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REVIEW PAPER

Diverse functional interactions between nitric oxide and abscisic acid in plant development and responses to stress

José León[1],*, Mari Cruz Castillo[1], Alberto Coego[1], Jorge Lozano-Juste[1,2] and Ricardo Mir[1]

[1] Plant Development and Hormone Action, Instituto de Biología Molecular y Celular de Plantas (CSIC-Universidad Politécnica de Valencia), Spain
[2] Department of Botany and Plant Sciences and Center for Plant Cell Biology and Institute for Integrative Genome Biology, University of California, Riverside, CA, USA

* To whom correspondence should be addressed. E-mail: jleon@ibmcp.upv.es

Abstract

The extensive support for abscisic acid (ABA) involvement in the complex regulatory networks controlling stress responses and development in plants contrasts with the relatively recent role assigned to nitric oxide (NO). Because treatment with exogenous ABA leads to enhanced production of NO, it has been widely considered that NO participates downstream of ABA in controlling processes such as stomata movement, seed dormancy, and germination. However, data on leaf senescence and responses to stress suggest that the functional interaction between ABA and NO is more complex than previously thought, including not only cooperation but also antagonism. The functional relationship is probably determined by several factors including the time- and place-dependent pattern of accumulation of both molecules, the threshold levels, and the regulatory factors important for perception. These factors will determine the actions exerted by each regulator. Here, several examples of well-documented functional interactions between NO and ABA are analysed in light of the most recent reported data on seed dormancy and germination, stomata movements, leaf senescence, and responses to abiotic and biotic stresses.

Key words: ABA, abiotic stress, defence, leaf senescence, nitric oxide, seed germination, stomatal closure.

Introduction

Plant growth is the successful consequence of a finely regulated network of hormone-controlled metabolic processes. Endogenous metabolic cues and exogenous environmental factors are transmitted through intricate signalling pathways that involve the perception of different stimuli and the downstream alteration of multiple processes. Changes in ion channels, enzyme activities, levels of reactive molecules, post-translational modification, and localization of proteins are often linked to modification of gene expression. The proper execution of such a complex scenario of regulatory events allows the plant to complete developmental transitions throughout its life cycle. However, plants do not perceive only

the effects of positive environmental stimuli. Some environmental stress conditions of both biotic and abiotic origin affect plants negatively. Because plants cannot travel and their capacity for movement is limited, they rely on the plasticity of metabolism and the versatility of hormonal regulation to avoid detrimental effects of stress.

The small molecule nitric oxide (NO) has recently been characterized as a co-regulator of many plant processes. This small molecule has the peculiarity of being a gas and also of being a free radical. These physicochemical features determine NO regulatory functions as a result of the balance between diffusivity from the biosynthesis site, and reactivity

with components of the cellular microenvironment surrounding its production site. Its regulatory role is often exerted in connection with the classical hormones auxins, cytokinins, gibberellins (GAs), ethylene, and abscisic acid (ABA), as well as the more recently characterized jasmonates, salicylates, brassinosteroids, and strigolactones. Functional interactions between most of the hormones and regulators mentioned above have been reported in plant development and responses to stress (Durbak *et al.*, 2012; Freschi 2013; Simontacchi *et al.*, 2013). Because some of the NO effects on plant physiology have been particularly well studied in processes such as seed germination and guard-cell movement controlling stomatal closure, both being critically regulated by ABA, this review will focus on the functional connections between ABA and NO. Although both molecules have mostly been considered as functioning in the same direction, with NO acting as a second messenger of ABA (Hancock *et al.*, 2011), there is much evidence suggesting that NO and ABA do not always function in this way. In fact, NO may exert a general negative regulation on ABA perception that may function as a feedback-loop mechanism to fine tune the magnitude or intensity of ABA-triggered responses (Lozano-Juste and León, 2010b). Special emphasis will be placed on these cases in this review, with the final goal of discussing how the diverse functional interactions between NO and ABA fit with our current knowledge of different plant physiological processes ranging from seed germination to different responses to stress.

Controversy surrounding NO biosynthesis and its connection to ABA

Pharmacological and genetic approaches have been used to elucidate the existence of different NO sources in plants. However, significant controversy still remains on our current knowledge of how NO is synthesized in plant cells. It has been reported that ABA induces NO production in plants (Guo *et al.*, 2003), and also that NO synthesis requires enhanced production of H_2O_2 (Bright *et al.*, 2006). The use of mammalian NO synthase (NOS) inhibitors, such as L-NG-nitroarginine methyl ester (L-NAME), has been reported to reduce NO levels (Corpas *et al.*, 2006), thus suggesting the existence or arginine-dependent production of NO in plants. In *Arabidopsis thaliana*, there are contradictory published data, either describing no inhibition (Desikan *et al.*, 2002) or L-NAME-inhibited NO production by ABA (Guo *et al.*, 2003; Bright *et al.*, 2006). Alternatively, by using nitrate reductase (NR) inhibitors, the role of this enzyme in ABA-induced NO production has also been probed (Neill *et al.*, 2003). In parallel, several genetic studies have been carried out to uncover the source of ABA-induced NO production. First, the *nia1nia2* double mutant, with less than 1% of NR activity, showed a reduced accumulation of NO in guard cells (Desikan *et al.*, 2002). Later work suggested that NIA1 but not NIA2 is required for NO production in response to ABA (Ribeiro *et al.*, 2009). Secondly, Guo *et al.*, (2003) showed that the *Atnoa1* mutant has reduced accumulation of NO in response to ABA in both the root tip and the guard cells, thus

pointing to cell-type-independent impaired responses to NO in this mutant. Here, it should nevertheless be highlighted that either pharmacological or genetic approaches used for elucidating NO biosynthesis in plants have also reported weaknesses that should be borne in mind when analysing NO production. Interestingly, L-NAME has recently been reported to inhibit the oligogalacturonide-induced NR activity and consequent nitrite-dependent NO production (Rasul *et al.*, 2012b). However, NO production was still detected in L-NAME-treated *nia1nia2* mutant plants (Rasul *et al.*, 2012b), indicating complex interactions between NO derived from arginine or from nitrite. Despite L-NAME has being reported to inhibit NR activity only in the context of oligogalacturonide-activated defence responses, this effect might also be functional in other circumstances. On the other hand, it has been reported that the *nia1nia2* plants contained low endogenous levels or arginine, thus also suggesting the possible defective arginine-dependent production of NO in NR mutant plants (Modolo *et al.*, 2006). The *NOA1* gene codes for a cGTPase (Moreau *et al.*, 2008) and not for a NOS-like enzyme as considered previously. It has been proposed that the reduced NO content in the *Atnoa1* mutant is the result of indirect effects derived from altered protein synthesis in chloroplasts (Liu H *et al.*, 2010a). Finally, the generation of a triple *nia1nia2noa1-2* mutant affected in both NR activity and NOA1 function revealed that both pathways are independent and contribute to NO production in response to ABA in *Arabidopsis* (Lozano-Juste and León, 2010a). Therefore, despite the possible interaction between both genetic pathways, other pathways have also been proposed to be involved in NO production. Several reviews published recently have addressed in detail the different origins and biosynthetic pathways of NO in plants (Moreau *et al.*, 2010; Gupta *et al.*, 2011; Mur *et al.*, 2013). Consequently, emphasis should be placed on the assessment of advantages/weaknesses of pharmacological and genetic approaches when studying NO function in plants. Moreover, additional caution should be taken when the analysis of NO production is performed in different plant organs or under different physiological conditions, where the contribution of different components and pathways to NO synthesis might also be different. As an example, ABA-triggered production of NO might be different in roots and shoots of the same plant, or even when comparing mesophyll with guard cells on the same leaf.

Role of NO in ABA-inhibited seed germination

The germination of seeds occurs only when dormancy, which negatively controls germination, is released. It is widely assumed that dormancy is directly promoted by endogenous levels of ABA and also that germination is enhanced by GAs (Finch-Savage and Leubner-Metzger, 2006). Both hormones interplay with other components such as ethylene to control seed germination (Finkelstein *et al.*, 2008; Holdsworth *et al.*, 2008; Graeber *et al.*, 2012). Although dormancy can be seen as a negative input for plant development, it is, in turn, an

essential quality of seeds of many species that allow them to keep quiescent until environmental conditions allow a future successful development.

In many plant species, endogenous cues as well as environmental factors favour seed germination through the mobilization and use of endosperm carbohydrate and lipid reserves. These catabolic processes allow embryo root to grow and emerge through the seed coat. Besides the positive and negative effects exerted by GAs and ABA on these events, several other regulatory components have been identified that influence seed germination. Nitrate, a major nitrogen source for plants, as well as nitrite have been reported to promote seed dormancy release (Bethke *et al.*, 2006*b*). Exogenously supplied nitrate seems to promote seed germination (Alboresi *et al.*, 2005) through the regulation of phytochrome signalling (Batak *et al.*, 2002). Because seed levels of nitrate and ABA are negatively correlated, and the expression of ABA catabolic genes is positively regulated by NO (Matakiadis *et al.*, 2009), nitrate effects on seed dormancy and germination are probably exerted through the stimulation of NO-mediated ABA catabolism. A more complex regulatory model including nitrogen and reactive oxygen species (ROS), such as NO and H_2O_2, respectively, has emerged to better explain the transition from dormancy to germination. These types of reactive species would act as synergistic effectors in releasing dormancy by acting upstream of ABA. Despite the fact that ABA induces NO production, it remains controversial which reductive or oxidative pathway for NO biosynthesis is functional in seeds (recently reviewed by Arc *et al.*, 2013). Nevertheless, it is well known that NO does not cooperate with ABA in inhibiting seed germination but, in contrast, promotes it (Beligni and Lamattina, 2000; Libourel *et al.*, 2006; Bethke *et al.*, 2007). Because treatment with NO scavengers reduces germination in dormant but not in non-dormant *Arabidopsis* seeds, the positive effect exerted by NO seems to be due mainly to released dormancy (Bethke *et al.*, 2006*a*, *b*). NO scavengers have been also reported to prevent germination of imbibed tomato seeds treated with the ABA biosynthesis inhibitor fluridone (Piterková *et al.*, 2012). In turn, NO donors potentiated the germination induced by the ABA biosynthesis inhibitor norflurazon and reduced the sensitivity to ABA in *Arabidopsis* seeds (Bethke *et al.*, 2006*a*). In agreement with this pharmacological approach, seeds of NO-deficient *Arabidopsis* mutants are hypersensitive to ABA and have enhanced dormancy and reduced germination potential (Lozano-Juste and León, 2010*a*). Moreover, increased production of NO due to both NR- and NOS-like activities has been described in embryos upon imbibition of seeds (Simontacchi *et al.*, 2007). NO derived from the storage form nitrosoglutathione (GSNO) also seems to be relevant to control seed germination, as demonstrated by the reduced germination of *atgsnor1-3* mutant seeds with loss of GSNO reductase function (Kwon *et al.*, 2012). Although still far from being completely known, the mechanism underlying the releasing effect exerted by NO on dormancy seems to involve the production and perception of NO in aleurone cells (Bethke, 2009), the promotion of GA synthesis and downstream signalling in the embryo, and a decrease

in ABA content by upregulating the metabolic *CYP707A2* gene encoding an enzyme with (+)-abscisic acid 8′-hydroxylase activity (Liu *et al.*, 2009). Both negative and positive effects exerted on ABA and GA accumulation and signalling, respectively, are the result of H_2O_2-triggered events that are mediated by NO (Liu Y *et al.*, 2010*b*). Despite the existence of extensive experimental data supporting the role of NO in promoting ABA catabolism, it has also been proposed that NO might function by decreasing the sensitivity of seeds to ABA (Bethke *et al.*, 2006*a*, *b*; Lozano-Juste and León, 2010*b*). On the other hand, aquaporin-encoding genes have been characterized as NO-inducible targets promoting seed germination in rice (Liu *et al.*, 2007), thus supporting the importance of NO-regulated water transport during seed germination. An extensive review describing the current knowledge on how NO and ethylene counteract ABA action, thus releasing dormancy and promoting seed germination, was published recently (Arc *et al.*, 2013).

NO-mediated post-translational modification is a field of growing interest because both cysteine *S*-nitrosylation (Lindermayr *et al.*, 2005) and tyrosine nitration (Lozano-Juste *et al.*, 2011) of proteins have been identified and characterized as an important level of regulation in NO-modulated processes. Interestingly, a functional connection between NO and ABA has been deduced from the proteomic identification of several nitrated proteins including the E3 SUMO-protein ligase SIZ1, and the molybdenum co-factor (Moco) sulfurase encoded by *ABA3* gene (Lozano-Juste *et al.*, 2011), both reported to be related to ABA signalling and biosynthesis. The SUMO E3 ligase SIZ1 has been reported to control ABA-related responses through sumoylation of the transcription factors ABI5 and MYB30 (Miura *et al.*, 2009; Zheng *et al.*, 2012). Moreover, the ABA3/Moco sulfurase is involved in catalysing the conversion between the sulfo- and desulfo-forms of Moco, which are the co-factors of aldehyde oxidase and NRs (Mendel and Hänsch, 2002), involved in ABA and NO biosynthesis, respectively. The identification of nitrated forms of the *S*-adenosylmethionine synthases METK1 and METK2 (Lozano-Juste *et al.*, 2011) involved in the biosynthesis of both ethylene and polyamines opens another interesting functional link between polyamine-triggered NO production, ethylene biosynthesis, and ABA-related signalling (Wimalasekera *et al.*, 2011).

All data available on NO-mediated promotion of seed germination fit with a model (Fig. 1) where NO produced and perceived in the aleurone/endosperm cell layer is followed by increased ABA catabolism in the aleurone and by the synthesis of active GAs in the embryo. The signalling cascade is then directed back to the aleurone where GA-inducible cell-wall degrading enzymes facilitate weakening of the physical barrier that encloses the root embryo, allowing root emergence and thus germination. In this series of events, the above-presented data strongly suggest that NO has a negative role in ABA signalling exerted at least at the ABA homeostasis checkpoint. How NO controls the expression of ABA catabolic genes is still unknown, although the post-translational modification of key proteins is certainly a possibility. The same situation is valid to explain the NO regulation of ABA
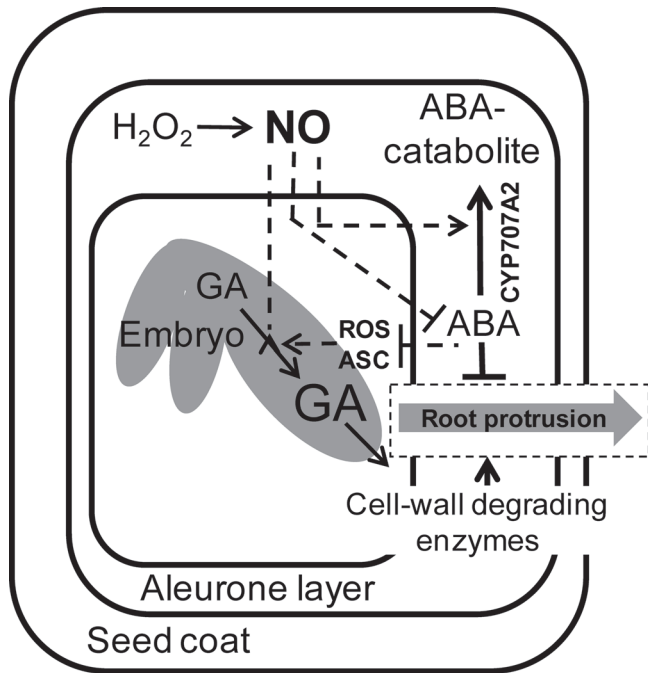
Fig. 1. Functional interactions of NO and ABA during seed germination. Dashed lines indicate positive (arrowheads) or negative (blunt-ended lines) regulatory functions exerted by the different components. ASC, ascorbate.

sensitivity, where nitration of regulatory proteins could affect transcription factors required for ABA signalling.

NO–ABA interactions in the control of stomata movements

Guard cells are unique specialized epidermal plant cells that, in pairs, form the stomata, which are involved in the precise control of gas exchange in leaves. Stomatal closure is the result of water-content-driven changes in the turgor of guard cells. ABA has been considered as a major component controlling guard cell signalling acting in close connection with other hormones, and NO has emerged lately as an important signalling molecule in the control of stomata movements (Daszkowska-Golec and Szarejko, 2013). It has been reported that ABA induces an increase in NO that is necessary for ABA-triggered stomatal closure or inhibition of opening (Desikan *et al.*, 2002). An extensive body of evidences has supported an established model assigning a positive role for NO in ABA signalling controlling stomata movements. However, there are some recently published data that contradict this assumption. It is important for NO researchers to be aware of such controversy and to keep it in mind for a careful interpretation of future work. We will highlight these controversial issues in this review.

A pharmacological approach led to the finding that NO, released by the application of NO donors, such as sodium nitroprusside (SNP), *S*-nitroso-*N*-acetyl-DL-penicillamine and GSNO, induced stomatal closure in different plant species. Concomitantly, treatment with the NO scavenger

2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyL-3-oxide (cPTIO) prevented ABA-induced stomatal closure (Garcia-Mata and Lamattina, 2001, 2002; Desikan *et al.*, 2002; Neill *et al.*, 2002*a*). Although NO scavenging by cPTIO attenuated stomatal closure induced by ABA, this scavenger did not block stomatal closure completely. These data suggest the existence of ABA-dependent NO-independent stomatal closure pathways, although it cannot be ruled out that incomplete NO scavenging may be the cause of partial stomata responses. Supporting a NO regulatory role in stomata movements, pharmacological and genetic approaches have demonstrated that NO is synthesized in guard cells. However, there is still some controversy regarding data derived from pharmacological approaches that deserves more attention. By using NR inhibitors such as tungstate, the role of this enzyme in ABA-induced NO production as well as its effect on stomatal closure has been demonstrated (Neill *et al.*, 2003). The use of mammalian NOS inhibitors, such as L-NAME, was also reported to reduce NO levels and stomatal closure in plants (Neill *et al.*, 2002*a*, *b*). These data support the contribution of NO biosynthetic pathways to stomata movements. However, it has been reported that NO donors such SNP or GSNO also inhibited NR activity in wheat leaves (Rosales *et al.*, 2011). Both inhibition and activation of NR activity by L-NAME treatment have been reported in *Arabidopsis* (Rasul *et al.*, 2012*b*) and wheat (Rosales *et al.*, 2011), respectively, providing concern as to how this compound alters NO synthesis in plants, and also about the usefulness of these pharmacological approaches. Fortunately, the genetic approach serves as an alternative strategy to overcome doubts generated by the pharmacological characterization. The double *nia1nia2* and the *noa1-1* mutants showed reduced accumulation of NO in guard cells accompanied by impaired stomatal closure in response to ABA (Desikan *et al.*, 2002; Guo *et al.*, 2003). Moreover, the generation of a triple mutant affected in NR activity and NOA1 function revealed that both pathways are independent and contribute to NO production (Lozano-Juste and León, 2010*a*). The accumulation of NO in the guard cells of this triple mutant was undetectable even in the presence of ABA (Lozano-Juste and León, 2010*a*). Therefore, genetic analysis of NO production in guard cells clearly shows that NO is produced in guard cells through both NO biosynthetic pathways. These studies also proved that genetic analysis might be a determinant, especially when pharmacological approaches are not clear enough.

The transport of osmotically active ions and malate across membranes and subsequent membrane depolarization are essential processes in controlling stomatal closure (Pandey *et al.*, 2007). The slow (S)-type anion channel SLAC1 and the potassium inward rectifier channel KAT1 have been characterized as key components in regulating membrane depolarization and stomatal closure, and both are targets of the ABA-related OST1/SnRK2.6 kinase (Sato *et al.*, 2009; Vahisalu *et al.*, 2010). Interestingly, the *slac1* mutant displayed reduced stomatal closure in response to factors different to ABA, including CO_2, ozone, transition from light to darkness, Ca^{2+}, H_2O_2, and NO (Vahisalu *et al.*, 2008). It has also been reported that NO participates in ABA-induced

stomatal closure through the regulation of inward-rectifying K^+ and anion channels (Garcia-Mata et al., 2003). In this context, NO negatively regulates inward-rectifying K^+ channels, while it exerts a positive effect on anion channels (Garcia-Mata et al., 2003). This regulation over inward K^+ channels promotes an increase in cytoplasmic Ca^{2+} (Garcia-Mata et al., 2003). However, because Ca^{2+} is required for NO production but not for NO-induced inhibition of stomatal opening, it seems that Ca^{2+} acts upstream of NO production for the inhibition of stomatal opening by ABA but downstream during ABA-induced stomatal closure (Garcia-Mata and Lamattina, 2007). It is noteworthy that NO also regulates the activity of K^+ efflux channels perhaps through the nitrosylation of cysteine sulfhydryl groups either of the K^+ channel or of a closely associated regulatory protein (Sokolovski and Blatt, 2004).

Several molecules including H_2O_2, cyclic GMP (cGMP), cyclic ADP ribose (cADRP), and Ca^{2+} have been characterized as working together with NO as part of ABA signalling in guard cells. Whereas in wild-type guard cells ABA induces a burst of H_2O_2 and NO, in the *rbohD/F* double mutant affected in NADPH oxidases D and F, H_2O_2 and NO production as well as ABA-induced stomatal closure were impaired (Bright et al., 2006). This points to an ABA-dependent and rbohD/ rbohF-mediated production of NO in guard cells. Moreover, the *nia1nia2* double mutant, but not the *noa1* mutant, is severely affected in NO production in response to H_2O_2 in guard cells (Bright et al., 2006), suggesting that H_2O_2 directly or indirectly affects NR-dependent but not NOA1-related NO production in guard cells. In root cells, H_2O_2 regulation of NR activity involves mitogen-activated protein kinase (MAPK) signalling, in such a way that MAPK6 phosphorylates NIA2 to induce NO production (Wang et al., 2010b). However, in guard cells, *nia2-5* but not *nia1-2* mutant retained wild-type levels of NO production after H_2O_2 treatment (He et al., 2013), suggesting that NIA1 seems to be the isoform required for NO production in response to H_2O_2 in stomata. Whether differences in the NR isoform required for H_2O_2-induced NO production between guard and root cells responds to a tissue-specific H_2O_2–NO signalling is an important and unexplored question. It is noteworthy that crosstalk between these two molecules is likely to be more complex than described above. NO seems to regulate H_2O_2 content by inducing its metabolism (Hasanuzzaman and Fujita, 2013) or by inhibiting its production through NAPDH oxidase *S*-nitrosylation (Yun et al., 2011). This process would represent a negative-feedback loop mechanism by which ABA could induce H_2O_2 production that activates NO biosynthesis that then reduces H_2O_2 levels. Taking into account that H_2O_2 is a positive regulator of ABA-induced stomatal closure, NO could have a dual role in this process, exerting a positive effect probably in fast responses, but acting later as a negative modulator of protein function through post-translational modifications of enzymes or proteins with signalling potential. Such an expanding panorama on NO–ABA interactions weakens the current established model considering NO just as a downstream intermediate in ABA signalling.

As mentioned above, Ca^{2+}, cADPR and cGMP are also involved in NO signalling in guard cells. Even though stomatal closure in the absence of calcium has also been described (Roelfsema and Hedrich, 2010), the entry of Ca^{2+} from the extracellular space as well as its release from intracellular reservoirs are required for a correct response to ABA in guard cells (MacRobbie, 2000). Extracellular Ca^{2+} could be perceived by a membrane receptor in guard cells (Han et al., 2003) or by extracellular calmodulin activating a signalling pathway that includes the α-subunit of G protein (Li et al., 2009). Perception by calmodulin activates AtrbohD/F-dependent H_2O_2 production and, subsequently, NOA1-related NO production (Li et al., 2009). On the other hand, it has also been reported that ABA-induced cytoplasmic Ca^{2+} release might be mediated either by cGMP production (Dubovskaya et al., 2011) or by changes in cADPR levels (Meimoun et al., 2009). Treatment with the cADPR synthesis inhibitor nicotinamide reduces ABA-induced stomatal closure and ion flux through the plasma membrane (Leckie et al., 1998; MacRobbie, 2000) and inhibited ABA and NO-induced stomatal closure in pea (Neill et al., 2002a). NO promotes intracellular Ca^{2+} release and thereby regulates guard cell ion channels via a subset of signalling events provoked by ABA that involve cGMP function (Garcia-Mata et al., 2003). A recent report has described that, in the presence of ROS, ABA and NO trigger the nitration of cGMP to produce 8-nitro-cGMP, which acts as a NO-derived second messenger that promotes stomatal closure in light (Joudoi et al., 2013). On the other hand, the NO-dependent guanylate cyclase mutant (*nogc*) that is impaired in cGMP production induced by NO is also impaired in ABA- and NO-induced stomatal closure (Joudoi et al., 2013). Because 8-bromo-cGMP, a membrane-permeating analogue of cGMP, did not trigger stomatal closure in light and instead promoted stomatal opening in darkness, cGMP and its nitrated derivative probably play different roles in the control of stomatal opening and closure (Joudoi et al., 2013). Thus, both agonistic and antagonistic effects can be depicted in the NO–ABA functional interaction in controlling stomatal closure through secondary metabolites such as cGMP and its derivatives. Interestingly enough, it has been reported that mutant plants severely compromised in NO production are hypersensitive to ABA in stomatal closure and also display a non-wilted phenotype under water shortage and a strong resistance to drought (Lozano-Juste and León, 2010a). Because NO-dependent guanylate cyclase-mediated production of cGMP will be severely reduced in NO-deficient plants, its proposed role in stomatal opening under darkness would also be diminished, thus also leading to closed stomata during the dark periods. Based on these data, the assumption of NO as a simple positive regulator of stomata movements acting downstream of ABA needs to be reassessed. A detailed study of cGMP's role on stomatal closure and the NO-deficient mutant phenotypes points to a new role of NO negatively regulating stomatal closure (Lozano-Juste and León, 2010a; Joudoi et al., 2013). However, more work is needed to fully understand the complex functional interaction between NO and ABA in the stomatal context.

Despite a large amount of information supporting an involvement of NO in the regulation of stomatal closure, only a few reports describe this regulation at the whole-plant level. The majority of papers analysing this response are based on studies with epidermal peelings of leaves. If ABA-induced stomatal closure is mediated by NO, could we tune stomatal closure and therefore drought stress tolerance by regulating NO levels? Desikan et al (2002) reported that they could not observe a wilting phenotype in the *nia1nia2* mutant as expected for a mutant totally blocked in ABA-induced stomatal closure. Furthermore, NO generation seems to be required for the ABA-induced closure of stomata in turgid leaves but not in dehydrated leaves (Ribeiro *et al.*, 2009). Besides, neither wild-type plants treated with NO donors or scavengers nor NO-deficient *nia* mutants showed a significant alteration in the ABA-induced stomatal closure of leaves undergoing water deficit (Ribeiro *et al.*, 2009). Again, this data clearly suggests that NO is not a mere positive regulator of ABA-induced stomatal closure. Actually, these studies described a role of NO on stomatal closure in epidermal peels that did not fit well with the behaviour of whole plants deficient in NO production (Desikan *et al.*, 2002). Additionally it seems that this is related to the plant water content and to the drought conditions (Ribeiro *et al.*, 2009). Taken together, we propose that NO synthesis and action are not required for ABA-mediated stomatal closure during drought responses in whole plants. Moreover, because NO-deficient plants are hypersensitive to ABA (Lozano-Juste and León, 2010*a*), it is logical to propose that, under certain conditions, NO might actually act as an early negative modulator of ABA action, maybe by altering hormone perception (Lozano-Juste and León, 2010*b*).

To summarize, the role that NO has in ABA-induced stomatal closure is still far from being clearly defined. Figure 2 provides a schematic diagram including some, but not all, the regulatory components and their functional interactions in promoting stomatal closure or opening depending on the environmental conditions. The complexity of the NO–ABA interactions is shown by both positive and negative effects exerted by NO on different ABA-regulated targets. The difficulty in matching data from epidermal peels and whole plants makes it difficult to provide conclusive evidence for the role of NO–ABA interaction in regulating stomatal closure. Based on pharmacological approaches in a decontextualized experimental system such as epidermal peels, a large body of data suggest that NO acts downstream of ABA in promoting stomatal closure. However, genetic approaches applied on whole plants under different watering conditions suggest that the role of NO interacting with ABA in guard-cell signalling is primarily devoted to regulating stomatal closure during the light-to-dark transition under non-stressed conditions. In fact, some recent reports suggest, as mentioned above, a negative role of NO in ABA-induced stomatal closure during the night. This contradictory scenario will certainly be clarified when exhaustive molecular and cellular studies are performed on previously identified direct targets of NO.
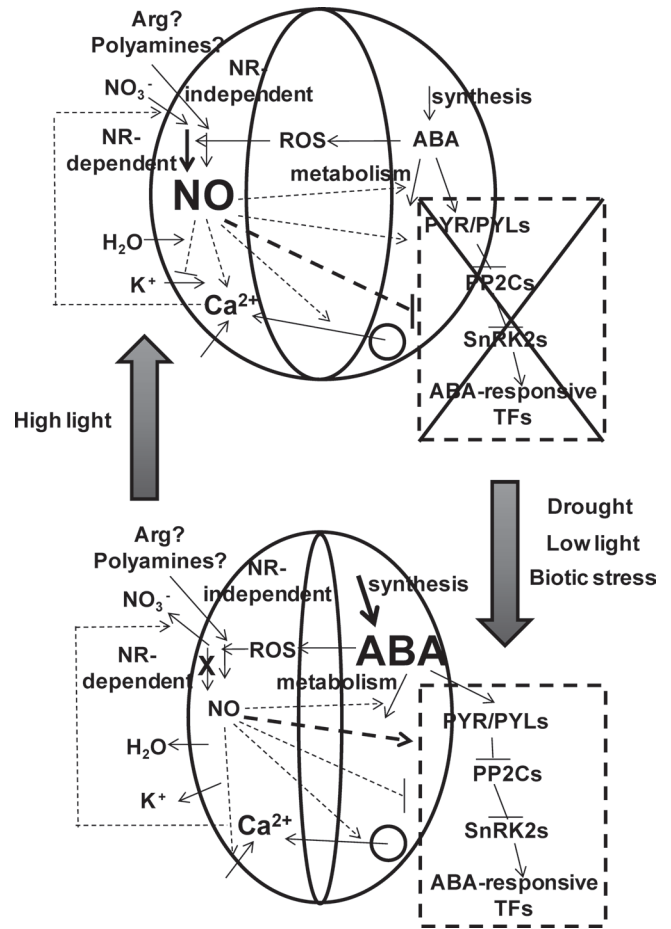

Fig. 2. Stomatal closure and opening are controlled by complex interactions between NO and ABA under different environmental conditions. Dashed lines indicate positive (arrowheads) or negative (blunt-ended lines) regulatory functions exerted by the different components. TFs, transcription factors.

NO and ABA in senescence

Senescence is an active genetically controlled process that affects cells, tissues, organs, and even entire plants during the last developmental stages. It is characterized by a sharp decline in photosynthetic capacity, chlorophyll degradation, visible leaf yellowing, and a decrease in total RNA and protein content. A large number of factors influencing senescence have been described including age, developmental stage, nutrient supply, light, environmental interactions, and classical and newly characterized hormones and growth regulators, as well as varied metabolites (Fischer, 2012). Li *et al.* (2012) recently built gene networks with *A. thaliana* genes promoting or delaying senescence to identify common regulators. Their results demonstrated that cytokinin, auxin, and NO delay leaf senescence, whereas ethylene, ABA, salicylic acid (SA) and jasmonic acid (JA) promote leaf senescence. Many of these regulators might control leaf senescence in coordination with environmental and developmental cues, showing divergence in early senescence initiation-related gene expression but convergence in senescence execution-related molecular processes (Guo and Gan, 2012). Although the putative

connection of NO with ethylene, SA, and JA is very interesting, we are going to focus the next section on the NO–ABA interaction during the senescence process.

Several studies support a negative regulatory role of NO in plant senescence (Guo and Crawford, 2005; de Michele *et al.*, 2009; Procházková and Wilhelmová, 2011). It has been reported that NO production decreases during maturation of plant organs (Leshem *et al.*, 1998) and also that peroxisomes isolated from senescent pea leaves contain lower NO content than that of young leaves (Corpas *et al.*, 2004). Accordingly, NO application has been demonstrated to delay yellowing and to retard the onset of chlorophyll degradation (Laxalt *et al.*, 1997). By contrast, leaf senescence is promoted by reducing NO production in either loss-of-function mutants or upon heterologous inducible expression in *Arabidopsis* of a NO-degrading dioxygenase from *Escherichia coli* (Guo and Crawford, 2005; Mishina *et al.*, 2007; Liu and Guo, 2013). Despite the experimentally supported role for NO in regulating senescence in plants, our knowledge about the dynamics of changes in NO and reactive nitrogen species as well as relevant enzymes during ageing and senescence is still deficient (Procházková and Wilhelmová, 2011). The contradictory effects observed for NO in regulating senescence at different concentrations might be, at least in part, explained by the complex interaction of NO with ROS that has also been characterized as important in regulating cell-death-related senescence symptoms (Li *et al.*, 2012; Wang *et al.*, 2013).

In contrast to the negative regulation exerted by NO on senescence, ABA is one of the most efficient promoters of leaf senescence. Accordingly, increased endogenous ABA levels have been detected in senescent plants, and exogenously applied ABA promotes leaf senescence and induces expression of senescence-related genes in several plant species (Even-Chen and Itai, 1975; Gepstein and Thimann, 1980; Smart, 1994; Yang *et al.*, 2002, 2003). Moreover, several genes involved in ABA synthesis, metabolism, and signalling are upregulated during senescence (Buchanan-Wollaston *et al.*, 2005). As mentioned before for NO, ABA also displays an extensive interaction with ROS in promoting leaf senescence. Elevated ABA levels cause an increased generation of O_2^- and H_2O_2 and the enhanced expression of antioxidant genes and their corresponding enzyme activities, thus protecting the cellular functions required for the onset of senescence (Hung and Kao, 2003). The balance between cellular protection and senescence activities regulated by ABA seems to be determinant in controlling progression of leaf senescence, probably in connection with other senescence-affecting factors such as age (Lim *et al.*, 2007; Khanna-Chopra, 2012). Despite the fact that ABA and NO are thought to act together in multiple plant responses, the scenario is opposite in senescence (Fig. 3A). NO prevents increases in H_2O_2 levels and lipid peroxidation and has a protective effect against ABA-, methyl jasmonate- and H_2O_2-promoted senescence of rice leaves (Hung and Kao, 2003, 2004, 2005). This effect of NO is presumably due to its scavenging activity on H_2O_2 and other ROS, thus suggesting that antioxidant properties of NO are operating to counteract oxidative stress in rice
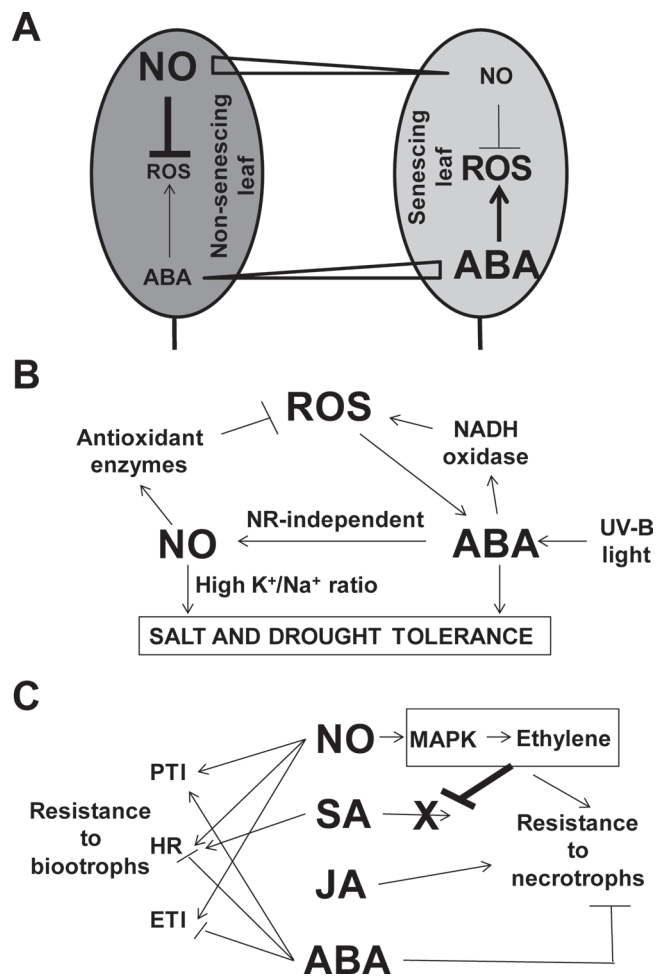

Fig. 3. Diverse contribution of NO and ABA to the control of (A) senescence, (B) abiotic stress and (C) resistance to pathogens. PTI, PAMP-triggered immunity; ETI, effector-triggered immunity; HR, hypersensitive response.

leaves (Hung and Kao, 2003). It remains unclear whether the increased ABA levels in senescent leaves does not lead to increased NO accumulation, as occurs in non-senescent leaves, because of the antioxidant activity of NO, thus quickly reacting with ROS, or whether it is due to defective production in senescent tissues. Nevertheless, these data represent another example of how the environment and endogenous plant cues might alter the functional interaction between ABA and NO in different processes. Additionally, as the senescence programme is activated at the whole-plant level, the tissues responsible for NO signalling in this case are different from those on seed germination or stomatal movement pointing to a tissue-dependent mode of action of NO in plants.

NO and ABA in plant responses to abiotic stress

Extensive experimental support accumulated during the last decade suggesting that NO plays an important role in a

broad spectrum of plant responses to abiotic stress together with ABA and other hormones. Under adverse environmental conditions, altered balance of NO production and metabolism causes nitrosative stress that influences both the responses to oxidative cues and the development and stress responses of plants (Corpas *et al.*, 2011). The antioxidant properties of NO as well as its potential in activating ROS-scavenging enzyme activities under abiotic stress may explain many of their regulatory functions (Siddiqui *et al.*, 2011; Fig. 3B). However, the mechanism(s) by which NO, in coordination with other hormones such as ABA, modulates the impact of abiotic stress often remains unclear.

Drought

Drought is one of the major constraints in limiting crop yield. ABA has been characterized extensively as a principal regulator of plant response to drought by inducing stomatal closure, therefore reducing water loss via transpiration (Davies and Zhang, 1991; Zhu, 2002). It has been received wisdom that NO cooperates in ABA-induced stomatal closure (Neill *et al.*, 2003). Accordingly, the application of the NO donor SNP enhanced plant tolerance to drought stress by reducing water stress, ion leakage, transpiration rate and by inducing stomatal closure (Garcia-Mata and Lamattina, 2001). The activated antioxidant systems also contribute to a better plant performance under water stress by removing excess ROS and by inhibiting lipid peroxidation. It has been proposed that NO and ROS enhance plant tolerance to water stress by regulating ABA biosynthesis (Zhao *et al.*, 2001; Xing *et al.*, 2004). Moreover, it has been also reported that the brassinosteroid (BR)-induced increase in ABA biosynthesis and tolerance to oxidative damage caused by water stress in maize leaves is, at least in part, due to the production of NO induced by BR (Zhang *et al.*, 2011). The complexity of the functional interaction between NO and ABA in regulating responses to water stress is evident by the fact that, reciprocally, ABA induces the accumulation of NO, which, in turn, activates MAPK and upregulates the expression of genes coding for antioxidant enzymes (Lamattina *et al.*, 2003; Zhang *et al.*, 2007; Lu *et al.*, 2009). NO, but not ABA or H_2O_2, has recently been reported to be essential in the cold- and dehydration-induced expression of a gene coding for a hybrid proline- and cysteine-rich protein from *Medicago falcata* that confers resistance to abiotic stress (Tan *et al.*, 2013a). Another gene coding for *myo*-inositol phosphate synthase, which also confers enhanced resistance to chilling, drought, and salt stresses, was induced by H_2O_2 and NO but was not responsive to ABA (Tan *et al.*, 2013b). These data point to the existence of different signalling pathways in drought responses, depending either on the coordinated action of NO, H_2O_2, and ABA, or, by contrast, only on subsets.

High salt

Nearly half of irrigated land and around 20% of cultivated land is currently affected by salinity (Misra *et al.*, 2001). A high salt concentration causes osmotic and ionic stress in plants. It limits the growth and development of plants by affecting several key metabolic processes (Hasegawa *et al.*, 2000). Much of the injury at the cellular level caused by salinity stress is associated with oxidative damage due to ROS. Plants appear to possess a wide array of defence strategies to protect themselves from oxidative damage. However, less is known about NO involvement in salt stress tolerance in plants. The application of NO stimulated the expression of the plasma membrane H^+-ATPase, a well-characterized target in ABA signalling and high-salt-triggered responses (Cerana *et al.*, 2006; Janicka-Russak and Klobus, 2007). It has been proposed that NO serves as a signal, inducing salt resistance by increasing the K^+/Na^+ ratio (Zhao *et al.*, 2004). Tolerant plants typically maintain high potassium (K^+) and low sodium (Na^+) in the cytosol of cells under salinity. This mechanism is mediated by H^+-ATPase, carriers (symporters and antiporters), and channels associated with plasma membranes and tonoplasts. It has been recently reported that energization of plasma membranes is a determinant for K^+ uptake (Haruta and Sussman, 2012). Also, the key role exerted by the tonoplast in controlling K^+ uptake has been documented extensively (Zhao *et al.*, 2004; Zhang *et al.*, 2006). Guo *et al.* (2009) suggested that NO might confer salt tolerance to plants by preventing both oxidative membrane damage and translocation of Na^+ from root to shoots (Fig. 3B). It has been also proposed that plants possess priming-like mechanisms that allow them to memorize previous NO exposure events and generate defence responses following salt stress (Molassiotis *et al.*, 2010). The increased drought and salt tolerance in transgenic tobacco plants overexpressing the ABA-biosynthetic enzyme 9-*cis*-epoxy-carotenoid dioxygenase is associated with the ABA-induced production of H_2O_2, via NADPH oxidase, and NO, via NOS-like activity, and the subsequent induction of antioxidant enzymes (Zhang *et al.*, 2009).

UV radiation stress

UV-B radiation has been shown to trigger an increase in ABA concentration in plants (Tossi *et al.*, 2009). This increase activates NADPH oxidase and H_2O_2 generation, and also the arginine-dependent production of NO that maintains cell homeostasis and attenuates UV-B-derived cell damage (Mackerness *et al.*, 1999; Tossi *et al.*, 2009). Moreover, NO generated from NOS-like activity appears to act synergistically with ROS in inducing ethylene synthesis in the defence response under UV-B radiation in maize leaves (Wang *et al.*, 2006). Shi *et al.* (2005) reported that addition of SNP can partially alleviate the UV-B-induced decrease in chlorophyll content, and the oxidative damage to the thylakoid membrane in bean leaves. It has been recently proposed that high doses of UV-B light seem to induce common signalling components such as ABA, NO, and Ca^{2+} in plants and other multicellular organisms (Tossi *et al.*, 2012), thus suggesting that ABA and NO function cooperatively in UV-B-triggered responses (Fig. 3B).

NO and ABA in PAMP-triggered immunity (PTI), effector-triggered immunity (ETI) and defence responses against necrotrophs

SA-related or JA/ethylene-related signalling events have traditionally been described as the major pathways in response to pathogen attack in plants (Glazebrook, 2005). However, the final response is mediated by a complex interconnected network comprising different phytohormones and other regulators (Robert-Seilaniantz *et al.*, 2011). The regulatory roles exerted by NO and ABA in plant defence against pathogens have been the focus of recent work (reviewed by Asselbergh *et al.*, 2008; Cao *et al.*, 2011; Gaupels *et al.*, 2011; Arasimowicz-Jelonek and Floryszak-Wieczorek, 2013; Bellin *et al.*, 2013). NO and ABA contribute to defence against pathogens both as alternative pathways to those centrally regulated through SA-related or JA/ethylene-related signalling events (Robert-Seilaniantz *et al.*, 2011), or in coordination with these classical pathways.

Lipopolysaccharide-triggered NO production contributes to *Arabidopsis* resistance to *Pseudomonas syringae* pv. Tomato strain DC3000 as demonstrated by the increased susceptibility observed in the NO-deficient *noa1* mutant (Zeidler *et al.*, 2004). Moreover, upon perception of pathogen-associated molecular patterns (PAMPs), plant leaves close their stomata (Fig. 2). This process leading to enhanced resistance to *P. syringae* is mediated by the FLS2 receptor and requires ABA and NO production and the function of the ABA-related OST1 kinase (Melotto *et al.*, 2006). These data established an important role for stomata as a defensive structure of plants linking ABA- and NO-related signalling to PTI against phytopathogenic bacteria. Both NO and ABA seem to play positive roles in activating PTI.

By contrast, antagonistic roles for NO and ABA have been reported in the activation of ETI. ETI is a subclass of plant immunity that is activated in response to pathogen effectors recognized by R proteins in the plant. NO functions as a positive regulator of ETI, as demonstrated by the enhanced susceptibility to *P.syringae avrRpm1* and *avrRps4* observed in wild-type *Arabidopsis* plants treated with inhibitors of NO production (Delledonne *et al.*, 1998), and in NO-deficient *Arabidopsis* mutants (Mandal *et al.*, 2012). In turn, ABA treatment leads to increased susceptibility of *Arabidopsis* plants to avirulent *P. syringae*, suggesting that ABA is a negative regulator of ETI (Mohr and Cahill, 2003). *Arabidopsis abi1-1* and *abi2-1* mutants, which are insensitive to ABA, and the *era1* hypersensitive mutant, were more resistant and susceptible, respectively, to virulent *P. syringae* (de Torres-Zabala *et al.*, 2007). ABA accumulation also compromised resistance to the biotrophic oomycete *Hyaloperonospora arabidopsidis* (Fan *et al.*, 2009). A large body of evidence supports the hypothesis that pathogen-modulated ABA signalling rapidly antagonizes SA-mediated defence (de Torres-Zabala *et al.*, 2009; Kim *et al.*, 2011). However, it has been reported recently that constitutive expression of a mammalian NOS in tobacco triggers NO accumulation, resistance to a wide array of pathogens and activation of the expression of SA-related genes (Chun *et al.*, 2012), pointing to NO as an agonist of

SA-related responses. The role of NO in plant immunity has been associated with changes in intracellular Ca^{2+} and cyclic nucleotide levels that exert a signalling function both upstream and downstream of NO (Ma, 2011). NO and ABA have also been reported to modulate cell death typical of the hypersensitive response in incompatible plant–pathogen interactions. ABA-RESPONSIVE1 (ABR1) has been characterized as a negative regulator of ABA signalling and its nuclear pool seems to be essential for cell death induction associated with ABA–SA antagonism (Choi and Hwang, 2011). On the other hand, treatment of *Arabidopsis* plants with either an inhibitor of NO synthesis or with NO scavengers impaired the proper activation of hypersensitive responses (Delledonne *et al.*, 2001), pointing to NO together with H_2O_2 as essential components in regulating cell death in pathogen-triggered responses. However, NO seems to have both promoting and suppressing effects on cell death, depending on cell type, cellular redox status, and dose (Wang *et al.*, 2010a).

Necrotrophic pathogens display a wide range of aggressive virulence strategies that promote host cell death to enable feeding from dead cells. This lifestyle strongly contrasts with immunity responses raised against biotrophic pathogens. As no resistance R gene has been associated with resistance to necrotrophs, ETI and its accompanying immune responses are primarily not effective regulators of resistance to necrotrophs. In turn, PTI has been reported to be efficient in triggering resistance to necrotrophs through a sequential signalling process involving MAPKs, camalexin biosynthesis, and ethylene that ends with suppression of SA signalling (Mengiste, 2012). Necrotrophic pathogens commonly trigger JA and ethylene signalling to activate plant defence responses (Glazebrook, 2005), although they can alter SA signalling to promote disease symptoms in the plant (Rahman *et al.*, 2012). It has been reported recently that ABA signalling modulates basal resistance in *Arabidopsis* through negative regulation of SA/JA/ethylene-mediated resistance to necrotrophic fungi (Sánchez-Vallet *et al.*, 2012). Moreover, resistance to *Botrytis cinerea* is enhanced by the increased permeability of the leaf cuticle, and is strongly linked to ROS formation through a process negatively regulated by ABA (L'Haridon *et al.*, 2011). On the other hand, infection with *Botrytis* caused a rapid increase in NO levels, and NO seems to cooperate in activating resistance, as treatment of plants with either biosynthesis inhibitors or scavengers of NO or mutant plants impaired in NO biosynthetic genes led to enhanced susceptibility to this necrotrophic fungi (Asai and Yoshioka, 2009; Rasul *et al.*, 2012a).

Taken together, current knowledge on plant immunity proposes that ABA and NO can act positively in promoting PTI (Fig. 3C). It seems that ABA can be very efficient in impeding pathogen entrance through stomata, although it is unclear whether NO cooperates in this process or whether it plays an additional role in defence mechanisms. By contrast, the role of ABA and NO in ETI or in defence against necrotrophic pathogens indicates an antagonism mode of action between these molecules (Fig. 3C). While ABA negatively regulates resistance, NO positively contributes to plant defence during ETI and necrotrophs attack.

Perspectives

Although during the last two decades an extensive experimental support has been conducted on multiple aspects of plant biology involving NO and ABA, many unsolved processes remain. Some topics, such as stomatal closure, would largely benefit from better contextualized experimental systems. Regarding this, work on deconstructed systems should be backed up by whole-plant studies. A large number of these processes would also benefit from better analytical tools allowing the quantification of NO and ABA as well as other hormones and growth regulators at tissue or even cellular levels. These tools should be developed in the next few years to allow more precise approaches. Pharmacologically based studies should be performed carefully to avoid misinterpretations due to unknown side effects of chemicals. An extensive characterization of chemicals used in pharmacological strategies and the use of only those displaying better specificity will help in clarifying our understanding of how functional interactions between plant regulators occur. Moreover, this sort of approach must be complemented with double experimental support. First, genetic studies using well-characterized mutant or transgenic plants will allow us to define crosstalk signalling by identifying epistatic or additive effects. Secondly, the identification of direct NO targets and their characterization, at both gene and protein levels, will be the main challenges for the future.

Future work will need to pay special attention to the characterization of protein modifications altering the function of proteins involved in the perception and downstream signalling of NO and ABA. All these considerations will help us to define whether NO exerts mainly a co-regulatory effect on other regulatory molecules, as extensively documented to date, or whether it might perform specific roles in controlling some aspects of plant physiology.

Another important issue to solve that is derived from the above-described data is the importance of the tissue-dependent mode of action of NO. It is becoming evident that NO can have a different regulatory role depending on the tissue or cellular type where it is generated. It is therefore feasible that its interaction with other regulators (i.e. ABA) will also be different. The tissue- and/or cell-type-specific NO role should be deciphered, and its interaction with specific regulatory networks within this cellular space will help to address current controversy and move forward for a better understanding of this fascinating molecule in plant physiology and the response to stress.

Acknowledgements

References

Alboresi A, Gestin C, Leydecker MT, Bedu M, Meyer C, Truong HM. 2005. Nitrate, a signal relieving seed dormancy in Arabidopsis. *Plant, Cell & Environment* **28,** 500–512.

Arasimowicz-Jelonek M, Floryszak-Wieczorek J. 2013. Nitric oxide: an effective weapon of the plant or the pathogen? *Molecular Plant Pathology* doi: 10.1111/mpp.12095.

Arc E, Sechet J, Corbineau F, Rajjou L, Marion-Poll A. 2013. ABA crosstalk with ethylene and nitric oxide in seed dormancy and germination. *Frontiers in Plant Science* **4,** 63.

Asai S, Yoshioka H. 2009. Nitric oxide as a partner of reactive oxygen species participates in disease resistance to nectrotophic pathogen *Botryis cinerea* in *Nicotiana benthamiana*. *Molecular Plant–Microbe Interactions* **22,** 619–629.

Asselbergh B, De Vleesschauwer D, Höfte M. 2008. Global switches and fine-tuning-ABA modulates plant pathogen defense. *Molecular Plant–Microbe Interactions* **21,** 709–719.

Batak I, Dević M, Gibal Z, Grubišić D, Poff KL, Konjević R. 2002. The effects of potassium nitrate and NO-donors on phytochrome A- and phytochrome B-specific induced germination of *Arabidopsis thaliana* seeds. *Seed Science Research* **12,** 253–259.

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. *Molecular Plant–Microbe Interactions* **26,** 271–277.

Bethke PC. 2009. Rebirth and death: nitric oxide and reactive oxygen species in seeds. *SEB Experimental Biology Series* **62,** 17–30.

Bethke PC, Libourel IG, Aoyama N, Chung YY, Still DW, Jones RL. 2007. The Arabidopsis aleurone layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. *Plant Physiology* **143,** 1173–1188.

Bethke PC, Libourel IG, Jones RL. 2006a. Nitric oxide reduces seed dormancy in Arabidopsis. *Journal of Experimental Botany* **57,** 517–526.

Bethke PC, Libourel IG, Reinöhl V, Jones RL. 2006b. Sodium nitroprusside, cyanide, nitrite, and nitrate break Arabidopsis seed dormancy in a nitric oxide-dependent manner. *Planta* **223,** 805–812.

Bright J, Desikan R, Hancock JT, Weir IS, Neill SJ. 2006. ABA-induced NO generation and stomatal closure in Arabidopsis are dependent on H_2O_2 synthesis. *The Plant Journal* **45,** 113–122.

Buchanan-Wollaston V, Page T, *et al.*. 2005. Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation induced senescence in Arabidopsis. *The Plant Journal* **42,** 567–585.

Cao FY, Yoshioka K, Desveaux D. 2011. The roles of ABA in plant-pathogen interactions. *Journal of Plant Research* **124,** 489–499.

Cerana M, Bonza MC, Harris R, Sanders D, De Michelis MI. 2006. Abscisic acid stimulates the expression of two isoforms of plasma membrane Ca^{2+}-ATPase in *Arabidopsis thaliana* seedlings. *Plant Biology (Stuttgart)* **8,** 572–578.

Choi DS, Hwang BK. 2011. Proteomics and functional analyses of pepper abscisic acid-responsive 1 (ABR1), which is involved in cell death and defense signaling. *Plant Cell* **23,** 823–842.

Chun HJ, Park HC, Koo SC, et al. 2012. Constitutive expression of mammalian nitric oxide synthase in tobacco plants triggers disease resistance to pathogens. *Molecular Cells* **34,** 463–471.

Corpas FJ, Barroso JB, Carreras A, et al. 2004. Cellular and subcellular localization of endogenous nitric oxide in young and senescent pea plants. *Plant Physiology* **136,** 2722–2733.

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

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 Science* **181,** 604–611.

Daszkowska-Golec A, Szarejko I. 2013. Open or close the gate—stomata action under the control of phytohormones in drought stress conditions. *Frontiers in Plant Science* **4,** 138.

Davies W, Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42,** 55–76.

De Michele R, Formentin E, Todesco M, et al.. 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 Phytologists* **181,** 563–575.

de Torres-Zabala M, Bennett MH, Truman WH, Grant MR. 2009. Antagonism between salicylic and abscisic acid reflects early host-pathogen conflict and moulds plant defence responses. *The Plant Journal* **59,** 375–386.

de Torres-Zabala M, Truman W, Bennett MH, Lafforgue G, Mansfield JW, Rodriguez P, Bögre L, Grant M. 2007. *Pseudomonas syringae* pv. tomato hijacks the Arabidopsis abscisic acid signalling pathway to cause disease. *EMBO Journal* **26,** 1434–1443.

Delledonne M, Xia YJ, Dixon R, 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. *Proceedings of the National Academy of Sciences,USA* **98,** 13454–13459.

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*. *Proceedings of the National Academy of Sciences,USA* **99,** 16314–16318.

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 Phytologist* **191,** 57–69.

Durbak A, Yao H, McSteen P. 2012. Hormone signaling in plant development. *Current Opinion in Plant Biology* **15,** 92–96.

Even-Chen Z, Itai C. 1975. The role of abscisic acid in senescence of detached tobacco leaves. *Physiologia Plantarum* **34,** 97–100.

Fan J, Hill L, Crooks C, Doerner P, Lamb C. 2009. Abscisic acid has a key role in modulating diverse plant–pathogen interactions. *Plant Physiology* **150,** 1750–1761.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. *New Phytologist* **171,** 501–523.

Finkelstein R, Reeves W, Ariizumi T, Steber C. 2008. Molecular aspects of seed dormancy. *Annual Review of Plant Biology* **59,** 387–415.

Fischer AM. 2012. The complex regulation of senescence. *Critical Reviews in Plant Sciences* **31,** 124–147.

Freschi L. 2013. Nitric oxide and phytohormone interactions: current status and perspectives. *Frontiers in Plant Science* **4,** 398.

Garcia-Mata C, Gay R, Sokolovski S, Hills A, Lamattina L, Blatt MR. 2003. Nitric oxide regulates K+ and Cl⁻ channels in guard cells through a subset of abscisic acid-evoked signaling pathways. *Proceedings of the National Academy of Sciences, USA* **100,** 11116–11121.

Garcia-Mata C, Lamattina L. 2001. Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiology* **126,** 1196–1204.

Garcia-Mata C, Lamattina L. 2002. Nitric oxide and abscisic acid cross talk in guard cells. *Plant Physiology* **128,** 790–792.

Garcia-Mata C, Lamattina L. 2007. Abscisic acid (ABA) inhibits light-induced stomatal opening through calcium- and nitric oxide-mediated signaling pathways. *Nitric Oxide* **17,** 143–151.

Gaupels F, Kuruthukulangarakoola GT, Durner J. 2011. Upstream and downstream signals of nitric oxide in pathogen defence. *Current Opinion in Plant Biology* **14,** 707–714.

Gepstein S, Thimann KV. 1980. Changes in the abscisic acid content of oat leaves during senescence. *Proceedings of the National Academy of Sciences, USA* **77,** 2050–2053.

Glazebrook J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* **43,** 205–227.

Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJ. 2012. Molecular mechanisms of seed dormancy. *Plant, Cell & Environment* **35,** 1769–1786.

Guo FQ, Crawford NM. 2005. Arabidopsis nitric oxide synthase 1 is targeted to mitochondria and protects against oxidative damage and dark-induced senescence. *Plant Cell* **17,** 3436–3450.

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

Guo Y, Gan S. 2012. Convergence and divergence in gene expression profiles induced by leaf senescence and 27

senescence-promoting hormonal, pathological and environmental stress treatments. *Plant,Cell and Environment* **35,** 644–655.

Guo Y, Tian Z, Yan D, Zhang J, Qin P. 2009. Effects of nitric oxide on salt stress tolerance in *Kosteletzkya virginica*. *Life Science Journal* **6,** 67–75.

Gupta KJ, Fernie AR, Kaiser WM, van Dongen JT. 2011. On the origins of nitric oxide. *Trends in Plant Science* **16,** 160–168.

Han S, Tang R, Anderson LK, Woerner TE, Pei ZM. 2003. A cell surface receptor mediates extracellular Ca^{2+} sensing in guard cells. *Nature* **425,** 196–200.

Hancock JT, Neill SJ, Wilson ID. 2011. Nitric oxide and ABA in the control of plant function. *Plant Science* **181,** 555–559.

Haruta M, Sussman MR. 2012. The effect of a genetically reduced plasma membrane protonmotive force on vegetative growth of Arabidopsis. *Plant Physiology* **158,** 1158–1171.

Hasanuzzaman M, Fujita M. 2013. Exogenous sodium nitroprusside alleviates arsenic-induced oxidative stress in wheat (*Triticum aestivum* L.) seedlings by enhancing antioxidant defense and glyoxalase system. *Ecotoxicology* **22,** 584–596.

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. 2000. Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* **51,** 463–499.

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

Holdsworth MJ, Bentsink L, Soppe WJJ. 2008. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. *New Phytologist* **179,** 33–54.

Hung KT, Kao CH. 2003. Nitric oxide counteracts the senescence of rice leaves induced by abscisic acid. *Journal of Plant Physiology* **160,** 871–879.

Hung KT, Kao CH. 2004. Nitric oxide acts as an antioxidant and delays methyl jasmonate-induced senescence of rice leaves. *Journal of Plant Physiology* **161,** 43–52.

Hung KT, Kao CH. 2005. Nitric oxide counteracts the senescence of rice leaves induced by hydrogen peroxide. *Botanical Bulletin of the Academia Sinica* **46,** 21–28.

Janicka-Russak M, Kłobus G. 2007. Modification of plasma membrane and vacuolar H^+-ATPases in response to NaCL and ABA. *Journal of Plant Physiology* **164,** 295–302.

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.

Khanna-Chopra R. 2012. Leaf senescence and abiotic stresses share reactive oxygen species-mediated chloroplast degradation. *Protoplasma* **249,** 469–481.

Kim TH, Hauser F, Ha T, *et al.* 2011. Chemical genetics reveals negative regulation of abscisic acid signaling by a plant immune response pathway. *Current Biology* **21,** 990–997.

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.

L'Haridon F, Besson-Bard A, Binda M, *et al.* 2011. A permeable cuticle is associated with the release of reactive oxygen species and induction of innate immunity. *PLoS Pathogens* **7,** e1002148.

Lamattina L, Garcia-Mata C, Graziano M, Pagnussat G. 2003. Nitric oxide: the versatility of an extensive signal molecule. *Annual Review of Plant Biology* **54,** 109–136.

Laxalt AM, Beligni MV, Lamattina L. 1997. Nitric oxide preserves the level of chlorophyll in potato leaves infected by *Phytophthora infestans*. *European Journal of Plant Pathology* **103,** 643–651.

Leckie CP, McAinsh MR, Allen GJ, Sanders D, Hetherington AM. 1998. Abscisic acid-induced stomatal closure mediated by cyclic ADP-ribose. *Proceedings of the National Academy of Sciences, USA* **95,** 15837–15842.

Leshem YY, Wills RBH, Veng Va Ku V. 1998. Evidence for the function of the free radical gas nitric oxide (NO) as an endogenous maturation and senescence regulation factor in higher plants. *Plant Physiology and Biochemistry* **36,** 825–833.

Li JH, Liu YQ, Lü P, Lin HF, Bai Y, Wang XC, Chen YL. 2009. A signaling pathway linking nitric oxide production to heterotrimeric G protein and hydrogen peroxide regulates extracellular calmodulin induction of stomatal closure in Arabidopsis. *Plant Physiology* **150,** 114–124.

Li Z, Peng J, Wen X, Guo H. 2012. Gene network analysis and functional studies of senescence-associated genes reveal novel regulators of *Arabidopsis* leaf senescence. *Journal of Integrative Plant Biology* **54,** 526–539.

Libourel IG, Bethke PC, De Michele R, Jones RL. 2006. Nitric oxide gas stimulates germination of dormant Arabidopsis seeds: use of a flow-through apparatus for delivery of nitric oxide. *Planta* **223,** 813–820.

Lim PO, Kim HJ, Nam HG. 2007. Leaf senescence. *Annual Review of Plant Biology* **58,** 115–136.

Lindermayr C, Saalbach G, Durner J. 2005. Proteomic identification of *S*-nitrosylated proteins in Arabidopsis. *Plant Physiology* **137,** 921–930.

Liu F, Guo FQ. 2013. Nitric oxide deficiency accelerates chlorophyll breakdown and stability loss of thylakoid membranes during dark-induced leaf senescence in Arabidopsis. *PLoS One* **8,** e56345.

Liu H, Lau E, Lam MP, *et al.* 2010. OsNOA1/RIF1 is a functional homolog of AtNOA1/RIF1: implication for a highly conserved plant cGTPase essential for chloroplast function. *New Phytologists* **187,** 83–105.

Liu HY, Yu X, Cui DY, Sun MH, Sun WN, Tang ZC, Kwak SS, Su WA. 2007. The role of water channel proteins and nitric oxide signaling in rice seed germination. *Cell Research* **17,** 638–649.

Liu Y, Shi L, Ye N, Liu R, Jia W, Zhang J. 2009. Nitric oxide-induced rapid decrease of abscisic acid concentration is required in breaking seed dormancy in Arabidopsis. *New Phytologists* **183,** 1030–1042.

Liu Y, Ye N, Liu R, Chen M, Zhang J. 2010. H_2O_2 mediates the regulation of ABA catabolism and GA biosynthesis in Arabidopsis

seed dormancy and germination. *Journal of Experimental Botany* **61,** 2979–2990.

Lozano-Juste J, Colom-Moreno R, León J. 2011. *In vivo* protein tyrosine nitration in *Arabidopsis thaliana*. *Journal of Experimental Botany* **62,** 3501–3517.

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

Lozano-Juste J, León J. 2010b. Nitric oxide modulates sensitivity to ABA. *Plant Signaling and Behavior* **5,** 314–316.

Lu S, Su W, Li H, Gou Z. 2009. Abscisic acid improves drought tolerance of triploid bermudagrass and involves H_2O_2- and NO-induced antioxidant enzyme activities. *Plant Physiology and Biochemistry* **47,** 132–138.

Ma W. 2011. Roles of Ca^{2+} and cyclic nucleotide gated channel in plant innate immunity. *Plant Science* **181,** 342–346.

Mackerness SAH, Surplus SL, Blake P, John CF, Buchanan-Wollaston V, Jordan BR, Thomas B. 1999. Ultraviolet-B induced stress and changes in gene expression in *Arabidopsis thaliana*: role of signaling pathways controlled by jasmonic acid, ethylene and reactive oxygen species. *Plant ,Cell and Environment* **22,** 1413–1423.

MacRobbie EA. 2000. ABA activates multiple Ca^{2+} fluxes in stomatal guard cells, triggering vacuolar $K^+(Rb^+)$ release. *Proceedings of the National Academy of Sciences, USA* **97,** 12361–12368.

Mandal MK, Chandra-Shekara AC, Jeong RD, Yu K, Zhu S, Chanda B, Navarre D, Kachroo A, Kachroo P. 2012. Oleic acid-dependent modulation of NITRIC OXIDE ASSOCIATED1 protein levels regulates nitric oxide-mediated defense signaling in Arabidopsis. *Plant Cell* **24,** 1654–1674.

Matakiadis T, Alboresi A, Jikumaru Y, Tatematsu K, Pichon O, Renou JP, Kamiya Y, Nambara E, Truong HN. 2009. The Arabidopsis abscisic acid catabolic gene *CYP707A2* plays a key role in nitrate control of seed dormancy. *Plant Physiology* **149,** 949–960.

Meimoun P, Vidal G, Bohrer AS, Lehner A, Tran D, Briand J, Bouteau F, Rona JP. 2009. Intracellular Ca^{2+} stores could participate to abscisic acid-induced depolarization and stomatal closure in *Arabidopsis thaliana*. *Plant Signaling and Behavior* **4,** 830–835.

Melotto M, Underwood W, Koczan J, Nomura K, He SY. 2006. Plant stomata function in innate immunity against bacterial invasion. *Cell* **126,** 969–980.

Mendel RR, Hänsch R. 2002. Molybdoenzymes and molybdenum cofactor in plants. *Journal of Experimental Botany* **53,** 1689–1698.

Mengiste T. 2012. Plant immunity to necrotrophs. *Annual Review of Phytopathology* **50,** 267–294.

Mishina T, Lamb C, Zeier C. 2007. Expression of a nitric oxide degrading enzyme induces a senescence programme in *Arabidopsis*. *Plant, Cell & Environment* **30,** 39–52.

Misra AN, Srivastava A, Strasser RJ. 2001. Utilization of fast chlorophyll *a* fluorescence technique in assessing the salt/ion sensitivity of mung bean and brassica seedlings. *Journal of Plant Physiology* **158,** 1173–1181.

Miura K, Lee J, Jin JB, Yoo CY, Miura T, Hasegawa PM. 2009. Sumoylation of ABI5 by the Arabidopsis SUMO E3 ligase SIZ1

negatively regulates abscisic acid signaling. *Proceedings of the National Academy of Sciences, USA* **106,** 5418–5423.

Modolo LV, Augusto O, Almeida IMG, Pinto-Maglio CAF, Oliveira HC, Seligman K, Salgado, I. 2006. Decreased arginine and nitrite levels in nitrate reductase-deficient *Arabidopsis thaliana* plants impair nitric oxide synthesis and the hypersensitive response to *Pseudomonas syringae*. *Plant Science* **171,** 34–40.

Mohr PG, Cahill DM. 2003. Abscisic acid influences the susceptibility of *Arabidopsis thaliana* to *Pseudomonas syringae* pv. Tomato and *Peronospora parasitica*. *Functional Plant Biology* **30,** 461–469.

Molassiotis A, Tanou G, Diamantidis G. 2010. NO says more than 'YES' to salt tolerance: salt priming and systemic nitric oxide signaling in plants. *Plant Signaling and Behavior* **5,** 209–212.

Moreau M, Lee GI, Wang Y, Crane BR, Klessig DF. 2008. AtNOS/AtNOA1 is a functional *Arabidopsis thaliana* cGTPase and not a nitric-oxide synthase. *Journal of Biological Chemistry* **283,** 32957–32967.

Moreau M, Lindermayr C, Durner J, Klessig, DF. 2010. NO synthesis and signaling in plants—where do we stand? *Physiologia Plantarum* **138,** 372–383.

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 Plants* **5,** pls052.

Neill S, Desikan R, Hancock JT. 2003. Nitric oxide signaling in plants. *New Phytologist* **159,** 11–35.

Neill SJ, Desikan R, Clarke A, Hancock JT. 2002a. Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. *Plant Physiology* **128,** 13–16.

Neill SJ, Desikan R, Clarke A, Hurst RD, Hancock JT. 2002b. Hydrogen peroxide and nitric oxide as signalling molecules in plants. *Journal of Experimental Botany* **53,** 1237–1247.

Pandey S, Zhang W, Assmann SM. 2007. Roles of ion channels and transporters in guard cell signal transduction. *FEBS Letters* **581,** 2325–2336.

Piterková J, Luhová L, Hofman J, Tureckova V, Novák O, Petrivalsky M, Fellner, M. 2012. Nitric oxide is involved in light-specific responses of tomato during germination under normal and osmotic stress conditions. *Annals of Botany* **110,** 767–776.

Procházková D, Wilhelmová N. 2011. Nitric oxide, reactive nitrogen species and associated enzymes during plant senescence. *Nitric Oxide* **24,** 61–65.

Rahman TA, Oirdi ME, Gonzalez-Lamothe R, Bouarab K. 2012. Necrotrophic pathogens use the salicylic acid signaling pathway to promote disease development in tomato. *Molecular Plant–Microbe Interactions* **25,** 1584–1593.

Rasul S, Dubreuil-Maurizi C, Lamotte O, Koen E, Poinssot B, Alcaraz G, Wendehenne D, Jeandroz S. 2012a. Nitric oxide production mediates oligogalacturonide-triggered immunity and resistance to *Botrytis cinerea* in *Arabidopsis thaliana*. *Plant, Cell & Environment* **35,** 1483–1499.

Rasul S, Wendehenne D, Jeandroz S. 2012b. Study of oligogalacturonides-triggered nitric oxide (NO) production provokes new questioning about the origin of NO biosynthesis in plants. *Plant Signaling and Behavior* **7,** 1031–1033.

Ribeiro DM, Desikan R, Bright J, Confraria A, Harrison J, Hancock JT, Barros RS, Neill SJ, Wilson ID. 2009. Differential requirement for NO during ABA-induced stomatal closure in turgid and wilted leaves. *Plant, Cell & Environment* **32,** 46–57.

Robert-Seilaniantz A, Grant M, Jones JD. 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology* **49,** 317–343.

Roelfsema MR, Hedrich R. 2010. Making sense out of Ca^{2+} signals: their role in regulating stomatal movements. *Plant, Cell & Environment* **33,** 305–321.

Rosales EP, Iannone MF, Groppa MD, Benavides MP. 2011. Nitric oxide inhibits nitrate reductase activity in wheat leaves. *Plant Physiology and Biochemistry* **49,** 124–130.

Sánchez-Vallet A, López G, Ramos B, et al. 2012. Disruption of abscisic acid signaling constitutively activates Arabidopsis resistance to the necrotrophic fungus *Plectosphaerella cucumerina*. *Plant Physiology* **160,** 2109–2124.

Sato A, Sato Y, Fukao Y, et al. 2009. Threonine at position 306 of the KAT1 potassium channel is essential for channel activity and is a target site for ABA-activated SnRK2/OST1/SnRK2.6 protein kinase. *Biochemical Journal* **424,** 439–448.

Shi S, Wang G, Wang Y, Zhang L, Zhang L. 2005. Protective effect of nitric oxide against oxidative stress under ultraviolet-B radiation. *Nitric Oxide* **13,** 1–9.

Siddiqui MH, Al-Whaibi MH, Basalah MO. 2011. Role of nitric oxide in tolerance of plants to abiotic stress. *Protoplasma* **248,** 447–455.

Simontacchi M, Garcia-Mata C, Bartoli CG, Santa-María GE, Lamattina L. 2013. Nitric oxide as a key component in hormone-regulated processes. *Plant Cell Reports* **32,** 853–866.

Simontacchi M, Jasid S, Puntarulo S. 2007. Enzymatic sources of nitric oxide during seed germination. In: Lamattina L, Polacco J, eds. *Nitric oxide in plant growth, development and stress physiology* . Berlin: Springer, 73–90.

Smart CM. 1994. Gene expression during leaf senescence. *New Phytologist* **126,** 419–448.

Sokolovski S, Blatt MR. 2004. Nitric oxide block of outward-rectifying K^+ channels indicates direct control by protein nitrosylation in guard cells. *Plant Physiology* **136,** 4275–4284.

Tan J, Wang C, Xiang B, Han R, Guo, Z. 2013b. Hydrogen peroxide and nitric oxide mediated cold- and dehydration-induced myo-inositol phosphate synthase that confers multiple resistances to abiotic stresses. *Plant, Cell & Environment* **36,** 288–299.

Tan J, Zhuo C, Guo Z. 2013a. Nitric oxide mediates cold- and dehydration-induced expression of a novel MfHyPRP that confers tolerance to abiotic stress. *Physiologia Plantarum* **149,** 310–320.

Tossi V, Cassia R, Bruzzone S, Zocchi E, Lamattina L. 2012. ABA says NO to UV-B: a universal response? *Trends in Plant Science* **17,** 510–517.

Tossi V, Lamattina L, Cassia R. 2009. An increase in the concentration of abscisic acid is critical for nitric oxide mediated plant adaptive responses to UV-B irradiation. *New Phytologist* **181,** 871–879.

Vahisalu T, Kollist H, Wang YF, et al.. 2008. SLAC1 is required for plant guard cell S-type anion channel function in stomatal signaling. *Nature* **452,** 487–491.

Vahisalu T, Puzõrjova I, Broschés M, et al. 2010. Ozone-triggered rapid stomatal response involves the production of reactive oxygen species, and is controlled by SLAC1 and OST1. *The Plant Journal* **62,** 442–453.

Wang P, Du Y, Li Y, Ren D, Song CP. 2010b. Hydrogen peroxide-mediated activation of MAP kinase 6 modulates nitric oxide biosynthesis and signal transduction in Arabidopsis. *Plant Cell* **22,** 2981–2998.

Wang Y, Chen C, Loake GJ, Chu C. 2010a. Nitric oxide: promoter or suppressor of programmed cell death? *Protein Cell* **1,** 133–142.

Wang Y, Feng H, Qu Y, Cheng J, Zhao Z, Zhang M, Wang X, An L. 2006. The relationship between reactive oxygen species and nitric oxide in ultraviolet-B-induced ethylene production in leaves of maize seedlings. *Environmental and. Experimental Botany* **57,** 51–61.

Wang Y, Lin A, Loake GJ, Chu C. 2013. H_2O_2 -induced leaf cell death and the crosstalk of reactive nitric/oxygen species. *Journal of Integrative Plant Biology* **55,** 202–208.

Wimalasekera R, Tebartz F, Scherer GF. 2011. Polyamines, polyamine oxidases and nitric oxide in development, abiotic and biotic stresses. *Plant Science* **181,** 593–603.

Xing H, Tan L, An L, Zhao Z, Wang S, Zhang C. 2004. Evidence for the involvement of nitric oxide and reactive oxygen species in osmotic stress tolerance of wheat seedlings: inverse correlation between leaf abscisic acid accumulation and leaf water loss. *Plant Growth Regulation* **42,** 61–68.

Yang J, Zhang J, Wang Z, Zhu Q, Liu L. 2002. Abscisic acid and cytokinins in the root exudates and leaves and their relationship to senescence a remobilization of carbon reserves in rice subjected to water stress during grain filling. *Planta* **215,** 645–652.

Yang JC, Zhang JH, Wang ZQ, Zhu QS, Liu LJ. 2003. Involvement of abscisic acid and cytokinins in the senescence and remobilization of carbon reserves in wheat subjected to water stress during grain filling. *Plant, Cell & Environment* **26,** 1621–1631.

Yun BW, Feechan A, Yin M, et al. 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. *Proceedings of the National Academy of Sciences, USA* **101,** 15811–15816.

Zhang A, Jiang M, Zhang J, Ding H, Xu S, Hu X, Tan M. 2007. Nitric oxide induced by hydrogen peroxidase mediates abscisic acid-induced activation of the mitogen-activated protein kinase cascade involved in antioxidant defense in maize leaves. *New Phytologist* **175,** 36–50.

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 and Cell Physiology* **52,** 181–92.

Zhang Y, Tan J, Guo Z, Lu S, He S, Shu W, Zhou B. 2009. Increased abscisic acid levels in transgenic tobacco over-expressing

9 cis-epoxycarotenoid dioxygenase influence H_2O_2 and NO production and antioxidant defences. *Plant, Cell & Environment* **32,** 509–519.

Zhang Y, 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 the tonoplast. *Planta* **224,** 545–555.

Zhao L, Zhang F, Guo J, Yang Y, Li B, Zhang L. 2004. Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. *Plant Physiology* **134,** 849–857.

Zhao Z, Chen G, Zhang C. 2001. Interaction between reactive oxygen species and nitric oxide in drought-induced abscisic acid synthesis in root tips of wheat seedlings. *Australian Journal of Plant Physiology* **28,** 1055–1061.

Zheng Y, Schumaker KS, Guo Y. 2012. Sumoylation of transcription factor MYB30 by the small ubiquitin-like modifier E3 ligase SIZ1 mediates abscisic acid response in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **109,** 12822–12827.

Zhu JK. 2002. Salt and drougtht stress signal transduction in plants. *Annual Review of Plant Biology* **53,** 247–273.