NITRIC OXIDE FUNCTION DURING OXYGEN DEPRIVATION IN PHYSIOLOGICAL AND STRESS PROCESSES.

Isabel Manrique-Gil¹, Inmaculada Sánchez-Vicente¹, Isabel Torres-Quezada¹, and Oscar Lorenzo*

Dpto. de Botánica y Fisiología Vegetal. Instituto Hispano-Luso de Investigaciones Agrarias (CIALE). Facultad de Biología. Universidad de Salamanca. C/ Río Duero 12, 37185 Salamanca, Spain

¹ These authors contributed equally to this work

* Corresponding author. E-mail oslo@usal.es; Tel. 34-923-294500-Ext.5117; fax 34-923-294790

© The Author(s) 2020. Published by Oxford University Press on behalf of the Society for Experimental Biology. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.
HIGHLIGHT

This review provides insights into nitric oxide (NO) function during plant developmental cues and stress responses when oxygen levels are scarce and with special emphasis to the difference between physiological hypoxia and stress-induced hypoxia.
ABSTRACT

Plants are aerobic organisms that have evolved to maintain specific requirements for oxygen (O$_2$) leading to a correct respiratory energy supply during growth and development. There are certain plant developmental cues and biotic or abiotic stress responses where O$_2$ levels are scarce. This O$_2$ deprivation known as hypoxia may occur in hypoxic niches of plant specific tissues and during adverse environmental cues like pathogen attack and flooding. In general, plants respond to hypoxia through a complex reprogramming of their molecular activities with the aim of reducing the impact of stress on their physiological and cellular homeostasis. This review focuses on the fine-tuned regulation of hypoxia triggered by a network of gaseous compounds that includes O$_2$, ethylene (ET) and nitric oxide (NO). In view of recent scientific advances, we aim to compile those molecular mechanisms mediated by phytoglobins (PGBs) and by the N-degron proteolytic pathway, with special emphasis on embryogenesis, seed imbibition and germination, and also specific structures, most notably root apical (RAM) and shoot apical (SAM) meristems. In addition, those biotic and abiotic stresses that comprise hypoxia are also highlighted.

Keywords: developmental cues, hypoxic stress, N-degron pathway, nitric oxide, oxygen, phytoglobins
INTRODUCTION

Nitric oxide (NO) has important features as a key signaling molecule in plants since it is rapidly synthesized, induces defined effects within the cells and is also scavenged quickly when no longer required.

NO is an essential component of the gaseous network described to modulate pre-adaptation to hypoxic conditions, a system that also comprises O₂, ethylene (ET) and carbon dioxide (CO₂) (reviewed in Sasidharan et al., 2018). An optimal balance of controlled levels of reactive oxygen species (ROS) is required for plant survival. Therefore, a tightly dynamic circuit of flooding signals is essential for suitable plant responses. Diverse processes occur during this situation, such as metabolic adjustments and physiological changes, leading to plant survival. Hypoxia includes both developmental and stress-related conditions. It is important to make a difference between stress-induced hypoxia (stress hypoxia) from constitutively generated chronic hypoxia (physiological hypoxia) (Weits et al., 2020). During stress hypoxia, a prompt decrease in O₂ concentration and a NO burst occur as a result of an environmental stress (e.g. flooding), among others changes in the cellular state. This hypoxia led to different adaptive responses, mainly controlled through ERFVII group. Physiological hypoxia refers to specific tissues where O₂ concentrations are constitutively low. This type of hypoxia is found in the so-called “hypoxic niches” and does not constitute a stress. Hypoxic niches have specific attributes that keep the O₂ concentrations low, including high respiration rates and the inability to release O₂ since they are heterotrophic tissues. Among them, various growth situations are governed by lower O₂ levels, such as embryogenesis, seed imbibition and germination, and also specific structures, most notably root apical (RAM) and shoot apical (SAM) meristems. In addition, some biotic and abiotic stresses also comprise hypoxia, highlighting pathogen attack and flooding.

To endure O₂ deprivation, plants have developed sensing mechanisms, leading to transcriptional reprogramming to allow hypoxia responses. Here, we outline the NO influence during the molecular crosstalk that underlies perception and acclimation processes. More than one source of NO is involved in the response during hypoxia, headlining nitrate reductase and plant mitochondrial activities (Gupta et al., 2005; Igamberdiev et al., 2005; Planchet et al., 2005). The NO burst that occurs during O₂ deprivation is not an undesirable trait and there are some data among different plant species supporting the role of NO in the plant acclimation to hypoxia (reviewed in Sasidharan et al., 2018; Armstrong et al., 2019).

NITRIC OXIDE AND HYPOXIC STRESS CROSSTALK

As aerobic organisms, plants have evolved to maintain specific requirements for oxygen (O₂) that lead to a correct respiratory energy supply. A close relationship between both O₂ and NO sensing is mediated by phytoglobins (PGBs), which are able to modulate the level of diatomic gases such carbon monoxide (CO), NO and O₂, and by the N-degron pathway, which perceives the fluctuations of these gases and activates transcriptional response through N-terminal
recognition that target proteins for degradation (Figure 1). Hypoxic conditions lead to an increase in NO levels suggesting a key role for the NO/O₂ balance during this stress (Dordas et al., 2003, Borisjuk et al., 2007; Ma et al., 2016).

Phytoglobins modulate the balance between nitric oxide and oxygen

Maintenance of correct spatiotemporal gradients in O₂ and NO becomes a crucial factor to determine the cellular redox status, necessary for the regulation of plant developmental and stress processes. Non-symbiotic plant hemoglobins, recently renamed as phytoglobins (PGBs) (Hill et al., 2016), are globular proteins able to bind small gaseous molecules such O₂, NO, CO and hydrogen sulfide (H₂S). This huge binding capacity suggests an important role during sensing of gaseous molecules and regulatory mechanisms in diverse organisms from all living kingdoms, such as photosynthetic organisms, animals, fungi or bacteria.

Phytoglobins

Hemoglobins use heme as a cofactor (Hoy and Hargove, 2008; reviewed in Gupta et al., 2011), which can bind the mentioned substrates, controlling their storage, transport, scavenging, and detoxification along the tissues (Arredondo-Peter et al., 1998). In plants, based on sequence cladistics three classes of PGBs exist, symbiotic (SymPGB and Lb), non-symbiotic (PGB0, 1 and 2) and truncated (PGB3) (Hoy and Hargrove, 2008). Depending on the ligands, the expression pattern and their physiological functions are categorized in symbiotic and non-symbiotic (Table 1). During stress hypoxia, caused by flooding or pathogen attack, the presence of PGBs exerts a protective role, modulating NO levels (Hartman et al., 2019).

Specifically, PGBs from Class 1 and 2 are key players on the crossroad between O₂ and NO, since the former regulates NO turnover and the later controls more deeply O₂ delivery and buffering along the tissues. These proteins are also involved in the Hemoglobin-NO cycle under hypoxia, which has been proposed to relieve the inhibition of mitochondrial transport chain by O₂ deficiency (Dordas et al., 2004; Perazzoli et al., 2004; Igamberdiev et al., 2005; Hebelstrup et al., 2006). This cycle increases energy status by oxidizing NAD(P)H to enhance the proton flow, resulting in ATP production. Protection against the severe effects of hypoxia depends on the binding capacity to ligands such as O₂ or NO, since plants that overexpress a PGB1 mutated with lower O₂ affinity are as susceptible to hypoxia as wild type (Hunt et al., 2002). It is also proposed that PGBs 1 and 2 might function as nitrite reductases under certain conditions of extreme hypoxia (Tiso et al., 2012). NO also controls PGBs posttranslationally to determine a fine-tune redox balance and energy status, as will be discussed later in this review.

The N-degron pathway operates as nitric oxide and oxygen sensor

The plant N-degron pathway is a proteolytic system that recognizes proteins containing certain N-terminal degradation signals, called “N-degrons”, and polyubiquitinates them for their degradation through the 26S proteasome (Bachmair et al., 1989; Varshavsky, 2011). This proteolytic pathway exists in prokaryotes and eukaryotes and the enzyme system responsible for substrate degradation in plants is conserved with higher animals (Graciet et
In plants, there are so far, two different N-degron pathways based on the E3 ligase, PROTEOLYSIS1 and 6 (PRT1 and PRT6), that recognize non-overlapping sets of N-terminal residues.

The PCO branch of the PRT6 N-degron pathway functions as both O₂ and NO sensor, as these two gases are required for the degradation of PRT6 substrates (Gibbs et al., 2011; Gibbs et al., 2014; Licausi et al., 2011) (Figure 1). Methionine-Cysteine (Met-Cys-) initiating substrates undergo four enzymatic reactions prior to its degradation through the proteasome, namely Met excision (carried out by methionine aminopeptidases, MAPs), Cys oxidation (plant cysteine oxidases, PCOs), arginylation (arginyl-tRNA-transferases, ATEs) and polyubiquitination (PRT6).

The O₂ sensors in plants are thought to be the PCOs (Weits et al., 2014), since these iron-dependent dioxygenases use molecular oxygen to catalyze the Cys oxidation and their $K_{m}^{app}$ (O₂) values are within a physiologically relevant range for response to both external and internal O₂ deficit that enable them to sensitively react to changes in O₂ availability (White et al., 2018). Similarly in mammals, the ADO enzyme is required for O₂-dependent degradation of N-degron substrates in human cells (Masson et al., 2019). This enzyme, which was previously assigned as cysteamine dioxygenase, is a thiol oxidase that is functionally identical to PCOs in plants, catalyzing the conversion of the N-terminal Cys to Cys sulfinic acid. Remarkably, when human ADO is expressed under control of the PCO1 promoter is able to complement the pco1/2/3/4 Arabidopsis mutant and plants can develop normally. It, therefore, remains to be explained how NO positively influences the substrate degradation of the PCO branch of the PRT6 N-degron pathway. NO itself could affect the activities of enzymatic components of the pathway (Zarban et al., 2019) or alter the cellular energy balance in an indirect manner (Armstrong et al., 2019).

The group VII of the Ethylene Response Factors (ERFVII) was the first substrate of the PCO branch of PRT6 N-degron pathway described in plants (Gibbs et al., 2011; Licausi et al., 2011) followed by the transcriptional regulators polycomb repressive complex 2 subunit VERNALIZATION 2 (VRN2) (Gibbs et al., 2018) and LITTLE ZIPPER 2 (ZPR2) (Weits et al., 2019). N-degron pathway substrates regulate important aspects of plant development such as seed storage mobilization (Zhang et al., 2018a; 2018b), germination (Holman et al., 2009; Gibbs et al., 2014), photomorphogenesis (Abbas et al., 2015), stomatal closure (Gibbs et al., 2014), shoot and leaf development (Graciet et al., 2009), root architecture (Shukla et al., 2019), shoot apical meristem function (Weits et al., 2019), vernalization (Gibbs et al., 2018; Labandera et al., 2020), flowering (Vicente et al., 2017) or leaf senescence (Yoshida et al., 2002) and also regulates stress responses such as flooding (Hartman et al., 2019) or pathogen attack (De Marchi et al., 2016; Vicente et al., 2019; Till et al., 2019).

In the case of VRN2 and ZPR2, these transcriptional regulators are located in hypoxic niches. ZPR2 is found in the SAM where controls the meristem maintenance; VRN2, besides SAM, is also placed in young leaf primordia and root meristematic zones and has a role in vernalization and root architecture (Weits et al., 2019; Labandera et al., 2020). The physiological hypoxia that exists in these zones prevents these proteins from degradation through N-degron pathway.
A different regulation occurs in the case of ERFVII group during normoxia or non-stressed growth conditions, where these transcription factors are attached to the plasma membrane, avoiding their degradation (Licausi et al., 2011). When hypoxia stress occurs (e.g. flooding) stable ERFVIIIs migrate to the nucleus and activate different hypoxia response genes. When flooded, plants rapid accumulate ET and increase the levels of the NO-scavenger PHYTOGLOBIN1 (PGB1). This ET-mediated NO depletion, besides hypoxia, promotes ERFVII accumulation and pre-adapts plants to survive subsequent hypoxia (Hartman et al., 2019).

These results confirm the key function of N-degron pathway in the regulation of genetic and molecular networks through NO/O$_2$ balance sensing.

### Nitric oxide posttranslational modifications of key hypoxia molecular players

A landmark in NO biology is the ability to modulate protein function and/or stability through three posttranslational mechanisms, the nitration of Tyr residues, the S-nitrosation of Cys residues and the nitrosylation of transition metals (reviewed in Sanz et al., 2015, Sánchez-Vicente et al., 2019b). A higher accumulation of S-nitrosothiols under hypoxic conditions points to this modification as a key feature by which NO exerts its responses (Hebelstrup et al., 2012). GSNOR is a master modulator of the intracellular levels of NO and, consequently, controls the concentration of S-nitrosothiols along the cell (Liu et al., 2001). Autophagy constitutes an important recycling process for normal growth and also under stress conditions, including hypoxia (Chen et al., 2015). It has been recently reported that NO is also coupled to hypoxia-related autophagy events through selective S-nitrosation of GSNO reductase (GSNOR) (Zhan et al., 2018). Several key molecular players during hypoxia adaptive response are described to be controlled by NO. Previous reports indicate that this gasotransmitter inhibits cytochrome c oxidase (COX) (Millar and Day, 1996), aconitase (Gupta et al., 2012) and ascorbate peroxidase 1 (APX1) (Begara-Morales et al., 2014). Consequently, the altered enzymatic activity is reorganized to modulate O$_2$ consumption, optimizing energy usage and supply.

The phytohormone ET, NO, and PGB1 are all associated with flooding-induced hypoxia since all of them are induced under O$_2$ deficiency (Hebelstrup et al., 2012; Hartman et al., 2019). Increased NO levels are associated with Nitrate reductase (NR) activity under nitrite accumulation (Planchet et al., 2005; Mugnai et al., 2012; reviewed in Gupta and Igamberdiev, 2016). PGB1, critical for plant survival during O$_2$ depletion, is also posttranslationally controlled by NO through Cys nitrosation (Perazzolli et al., 2004; Rubio et al., 2019), metal nitrosylation (Perazzolli et al., 2004) and Tyr nitration (Sainz et al., 2015). Interestingly, the binding of NO to the heme group of PGBs affects the scavenging of this molecule (Gupta et al., 2011). The interplay between NO and ET also impacts plant responses. Previous reports proved both gases may affect each other, depending on the developmental stage and stress conditions studied (Magalhaes et al., 2000; Li et al., 2016; Liu et al., 2017; Singh and Bhatla, 2018). Recently, these molecules were linked to PGB1 during flooding events, establishing a complex cycle that involved the requirement of all of them for the correct plant adaptation (Hebelstrup et al., 2012; Hartman et al., 2019). This overview showed us the intricate network governing hypoxia dynamic responses, mainly directed by the connection and coordination among PGB1, ET, and NO to maintain the energy state.
ABA also participates in the response to hypoxic conditions, such as root flooding (Hsu et al., 2011) or seed environment before germination (Benech-Arnold et al., 2006) and its exogenous application promotes hypoxia tolerance in roots (Ellis et al., 1999). In fact, ABA perception and signaling constitute a key hormonal network affected by the N-degron pathway (Holman et al., 2009; Vicente et al., 2017).

NITRIC OXIDE IMPACT ON SOMATIC EMBRYOGENESIS AND SEED GERMINATION UNDER LOW OXYGEN CONDITIONS

Somatic embryogenesis is the initiation of autonomous embryo development in somatic cells in response to exogenous and/or endogenous signals (Fehér, 2014), and is considered to be one of the most extreme examples of flexibility in plant development (Fehér et al. 2003). The phases of somatic embryogenesis as a morphogenic phenomenon are characterized by distinct biochemical and molecular events (Suprasanna and Bapat, 2005). The first phase is the induction stage in which differentiated somatic cells acquire embryogenic competence. This phase is followed by the expression or initiation of somatic embryogenesis in which competent cells or proembryos start developing. Finally, during maturation, somatic embryos anticipate germination by desiccation and reserve accumulation (Jiménez, 2001).

Two categories of inductive conditions which allow differentiated cells to develop into competent dedifferentiated cells are now recognized. These include plant growth regulators and stress factors (revised in Zavattieri et al., 2010). It has been described that this process is generally favored by mild hypoxia (Thorpe and Stasolla, 2001), which mimics the low O₂ environment accompanying zygotic embryo development (Rolletschek et al., 2003) (Figure 2).

An increasing number of publications link ROS and somatic embryogenesis. Oxidative stress-inducing compounds increase the cell endogenous auxin levels and promote dedifferentiation (Pasternak et al. 2002; Correa-Aragunde et al. 2006). Ötvös et al. (2005), working with alfalfa cell cultures, showed that H₂O₂ and NO have a promoting effect on somatic embryogenesis. NO stimulates the activation of cell division and embryogenic cell formation in leaf-protoplast cells of alfalfa in the presence of auxins.

In Arabidopsis PGB1 scavenges NO produced under severe hypoxia, thus fulfilling a protective role during stress conditions (Dordas et al., 2003; Dordas et al., 2004; Perazzolli et al., 2004). Like PGB1, overexpression of PGB2 enhances survival under hypoxic conditions through removal of cellular NO (Hebelstrup et al., 2006; Hebelstrup and Jensen, 2008; Hebelstrup et al., 2012). Mutation of PGB2 increases the number of Arabidopsis somatic embryos by suppressing the expression of MYC2, a repressor of auxin synthesis, and inducing the transcription of several indole-3-acetic acid (IAA) biosynthetic genes (Elhiti et al., 2013). An experimental reduction of NO through pharmacological treatments reverses the effects of PGB2 suppression on somatic embryogenesis (Elhiti et al., 2013). This phenotype
can be reversed by the re-introduction of PGB2 in the nucleus but not in the cytoplasm; this promotive effect can be attenuated by reducing the level of NO (Godee et al., 2017).

Embryo production in Arabidopsis appears to be susceptible to NO levels, as it is increased in the presence of the NO donors SNP and SNAP and is decreased after scavenging with PTIO and cPTIO (Elhiti et al. 2013). Hypoxia is also linked to non-stress conditions, but specific developmental stages such as seed imbibition and germination. The outermost layers of seed restrict O₂ diffusion, leading to hypoxic or even almost anoxia states of inner seed tissues (Borisjuk et al., 2007). It was described the NO accumulation in response to O₂ deficiency, avoiding endogenous anoxia and fermentation (Borisjuk et al., 2007). This gasotransmitter mediates a reversible O₂ balance through respiratory fluxes modulation, facilitating energy supply for the synthesis of storage compounds. PGB1 and 2 overexpression also promotes the metabolic reprogramming and lower NO content in the seed (Vigeolas et al., 2011; Thiel et al., 2011), highlighting again the importance of the molecular team composed by O₂, NO and PGBs.

NO burst was also described at early seed germination events (Simontacchi et al., 2004; Albertos et al., 2015). This NO free gas is absolutely necessary for germination completion at different molecular levels, converging into the bZIP transcription factor (TF) ABI5 (reviewed in Sánchez-Vicente et al., 2019a, b). This TF represents a molecular hub during germination repression mediated by abscisic acid (ABA) (Finkelstein and Lynch, 2000; Lopez-Molina et al., 2001). NO binds directly to ABI5, through Cys S-nitrosation of Cys153, promoting the interaction with CULLIN4-based and KEEP ON GOING E3 ligases and consequently its degradation by the proteasome (Albertos et al., 2015). Additional posttranslational levels of ABI5 regulation by NO correspond to the SUMO E3 ligase SIZ1, which is considered a Tyr nitration target (Lozano-Juste et al., 2011), and to the SNF1-RELATED PROTEIN KINASE2 (SnRK2), whose activity is inhibited by S-nitrosation (Wang et al., 2015). At the transcriptional level, ABI5 is also modulated by NO and O₂ through the N-degron pathway. Members of the ERFVII group were identified as ABI5 transcriptional activators (Gibbs et al., 2014). Additionally, the ERFVII group controls the ABI5 transcriptional repressor BRAHMA (Vicente et al., 2017).

The network integrated by NO, O₂, and PGBs tightly regulates ABI5, both at the transcriptional and posttranslational levels, highlighting fine-tune mechanisms controlling early developmental stages, which are governed by low O₂ abundance.

**NITRIC OXIDE FUNCTION IN THE RAM AND SAM, PLACES WITH SCARCE OXYGEN CONCENTRATION**

Meristems are populations of small, isodiametric cells with embryonic characteristics. Vegetative meristems are self-perpetuating, not only do they produce all tissues and organs, but they also retain their embryonic character indefinitely (Taiz et al., 2014). Previous studies have measured and defined the O₂ concentration profile in maize root apical meristem (RAM) (Gibbs et al., 1998; Darwent et al., 2003) and more recently, Weits et al. (2019) shaped...
the O$_2$ profile in Arabidopsis SAM, using a micro-scale Clark-type oxygen sensor. Both meristems display a decrease in O$_2$ concentration in the central zone, the area committed to the maintenance of the stem cells that sustain growth and development.

Besides O$_2$ levels, NO has an important role in the maintenance of the meristems and alteration in NO homeostasis is sufficient to influence whole meristems fate. NO is necessary for normal RAM organization (Sanz et al., 2014), however high levels of NO reduce auxin transport in a PIN1-dependent mechanism and RAM activity is reduced concomitantly (Fernández-Marcos et al., 2011; Sanz et al., 2014).

Some substrates of the N-degron pathway are found in meristems, where they have important functions (Gibbs et al., 2018; Weits et al., 2019; Labandera et al., 2020). The physiological hypoxia that exists in the meristems prevents its degradation through the N-degron pathway. Among hypoxia, NO levels must be also kept low to prevent N-degron pathway activation. ZPR2 sustains leaf production in the SAM (Weits et al., 2019), VRN2 is found in the SAM, RAM and lateral root primordia (LRP) where contributes to vernalization (cold-induced flowering) and hypoxia tolerance (Gendall et al., 2001; Gibbs et al., 2018). In LRP, stabilized RAP2.12 (member of ERFVII group) induce expression of core hypoxia responsive genes, promoting LRP stabilization by attenuating auxin signaling (Shukla et al., 2019). Remarkably, there is a differential gene regulation between LRP and RAM since these hypoxia responsive genes are not expressed in RAM. According to this, NO-donor treatments promote lateral root growth in a dose-dependent manner, while primary root (PR) growth is arrested (Correa-Aragunde et al., 2004). In LRP, NO could be promoting RAP2.12 degradation and thus reducing LRP stabilization.

PGBs expression patterns in meristems (Heckmann et al., 2006; Hebelstrup et al., 2007, 2012) may indicate that these proteins are facilitating alongside hypoxia the stabilization of N-degron pathway substrates by reducing NO levels. PGBs have also a central role in the protection of meristems during stress, specifically the RAM. This meristem is particularly susceptible to environmental perturbations (e.g. salinity, drought, flooding) since it is directly exposed to the soil. NO over-accumulates at the root tip under stress (Fernández-Marcos et al., 2011; Liu et al., 2015), risking RAM functionality. High levels of NO increase ET production to inhibit meristematic cell proliferation and to induce cell death through ROS (Mira et al., 2016b). PGBs have been reported to protect meristems during PEG-induced water stress (Mira et al., 2017) and hypoxia (Mira et al., 2016b) (Figure 3). Under these conditions, PGBs accumulate to reduce the programmed cell death (PCD) initiated by the high levels of NO and mediated by ET via ROS (Mira et al., 2016b, 2017). In addition, plants with jeopardized PGB1 gene expression show a number of shoot and leaf related phenotypes that include flowering delay, the tendency of SAM to reverse from bolting stage to rosette stage (Hebelstrup and Jensen, 2008), and stunted leaves with enlarged hydathodes (Hebelstrup et al., 2006). These phenotypes are coincident with NO accumulation in the affected organs, which hints at a role for PGBs in modulation of NO signaling during plant development (Hebelstrup et al., 2007).
ROLE OF NITRIC OXIDE SIGNALING BETWEEN LOW OXYGEN AND BIOTIC STRESS

Plants rely on a sophisticated network of signal transduction pathways to respond to pathogen attacks and unfavorable environmental conditions, which leads to metabolic and transcriptional reprogramming (Valeri et al., 2020). Several phytohormones have been related to plant defense, among them salicylic acid (SA) is predominantly associated with biotrophs; while jasmonic acid (JA) and ET are associated with necrotrophs (Wildermuth et al., 2001; Thaler et al. 2004; reviewed in Conrath, 2006; reviewed in Halim et al. 2006).

Although the role of NO in plants is strongly coming up to be involved in a great variety of cellular processes associated with growth and development (reviewed in Sanz et al., 2015), it was firstly described as a molecule involved in the plant immune response (Delledonne et al., 1998, Durner et al., 1998). Basal defenses and hypersensitive responses rely on NO (Mur et al., 2013). For instance, modulation of Pgb expression, which are naturally up-regulated by low oxygen tensions (Taylor et al., 1994; Hunt et al., 2002) has been shown to influence plant responses to a variety of pathogens, and suppression of Pgb resulted in elevated levels of NO, hydrogen peroxide, and JA in Arabidopsis plants infected with Botrytis cinerea (Mur et al., 2012; reviewed in Mira et al., 2016a).

One of the earliest cellular responses following successful pathogen recognition is the so-called oxidative burst, which is a rapid, transient production of ROS via consumption of O₂, that can trigger hypersensitive cell death (Wojtaszek, 1997; Govrin and Levine 2000; Torres et al., 2006). In this context, results difficult to separate NO from ROS, considering their signaling pathways in plant biotic interactions are closely connected (Scheler et al., 2013; reviewed in Sánchez-Vicente et al., 2019b).

NO also plays a major role in the signaling pathways of phytopathogenic fungi. For instance, the expression of the Botrytis cinerea flavohemoglobin gene (Bcfhg1), which is the main NO detoxification method in the fungus, is developmentally regulated, with peak expression levels during germination of conidia, and is enhanced very quickly upon exposure to NO of germinating conidia. It is believed that the production of NO by B. cinerea is probably modulated to promote fungal colonization of the plant tissue (Turrión-Gómez et al., 2010; Turrión Gómez and Benito, 2011). Furthermore, the application of external NO to Colletotrichum coccodes defers spore germination, whilst treatment with NO scavengers stimulate spore germination (Wang and Higgins, 2005).

Moreover, low O₂ predisposes plants to infection by soilborne pathogens (Figure 3). For instance, oxygen-deficient soils stress plants and predispose them to infection by water moulds such as Pythium and Phytophthora cinnamomi (Davison et al. 1993), and O₂ deprived roots leak greater amounts of soluble metabolites and ethanol attracting zoospores (Badri and Vivanco, 2009; Kozlowski, 1997). Thus, as an aerobic organism and in a water-saturated growing medium, P. cinnamomi zoospores will infect roots near the surface where there is enough O₂.

The inactivation of different components of the Arg/N-degron pathway results in greater susceptibility of Arabidopsis to necrotrophic pathogens. Thus, it has been shown that induction of components of the hypoxia response, controlled by the ERFVIIIs, enhanced clubroot disease progress, indicating that the protist hijacks the N-end
rule ERFVII regulation system to enhance infection (Gravot et al., 2016). Early studies indicate that RAP2.3, and maybe other ERFVII transcription factors, might be key regulators both in the low-oxygen and plant biotic stress responses (Valeri et al., 2020). Kim et al. (2018) results show that OCTADECANOID-RESPONSIVE ARABIDOPSIS 59 (ORA59), one of the best characterized ERF transcription factors involved in B. cinerea resistance, interacts with RAP2.3, and its expression is induced synergistically by JA and ET confirming its importance in the JA and ET signaling pathway (Pré et al., 2008). To this regard, Arabidopsis plants overexpressing RAP2.2 and a mutant line showed higher resistance and more susceptibility respectively, suggesting an important role of RAP2.2 against the infection by the necrotroph (Zhao et al., 2012). A recent study conducted by Valeri et al. (2020) indicates that infection by B. cinerea induces increased respiration, leading to a drastic drop in the O2 level in the leaf and that the establishment of this local hypoxic area results in stabilization and nuclear relocalization of RAP2.12. As a consequence, this nuclear relocalization activates the hypoxia-responsive gene network, implying that ERF-VII proteins can get stabilized in infected tissue and have influence in pathogen resistance, allowing RAP2.3 to form a complex with ORA59 to regulate plant defense genes (Kim et al., 2018) or influencing other proteins with a hypoxia dependent stabilization.

CONCLUDING REMARKS

Among the challenges imposed by global warming, the forecast of unexpected and increased floods will cause limitations in plant normal development and productivity for agricultural purposes. Therefore, the control of plant responses to this hypoxia scenario is a landmark aspect for future research, as it critically impacts on seed germination, plant development and establishment and, consequently, on plant productivity. The identification of the elements and the molecular bases that participate in hypoxic stress responses is essential to understand their function in the plant, which is a prerequisite for its genetic improvement. Thus, advances in the study of plant priming using NO-related compounds to enhance hypoxia tolerance could be achieved in a similar way to ET and ABA pre-treatments (Ellis et al., 1999; Hartman et al., 2019).

In parallel, this environmental modification can favor the development of new plant-pest and pathogens or increase the incidence levels of the today existents. Nowadays some controversy still surrounds the NO homeostasis in plant immunity, both at the level of production and turnover (reviewed in Vandelle et al., 2016), that needs to be solved for a better pest control.

The N-degron pathway was identified as a new NO sensor that functions through its ability to destroy specific regulatory proteins bearing N-terminal cysteine in mammals (Hu et al., 2005; Masson et al., 2019). In plants, apart from the evidences reported by Gibbs et al., 2014 and Gibbs et al., 2018 on the proteolytic control of group VII ERF transcription factors and polycomb repressive complex 2 subunit VRN2, respectively, no other target has been related with NO directly. Deciphering the mechanism of NO sensing, by direct binding of the molecule, and the posttranslational regulation of molecular targets across the different components of the N-degron pathway will shed light to control hypoxia, what is detrimental for plant survival.
ACKNOWLEDGMENTS

This work was financed by grants BIO2017-85758-R from the Ministerio de Ciencia, Innovación y Universidades (MICIU), SA313P18 from Junta de Castilla y León and Escalera de Excelencia CLU-2018-04 co-funded by the P.O. FEDER of Castilla y León 2014–2020 Spain (to O.L.) and FS/26-2017 and 2019 from Fundación Solórzano (to I. S-V). I. M-G is supported by a FPU grant. We thank BIO2015-68957-REDT and RED2018-102397-T Spanish network for stimulating discussions.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the visualization, writing of original draft, review and editing. O.L. conceived the study and is responsible for supervision and funding acquisition.
REFERENCES

Abbas M, Berckhan S, Rooney DJ, et al. 2015. Oxygen sensing coordinates photomorphogenesis to facilitate seedling survival. Current Biology 25, 1483–1488.

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. Nature Communications 6, 1–10.

Appleby CA, Tjepkema JD, Trinick MJ. 1983. Hemoglobin in a nonleguminous plant, Parasponia: possible genetic origin and function in nitrogen fixation. Science 220, 951–953.

Armstrong W, Beckett PM, Colmer TD, Setter TL, Greenway H. 2019. Tolerance of roots to low oxygen: ‘Anoxic’ cores, the phytoglobin-nitric oxide cycle, and energy or oxygen sensing. Journal of Plant Physiology 239, 92–108.

Arredondo-Peter R, Hargrove MS, Moran JF, Sarath G, Klucas R V. 1998. Plant hemoglobins. Plant Physiology 118, 1121–1125.

Bachmair A, Finley D, Varshavsky A. 1986. In vivo half-life of a protein is a function of its amino-terminal residue. Science 234, 179–186.

Badri DV, Vivanco JM. 2009. Regulation and function of root exudates. Plant, Cell & Environment 32, 666–681.

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

Benech-Arnold RL, Gualano N, Leymarie J, Côme D, Corbineau F. 2006. Hypoxia interferes with ABA metabolism and increases ABA sensitivity in embryos of dormant barley grains. Journal of Experimental Botany 57, 1423–1430.

Borisjuk I, Macherel D, Benamar A, Wobus U, Rolletschek H. 2007. Low oxygen sensing and balancing in plant seeds: A role for nitric oxide. New Phytologist 176, 813–823.
Cantrel C, Vazquez T, Puyaubert J, Rezé N, Lesch M, Kaiser WM, Dutilleul C, Guillais I, Zachowski A, Baudouin E. 2011. Nitric oxide participates in cold-responsive phosphosphingolipid formation and gene expression in Arabidopsis thaliana. New Phytologist 189, 415–427.

Chen L, Liao B, Qi H, et al. 2015. Autophagy contributes to regulation of the hypoxia response during submergence in Arabidopsis thaliana. Autophagy 11, 2233–2246.

Conrath U. 2006 Systemic Acquired Resistance. Plant Signaling & Behavior 1, 179-184.

Correa-Aragunde N, Graziano M, Lamattina L. 2004. Nitric oxide plays a central role in determining lateral root development in tomato. Planta 218, 900–905.

Correa-Aragunde N, Lanteri ML, García-Mata C, ten Have A, Laxalt AM, Graziano M, Lamattina L. 2006. Nitric oxide functions as intermediate in auxin, abscisic acid, and lipid signaling pathways. Nitric oxide in plant growth, development and stress physiology. 57, 581–588.

Darwent MJ, Armstrong W, Armstrong J, Beckett PM. 2003. Exploring the radial and longitudinal aeration of primary maize roots by means of clark-type oxygen microelectrodes. Russian Journal of Plant Physiology.722–732.

Davison EM, Stukely MJC, Crane CE, Tay FCS. 1993. Invasion of phloem and xylem of woody stems and roots of Eucalyptus marginata and Pinus radiata by Phytophthora cinnamomi. Phytopathology. 84, 335–340.

De Marchi R, Sorel M, Mooney B, et al. 2016. The N-end rule pathway regulates pathogen responses in plants. Scientific Reports 6.

Delledonne M, Xia Y, Dixon RA, Lamb C. 1998. Nitric oxide functions as a signal in plant disease resistance. 394, 585.

Dordas C, Hasinoff BB, Igamberdiev AU, Manac’h N, Rivoal J, Hill RD. 2003. Expression of a stress-induced hemoglobin affects NO levels produced by alfalfa root cultures under hypoxic stress. Plant Journal 35, 763–770.

Dordas C, Hasinoff BB, Rivoal J, Hill RD. 2004. Class-1 hemoglobins, nitrate and NO levels in anoxic maize cell-suspension cultures. Planta 219, 66–72.

Durner J, Wendehenne D, Klessig DF. 1998. Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose. Proceedings of the National Academy of Sciences 95, 10328–10333.

Elhiti M, Huang S, Mira MM, Hill RD, Stasolla C. 2018. Redirecting cell fate during in vitro embryogenesis: Phytoglobins as molecular switches. Frontiers in Plant Science 9, 1–4.

Elhiti M, Stasolla C, Wang A. 2013. Molecular regulation of plant somatic embryogenesis. In Vitro Cellular & Developmental Biology – Plant 49, 631–642.

Ellis MH, Dennis ES, Peacock WJ. 1999. Arabidopsis roots and shoots have different mechanisms for hypoxic stress tolerance. Plant physiology 119, 57–64.
Fehér A, Pasternak TP, Dudits D. 2003. Transition of somatic plant cells to an embryogenic state. Plant Cell, Tissue and Organ Culture 74, 201–228.

Fehér A. 2014. Somatic embryogenesis – Stress-induced remodeling of plant cell fate. Biochimica et Biophysica Acta (BBA) – Gene Regulatory Mechanisms 1849, 385–402.

Fernández-Marcos M, Sanz L, Lewis DR, Muday GK, Lorenzo O. 2011. Nitric oxide causes root apical meristem defects and growth inhibition while reducing PIN-FORMED 1 (PIN1)-dependent acropetal auxin transport. Proceedings of the National Academy of Sciences of the United States of America 108, 18506–18511.

Finkelstein RR, Lynch TJ. 2000. The Arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. Plant Cell 12, 599–609.

Garrocho-Villegas V, Arredondo-Peter R. 2008. Molecular cloning and characterization of a moss (Ceratodon purpureus) nonsymbiotic hemoglobin provides insight into the early evolution of plant nonsymbiotic hemoglobins. Molecular Biology and Evolution 25, 1482–1487.

Gendall AR, Levy YY, Wilson A, Dean C. 2001. The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in Arabidopsis. Cell 107, 525–35.

Gibbs DJ, Lee SC, Md Isa N, et al. 2011. Homeostatic response to hypoxia is regulated by the N-end rule pathway in plants. Nature 479, 415–418.

Gibbs DJ, Mdlsa N, Movahedi M, et al. 2014. Nitric oxide sensing in plants is mediated by proteolytic control of Group VII ERF transcription factors. Molecular Cell 53, 369–379.

Gibbs DJ, Tedds HM, Labandera AM, et al. 2018. Oxygen-dependent proteolysis regulates the stability of angiosperm polycomb repressive complex 2 subunit VERNALIZATION 2. Nature Communications 9, 5438.

Gibbs J, Turner DW, Armstrong W, Darwent MJ, Greenway H. 1998. Response to oxygen deficiency in primary maize roots. I. Development of oxygen deficiency in the stele reduces radial solute transport to the xylem. Australian Journal of Plant Physiology 25, 745–758.

Godee C, Mira MM, Wally O, Hill RD, Stasolla C. 2017. Cellular localization of the Arabidopsis class 2 phytoglobin influences somatic embryogenesis. Journal of Experimental Botany 68, 1013–1023.

Gopalasubramaniam SK, Kovacs F, Violante-Mota F, Twigg P, Arredondo-Peter R, Sarath G. 2008. Cloning and characterization of a caesalpinoid (Chamaecrista fasciculata) hemoglobin: The structural transition from a nonsymbiotic hemoglobin to a leghemoglobin. Proteins: Structure, Function and Genetics 72, 252–260.

Govrin EM, Levine A. 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen Botrytis cinerea. Current Biology 10, 751–757.
Graciet E, Mesiti F, Wellmer F. 2010. Structure and evolutionary conservation of the plant N-end rule pathway. The Plant Journal 61, 741–751.

Graciet E, Walter F, Maoiléidigh DÓ, Pollmann S, Meyerowitz EM, Varshavsky A, Wellmer F. 2009. The N-end rule pathway controls multiple functions during Arabidopsis shoot and leaf development. Proceedings of the National Academy of Sciences of the United States of America 106, 13618–13623.

Gravot A, Richard G, Lime T, Lemarié S, Jubault M, Lariagon C, Lemoine J, Vicente J, Robert-Seilaniantz A, Holdsworth MJ, Manzanares-Dauleux MJ. 2016. Hypoxia response in Arabidopsis roots infected by Plasmodiophora brassicae supports the development of clubroot. BMC Plant Biology 16, 25.

Gupta KJ, Hebelstrup KH, Mur LAJ, Igamberdiev AU. 2011. Plant hemoglobins: Important players at the crossroads between oxygen and nitric oxide. FEBS Letters 585, 3843–3849.

Gupta KJ, Igamberdiev AU. 2016. Reactive nitrogen species in mitochondria and their implications in plant energy status and hypoxic stress tolerance. Frontiers in Plant Science 7, 1–6.

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

Gupta KJ, Stoimenova M, Kaiser WM. 2005. In higher plants, only root mitochondria, but not leaf mitochondria reduce nitrite to NO, in vitro and in situ. Journal of experimental botany 56, 2601–2609.

Halim VA, Vess A, Scheel D, Rosahl S. 2006. The role of Salicylic Acid and Jasmonic Acid in pathogen defense. Plant Biology 8, 307–313.

Hargrove MS, Barry JK, Brucker EA, Berry MB, Phillips GN, Olson JS, Arredondo-Peter R, Dean JM, Klucas R V., Sarath G. 1997. Characterization of recombinant soybean leghemoglobin a and apolar distal histidine mutants. Journal of Molecular Biology 266, 1032–1042.

Hartman S, Liu Z, van Veen H, et al. 2019. Ethylene-mediated nitric oxide depletion pre-adapts plants to hypoxia stress. Nature Communications 10, 1–9.

Hebelstrup KH, Hunt P, Dennis E, Jensen SB, Jensen EØ. 2006. Hemoglobin is essential for normal growth of Arabidopsis organs. Physiologia Plantarum 127, 157–166.

Hebelstrup KH, Igamberdiev AU, Hill RD. 2007. Metabolic effects of hemoglobin gene expression in plants. Gene 398, 86–93.

Hebelstrup KH, Jensen EO. 2008. Expression of NO scavenging hemoglobin is involved in the timing of bolting in Arabidopsis thaliana. Planta 227, 917–927.
Hebelstrup KH, van Zanten M, Mandon J, Voesenek LACJ, Harren FJM, Cristescu SM, Möller IM, Mur LAJ. 2012. Haemoglobin modulates NO emission and hyponasty under hypoxia-related stress in Arabidopsis thaliana. Journal of Experimental Botany 63, 5581–5591.

Heckmann AB, Hebelstrup KH, Larsen K, Micaelo NM, Jensen E. 2006. A single hemoglobin gene in Myrica gale retains both symbiotic and non-symbiotic specificity. Plant Molecular Biology 61, 769–779.

Hill R, Hargrove M, Arredondo-Peter R. 2016. Phytoglobin: A novel nomenclature for plant globins accepted by the globin community at the 2014 XVIII conference on Oxygen-Binding and Sensing Proteins. F1000Research 5, 1–8.

Holman TJ, Jones PD, Russell L, et al. 2009. The N-end rule pathway promotes seed germination and establishment through removal of ABA sensitivity in Arabidopsis. Proceedings of the National Academy of Sciences 106, 4549–4554.

Hoy JA, Hargrove MS. 2008. The structure and function of plant hemoglobins. Plant Physiology and Biochemistry 46, 371–379.

Hsu FC, Chou MY, Peng HP, Chou SJ, Shih MC. 2011. Insights into hypoxic systemic responses based on analyses of transcriptional regulation in Arabidopsis. PloS One 6, e28888.

Hu RG, Sheng J, Qi X, Xu Z, Takahashi TT, Varshavsky A. 2005. The N-end rule pathway as a nitric oxide sensor controlling the levels of multiple regulators. Nature 437, 981–986.

Hunt PW, Klok EJ, Trevaskis B, Watts RA, Ellis MH, Peacock WJ, Dennis ES. 2002. Increased level of hemoglobin 1 enhances survival of hypoxic stress and promotes early growth in Arabidopsis thaliana. Proceedings of the National Academy of Sciences of the United States of America 99, 17197–17202.

Hunt PW, Watts RA, Trevaskis B, Llewelyn DJ, Burnell J, Dennis ES, Peacock WJ. 2001. Expression and evolution of functionally distinct haemoglobin genes in plants. Plant Molecular Biology 47, 677–692.

Igamberdiev AU, Baron K, Manac’h-Little N, Stoimenova M, Hill RD. 2005. The haemoglobin/nitric oxide cycle: Involvement in flooding stress and effects on hormone signalling. Annals of Botany 96, 557–564.

Jacobsen-Lyon K, Jensen EO, Jorgensen JE, Marcker KA, Peacock WJ, Dennis ES. 1995. Symbiotic and nonsymbiotic hemoglobin genes of Casuarina glauca. Plant Cell 7, 213–223.

Jiménez VM. 2001. Regulation of in vitro somatic embryogenesis with emphasis on the role of endogenous hormones. Revista Brasileira de Fisiologia Vegetal. 13, 196-223.

Kim NY, Jang YJ, Park OK. 2018. AP2/ERF family transcription factors ORA59 and RAP2.3 interact in the nucleus and function together in ethylene responses. Frontiers in Plant Science 9, 1675.

Kozlowski TT. 1997. Responses of woody plants to flooding and salinity. Tree Physiology 17, 490–490.
Labandera A, Tedds HM, Bailey M, Sprigg C, Etherington RD, Akintewe O, Kallechurn G, Holdsworth MJ, Gibbs DJ. 2020. The PRT6 N-degron pathway restricts VERNALIZATION 2 to endogenous hypoxic niches to modulate plant development. New Phytologist, nph.16477.

Li X, Pan Y, Chang B, Wang Y, Tang Z. 2016. NO promotes seed germination and seedling growth under high salt may depend on EIN3 protein in arabidopsis. Frontiers in Plant Science 6, 1–10.

Licausi F, Kosmacz M, Weits DA, Giuntoli B, Giorgi FM, Voesenek LACJ, Perata P, Van Dongen JT. 2011. Oxygen sensing in plants is mediated by an N-end rule pathway for protein destabilization. Nature 479, 419–422.

Lira-Ruan V, Ross EJH, Sarath G, Klucas R V., Arredondo-Peter R. 2002. Mapping and analysis of a hemoglobin gene family from Oryza sativa. Plant Physiology and Biochemistry 40, 199–202.

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.

Liu M, Liu XX, He XL, Liu LJ, Wu H, Tang CX, Zhang YS, Jin CW. 2017. Ethylene and nitric oxide interact to regulate the magnesium deficiency-induced root hair development in Arabidopsis. New Phytologist 213, 1242–1256.

Liu W, Li RJ, Han TT, Cai W, Fu ZW, Lu YT. 2015. Salt stress reduces root meristem size by nitric oxidemediated modulation of auxin accumulation and signaling in Arabidopsis. Plant Physiology 168, 343–356.

Lopez-Molina L, Mongrand S, Chua NH. 2001. A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. Proceedings of the National Academy of Sciences of the United States of America 98, 4782–4787.

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

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

Magalhaes JR, Monte DC, Durzan D. 2000. Nitric oxide and ethylene emission in Arabidopsis thaliana. Physiology and Molecular Biology of Plants 6, 117–127.

Masson N, Keeley TP, Giuntoli B, White MD, Lavilla Puerta M, Perata P, Hopkinson RJ, Flashman E, Licausi F, Ratcliffe PJ. 2019. Conserved N-terminal cysteine dioxygenases transduce responses to hypoxia in animals and plants. Science 364, 65–69.

Millar HA, Day DA. 1996. Nitric oxide inhibits the cytochrome oxidase but not the alternative oxidase of plant mitochondria. FEBS Letters 398, 155–158.
Mira MM, Hill RD, Stasolla C. 2016a. Regulation of programmed cell death by phytoglobins. Journal of Experimental Botany 67, 5901–5908.

Mira MM, Hill RD, Stasolla C. 2016b. Phytoglobins improve hypoxic root growth by alleviating apical meristem cell death. Plant Physiology 172, 2044–2056.

Mira MM, Huang S, Kapoor K, Hammond C, Hill RD, Stasolla C. 2017. Expression of Arabidopsis class 1 phytoglobin (AtPgb1) delays death and degradation of the root apical meristem during severe PEG-induced water deficit. Journal of Experimental Botany 68, 5653–5668.

Mot AC, Puscas C, Miclea P, Naumova-Letia G, Dorneanu S, Dismeyer N, Silaghi-Dumitrescu R. 2018. Redox control and autoxidation of class 1, 2 and 3 phytoglobins from Arabidopsis thaliana. Scientific Reports 8, 1–13.

Mugnai S, Azzarello E, Baluka F, Mancuso S. 2012. Local root apex hypoxia induces n0-mediated hypoxic acclimation of the entire root. Plant and Cell Physiology 53, 912–920

Mukhi N, Kundu S, Kaur J. 2017. NO dioxygenase- and peroxidase-like activity of Arabidopsis phytoglobin 3 and its role in Sclerotinia sclerotiorum defense. Nitric Oxide – Biology and Chemistry 68, 150–162.

Mur LAJ, Prats E, Pierre S, Hall MA, Hebelstrup KH. 2013. Integrating nitric oxide into salicylic acid and jasmonic acid/ethylene plant defense pathways. Frontiers in Plant Science 4:215.

Mur LAJ, Sivakumaran A, Mandon J, Cristescu SM, Harren FJM, Hebelstrup KH. 2012. Haemoglobin modulates salicylate and jasmonate/ethylene-mediated resistance mechanisms against pathogens. Journal of Experimental Botany 63, 4375–4387.

Ott T, Van Dongen JT, Günther C, Krusell L, Desbrosses G, Vigeolas H, Bock V, Czechowski T, Geigenberger P, Udvardi MK. 2005. Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development. Current Biology 15, 531–535.

Ötvös K, Pasternak TP, Miskolczi P, Domoki M, Dorjgotov D, Szőcs A, Bottka S, Dudits D, Fehér A. 2005. Nitric oxide is required for, and promotes auxin-mediated activation of, cell division and embryogenic cell formation but does not influence cell cycle progression in alfalfa cell cultures: NO in cell division. The Plant Journal 43, 849–860.

Pasternak TP, Prinsen E, Ayaydin F, Miskolczi P, Potters G, Asard H, Van Onckelen HA, Dudits D, Fehér A. 2002. The role of auxin, pH, and stress in the activation of embryogenic cell division in leaf protoplast-derived cells of Alfalfa. Plant Physiology 129, 1807–1819.

Perazzoli M, Dominici P, Romero-Puertas MC, Zago E, Zeier J, Sonoda M, Lamb C, Delledonne M. 2004. Arabidopsis nonsymbiotic hemoglobin Ahb1 modulates nitric oxide bioactivity. Plant Cell 16, 2785-2794.
Planchet E, Gupta KJ, Sonoda M, Kaiser WM. 2005. Nitric oxide emission from tobacco leaves and cell suspensions: Rate limiting factors and evidence for the involvement of mitochondrial electron transport. Plant Journal 41, 732–743.

Pré M, Atallah M, Champion A, De Vos M, Pieterse CMJ, Memelink J. 2008. The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiology 147, 1347–1357.

Rivoal J, Hill RD, Dordas C, Hasinoff BB. 2004. Class-1 hemoglobin, nitrate and NO levels in anoxic maize cell-suspension cultures. Planta 219, 66–72.

Rolletschek H, Weber H, Borisjuk L. 2003. Energy status and its control on embryogenesis of legumes. Embryo photosynthesis contributes to oxygen supply and is coupled to biosynthetic fluxes. Plant Physiology 132, 1196–1206.

Rubio MC, Calvo-Begueria L, Díaz-Mendoza M, et al. 2019. Phytoglobins in the nuclei, cytoplasm and chloroplasts modulate nitric oxide signaling and interact with abscisic acid. Plant Journal 100, 38–54.

Sainz M, Calvo-Begueria L, Pérez-Rontomé C, Wienkoop S, Abián J, Staudinger C, Bartesaghi S, Radi R. 2015. Leghemoglobin is nitrated in functional legume nodules in a tyrosine residue within the heme cavity by a nitrite/peroxide-dependent mechanism. 81, 723–735.

Sánchez-Vicente I, Albertos P, Lorenzo O. 2019a. Protein shuttle between nucleus and cytoplasm: new paradigms in the ABI5-dependent ABA Responses. Molecular Plant 12, 1425–1427.

Sánchez-Vicente I, Fernández-Espinosa MG, Lorenzo O. 2019b. Nitric oxide molecular targets: Reprogramming plant development upon stress. Journal of Experimental Botany 70, 4441–4460.

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

Sanz L, Fernández-Marcos M, Modrego A, Lewis DR, Muday GK, Pollmann S, Dueñas M, Santos-Buelga C, Lorenzo O. 2014. Nitric oxide plays a role in stem cell niche homeostasis through its interaction with auxin. Plant Physiology 166, 1972–1984.

Sasidharan R, Hartman S, Liu Z, Martopawiro S, Sajeev N, Van Veen H, Yeung E, Voesenek LACJ. 2018. Signal dynamics and interactions during flooding stress. Plant Physiology 176, 1106–1117.

Scheler C, Durner J, Astier J. 2013. Nitric oxide and reactive oxygen species in plant biotic interactions. Current Opinion in Plant Biology 16, 534–539.

Shukla V, Lombardi L, Iacopino S, Pencik A, Novak O, Perata P, Giuntoli B, Licausi F. 2019. Endogenous hypoxia in lateral root primordia controls root architecture by antagonizing auxin signaling in Arabidopsis. Molecular Plant 12, 538–551.
Simontacchi M, Jasid S, Puntarulo S. 2004. Nitric oxide generation during early germination of sorghum seeds. Plant Science 167, 839–847.

Singh N, Bhatla SC. 2018. Nitric oxide regulates lateral root formation through modulation of ACC oxidase activity in sunflower seedlings under salt stress. Plant Signaling and Behavior 13, 1–7.

Spyrakis F, Bruno S, Bidon-Chanal A, Luque FJ, Abbruzzetti S, Viappiani C, Dominici P, Mozzarelli A. 2011. Oxygen binding to Arabidopsis thaliana Ahb2 nonsymbiotic hemoglobin: Evidence for a role in oxygen transport. IUBMB Life 63, 355–362.

Suprasanna P, Bapat, VA. 2005. Differential gene expression during somatic embryogenesis. Plant Cell Monographs 2, 305–320.

Taiz L, Zeiger E, Møller IM, Murphy A. 2014. Plant Physiology. Sinauer.

Taylor ER, Nie XZ, MacGregor AW, Hill RD. 1994. A cereal haemoglobin gene is expressed in seed and root tissues under anaerobic conditions. Plant Molecular Biology 24, 853–862.

Thaler JS, Owen B, Higgins VJ. 2004. The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. Plant Physiology 135, 530–538.

Thiel J, Rolletschek H, Friedel S, Lunn JE, Nguyen TH, Feil R, Tschiersch H, Müller M, Borisjuk L. 2011. Seed-specific elevation of non-symbiotic hemoglobin AtHb1: Beneficial effects and underlying molecular networks in Arabidopsis thaliana. BMC Plant Biology 11, 48.

Thorpe, TA, Stasolla, C. 2001. Somatic embryogenesis. Current trends in the embryology of Angiosperms, 279–336.

Till CJ, Vicente J, Zhang H, Oszvald M, Deery MJ, Pastor V, Lilley KS, Ray R V., Theodoulou Fl, Holdsworth MJ. 2019. The Arabidopsis thaliana N-receognin E3 ligase PROTEOLYSIS1 influences the immune response. Plant Direct 3.

Tiso M, Tejero J, Kenney C, Frizzell S, Gladwin MT. 2012. Nitrite reductase activity of nonsymbiotic hemoglobins from Arabidopsis thaliana. Biochemistry 51, 5285–5292.

Torres MA, Jones JDG, Dangl JL. 2006. Reactive oxygen species signaling in response to pathogens. Plant Physiology 141, 373–378.

Trevaskis B, Watts RA, Andersson CR, Llewellyn DJ, Hargrove MS, Olson JS, Dennis ES, Peacock WJ. 1997. Two hemoglobin genes in Arabidopsis thaliana: The evolutionary origins of leghemoglobins. Proceedings of the National Academy of Sciences of the United States of America 94, 12230–12234.

Turrión-Gómez JL, Benito EP. 2011. Flux of nitric oxide between the necrotrophic pathogen Botrytis cinerea and the host plant. Molecular Plant Pathology 12, 606–616.
Turrión-Gómez JL, Eslava AP, Benito EP. 2010. The flavohemoglobin BCFHG1 is the main NO detoxification system and confers protection against nitrosative conditions but is not a virulence factor in the fungal necrotoph Botrytis cinerea. Fungal Genetics and Biology 47, 484–496.

Valeri MC, Novi G, Weits DA, Mensuali A, Perata P, Loreti E. 2020. Botrytis cinerea induces local hypoxia in Arabidopsis leaves. New Phytologist, nph.16513.

Vandelle E, Ling T, Imanifard Z, Liu R, Delledonne M, Bellin D. 2016. Nitric oxide signaling during the hypersensitive disease resistance response. Advances in Botanical Research, Academic Press 77, 219-243.

Varshavsky A. 2011. The N-end rule pathway and regulation by proteolysis. Protein Science 20, 1298–1345.

Vicente J, Mendiondo GM, Movahedi M, et al. 2017. The Cys-Arg/N-End Rule Pathway is a general sensor of abiotic stress in flowering plants. Current Biology 27, 3183-3190.e4.

Vicente J, Mendiondo GM, Pauwels J, et al. 2019. Distinct branches of the N-end rule pathway modulate the plant immune response. New Phytologist 221, 988–1000.

Vigeolas H, Hühn DH, Geigenberger P. 2011. Nonsymbiotic hemoglobin-2 leads to an elevated energy state and to a combined increase in polyunsaturated fatty acids and total oil content when overexpressed in developing seeds of transgenic arabidopsis plants. Plant Physiology 155, 1435–1444.

Wang J, Higgins VJ. 2005. Nitric oxide has a regulatory effect in the germination of conidia of Colletotrichum coccodes. Fungal Genetics and Biology 42, 284–292.

Wang P, Zhu JK, Lang Z. 2015. Nitric oxide suppresses the inhibitory effect of abscisic acid on seed germination by S-nitrosylation of SnRK2 proteins. Plant Signaling and Behavior 10, 6–8.

Wang R, Guegler K, LaBrie ST, Crawford NM. 2000. Genomic analysis of a nutrient response in arabidopsis reveals diverse expression patterns and novel metabolic and potential regulatory genes induced by nitrate. Plant Cell 12, 1491–1509.

Watts RA, Hunt PW, Hvitved AN, Hargrove MS, Peacock WJ, Dennis ES. 2001. A hemoglobin from plants homologous to truncated hemoglobins of microorganisms. Proceedings of the National Academy of Sciences of the United States of America 98, 10119–10124.

Weits DA, Giuntoli B, Kosmacz M, Parlanti S, Hubberten H-M, Riegler H, Hoeftgen R, Perata P, van Dongen JT, Licausi F. 2014. Plant cysteine oxidases control the oxygen-dependent branch of the N-end-rule pathway. Nature Communications 5, 3425.

Weits DA, Kunkowska AB, Kamps NCW, et al. 2019. An apical hypoxic niche sets the pace of shoot meristem activity. Nature 569, 714–717.
Weits DA, van Dongen JT, Licausi F. 2020. Molecular oxygen as a signaling component in plant development. New Phytologist, nph.16424.

White MD, Kamps JJAG, East S, Taylor Kearney LJ, Flashman E. 2018. The plant cysteine oxidases from Arabidopsis thaliana are kinetically tailored to act as oxygen sensors. Journal of Biological Chemistry 293, 11786–11795.

Wildermuth MC, Dewdney J, Wu G, Ausubel FM. 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414, 562–565.

Wittenberg JB, Appleby CA, Bergersen FJ, Turner GL. 1975. Leghemoglobin: the role of hemoglobin in the nitrogen-fixing legume root nodule. Annals of the New York Academy of Sciences 244, 28–34.

Wojtaszek P. 1997. Oxidative burst: an early plant response to pathogen infection. Biochemical Journal 322, 681–692.

Yoshida S, Ito M, Callis J, Nishida I, Watanabe A. 2002. A delayed leaf senescence mutant is defective in arginyl-tRNA:protein arginyltransferase, a component of the N-end rule pathway in Arabidopsis. Plant Journal 32, 129–137.

Zarban R, Vogler M, Wong A, Eppinger J, Al-Babili S, Gehring C. 2019. Discovery of a nitric oxide-responsive protein in Arabidopsis thaliana. Molecules 24, 2691.

Zavattieri MA, Frederico AM, Lima M, Sabino R, Arnholdt-Schmitt B. 2010. Induction of somatic embryogenesis as an example of stress-related plant reactions. Electronic Journal of Biotechnology 13, 0–0.

Zhan N, Wang C, Chen L, et al. 2018. S-Nitrosylation targets GSNO Reductase for selective autophagy during hypoxia responses in plants. Molecular Cell 71, 142-154.e6.

Zhang H, Gannon L, Hassall KL, Deery MJ, Gibbs DJ, Holdsworth MJ, van der Hoorn RAL, Lilley KS, Theodoulou FL. 2018a. N-terminomics reveals control of Arabidopsis seed storage proteins and proteases by the Arg/N-end rule pathway. New Phytologist 218, 1106–1126.

Zhang H, Gannon L, Jones PD, Rundle CA, Hassall KL, Gibbs DJ, Holdsworth MJ, Theodoulou FL. 2018b. Genetic interactions between ABA signalling and the Arg/N-end rule pathway during Arabidopsis seedling establishment. Scientific Reports 8, 1–12.

Zhao Y, Wei T, Yin K-Q, Chen Z, Gu H, Qu L-J, Qin G. 2012. Arabidopsis RAP2.2 plays an important role in plant resistance to Botrytis cinerea and ethylene responses. New Phytologist 195, 450–460.
Table 1. Overview of the phytoglobins described in plants.

| NAME | TISSUE SPECIFICITY | EXPRESSION PATTERN | PROCESSES REGULATED | BOUNDING CAPACITY | BIOPHYSICAL ROLE | REFERENCES |
|------|--------------------|--------------------|---------------------|-------------------|-----------------|------------|
| Symbiotic phytoglobin (SymPhytogb) | Root nodules | Nodule-specific expression pattern | $O_2$ transport and release during $N_2$ fixation to maintain the flux for respiration | High affinity for $O_2$ | Facilitate $O_2$ diffusion | Appleby et al., 1983; Jacobsen-Lyon et al., 1995; Gopalasubramaniam et al., 2008 |
| Symbiotic hemoglobin (sHb) | | | | | | |
| Phytoglobin0 (Phytogb0) | Whole plant | Higher expression in gametophytes; induction under hot and cold stresses, exposure to nitrate and increased sucrose supply | NO detoxification under hypoxia stress | High affinity for $O_2$ | NO scavenging | Garrocho-Villegas and Arredondo-Peter, 2008 |
| Nonsymbiotic hemoglobin (nsHb) | | | | | | |
| Class/type 1 nonsymbiotic hemoglobin (nsHb-1) | Embryonic and vegetative organs | Induction under hypoxia, ethylene, exposure to nitrate and increased sucrose supply in roots and rosette leaves, and upon NO and $H_2O_2$ treatments | Maintenance of NO and $O_2$ levels during cellular hypoxic conditions to modulate energy status | Highest affinity for $O_2$, low dissociation rate | $O_2$ and NO scavenging, NO dioxygenase activity | Trevaskis et al., 1997; Wang et al., 2000; Lira-Ruan et al., 2002; Dordas et al., 2003, 2004; Hunt et al., 2001, 2002; Perazzolli et al., 2004; Cantrel et al., 2011; Thiel et al., 2011; Hartman et al., |
| Protein                  | Class/Type | Description                                        | Induction/Expression Conditions                                                                 | Function                                                                 | References                                      |
|-------------------------|------------|----------------------------------------------------|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|------------------------------------------------|
| Phytoglobin2 (Phytogb2) | Class/Type 2 | Nonsymbiotic hemoglobin                           | Induction under cytokinin treatment and low temperature                                       | Maintenance of NO and O₂ levels during cellular hypoxic conditions and during embryogenesis; regulation of oil and sucrose accumulation in seeds | Trevaskis et al., 1997; Hunt et al., 2001; Dordas et al., 2003, 2004; Spyrakis et al., 2011; Vigeolas et al., 2011; Elhiti et al., 2018 |
| Phytoglobin3 (Phytogb3) | Class/Type 3 | Nonsymbiotic hemoglobin/Truncated hemoglobin (tHb) | Inhibition under hypoxia; induction upon auxin, NO, and H₂O₂ treatments and biotic stress | NO and ROS levels modulation during biotic stress; CO and O₂ in a reversible manner, low O₂ affinity | Watts et al., 2001; Mukhi et al., 2017 |
| Leghemoglobin (Lb)      |        | Legume root nodules                               | Nodule-specific expression pattern                                                             | O₂ transport and release during N₂ fixation to maintain the flux for respiration; High affinity for O₂ Facilitate O₂ diffusion | Wittenberg et al., 1975; Hargrove et al., 1997; Ott et al., 2005 |
FIGURES AND FIGURE LEGENDS

Figure 1. NO and O\textsubscript{2} involvement along the different steps of the N-degron pathway in plants and PGBs implication. The stability of N-degron substrates is controlled by NO/O\textsubscript{2} levels, which balance is modulated by Phytoglobins (PGBs). Under normoxia (1), these substrates are degraded by the action of different enzymes consecutively along the PCO-branch. When plants suffer a hypoxic-related stress (2), this pathway becomes inhibited, triggering the transcriptional responses. During stress, PGBs play a key role, scavenging free NO (3), which in turns is able to modify PGBs posttranslationally, to determine a fine-tune redox balance and energy status (created with BioRender.com).

MAPs (Methionine aminopeptidases); PCOs (Plant cysteine oxidases); ATEs (Arginyl-tRNA-transferases); PRT6 (PROTEOLYSIS 6); NR (Nitrate reductase); ZPR2 (Protein LITTLE ZIPPER 2); VRN2 (VERNALIZATION 2); ERFVII (Group VII Ethylene response factors).

Figure 2. Network of NO and low oxygen interactions in a developmental stage-based context. Somatic embryogenesis, seed germination, RAM and SAM. a) SAM displays a state of physiological hypoxia which prevents N-degron pathway activation, that is also influenced by low NO levels. VRN2 contributes to vernalization and hypoxia tolerance, while ZPR2 sustains leaf production in the SAM. b) SE is generally favored by mild hypoxia and oxidative stress-inducing compounds promote dedifferentiation by increasing endogenous auxin levels. NO stimulates the activation of cell division and embryogenic cell formation in some systems. Mutation of \textit{PGB2} increases the number of somatic embryos by suppressing the expression of \textit{MYC2} and induces the transcription of several IAA biosynthetic genes promoting SE. c) NO is necessary for germination completion, NO binds to ABI5, through Cys S-nitrosation of Cys153, and promotes the interaction with CULLIN4-based and KEEP ON GOING E3 ligases and consequently its degradation by the proteasome. \textit{ABI5} is modulated by NO and O\textsubscript{2} through the N-degron pathway. Members of the ERFVII group have been identified as \textit{ABI5} transcriptional activators. d) NO is necessary for normal RAM organization, however high levels of NO reduce PIN1 dependent auxin transport, reducing RAM activity. NO influences meristem size and promotes PR root growth by
preventing N-degron pathway activation. e) NO-donor treatments promote lateral root growth in a dose-dependent manner, NO could be promoting RAP2.12 degradation and thus reducing LRP stabilization and inhibiting LR density. Arrows and bars indicate positive and inhibitory effects, respectively. Dotted arrows and bars indicate putative regulations (created with BioRender.com).

ZPR2 (Protein LITTLE ZIPPER 2); VRN2 (VERNALIZATION 2); PGB2 (PHYTOGLOBIN 2); MYC2 (BASIC HELIX-LOOP-HELIX PROTEIN 6); IAA (Indole-3-acetic acid); ABA (abscisic acid); ABI5 (ABA INSENSITIVE 5); ERFVII (Group VII Ethylene response factors); PRT6 (PROTEOLYSIS 6); PIN1 (PIN-FORMED 1); SAM (shoot apical meristem); SE (somatic embryogenesis); RAM (root apical meristem); PR (primary root); LR (lateral root).

Figure 3. Network of NO and low oxygen interactions in a stress-based context. Abiotic and biotic.

Left side: abiotic stress such as flooding causes NO and ethylene (ET) accumulation. ET signaling promotes enhanced levels of the NO-scavenger PHYTOGLOBIN 1 (PGB1), limiting group VII Ethylene response factors (ERFVII) degradation through inactivation of the PRT6 N-degron pathway. ERFVII members induce expression of core hypoxia-response genes. In roots, PGBs also protect meristems during PEG-induced water stress and hypoxia, since they scavenge NO to prevent programmed cell death (PCD), process initiated by the over-accumulation of NO and mediated by ET and ROS. Right side: after certain pathogen attacks, respiration rate increases in the plant, leading to local hypoxia that promotes ERFVII members accumulation. The oxidative burst (ROS and NO) as a response to the infection can trigger PCD and promotes, along with ERFVII members, activation of defense genes. Besides, flooded soils predispose root plants to infections by soilborne pathogens (created with BioRender.com).

EIN2 (ETHYLENE INSENSITIVE 2); PGB1 (PHYTOGLOBIN 1); ERFVII (group VII ethylene response factors); ROS (reactive oxygen species); PCD (programmed cell death).
