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

J. C. Begara-Morales · M. Chaki · R. Valderrama · C. Mata-Pérez · M. Padilla · J. B. Barroso (✉)
Group of Biochemistry and Cell Signaling in Nitric Oxide, Department of Experimental Biology, Center for Advanced Studies in Olive Grove and Olive Oils, Faculty of Experimental Sciences, Campus Universitario "Las Lagunillas" s/n, University of Jaén, Jaén, Spain
e-mail: jbarroso@ujaen.es

D. K. Gupta et al. (eds.), *Nitric Oxide and Hydrogen Peroxide Signaling in Higher Plants*, https://doi.org/10.1007/978-3-030-11129-8_4

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

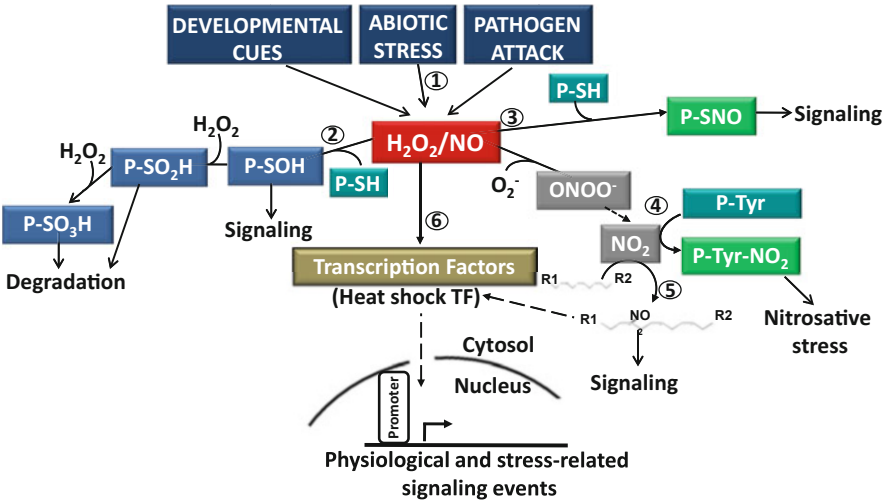

Fig. 1 Hydrogen peroxide and nitric oxide-dependent signaling events. Different molecular cues derived from developmental processes or different stress situations, among others, can induce the production of hydrogen peroxide (H_2O_2) and nitric oxide (NO) (1). These signaling molecules can transmit their 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.

Acknowledgments JCBM wishes to thank the Ministry of Economy and Competitiveness (Spain) for postdoctoral research funding within the Juan de la Cierva-Incorporación program. The work in our lab is supported by the ERDF grants co-financed by the Ministry of Economy and Competitiveness (projects BIO2015-66390-P) and the Junta de Andalucía (group BIO286) in Spain.

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