Weidert et al. 2014). In root apoplast space, NO_2^- conversion to NO is performed by plasma membrane-associated nitrite-nitric oxide reductase (Ni-NOR), which is closely related to plasma membrane nitrate reductase (PM-NR) (Stöhr and Ullrich 2002; Eick and Stöhr 2012). The oxidative pathways include the enzymatic production of NO from L-arginine. In animals, nitric oxide synthases (NOS) oxidizing L-arginine to NO via L-citrulline are well recognized. Despite many years' explorations, there is no direct molecular evidence for existence of homologous NOS in higher plants, although NO production sensitive to mammalian NOS inhibitors has been detected (Corpas et al. 2009; Santolini et al. 2017).

The action mechanism of nitric oxide in plant response to abiotic stresses can be manifested through affecting the function and activity of numerous proteins due to their gene expression and/or protein posttranslational modifications (PTM). S-nitrosylation of cysteine residue, nitration of tyrosine, and metal nitrosylation are the main ones (Astier and Lindermayr 2012). *S*-nitrosylation consists a reversible covalent binding of NO moiety to the thiol group of cysteine (Cys) residue of target proteins. This reaction is resulting in the formation of *S*-nitrosothiol (Astier et al. 2011). *S*-nitrosylation can alter proteins activity, stability, conformation, subcellular localization, and protein-protein interactions (Farnese et al. 2016). The evidences indicate that this PTM is a crucial component of primary metabolism and photosynthesis and contributes to plant signaling and responses to abiotic stresses (Yu et al. 2014; Sami et al. 2018). To date, several proteins, such as H^+-ATPase, NADPH oxidase, ascorbate peroxidase glutathione reductase, or nitrosoglutathione reductase, concerned with stress reaction or ROS production and/or detoxification undergo modifications through *S*-nitrosylation (Tanou et al. 2009; Yun et al. 2011; Romero-Puertas et al. 2013).

Tyrosine (Tyr) nitration is another NO-mediated protein modification which involves addition of nitro group ($-NO_2$) transferring from peroxynitrite ($ONOO^-$) to the aromatic ring of Tyr residue (Corpas et al. 2009). Tyr nitration leads to structural and functional changes in target proteins and leads to altered cell and tissue homeostasis. Several stress-related proteins, such as some antioxidant enzymes, were identified to undergo Tyr nitration (Mata-Pérez et al. 2016). Metal nitrosylation consists of reversible NO binding to transitions metal, e.g., iron, zinc, and copper, in metalloproteins to form metal-nitrosyl complexes (Astier and Lindermayr 2012; Farnese et al. 2016). This PTM induces changes of protein conformation that affects the activity or reactivity of target proteins (Astier and Lindermayr 2012). Besides posttranslational proteins modifications, NO can affect gene expression of several protein related to and involved in plant response to abiotic stresses. Other studies showed that exogenous NO induces the expression of plasma membrane H^+-ATPase in plants under salt stress (Siddiqui et al. 2010).

Many studies have documented the essential role of NO for the plant's tolerance to elevated salt concentrations. Generally, it was shown that during salt treatment, NO improved biomass of shoots and roots (Egbichi et al. 2014), enhanced relative

water content and chlorophyll level, decrease leakage of electrolyte, and protect from and reduces membrane injury and lipid peroxidation (Liu et al. 2016). Plant's exposure to raised NaCl concentration leads to accumulation of NO in cucumber tissues (Janicka et al. 2018). Salt-induced NO synthesis is associated with NR-dependent and/or NOS-like-dependent NO biosynthesis pathway (Zhao et al. 2007; Reda et al. 2018). Moreover, NO enhances K^+/Na^+ ratio by increasing activity of plasma membrane and tonoplast proton pumps and Na^+/H^+ antiport (Zhang et al. 2006; Kabała and Janicka-Russak 2012). In plants, both extremely high and low temperatures lead to accumulation of *S*-nitrosothiols and rise of protein nitration (Corpas et al. 2011; Farnese et al. 2016; Sami et al. 2018). To date, extreme temperatures usually promote a rapid increase in the endogenous NO and other RNS levels (Corpas et al. 2011; Li et al. 2013; Yu et al. 2014; Janicka et al. 2018). Moreover, exogenous application of NO to plants subjected to high temperature stress enhances the activity of several antioxidant enzymes (Sami et al. 2018). It was shown that activation of plasma membrane H^+-ATPase occurring during low temperature acclimation is NO mediated (Janicka et al. 2018). Additionally, action of NO on plasma membrane proton pump was tightly associated with H_2O_2 (Janicka et al. 2018). A growing body of evidence shows changes in NO generation in various plants subjected to heavy metals. However, intensity of NO production seems to be strictly dependent on form and concentration of metal used (Chmielowska-Bąk et al. 2014). Exogenously applied NO alleviates Cd toxicity by increasing pectin and cellulose content in cell walls leading to increasing Cd deposition in root cell walls (Xiong et al. 2009). NO can also promote incorporation of metals into vacuole by increasing phytochelatin synthesis (Arasimowicz-Jelonek et al. 2011) or metal removal from the cell by enhancing the activity of plasma membrane proton pump (Janicka-Russak et al. 2012a, b).

6 Conclusion

Recent studies show that NO and H_2O_2 interact to alleviate the negative influence of abiotic stress on plant growth, metabolism, and development. Both NO and H_2O_2 are generated in plant cells to activate different resistance mechanisms. As the plasma membrane is the main place of the signal perception related to unfavorable conditions, the PM-bound H^+-ATPase, responsible for proton gradient generation, seems to be a key enzyme involved in plant adaptation to changing environment. The activity, phosphorylation status, and gene expression of plasma membrane proton pump are modulated under abiotic stress conditions. It was demonstrated that enhanced PM H^+-ATPase functioning is one of the main adaptive mechanisms enabling plant survival under salinity, heavy metals, and low and high temperatures. Recently, NO and H_2O_2 were recognized as signaling molecules, which can affect PM proton pump action. Therefore, it is postulated that both NO and H_2O_2 signaling pathways are involved in the regulation of this enzyme and improvement of stress-induced cell disorders (Fig. 1).

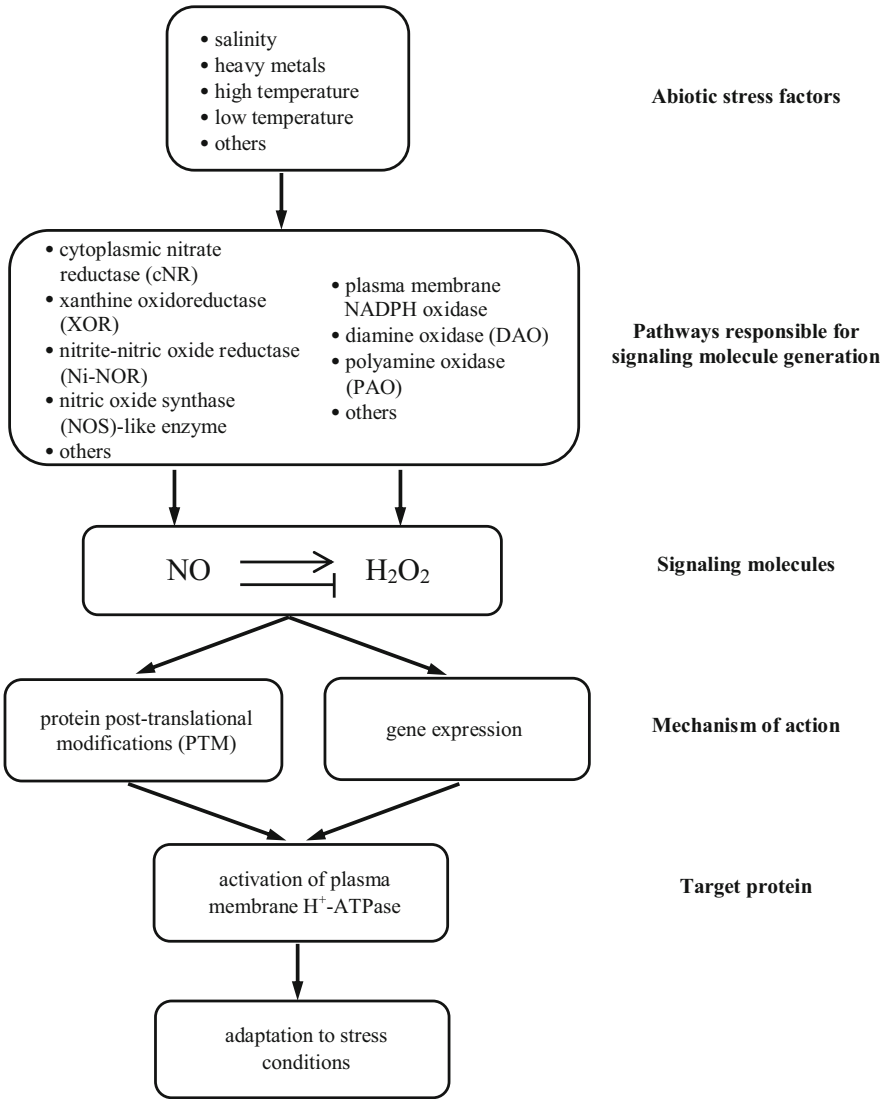

Fig. 1 Model of NO and H_2O_2 signaling pathways, induced under abiotic stress conditions, responsible for activation of plasma membrane H^+-ATPase involved in plant stress tolerance. Abiotic stresses, including salinity, heavy metals, and low as well as high temperatures, are known to induce NO and H_2O_2 generation. Increase in NO and H_2O_2 levels occurs as a result of enhanced activity of different enzymatic systems existing in plant cells. Additionally, NO can affect H_2O_2 accumulation, elevating or inhibiting it. Both NO and H_2O_2 act as signaling molecules which modulate the function and activity of numerous proteins due to their gene expression and/or posttranslational modifications (PTM), such as reversible phosphorylation, *S*-nitrosylation, or Tyr-nitration. The PM-bound H^+-ATPase, responsible for proton gradient generation, is a key enzyme involved in plant adaptation to environmental stresses. NO as well H_2O_2 can activate PM proton pump at both genetic and protein levels and in this way participate in plant tolerance

References

Ahn SJ, Yang IJ, Chung GC, Cho BH (1999) Inducible expression of plasma membrane H^+-ATPase in the roots of figleaf gourd plants under chilling root temperature. Physiol Plant 106:35–40

Alvarez I, Tomaro LM, Bernavides PM (2003) Changes in polyamines, proline and ethylene in sunflower calluses treated with NaCl. Plant Cell Tissue Organ Cult 74:51–59

Alscher RG, Donahue JL, Cramer CL (1997) Reactive oxygen species and antioxidants: relationship in green cells. Physiol Plant 100:224–233

Arasimowicz-Jelonek M, Floryszak-Wieczorek J, Gwóźdź EA (2011) The message of nitric oxide in cadmium challenged plants. Plant Sci 181:612–620

Arora D, Jain P, Singh N, Kaur H, Bhatla SC (2016) Mechanisms of nitric oxide crosstalk with reactive oxygen species scavenging enzymes during abiotic stress tolerance in plants. Free Radic Res 50:291–303

Astier J, Lindermayr C (2012) Nitric oxide-dependent posttranslational modification in plants: an update. Int J Mol Sci 13:15193–15208

Astier J, Rasul S, Koen E, Manzoor H, Besson-Bard A, Lamotte O, Jeandroz S, Durner J, Lindermayr C, Wendehenne D (2011) *S*-nitrosylation: an emerging post-translational protein modification in plants. Plant Sci 181:527–533

Astolfi S, Zuchi S, Chiani A, Passera C (2003) In vivo and in vitro effects of cadmium on H^+-ATPase activity of plasma membrane vesicles from oat (*Avena sativa* L.) roots. J Plant Physiol 160:387–393

Astolfi S, Zuchi S, Passera C (2005) Effect of cadmium on H^+-ATPase activity of plasma memebrane vesicles isolated from roots of different S-supplied maize (*Zea mays* L.) plants. Plant Sci 169:361–368

Bolwell GP, Bindschedler LV, Blee KA, Butt VS, Davies DR, Gardner SL, Gerrish C, Minibayeva F (2002) The apoplastic oxidative burst in response to biotic stress in plants: a tree component system. J Exp Bot 53:1367–1137

Breckle SW, Kahle H (1991) Effects of toxic heavy metals (Cd, Pb) on growth and mineral nutrition on beech (*Fagus sylvatica* L). Plant Ecol 101:43–53

Chen Z, Pottosin II, Cuin TA, Fuglsang AT, Tester M, Jha D, Zepeda-Jazo I, Zhou M, Palmgren MG, Newman IA, Shabala S (2007) Root plasma membrane transporters controlling K^+/Na^+ homeostasis in salt-stressed barley. Plant Physiol 145:1714–1725

Chinnusamy V, Jagendorf A, Zhu J (2005) Understanding and improving salt tolerance in plants. Crop Sci 45:437–448

Chinnusamy V, Zhu J, Zhu J (2006) Gene regulation during cold acclimation in plants. Physiol Plant 126:52–56

Chmielowska-Bąk J, Gzyl J, Rucińska-Sobkowiak R, Arasimowicz-Jelonek M, Deckert J (2014) The new insight into cadmium sensing. Front Plant Sci 5:245

Clarke D, Durner J, Navarre DA, Klessig DF (2000) Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. Mol Plant-Microbe Interact 13:1380–1384

Corpas FJ, Leterrier M, Valderrema R, Airaki M, Chaki M, Palma JM, Barroso JB (2011) Nitric oxide imbalance provokes a nitrosative response in plants under abiotic stress. Plant Sci 181:604–611

Corpas FJ, Palma JM, del Rio LA, Barroso JB (2009) Evidence supporting the existence of L-arginine-dependent nitric oxide synthase activity in plants. New Phytol 184:9–14

del Rio LA, Corpas FJ, Sandalio LM (2003) Plant peroxisomes, reactive oxygen metabolism and nitric oxide. IUBMB Life 55:71–81

Demidchik V, Sokolik A, Yurin V (1997) The effect of Cu^{2+} ion transport systems of the plant cell plasmalemma. Plant Physiol 114:1313–1325

Devi S, Prasad M (1999) Membrane lipid alterations in heavy metal exposed plants. In: Prasad MNV, Hagemeyer J (eds) Heavy metal stress in plants. From molecules to ecosystems. Springer, Berlin, pp 99–116

Domingos P, Pardo AM, Wong A, Gehring C, Feijo JA (2015) Nitric oxide: a multitasked signaling gas in plants. Mol Plant 8:506–520

Egbichi I, Keyster M, Ludidi N (2014) Effect of exogenous application of nitric oxide on salt stress responses of soybean. South Afr J Bot 90:131–136

Eick M, Stöhr C (2012) Denitrification by plant roots? New aspects of plant plasma membrane-bound nitrate reductase. Protoplasma 249:909–918

Farnese FS, Menezes-Silva PE, Gusman GS, Oliveira JA (2016) When bad guys become good ones: the key role of reactive oxygen species and nitric oxide in the plant responses to abiotic stress. Front Plant Sci 7:471

Foyer CH, Lopez-Delgado H, Dat JF, Scott IM (1997) Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. Physiol Plant 100:241–254

Foyer CH, Noctor G (2009) Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. Antioxid Redox Signal 11:861–890

Fuglsang A, Tulinius G, Ciu N, Palmgren M (2006) Protein phosphatases 2A scaffolding subunit A interacts with plasma membrane H^+-ATPase C-terminus in the same region as 14-3-3 protein. Physiol Plant 128:334–340

Gill SS, Hasanuzzaman M, Nahar K, Macovei A, Tuteja N (2013) Importance of nitric oxide in cadmium stress tolerance in crop plants. Plant Physiol Biochem 63:254–261

Gong M, Li YJ, Chen SZ (2002) Abscisic acid-induced thermotolerance in maize seedlings is mediated by calcium and associated with antioxidant systems. J Plant Physiol 153:488–496

Groβ F, Durner J, Gaupels F (2013) Nitric oxide, antioxidants and prooxidants in plant defence responses. Front Plant Sci 4:419

Gupta KJ, Igamberdiev AU (2011) The anoxic plant mitochondrion as a nitrite: NO reductase. Mitochondrion 11:537–543

Guy C (1990) Cold acclimation and freezing stress tolerance: role of protein metabolism. Annu Rev Plant Physiol Plant Mol Biol 41:187–223

Hancock JT (2012) NO synthase? Generation of nitric oxide in plants. Period Biol 114:19–24

Hossain MA, Piyatida P, da Silva JAT, Fujita M (2012) Molecular mechanism of heavy metal toxicity and tolerance in plants: central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. J Bot 2012:37

Howlett NG, Avery SV (1997) Relationship between cadmium sensitivity and degree of plasma membrane fatty acid unsaturation in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 48:539–545

Hu X, Jiang M, Zhang A, Lu J (2005) Abscisic acid induced apoplastic H_2O_2 accumulation up-regulates the activities of chloroplastic and cytosolic antioxidant enzymes in maize leaves. Planta 223:57–68

Hu X, Zhang A, Zhang J, Jiang M (2006) Abscisic acid is a key inducer of hydrogen peroxide production in leaves of maize plants exposed to water stress. Plant Cell Physiol 47:1484–1495

Jakubowska D, Janicka-Russak M, Kabała K, Migocka M, Reda M (2015) Modification of plasma membrane NADPH oxidase activity in cucumber seedling roots in response to cadmium stress. Plant Sci 234:50–59

Janicka M, Reda M, Czyżewska K, Kabała K (2018) Involvement of signalling molecules NO, H_2O_2 and H_2S in modification of plasma membrane proton pump in cucumber roots subjected to salt or low temperature stress. Funct Plant Biol 45:428–439

Janicka-Russak M (2011) Plant plasma membrane H+-ATPase in adaptation of plants to abiotic stresses. In: Shanker A, Venkateswarlu B (eds) Abiotic stress response in plants- physiological, biochemical and genetic perspectives. InTech, Rijeka, pp 197–218

Janicka-Russak M, Kabała K, Burzyński M, Kłobus G (2008) Response of plasma membrane H^+-ATPase to heavy metal stress in *Cucumis sativus* roots. J Exp Bot 59:3721–3728

Janicka-Russak M, Kabała K (2012) Abscisic acid and hydrogen peroxide induce modification of plasma membrane H^+-ATPase from *Cucumis sativus* L. roots under heat shock. J Plant Physiol 1969:1607–1614

Janicka-Russak M, Kabała K, Wdowikowska A, Kłobus G (2012a) Response of plasma membrane H^+-ATPase to low temperature in cucumber roots. J Plant Res 125:291–300

Janicka-Russak M, Kabała K, Burzyński M (2012b) Different effect of cadmium and copper on H^+-ATPase activity in plasma membrane vesicles from *Cucumis sativus* roots. J Exp Bot 63:4133–4142

Janicka-Russak M, Kabała K, Wdowikowska A, Kłobus G (2013) Modification of plasma membrane proton pumps in cucumber roots as an adaptation mechanism to salt stress. J Plant Physiol 170:915–922

Janicka-Russak M, Kłobus G (2007) Modification of plasma membrane and vacuolar H+-ATPase in response to NaCl and ABA. J Plant Physiol 164:295–302

Jasid S, Simontacchi M, Bartoli CG, Puntarulo S (2006) Chloroplasts as a nitric oxide cellular source. Effect of reactive nitrogen species on chloroplastic lipids and proteins. Plant Physiol 142:1246–1255

Jiang M, Zhang J (2002) Involvement of plasma membrane NADPH oxidase in abscisic acid-and water stress-induced antioxidant defense in leaves of maize seedlings. Planta 215:1022–1030

Johnston MK, Jacob NP, Brodl MR (2007) Heat shock-induced changes in lipid and protein metabolism in the endoplasmic reticulum of barley aleurone layers. Plant Cell Physiol 48:31–41

Jones HG, Jones MB (1989) Introduction: some terminology and common mechanisms. In: Jones HG, Flowers TJ, Jones MB (eds) Plants under stress. Cambridge University Press, Cambridge, pp 1–10

Kabała K, Janicka-Russak M (2012) Na^+/H^+ antiport activity in plasma membrane and tonoplast vesicles isolated from NaCl treated cucumber roots. Biol Plant 56:377–382

Kabała K, Janicka-Russak M, Burzyński M, Kłobus G (2008) Comparison of heavy metal effect on the proton pumps of plasma membrane and tonoplast in cucumber root cells. J Plant Physiol 165:278–288

Kanczewska J, Marco S, Vandermeeren C, Maudoux O, Rigaud J, Boutry M (2005) Activation of the plant plasma membrane H^+-ATPase by phosphorylation and binding of 14-3-3 proteins convert a dimer into a hexamer. Proc Natl Acad Sci U S A 102:11675–11680

Kasamo K (2003) Regulation of plasma membrane H^+-ATPase activity by the membrane environment. J Plant Res 116:517–523

Kłobus G, Janicka-Russak M (2004) Modulation by cytosolic components of proton pump activities in plasma membrane and tonoplast from *Cucumis sativus* roots during salt stress. Physiol Plant 121:84–92

Knight H, Trewavas AJ, Knight MR (1996) Cold calcium signaling in *Arabidopsis* involves two cellular pools and a change in calcium signature after acclimation. Plant Cell 8:489–503

Kolbert Z, Ortega L, Erdei L (2010) Involvement of nitrate reductase (NR) in osmotic stress-induced NO generation of *Arabidopsis thaliana* L. roots. J Plant Physiol 167:77–80

Kocsy G, Galiba G, Brunold C (2001) Role of glutathione in adaptation and signaling during chilling and cold acclimation in plants. Physiol Plant 113:158–164

Königshofer H, Tromballa HW, Löppert HG (2008) Early events in signaling high temperature stress in tobacco BY2 cells involve alterations in membrane fluidity and enhanced hydrogen peroxide production. Plant Cell Environ 31:1771–1780

Krishna P, Sacco M, Cherutti JF, Hill S (1995) Cold-induced accumulation of hsp-90 transcripts in *Brassica napus*. Plant Physiol 107:915–923

Kubiś J (2003) Polyamines and "scavenging system": influence of exogenous spermidine on catalase and guaiacol peroxidase activities, and free polyamines level in barley leaves under water deficit. Acta Physiol Plant 25:337–343

Laloi C, Apel K, Danon A (2004) Reactive oxygen signalling: the latest news. Curr Opin Plant Biol 7:323–328

Larkindale J, Huang B (2004) Changes of lipid composition and saturation level in leaves and roots for heat-stress and heat-acclimated creeping bentgrass (*Agrostis stolonifera*). Environ Exp Bot 51:57–67

Larkindale J, Knight MR (2002) Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene and salicylic acid. Plant Physiol 128:682–695

Li W, Wei Z, Qiao Z, Wu Z, Cheng L, Wang Y (2013) Proteomics analysis of alfalfa response to heat stress. PLoS One 8:82725

Liu A, Fan J, Gitau MM, Chen L, Fu J (2016) Nitric oxide involvement in Bermuda grass response to salt stress. J Amer Soc Hort Sci 141:425–443

Lopez-Pérez L, Martínez Ballesta M, Maurel C, Carvajal M (2009) Changes in plasma membrane lipids, aquaporins and proton pump of broccoli roots, as an adaptation mechanism to salinity. Phytochemistry 70:492–500

Mahajan S, Tuteja N (2005) Cold, salinity and drought stress: an overview. Arch Biochem Biophys 444:139–158

Martz F, Sutinen M, Kiviniemi S, Palta J (2006) Changes in freezing tolerance, plasma membrane H^+-ATPase activity and fatty acid composition in *Pinus resinosa* needles during cold acclimation and de-acclimation. Tree Physiol 26:783–790

Mata-Pérez C, Begara-Morales JC, Chaki M, Sánchez-Calvo B, Valderrama R, Padilla MN, Corpas FJ, Barroso JB (2016) Protein tyrosine nitration during development and abiotic stress response in plants. Front Plant Sci 7:1699

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

Moschou PN, Paschalidis AK, Delis ID, Andriopoulou AH, Lagiotis GD, Yakoumakis DI, Roubelakis-Angelakis KA (2008) Spermidine exodus and oxidation in the apoplast induced by abiotic stress is responsible for H_2O_2 signatures that direct tolerance responses in tobacco. Plant Cell 20:1708–1724

Murphy A, Taiz L (1997) Correlation between potassium efflux and copper sensitivity in 10 *Arabidopsis* ecotypes. New Phytol 136:211–222

Murphy A, Eisinger WR, Shaff JE, Kochian LV, Taiz L (1999) Early copper-induced leakage of K^+ from *Arabidopsis* seedlings is mediated by ion channels and coupled to citrate efflux. Plant Physiol 121:1375–1382

Neil S, Barros R, Bright J, Desikan R, Hancock J, Harrison J et al (2008) Nitroc oxide, stomatal closure, and abiotic stress. J Exp Bot 59:165–176

Neill SJ, Desikan D, Clarke A, Hurst RD, Hancock JT (2002) Hydrogen peroxide and nitric oxide as signaling molecules in plants. J Exp Bot 53:1237–1242

Niu X, Narasimhan M, Salzman R (1993) NaCl regulation of plasma membrane H^+-ATPase gene expression in *Glycophyte* and *Halophyte*. Plant Physiol 103:712–718

Oufattole M, Arango M, Boutry M (2000) Identification and expression of three new *Nicotiana plumbaginifolia* genes which encode isoforms of a plasma membrane H^+-ATPase, and one which is induced by mechanical stress. Planta 210:715–722

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Saf 60:324–349

Pál M, Horwáth E, Janda T, Páldi E, Szalai G (2005) Cadmium stimulates the accumulation of salicylic acid and its putative precursors in maize (*Zea mays*) plants. Physiol Plant 125:356–364

Paschalidis AK, Toumi I, Moschou NP, Kalliopi A, Roubelakis-Angelakis A (2010) ABA-dependent amine oxidases-derived H_2O_2 affects stomata conductance. Plant Signal Behav 5:1153–1156

Pei ZM, Murata Y, Benning G, Thomine S, Klüsener B, Allen GJ, Grill E, Schroeder JI (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature 17:731–734

Pérez de Juan J, Irigoyen J, Sánchez-Díaz M (1997) Chilling of drought-hardened and non-hardened plants of different chilling-sensitive maize lines changes in water relations and ABA contents. Plant Sci 122:71–79

Perez-Prat E, Narasimhan M, Niu X, Botella M, Bressan R, Valupesta V, Hasegawa PM, Binzel ML (1994) Growth cycle stage dependent NaCl induction of plasma membrane H^+-ATPase mRNA accumulation in de-adapted tobacco cells. Plant Cell Environ 17:327–333

Piotrovskii MS, Shevyreva TA, Zhestkova IM, Trofimova MS (2011) Activation of plasmalemmal NADPH oxidase in etiolated maize seedlings exposed to chilling temperatures. Russ J Plant Physiol 58:234–242

Polisensky DH, Braam J (1996) Cold shock regulation of the *Arabidopsis* TCH genes and the effects of modulating intracellular calcium levels. Plant Physiol 111:1271–1279

Qiao W, Faan LM (2008) Nitric oxide signaling in plant responses to abiotic stresses. J Integr Plant Biol 50:1238–1246

Qiao W, Li C, Fan LM (2014) Cross-talk between nitric oxide and hydrogen peroxide in plant responses to abiotic stresses. Environ Exp Bot 100:84–93

Quan LJ, Zhang B, Shi WW, Li HY (2008) Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. J Integr Plant Biol 50:2–18

Queval G, Hager J, Gakière B, Noctor G (2008) Why are literature data for H_2O_2 contents so variable? A discussion of potential difficulties in quantitative assays of leaf extracts. J Exp Bot 59:135–146

Reda M, Golicka A, Kabała K, Janicka M (2018) Involvement of NR and PM-NR in NO biosynthesis in cucumber plants subjected to salt stress. Plant Sci 267:55–64

Robertson AJ, Ishikawa M, Gusta LV, MacKenzie SL (1994) Abscisic acid induced heat tolerance in *Bromus inermis* lees cell suspension cultures. Plant Physiol 105:181–190

Romero-Puertas MC, Rodríguez-Serrano M, Sandalio ML (2013) Protein S-nitrosylation in plants under abiotic stress: an overview. Front Plant Sci 4:373

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

Sahay S, Gupta M (2017) An update on nitric oxide and its benign role in plant responses under metal stress. Nitric Oxide 67:39–52

Sahi C, Singh A, Blumwald E, Grover A (2006) Beyond osmolytes and transporters novel plant salt-stress tolerance-related genes from transcriptional profiling data. Physiol Plant 127:1–9

Sahu BB, Shaw BP (2009) Salt-inducible isoform of plasma membrane H^+-ATPase gene in rice remains constitutively expressed in natural halophyte, *Suaeda maritima*. J Plant Physiol 166:1077–1089

Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Physiol Plant Mol Biol 49:643–668

Sami F, Faizan M, Faraz A, Siddiqui F, Yusuf M, Hayat S (2018) Nitric oxide-mediated integrative alterations in plant metabolism to confer abiotic stress tolerance, NO crosstalk with phytohormones and NO-mediated post translational modifications in modulating diverse plant stress. Nitric Oxide 73:22–38

Santolini J, André F, Jeandroz S, Wendehenne D (2017) Nitric oxide synthase in plants: where do we stand? Nitric Oxide 63:30–38

Serrano R (1989) Structure and function of plasma membrane ATPase. Annu Rev Plant Physiol Plant Mol Biol 40:61–94

Shapiro AD (2005) Nitric oxide signaling in plants. Vitam Horm 72:339–398

Siddiqui MH, Al-Whaibi MH, Basalah MO (2010) Role of nitric oxide in tolerance of plants to abiotic stress. Protoplasma 248:447–455

Silva P, Geros H (2009) Regulation by salt of vacuolar H^+-ATPase and H^+-pyrophosphatase activities and Na^+/H^+ exchange. Plant Signal Behav 4:718–726

Stöhr C, Stremlau S (2006) Formation and possible roles of nitric oxide in plant roots. J Exp Bot 57:463–470

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

Sun C, Lu L, Liu L, Liu W, Yu Y, Liu X, Hu Y, Jin C, Lin X (2014) Nitrate reductase-mediated early nitric oxide burst alleviates oxidative damage induced by aluminum through enhancement of antioxidant defenses in roots of wheat (*Triticum aestivum*). New Phytol 201:1240–1250

Suzuki N, Mittler R (2006) Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. Physiol Plant 26:45–51

Svennelid F, Olsson A, Piotrowski M, Rosenquist M, Ottman C, Larsson C, Oecking C, Sommarin M (1999) Phosphorylation of Thr-948 at the C-terminus of the plasma membrane H^+-ATPase creates a binding site for regulatory 14-3-3 protein. Plant Cell 11:2379–2391

Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Molassiotis A, Job D (2009) Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. Plant J 60:795–805

Timperio AM, Egidi MG, Zolla L (2008) Proteomics applied on plant abiotic stresses: role of heat shock proteins (HSP). J Proteom 71:391–411

Tischner R, Planchet E, Kaiser WM (2004) Mitochondrial electron transport as a source for nitric oxide in the unicellular green alga *Chlorella sorokiniana*. FEBS Lett 576:151–155

Toumi I, Moschou PN, Paschalidis KA, Bouamama B, Ben Salem-Fnayou A, Ghorbel AW, Mliki A, Roubelakis-Angelakis KA (2010) Abscisic acid signals reorientation of polyamine metabolism to orchestrate stress responses via the polyamine exodus pathway in grapevine. J Plant Physiol 167:519–525

Uchida A, Jagendorf AT, Hibino T, Takabe T, Takabe T (2002) Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci 163:515–523

Van Gestelen P, Asard H, Caubergs RJ (1997) Solubilization and separation of a plant plasma membrane NADPH-superoxide ($O^{\cdot}{}_2^-$)-synthase from other NAD(P)H-oxidoreductases. Plant Physiol 115:543–550

Vandenabeele S, Van Der Kelen K, Dat J, Gadjev I, Boonefaes T, Morsa S, Rottiers P, Slooten L, van Montagu M, Zabeau M, Inze D, van Breusegem F (2003) A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. Proc Natl Acad Sci U S A 100:16113–16118

Vera-Estrella R, Barkla B, Higgins V, Blumwald E (1994) Plant defense response to fungal pathogens (activation of host-plasma membrane H^+-ATPase by elicitor-induced enzyme dephosphorylation). Plant Physiol 104:209–215

Volkov RA, Panchuk II, Mullineaux PM, Schoffl F (2006) Heat stress-induced H_2O_2 is required for effective expression of heat shock genes in *Arabidopsis*. Plant Mol Biol 61:733–746

Waie B, Rajam M (2003) Effect of increased polyamine biosynthesis on stress responses in transgenic tobacco by introduction of human S-adenosylmethionine gene. Plant Sci 164:727–734

Wang W, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trend Plant Sci 9:244–253

Wang X, Ma Y, Huang C, Li J, Wan Q, Bi Y (2008) Involvement of glucose-6-phosphate dehydrogenase in reduced glutathione maintenance and hydrogen peroxide signal under salt stress. Plant Signal Behav 3:394–395

Weidert ER, Schoenborn SO, Cantu-Medellin N, Choughule KV, Jones JP, Kelly EE (2014) Inhibition of xanthine oxidase by the aldehyde oxidase inhibitor raloxifene; implications for identifying molybdenum nitrite reductases. Nitric Oxide 37:41–45

Xiong J, An L, Lu H, Zhu C (2009) Exogenous nitric oxide enhances cadmium tolerance of rice by increasing pectin and hemicellulose contents in root cell wall. Planta 230:755–765

Xiong L, Zhu J (2001) Abiotic stress signal transduction in plants: molecular and genetic perspectives. Physiol Plant 112:152–166

Yang Y, Xu S, An L, Chen N (2007) NADPH oxidase-dependent hydrogen peroxide production, induced by salinity stress, may be involved in the regulation of total calcium in roots of wheat. J Plant Physiol 164:1429–1435

Yu M, Lamattina L, Spoel SH, Loake GJ (2014) Nitric oxide function in plant biology: a redox cue in deconvolution. New Phytol 202:1142–1156

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

Zhang JZ, Wang L, Liu Y, Zhang Q, Wei Q, Zhang W (2006) Nitric oxide enhances salt tolerance in maize seedlings through increasing activities of proton pump and Na^+/H^+ antiport in tonoplast. Planta 224:545–555

Zhang F, Wang Y, Yang Y, Wu H, Wang D, Liu J (2007) Involvement of hydrogen peroxide and nitric oxide in salt resistance in the calluses from *Populus euphratica*. Plant Cell Environ 30:775–785

Zhao MG, Tian QY, Zhang WH (2007) Nitric oxide synthase-dependent nitric oxide production is associated with salt tolerance in *Arabidopsis*. Plant Physiol 144:206–217

Zhu JK (2003) Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 6:1–5

Nitric Oxide and Hydrogen Peroxide in Plant Response to Biotic Stress

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Contents

Abstract NO and H_2O_2 act as key regulators in a broad range of physiological processes in algae and higher plants. A large amount of research highlights multiple roles for NO/H_2O_2 in plant defence. They function as protectants but also as signaling molecules that mediate various metabolic processes and activate further systematic plant defence reactions through the regulation of genes involved in pathogen defence. This chapter summarises the current knowledge on NO and H_2O_2 necessity in plant cell resistance response to biotic stressors.

Keywords Reactive oxygen species · Reactive nitrogen species · Signal molecule · Biotic stress · Gene expression · Crosstalk

1 Introduction

Plants and algae possess different signaling molecules (e.g. growth regulators, proteins, amino acids, nucleotides, hormones) which are essential for their growth, development as well as for their response and adaptation to a variety of abiotic and

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biotic stresses (Agostoni and Montgomery 2014; Bickerton et al. 2016; Dobrikova 2017; Górka and Wieczorek 2017). In view of the increasing pollution and more pronounced climate changes that affect plant organisms at the level of molecular function, developmental processes, morphological traits, and physiology (Gray and Brady 2016), influence the severity of plant disease and further coevolution of plants and their pathogens, the investigation of signaling molecules in algae and plants is becoming increasingly important.

Nitric oxide (NO) has been recognised as one of the central players in the cell signaling and attracted great attention of researchers (Mallick et al. 2002; Chung et al. 2008; Neill et al. 2002; Jeandroz et al. 2016). NO is a small gaseous molecule with low density and lipophilic quality (Cevahir et al. 2007). Its production arises from different enzymatic and nonenzymatic pathways (Rőszer 2014; Astier et al. 2018a). The well documented reductive NO production routes in plants are nitrate reductase (NR) and the plasma membrane-bound nitrite: NO reductase (NiNOR) systems. Also, NO can be produced in an arginine-dependent pathway, similar to the nitric oxide synthase (NOS) activity present in animals. Recent investigations showed that terrestrial plants do not possess animal NOS-like enzymes, while these enzymes were found in few algal species (Santolini et al. 2017; Weisslocker-Schaetzel et al. 2017; Astier et al. 2018b). Foresi et al. (2010) isolated protein from the alga *Ostreococcus tauri* (OtNOS) that is about 45% similar to human NOS enzyme and has in vitro NOS activity. Kumar et al. (2015) found NOS-like sequences from the marine green algae *Bathycoccus prasinos* and *Ostreococcus lucimarinus* that exhibited 62% similarity with OtNOS and several conserved residues in cofactor-binding sites. Each monomer of OtNOS homodimer enzyme possesses two domains: a C-terminal reductase domain (NOSred) and an N-oxygenase domain (NOSoxy) which harbours the catalytic site. Furthermore, Weisslocker-Schaetzel et al. (2017) made in-depth structural and functional analysis of OtNOS NOSoxy domain and found weak homology with NOS enzymes from other clades. Still, residues that form the catalytic core of the domain were conserved.

Nonenzymatic pathways include NO synthesis from nitrite NO_2^- under acidic conditions or from hydroxylamine and salicylhydroxamate (Gupta et al. 2011a; Procházková et al. 2014). NO generation has been clearly demonstrated in a several different cell organelles including protoplasts, chloroplasts, mitochondria and peroxisomes (Gupta et al. 2011b; Tewari et al. 2013; Corpas and Barroso 2014), but it also could be synthesised in the cytoplasm, cell membrane, endoplasmic reticulum as well as apoplast (Frohlich and Durner 2011; Sahay and Gupta 2017). Due to the simple structure and small dimensions, NO freely diffuses through biological membranes provoking both beneficial and harmful effects in algae and plant cells (Arasimowicz and Floryszak-Wieczorek 2007). This dual role depends on the NO local concentration, as well as its ability to directly interact with other molecules and signals. Generally, at low concentrations, NO is a signaling molecule with a variety of physiological roles (Galatro and Puntarulo 2014). It plays an important role in many developmental processes of plants (Mur et al. 2013) including seed and pollen germination (Beligni and Lamattina 2000; Pasqualini et al. 2015), pollen tube growth (Prado et al. 2004), root growth (Corpas and Barroso 2015; Moni et al.

2018; Singh and Bhatla 2018; Sun et al. 2018), flowering (Senthil Kumar et al. 2016; Salachna and Zawadzińska 2018), photomorphogenesis (Lozano-Juste and León 2011; Melo et al. 2016), stomatal closure (García-Mata and Lamattina 2001), leaf senescence (Yang et al. 2018), gravitropism (Hu et al. 2005; París et al. 2018) and fertilisation (Domingos et al. 2015). Furthermore, it acts as a signal molecule for the activation of plant and algal defence response. The antioxidant role of NO is mainly based on its ability to maintain the cellular redox homeostasis and regulate the toxicity of reactive oxidative species (ROS) (Sheokand and Kumari 2015). For example, peroxynitrite ($OONO^-$) results from the reaction between NO and superoxide radical ($O_2^{\cdot -}$). It is a relatively short-lived reactive nitrogen species (RNS) which may readily migrate through biological membranes and interact with proteins, lipids and DNA via direct oxidation reactions or indirectly through the formation of highly reactive radicals (Radi 2013; Procházková et al. 2015). However, in systems where the toxicity is predominantly from peroxides, these compounds are much more toxic than NO and $OONO^-$, making NO protective against them (Misra et al. 2011).

ROS have also been shown to play an important role as signaling molecules in response to environmental conditions (Mallick and Mohn 2000; del Río 2015; Mullineaux et al. 2018). Among the ROS compounds, H_2O_2 is generally recognised as one of the most important signaling molecules which has relatively long half-life and small size and thus may migrate in different cell compartments through aquaporin channels (Hooijmaijers et al. 2012; Srivastava et al. 2014; Rodrigues et al. 2017). H_2O_2 is a product of aerobic metabolism in plants and could be generated by several pathways such as photorespiration, electron transport chains and redox reaction (Møller 2001; Voss et al. 2013). It can be synthesised by different enzymes including cell wall peroxidases (Mittler 2002), oxalate oxidases (Hu et al. 2003), amine oxidases (An et al. 2008) and flavin-containing enzymes (Cona et al. 2006), as well as by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases coupled with superoxide dismutases (SOD; Vianello and Macri 1991). At lower concentrations, it acts as a long-distance signaling molecule (Matilla-Vázquez and Matilla 2014) which has a role in various physiological processes, including photosynthesis, respiration, translocation and transpiration (Vranová et al. 2002; Ślesak et al. 2007; Ismail et al. 2015). It is involved in the formation and development of adventitious roots (Li et al. 2007), stomatal closure (Zhang et al. 2001), senescence process (Bieker et al. 2012), protection against pathogen attack (Shetty et al. 2008) as well as in the regulation of various abiotic stresses (Mittler and Berkowitz 2001; Cuypers et al. 2016).

Abiotic and biotic factors induce rapid accumulation of variety of reactive nitrogen species (e.g. nitrosonium cation (NO^+), nitroxyl anion (NO^-), free radical ($NO^{\cdot}$), dinitrogen trioxide (N_2O_3), nitrogen dioxide (NO_2) and *S*-nitrosothiols (SNOs)) causing nitrosative stress (Corpas et al. 2007; Procházková et al. 2015). In response to different stress factors including infections by pathogenic fungi, bacteria, and viruses, rapid accumulation of a variety of ROS also occurs (Mittler 2017). One of the earliest events following elicitation characterised by high ROS and NO concentrations is generally known as oxidative burst (Wojtaszek 1997). H_2O_2

generated during oxidative bursts under biotic stress may reduce pathogen growth or may cause the expression of genes encoding proteins involved in antioxidant and defensive processes but also may induce programmed cell death (PCD) that occurs during hypersensitive response (HR) in plants (Bhattacharjee 2005).

At higher concentration, all reactive species including NO and H_2O_2 interact with different macromolecules affecting their functionalities (Gadjev et al. 2008; Habibi 2014; Corpas and Palma 2018). The differential reactivity of RNS defines the diversity of the NO potential target molecules and its chemical reactions including nitrosylation, nitration and oxidation (Arasimowicz and Floryszak-Wieczorek 2007; Krasylenko et al. 2017).

Plants have developed an antioxidative system that encompasses both the enzymatic and nonenzymatic components to minimise nitro-oxidative stress caused by RNS and ROS (Corpas and Barroso 2013). The enzymatic components include very efficient various enzymes such as SOD, catalase (CAT), glutathione peroxidase (GPX), glutathione *S*-transferase (GST), guaiacol peroxidase (GPOX) and the ascorbate-glutathione cycle enzymes: ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDAR) and glutathione reductase (GR). Nonenzymatic antioxidants such as ascorbic acid (ASH), glutathione (GSH), phenolic compounds, alkaloids, nonprotein amino acids and α-tocopherols can quench all kinds of ROS directly but also may assist the enzymes to efficiently detoxify ROS (Perl-Treves and Perl 2002; Gill and Tuteja 2010; Štolfa et al. 2016, 2018).

In response to development and different abiotic and biotic factors, both NO and H_2O_2 may interact with a variety of other signaling molecules and phytohormones (Ferreira and Cataneo 2010; Mostofa et al. 2015; Saxena et al. 2016) (Fig. 1). Also, crosstalk between the NO and H_2O_2 has been demonstrated in plants and algae (González et al. 2012; Niu and Liao 2016). These molecules may interact in their different developmental and physiological processes. Thus, it has been found that NO and H_2O_2 are essential signal molecules that mediate abscisic acid (ABA)-induced stomatal closure (Li et al. 2017) and that H_2O_2 and NO signaling pathways are involved in adventitious rooting in mung bean seedlings (Li and Xue 2010) as well as in cell cycle of green alga *Chlamydomonas reinhardtii* (Pokora et al. 2017). During the last decades, the roles of NO and H_2O_2, as well as their crosstalk in mediating plant defence mechanisms, have been largely studied. For example, it has been found that H_2O_2 and NO-signaling pathway overlap during the citrus plant acclimation on salinity (Tanou et al. 2009).

The mechanisms of stresses response triggered by NO and H_2O_2 include the enhancement of antioxidant systems and specific stress mechanisms, depending on the stress type (e.g. drought, temperature, heavy metals, etc.), and demand the interaction with other signaling molecules, such as mitogen-activated protein kinase (MAPK), plant hormones and calcium (Molassiotis and Fotopoulos 2011; Farnese et al. 2016). MAPK cascades are present in plants and algae (Mohanta et al. 2015) and consist of a few protein kinase modules including MAP kinase kinase (MAP2Ks, also called MKKs, MEKs or MAPKK), MAP kinase kinase kinases (MAP3Ks, also called MAPKKKs or MEKKs) and MAP kinase kinase kinase kinases (MAP4K; Rodriguez et al. 2010; Çakır and Kılıçkaya 2015). Generally,

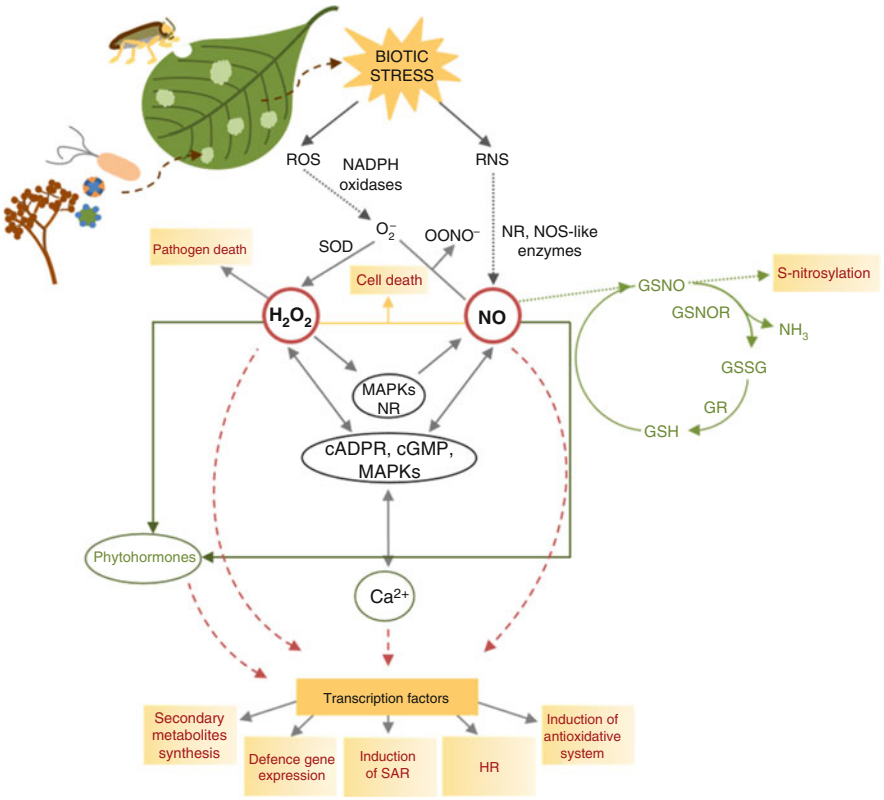

Fig. 1 The signal cascade mediated by NO and H_2O_2 in plant pathogen defence

MAPK is activated via phosphorylation of threonine and tyrosine residues in the catalytic subdomain (Ichimura et al. 2002) and then translocated into the cytoplasm or nucleus to trigger the cellular responses (Nadarajah and Sidek 2010). They are involved in cellular responses to hormones, plant growth and development, regulation of the cell cycle and responses to biotic and abiotic stresses (Sinha et al. 2011; Zhao et al. 2013; Hettenhausen et al. 2015; Chardin et al. 2017). MAPKs can phosphorylate many target molecules including other kinases, enzymes and transcription factors (TFs). The responses mediated by MAPKs involve the induction of a variety of enzymes and may attenuate or amplify the original signal triggered by ROS and NO (Asai et al. 2008; Opdenakker et al. 2012). NO and H_2O_2 may also increase the concentration of some other signaling molecules such as cyclic guanosine 3′,5′-monophosphate (cGMP). This cyclic nucleotide is generated from guanosine 5′-triphosphate (GTP) by enzymes with guanylate cyclase (GCs) activity (Mulaudzi et al. 2011). These molecules have long been known to be present in plants and algae, playing important roles in many biological processes including growth and differentiation, photosynthesis as well as biotic and abiotic defence (Dubovskaya et al. 2011; Nan et al. 2014; Gehring and Turek 2017; Demidchik

et al. 2018). Responses associated with cGMP depend on the time between the perception of the stimulus and the peak in nucleotide concentration and consequently involve fast responses, which include the ion channels modulation (e.g. Ca^{2+} channels) or long-term adaptive responses, which result in changes in the transcriptome and in the proteome (Pasqualini et al. 2009).

Thus, considering the very important role of NO and H_2O_2 molecules in a variety of biological process in plant and algae as well as in their response and adaptation to the environmental changes, the present review aims to summarise the recent data concerning the role of NO and H_2O_2 in plant defence mechanisms.

2 Enrolment of NO and H_2O_2 in Plant Stress Response

2.1 Sources, Signaling and Interaction

Elicitation by abiotic or biotic stress factors induces oxidative burst as one of the earliest events following plant challenge with pathogenic micro-organisms, including fungi, bacteria and viruses, as well as in cultured cells treated with elicitor preparations, pathogens or plant cell-wall fragments or in response to mechanical wounding (Wojtaszek 1997). To protect themselves against pathogens and herbivores, plants have evolved a complex network of immune responses – HR. It is defined as a programmed execution of infected cells and sometimes additional surrounding cells with a function to restrict pathogen infection (Lamb and Dixon 1997; Greenberg and Yao 2004). Beside local HR, plants react to pathogen attack by establishing systemic-acquired resistance (SAR), a long-lasting systemic immunity that protects the entire plant from the subsequent invasion of a broad range of pathogens associated with the systemic expression of defence gene families encoding pathogenesis-related proteins (Yu et al. 2014). One of the earliest events in the HR is the rapid accumulation of ROS (Zurbriggen et al. 2010) and NO (Delledonne et al. 1998; Durner et al. 1998). Plant species differentiate according to the major ROS building the oxidative burst. New and highly sensitive methods enabled the real-time detection of ROS generation in response to stress. Recently, the use of catalytic amperometric biosensors tested on in vivo spinach (*Spinacia oleracea*) leaf sample showed the continuous generation of $O_2^{\cdot -}$ for minutes after wounding, followed by a decline, while the production increased considerably with the dose of mechanical injury. The total $O_2^{\cdot -}$ concentration was found to be equivalent to 40 nM and 200 nM for a minimal dose of injury and injury at multiple sites, respectively (Prasad et al. 2017). Seaweed species released strong oxidants within 1 min of wounding and/or showed cellular accumulation of strong oxidants over an hour post-wounding (McDowell et al. 2014). However, the inherent interrelationship between H_2O_2 and $O_2^{\cdot -}$ makes it sometimes difficult to clearly identify the ROS behind the oxidative burst (Wojtaszek 1997). Stress treatment induces an oxidative burst in barley (*Hordeum vulgare*) anthers, as revealed by the formation of $O_2^{\cdot -}$ that gives rise to H_2O_2. Among ROS, H_2O_2 may have a central role in anthers

as a diffusible signal molecule allowing selective induction of defence-related genes (Varnova et al. 2002).

The bulk of active oxygen species, mainly $O_2^{\cdot -}$, H_2O_2, singlet oxygen ($^1O_2$) as well as NO, are produced to serve not only as protectants but also as signals activating further plant defence reactions (Bolwell 1999). H_2O_2 production is indispensable during plant growth, development and resistance responses. Its great capacity to buffer other ROS molecules and balance the capacity of oxygen scavenging enzymes changes oxygen's relative impact on cells altering its resistance to stress (Quan et al. 2008). However, its beneficial role in integrating signaling network in response to biotic and abiotic stress and during developmental processes is well established (Wojtyla et al. 2016). Due to the large plethora of different signal messages triggered by H_2O_2, plants can sense, transport and induce cellular responses (Quan et al. 2008). The network of signals includes other reactive species such as NO and H_2S, plant phytohormones jasmonic acid (JA), salicylic acid (SA), ABA, ethylene, auxin and gibberellins, as well as ions regulating diverse cellular processes like Ca^{2+} and K^+ (Quan et al. 2008; Kaurilind et al. 2015).

One of the most important roles of H_2O_2 is in the reaction with the invading organisms. After the detection of an invader at the cell wall, the NADPH oxidase at the plasma membrane adjacent to the invasion site produces $O_2^{\cdot -}$ and ultimately H_2O_2 outside the plasma membrane.

Aquaporins in the plasma membrane are crucial for the efficiency of H_2O_2 signaling between cells. Their capacity to facilitate the H_2O_2 diffusion may have physiological significance in many organisms and be important in communication between different species (Bienert et al. 2006).

A number of possible functions for H_2O_2 have been proposed in plant-pathogen reactions: direct killing of pathogens, involvement in cell-wall reinforcing processes, promotion of PCD at the site of infection, phytoalexin synthesis, induction of defence gene expression and signaling in the induction of SAR (Bolwell 1999; Kuźniak and Urbanek 2000). During the pathogen attack on the tea plant (*Camellia sinensis*) leaves, H_2O_2 can accumulate in mesophyll and epidermal cells (Wang et al. 2018). Cellular and subcellular localisation analyses revealed that H_2O_2 was mainly localised in wound zones and spread throughout the veins and tissues. Preferentially, H_2O_2 was found in cell walls of spongy and mesophyll cells facing intercellular spaces in the herbivore-wounded lima bean (*Phaseolus lunatus*) leaves, even though confocal laser scanning microscopy analyses also revealed the presence of H_2O_2 in mitochondria/peroxisomes (Maffei et al. 2006). Like in the case of wounding, H_2O_2 also accumulates in the *Capsicum* leaf abscission zone throughout plant growth, it increases with age and during the execution phase, and its role in leaf abscission is associated with ethylene both in vitro and in planta (Sakamoto et al. 2008).

In the unicellular green alga *Micrasterias denticulata*, chloroplasts and mitochondria are generally the main production sites of H_2O_2 independent of the treatment (salt and osmotic stress), followed by cytoplasm, while levels remained unchanged or even slightly decreased in cell walls of treated cells and elevated concentration at the plasma membrane of KCl-treated cells (Darehshouri and Lütz-Meindl 2010). Increased H_2O_2 production can also be a defensive mechanism against epiphytism in

algae. Green macroalga *Caulerpa taxifolia* enhanced the production of H_2O_2 and toxin caulerpenyne as well as the antioxidant enzymes activities to compete against epiphytic rhodophyte *Lophocladia lallemandii* (Box et al. 2008). H_2O_2 is also engaged in signal transduction and defence reactions in the nonhost-pathogen interactions, like the formation of callose-containing papilla for cell wall reinforcement and HR. HR and papilla deposition was highly associated with H_2O_2 accumulation in pepper epidermal cells, which has a key role in nonhost resistance against *Blumeria graminis* f. sp. *tritici* preventing its colonisation and disease development (Hao et al. 2011). Although being the most prominent oxidant in the algal oxidative burst cocktail (Potin 2008), some seaweeds show the smaller role of H_2O_2 upon wounding and oxidant release. For example, in several species of Antarctic brown and red macroalgae, other oxidant(s) besides H_2O_2 are released upon wounding (McDowell et al. 2014). H_2O_2 was a component of immediate oxidant release in one of five species, while in some it was not detected at all. Even the two close sister algal taxa may produce different amounts of ROS and have a different composition of chemical species in the oxidative burst. While after sonic injury endosymbiotic dinoflagellate *Symbiodinium* sp. produced a small oxidative burst of 0.042 ± 0.0045 pmol H_2O_2 min^{-1} $cell^{-1}$ composed of nearly 100% H_2O_2, the oxidative burst in *Heterocapsa pygmaea*, the species similar in size and genetically related, was nearly 80 times greater (3.37 ± 0.26 pmol H_2O_2 min^{-1} $cell^{-1}$) and also produced a fraction of other free radicals (Mydlarz and Jacobs 2004). The amount of produced H_2O_2 can vary with the different type of wounding, e.g. herbivore-wounded lima bean (*Phaseolus lunatus*) leaves produced higher H_2O_2 concentrations compared to mechanically damaged leaves (Maffei et al. 2006). As a response to oxidative burst, many species of marine algae produce volatile organic compounds, such as halogenated, sulphur containing, aldehydes, non-methane hydrocarbons and oxygenated species as one of the ways to lower the content of produced H_2O_2 (Potin 2008). An oxidative burst of H_2O_2 and the consequent production of volatile organic compounds in brown alga *Laminaria digitata* after elicitation induce defence response which can even be transmitted as a warning message to neighbouring conspecifics (Thomas et al. 2011).

Exogenously applied H_2O_2 is known as a good microbicidal agent which can benefit seed health and performance. Soaking the zinnia (*Zinnia elegans*) seeds regardless of time (10, 20, 30 and 60 min) and H_2O_2 concentration (3, 6, 9 and 12%, respectively) significantly reduced seed infestation with *Alternaria* spp. and *Fusarium* spp. fungi, while 3% solution positively influenced seed germination and vigour at 20 min and 10 min application, respectively (Szopińska 2014).

Foliar application of H_2O_2 (18 mM) in chili pepper plants (*Capsicum chinense*) significantly increased the amount of phenolics, flavonoids and capsaicinoids contents in fruits, thus concomitantly increasing antimicrobial activity of its extracts against bacteria and yeast (Vargas-Hernández et al. 2017). H_2O_2 is also utilised as an efficient algicide for the management of waste waters. The dose of 3.0×10^{-3} g H_2O_2 μg^{-1} phytoplankton chlorophyll-a was the most effective in the removal of nuisance phytoplankton, including toxic cyanobacteria (Barrington and Ghadouani 2008). Sensing the increased H_2O_2 concentrations can also be one of the

phytoplankton survival strategies under unfavourable environmental conditions. The encystment of some dinoflagellate species, an effective strategy that enables them to survive, is redox-mediated, presumably by H_2O_2 (Ganini et al. 2013).

NO burst can be detected very early after inoculation with pathogens and directly precedes H_2O_2 generation. NO production in affected cells can be monitored with the application of fluorescent dyes (Foissner et al. 2000; Prats et al. 2005).

One of the most important NO signaling mechanism during plant defence is based on its chemical reaction with proteins (Leitner et al. 2009). The fine balance between *S*-nitrosylation and denitrosylation is critical for NO signaling. *S*-Nitrosylation regulates numerous defence-related proteins such as enzymes, transcriptional activators, or co-activators involved in plant immune response in the way of protein relocalisation or activity modulation (Bellin et al. 2013). *S*-nitrosylation is a chemical reaction in which a NO moiety is covalently added to the sulfhydryl group of reactive cysteine residues in target proteins to form an *S*-nitrosothiol (SNO) (Stamler et al. 2001). The SNO turnover represents an alternative mechanism to control the process of protein *S*-nitrosylation. Namely, the main enzymatic systems which mediate denitrosylation in plants are described: the glutathione/*S*-nitrosoglutathione reductase (GSH/GSNOR) and the thioredoxin-*h*5 (Trx-*h*5) (Kneeshaw et al. 2014). An *S*-nitrosylated form of glutathione, *S*-nitrosoglutathione (GSNO) is a global reservoir of NO bioactivity (Liu 2001). Experiments with *Arabidopsis* knockout mutants of this enzyme, known as GSNO Reductase 1 (GSNOR1), accumulate high levels of protein-SNO and consequently have impaired SA-dependent immune signaling and are highly disease susceptible (Feechan et al. 2005). In contrast, plants expressing antisense GSNOR1 show enhanced SAR, likely due to a partial reduction in GSNOR1 activity, which increases NO levels to the extent that is ideal for stimulating SAR (Rustérucci et al. 2007; Espunya et al. 2012). In plants, GSNOR has been found to be important in resistance to bacterial and fungal pathogens like *Blumeria graminis*, *Hyaloperonospora parasitica*, and *Pseudomonas syringae* (Feechan et al. 2005) and possibly also involved in plant-herbivore interactions (Wünsche et al. 2011). The enzyme GSNOR does not directly reverse the *S*-nitrosylation of Cys residue but rather turns over GSNO thereby reducing the cells store of NO bioactivity and controls intracellular levels of both GSNO and SNO affecting the global level of *S*-nitrosylation (Feechan et al. 2005; Malik et al. 2011). On the other hand, the mechanism of Trx denitrosylation directly interacts with SNO-proteins by the formation of an intermolecular disulphide intermediate in which Trx is covalently linked to the substrate protein through a disulphide bridge or transnitrosylation in which Trx is transiently *S*-nitrosylated. This mechanism is described in detail in animal cells (Benhar et al. 2008), while Kneeshaw et al. (2014) proved the similar mechanism of Trx-h5 in plant cells.

A number of experiments using NO donors, scavengers and NOS inhibitors proved that NO plays a central role in plant defence against biotic stress, in combination with ROS (Delledonne et al. 1998, 2001; Yun et al. 2011). Keshavarz-Tohid et al. (2016) found the positive interaction of NO and H_2O_2 in bean plant defence against *Pseudomonas fluorescens* and *Rhizoctonia solani*. Namely, NO donor *S*-nitroso-N-acetyl D-penicillamine increased the production of

H_2O_2, thereby increasing the activity of antioxidant enzymes as well as the activity of phenylalanine ammonia lyase (PAL). NO action upstream of H_2O_2 was also shown in plants responses to herbivore attacks and *Tobacco Mosaic Virus* infection (Orozco-Cárdenas and Ryan 2002; Liao et al. 2013). Reversely, Qiao et al. (2015) found that Ca^{2+} and H_2O_2 are involved in upstream of NO production to induce the HR cell death in the wheat cells during *Puccinia triticina* infection.

H_2O_2 and NO display both prooxidant and antioxidant properties. For example, they serve as prooxidant agents and putative redox signals for in vitro encystment of the dinoflagellate *Lingulodinium polyedrum*. Oxidative stress induced by high H_2O_2 dose (500 mM) forced *L. polyedrum* cells to rapidly encyst (<30 min), while slower cyst formation was observed in lower H_2O_2 concentrations (Ganini et al. 2013). NO may induce scavenging of excess H_2O_2 and inhibit peroxide signaling pathways but also may collaborate with H_2O_2 to switch on SAR or stress tolerance. Two faced NO molecule can act as an antioxidant and an antiapoptotic modulator that prevents cell death and in the same time can have a cytotoxic effect. These cytotoxic and protective effects of NO are often concentration dependent (Wink and Mitchell 1998).

The mechanism of NO/H_2O_2 interaction in the induction of cell death is largely unknown. The in vitro chemical reaction between NO and H_2O_2 produces either $^1O_2$ or hydroxyl radicals ($^{\cdot}OH$) which can mediate cell killing (Noronha-Dutra et al. 1993). Although $^1O_2$ formation in plant system is merely dependent on light, especially pronounced in the highlight, temperature, heavy metal stress or wounding, its light-independent generation in plant cells under multiple stresses has also been proposed (Mor et al. 2014; Chen and Fluhr 2018; Prasad et al. 2018). NO can also interact with $O_2^{\cdot -}$ to produce another highly reactive species, $OONO^-$. Alamillo and Garcia-Olmedo (2001) found that direct application of $OONO^-$ induced plant cell death, which was not observed in the case when urea ($OONO^-$ scavenger) was added. $OONO^-$ may have a toxic effect on microorganisms, although so far it has not been clarified whether NO and its derivatives are directly toxic to pathogens in plants (Garcia-Olmedo et al. 2001). In tobacco cell suspensions, only a simultaneous increase in NO and H_2O_2 activated cell death, whereas an independent increase of only one of the factors mentioned above induced cell death only slightly (de Pinto et al. 2002). Moreover, only the synergistic effect of H_2O_2 and NO was effective in control of tomato bacterial wilt (Hong et al. 2013).

2.2 Regulation of Gene Expression

Both NO and H_2O_2 influence gene expression from transcription to protein synthesis. They regulate genes involved in the induction of pathogen defence, such as genes encoding pathogenesis-related (PR) proteins, genes involved in accumulation of phenylpropanoid compounds, genes encoding H_2O_2 detoxifying enzymes, enzymes involved in JA biosynthesis (Jacquard et al. 2009), upregulate cellulase expression (Sakamoto et al. 2008) and control genes involved in the hypersensitive

cell death (Kaurilind et al. 2015). The large-scale gene expression analysis in *Arabidopsis* performed using the paired-end RNA-seq technology showed a different response to GSNO treatment in leaves and roots, suggesting that NO signaling mechanisms are organ specific (Begara-Morales et al. 2014). Another study on *Arabidopsis* transcriptome also showed that NO modulates gene expression in a concentration-dependent manner (Parani et al. 2004).

NO and ROS signaling is important in the establishment of plant disease resistance through modulation of defence-related genes encoding phenylalanine ammonia lyase (PAL) and pathogenesis-related protein 1 (PR1), markers for phenylpropanoid biosynthesis and SA-mediated signaling, respectively.

The key redox control of SAR exerts through nitrosylation of NPR1 (pathogenesis-related (PR)1), the key protein in plant immunity that co-activates defence gene expression and is an important component of SA-mediated signal transduction (Mou et al. 2003; Tada et al. 2008). Moreover, Wu et al. (2012) showed this protein to bind SA, working as an SA receptor directly. In unaffected cells, NPR1 is normally present as a high molecular weight oligomer formed with intermolecular disulphide bridges. After the pathogen attack as well as accumulation of SA, changes in cellular redox potential lead to the reduction of cysteines through the activity of thioredoxins (TRX-h3 and TRX-h5) and translocation of NPR1 monomers to the nucleus. In the nucleus, NPR1 interacts with the TGACG motif-binding factor (TGA) that binds to elements of the PR1 promoter, promoting PR gene expression and defence (Tada et al. 2008; Bellin et al. 2013). On the other hand, Tada et al. (2008) also found that S-nitrosylation in vivo promotes disulphide bond formation and oligomerisation. This reaction may be required to maintain NPR1 oligomer/monomer homeostasis, thereby facilitating the steady supply of monomeric protein to support SA-dependent gene expression. Beside NO, increased levels of H_2O_2 also inhibit NPR1 translocation (Peleg-Grossman et al. 2010). On the other hand, an oxidative burst and accumulation of H_2O_2 induced by the fungus *C. gloeosporioides* in the resistant cowpea genotype TE97 enhanced the levels of PR proteins (GLU and CHI) as a defence strategy against pathogen attack (Oliveira et al. 2014).

However, Lindermayr et al. (2010) found that NO also promotes NPR1-dependent defence responses by facilitating the translocation of NPR1 into the nucleus. Notably, TGA, an interaction partner of NPR1, can also be oxidised and S-nitrosylated. The oxidised form carries disulphide bonds that block interactions with NPR1, while the GSNO-mediated S-nitrosylation of cysteine residues protects TGA proteins from oxidative modification and improves its binding activity to PR1 promoter region (Lindermayr et al. 2010). This additional positive regulatory mechanism also involves the NO-dependent regulation of GSH biosynthesis and accumulation, which increases SA levels and thus activates NPR1-dependent defence responses (Kovacs et al. 2015). However, the nuclear translocation of NPR1 was much slower when driven by GSNO instead of SA. This finding suggests that this process was not the direct consequence of NPR1 S-nitrosylation but, instead, was dependent on a signaling pathway involving GSH biosynthesis. These findings

revealed evidence of additional crosstalks among NO, GSH and SA pathways in the establishment of immunity in plants.

Besides NPR, *S*-nitrosylation modulates the activity of another SA-binding protein AtSABP3 (*A. thaliana* SA-binding protein 3). Namely, it suppresses both binding of SA and also its carbonic anhydrase activity that could contribute to negative feedback that modulates plant defence response and cell death (Wang et al. 2009).

Another important defence-related gene PAL is also modulated via NO signaling (Durner et al. 1998; de Pinto et al. 2002). Moreover, inhibition of NOS activity significantly reduces the accumulation of transcripts encoding PAL and chalcone synthase enzymes important for flavonoids and isoflavonoid synthesis (Delledonne et al. 1998). Additionally, an increase in cinnamate-4-hydroxylase transcripts, a key enzyme in the synthesis of phenolic compounds, has been found in *Arabidopsis* treated with a NO donor (Polverari et al. 2003). Likewise, exogenous H_2O_2 treatment induces *PAL1* mRNA expression in *Arabidopsis* where this enzyme mediates biosynthesis of lignin and SA (Desikan et al. 1998).

NO is involved in the regulation of gene expression of NO-responsive TFs such as MYB, WRKY, C2H2, Aux/IAA, bZIP, etc. included in the mediation of abiotic and biotic stress responses (Imran et al. 2018). Novel information about a large number of genes involved in NO signaling is discovered with the use of next-generation sequencing (NGS) technologies. Imran et al. (2018) showed that almost 30% of total NO-responsive TFs were related to stress tolerance, among which 95.5% were related to biotic stress. For example, bZIP-binding elements associated with defence formed by octopine synthase (OCS) are very important for the expression of above-mentioned PR1 gene in *Arabidopsis* where OCS element-binding factor interacts with NPR1 (Lebel et al. 1998; Zhou et al. 2000). Likewise, WRKY transcription factors involved in plant response to wounding and pathogen infection were upregulated in seedlings of *Larix olgensis* Henry treated with NO (Hu et al. 2015). Expression of transcription factor WRKY8 can also be activated through ROS-mediated signaling mechanism (Chen et al. 2010). Also, a large number of genes encoding TFs involved in NO signaling are characterised by RNA-Seq in root nodules formed in the symbiotic relationship of *Medicago truncatula* and its bacterial symbiont *Sinorhizobium meliloti* (Boscari et al. 2013).

The NO/ROS crosstalk during plant defence was also reflected through modulation of NO reactivity by *S*-nitrosylation of ROS producers and ROS or RNS scavengers. NADPH oxidase AtRBOHD is a major participant in ROS production induced by pathogens in *Arabidopsis* (Torres et al. 2005) and in grass roots infected with fungal phytopathogen *Rhizoctonia solani* (Liu et al. 2018). Yun et al. (2011) found that cysteine 890 S-nitrosylation of AtRBOHD during plant defence decreases NADPH oxidase activity and consequently reduces ROS accumulation, limiting the later stages of HR cell death. Also, different NO-responsive TFs knockout *Arabidopsis* mutants showed significantly higher expression of the *NADPH oxidase 1* (*NOX1*) and consequently more ROS accumulation. However, these mutants also showed higher expression of important antioxidative enzyme genes *CAT1*, *2* and *3* and *APX1* and *2* (Imran et al. 2018). Another way of oxidative burst inhibition

triggered by NO is through its reaction with $O_2^{\cdot-}$ to form $ONOO^-$ and thus work directly as an antioxidant. Through this interaction, NO direct competes with SOD activity that could, therefore, inhibit the oxidative burst and decrease the accumulation of H_2O_2. Also, Holzmeister et al. (2015) found that NO modifies and inhibits three of the seven *A. thaliana* SODs by tyrosine nitration. The relevance of this modification in plant defence is still undefined, but it could be important for regulating ROS levels in HR cell death. Also, different antioxidant enzymes involved in H_2O_2 detoxification are exposed to S-nitrosylation or nitration according to in vitro studies, like APX, MDAR, CAT and peroxiredoxins, all of which are involved in H_2O_2 detoxification (Romero-Puertas et al. 2007; de Pinto et al. 2013; Begara-Morales et al. 2015; Camejo et al. 2015). Another antioxidative enzyme, Peroxidase 2 (PA 2) gene, may be a negative regulator of H_2O_2 production and is suppressed by an unknown TF to increase H_2O_2 levels in an anthracnose-resistant tea plant (*Camellia sinensis*) cultivar as a defence to *Colletotrichum fructicola*. H_2O_2 also upregulated a cell signaling receptor (wall-associated kinase 3) connected with cell wall reinforcement in *C. sinensis* at the penetration sites of pathogen hyphae (Wang et al. 2018). Activated resistance genes also regulate the thickening of cell wall tissue to defend against hyphal growth.

H_2O_2 accumulation also regulates the expression of cellulase genes. H_2O_2 is involved in the abscission signaling downstream of ethylene to upregulate cellulase expression. Moreover, salinity induced the production of H_2O_2 before leaf abscission, indicating that H_2O_2 might generally be involved in stress-induced leaf abscission (Sakamoto et al. 2008). Increased H_2O_2 accumulation in herbivore-wounded leaves was also correlated with increased SOD enzyme activity and gene expression (Maffei et al. 2006).

Besides NO, ROS metabolism localised in the peroxisome is usually controlled by the protein peroxin 11a (PEX11a) under stress conditions which was distinctly upregulated in the resistant tea plant during *C. fructicola* infection and may also be associated with H_2O_2 production (Wang et al. 2018).

In the recent years, the physiological relevance of H_2S signaling and its tight connection with H_2O_2 and NO gained considerable interest (Zhang 2016). As a part of ROS/NO antioxidative network, H_2S is involved in plant responses to different abiotic stress factors, although its role in plant response to a pathogen is still largely unknown (Hancock 2018). For example, spermidine-induced H_2O_2 in leaves of white clover could be an upstream signal molecule of NO and H_2S, and derived H_2S might act as the downstream signal of NO. Moreover, accumulation of H_2S can significantly improve antioxidant enzyme (SOD, CAT, GPOX, APX, GR, DAR and MDAR) activities and transcript levels of dehydration-regulated TFs (bZIP37, bZIP107, DREB2, DREB4 and WRKY108715) and genes encoding antioxidant enzymes (Li et al. 2017).

3 Conclusion

The review highlights important roles and tight relationship between NO and H_2O_2 signaling in plants subjected to biotic stress. Stressors (e.g. pathogen attack, wounding) initiate a myriad of reactions resulting in the oxidative burst, i.e. bulk production of reactive species such as NO and H_2O_2. These trigger the defence signaling through different pathways including phytohormones, MAPKs, NR, cGMP, cADPR and Ca^{2+}, and further activate transcriptional factors inducing the expression of genes involved in plant defence (secondary metabolism, SAR, HR, antioxidative system). New studies are awaited to identify further biochemical and functional characteristics of NO and H_2O_2, their interactions and mechanisms which lead to plant defence responses.

References

Agostoni M, Montgomery BL (2014) Survival strategies in the aquatic and terrestrial world: the impact of second messengers on cyanobacterial processes. Life 4:745–769

Alamillo JM, Garcia-Olmedo F (2001) Effects of urate, a natural inhibitor of peroxynitrite-mediated toxicity, in the response of *Arabidopsis thaliana* to the bacterial pathogen *Pseudomonas syringae*. Plant J 125:529–540

An Z, Jing W, Liu Y, Zhang W (2008) Hydrogen peroxide generated by copper amine oxidase is involved in abscisic acid-induced stomatal closure in *Vicia faba*. J Exp Bot 59:815–825

Arasimowicz M, Floryszak-Wieczorek J (2007) Nitric oxide as a bioactive signalling molecule in plant stress responses. Plant Sci 172:876–887

Asai S, Ohta K, Yoshioka H (2008) MAPK signaling regulates nitric oxide and NADPH oxidase-dependent oxidative bursts in *Nicotiana benthamiana*. Plant Cell 20:390–1406

Astier J, Gross I, Durner J (2018a) Nitric oxide production in plants: an update. J Exp Bot 69:3401–3411

Astier J, Jeandroz S, Wendehenne D (2018b) Nitric oxide synthase in plants: the surprise from algae. Plant Sci 268:64–66

Barrington DJ, Ghadouani A (2008) Application of hydrogen peroxide for the removal of toxic cyanobacteria and other phytoplankton from wastewater. Environ Sci Technol 42:8916–8921

Begara-Morales JC, Sánchez-Calvo B, Luque F, Leyva-Pérez MO, Leterrier M, Corpas FJ, Barroso JB (2014) Differential transcriptomic analysis by RNA-Seq of GSNO-responsive genes between *Arabidopsis* roots and leaves. Plant Cell Physiol 55:1080–1095

Begara-Morales JC, Sánchez-Calvo B, Chaki M, Mata-Pérez C, Valderrama RP, Adilla MN, López-Jaramillo J, Luque F, Corpas FJ, Barroso JB (2015) Differential molecular response of monodehydroascorbate reductase and glutathione reductase by nitration and S-nitrosylation. J Exp Bot 66:5983–5996

Beligni MV, Lamattina L (2000) Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. Planta 210:215–221

Bellin D, Asai S, Delledonne M, Yoshioka H (2013) Nitric oxide as a mediator for defense responses. Mol Plant Microb Interact 26:271–277

Benhar M, Forrester MT, Hess DT, Stamler JS (2008) Regulated protein denitrosylation by cytosolic and mitochondrial thioredoxins. Science 320:1050–1054

Bhattacharjee S (2005) Reactive oxygen species and oxidative burst: roles in stress, senescence and signal transduction in plants. Curr Sci 89:1113–1121

Bickerton P, Sello S, Brownlee C, Pittman JK, Wheeler GL (2016) Spatial and temporal specificity of Ca^{2+} signalling in *Chlamydomonas reinhardtii* in response to osmotic stress. New Phytol 212:920–933

Bieker S, Riester L, Stahl M, Franzaring J, Zentgraf U (2012) Senescence-specific alteration of hydrogen peroxide levels in *Arabidopsis thaliana* and oilseed rape spring variety *Brassica napus* L. cv. Mozart. J Integr Plant Biol 54:540–554

Bienert GP, Schjoerring JK, Jahn TP (2006) Membrane transport of hydrogen peroxide. Biochim Biophys Acta 1758:994–1003

Bolwell GP (1999) Role of active oxygen species and NO in plant defence responses. Curr Opin Plant Biol 2:287–294

Boscari A, Del Giudice J, Ferrarini A, Venturini L, Zaffini AL, Delledonne M, Puppo A (2013) Expression dynamics of the *Medicago truncatula* transcriptome during the symbiotic interaction with *Sinorhizobium meliloti*: which role for nitric oxide? Plant Physiol 161:425–439

Box A, Sureda A, Terrados J, Pons A, Deudero S (2008) Antioxidant response and caulerpenyne production of the alien *Caulerpa taxifolia* (Vahl) epiphytized by the invasive algae *Lophocladia lallemandii* (Montagne). J Exp Mar Biol Ecol 364:24–28

Çakır B, Kılıçkaya O (2015) Mitogen-activated protein kinase cascades in *Vitis vinifera*. Front Plant Sci 6:556

Camejo D, Ortiz-Espin A, Lazaro JJ, Romero-Puertas MC, Lazaro-Payo A, Sevilla F, Jiménez A (2015) Functional and structural changes in plant mitochondrial PrxII F caused by NO. J Protom 119:112–125

Cevahir G, Aytamka E, Erol Ç (2007) The role of nitric oxide in plants. Biotechnol Biotechnol Equip 21:13–17

Chardin C, Schenk ST, Hirt H, Colcombet J, Krapp A (2017) Review: Mitogen-activated protein kinases in nutritional signaling in *Arabidopsis*. Plant Sci 260:101–108

Chen T, Fluhr R (2018) Singlet oxygen plays an essential role in the root's response to osmotic stress. Plant Physiol 177:1717–1727

Chen L, Zhang L, Yu D (2010) Wounding-induced WRKY8 is involved in basal defense in *Arabidopsis*. Mol Plant Microb Int 23:558–565

Chung CC, Hwang SPL, Chang J (2008) Nitric oxide as a signaling factor to upregulate the death-specific protein in a marine diatom, *Skeletonema costatum*, during blockage of electron flow in photosynthesis. Appl Environ Microbiol 74:6521–6527

Cona A, Rea G, Botta M, Corelli F, Federico R, Angelini R (2006) Flavin-containing polyamine oxidase is a hydrogen peroxide source in the oxidative response to the protein phosphatase inhibitor cantharidin in *Zea mays* L. J Exp Bot 57:2277–2289

Corpas FJ, Barroso JB (2013) Nitro-oxidative stress vs oxidative or nitrosative stress in higher plants. New Phytol 199:633–635

Corpas FJ, Barroso JB (2014) Peroxisomal plant nitric oxide synthase (NOS) protein is imported by peroxisomal targeting signal type 2 (PTS2) in a process that depends on the cytosolic receptor PEX7 and calmodulin. FEBS Lett 588:2049–2054

Corpas FJ, Barroso JB (2015) Functions of nitric oxide (NO) in roots during development and under adverse stress conditions. Plants 4:240–252

Corpas FJ, Palma JM (2018) Assessing nitric oxide (NO) in higher plants: an outline. Nitrogen 1:12–20

Corpas FJ, Carreras A, Valderrama R, Chaki M, Palma JM, del Río LA, Barroso JB (2007) Reactive nitrogen species and nitrosative stress in plants. Plant Stress 1:37–41

Cuypers A, Hendrix S, Amaral dos Reis R, De Smet S, Deckers J, Gielen H, Jozefczak M, Loix C, Vercampt H, Vangronsveld J, Keunen E (2016) Hydrogen peroxide, signaling in disguise during metal phytotoxicity. Front Plant Sci 7:470

Darehshouri A, Lütz-Meindl U (2010) H_2O_2 localization in the green alga *Micrasterias* after salt and osmotic stress by TEM-coupled electron energy loss spectroscopy. Protoplasma 239:49–56

de Pinto MC, Tomassi F, de Gara L (2002) Changes in the antioxidant systems as a part of the signaling pathway responsible for the programmed cell death activated by nitric oxide and reactive oxygen species in tobacco bright-yellow 2 cells. Plant Physiol 130:689–708

de Pinto MC, Locato V, Sgobba A, Romero-Puertas M d C, 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

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

Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. Nature 394:585–588

Delledonne M, Zeier J, Marocco A, Lamb C (2001) Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci U S A 98:13454–13459

Demidchik V, Maathuis F, Voitsekhovskaja O (2018) Unravelling the plant signalling machinery: An update on the cellular and genetic basis of plant signal transduction. Funct Plant Biol 45:1–8

Desikan R, Reynolds A, Hancock JT, Neill SJ (1998) Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on defence gene expression in *Arabidopsis* suspension cultures. Biochem J 330:115–120

Dobrikova AG (2017) Signaling molecules in plants: exogenous application. Acta Sci Agri 1:38–41

Domingos P, Prado AM, Wong A, Gehring C, Feijo JA (2015) Nitric oxide: A multitasked signaling gas in plants. Mol Plant 8:506–520

Dubovskaya LV, Bakakina YS, Kolesneva EV, Sodel DL, McAinsh MR, Hetherington AM, Volotovski ID (2011) cGMP-dependent ABA-induced stomatal closure in the ABA-insensitive *Arabidopsis* mutant abi1-1. New Phytol 191:57–69

Durner J, Wendehenne D, Klessig DF (1998) Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proc Natl Acad Sci U S A 95:10328–10333

Espunya MC, De MRG-CA, Martinez M (2012) S-nitrosoglutathione is a component of wound- and salicylic acid induced systemic responses in *Arabidopsis thaliana*. J Exp Bot 63:3219–3227

Farnese FS, Menezes-Silva PE, Gusman GS, Oliveira JA (2016) When bad guys become good ones: the key role of reactive oxygen species and nitric oxide in the plant responses to abiotic Stress. Front Plant Sci 7:471

Feechan A, Kwon E, Yun BW, Wang Y, Pallas JA, Loake GJ (2005) A central role for S-nitrosothiols in plant disease resistance. Proc Natl Acad Sci U S A 102:8054–8059

Ferreira LC, Cataneo AC (2010) Nitric oxide in plants: A brief discussion on this multifunctional molecule. Sci Agric (Piracicaba Braz) 67:236–243

Foissner ID, Wendehenne D, Langebartels C, Durner J (2000) In vivo imaging of an elicitor-induced nitric oxide burst in tobacco. Plant J 23:817–824

Foresi N, Correa-Aragunde N, Parisi G, Caló G, Salerno G, Lamattina L (2010) Characterization of a nitric oxide synthase from the plant kingdom: NO generation from the green alga *Ostreococcus tauri* is light irradiance and growth phase dependent. Plant Cell 22:3816–3830

Frohlich A, Durner J (2011) The hunt for plant nitric oxide synthase (NOS): is one really needed? Plant Sci 181:401–404

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

Galatro A, Puntarulo S (2014) An update to the understanding of nitric oxide metabolism in plants. In: Khan MN, Mobin M, Mohammad F, Corpas JF (eds) Nitric oxide in plants: metabolism and role in stress physiology. Springer, Basel, pp 3–16

Ganini D, Hollnagel HC, Colepicolo P, Barros MP (2013) Hydrogen peroxide and nitric oxide trigger redox-related cyst formation in cultures of the dinoflagellate *Lingulodinium polyedrum*. Harmful Algae 27:121–129

García-Mata C, Lamattina L (2001) Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. Plant Physiol 126:1196–1204

Garcia-Olmedo F, Rodrigguez-Palenzulea P, Molina A, Alamillo JM, Lopez-Solanilla E, Berrocal-Lobo M, Poza-Carrion C (2001) Antibiotic activities of peptides, hydrogen peroxide and peroxynitrite in plant defence. FEBS Lett 489:219–222

Gehring C, Turek IS (2017) Cyclic nucleotide monophosphates and their cyclases in plant signaling. Front Plant Sci 8:1704

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

González A, de los Ángeles Cabrera M, Henríquez MJ, Contreras RA, Morales B, Moenne A (2012) Cross talk among calcium, hydrogen peroxide, and nitric oxide and activation of gene expression involving calmodulins and calcium-dependent protein kinases in *Ulva compressa* exposed to copper excess. Plant Physiol 158:1451–1462

Górka B, Wieczorek PP (2017) Simultaneous determination of nine phytohormones in seaweed and algae extracts by HPLC-PDA. J Chromatogr B Analyt Technol Biomed Life Sci 1057:32–39

Gray SB, Brady SM (2016) Plant developmental responses to climate change. Dev Biol 419:64–77

Greenberg JT, Yao N (2004) The role and regulation of programmed cell death in plant-pathogen interactions. Cell Microbiol 6:201–211

Gupta KJ, Fernie AR, Kaiser WM, van Dongen JT (2011a) On the origins of nitric oxide. Trend Plant Sci 16:160–168

Gupta KJ, Igamberdiev AU, Manjunatha G, Segu S, Moran JF, Neelawarne B, Bauwe H, Kaiser WM (2011b) The emerging roles of nitric oxide (NO) in plant mitochondria. Plant Sci 181:520–526

Habibi G (2014) Hydrogen peroxide (H_2O_2) generation, scavenging and signaling in plants. In: Ahmad P (ed) Oxidative damage to plants: antioxidant networks and signaling. Academic, San Diego, pp 557–584

Hancock JT (2018) Hydrogen sulfide and environmental stresses. Environ Exp Bot. https://doi.org/10.1016/j.envexpbot.2018.08.034

Hao X, Yu K, Ma Q, Song X, Li H, Wang M (2011) Histochemical studies on the accumulation of H_2O_2 and hypersensitive cell death in the nonhost resistance of pepper against *Blumeria graminis* f. sp. *tritici*. Physiol Mol Plant Pathol 76:104–111

Hettenhausen C, Schuman MC, Wu J (2015) MAPK signalling: a key element in plant defense response to insects. Insect Sci 22:157–164

Holzmeister C, Gaupels F, Geerlof A, Sarioglu H, Sattler M, Durner J, Lindermayr C (2015) Differential inhibition of *Arabidopsis* superoxide dismutases by peroxynitrite-mediated tyrosine nitration. J Exp Bot 66:989–999

Hong JK, Kang SR, Kim YH, Yoon DJ, Kim DH, Kim HJ, Sung CH, Kang HS, Choi CW, Kim DH, Kim YS (2013) Hydrogen peroxide- and nitric oxide-mediated disease control of bacterial wilt in tomato plants. Plant Pathol J 29:386–396

Hooijmaijers C, Rhee JY, Kwak KJ, Chung GC, Horie T, Katsuhara M, Kang H (2012) Hydrogen peroxide permeability of plasma membrane aquaporins of *Arabidopsis thaliana*. J Plant Res 125:147–153

Hu X, Bidney DL, Yalpani N, Duvick JP, Crasta O, Folkerts O, Lu G (2003) Overexpression of a gene encoding hydrogen peroxide-generating oxalate oxidase evokes defense responses in sunflower. Plant Physiol 133:170–181

Hu X, Neill SJ, Tang Z, Cai W (2005) Nitric oxide mediates gravitropic bending in soybean roots. Plant Physiol 137:663–670

Hu X, Yang J, Li C (2015) Transcriptomic response to nitric oxide treatment in *Larix olgensis* Henry. Int J Mol Sci 16:28582–28597

Ichimura K, Shinozaki K, Tena G, Sheen J, Henry Y, Champion A, Kreis M, Zhang S, Hirt H, Wilson C, Heberle-Bors E, Ellis BE, Morris PC, Innes RW, Ecker JR, Scheel D, Klessig DF, Machida Y, Mundy J, Ohashi Y, Walker JC (2002) Mitogen-activated protein kinase cascades in plants: a new nomenclature. Trend Plant Sci 27:301–308

Imran QM, Hussain A, Lee SU, Mun BG, Falak N, Loake GJ, Yun BW (2018) Transcriptome profile of NO-induced *Arabidopsis* transcription factor genes suggests their putative regulatory role in multiple biological processes. Sci Rep 8:771

Ismail SZ, Khandaker MM, Mat N, Boyce AN (2015) Effects of hydrogen peroxide on growth, development and quality of fruits: a review. J Agron 14:331–336

Jacquard C, Mazeyrat-Gourbeyre F, Devaux P, Boutilier K, Baillieul F, Clément C (2009) Microspore embryogenesis in barley: anther pre-treatment stimulates plant defence gene expression. Planta 229:393–402

Jeandroz S, Wipf D, Stuehr DJ, Lamattina L, Melkonian M, Tian Z, Zhu Y, Carpenter EJ, Wong GKS, Wendehenne D (2016) Occurrence, structure, and evolution of nitric oxide synthase–like proteins in the plant kingdom. Sci Signal 9:re2

Kaurilind E, Xu E, Brosché M (2015) A genetic framework for H_2O_2 induced cell death in *Arabidopsis thaliana*. BMC Genom 16:837

Keshavarz-Tohid V, Taheri P, Taghavi SM, Tarighi S (2016) The role of nitric oxide in basal and induced resistance in relation with hydrogen peroxide and antioxidant enzymes. J Plant Physiol 199:29–38

Kneeshaw S, Gelineau S, Tada Y, Loake GJ, Spoel SH (2014) Selective protein denitrosylation activity of thioredoxin-h5 modulates plant immunity. Mol Cell 56:153–162

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

Krasylenko YA, Yemets AI, Blume YB (2017) Cell mechanisms of nitric oxide signaling in plants under abiotic stress conditions. In: Pandey G (ed) Mechanism of plant hormone signaling under stress. Wiley, Hoboken, NJ

Kumar A, Castellano I, Patti FP, Palumbo A, Buia MC (2015) Nitric oxide in marine photosynthetic organisms. Nitric Oxide 47:34–39

Kuźniak E, Urbanek H (2000) The involvement of hydrogen peroxide in plant responses to stresses. Acta Physiol Plant 22:195–203

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Ann Rev Plant Physiol Plant Mol Biol 48:251–275

Lebel E, Heifetz P, Thorne L, Uknes S, Ryals J, Ward E (1998) Functional analysis of regulatory sequences controlling PR-1 gene expression in *Arabidopsis*. Plant J 16:223–233

Leitner M, Vandelle E, Gaupels F, Bellin D, Delledonne M (2009) NO signals in the haze: nitric oxide signalling in plant defence. Curr Opin Plant Biol 12:451–458

Li SW, Xue L (2010) The interaction between H_2O_2 and NO, Ca^{2+}, cGMP, and MAPKs during adventitious rooting in mung bean seedlings. In Vitro Cell Dev Biol Plant 46:142–148

Li SW, Xue L, Xu S, Feng H, An L (2007) Hydrogen peroxide involvement in formation and development of adventitious roots in cucumber. Plant Growth Regul 52:173–180

Li Q, Wang YJ, Liu CK, Pei ZM, Shi WL (2017) The crosstalk between ABA, nitric oxide, hydrogen peroxide, and calcium in stomatal closing of *Arabidopsis thaliana*. Biologia 72:1140–1146

Liao YWK, Sun ZH, Zhou YH, Shi K, Li X, Zhang GQ, Xia XJ, Chen ZX, Yu JQ (2013) The role of hydrogen peroxide and nitric oxide in the induction of plant-encoded RNA-dependent RNA polymerase 1 in the basal defense against *Tobacco Mosaic Virus*. PLoS One 8:e76090

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

Liu L (2001) A metabolic enzyme for S-nitrosothiol conserved from bacteria to humans. Nature 410:490–494

Liu B, Wang H, Ma Z, Gai X, Sun Y, He S, Liu X, Wang Y, Xuan Y, Gao Z (2018) Transcriptomic evidence for involvement of reactive oxygen species in *Rhizoctonia solani* AG1 IA sclerotia maturation. Peer J 6:e5103

Lozano-Juste J, León J (2011) Nitric oxide regulates DELLA content and PIF expression to promote photomorphogenesis in *Arabidopsis*. Plant Physiol 156:1410–1423
Maffei ME, Mithöfer A, Arimura G, Uchtenhagen H, Bossi S, Bertea CM, Starvaggi Cucuzza L, Novero M, Volpe V, Quadro S, Boland W (2006) Effects of feeding *Spodoptera littoralis* on lima bean leaves. III. Membrane depolarization and involvement of hydrogen peroxide. Plant Physiol 140:1022–1035
Malik SI, Hussain A, Yun BW, Spoel SH, Loake GJ (2011) GSNOR-mediated de-nitrosylation in the plant defence response. Plant Sci 181:540–544
Mallick N, Mohn FH (2000) Reactive oxygen species: response of algal cells. J Plant Physiol 157:183–193
Mallick N, Mohn FH, Soeder CJ, Grobbelaar JU (2002) Ameliorative role of nitric oxide on H_2O_2 toxicity to a chlorophycean alga *Scenedesmus obliquus*. J Gen Appl Microbiol 48:1–7
Matilla-Vázquez MA, Matilla AJ (2014) Role of H_2O_2 as signaling molecule in plants. In: Ahmad P, Prasad MNV (eds) Environmental adaptations and stress tolerance of plants in the era of climate change. Springer, Heidelberg, pp 361–380
McDowell RE, Amsler CD, Dickinson DA, McClintock JB, Baker BJ (2014) Reactive oxygen species and the Antarctic macroalgal wound response. J Phycol 50:71–80
Melo NKG, Bianchetti RE, Lira BS, Oliveir PMR, Zuccarelli R, Dias DLO, Demarco D, Peres LEP, Rossi M, Freschi L (2016) Nitric oxide, ethylene, and auxin cross talk mediates greening and plastid development in deetiolating tomato seedlings. Plant Physiol 170:2278–2294
Misra AN, Misra M, Singh R (2011) Nitric oxide ameliorates stress responses in plants. Plant Soil Environ 57:95–100
Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trend Plant Sci 7:405–410
Mittler R (2017) ROS are good. Trend Plant Sci 22:11–19
Mittler R, Berkowitz G (2001) Hydrogen peroxide, a messenger with too many roles? Redox Rep 6:69–72
Mohanta TK, Arora PK, Mohanta N, Parida P, Bae H (2015) Identification of new members of the MAPK gene family in plants shows diverse conserved domains and novel activation loop variants. BMC Genom 16:58
Molassiotis A, Fotopoulos V (2011) Oxidative and nitrosative signaling in plants two branches in the same tree? Plant Signal Behav 6:210–214
Møller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol 52:561–591
Moni A, Islam MN, Uddin MJ (2018) Role of auxin and nitric oxide on growth and development of lateral root of plants: possible involvement of exogenously induced Phot1. J Adv Biotechnol Exp Ther 1:61–64
Mor A, Koh E, Weiner L, Rosenwasser S, Sibony-Benyamini H, Fluhr R (2014) Singlet oxygen signatures are detected independent of light or chloroplasts in response to multiple stresses. Plant Physiol 165:249–261
Mostofa MG, Fujita M, Tran LSP (2015) Nitric oxide mediates hydrogen peroxide- and salicylic acid induced salt tolerance in rice (*Oryza sativa* L.) seedlings. Plant Growth Regul 77:265–277
Mou Z, Fan W, Dong X (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell 113:935–944
Mulaudzi N, Ludidi O, Ruzvidzo M, Morse N, Hendricks E, Iwuoha C, Gehring C (2011) Identification of a novel *Arabidopsis thaliana* nitric oxide-binding molecule with guanylate cyclase activity in vitro. FEBS Lett 585:2693–2697
Mullineaux PM, Exposito-Rodriguez M, Laissue PP, Smirnoff N (2018) ROS-dependent signalling pathways in plants and algae exposed to high light: comparisons with other eukaryotes. Free Radic Biol Med 122:52–64
Mur LAJ, Mandon J, Persijn S, Cristescu SM, Moshkov IE, Novikova GV, Hall MA, Harren FJM, Hebelstrup KH, Gupta KJ (2013) Nitric oxide in plants: an assessment of the current state of knowledge. AoB Plant 5:pls052

Mydlarz LD, Jacobs RS (2004) Comparison of an inducible oxidative burst in freeliving and symbiotic dinoflagellates reveals properties of the pseudopterosins. Phytochemistry 65:3231–3241

Nadarajah K, Sidek H (2010) The green MAPKS. Asian J Plant Sci 9:1–10

Nan W, Wang X, Yang L, Hu Y, Wei Y, Liang X, Mao L, Bi Y (2014) Cyclic GMP is involved in auxin signalling during *Arabidopsis* root growth and development. J Exp Bot 65:1571–1583

Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT (2002) Hydrogen peroxide and nitric oxide as signalling molecules in plants. J Exp Bot 53:1237–1247

Niu L, Liao W (2016) Hydrogen peroxide signaling in plant development and abiotic responses: crosstalk with nitric oxide and calcium. Front Plant Sci 7:230

Noronha-Dutra AA, Epperlein MM, Woolf N (1993) Reaction of nitric oxide with hydrogen peroxide to produce potentially cytotoxic singlet oxygen as a model for nitric oxide-mediated killing. FEBS Lett 321:59–62

Oliveira JTA, Barreto ALH, Vasconcelos IM, Eloy YRG, Gondim DMF, Fernandes CF, Freire-Filho FR (2014) Role of antioxidant enzymes, hydrogen peroxide and PR-proteins in the compatible and incompatible interactions of cowpea (*Vigna unguiculata*) genotypes with the fungus *Colletotrichum gloeosporioides*. J Plant Physiol Pathol 2:3

Opdenakker K, Remans T, Vangronsveld J, Cuypers A (2012) Mitogenactivated protein (map) kinases in plant metal stress: regulation and responses in comparison to other biotic and abiotic stresses. Int J Mol Sci 13:7828–7853

Orozco-Cárdenas ML, Ryan CA (2002) Nitric oxide negatively modulates wound signaling in tomato plants. Plant Physiol 130:487–493

Parani M, Rudrabhatla S, Myers R, Weirich H, Smith B, Leaman DW, Goldman SL (2004) Microarray analysis of nitric oxide responsive transcripts in *Arabidopsis*. Plant Biotechnol J 2:359–366

París R, Vazquez MM, Graziano M, Terrile MC, Miller ND, Spalding EP, Otegui MS, Casalongué CA (2018) Distribution of endogenous NO regulates early gravitropic response and PIN2 localization in *Arabidopsis* roots. Front Plant Sci 9:495

Pasqualini S, Meier S, Gehring C, Madeo L, Fornaciari M, Romano B, Ederli L (2009) Ozone and nitric oxide induce cGMP-dependent and independent transcription of defence genes in tobacco. New Phytol 181:860–870

Pasqualini S, Cresti M, Del Casino C, Faleri C, Frenguelli G, Tedeschini E, Ederli L (2015) Roles for NO and ROS signalling in pollen germination and pollen-tube elongation in *Cupressus arizonica*. Biol Plant 59:735–744

Peleg-Grossman S, Melamed-Book N, Cohen G, Levine A (2010) Cytoplasmic H_2O_2 prevents translocation of NPR1 to the nucleus and inhibits the induction of PR genes in *Arabidopsis*. Plant Signal Behav 5:1401–1406

Perl-Treves R, Perl A (2002) Oxidative stress: an introduction. In: Inze D, Montago M (eds) Oxidative stress in plants. Taylor and Francis, New York, NY, pp 1–32

Pokora W, Aksmann A, Baścik-Remisiewicz A, Dettlaff-Pokora A, Rykaczewski M, Gappa M, Tukaj Z (2017) Changes in nitric oxide/hydrogen peroxide content and cell cycle progression: study with synchronized cultures of green alga *Chlamydomonas reinhardtii*. J Plant Physiol 208:84–93

Polverari A, Molesini B, Pezzotti M, Buonaurio R, Marte M, Delledonne M (2003) Nitric oxide-mediated transcriptional changes in *Arabidopsis thaliana*. Mol Plant Mic Int 16:1094–1105

Potin P (2008) Oxidative burst and related responses in biotic interactions of algae. In: Amsler CD (ed) Algal chemical ecology. Springer, Heidelberg

Prado AM, Marshall Porterfield D, Feijó JA (2004) Nitric oxide is involved in growth regulation and re-orientation of pollen tubes. Development 131:2707–2714

Prasad A, Kumar A, Matsuoka R, Takahashi A, Fujii R, Sugiura Y, Kikuchi H, Aoyagi S, Aikawa T, Kondo T, Yuasa M, Pospíšil P, Kasai S (2017) Real-time monitoring of superoxide anion radical generation in response to wounding: electrochemical study. Peer J 5:e3050

Prasad A, Sedlářová M, Pospíšil P (2018) Singlet oxygen imaging using fuorescent probe singlet oxygen sensor green in photosynthetic organisms. Sci Rep 8:13685

Prats E, Mur LAJ, Sanderson R, Carver TLW (2005) Nitric oxide contributes both to papilla-based resistance and the hypersensitive response in barley attacked by *Blumeria graminis* f. sp. *hordei*. Mol Plant Pathol 6:65–78

Procházková D, Haisel D, Pavlíková D (2014) Nitric oxide biosynthesis in plants – the short overview. Plant Soil Environ 60:129–134

Procházková D, Wilhelmová N, Pavlík M (2015) Reactive nitrogen species and nitric oxide. In: Khan MN, Mobin M, Mohammad F, Corpas FJ (eds) Nitric oxide action in abiotic stress responses in plants. Springer, Heidelberg, pp 3–19

Qiao M, Sun J, Liu N, Sun T, Liu G, Han S, Hou C, Wang D (2015) Changes of nitric oxide and its relationship with H_2O_2 and Ca^{2+} in defense interactions between wheat and *Puccinia triticina*. PLoS One 10:e0132265

Quan LJ, Zhang B, Shi WW, Li HY (2008) Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. J Integr Plant Biol 50:2–18

Radi R (2013) Peroxynitrite, a stealthy biological oxidant. J Biol Chem 288:26464–26472

Rodrigues O, Reshetnyak G, Grondin A, SaijoY LN, Maurel C, Verdoucq L (2017) Aquaporins facilitate hydrogen peroxide entry into guard cells to mediate ABA- and pathogen-triggered stomatal closure. Proc Natl Acad Sci U S A 114:9200–9205

Rodriguez MCS, Petersen M, Mundy J (2010) Mitogen-activated protein kinase signaling in plants. Annu Rev Plant Biol 61:621–649

Romero-Puertas MC, Laxa M, Matte A, Zaninotto F, Finkemeier I, Jones AM, Perazzolli M, Vandelle E, Dietz KJ, Delledonne M (2007) S-nitrosylation of peroxiredoxin II E promotes peroxynitrite mediated tyrosine nitration. Plant Cell 19:4120–4130

Rőszer T (2014) Biosynthesis of nitric oxide in plants. In: Khan MN, Mobin M, Mohammad F, Corpas JF (eds) Nitric oxide in plants: metabolism and role in stress physiology. Springer, Heidelberg, pp 17–33

Rustérucci C, Espunya MC, Díaz M, Chabannes M, Martínez MC (2007) S-nitrosoglutathione reductase affords protection against pathogens in *Arabidopsis*, both locally and systemically. Plant Physiol 143:1282–1292

Sahay S, Gupta M (2017) An update on nitric oxide and its benign role in plant responses under metal stress. Nitric Oxide 67:39–52

Sakamoto M, Munemura I, Tomita R, Kobayashi K (2008) Involvement of hydrogen peroxide in leaf abscission signaling, revealed by analysis with an in vitro abscission system in Capsicum plants. Plant J 56:13–27

Salachna P, Zawadzińska A (2018) Effect of nitric oxide on growth, flowering and bulb yield of *Eucomis autumnalis*. Acta Hortic 1201:635–640

Santolini J, André F, Jeandroz S, Wendehenne D (2017) Nitric oxide synthase in plants: where do we stand? Nitric Oxide 63:30–38

Saxena I, Srikanth S, Chen Z (2016) Cross talk between H_2O_2 and interacting signal molecules under plant stress response. Front Plant Sci 7:570

Senthil Kumar R, Shen CH, Wu PY, Suresh Kumar S, Sang Hua M, Yeh KW (2016) Nitric oxide participates in plant flowering repression by ascorbate. Sci Rep 6:35246

Sheokand S, Kumari A (2015) Nitric oxide and abiotic stress-induced oxidative stress. In: Khan M, Mobin M, Mohammad F, Corpas F (eds) Nitric oxide action in abiotic stress responses in plants. Springer, Heidelberg

Shetty NP, Jørgensen HJL, Jensen JD, Collinge DB, Shetty HS (2008) Roles of reactive oxygen species in interactions between plants and pathogens. Eur J Plant Pathol 121:267–280

Singh N, Bhatla SC (2018) Nitric oxide regulates lateral root formation through modulation of ACC oxidase activity in sunflower seedlings under salt stress. Plant Signal Behav 25:1–7

Sinha AK, Jagg M, Raghuram B, Tuteja N (2011) Mitogen-activated protein kinase signalling in plants under abiotic stress. Plant Signal Behav 6:196–203

Ślesak I, Libik M, Karpinska B, Karpinski S, Miszalski Z (2007) The role of hydrogen peroxide in regulation of plant metabolism and cellular signaling in response to environmental stresses. Acta Biochim Pol 54:39–50

Srivastava AK, Penna S, Van Nguyen D, Tran LSP (2014) Multifaceted roles of aquaporins as molecular conduits in plant responses to abiotic stresses. Crit Rev Biotechnol 28:1–10

Stamler JS, Lamas S, Fang FC (2001) Nitrosylation: the prototypic redox-based signaling mechanism. Cell 106:675–683

Štolfa CI, Špoljarić MD, Žuna PT, Lončarić Z (2016) Glutathione and related enzymes in response to abiotic stress. In: Gupta DK, Palma JM, Corpas FJ (eds) Redox state as a central regulator of plant cell stress responses. Springer, Heidelberg, pp 183–211

Štolfa ČI, Žuna PT, Špoljarić MD (2018) Abiotic stress response in plants: the relevance of tocopherols. In: Gupta DK, Palma JM, Corpas FJ (eds) Antioxidants and antioxidant enzymes in higher plants. Springer, Cham, pp 233–251

Sun H, Feng F, Liu J, Zhao Q (2018) Nitric oxide affects rice root growth by regulating auxin transport under nitrate supply. Front Plant Sci 9:659

Szopińska D (2014) Effects of hydrogen peroxide treatment on the germination, vigour and health of *Zinnia elegans* seeds. Folia Hort 26:19–29

Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X (2008) Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. Science 321:952–956

Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Molassiotis A, Job D (2009) Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. Plant J 60:795–804

Tewari RK, Prommer J, Watanabe M (2013) Endogenous nitric oxide generation in protoplast chloroplasts. Plant Cell Rep 32:31–44

Thomas F, Cosse A, Goulitquer S, Raimund S, Morin P, Valero M, Leblanc C, Potin P (2011) Waterborne signaling primes the expression of elicitor-induced genes and buffers the oxidative responses in the brown alga *Laminaria digitata*. PLoS One 6:e21475

Torres MA, Jones JD, Dangl JL (2005) Pathogen-induced, NADPH oxidase-derived reactive oxygen intermediates suppress spread of cell death in *Arabidopsis thaliana*. Nat Genet 37:1130–1134

Vargas-Hernández M, Torres-Pacheco I, Gautier F, Álvarez-Mayorga B, Cruz-Hernández A, García-Mier L, Jiménez-García SN, Ocampo-Velázquez RV, Feregrino-Perez AA, Guevara-Gonzalez RG (2017) Influence of hydrogen peroxide foliar applications on in vitro antimicrobial activity in *Capsicum chinense* Jacq. Plant Biosys 151:269–275

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

Vianello A, Macri FJ (1991) Generation of superoxide anion and hydrogen peroxide at the surface of plant cells. Bioenerg Biomemb 23:409–423

Voss I, Sunil B, Scheibe R, Raghavendra AS (2013) Emerging concept for the role of photorespiration as an important part of abiotic stress response. Plant Biol 5:713–722

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

Wang YQ, Feechan A, Yun BW, Shafiei R, Hofmann A, Taylor P, Xue P, Yang FQ, Xie ZS, Pallas JA, Chu CC, Loake GJ (2009) *S*-nitrosylation of AtSABP3 antagonizes the expression of plant immunity. J Biol Chem 284:2131–2137

Wang Y, Hao X, Lu Q, Wang L, Qian W, Li N, Ding C, Wang X, Yang Y (2018) Transcriptional analysis and histochemistry reveal that hypersensitive cell death and H_2O_2 have crucial roles in the resistance of tea plant (*Camellia sinensis* (L.) O. Kuntze) to anthracnose. Hortic Res 5:18

Weisslocker-Schaetzel M, André F, Touazi N, Foresi N, Lembrouk M, Dorlet P, Frelet-Barrand A, Lamattina L, Santolini J (2017) The NOS-like protein from the microalgae *Ostreococcus tauri* is a genuine and ultrafast NO-producing enzyme. Plant Sci 265:100–111

Wink DA, Mitchell JB (1998) Chemical biology of nitric oxide: Insights into regulatory, cytotoxic, and cytoprotective mechanisms of nitric oxide. Free Radic Biol Med 25:434–456

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

Wojtyla L, Lechowska K, Kubala S, Garnczarsk M (2016) Different modes of hydrogen peroxide action during seed germination. Front Plant Sci 7:66

Wu Y, Zhang D, Chu JY, Boyle P, Wang Y, Brindle ID, De Luca V, Després C (2012) The Arabidopsis NPR1 protein is a receptor for the plant defense hormone salicylic acid. Cell Rep 8:639–647

Wünsche H, Baldwin IT, Wu J (2011) S-Nitrosoglutathione reductase (GSNOR) mediates the biosynthesis of jasmonic acid and ethylene induced by feeding of the insect herbivore *Manduca sexta* and is important for jasmonate-elicited responses in *Nicotiana attenuata*. J Exp Bot 62:4605–4616

Yang H, Kim HJ, Chen H, Lu Y, Lu X, Wang C, Zhou B (2018) Reactive oxygen species and nitric oxide induce senescence of rudimentary leaves and the expression profiles of the related genes in *Litchi chinensis*. Hort Res 5:23

Yu M, Lamattina L, Spoe SH, Loake GJ (2014) Nitric oxide function in plant biology: a redox cue in deconvolution. New Phytol 202:1142–1156

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

Zhang H (2016) Hydrogen sulfide in plant biology. In: Lamattina L, García-Mata C (eds) Gasotransmitters in plants. Signaling and communication in plants. Springer, Cham

Zhang X, Zhang L, Dong F, Gao J, Galbraith DW, Song CP (2001) Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba*. Plant Physiol 126:1438–1448

Zhao FY, Hu F, Zhang SY, Wang K, Zhang CR, Liu T (2013) MAPKs regulate root growth by influencing auxin signaling and cell cycle-related gene expression in cadmium-stressed rice. Environ Sci Pollut Res 28:5449–5460

Zhou JM, Trifa Y, Silva H, Pontier D, Lam E, Shah J, Klessig DF (2000) NPR1 differentially interacts with members of the TGA/OBF family of transcription factors that bind an element of the *PR-1* gene required for induction by salicylic acid. Mol Plant Microbe 13:191–202

Zurbriggen MD, Carrillo N, Hajirezaei MR (2010) ROS signaling in the hypersensitive response: when, where and what for? Plant Signal Behav 5:393–396

Biotechnological Application of Nitric Oxide and Hydrogen Peroxide in Plants

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Contents

Abstract Over the last decades, hydrogen peroxide (H_2O_2) and nitric oxide (NO) have emerged as critical signaling molecules in plants, controlling numerous developmental and stress-related responses. In this chapter, we discuss the current and potential biotechnological uses of these reactive molecules in agriculture, with particular emphasis on their involvement in regulating the postharvest shelf life and quality of the horticultural products as well as plant development and adaptation to environmental stresses. The multiple methods used to deliver H_2O_2 and NO to plants were compared in light of their effectiveness in controlling plant responses associated with desirable crop traits. As both H_2O_2 and NO levels in plant cells are regulated by multiple and overlapping routes of production, scavenging, and removal, numerous candidate genes have been selected for genetic manipulation in both model and crop species. The encouraging results obtained in most of these

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attempts to manipulate the endogenous levels of these reactive molecules in transgenic plants are presented and discussed, highlighting the enormous potential that adjusting H_2O_2/NO metabolism may have in further improving crop performance under both optimal and unfavorable environmental conditions.

Keywords Hydrogen peroxide · Nitric oxide · Postharvest · Priming · Transgenics · Stress resistance

1 Initial Considerations

Reactive oxygen and nitrogen species (ROS and RNS, respectively) are by-products of diverse biochemical processes, and under severe stressful conditions, imbalances in their production and removal rates may trigger nitro-oxidative stress, consequently leading to cellular damage (Gill and Tuteja 2010; Asgher et al. 2017). However, over the course of evolution, ROS/RNS have acquired novel signaling functions as plants become capable of deliberately modulating the level of these substances to regulate a range of biological processes such as development, growth, and environmental adaptation (Mittler et al. 2011; Schieber and Chandel 2014; Domingos et al. 2015). Accumulating evidence indicates that hydrogen peroxide (H_2O_2) and nitric oxide (NO) are the most probable candidates as second messengers during responses mediated by ROS/RNS due to their relatively long life, lower damage capacity, and higher permeability across biological membranes when compared with other reactive species (Mittler 2017). Plant physiological events as diverse as seed germination, root growth, cell wall modification, xylem differentiation, stomatal closure, fruit ripening, and plant responses to abiotic and biotic stresses are regulated by both H_2O_2 and NO signaling (Asgher et al. 2017; Gong et al. 2018).

Both H_2O_2 and NO display complex networks of biosynthesis and scavenging, which involve several cell compartments and both enzymatic and nonenzymatic pathways. H_2O_2 can be generated as a by-product of several essential plant cell reactions such as electron transport chains, photorespiration, and many other enzymatic reactions (e.g., wall peroxidases, flavin-containing oxidases, and superoxide dismutases, among others) (Palma et al. 2013; Niu and Liao 2016). To maintain H_2O_2 levels under control, plant cells employ a complex array of antioxidant enzymes, mainly including superoxide dismutase (SOD) (del Río et al. 2018), catalase (CAT) (del Río 2015; Sofo et al. 2015), ascorbate peroxidase (APX) (Sofo et al. 2015), glutathione peroxidase (GP) (Noctor et al. 2018), and peroxidases (POX) (Saraiva et al. 2010). These enzymes act together by removing the excess of H_2O_2 and preventing the formation of hydroxyl radical ($^{\bullet}OH$), which is highly toxic to plant cells. In nonenzymatic pathways, ascorbate (AsA) and glutathione (GSH) are responsible for directly reducing H_2O_2 cellular content (Niu and Liao 2016). The NO production and scavenging pathways in plant cells are equally diverse. Besides the nonenzymatic NO production in the apoplast (Bethke et al. 2004), at least seven potential biosynthetic sources of NO have already been described in plants (Corpas

and Barroso 2017). Among them, nitrate reductase (NR)- and nitric oxide synthase-like (NOS-like)-dependent reactions apparently are the most likely candidates for the production of NO under physiologically relevant conditions (Neill et al. 2008; Mur et al. 2013; Corpas and Barroso 2017). After being generated, NO can bind to the thiol group of proteins and peptides leading to the formation of S-nitrosylated species, which are considered a central mechanism for NO control and action in biological systems (Corpas et al. 2015). Of particular relevance is the reversible reaction between NO and GSH forming *S*-nitrosoglutathione (GSNO) as this S-nitrosylated species can either serve as a cellular reservoir of NO or an intermediate for NO removal via GSNO reductase (GSNOR) activity (Corpas et al. 2015).

In this chapter, we will focus on the current and potential biotechnological uses of H_2O and NO manipulation as a means to adjust postharvest shelf life of fruits and vegetables as well as plant development and adaptation to environmental stresses. The challenges and effectiveness of pharmacological approaches (e.g., chemical donors, nanoparticles) to regulate the levels of these reactive substances will be compared with the outcomes of recent attempts to genetically engineer H_2O_2 and NO production and scavenging in plant systems. Finally, some future perspectives toward a more judicious manipulation of H_2O_2 and NO levels in plant tissues will also be presented and discussed.

2 Pharmacological Manipulation of H_2O_2 and NO Levels in Plant Tissues

Compared to the widespread use of plant growth regulators (PGRs) in contemporary agriculture, the biotechnological use of exogenous treatments with H_2O_2 and NO in plant production and postharvest conservation of fruits and vegetables is still in its infancy. Nevertheless, due to the ubiquitous action of these molecules on living organisms, it is not surprising that increasing attention has been devoted to employing H_2O_2 and NO to control different aspects of plant biology. As for other PGRs, low-to-medium H_2O_2 and NO doses usually are sufficient to trigger responses associated with desirable agricultural traits; however, the large-scale application of H_2O_2 and NO under field conditions requires additional safety concerns due to the reactive nature of these molecules as well as possible limitations in cost-effectiveness.

2.1 Methods for the Delivery of H_2O_2 and NO to Plant Tissues

Whereas H_2O_2 can be delivered to plant tissues essentially via immersion/irrigation or through the exposure to hydrogen peroxide vapor (HPV), considerably more

diversified methods have been employed so far to deliver NO to plants (Marvasi 2017).

Under laboratory conditions, fumigation with gaseous NO has long been demonstrated to be effective in triggering plant responses (Wills et al. 2000; Soegiarto and Wills 2004; Singh et al. 2009; Zhu et al. 2009). However, many aspects can interfere with the effectiveness of the delivery of NO via fumigation. In the presence of oxygen, NO can lead to the formation of nitrogen dioxide (NO_2); therefore, some authors argue that NO delivery to plant tissues must occur under anoxic conditions or ultralow oxygen (ULO) atmospheres to prevent the formation of NO_2, which is toxic to plant tissues at high concentrations (Saltveit 2003; Liu et al. 2016; Liu and Yang 2016). However, besides adding further complexity to the treatment methods, the delivery of NO under ULO atmosphere entails exposing the plant cells to transitory hypoxic or anoxic conditions, which may trigger additional physiological responses not directly related with the presence of NO itself. In addition to that, meticulous control of NO dosages during delivery and monitoring of by-products formation must be considered to ensure the induction of the desired response without causing unwanted deleterious effects (Anderson and Mansfield 1979).

Therefore, due to economic and safety reasons, it is currently accepted that the large-scale biotechnological applications of NO in agriculture rely on the use of reactive precursors, the so-called NO donors (Marvasi 2017). The kinetics and intensity of the decomposition of each NO donor into NO and by-products dramatically vary depending on its chemical structure and concentration in the medium as well as environmental factors such as light intensity and quality, pH, temperature, and the presence of metals, plant metabolites, reducing agents, and other chemicals. Overall, the decomposition products of NO donors (i.e., by-products) greatly vary in their toxicity to plant tissues and the environment.

NO donors are generally unstable, and their decomposition rate is usually increased by high temperatures and light exposition, leading to rapid NO release, which may result in toxic effect (Oliveira et al. 2016). In this context, a new alternative for the large-scale application of NO in agriculture has recently emerged, which is based on encapsulating NO donors into nanoparticles (NPs) (Seabra et al. 2014; Oliveira et al. 2016). This technology brings the promise of offering an efficient and safe way of delivering NO to the plant tissues under field conditions because NO-releasing NPs are usually more stable than NO donors, thereby providing more controlled NO release rates, which are less influenced by the surrounding environmental conditions. This may reduce the doses required to trigger physiological responses, consequently decreasing cost and minimizing environment contamination (Seabra et al. 2014; Oliveira et al. 2016). Among the several nanoparticles currently available (e.g., carbon nanotubes, liposomes, dendrimers, as well as silica, polymeric, chitosan, iron, silver, and gold nanoparticles), only a few have already been tested in plants. In one of the few studies in this research area, Oliveira et al. (2016) synthesized and characterized chitosan nanoparticles (CS NPs) containing the NO donor *S*-nitroso-mercaptosuccinic acid (*S*-nitroso-MSA).

Data showed that these NO-releasing NPs (i.e., *S*-nitroso-MSA-CS NPs) were significantly more efficient than was the non-encapsulated NO donor in mitigating

the effects of salt stress in maize plants. Interestingly, the MSA-CS NPs were synthesized through ionotropic gelation, which is a simple, efficient, and cost-effective process to obtain CS NPs for a wide range of applications (Oliveira et al. 2016). Attempts to encapsulate GSNO in polymeric NPs (Wu et al. 2015) and alginate/chitosan NPs (Pereira et al. 2015) have also been developed aiming at providing controlled NO release to plant tissues.

2.2 *Impacts of Exogenous H_2O_2 and NO on the Shelf Life of Fruit and Vegetables*

The development of new techniques to improve the quality, nutritional value, and shelf life of the horticultural products has been steadily increasing over the years motivated by the consumer demands (Gong et al. 2018). Among these technologies, treatments with exogenous H_2O_2 or NO have emerged as promising alternatives to extend the postharvest shelf life and quality of fruits and vegetables (Manjunatha et al. 2010; Freschi 2013; Ali et al. 2017; Corpas and Palma 2018).

In line with its reactive nature, H_2O_2 is currently considered an effective antimicrobial agent in postharvest and food conservation. In fact, H_2O_2 has been recognized by the USA as a GRAS (generally regarded as safe) substance for use in various food products (e.g., milk, dried egg, wine, starch, and instant tea) as a bleaching, oxidizing, and antimicrobial agent in a concentration range of 0.04–1.25% (Ali et al. 2017). Therefore, H_2O_2 may replace other chemicals, such as chlorine, in treatments aiming at extending the storability and shelf life of fresh-cut fruits and vegetables by acting as a surface disinfectant (Suslow 1997; Afek et al. 1999). As summarized in Table 1, the use of this molecule as an antimicrobial agent against food spoilage and pathogenic microorganisms has been conducted on fresh mushrooms (Brennan et al. 2000; Chikthimmah et al. 2005; Cliffe-Byrnes and O'Beirne 2008), blueberry (Li and Wu 2013), lettuce (Back et al. 2014), baby spinach (Huang et al. 2012), lemon (Cerioni et al. 2013), red bell pepper, strawberry, watercress (Alexandre et al. 2012), among others. One of the advantages of H_2O_2 treatment such as antimicrobial or sanitizer consists on the capacity of hydrogen peroxide to be decomposed into oxygen and water by enzymes such as catalase, which are naturally found in plants, thereby not leading to the formation of carcinogenic residues such as chlorine (Ali et al. 2017). Moreover, residual H_2O_2 can also be easily removed via the application of antioxidants, such as ascorbic acid, to avoid reaction with food constituents (Ali et al. 2017).

Postharvest treatment with H_2O_2, either isolated or in combination with calcium chloride or isoascorbate, was shown to extend the shelf life of fresh mushrooms at low temperature, facilitating the control of the bacterial population and providing better retention of whiteness (Brennan et al. 2000; Chikthimmah et al. 2005; Cliffe-Byrnes and O'Beirne 2008). Similarly, the immersion of pepper fruits in H_2O_2 solution (0–15 mM) prior storage at low temperature (10 and 20 °C) was shown to

Table 1 Effect of exogenous H_2O_2 application on the shelf life of horticultural products

Species[a] (organ)	Treatment	Consequences	References
Tomato (fruits)	0.4 M H_2O_2 for 1 min	Reduced microbial population	Kim et al. (2007)
Pepper (fruits)	1, 5, and 15 mM H_2O_2 for 30 min	Promoted ascorbate and antioxidant enzymes, reduced weight loss, rot rate index, and nitrate content	Bayoumi (2008)
Pears (fruits)	0.3% H_2O_2 and UV-C	Reduced microbial population but stimulated browning	Schenk et al. (2012)
Blueberries (fruits)	200 ppm H_2O_2 plus 500 ppm SDS	Reduced *Salmonella*, mold, and yeast population	Li and Wu (2013)
Lemon (fruits)	20 g L^{-1} H_2O_2 plus 6 mM copper sulfate for 1 min	Controlled postharvest disease	Cerioni et al. (2013)
Pepper, Strawberry (fruits)	1% H_2O_2 for 2 min	Reduced microbial population	Alexandre et al. (2012)
Water chestnut (seeds/ fruits)	0.9% H_2O_2 for 2 min	Delayed microorganism-induced diseases and reduced discoloration	Peng et al. (2008)
Melon (fruits)	50 mg L^{-1} H_2O_2 for 1 min	Reduced microbial population	Silveira et al. (2008)
Melon (fruits)	1–2.5% H_2O_2 for 5 min	Inhibited growth of spoilage and pathogenic microorganisms	Ukuku (2004); Ukuku et al. (2005)
Mushroom (fruiting body)	1.5% H_2O_2 for 10 min	Reduced microbial population and improved product quality	Brennan et al. (2000)
Mushroom (fruiting body)	0.75% H_2O_2 and 0.3% calcium chloride	Extended the shelf life	Chikthimmah et al. (2005)
Mushroom (fruiting body)	3% H_2O_2 and 4% isoascorbate for 1 min	Reduced surface browning and extended shelf life	Cliffe-Byrnes and O'Beirne (2008)
Mushroom (fruiting body)	0.3% H_2O_2 and UV-C	Reduced microbial population and extended shelf life	Guan et al. (2013)
Lettuce (leaves)	10% vaporized H_2O_2 for 10 min	Reduced microbial population	Back et al. (2014)
Spinach (leaves)	3% H_2O_2 for 5 min	Reduced microbial population	Huang et al. (2012)

[a]*Capsicum annuum* (pepper); *Citrus limon* (L.) Burm (lemon); *Cucumis melo* (melon); *Cyanococcus* (blueberries); *Eleocharis dulcis* (water chestnut); *Fragaria* × *ananassa* (strawberry); *Lactuca sativa* (lettuce); *Pyrus communis* (pears); *Solanum lycopersicum* (tomato); *Spinacia oleracea* (spinach)

minimize weight loss, reduce nitrate content, improve both of general appearance and fruit shelf life, and also increase the fruit nutritional quality by promoting ascorbate accumulation (Bayoumi 2008). In fresh-cut Chinese water chestnut, H_2O_2 treatment before cold storage reduced pericarp browning by inhibiting the activity of phenolic-related metabolic enzymes such as peroxidases (POD), polyphenol oxidase (PPO), and phenylalanine ammonia lyase (PAL) (Peng et al. 2008).

The antimicrobial action of H_2O_2 treatment can be further promoted by its combination with UV-C irradiation as demonstrated for mushroom (Guan et al. 2013) and pears (Schenk et al. 2012), even though in the case of pears, the H_2O_2 plus UV-C treatment stimulated fruit browning as an adverse collateral effect. Besides controlling plant pathogenic microorganisms, H_2O_2 treatment also seems to be an efficient mechanism to reduce the contamination of horticultural products with human pathogens, such as *Salmonella*, as demonstrated for blueberries (Martin and Maris 2012), fresh-cut tomato, and melon slices (Ukuku 2004; Ukuku et al. 2004). Therefore, the overall positive influence of the H_2O_2 treatment on the postharvest shelf life of fruits and vegetables is mainly associated with the action of this molecule as an antimicrobial agent, thereby reducing the attack by microorganisms.

In contrast, accumulating data indicate that the effect of NO treatment on the shelf life of fruits, flowers, and vegetables relies on NO-triggered changes in plant metabolism and hormonal physiology (Manjunatha et al. 2010; Freschi 2013; Corpas and Palma 2018). As extensively reviewed in the literature (Manjunatha et al. 2010; Freschi 2013; Asgher et al. 2017; Corpas and Palma 2018), either fumigation with gaseous NO or treatments with NO donors can delay the senescence of both vegetative and reproductive organs by transcriptionally and posttranslationally regulating ethylene biosynthetic enzymes (Leshem and Haramaty 1996; Leshem et al. 1998; Wills et al. 2000; Zhu and Zhou 2006; Manjunatha et al. 2010, 2012). In line with these findings, an inverse correlation between the NO and ethylene production has been described during fruit ripening and leaf senescence (Leshem et al. 1998; Leshem and Pinchasov 2000; Magalhaes et al. 2000; Corpas et al. 2004; Parra-Lobato and Gómez-Jiménez 2011). Table 2 shows some examples of the influence of NO treatment, either via fumigation or NO-releasing chemicals, in postharvest fruits and vegetable. Besides regulating ethylene production, exogenous NO application has also been demonstrated to affect other metabolic processes. For instance, the delayed ripening progression observed in NO-fumigated pepper fruits was associated with an increment in nitrosothiol and nitroprotein levels (Chaki et al. 2015) as well as significant increases in ascorbate (vitamin C) due to the activation of mitochondrial L-galactono-1,4-lactone dehydrogenase (GalLDH) activity and gene expression (Rodríguez-Ruiz et al. 2017a).

Moreover, Pristijono et al. (2008) demonstrated that apple slices treated with the NO donor DETANO were less affected by surface browning and microorganism attack. Data also showed that dipping fruit tissues in DETANO solution was more effective in inhibiting surface browning than gaseous NO fumigation, and this response was associated with a reduction in phenol content, PPO activity, ion leakage, and respiration (Huque et al. 2013). Similarly, in Chinese bayberry fruits,

Table 2 Effect of exogenous NO application on the shelf life of horticultural products

Species[a] (organ)	Treatment[b]	Consequence	References
Mango (fruits)	20 μL L^{-1} NO gas for 2 h	Inhibited ethylene biosynthesis	Zaharah and Singh (2011)
Bayberry (fruits)	20 μL L^{-1} NO gas for 2 h	Inhibited ethylene production, delayed changes in firmness, membrane permeability, lipid peroxidation, and H_2O_2 and $O_2^{\bullet-}$ production	Wu et al. (2012)
Pepper (fruits)	5 ppm NO gas for 1 h	Delayed ripening, increased ascorbate content and GalLDH expression/activity	Chaki et al. (2015); Rodríguez-Ruiz et al. (2017a)
Papaya (fruits)	60 μL L^{-1} NO for 3 h	Delayed ripening and softening via the regulation of cell wall softening-related enzymes, lower ethylene production	Guo et al. (2014)
Peach (fruits)	15 μM NO	Activated the phenylpropanoid pathway, increased phenolic, flavonoids and lignin content, higher resistance against pathogens	Li et al. (2017)
Peach (fruits)	15 μL L^{-1} NO gas and stored in an intermittent warming of two cycles	Alleviated chilling injury and maintained fruit quality	Zhu et al. (2010)
Orange (fruits)	50 μM SNP solution for 10 min	Enhanced resistance against postharvest anthracnose. Increased total phenolic content, induced antioxidant- and phenylpropanoid-related enzymes	Zhou et al. (2016b)
Strawberry (fruits)	1, 5, and 10 μM SNP	Extend postharvest shelf life	Zhu and Zhou (2007)
Apple (fruit)	10 mg L^{-1} DETANO	Inhibited browning, reduced phenol content, inhibited PPO activity, reduced ion leakage and respiration	Pristijono et al. (2008); Huque et al. (2013)
Lettuce (leaves)	500 mg L^{-1} DETANO solution and 500 μL L^{-1} fumigation	Inhibited browning	Wills et al. (2008)
Bamboo (shoot)	70 μM SNP aqueous solution	Enhanced chilling tolerance, activated polyamine, γ-aminobutyric acid, and proline metabolism	Wang et al. (2017)
Mushroom (fruiting body)	1 mM DETANO for 10 min	Delayed browning, promoted phenol and ascorbic acid content, modulated respiration and H_2O_2 levels, induced the activity of antioxidant enzymes	Jiang et al. (2011)

[a]*Capsicum annuum* (pepper); *Carica papaya* (papaya); *Citrus sinensis* (orange); *Fragaria* × *ananassa* (strawberry); *Lactuca sativa* (lettuce); *Malus* × *domestica* (apple); *Mangifera indica* (mango); *Morella rubra* (Chinese bayberry); *Prunus persica* (peach)
[b]Sodium nitroprusside (SNP); diethylenetriamine/nitric oxide (DETANO)

NO fumigation was shown to inhibit disease incidence, maintain fruit firmness, modify the total phenolic content, reduce the increases in membrane permeability and lipid peroxidation, and regulate $O_2^{\bullet-}$ and H_2O_2 levels via increments in SOD, CAT, and APX activities (Wu et al. 2012). In peach fruits, NO treatment also strongly activated the phenylpropanoid pathway, consequently improving biotic stress resistance during postharvest storage (Li et al. 2017). Enhanced resistance against fruit pathogens has also been observed in other NO-treated fruits, such as tomato and strawberries (Zhu and Zhou 2007; Lai et al. 2011). Interestingly, for some other horticulture products, such as fresh-cut iceberg lettuce, the immersion in DETANE in combination with NO fumigation was shown to be more effective than the NO gas alone in minimizing postharvest problems such as surface browning and pathogen attack (Wills et al. 2008). Among the different NO donors currently available, only a few have been already tested in postharvest (e.g., Piloty's acid, *N-tert*-butyl-α-phenylnitrone (PBN), DETANE), and their effectiveness has been shown to vary significantly depending on the horticultural product under consideration (Huque et al. 2013).

2.3 *Impacts of Exogenous H_2O_2 and NO on Plant Development and Stress Resistance*

It is currently accepted that H_2O_2 and NO are essential signaling molecules involved in controlling multiple aspects of plant development and responses to biotic and abiotic stress (Saxena et al. 2016). Therefore, the exogenous application of these molecules can promote multiple physiological responses associated with desired plant traits, thereby holding the potential to be used as a means to adjust the growth, development, and metabolism of crops (Fig. 1).

Under laboratory conditions, both H_2O_2 and NO treatments have been shown to promote root formation via an intricated cross talk with auxins (Takáč et al. 2016). Moreover, as reviewed by Marvasi (2017), treatments with NO donors have the potential to be used in agriculture to regulate seed vigor and dormancy, wound healing, and plant nutrition, even though field trials are still required to determine the feasibility of such biotechnological application of NO treatments.

In addition, multiple studies indicate that the application of H_2O_2 or NO at optimal concentrations can improve plant tolerance to abiotic stresses (Terzi et al. 2014), such as drought (He and Gao 2009; Liu et al. 2010; Sun et al. 2016), heat (Gao et al. 2010), heavy metal stresses (Hu et al. 2009; Hasanuzzaman et al. 2017), among others.

Many of the stress-related responses controlled by H_2O_2 and NO require the induction of a so-called primed state, which consists in exposing specific tissues or the whole plant to a condition or substance capable of activating some biological systems related to defense and acclimatization (Savvides et al. 2016). In agreement, accumulating evidence indicates that both RNS (in the form of NO) and ROS (in the

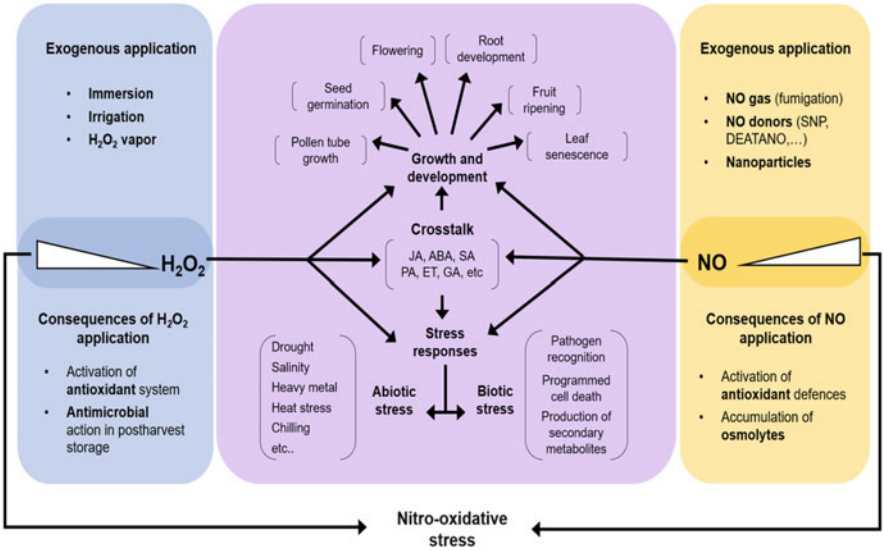

Fig. 1 Role of H_2O_2 and NO in plants. Both H_2O_2 and NO act as signaling molecules controlling a vast range of developmental and metabolic processes either directly or via cross talk with other signaling molecules (e.g., plant hormones, second messengers). Despite acting as critical signaling molecules, excessive levels of H_2O_2 and NO can induce nitro-oxidative stress. *JA* jasmonic acid, *ABA* abscisic acid, *SA* salicylic acid, *PA* polyamines, *ET* ethylene, *GA* gibberellins, *NO* nitric oxide, *H_2O_2* hydrogen peroxide

form of H_2O_2) can be involved in the priming phenomena both in annual and perennial species (Leshem et al. 1998; del Río 2015). Overall, the activation of the plant antioxidant defenses seems to be a central process behind the induction of the primed state in response to H_2O_2 and NO treatment (Hu et al. 2009; Liu et al. 2010; Golemiec et al. 2014; Sun et al. 2016; Hasanuzzaman et al. 2017). By boosting the plant antioxidative defense system, both the H_2O_2 and NO treatments increase the capacity of plant cells to alleviate membrane lipid peroxidation and other damages frequently triggered by stressful environmental conditions, such as drought, heat, colt, high light, and heavy metal stress (Giovannoni et al. 2017; Marvasi 2017).

Besides regulating the antioxidant defenses, H_2O_2 and NO may also trigger additional physiological responses that facilitate plant survival under stress conditions. For instance, H_2O_2 treatment can alleviate water loss and induce osmotic stress resistance by increasing the levels of soluble sugars, proline, polyamines (PA), and abscisic acid (ABA) (Terzi et al. 2014). Moreover, both H_2O_2 and NO are known to participate in the regulation of stomatal closure, thereby playing a central role during drought (Guan et al. 2000; Saxena et al. 2016). NO donors have also been demonstrated to enhance chilling tolerance by activating PA, γ-aminobutyric acid (GABA), and proline metabolism and inducing the activity of enzymes associated with RNS metabolism (Wang et al. 2017).

3 Genetic Manipulation of H_2O_2 and NO Metabolism

Genetic manipulation of H_2O_2 and NO metabolisms to adjust plant development and adaptation to environmental stresses represent a significant challenge in plant biotechnology given the multiple and overlapping routes of ROS and RNS production, scavenging, and interaction in plant systems. However, despite the methodological difficulties and conceptual complexity intrinsically involved in modifying the plant redox metabolism, some cutting-edge insights into the impact of the manipulation of critical components of ROS/RNS metabolism have recently been achieved. As discussed in this section and summarized in Table 3, many attempts to manipulate enzymes involved in H_2O_2 and NO metabolism resulted in significant alterations in plant development and resistance to abiotic and biotic stresses. This indicates that functional analysis of ROS/RNS-related genes will probably continue to be an approach of utmost relevance over the next years as it represents an essential step in identifying the best candidates for biotechnological use in crops.

3.1 Genetic Manipulation of H_2O_2 Metabolism

As highlighted in this chapter, H_2O_2 can be produced via multiple enzymatically and nonenzymatically pathways in plants. Therefore, instead of targeting H_2O_2 production, most studies have focused on overexpressing enzymes involved in the removal of this reactive molecule (e.g., SOD, APX, CAT) (Allen et al. 1997). Overall, improved plant resistance to abiotic stress, including drought, cold, heavy metals, high light, and salt stress, has been achieved via these genetic manipulations (Table 3).

In line with its crucial protective role against oxidative stress, SODs have been some of the first ROS-related enzymes genetically manipulated in plants (Gupta et al. 1993a; Zelko et al. 2002). In multiple plant species, the constitutive overexpression of SOD-encoding genes has been shown to promote photosynthetic rates under chilling and high light intensity, to stimulate APX activity, and to increase tolerance to water deficit, high light, cold, salinity, and herbicides, such as methyl viologen (MV) (Gupta et al. 1993a, b; Hamid Badawi et al. 2004; Jing et al. 2015). However, considerable differences have been observed depending on the SOD isoform selected for the genetic manipulation. Plant SODs are grouped into three classes based on differences in their protein structure, intracellular localization, and metal cofactor, i.e., Cu/Zn-SOD, Fe-SOD, and Mn-SOD (Palma et al. 2013; Gill et al. 2015; del Río et al. 2018). Whereas Fe-SOD activity is inhibited by high H_2O_2 levels, the Mn-SOD activity is not influenced by this ROS. In line with this, the overexpression of a recombinant chloroplast-targeted Mn-SOD sequence was particularly effective in promoting resistance to abiotic stress (Alscher et al. 2002). Data also indicate that transgenic plants overexpressing both mitochondrial- and chloroplast-targeted Mn-SOD displayed a stress resistance phenotype equivalent to

Table 3 Manipulation of enzymes involved in H_2O_2 and NO metabolism

Enzyme (manipulation)	Species[a]	Consequences/mechanism	References
SOD (overexpression)	Tobacco, wheat	Increased resistance to cold, high light, and salinity. Enhanced removal of $O_2^{\cdot-}$ and induction of APX activity	Alscher et al. (2002); Gupta et al. (1993a, b); Hamid Badawi et al. (2004); Jing et al. (2015)
SOD and APX (overexpression)	Potato, cassava, sweet potato, tall fescue	Increased resistance to MV, H_2O_2, cadmium, arsenic, copper, SO_2, and high light stress Increased removal $O_2^{\cdot-}$ and H_2O_2	Tang et al. (2006); Lee et al. (2007); Xu et al. (2014); Kim et al. (2015); Yan et al. (2016)
SOD and CAT (overexpression)	Chinese cabbage	Increased resistance to SO_2 and salt stress Increased removal $O_2^{\cdot-}$ and H_2O_2	Tseng et al. (2007); Yiu and Tseng (2005)
CAT and APX (silencing)	*Arabidopsis*	Increased resistance to MV Activation of chloroplast alternative oxidase	Rizhsky et al. (2002)
APX (overexpression)	*Arabidopsis*, rice, tobacco, alfalfa	Increased resistance to cold, salt, and aminotriazole Increased removal of H_2O_2	Wang et al. (1999); Yan et al. (2003); Xu et al. (2008); Sato et al. (2011); Zhang et al. (2014)
CAT (overexpression)	Tobacco	Increased resistance to cold, salt, oxidative stress, and water deficit Increased removal of H_2O_2	Brisson et al. (1998); Shikanai et al. (1998); Guan et al. (2009)
CAT (silencing)	Tobacco	Increased levels of pathogen-related protein (PR-1) Increase in H_2O_2 content	Chamnongpol et al. (1996); Takahashi et al. (1997)
Fungal-derived glucose oxidase (overexpression)	Tobacco	Increased resistance to pathogens Increase in H_2O_2 content	Wu et al. (1995, 1997)
NOS (overexpression)	*Arabidopsis*, rice	Increased resistance to salt and drought Increased NO content and increase in *CAT* and *POX* genes and reduced levels of H_2O_2	Guo and Crawford (2005); Shi et al. (2012); Cai et al. (2015); Foresi et al. (2015)
Spermidine synthase (overexpression)	*Arabidopsis*, rice, tobacco, eggplant, sweet potato	Increased resistance to multiple environmental stresses Increased NO content and upregulation of stress-related genes	Kasukabe et al. (2004); Roy and Wu (2002); Marco et al. (2015)
GSNOR (overexpression)	Tomato	Increased resistance to alkali stress Increased NO removal	Gong et al. (2015)

(continued)

Table 3 (continued)

Enzyme (manipulation)	Species[a]	Consequences/mechanism	References
Hemoglobin (overexpression)	Barley	Increased drought tolerance Increased NO removal	Montilla-Bascón et al. (2017)

[a]*Arabidopsis thaliana* (*Arabidopsis*); *Brassica rapa* subsp. *pekinensis* (Chinese cabbage); *Hordeum vulgare* (barley); *Ipomoea batatas* (sweet potato); *Festuca arundinacea* (tall fescue); *Manihot esculenta* (cassava); *Medicago sativa* (alfafa); *Nicotiana tabacum* (tobacco); *Oryza sativa* (rice); *Solanum lycopersicum* (tomato); *Solanum melongena* (eggplant); *Solanum tuberosum* (potato); *Triticum aestivum* (wheat)

plants overexpressing only the chloroplast-targeted Mn-SOD isoform (Samis et al. 2002).

Similarly, *APX* overexpression also resulted in stress resistance, as exemplified by the positive impact of this manipulation on the tolerance against cold in rice (Sato et al. 2011), salt in alfalfa and *Arabidopsis* (Xu et al. 2008; Zhang et al. 2014), oxidative stress induced by the herbicide aminotriazole in *Arabidopsis* (Wang et al. 1999), and water deficit in tobacco (Yan et al. 2003). Similarly, overexpressing CAT-encoding gene in tobacco promoted resistance to cadmium (Guan et al. 2009) and preserved the photosynthetic efficiency at high temperatures (Brisson et al. 1998). Moreover, when transgenic tobacco plants overexpressing *Escherichia coli* catalase in chloroplasts were simultaneously exposed to both high irradiance and drought, tolerance to these stresses was observed, but APX activity was totally inactivated in both transgenic and wild-type tobacco plants (Shikanai et al. 1998). Thus, the authors concluded that qualitative rather than quantitative manipulations of the antioxidant system via transgenic approaches are more significant to improve plant tolerance against oxidative stress (Shikanai et al. 1998).

In agreement with their coordinated reactions, the simultaneous overexpression of genes encoding SOD and APX or CAT has also been demonstrated to improve plant resistance against abiotic stress. The simultaneous overexpressing of chloroplast-targeted SOD and APX under the control of stress-inducible promoter *SWPA2* in potato produced plants with increased resistance to MV and higher photosynthetic rates under stress (Tang et al. 2006). Similarly, the concomitant overexpression of both *SOD*- and *APX*-encoding genes in other species, including cassava, sweet potato, and tall fescue, resulted in plants with increased resistance to a wide range of abiotic stress, such as heavy metals (Lee et al. 2007), chilling (Xu et al. 2014), salt (Yan et al. 2016), and SO_2 (Kim et al. 2015). Increased resistance to SO_2 and salt stress was also observed when *SOD* and *CAT* genes were simultaneously overexpressed in *Brassica rapa* (Yiu and Tseng 2005; Tseng et al. 2007). Paradoxically, however, the antisense-mediated silencing of both *APX* and *CAT* resulted in *Arabidopsis* plants with increased resistance to MV (Rizhsky et al. 2002), a response associated with the suppression of photosynthesis and induction of the chloroplast alternative oxidase. One plausible explanation may also be the redundancy of the antioxidant systems that generally relies on different isoenzymes, so the

disappearance of one isozyme can be compensated by the induction of another equivalent in the same or another subcellular compartment.

Although usually presenting improved tolerance against abiotic stress, transgenic plants exhibiting increased H_2O_2 removal capability may be disturbed in other physiological processes, such as pathogen resistance. This aspect remains poorly investigated, but some reports seem to support this possibility. For example, transgenic tobacco plants with reduced CAT levels presented severe leaf damage under high light but exhibited increased pathogenesis-related (PR)-1 protein synthesis (Chamnongpol et al. 1996; Takahashi et al. 1997). Accordingly, transgenic potato plants expressing a fungal H_2O_2-generation glucose oxidase showed increased H_2O_2 levels and enhanced resistance to a broad range of pathogens (Wu et al. 1995; Wu et al. 1997). In line with these findings, H_2O_2 and other ROS generated by plasma membrane NADPH oxidases have long been associated with the rapid increase in apoplast $O_2^{\cdot-}$ and H_2O_2 levels, which promotes pathogen killing by oxidative stress (Sagi and Fluhr 2006) and programmed cell death (PCD) that may limit the propagation of the pathogen infection (Greenberg and Yao 2004).

3.2 Genetic Manipulation of NO Metabolism

The diverse sources of NO production in plants implicate that many different metabolic pathways can be modulated to adjust RNS endogenous levels in plant cells. For example, the overexpression of the rat gene encoding a neuronal NO synthase (NOS) in rice resulted in increased NO levels, higher salt and drought tolerance, and upregulation of *CAT* and *POX* expression, thereby reducing H_2O_2 levels (Cai et al. 2015). A similar manipulation in *Arabidopsis* resulted in higher accumulation of osmolytes, such as proline and sucrose, and increased antioxidant enzyme activities (Shi et al. 2012). Likewise, transgenic *Arabidopsis* plants overexpressing the *NOS*-like gene from the unicellular marine alga *Ostreococcus tauri* (OtNOS) displayed similar responses. These transgenic plants had higher NO content and showed increased tolerance to several stresses including salinity, drought, and oxidative stress generated by the herbicide MV (Foresi et al. 2015).

Overexpression of NR-encoding genes has also been explored as an alternative to producing plants with increased NO levels. However, the *NR* mRNA levels are under strict cellular regulation by the plant cells and attempts to overexpress this gene predominantly resulted in gene co-suppression. Therefore, the constitutive overexpression of *NR* is not a promising avenue to obtain NO-overproducing plants (Dorlhac de Borne et al. 1994; Vaucheret et al. 1997; Ferrario-Méry et al. 1998; Dubois et al. 2005). However, NR activity can be modulated by substituting the regulatory serine (Ser 521 in tobacco) by an arginine. This serine-to-arginine substitution results in a constitutively active form of the enzyme, whose activity is no longer inhibited via phosphorylation, thereby exacerbating NO production by the plant cells (Lea et al. 2004). Despite the difficulties of increasing NO production via NR manipulation, this enzyme has been shown to be a significant source of NO in

multiple plant responses (Chamizo-Ampudia et al. 2017), including stomatal movements (Desikan et al. 2002; Garcia-Mata and Lamattina 2003), immunity (Jian et al. 2015; Floryszak-Wieczorek et al. 2016), de-etiolation (Melo et al. 2016), ABA signaling (Zhao et al. 2016), and cold and salt tolerance (Zhao et al. 2009; Xie et al. 2013), among others.

Evidence also suggests that NO can be generated in plants from the oxidation of polyamines (Tun et al. 2006). Overall, PA biosynthesis and degradation are correlated with the increase and decrease, respectively, in NO levels (Kusano and Suzuki 2015), and the supplementation with PA can promote NO production in certain plant species (Tun et al. 2006). Therefore, the overexpression of PA biosynthesis-related enzymes, such as spermidine synthase, has been proposed as a viable alternative to increase endogenous NO content and promote tolerance to multiple environmental stresses (Kasukabe et al. 2004; Roy and Wu 2002; Marco et al. 2015). It is also important to highlight that PA catabolism induces H_2O_2 formation (Moschou et al. 2008), which may further impact plant responses to stressful conditions as already discussed in this chapter.

Given the central role played by GSNOR on NO homeostasis, it is not surprising that many studies have investigated the relevance of this enzyme in plant responses to biotic and abiotic stress (Barroso et al. 2006; Lee et al. 2008; Leterrier et al. 2011; Malik et al. 2011). A decline in GSNOR activity followed by an increase of *S*-nitrosothiols is typically observed in response to abiotic stress, such as heavy metals (Barroso et al. 2006), hypoxia (Zhan et al. 2018), mechanical wounding (Chaki et al. 2011), and plant disease resistance (Chaki et al. 2009; Malik et al. 2011; Tichá et al. 2018). Similarly, downregulation of GSNOR activity with a concomitant increase of protein *S*-nitrosothiols has also been reported during pepper (*Capsicum annuum*) fruit ripening (Rodríguez-Ruiz et al. 2017b), which has been interpreted as an adaption mechanism to the physiological nitro-oxidative stress associated with fruit ripening (Corpas et al. 2018).

In line with this, the *GSNOR*-deficient *Arabidopsis* mutant *hot5* exhibits increased levels of NO and *S*-nitrosylated species and reduced disease resistance and cannot acclimate under high temperature (Xu et al. 2013). In contrast, *Arabidopsis GSNOR*-silenced lines showed higher pathogen infection resistance despite their increased endogenous NO and *S*-nitrosothiol levels compared to the wild type (Rustérucci et al. 2007). Also intriguing, *GSNOR* overexpression in tomato increased tolerance to alkaline stress compared to the wild type (Gong et al. 2015).

Collectively, these findings support the idea that the consequences of *GSNOR* genetic manipulation in plants are highly dependent on the environmental context and plant species and still need more clarifications. Further elucidating the physiological relevance of posttranslational regulatory mechanisms controlling GSNOR activity, such as *S*-nitrosylation (Frungillo et al. 2014; Guerra et al. 2016) and calmodulin (Zhou et al. 2016a), may bring essential insights to clarify the complex responses triggered by GSNOR manipulation.

In conjunction with GSNOR, plant hemoglobins (i.e., phytoglobins) play a significant role in controlling NO levels, particularly under flood and hypoxic/anoxic conditions (Gardner 2012). Transgenic plants overexpressing hemoglobins

frequently exhibit reduced NO content; however, similar to the discussed above for GSNOR, the effectiveness of hemoglobin manipulation remains controversial in plants. For example, *Arabidopsis* overexpressing hemoglobin are less tolerant to abiotic stress than the wild type (Bai et al. 2016), whereas increased drought tolerance was observed when barley plants were manipulated in the same way (Montilla-Bascón et al. 2017).

Adding further complexity, both thioredoxins (Trx) and peroxiredoxin (Prx) have also been shown to denitrosylate plant proteins (Tada et al. 2008; Sevilla et al. 2015). Therefore, additional opportunities to manipulate NO removal mechanisms may arise in the upcoming years.

4 Concluding Remarks

Despite encouraging results under laboratory conditions, the large-scale use of exogenous NO and H_2O_2 application under field conditions still requires detailed methodological and economic viability analysis. Basically, the efficiency of such biotechnological application lies between a narrow window of dosage and exposure time, whose borders can have, in the best scenario, an insufficient response and, in the worst case, a toxic and deleterious effect. As many variables such as temperature, humidity, and light exposure can interfere with the NO release rates and intensity by NO donors, it seems clear that investigating new alternatives to deliver NO to plants in a more controlled way (e.g., new donors, nanoparticles) represents a promising venue for facilitating the biotechnological use of this reactive molecule under field conditions.

Besides interacting with each other, H_2O_2 and NO also extensively interplay with plant hormones and other signaling molecules (Freschi 2013; Saxena et al. 2016; Asgher et al. 2017; Corpas and Palma 2018). Moreover, NO and H_2O_2 metabolism lead to the formation of other, more dangerous, reactive species, such as peroxynitrite (del Río 2015; Bartesaghi and Radi 2018). Keeping this in mind, it seems reasonable to expect unwanted collateral effects whether the NO and H_2O_2 are delivered to the plant tissues with insufficient temporal, spatial, and quantitative control.

In this context, the genetic manipulation of NO and H_2O_2 metabolism via transgenesis or genome editing tools holds great potential in adjusting the production and removal of these molecules in the correct cells and at the adequate moments of the life cycle. However, this line of research is still at an exploratory stage, and further gene functional analysis is required to compile the list of candidate genes and how they should be manipulated. For example, particular attention has been given to the role of H_2O_2 and NO on plant resistance and survival under stress conditions; however, many other physiological events of also great biotechnological interest are equally influenced by these reactive molecules, including the PCD and cell differentiation in wood and fiber production (Potikha et al. 1999; Gabaldon et al. 2005),

secondary metabolite production, nutrient accumulation (Graziano et al. 2002), and vegetative-to-reproductive transition, among others.

It is also important to highlight that many new molecular tools have been developed in recent years, including genome editing technologies (e.g., CRISPR/Cas9) and synthetic biology approaches (e.g., multigene constructs), which offer unprecedented opportunities to create plants with much more precise alterations in H_2O_2 and NO metabolisms. Among these tools, the use of tissue-specific, developmental stage-specific, and stress-inducible promoters to drive the expression of ROS- and RNS-related transgenes may provide a much more controlled way to manipulate H_2O_2 and NO levels inside plant cells compared to the widely employed constitutive cauliflower mosaic virus *35S* promoter. This refinement may prove crucial to minimize yield penalties or other negative collateral consequences potentially associated with the genetic manipulation of plant antioxidant metabolism. Finally, we must remain open-minded to conceive more extensive reengineering of plant metabolism in the upcoming years, in which the manipulation of H_2O_2 and NO cellular levels may be only part of a suite of strategies to create more stress resistance crops that also fulfill other consumer and environmental demands, such as nutritional enrichment, increased yield, and higher efficiency in water and nutrient use.

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References

Afek U, Orenstein J, Nuriel E (1999) Fogging disinfectants inside storage rooms against pathogens of potatoes and sweet potatoes. Crop Prot 18:111–114

Alexandre EMC, Brandão TRS, Silva CLM (2012) Assessment of the impact of hydrogen peroxide solutions on microbial loads and quality factors of red bell peppers, strawberries and watercress. Food Control 27:362–368

Ali A, Yeoh WK, Forney C, Siddiqui MW (2017) Advances in postharvest technologies to extend the storage life of minimally processed fruits and vegetables. Crit Rev Food Sci Nutr 26:1–18

Allen RD, Webb RP, Schake SA (1997) Use of transgenic plants to study antioxidant defenses. Free Radic Biol Med 23:473–479

Alscher GR, Erturk N, Heath LS (2002) Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J Exp Bot 53:1331–1342

Anderson LS, Mansfield TA (1979) The effects of nitric oxide pollution on the growth of tomato. Environ Pollut 20:113–121

Asgher M, Per TS, Masood A, Fatma M, Freschi L, Corpas FJ, Khan NA (2017) Nitric oxide signaling and its crosstalk with other plant growth regulators in plant responses to abiotic stress. Environ Sci Pollut Res Int 24:2273–2285

Back KH, Ha JW, Kang DH (2014) Effect of hydrogen peroxide vapor treatment for inactivating Salmonella typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce. Food Contr 44:78–85

Bai X, Long J, He X, Yan J, Chen X, Tan Y, Li K, Chen L, Xu H (2016) Overexpression of spinach non-symbiotic hemoglobin in *Arabidopsis* resulted in decreased NO content and lowered nitrate and other abiotic stresses tolerance. Sci Rep 6:26400

Barroso JB, Corpas FJ, Carreras A, Rodríguez-Serrano M, Esteban FJ, Fernández-Ocaña A, Chaki M, Romero-Puertas MC, Valderrama R, Sandalio LM, del Río LA (2006) Localization of *S*-nitrosoglutathione and expression of *S*-nitrosoglutathione reductase in pea plants under cadmium stress. J Exp Bot 57:1785–1793

Bartesaghi S, Radi R (2018) Fundamentals on the biochemistry of peroxynitrite and protein tyrosine nitration. Redox Biol 14:618–625

Bayoumi YA (2008) Improvement of postharvest keeping quality of white pepper fruits (*Capsicum annuum*, L.) by hydrogen peroxide treatment under storage conditions. Acta Biolog Szeged 52:7–15

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

Brennan M, Le Port G, Gormley R (2000) Post-harvest treatment with citric acid or hydrogen peroxide to extend the shelf life of fresh sliced mushrooms. Food Sci Technol 33:285–289

Brisson LF, Zelitch I, Havir EA (1998) Manipulation of catalase levels produces altered photosynthesis in transgenic tobacco plants. Plant Physiol 116:259–269

Cai W, Liu W, Wang WS, Fu ZW, Han TT, Lu YT (2015) Overexpression of rat neurons nitric oxide synthase in rice enhances drought and salt tolerance. PLoS One 10:e0131599

Cerioni L, Sepulveda M, Rubio-Ames Z, Volentini SI, Rodríguez-Montelongo L, Smilanick JL, Ramallo J, Rapisarda VA (2013) Control of lemon postharvest diseases by low-toxicity salts combined with hydrogen peroxide and heat. Posthar Biol Technol 83:17–21

Chaki M, Fernández-Ocaña AM, Valderrama R, Carreras A, Esteban FJ, Luque F, Gómez-Rodríguez MV, Begara-Morales JC, Corpas FJ, Barroso JB (2009) Involvement of reactive nitrogen and oxygen species (RNS and ROS) in sunflower-mildew interaction. Plant Cell Physiol 50:265–279

Chaki M, Valderrama R, Fernández-Ocaña AM, Carreras A, Gómez-Rodríguez MV, Pedrajas JR, Begara-Morales JC, Sánchez-Calvo B, Luque F, Leterrier M, Corpas FJ, Barroso JB (2011) Mechanical wounding induces a nitrosative stress by down-regulation of GSNO reductase and an increase in S-nitrosothiols in sunflower (*Helianthus annuus*) seedlings. J Exp Bot 62:1803–1813

Chaki M, Álvarez de Morales P, Ruíz 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. Ann Bot 116:637–647

Chamizo-Ampudia A, Sanz-Luque E, Llamas A, Galvan A, Fernandez E (2017) Nitrate reductase regulates plant nitric oxide homeostasis. Trend Plant Sci 22:163–174

Chamnongpol S, Willekens H, Langebartels C, Montagu M, Inzé D, Camp W (1996) Transgenic tobacco with a reduced catalase activity develops necrotic lesions and induces pathogenesis-related expression under high light. Plant J 10:491–503

Chikthimmah N, Borde LF, Beelman RB (2005) Hydrogen peroxide and calcium chloride added to irrigation water as a strategy to reduce bacterial populations and improve quality of fresh mushrooms. J Food Sci 70:273–278

Cliffe-Byrnes V, O'Beirne D (2008) Effects of washing treatment on microbial and sensory quality of modified atmosphere (MA) packaged fresh sliced mushroom (*Agaricus bisporus*). Posthar Biol Technol 48:283–294

Corpas FJ, Barroso JB (2017) Nitric oxide synthase-like activity in higher plants. Nitric Oxide Biol Chem 68:5–6

Corpas FJ, Palma JM (2018) Nitric oxide on/off in fruit ripening. Plant Biol (Stuttg) 20:805–807

Corpas FJ, Barroso JB, Carreras A, Quirós M, León AM, Romero-Puertas MC, Esteban FJ, Valderrama R, Palma JM, Sandalio LM, Gómez M, del Río LA (2004) Cellular and subcellular localization of endogenous nitric oxide in young and senescent pea plants. Plant Physiol 136:2722–2733

Corpas FJ, Begara-Morales JC, Sánchez-Calvo B, Chaki M, Barroso JB (2015) Nitration and S-Nitrosylation: two post-translational modifications (PTMs) mediated by reactive nitrogen species (RNS) and their role in signalling processes of plant cells. In: Gupta KJ, Igamberdiev AU (eds) Reactive oxygen and nitrogen species signaling and communication in plants. Springer, Cham, pp 267–281

Corpas FJ, Freschi L, Rodríguez-Ruiz M, Mioto PT, González-Gordo S, Palma JM (2018) Nitro-oxidative metabolism during fruit ripening. J Exp Bot 69:3449–3463

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

del Río LA, Corpas FJ, López-Huertas E, Palma JM (2018) Plant superoxide dismutases: function. In: Gupta DK, Palma JM, Corpas FJ (eds) Antioxidants and antioxidant enzymes in higher plants. Springer, Cham, pp 1–26

Desikan R, Griffiths R, Hancock J, Neill S (2002) A new role for an old enzyme: nitrate reductase-mediated nitric oxide generation is required for abscisic acid-induced stomatal closure in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 99:16314–16318

Domingos P, Prado AM, Wong A, Gehring C, Feijo JA (2015) Nitric oxide: a multitasked signaling gas in plants. Mol Plant 8:506–520

Dorlhac de Borne F, Vincentz M, Chupeau Y, Vaucheret H (1994) Co-suppression of nitrate reductase host genes and transgenes in transgenic tobacco plants. Mol Gen Genet 243:613–621

Dubois V, Botton E, Meyer C, Rieu A, Bedu M, Maisonneuve B, Mazier M (2005) Systematic silencing of a tobacco nitrate reductase transgene in lettuce (*Lactuca sativa* L.). J Exp Bot 56:2379–2388

Ferrario-Méry S, Valadier MH, Foyer CH (1998) Overexpression of nitrate reductase in tobacco delays drought-induced decreases in nitrate reductase activity and mRNA. Plant Physiol 117:293–302

Floryszak-Wieczorek J, Arasimowicz-Jelonek M, Izbianska K (2016) The combined nitrate reductase and nitrite-dependent route of NO synthesis in potato immunity to *Phytophthora infestans*. Plant Physiol Biochem 108:468–477

Foresi N, Mayta ML, Lodeyro AF, Scuffi D, Correa-Aragunde N, García-Mata C, Casalongué C, Carrillo N, Lamattina L (2015) Expression of the tetrahydrofolate-dependent nitric oxide synthase from the green alga *Ostreococcus tauri* increases tolerance to abiotic stresses and influences stomatal development in *Arabidopsis*. Plant J 82:806–821

Freschi L (2013) Nitric oxide and phytohormone interactions: current status and perspectives. Front Plant Sci 4:1–22

Frungillo L, Skelly MJ, Loake GJ, Spoel SH, Salgado I (2014) S-nitrosothiols regulate nitric oxide production and storage in plants through the nitrogen assimilation pathway. Nat Commun 5:5401

Gabaldon C, Gomez Ros LV, Pedreno MA, Ros Barcelo A (2005) Nitric oxide production by the differentiating xylem of *Zinnia elegans*. New Phytol 165:121–130

Gao Y, Guo YK, Lin SH, Fang YY, Bai JG (2010) Hydrogen peroxide pretreatment alters the activity of antioxidant enzymes and protects chloroplast ultrastructure in heat-stressed cucumber leaves. Sci Hortic 126:20–26

Garcia-Mata C, Lamattina L (2003) Abscisic acid, nitric oxide and stomatal closure—is nitrate reductase one of the missing links? Trend Plant Sci 8:20–26

Gardner PR (2012) Hemoglobin: a nitric-oxide dioxygenase. Scientifica 2012:34

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, 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 22:10375–10394

Giovannoni J, Nguyen C, Ampofo B, Zhong S, Fei Z (2017) The epigenome and transcriptional dynamics of fruit ripening. Annu Rev Plant Biol 68:61–84

Golemiec E, Tokarz K, Wielanek M, Niewiadomska E (2014) A dissection of the effects of ethylene, H_2O_2 and high irradiance on antioxidants and several genes associated with stress and senescence in tobacco leaves. J Plant Physiol 171:269–275

Gong B, Wen D, Wang X, Wei M, Yang F, Li Y, Shi Q (2015) S-nitrosoglutathione reductase-modulated redox signaling controls sodic alkaline stress responses in *Solanum lycopersicum* L. Plant Cell Physiol 56:790–802

Gong T, Li C, Bian B, Wu Y, Dawuda MM, Liao W (2018) Advances in application of small molecule compounds for extending the shelf life of perishable horticultural products: a review. Sci Hortic 230:25–34

Graziano M, Beligni MV, Lamattina L (2002) Nitric oxide improves internal iron availability in plants. Plant Physiol 130:1852–1859

Greenberg JT, Yao N (2004) The role and regulation of programmed cell death in plant-pathogen interactions. Cell Microbiol 6:201–211

Guan LM, Zhao J, Scandalios JG (2000) *Cis*-elements and *trans*-factors that regulate expression of the maize *Cat1* antioxidant gene in response to ABA and osmotic stress: H_2O_2 is the likely intermediary signaling molecule for the response. Plant J 22:87–95

Guan Z, Chai T, Zhang Y, Xu J, Wei W (2009) Enhancement of Cd tolerance in transgenic tobacco plants overexpressing a Cd-induced catalase cDNA. Chemosphere 76:623–630

Guan W, Fan X, Yan R (2013) Effect of combination of ultraviolet light and hydrogen peroxide on inactivation of *Escherichia coli* O157:H7, native microbial loads, and quality of button mushrooms. Food Contr 34:554–559

Guerra D, Ballard K, Truebridge I, Vierling E (2016) *S*-nitrosation of conserved cysteines modulates activity and stability of S-nitrosoglutathione reductase (GSNOR). Biochemistry 55:2452–2464

Guo FQ, Crawford NM (2005) *Arabidopsis* nitric oxide synthase1 is targeted to mitochondria and protects against oxidative damage and darkinduced senescence. Plant Cell 17:3436–3450

Guo Q, Wu B, Chen W, Zhang Y, Wang J, Li X (2014) Effects of nitric oxide treatment on the cell wall softening related enzymes and several hormones of papaya fruit during storage. Food Sci Technol Int 20:309–317

Gupta AS, Heinen JL, Holaday AS, Burke JJ, Allen RD (1993a) Increased resistance to oxidative stress in transgenic plants that overexpress chloroplastic Cu/Zn superoxide dismutase. Proc Natl Acad Sci USA 90:1629–1633

Gupta AS, Webb RP, Holaday AS, Allen RD (1993b) Overexpression of superoxide dismutase protects plants from oxidative stress (induction of ascorbate peroxidase in superoxide dismutase-overexpressing plants). Plant Physiol 103:1067–1073

Hamid Badawi G, Yamauchi Y, Shimada E, Sasaki R, Kawano N, Tanaka K, Tanaka K (2004) Enhanced tolerance to salt stress and water deficit by overexpressing superoxide dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. Plant Sci 166:919–928

Hasanuzzaman M, Nahar K, Gill SS, Alharby HF, Razafindrabe BHN, Fujita M (2017) Hydrogen peroxide pretreatment mitigates cadmium-induced oxidative stress in *Brassica napus* L.: an intrinsic study on antioxidant defense and glyoxalase systems. Front Plant Sci 8:115

He L, Gao Z (2009) Pretreatment of seed with H_2O_2 enhances drought tolerance of wheat (*Triticum aestivum* L.) seedlings. Afr J Biotechnol 8:6151–6157

Hu Y, Ge Y, Zhang C, Ju T, Cheng W (2009) Cadmium toxicity and translocation in rice seedlings are reduced by hydrogen peroxide pretreatment. Plant Growth Regul 59:51

Huang Y, Ye M, Chen H (2012) Efficacy of washing with hydrogen peroxide followed by aerosolized antimicrobials as a novel sanitizing process to inactivate *Escherichia coli* O157:H7 on baby spinach. Int J Food Microbiol 153:306–313

Huque R, Wills RBH, Pristijono P, Golding JB (2013) Effect of nitric oxide (NO) and associated control treatments on the metabolism of fresh-cut apple slices in relation to development of surface browning. Posthar Biol Technol 78:16–23

Jian W, Zhang D, Zhu F, Wang S, Zhu T, Pu X, Zheng T, Feng H, Lin H (2015) Nitrate reductase-dependent nitric oxide production is required for regulation alternative oxidase pathway

involved in the resistance to cucumber mosaic virus infection in *Arabidopsis*. Plant Growth Regul 77:99–107

Jiang T, Zheng X, Li J, Jing G, Cai L, Ying T (2011) Integrated application of nitric oxide and modified atmosphere packaging to improve quality retention of button mushroom (*Agaricus bisporus*). Food Chem 126:1693–1699

Jing X, Hou P, Lu Y, Deng S, Li N, Zhao R, Sun J, Wang Y, Han Y, Lang T, Ding M, Shen X, Chen S (2015) Overexpression of copper/zinc superoxide dismutase from mangrove Kandelia candel in tobacco enhances salinity tolerance by the reduction of reactive oxygen species in chloroplast. Front Plant Sci 6:23

Kasukabe Y, He L, Nada K, Misawa S, Ihara I, Tachibana S (2004) Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol 45:712–722

Kim HJ, Fonseca JM, Kubota C, Choi JH (2007) Effect of hydrogen peroxide on quality of fresh-cut tomato. J Food Sci 72:S463–S467

Kim YH, Lim S, Han SH, Lee JJ, Nam KJ, Jeong JC, Lee HS, Kwak SS (2015) Expression of both *CuZnSOD* and *APX* in chloroplasts enhances tolerance to sulfur dioxide in transgenic sweet potato plants. Comp Rend Biol 338:307–313

Kusano T, Suzuki H (2015) Polyamines: a universal molecular nexus for growth, survival, and specialized metabolism. Springer, New York

Lai T, Wang Y, Li B, Qin G, Tian S (2011) Defense responses of tomato fruit to exogenous nitric oxide during postharvest storage. Posthar Biol Technol 62:127–132

Lea US, Ten Hoopen F, Provan F, Kaiser WM, Meyer C, Lillo C (2004) Mutation of the regulatory phosphorylation site of tobacco nitrate reductase results in high nitrite excretion and NO emission from leaf and root tissue. Planta 219:59–65

Lee SH, Ahsan N, Lee KW, Kim DH, Lee DG, Kwak SS, Kwon SY, Kim TH, Lee BH (2007) Simultaneous overexpression of both CuZn superoxide dismutase and ascorbate peroxidase in transgenic tall fescue plants confers increased tolerance to a wide range of abiotic stresses. J Plant Physiol 164:1626–1638

Lee U, Wie C, Fernandez BO, Feelisch M, Vierling E (2008) Modulation of nitrosative stress by S-nitrosoglutathione reductase is critical for thermotolerance and plant growth in *Arabidopsis*. Plant Cell 20:786–802

Leshem YY, Haramaty E (1996) The characterization and contrasting effects of the nitric oxide free radical in vegetative stress and senescence of *Pisum sativum* Linn. foliage. J Plant Physiol 148:258–263

Leshem YY, Pinchasov Y (2000) Non-invasive photoacoustic spectroscopic determination of relative endogenous nitric oxide and ethylene content stoichiometry during the ripening of strawberries *Fragaria anannasa* (Duch.) and avocados *Persea americana* (Mill.). J Exp Bot 51:1471–1473

Leshem YY, Wills R, VengVa KV (1998) Evidence for the function of the free radical gas—nitric oxide ($NO^{\cdot}$)—as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiol Biochem 36:825–833

Leterrier M, Chaki M, Airaki M, Valderrama R, Palma JM, Barroso JB, Corpas FJ (2011) Function of *S*-nitrosoglutathione reductase (GSNOR) in plant development and under biotic/abiotic stress. Plant Signal Behav 6:789–793

Li Y, Wu C (2013) Enhanced inactivation of *Salmonella typhimurium* from blueberries by combinations of sodium dodecyl sulfate with organic acids or hydrogen peroxide. Food Res Int 54:1553–1559

Li G, Zhu S, Wu W, Zhang C, Peng Y, Wang Q, Shi J (2017) Exogenous nitric oxide induces disease resistance against *Monilinia fructicola* through activating the phenylpropanoid pathway in peach fruit. J Sci Food Agric 97:3030–3038

Liu YB, Yang X (2016) Prospect of nitric oxide as a new fumigant for postharvest pest control. In: Controlled atmosphere & fumigation in stored products international conference, pp 161–166

Liu ZJ, Guo YK, Bai JG (2010) Exogenous hydrogen peroxide changes antioxidant enzyme activity and protects ultrastructure in leaves of two cucumber ecotypes under osmotic stress. J Plant Growth Regul 29:171–183

Liu YB, Yang X, Simmons G (2016) Efficacy of nitric oxide fumigation for controlling codling moth in apples. Insects 7:71

Magalhaes J, Monte D, Durzan D (2000) Nitric oxide and ethylene emission in *Arabidopsis thaliana*. Physiol Mol Biol Plant 6:117–127

Malik SI, Hussain A, Yun BW, Spoel SH, Loake GJ (2011) GSNOR-mediated de-nitrosylation in the plant defence response. Plant Sci 181:540–544

Manjunatha G, Lokesh V, Neelwarne B (2010) Nitric oxide in fruit ripening: trends and opportunities. Biotechnol Adv 28:489–499

Manjunatha G, Gupta KJ, Lokesh V, Mur LA, Neelwarne B (2012) Nitric oxide counters ethylene effects on ripening fruits. Plant Signal Behav 7:476–483

Marco F, Bitrián M, Carrasco P, Alcázar R, Tiburcio AF (2015) Polyamine biosynthesis engineering as a tool to improve plant resistance to abiotic stress. In: Jaiwal PK, Singh RP, Dhankher OP (eds) Genetic manipulation in plants for mitigation of climate change. Springer, Cham, pp 103–116

Martin H, Maris P (2012) Synergism between hydrogen peroxide and seventeen acids against six bacterial strains. J Appl Microbiol 113:578–590

Marvasi M (2017) Potential use and perspectives of nitric oxide donors in agriculture. J Sci Food Agric 97:1065–1072

Melo NKG, Bianchetti RE, Lira BS, Oliveira PMR, Zuccarelli R, Dias DLO, Demarco D, Peres LEP, Rossi M, Freschi L (2016) Nitric oxide, ethylene and auxin crosstalk mediates greening and plastid development in deetiolating tomato seedlings. Plant Physiol 70:2278–2294

Mittler R (2017) ROS are good. Trend Plant Sci 22:11–19

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

Montilla-Bascón G, Rubiales D, Hebelstrup KH, Mandon J, Harren FJM, Cristescu SM, Mur LAJ, Prats E (2017) Reduced nitric oxide levels during drought stress promote drought tolerance in barley and is associated with elevated polyamine biosynthesis. Sci Rep 7:13311

Moschou PN, Paschalidis KA, Roubelakis-Angelakis KA (2008) Plant polyamine catabolism: the state of the art. Plant Signal Behav 3:1061–1066

Mur LA, Mandon J, Persijn S, Cristescu SM, Moshkov IE, Novikova GV, Hall MA, Harren FJ, Hebelstrup KH, Gupta KJ (2013) Nitric oxide in plants: an assessment of the current state of knowledge. AoB Plant 5:pls052

Neill S, Barros R, Bright J, Desikan R, Hancock J, Harrison J, Morris P, Ribeiro D, Wilson I (2008) Nitric oxide, stomatal closure, and abiotic stress. J Exp Bot 59:165–176

Niu L, Liao W (2016) Hydrogen peroxide signaling in plant development and abiotic responses: crosstalk with nitric oxide and calcium. Front Plant Sci 7:230

Noctor G, Reichheld JP, Foyer CH (2018) ROS-related redox regulation and signaling in plants. Semin Cell Dev Biol 80:3–12

Oliveira HC, Gomes BC, Pelegrino MT, Seabra AB (2016) Nitric oxide-releasing chitosan nanoparticles alleviate the effects of salt stress in maize plants. Nitric Oxide 61:10–19

Palma JM, Gupta DK, Corpas FJ (2013) Metalloenzymes involved in the metabolism of reactive oxygen species and heavy metal stress. In: Gupta DK, Palma JM, Corpas FJ (eds) Heavy metal stress in plants. Springer, Heidelberg, pp1-17.

Parra-Lobato MC, Gómez-Jiménez MC (2011) Polyamine-induced modulation of genes involved in ethylene biosynthesis and signalling pathways and nitric oxide production during olive mature fruit abscission. J Exp Bot 62:4447–4465

Peng L, Yang S, Li Q, Jiang Y, Joyce DC (2008) Hydrogen peroxide treatments inhibit the browning of fresh-cut Chinese water chestnut. Posthar Biol Technol 47:260–266

Pereira AES, Narciso AM, Seabra AB, Fraceto LF (2015) Evaluation of the effects of nitric oxide-releasing nanoparticles on plants. J Phys Conf Ser 617:012025

Potikha TS, Collins CC, Johnson DI, Delmer DP, Levine A (1999) The involvement of hydrogen peroxide in the differentiation of secondary walls in cotton fibers. Plant Physiol 119:849–858

Pristijono P, Wills RBH, Golding JB (2008) Use of the nitric oxide-donor compound, diethylenetriamine-nitric oxide (DETANO), as an inhibitor of browning in apple slices. J Hortic Sci Biotechnol 83:555–558

Rizhsky L, Hallak-Herr E, Van Breusegem F, Rachmilevitch S, Barr JE, Rodermel S, Inze D, Mittler R (2002) Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J 32:329–342

Rodríguez-Ruiz M, Mateos RM, Codesido V, Corpas FJ, Palma JM (2017a) Characterization of the galactono-1,4-lactone dehydrogenase from pepper fruits and its modulation in the ascorbate biosynthesis. Role of nitric oxide. Red Biol 12:171–181

Rodríguez-Ruiz M, Mioto P, Palma JM, Corpas FJ (2017b) *S*-nitrosoglutathione reductase (GSNOR) activity is down-regulated during pepper fruit ripening. Nitric Oxide 68:51–55

Roy M, Wu R (2002) Overexpression of *S*-adenosylmethionine decarboxylase gene in rice increases polyamine level and enhances sodium chloridestress tolerance. Plant Sci 163:987–992

Rustérucci C, Espunya MC, Díaz M, Chabannes M, Martínez MC (2007) *S*-nitrosoglutathione reductase affords protection against pathogens in *Arabidopsis*, both locally and systemically. Plant Physiol 143:1282–1292

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

Saltveit ME (2003) Is it possible to find an optimal controlled atmosphere? Posthar Biol Technol 27:3–13

Samis K, Bowley S, McKersie B (2002) Pyramiding Mn-superoxide dismutase transgenes to improve persistence and biomass production in alfalfa. J Exp Bot 53:1343–1350

Saraiva JA, Cláudia CS, Nunes S, Coimbra MA (2010) Purification and characterization of Olive (*Olea europaea* L.) Peroxidases A2—Preedy, Victor R. In: Watson RR (ed) Olives and olive oil in health and disease prevention. Academic, San Diego, pp 325–332

Sato Y, Masuta Y, Saito K, Murayama S, Ozawa K (2011) Enhanced chilling tolerance at the booting stage in rice by transgenic overexpression of the ascorbate peroxidase gene, OsAPXa. Plant Cell Rep 30:399–406

Savvides A, Ali S, Tester M, Fotopoulos V (2016) Chemical priming of plants against multiple abiotic stresses: mission possible? Trend Plant Sci 21:329–340

Saxena I, Srikanth S, Chen Z (2016) Cross Talk between H_2O_2 and interacting signal molecules under plant stress response. Front Plant Sci 7:570

Schenk M, García Loredo A, Raffellini S, Alzamora SM, Guerrero S (2012) The effect of UV-C in combination with H_2O_2 treatments on microbial response and quality parameters of fresh cut pear discs. Int J Food Sci Technol 47:1842–1851

Schieber M, Chandel NS (2014) ROS function in redox signaling and oxidative stress. Curr Biol 24: R453–R462

Seabra AB, Rai M, Durán N (2014) Nano carriers for nitric oxide delivery and its potential applications in plant physiological process: a mini review. J Plant Biochem Biotechnol 23:1–10

Sevilla F, Camejo D, Ortiz-Espin A, Calderon A, Lazaro JJ, Jimenez A (2015) The thioredoxin/peroxiredoxin/sulfiredoxin system: current overview on its redox function in plants and regulation by reactive oxygen and nitrogen species. J Exp Bot 66:2945–2955

Shi HT, Li RJ, Cai W, Liu W, Wang CL, Lu YT (2012) Increasing nitric oxide content in Arabidopsis thaliana by expressing rat neuronal nitric oxide synthase resulted in enhanced stress tolerance. Plant Cell Physiol 53:344–357

Shikanai T, Takeda T, Yamauchi H, Sano S, Tomizawa KI, Yokota A, Shigeoka S (1998) Inhibition of ascorbate peroxidase under oxidative stress in tobacco having bacterial catalase in chloroplasts. FEBS Lett 428:47–51

Silveira AC, Conesa A, Aguayo E, Artes F (2008) Alternative sanitizers to chlorine for use on fresh-cut "Galia" (*Cucumis melo* var. catalupensis) melon. J Food Sci 73:405–411

Singh S, Singh Z, Swinny E (2009) Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina* Lindell). Posthar Biol Technol 53:101–108

Soegiarto L, Wills R (2004) Short term fumigation with nitric oxide gas in air to extend the postharvest life of broccoli, green bean, and bok choy. Hortic Technol 14:538–540

Sofo A, Scopa A, Nuzzaci M, Vitti A (2015) Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. Int J Mol Sci 16:13561–13578

Sun Y, Wang H, Liu S, Peng X (2016) Exogenous application of hydrogen peroxide alleviates drought stress in cucumber seedlings. South Afr J Bot 106:23–28

Suslow T (1997) Postharvest chlorination: basic properties & key points for effective distribution. UC Agric Nat Resour 1–8

Tada Y, Spoel SH, Pajerowska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X (2008) Plant immunity requires conformational changes of NPR1 via *S*-nitrosylation and thioredoxins. Science 321:952–956

Takáč T, Obert B, Rolčík J, Šamaj J (2016) Improvement of adventitious root formation in flax using hydrogen peroxide. New Biotechnol 33:728–734

Takahashi H, Chen Z, Du H, Liu Y, Klessig DF (1997) Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels. Plant J 11:993–1005

Tang L, Kwon SY, Kim SH, Kim JS, Choi JS, Cho KY, Sung CK, Kwak SS, Lee HS (2006) Enhanced tolerance of transgenic potato plants expressing both superoxide dismutase and ascorbate peroxidase in chloroplasts against oxidative stress and high temperature. Plant Cell Rep 25:1380–1386

Terzi R, Kadioglu A, Kalaycioglu E, Saglam A (2014) Hydrogen peroxide pretreatment induces osmotic stress tolerance by influencing osmolyte and abscisic acid levels in maize leaves. J Plant Interact 9:559–565

Tichá T, Sedlářová M, Činčalová L, Trojanová ZD, Mieslerová B, Lebeda A, Luhová L, Petřivalský M (2018) Involvement of S-nitrosothiols modulation by S-nitrosoglutathione reductase in defence responses of lettuce and wild *Lactuca* spp. to biotrophic mildews. Planta 247:1203–1215

Tseng MJ, Liu CW, Yiu JC (2007) Enhanced tolerance to sulfur dioxide and salt stress of transgenic Chinese cabbage plants expressing both superoxide dismutase and catalase in chloroplasts. Plant Physiol Biochem 45:822–833

Tun NN, Santa-Catarina C, Begum T, Silveira V, Handro W, Floh EI, Scherer GF (2006) Polyamines induce rapid biosynthesis of nitric oxide (NO) in *Arabidopsis thaliana* seedlings. Plant Cell Physiol 47:346–354

Ukuku DO (2004) Effect of hydrogen peroxide treatment on microbial quality and appearance of whole and fresh-cut melons contaminated with *Salmonella* spp. Int J Food Microbiol 95:137–146

Ukuku DO, Pilizota V, Sapers GM (2004) Effect of hot water and hydrogen peroxide treatments on survival of *Salmonella* and microbial quality of whole and fresh-cut cantaloupe. J Food Prot 67:432–437

Ukuku DO, Bari ML, Kawamoto S, Isshiki K (2005) Use of hydrogen peroxide in combination with nisin, sodium lactate and citric acid for reducing transfer of bacterial pathogens from whole melon surfaces to fresh-cut pieces. Int J Food Microbiol 104:225–233

Vaucheret H, Palauqui JC, Mourrain P, Elmayan T (1997) Nitrate reductase and nitrite reductase as targets to study gene silencing phenomena in transgenic plants. Euphytica 93:195–200

Wang J, Zhang H, Allen RD (1999) Overexpression of an Arabidopsis peroxisomal ascorbate peroxidase gene in tobacco increases protection against oxidative stress. Plant Cell Physiol 40:725–732

Wang D, Li L, Xu Y, Limwachiranon J, Li D, Ban Z, Luo Z (2017) Effect of exogenous nitro oxide on chilling tolerance, polyamine, proline, and gamma-aminobutyric acid in bamboo shoots (*Phyllostachys praecox* f. *prevernalis*). J Agric Food Chem 65:5607–5613

Wills RBH, Ku VVV, Leshem YY (2000) Fumigation with nitric oxide to extend the postharvest life of strawberries. Posthar Biol Technol 18:75–79

Wills RBH, Pristijono P, Golding JB (2008) Browning on the surface of cut lettuce slices inhibited by short term exposure to nitric oxide (NO). Food Chem 107:1387–1392

Wu G, Shortt BJ, Lawrence EB, Levine EB, Fitzsimmons KC, Shah DM (1995) Disease resistance conferred by expression of a gene encoding H_2O_2-generating glucose oxidase in transgenic potato plants. Plant Cell 7:1357–1368

Wu G, Shortt BJ, Lawrence EB, Leon J, Fitzsimmons KC, Levine EB, Raskin I, Shah DM (1997) Activation of host defense mechanisms by elevated production of H_2O_2 in transgenic plants. Plant Physiol 115:427

Wu F, Yang H, Chang Y, Cheng J, Bai S, Yin J (2012) Effects of nitric oxide on reactive oxygen species and antioxidant capacity in Chinese Bayberry during storage. Sci Hortic 135:106–111

Wu W, Gaucher C, Fries I, Hu XM, Maincent P, Sapin-Minet A (2015) Polymer nanocomposite particles of S-nitrosoglutathione: a suitable formulation for protection and sustained oral delivery. Int J Pharm 495:354–361

Xie Y, Mao Y, Lai D, Zhang W, Zheng T, Shen W (2013) Roles of NIA/NR/NOA1-dependent nitric oxide production and HY1 expression in the modulation of *Arabidopsis* salt tolerance. J Exp Bot 64:3045–3060

Xu WF, Shi WM, Ueda A, Takabe T (2008) Mechanisms of salt tolerance in transgenic *Arabidopsis thaliana* carrying a peroxisomal ascorbate peroxidase gene from Barley11Project supported by a grant of CAS (Chinese Academy of Sciences), Research Program on Soil Biosystems and Agro-Product Safety (No. CXTD-Z2005-4) and the Knowledge Innovation Project of CAS (No. KZCX3-SW-439). Pedosphere 18:486-495.

Xu S, Guerra D, Lee U, Vierling E (2013) *S*-nitrosoglutathione reductases are low-copy number, cysteine-rich proteins in plants that control multiple developmental and defense responses in *Arabidopsis*. Front Plant Sci 4:1–13

Xu J, Yang J, Duan X, Jiang Y, Zhang P (2014) Increased expression of native cytosolic Cu/Zn superoxide dismutase and ascorbate peroxidase improves tolerance to oxidative and chilling stresses in cassava (*Manihot esculenta* Crantz). BMC Plant Biol 14:208–208

Yan J, Wang J, Tissue D, Holaday AS, Allen R, Zhang H (2003) Photosynthesis and seed production under water-deficit conditions in transgenic tobacco plants that overexpress an *Arabidopsis* ascorbate peroxidase gene. Crop Sci 43:1477–1483

Yan H, Li Q, Park SC, Wang X, Liu YJ, Zhang YG, Tang W, Kou M, Ma DF (2016) Overexpression of CuZnSOD and APX enhance salt stress tolerance in sweet potato. Plant Physiol Biochem 109:20–27

Yiu JC, Tseng MJ (2005) Manipulation of superoxide dismutase and catalase to enhance sulfur dioxide tolerance in transgenic Chinese cabbage. In: International Society for Horticultural Science (ISHS), Leuven, Belgium, pp 91–100

Zaharah SS, Singh Z (2011) Postharvest nitric oxide fumigation alleviates chilling injury, delays fruit ripening and maintains quality in cold-stored 'Kensington Pride' mango. Posthar Biol Technol 60:202–210

Zelko IN, Mariani TJ, Folz RJ (2002) Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radic Biol Med 33:337–349

Zhan N, Wang C, Chen L, Yang H, Feng J, Gong X, Ren B, Wu R, Mu J, Li Y, Liu Z, Zhou Y, Peng J, Wang K, Huang X, Xiao S, Zou J (2018) *S*-nitrosylation targets GSNO reductase for selective autophagy during hypoxia responses in plants. Mol Cell 71:142–154

Zhang Q, Ma C, Xue X, Xu M, Li J, Wu JX (2014) Overexpression of a cytosolic ascorbate peroxidase gene, osapx2, increases salt tolerance in transgenic alfalfa. J Integr Agric 13:2500–2507

Zhao MG, Chen L, Zhang LL, Zhang WH (2009) Nitric reductase-dependent nitric oxide production is involved in cold acclimation and freezing tolerance in *Arabidopsis*. Plant Physiol 151:755–767

Zhao C, Cai S, Wang Y, Chen ZH (2016) Loss of nitrate reductases NIA1 and NIA2 impairs stomatal closure by altering genes of core ABA signaling components in *Arabidopsis*. Plant Signal Behav 11:e1183088

Zhou S, Jia L, Chu H, Wu D, Peng X, Liu X, Zhang J, Zhao J, Chen K, Zhao L (2016a) *Arabidopsis* CaM1 and CaM4 promote nitric oxide production and salt resistance by inhibiting *S*-nitrosoglutathione reductase via direct binding. PLoS Genet 12:e1006255

Zhou Y, Li S, Zeng K (2016b) Exogenous nitric oxide-induced postharvest disease resistance in citrus fruit to *Colletotrichum gloeosporioides*. J Sci Food Agric 96:505–512

Zhu S, Zhou J (2006) Effects of nitric oxide on fatty acid composition in peach fruits during storage. J Agric Food Chem 54:9447–9452

Zhu SH, Zhou J (2007) Effect of nitric oxide on ethylene production in strawberry fruit during storage. Food Chem 100:1517–1522

Zhu LQ, Zhou J, Shu-Hua Z, Lai-Hui G (2009) Inhibition of browning on the surface of peach slices by short-term exposure to nitric oxide and ascorbic acid. Food Chem 114:174–179

Zhu LQ, Zhou J, Zhu SH (2010) Effect of a combination of nitric oxide treatment and intermittent warming on prevention of chilling injury of 'Feicheng' peach fruit during storage. Food Chem 121:165–170