Nitric oxide and plant mineral nutrition: current knowledge

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Highlight

This review describes the impact of nitric oxide over plant mineral nutrition focusing on nitrogen, phosphate, potassium and iron homeostasis. The mechanisms involved in nitric oxide action are also discussed.

Abstract

Plants under essential mineral deficiencies trigger signaling mechanisms involving common components. Among them, nitric oxide (NO) has been pointed out as a key participant in responses to changes in nutrient availability.

Usually, nutrient imbalances affect NO levels in specific plant tissues, caused by modifications in its synthesis or degradation rates. Changes in NO level affect plant morphology and/or trigger responses associated to nutrient homeostasis, mediated by its interaction with reactive oxygen species (ROS), phytohormones and through post-translational modifications to proteins. NO-related events constitute an exciting field of research to understand how plants adapt and respond to conditions of nutrient shortage. This review summarizes the current knowledge describing NO as a component of the multiple processes related to plant performance under conditions of deficiency in mineral nutrients focusing on macronutrients such as nitrogen, phosphate, potassium and magnesium, as well as micronutrients like iron and zinc.

Key words

Iron, Mineral nutrition, Nitric oxide, Nitrogen, Nutrient deficiency, Phosphate, Potassium

Abbreviations

ABA, abscisic acid; cPTIO, 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide; DAF-FM DA, 4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate; DEA/NONOate, diethylamine NONOate; DNIC, dinitrosyl-iron complexes; FIT, FER-like iron-deficiency-induced transcription factor; FRO, ferric reductase oxidase; GA, gibberellins; GSH, glutathione; GSNO, S-nitrosoglutathione; GSNOR, S-nitrosoglutathione reductase; IRT, iron regulated tranporter; L-NAME, Nω-nitro-L-arginine methyl ester; MNIC, mononitrosyl-iron complexes; NO, nitric oxide; NR, nitrate reductase; NOS, nitric oxide synthase; nsHbs, non-symbiotic haemoglobins; PLP, pyridoxal 5′-phosphate; PTM, post-translational modifications; ROS, reactive oxygen species; SNO, S-nitrosothiols; SNP, sodium nitroprusside.

Introduction

Plants are sessile organisms being exposed to continuing changes in environmental conditions. To survive, they have developed highly flexible and finely balanced mechanisms that allow them to sense and acclimate to multiple biotic and abiotic stress situations, such as variable soil nutrient concentrations. Deficiencies in essential mineral nutrients (macro- and micronutrients) lead to plant disorders related to the specific function of each nutrient in plant life, and trigger signaling mechanisms sharing pathways involving common components. Among them, nitric oxide (NO) has been involved in abiotic stress acclimation responses, such as the low nutrient supply (Meng *et al.*, 2012).

To act as a signal molecule, NO has to be synthesized in a specific tissue under certain conditions, to react with specific targets and eventually the signaling cascade will be turned off. NO is endogenously produced in plants, in different cellular and subcellular compartments, under physiological and stress conditions (Astier *et al.*, 2018). Although much progress has been made related to the knowledge of possible sources of NO in plants, the complete scenery is still elusive (Jeandroz *et al*., 2016; Chamizo-Ampudia *et al*., 2017; Corpas and Barroso, 2017).

Endogenously synthesized or exogenously applied NO, exerts its biological function, at least in part, due to protein modifications through i) reaction with tyrosine residues leading to tyrosine nitration, ii) binding to the thiol group of cysteinyl residues in a reaction so-called S-nitrosation (also termed as S-nitrosylation), or iii) interacting with metalloproteins, leading to conformational changes (reviewed in Astier and Lindermayr, 2012; Jain and Bhatla, 2018; Kolbert *et al*., 2017). In addition, NO is involved in a broad spectrum of biochemical events through the interaction with hormones, reactive oxygen species (ROS), and calcium (Garcia-Mata *et al*., 2003; Freschi, 2013; Domingos *et al*., 2015).

Not only proteins but also low molecular weight thiols can undergo S-nitrosation. The most abundant low molecular weight nitrosothiol is S-nitrosoglutathione (GSNO) which, in turn, is considered to be a form of storage and long distance transport of NO (Begara-Morales *et al*., 2018). The enzyme GSNO reductase (GSNOR) mediates GSNO turnover, giving ammonium and glutathione disulphide as products. The activity and the physiological role of GSNOR in plant metabolism have been recently reviewed

(Lindermayr, 2018). NO is also scavenged by non-symbiotic haemoglobins (nsHbs) through a NAD(P)H dependent mechanism (Perazzolli *et al*., 2004). The expression of nsHbs in higher plants is increased under stress conditions (reviewed in Perazzolli *et al*., 2006). Interestingly, nsHbs are also able to react with S-nitrosothiols (SNO) leading to denitrosation (Perazzolli *et al*., 2004).

To our knowledge, the first report suggesting a role for NO in plant mineral nutrition came from the response to exogenous NO supply in maize plants suffering from iron (Fe) deficiency (Graziano *et al*., 2002). Since then, extensive evidence supporting its participation in plant mineral nutrition disorders has been accumulated and reported for other essentials elements: nitrogen (N), phosphate (P), potassium (K), zinc (Zn), and magnesium (Mg), among others.

NO levels have been reported as increased in different plant tissues following alterations in nutrient supply due to modifications in its synthesis or degradation rates. The NO-related events that follow the NO increase constitute an exciting field of research to understand how plants may adapt to environmental conditions of nutrient shortage. This review will focus on NO as a key component of the multiple processes related to plant performance under several mineral nutrient deficiency conditions.

Nitric oxide affects nitrogen uptake and homeostasis

Nitrogen (N) is an essential macronutrient, a building block of biological molecules such as nucleotides, amino acids, and proteins, which is critical for plant growth and development, and as a consequence for crop yield (Wang *et al*., 2012; O'Brien *et al*., 2016). Nitrate (NO_3^-) and ammonium (NH_4^+) are preferred N forms taken up by land plants; however they can be found in a short supply in most ecosystems as well as in agricultural lands (O'Brien *et al*., 2016). In agricultural systems, crop production relies on the application of nitrogenous fertilizers, but a large fraction of the N is not absorbed by plants, being lost into the environment causing several environmental and pollution problems (O'Brien *et al*., 2016; Kant, 2018).

Nitrogen supply in soil can fluctuate, and the root is the site where nutrient perception and acquisition occurs through efficient sensing systems (Alvarez *et al*., 2012). Local signaling pathways involve sensors, signal transduction pathway components, and

effectors such as transcription factors that trigger N responses. Less is known about the systemic N signaling pathways in plants. It requires root-shoot-root communication, and in addition to NO_3^-, other N-metabolites may function as systemic signals. Additional systemic signals include phytohormones like cytokinins and auxins (Alvarez *et al*., 2012). Local and systemic regulatory pathways participate in the modulation of root architecture by NO_3^-, where locally concentrated NO_3^- promotes lateral root elongation, and on the contrary high NO_3^- applied to the whole root has an inhibitory effect on lateral root development (Zhang *et al*., 1999, Alvarez *et al*., 2012). Regarding the effect of NO_3^- supply on primary root growth, there have been observed some contradictory reports showing inhibition, stimulation or no effect whatsoever (reviewed by Trevisan *et al*., 2014). As NO has been involved in root growth modulation (Correa-Aragunde *et al*., 2004; 2006), and NO_3^- and NO are metabolically connected, the possibility that NO may participate in NO_3^--mediated root growth has been explored (Trevisan *et al*., 2014). A role for NO production in root response to NO_3^- was postulated due to the observation of a coordinate spatio-temporal expression of nitrate reductase (NR) and nsHbs, involved in NO synthesis and scavenging, respectively. These findings suggested that they could play an important role during the early perception and signaling of NO_3^- in the rhizosphere (Trevisan *et al*., 2011). The involvement of NR and NO in root response to NO_3^- or NO_3^-/ NH_4^+ (partial nitrate nutrition) has been confirmed with the use of chemical detection of NO *in situ*, and interfering with its synthesis (tungstate) or scavenging (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, cPTIO) in maize and rice (Manoli *et al*., 2014; Sun *et al*., 2015). An increase in the NO content in the lateral root region and the root tip of a high-nitrate-response rice cultivar (Nanguang) growing under partial NO_3^- nutrition conditions (14 d) as compared to NH_4^+ treatment was observed, as well as an improved N acquisition capacity through the modulation of lateral root initiation and the N uptake rate. NO accumulation resulted mainly from an NIA2-dependent NR source (Sun *et al*., 2015). In maize plants, an early response was observed when seedlings grown for 24 h without NO_3^- were re-supplied with NO_3^-. The NO_3^- supply caused an increase in DAF- FM DA (4-amino-5-methylamino-2′,7′-difluorofluorescein diacetate) fluorescence (corresponding to NO detection) in the first minutes after treatment that was mainly localized immediately above the meristematic apex, in the transition zone. NO was produced by NR as an early

response to NO_3^- supply and the coordinated induction of nsHbs could finely regulate steady state NO level. This mechanism may be implicated in the modulation of the root elongation in response to NO_3^- perception (Manoli *et al.*, 2014). The preferential localization and the strong transcriptional responsiveness of both NR and nsHbs in the transition zone of the apex strengthened the hypothesis of a role for this root portion in translating the environmental stimuli in developmental response (Trevisan *et al.*, 2014). Trevisan and co-workers (2015) employing transcriptome and proteome studies confirmed that the transition zone was critical in sensing NO_3^-, and the contribution of NO to the NO_3^--induced transcriptional response. However other NO_3^--derived signals also seem to contribute to this pathway. In addition, phytohormones, as auxin, seem to belong to the network of events involved in the adaptation to NO_3^- fluctuations. Therefore, it would seem possible to influence the pattern of root growth as well as the uptake capacity under specific conditions of N supply by manipulating elements involved in NO signaling (Simontacchi *et al.*, 2015).

Despite the differences between species and treatments, NO seems to be implicated in NO_3^- modulation of root system architecture in a series of events that imply local and systemic responses and a tight regulation of NO levels through its synthesis and scavenging or consumption. Future research in other higher plants will add information about the complex regulation mechanisms involved in N perception and uptake under fluctuating situations.

N assimilation and NO generation are closely connected. It has been described that not only the amount of N (Caro and Puntarulo, 1998; Jin *et al.*, 2009) but also the form of N supply (NO_3^- or/and NH_4^+) (Sun *et al.*, 2015; Zhu CQ *et al.*, 2016) impact on NO levels. In fact, nitrite (NO_2^-) and arginine, both derived from N assimilation and metabolism are the main substrates for NO synthesis. However, plants may have optimized the use of NO_2^- as a main source for NO (Jeandroz *et al.*, 2016; Santolini *et al.*, 2017). NR is a key enzyme in the N metabolism and a source of NO (Chamizo-Ampudia *et al.*, 2017), and in turn its activity may be affected by NO levels (Table 1). Regarding the possible mechanisms implicated in the modulation of NR activity, the absence of changes in the protein content and the conduction of *in vitro* experiments employing enzyme extracts lead to the idea of that the regulatory effect of NO on NR activity occurs at post-translational level (Jin *et al.*,

2009; Du *et al.*, 2008; Rosales *et al.*, 2011). In addition, in tomato roots the positive effect of NO on NR activity obtained in roots fed under low nitrate was reversed after the removal of NO, whereas the inhibition of NR from roots fed under high nitrate was irreversible (Jin *et al.*, 2009). Thus different mechanism could be operative under different situations depending on N supply that will require further investigation. However, nitrotyrosines were not detected in purified NR from wheat leaves, either from controls or NO-treated samples (Rosales *et al.*, 2011). A recent analysis indicates the possible sites of S-nitrosation in the protein (Fu *et al.*, 2018), showing that NR from different plant species can undergo this post-translational modification (PTM) in the presence of NO donors. The presence of NO could alter NR functionality and, as a consequence, N assimilation (Jin *et al.*, 2009; Rosales *et al.*, 2011). Recently Balotf *et al.* (2018) studied the expression and activity of some enzymes from N assimilation pathways in two Australian wheat cultivars, cv. *Spitfire* (high nitrogen use efficiency, NUE) and cv. *Westonia* (normal NUE), under different combinations and levels of nitrogen sources, and the NO donor SNP (sodium nitroprusside). SNP treatment affected the activity (Table 1) and the expression of NR, and other enzymes of nitrogen assimilation pathway, showing that NO may have an important role in transcriptional and post-transcriptional regulation of N assimilation pathway enzymes. The dependence of the effect on N concentrations may be a strategy of the plants to increase NUE, as inducible effects on assimilation enzymes were observed mainly at low N concentrations (Balotf *et al.*, 2018). Thus, different NO donors, such as SNP, GSNO, and diethylamine NONOate (DEA/NONOate), and the NO scavenger cPTIO were utilized to study the effects of NO supply on the NR activity in different species, tissues, and under different experimental conditions as N sources and supply. As it is summarized in Table 1, NO affects NR activity in a way that depends on the N source and level (as NO_3^- concentration in the growth medium), the level of NO (or GSNO) reached inside the cell, as well as the duration of treatment, tissues and the species and genotypes, which may lead to different responses. In C*hlamydomonas reinhardtii* (Sanz-Luque *et al.*, 2013), unlike other systems (Du *et al.*, 2008; Jin *et al.*, 2009), NO did not inhibit NR activity in extracts, but it did so in living cells, indicating that a cellular component or cell structure is necessary for NO inhibition of NR activity (Sanz-Luque *et al.*, 2013). An unquestionable role for NO in modulating NR activity may be proposed and it should ultimately affect N metabolism, as

NR is believed to be the rate-limiting step in NO_3^- assimilation pathway in plants. To add more complexity to this scenario, known mammalian nitric oxide synthase (NOS) inhibitors and polyamine treatments have modified NR activity (Rosales *et al.*, 2011; 2012), indicating that NO from other sources, not directly related with NO_3^- assimilation, can also modulate NR activity. Additionally, in *nox1* mutants, which display increased NO synthesis, a NR-independent NO overproduction decreased NO_3^- content in part by suppressing its transport, suggesting that a NR-independent NO production may contribute to NO_3^- homeostasis (Frungillo *et al*., 2014).

Furthermore, NO seems to modulate N uptake systems. Sanz-Luque and co-workers (2013) described for the algae (*Chlamydomonas reinhardtii*) the fast and reversible inhibition of high-affinity NH_4^+ transporter (HAAT) and high-affinity NO_3^- and NO_2^- transporter (HAN/NiT) by NO, suggesting a post-translational regulation. It was also demonstrated that NO and SNO can modulate N assimilation by inhibiting differently NO_3^- uptake and reduction (Frungillo *et al.*, 2014). Elevated NO and SNO levels induced a switch from high- to low-affinity NO_3^- transport. It has been proposed that GSNO inhibits NO_3^- uptake and reduction and NO inhibits GSNOR1, by S-nitrosation, preventing GSNO degradation. Inhibition of GSNOR1 may be necessary to amplify SNO signals as GSNO prolongs NO half-life through the formation of a more stable pool of NO, which can also regulate NO_3^- assimilation and finely tune N homeostasis (Frungillo *et al*., 2014).

Overall, the reactions and molecules involved in N assimilation and metabolism, as well as in the NO generation, in addition to their multiple interactions, feedback connections, and the specific variations in particular organisms propose a complex scenario (Fig. 1). Future research in this field especially with crop cultures will contribute to understand the role of this signaling molecule in N nutrition, including the possibility of improving N availability under scarcity by modulating endogenous NO levels.

Nitric oxide participates in acclimation to phosphorus restriction

Phosphorus (P) is a major essential nutrient. It acts as a structural component of nucleic acids and membranes, and it is also a key component of signal transduction and energy metabolism (Plaxton and Tran, 2011). Low P availability in soils imposes an important worldwide crop yield limitation, thus phosphoric rock-derived fertilizers are

extensively used to cope with P scarcity in agricultural systems, making P a key hit in food safety and ambient sustainability (Beardsley, 2011). The chemical form suitable for plant root uptake ($H_2PO_4^-$) is frequently found at low concentration in soil solution, below 10 µM (Shen *et al*., 2011), while the major P stock correspond to chemical forms that are unavailable for plants (precipitated, adsorbed to soil particles and organic P) (Holford, 1997; Raghothama 1999; Vance *et al*., 2003).

Plants are able to sense low P availability in soil and activate a complex signaling network to trigger several morphological and physiological responses, including the improvement of mutualistic relationships, in order to cope with P scarcity (Chiou and Lin, 2011; López-Arredondo *et al*., 2014; Zhang *et al*., 2014). Plants respond to P starvation in a variety of ways that include the release of P from vacuole; changes in membrane composition, replacing phospholipids with galactolipids and sulpholipids (Lambers *et al*., 2012); and the redistribution of P to the young actively growing tissues (Baker *et al*., 2015). At root level, the exudation of protons, organic anions and acid phosphatases increases P availability in the soil solution (Gaume *et al*., 2001; Brinch-Pedersen *et al*., 2002; Shen *et al*., 2006). Remodelling the root system and increasing P-transport activity improve both soil exploration and P uptake by plants (Raghothama and Karthikeyan, 2005; Lambers *et al*., 2006; 2011; Baker *et al*., 2015).

NO participation in modulation of several plant P starvation responses has been recently reported for different plant species, affecting both physiological processes and morphological traits (Wang *et al*., 2010; Zhu *et al*., 2017) (Fig. 2). In white lupin (*Lupinus albus*), Wang and co-workers (2010) described physiological changes associated to citrate exudation by roots, and morphological changes related to cluster root generation, both caused by P starvation and modulated by NO. In maize (*Zea mays*), employing the NO donor, GSNO, we found that in P-starved plants, NO increases the acid phosphatase activity in root tissues, the uptake of P from nutrient solution, and the decrease of pH in the external medium (Ramos-Artuso *et al*., 2018). In rice (*Oryza sativa*), changes in the internal P reutilization from cell walls under P starvation, has been described as a NO mediated process (Zhu CQ *et al*., 2016), in which NO acts upstream of ethylene (Zhu *et al*., 2017).

Under P starvation it was found an increase in root sensitivity to auxin, which may affect root system architecture (López-Bucio *et al.*, 2002; Nacry *et al.*, 2005; Bouain *et al.*, 2016). It was also postulated that P starvation results in increases or decreases in auxin accumulation at different parts of the root system (Nacry *et al.*, 2005; Sánchez-Calderón *et al.*, 2005). H^+-ATPase activity increases under P deficiency (Shen *et al.*, 2006), which leads to a higher P uptake capacity *via* an anion/H^+ co-transport process (Ullrich-Eberius *et al.*, 1984) lowering the rhizosphere pH. Auxins seems to have a role in the increase of H^+-ATPase activity (Frías *et al.*, 1996), then it is possible to speculate an interaction between NO and auxins in the modulation of H^+-ATPase activity, since NO levels rise under P starvation in root tissues (Wang *et al.*, 2010). According to this hypothesis, the addition of exogenous NO to *Phragmites communis* calluses produced a great increase in the activity and the expression of plasma membrane H^+-ATPase (Zhao *et al.*, 2004). However, it is worth to mention that there is a lack of experimental evidence for the interplay among NO-auxin in plants suffering from P deficiency.

The impact of P restriction on root architecture varies among plant species. In the case of *Arabidopsis*, a clear effect is the restriction of primary root growth (Sánchez-Calderón *et al.*, 2005; Niu *et al.*, 2013). One possible explanation for root length restrain is the antagonistic effect of NO with giberellins (GA) action. DELLA proteins act as inhibitors of plant growth, and the GA/GA receptor (GID1)/DELLA interaction stimulates its degradation through the proteasome, preventing DELLA-mediated growth inhibition (Harberd *et al.*, 2009), and thus promoting growth. Experimental support for the cross-talk NO-DELLAs came from the work performed by Lozano-Juste and León (2011) in plant responses to light, where exogenous NO increased the levels of DELLA proteins. It has been observed that DELLAs exert a restriction on primary root growth (Jiang *et al.*, 2007) and that NO exerts a similar effect (Fernández-Marcos *et al.*, 2011) in a DELLAs partially dependent mode (Fernández-Marcos *et al.*, 2012). In *Arabidopsis*, a complete frame for the convergence of P supply, NO and GA in the inhibition of primary root growth was reported (Wu *et al.*, 2014), demonstrating that the inhibitory effect of NO and low P on primary root growth depends on the DELLA pathway.

Soybean leaves exposed to short-term P-restriction exhibited increased NO levels (Ramos-Artuso *et al.*, 2019). Some hypothesis may be proposed regarding the source of

NO involved under low P availability: i) an increase of NR activity through dephosphorylation (Lillo *et al*., 2004) that could lead to a higher NO generation (Chamizo-Ampudia *et al*., 2017; Ramos-Artuso *et al.*, 2019), ii) an enhanced activity of xanthine oxido-reductase (XOR) (Wang *et al*., 2010), and/or iii) a higher content of the substrates arginine and nitrate (Rabe and Lovatt, 1986). The importance of the NR pathway for NO production under P restricted conditions has been demonstrated (Royo *et al.*, 2015). NO levels increased substantially in *Arabidopsis* wild type (WT) roots exposed to P restriction, but decreased in the roots of the *nia* mutant. Also, *nia* mutants were more sensitive to P deprivation than the WT plants indicating a role for NO in the adaptation to low P. In addition, P restriction has profound effects on mitochondrial electron flow increasing the pathway involving alternative oxidase (AOX) in a mechanism mediated by NO (Royo *et al.*, 2015).

Taken into account the effects exerted by NO on internal P reutilization, the release organic acids, acid phosphatases activity, P-uptake capacity, and the acidification of the growing medium, together with those affecting root architecture, it can be concluded that several acclimation mechanisms involved in P-acquisition under low P are positively influenced by NO, suggesting a critical role of this molecule during the acclimation to P-deficiency. Further research is needed to reveal the precise NO signalling mechanisms being involved in P deficiency response in different plant species.

Nitric oxide affects potassium homeostasis in plants

Potassium (K^+), a major nutrient, is accumulated up to 10% of the dry mass, being the major inorganic cation in plant cells. Inside the cells, K^+ cooperates in the formation of membrane potential and maintenance of cytosolic pH homeostasis. It interacts with charges of nucleic acids and proteins, and also acts as a cofactor activating specific enzymes (Maathuis, 2009; Dreyer and Uozomi, 2011). Maintaining K^+ homeostasis enables plants to operate metabolic pathways, but also contributes to set the osmotic potential and thus the turgor required for structure, plant growth and movements including stomatal aperture. Recent evidence suggests that cytosolic K^+ homeostasis is important as a signal in growth and development under stress conditions, mainly in redirecting energy from metabolic reactions to defense responses (Shabala, 2017). In the soil solution, K^+ concentration may

vary within a wide range which is usually about 2–4 orders of magnitude lower than the concentration within the plant. At sub-millimolar concentrations, K^+ is typically taken up from the solution bathing the roots through the activity of specific Shaker like potassium channels and by K^+-starvation induced KT-HAK-KUP transporters, AKT1 and AtHAK5 respectively; but other entities could also contribute to this transport process. The subsequent movement of K^+ within the cells and among plant organs involves several additional transporters (Véry *et al*., 2014; Santa-María *et al*., 2018).

There are many ways in which K^+ nutrition could be affected by the presence of NO, one of them is through the general effect of NO on root architecture, in addition to an specific effect over K^+ transport (Fig. 3). An important relationship between K^+ and NO came also from a particular cellular type, the stomata. Furthermore in the complex interaction between NO and K^+, some phytohormones are likely to play a key role.

Arabidopsis plants exposed to K^+ restriction reduced total root length and increased the density of second order lateral roots with no alteration in the root:shoot ratio (Gruber *et al*., 2013), while the root:shoot ratio increased in two wheat cultivars upon long term K^+ restriction (Moriconi *et al*., 2012). In tobacco plants, a low K^+-susceptible cultivar significantly decreased total root length, root volume and the number of first order lateral roots when exposed to K^+ restriction, whereas root morphology was not affected in a tolerant one (Song *et al*., 2018). For the susceptible cultivar current evidence suggests that NO plays an important role in modulating the growth of first-order lateral roots. Interestingly, roots of this cultivar exhibited increased levels of NO after K^+-restriction. Consistently, the addition of two NO donors (SNP and DEA/NONOate) acts in the same way inhibiting first order lateral roots. According to that, the addition of cPTIO, L-NAME (Nω-nitro-L-arginine methyl ester) or tungstate resulted in an increase of the first-order roots length (Song *et al*., 2018). Besides, indirect effects of NO on root elongation associated to defective K^+-nutrition are likely to occur as observed in plants lacking AKT1 activity (*akt1*) for which root length proved to be hypersensitive to the NO donor SNP (Xia *et al*., 2014).

As K^+ plays major roles in plant function, the K^+ transport capacity is, not surprisingly, subjected to multiple regulations (Amtmann and Blatt, 2009; Santa-María *et al*., 2018). Xia and co-workers (2014), in the search of a connection between the changes in

K^+ content and the altered levels of NO during salt stress, found that NO lowers K^+ channel AKT1 activity in *Xenopus* oocytes and protoplasts under conditions of adequate K^+ supply. As above mentioned, the AKT1 channel likely contributes to a major route for K^+ absorption in *Arabidopsis* in most environments (Véry *et al*., 2014). The mechanism proposed by which NO exerts its negative effect on AKT1 does not involve a direct action of NO on the channel. Instead high NO levels increase the content of pyridoxal 5′-phosphate (PLP), an active form of vitamin B6, which in turn inhibits the activity of AKT1 (Xia *et al*., 2014). Therefore NO mediates K^+ homeostasis by the negative regulation of K^+ uptake *via* AKT1. It remains unknown whether or not a similar negative effect involves the other major player in K^+ uptake from diluted K^+ solutions, AtHAK5. It is interesting to note that recent work with the *nia1nia2* mutant, defective in NR activity, unveiled that leaf K^+ content was impaired in those plants (Chen *et al*., 2016). While this result indirectly suggests that NO would be involved in the control of long-distance transport of K^+, it could be also interpreted in terms of the reciprocal interaction between K^+ and N nutrition.

Another NO-mediated alteration in K^+ homeostasis resulted from the work with *Arabidopsis* roots treated with excess iron (Fe) (Zhang *et al*., 2018). In the root tips, the levels of NO increased as a consequence of exposure to toxic Fe concentrations (Arnaud *et al*., 2006), leading to growth arrest, which in part is related to NO-induced alteration in K^+ homeostasis. In the search for the fluxes determining imbalanced K^+ homeostasis, the authors found a net loss of K^+ from apical root zones. K^+ efflux from roots is thought to be mediated by K^+-selective channels, nonselective cation channels and annexins (reviewed in Demidchik *et al*., 2014). The observation that Fe-induced K^+ efflux currents are diminished by the presence of Gd^{3+}, suggests that non-selective cation channels could be involved in the process studied. The addition of the NO donor SNP stimulates K^+ efflux, while that of the NO scavenger cPTIO reduces it, thus indicating that those currents are related with NO signaling. Furthermore, the authors obtained evidence that addition of PLP contributes to K^+ efflux. In the mechanism proposed by Zhang and coworkers (2018), NO-induces K^+ loss by nonselective cation channels (NSCCs), *via* regulation of the levels of PLP, thus establishing a parallelism with the findings above mentioned for AKT1 regulation (Xia *et al*., 2014). The significant loss of K^+ induced by exposure to high Fe levels, could likely exert its detrimental effect on root elongation either by reducing cell turgor thus limiting

cell expansion, by affecting metabolic processes or by eliciting a cell death process as found under stress conditions (Demidchik *et al.*, 2010).

It is known that NO enhances plant tolerance to drought by affecting stomatal closure evoked by abscisic acid (ABA) (Garcia-Mata and Lamattina, 2003). In *Vicia faba* guard cells, NO promotes intracellular Ca^{2+} release and thus regulates Ca^{2+}-sensitive K^+ inward channels at the plasma membrane (Garcia-Mata *et al.*, 2003). Later, a direct effect was proposed for NO locking down the outward-rectifying K^+ channels mediated by S-nitrosation of cysteinyl residues in the ion channel protein (Sokolovski and Blatt, 2004). Rise in NO levels following ABA perception by guard cells is proposed to rely on the activity of NR, as it was previously suggested (Scuffi *et al.*, 2014) and confirmed by the use of *nia1nia2 Arabidopsis* mutant, lacking the two genes coding for NR (Chen *et al.*, 2016). The double mutant showed reduced NO synthesis and lower leaf K^+ content, and stomata exhibited ABA insensitivity; however, they responded to exogenous NO addition. Alteration in K^+ homeostasis in plants with reduced NR activity correlates with a reduction in *KAT2*, *AKT2* and *KC1* transcripts, coding for channels involved in K^+ transport, as well as *GORK* transcripts coding for an outward rectifier K^+-channel involved in K^+ efflux, and increases in the accumulation of transcripts coding for the inward rectifier K^+ channels KAT1 and AKT1. According to the model offered by the authors, in *Arabidopsis* guard cells, ABA-induced stomatal closure involves NO derived from NR activity, which contributes to inhibit inward currents mediated by the KAT1 and AKT1 K^+ channels through a Ca^{2+}-dependent mechanism (Chen *et al.*, 2016).

Overall, the available evidence suggests a major role for NO in modulating K^+ accumulation in plants as well as the movements at cellular level (Fig. 3), which may be particularly relevant when plants face stress conditions.

Nitric oxide is a central player in iron homeostasis in plants

Iron (Fe) is an essential micronutrient for plants. Given its redox properties, Fe acts as an important cofactor in enzymes and component in proteins. Fe containing proteins take part in the electron transfer chain of mitochondrion and chloroplasts and in the chlorophyll biosynthetic pathway; evidencing its importance for plant physiology. Despite being one of the most abundant elements on Earth's crust, plants are frequently exposed to Fe deficiency

Due to its low bioavailability as a result of the poor solubility of Fe in the soil solution, (Ma and Ling, 2009). Fe deficiency causes symptoms in plants that severely affect growth and development. On the other hand, Fe overload and the presence of high Fe levels in tissues can lead to oxidative stress and damage owing to the generation of ROS through Fenton's reaction (Halliwell and Gutteridge, 1999). This situation is more likely to occur under waterlogging conditions (Nikolic and Pavlovic, 2018).

The mechanisms developed for higher plants to acquire Fe from soils could be classified into two strategies. Non-graminaceous plants have evolved Strategy I which consists in the induction of the activity of an H^+-ATPase pump associated to the plasma membrane that acidifies the rhizosphere, a ferric reductase oxidase (FRO) that localizes at the plasma membrane and catalyzes the reduction of Fe^{3+} to Fe^{2+}, and a Fe^{2+}-transporter, so-called iron regulated transporter (IRT) (Kobayashi and Nishizawa, 2012). The expression of these genes is regulated by a FER/FER-like transcription factor (Lucena *et al.*, 2006). Meanwhile graminaceous plants have adopted Strategy II which is based on the release of phytosiderophores to the rhizosphere to chelate Fe^{3+} and the activity of transporters that take Fe^{3+}-phytosiderophore complexes into the root symplast, called yellow stripe (YS) and yellow stripe-like (YSL) (Kobayashi and Nishizawa, 2012). In some plant species, these two strategies coexist, such as the case of rice (Ishimaru *et al*., 2006). NO is involved in the regulation of both Fe-uptake strategies.

It has been evidenced that in tomato and *Arabidopsis*, Fe deficiency causes an increase in NO levels evaluated by the employment of DAF-FM DA . Endogenous NO, and also exogenous NO (released by GSNO), reverts chlorosis (Graziano *et al*., 2002), positively modulates the expression of FIT (FER-like Iron-deficiency-induced transcription factor, in *Arabidopsis*) or FER (in tomato), and consequently, FRO and IRT (Graziano and Lamattina, 2007; Chen *et al*., 2010). In tomato, NR dependent pathway is likely involved in NO synthesis, given that plants under Fe-restriction treated with tungstate (a known NR inhibitor) have not shown NO accumulation and *nia* mutants (which display a 20% of NR activity present in WT plants) have a weaker induction of Strategy I gene expression (Graziano and Lamattina, 2007). Meanwhile, it has been proposed that in *Arabidopsis*, NO generated under Fe deficiency comes mainly from both NR and arginine-dependent synthesis (Chen *et al*., 2010). In addition, assays with cPTIO, an NO scavenger, confirmed

that NO induces the expression of FER and favours its stability by inhibiting its proteasomal degradation (Meiser *et al.*, 2011).

The effect of NO on FER/FIT accumulation is paralleled by auxins and ethylene. The interplay between NO and auxins has been addressed early whereas ethylene has recently emerged as a new player in conjunction with NO in plant mineral nutrition (García *et al.*, 2011). Chen and co-workers (2010) have proven that Fe deficiency triggers auxins accumulation that leads to a NO burst and the subsequent induction of FIT in *Arabidopsis*, while other works describe the involvement of ethylene in this response (Lucena *et al.*, 2006; García *et al.*, 2010). It has been evidenced by the assays performed in *Arabidopsis* and cucumber plants, using an ethylene precursor (1-aminocyclopropane-1-carboxylic acid), an inhibitor (silver thiosulphate), and an NO donor (GSNO) and scavenger (cPTIO), that Fe deficiency provokes NO accumulation that induces ethylene synthesis that, in turn, enhances NO levels (García *et al.*, 2011). Thus ethylene would be the responsible for FIT induction. Taken these data together, we can speculate a model of low Fe responses (Fig. 4) in which low Fe levels may induce the increase in auxins that result in NO accumulation and the subsequent ethylene raise leads to FIT regulated Strategy I gene expression. However, it is worth mentioning that ethylene and NO are also able to modulate auxins activity (Stepanova *et al.*, 2007; Terrile *et al.*, 2012). It would be interesting to study this proposed model in the light of the availability of *Arabidopsis* mutants for auxins, ethylene and NO.

Research focused on NO role in Fe acquisition by graminaceous plants is less abundant than that focusing on plants using Strategy I. Increased NO levels were found in rice roots in response to Fe-deficiency, assessed by DAF-FM DA fluorescence (Sun *et al.*, 2017; Zhu *et al.*, 2018). Interestingly, Fe-deficient plants showed increased NR expression and activity, supporting a role in NO biosynthesis in this condition (Sun *et al.*, 2017). Recently, it has been reported that rice plants grown under Fe-deficient supply in the presence of NH_4^+ showed enhanced NO levels and have higher expression levels of the Fe transporters OsIRT1 (corresponding to Strategy I) and OsYSL15 (corresponding to Strategy II) than plants grown under Fe-deficient supply but in the presence of NO_3^-, suggesting that an oxidative NO synthesis pathway could be also operative (Zhu *et al.*, 2018). The induced expression of the Fe transporters could be related to the differential NO

accumulation suggesting a role for NO in Fe-deficiency responses in graminaceous plants, although, more studies are required to confirm this hypothesis. It has also been established that changes in root architecture in response to Fe deficiency are mediated by NO and auxins. NO acts downstream of auxins, similarly to that observed in Strategy I plants (Sun *et al*., 2017).

Several studies have highlighted the importance of shoot-root communication for inducing Fe-deficiency responses (Durrett *et al*., 2007; García *et al*., 2013; Chen *et al*., 2018). García and colleagues (2013) revealed that foliar Fe fertilization blocks Fe-deficiency responses related to Fe-acquisition in different plant species and these results were confirmed in mutants expressing constitutively Fe-acquisition genes. One relevant transporter involved in Fe loading into the phloem is Oligopeptide Transporter 3 (OPT3) that is able to transport Fe, likely in a chelated form. The exact nature of the signal molecule implicated in this regulation has not been described yet but, recently, it has been reported that this signal requires functional expression of OPT3 for repression of ethylene synthesis and GSNOR activity in *Arabidopsis* roots (García *et al*., 2018). It is hypothesized that under Fe-deficient conditions, this signal is not sent from shoots to roots, inducing ethylene synthesis and GSNOR activity in roots, suggesting that low GSNO levels are required for ethylene and NO accumulation, leading to Fe deficiency responses. In this regard, it is relevant to note that GSNO levels in cells regulate the extent of S-nitrosation (Begara-Morales *et al.*, 2018). Even though García and co-workers (2018) did not determine NO and auxin levels, it could be suggested, considering all data, that at sufficient Fe supply, this iron signal could be repressing auxins in the proposed model.

As mentioned above, roots of *Arabidopsis* plants grown under Fe deficient conditions showed a low content of GSNO (Shanmugam *et al.*, 2015; García *et al.* 2018). However, it is worth to mention that it has been evidenced an increase of glutathione (GSH) levels in these conditions (Shanmugam *et al.*, 2015; García *et al.* 2018), and this increase could be required to trigger NO accumulation, as illustrated by the absence of DAF-FM fluorescence accumulation when plants were incubated with an inhibitor of GSH synthesis (buthionine sulfoximine) (Shanmugam *et al.*, 2015). This piece of evidence adds more complexity to Strategy I response regulation and it would be interesting to thoroughly study

GSH and NO interaction taking into account the Strategy I regulation by the phytohormones auxins and ethylene.

Moreover, NO also plays an important role in internal Fe homeostasis under Fe-sufficient and deficient supply. NO improves the internal availability of Fe (Graziano *et al*., 2002; Jasid *et al*., 2008; Simontacchi *et al*., 2012). This characteristic relies, at least in part, on the ability to form nitrosyl-Fe complexes. These complexes consist of one or two molecules of NO attached to low molecular weight thiol ligand and coordinated with an atom of Fe, resulting in mononitrosyl- or dinitrosyl Fe complexes (MNIC and DNIC, respectively), that contribute to the labile iron pool, the fraction readily bioavailable of total Fe (Simontacchi *et al*., 2012). Through the formation of this kind of complexes, Fe is kept in a redox safety and available form for plant metabolism (Jasid *et al*., 2008; Simontacchi *et al*., 2012).

On the other hand, NO has been described to mediate the accumulation of ferritin in response to Fe (Murgia *et al*., 2002) by triggering ubiquitinilation of ferritin repressor (Arnaud *et al*., 2006). Ferritins are ubiquitous proteins that storage Fe in a safely form, preventing Fenton reactions (Galatro and Puntarulo, 2007). More recently, it has been reported that NO leads to increased ferritin expression during senescence in *Lotus japonicus* nodules (Chungopast *et al*., 2017). Another protein relevant regarding Fe homeostasis is frataxin. This mitochondrial protein is involved in the assembly of Fe-S clusters. *Arabidopsis* frataxin knock-down plants displayed high Fe levels in mitochondria and plastids in roots, leading to oxidative damage (Busi *et al*., 2006). These plants showed increased NO that ameliorates oxidative stress either by inducing ferritin expression or by a direct antioxidant function (Martin *et al*., 2009). It would be interesting to explore the contribution of MNIC and DNIC formation to avoid high free Fe levels in these mutants.

Regarding the improvement of Fe availability, it could be pointed out a role for NO in Fe utilization efficiency. Reports evidencing a modulation of Fe remobilization by NO have been recently published. Putrescine induced NO accumulation in *Arabidopsis* triggers changes in cell-wall composition leading to Fe release from cell-walls and increasing Fe available levels (Zhu XF *et al*., 2016). Zhu and colleagues (2018) reached to the same conclusion in rice plants grown under Fe deficient supply but in the presence of NH_4^+.

It would be interesting for future research to deepen on the knowledge about NO regulation of Fe acquisition in Strategy II plants, and to investigate the role of NO in the modulation of the mechanisms implicated in Fe use efficiency for improve crops growth under Fe deficient conditions.

NO and other mineral nutrients homeostasis

There are fewer studies regarding the interaction between NO and other mineral nutrient deficiencies like magnesium (Mg) and zinc (Zn). Here we summarized some of the current information regarding NO role under some responses to these mineral disorders.

In *Arabidopsis* plants exposed to Mg deficiency, root hair development is promoted, and using mutants with altered levels of NO and ethylene, the participation of both species was recently analyzed (Liu *et al*., 2017). Upon Mg deficiency, a burst of NO was observed, as well as an increase in ethylene production. Elongation and development of root hairs in plants exposed to Mg deficiency was impaired in the mutants *nia1,2* and *noa1* (with lower NO levels) and *ein2-5* and *ein3-1* (ethylene-insensitive) (Liu *et al*., 2017). These findings were also confirmed by the use of a pharmacological approach, where the application of SNP to Mg sufficient wild type plants increased the length and density of root hairs, while the opposite effect was observed after the application of NO scavenger c-PTIO under Mg deficiency. In addition, the NOS inhibitor L-NAME and the NR inhibitor tungstate inhibited root hair development in Mg- deficient plants.

Plants can be exposed to excess or deficiency of Zn, and as a micronutrient both conditions affect plant growth and development. Most of the reports involving NO and Zn interaction described increases in NO levels in response to high Zn (Xu *et al*., 2010; Duan *et al*., 2015; Feigl *et al*., 2016). However, there is a lack of knowledge about NO participation in plants exposed to Zn deficiency. It has been reported that the addition of a NO-donor (GSNO) during recovery experiments after Zn-deprivation lead to a reduced net Zn uptake from a 2 μM Zn solution in wheat plants, suggesting that NO has the capacity to down-modulate the accumulation of Zn under conditions of adequate/high Zinc supply thus helping plants to prevent the rise of Zn within tissues (Buet *et al*., 2014). However, further studies are needed to disclose the specific effect of NO over Zn transport processes and on the transporters underlying them.

Mechanisms of NO participation in plant mineral nutrition

It is evident from several reports that the increase in NO levels is a common event when plants are exposed to suboptimal amount of different elements, thus it could be considered as a general response to mineral imbalances (Fig. 5) (Wang *et al.*, 2010; Buet and Simontacchi, 2015; Sun *et al.*, 2015; Liu *et al*., 2017). NO may be produced in higher plants from a variety of enzymatic and non-enzymatic sources, that have been extensively reviewed (Moreau *et al*., 2010; Fröhlich and Durner, 2011; Gupta *et al*., 2011; Mur *et al*., 2013; Astier *et al*., 2018) and may contribute to increase NO under mineral deficiencies.

NO has a general effect in the regulation of root system architecture. It is known that plants facing mineral deficiencies adapt their root morphology, physiology and metabolism in order to explore the soil, modify the physical-chemistry properties of the rhizosphere and enhance nutrient uptake. Even though modifications in root system due to mineral scarcity largely depend on the nutrient availability and vary among plant species or cultivars (Kellermeier *et al*., 2013), NO can be considered a common component of some observed responses. NO also participates in the formation of root hair upon mineral nutrient restriction (Lombardo *et al*., 2006), enhancing nutrient acquisition by the increase in the surface area of roots.

A positive correlation seems to be operative between NO and ethylene, where NO stimulates ethylene synthesis and *vice versa* (Wang *et al*., 2009; García *et al*., 2011; Zhu *et al*., 2017). Both NO and ethylene enhance auxin levels in roots exposed to Mg deficiency, likely affecting its transport (Liu *et al.*, 2018). In turn, elevated auxin levels exert a positive feedback loop, being necessary for the increased NO and ethylene levels in Mg-deficient plants (Liu *et al.*, 2018). It has also been shown the existence of a close interrelationship between DELLAs, a key effector in gibberellin signaling, and NO signaling in plant responses to P-deprivation (Wu *et al*., 2014). These findings pointed out the relevance of the complex interaction in which NO may influence phytohormone biosynthesis, catabolism, conjugation, transport, perception or transduction (Freschi, 2013).

PTM of proteins impact plant performance under physiological and stress conditions (Domingos *et al.*, 2015; Simontacchi *et al.*, 2015; Begara-Morales *et al.*, 2018). NO can react with superoxide anion to give peroxynitrite ($ONOO^-$), a highly reactive oxidant capable of adding a nitro group to the aromatic ring of tyrosine residues present in proteins. It is worth mentioning that despite being a result from a non-enzymatic reaction, nitrated proteins are present in low quantities under physiological conditions, representing only 1-2% of the total tyrosine pool (Radi 2004; Chaki *et al.*, 2009; Lozano-Juste *et al.*, 2011; Kolbert *et al.*, 2017) and tend to increase under stress conditions (David *et al.*, 2015; Feigl *et al.*, 2016). In soybean leaves, chloroplast proteins seem to be a target of this PTM, in coincidence with a proposed site for NO generation (Galatro *et al.*, 2013, Ramos-Artuso *et al.*, 2019). In *Brassica* qualitative differences in protein nitration patterns were observed between plant species with differences in plant performance under Zn toxicity conditions (Feigl *et al.*, 2016). However, under short term P-deprivation in soybean leaves, although NO levels were increased, tyrosine nitration was not drastically affected (Ramos-Artuso *et al.*, 2019).

S-nitrosation is another PTM by which NO modulates protein activity through the attachment of a nitrosyl group to cysteinyl residues (Astier *et al.*, 2011). S-nitrosation has emerged as a signal for plant growth and development processes and it has also been described that the level of S-nitrosation in proteins changes in response to abiotic stress (Yun *et al.*, 2011; Ortega-Galisteo *et al.*, 2012; Terrile *et al.*, 2012; Albertos *et al.*, 2015). The pattern of proteins showing this PTM was affected in mitochondria from plants exposed to NaCl (Camejo *et al.*, 2013). Biological functions of NO in plants are partially mediated by S-nitrosation of transcription factors, and it was recently described that other important step in transcriptional control, the chromatin state, is also affected by NO (Mengel *et al.*, 2017). The findings suggest that plant histone deacetylases might be targets of S-nitrosation or S-glutathionylation (incorporation of a glutathione molecule to a cysteinyl residue) resulting in the hyperacetylation of specific genes. This mechanism might operate facilitating the stress-induced transcription of genes. It has also been reported the modulation of a group of WRKY transcription factors, involved in abiotic stress tolerance, by NO (Imran *et al.*, 2018). Most of the NO-responsive WRKY transcription

factors have a cysteine or a tyrosine residue near to the WRKY domain, suggesting a mechanism of regulation by S-nitrosation or tyrosine nitration.

These common mechanisms help to explain the broad spectrum of NO actions that are put into play when plants are exposed to nutrient imbalances (Fig. 5), ranging from PTM in proteins, enzymes, transporters and transcription factors that contribute to perform some physiological responses, through the interaction with phytohormone action allowing to face with nutritional stress situations.

Concluding remarks

In light of the evidence regarding the role of NO in plant mineral nutrition presented here, it should be highlighted its involvement in some responses to low nutrient supply, including macro and micronutrients. In most of these responses, a strong interaction with hormones (mainly auxins and ethylene) has been evidenced. Also, changes associated with root morphology or to the expression of specific proteins involved in nutrient homeostasis (such as transport proteins involved in uptake and translocation), and redox control (such as antioxidants and proteins as ferritin involved in safe Fe storage) has been described. Some interactions may be mediated by NO PTM of regulatory and targets proteins and also by the promotion of gene expression. It would be interesting to deepen the studies that link proteomic changes with organelle specific ionome in plants under specific mineral nutrient imbalances.

The increase in NO levels triggered by the deficiency of a particular nutrient, also involves the regulation of the homeostasis of other nutrients as evidenced by the work of Meng and collaborators (2012). This evidence adds a greater complexity to the study of the responses modulated by NO under mineral nutrition, implying a regulatory network of plant ionome. The regulation of plant ionome by NO constitutes a field of study that remains largely unexplored and the advances in this area of research could lead to an integral knowledge about the regulation of plant mineral nutrition. In addition, there is a lack of knowledge, likely to due technical hitches, regarding NO signaling in local and systemic responses to microelement deficiencies.

From the studies performed employing NO donors, a caution note should be added regarding the use of SNP as the only NO donor in a high number of works analyzed. This

widely used NO donor is a metal-NO complex that contains Fe and may release cyanide during the exposition. So assays employing other NO donors or scavengers are needed to strongly sustain the relationship between NO and the reported responses.

Different mechanisms could be involved in NO generation due to changes in mineral nutrition, where NR seems to have a critical role. The specific level of NO required to exert a particular function may be finely tuned by the molecules involved in its synthesis, consumption, or stabilization (such as endogenous GSNO and nitrosyl iron compounds) that would finally modulate (or trigger) the observed response. In this context, the potential use of NO donors (Marvasi 2017), as well as the possibility to elicit endogenous levels by modulating the sources and mechanisms involved in its generation/consumption, opens a great field of research in the search for a sustainable agriculture.

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Table 1. Modulation of NR activity by NO level under different nitrogen supply

Species	Source of nitrogen	NO donor (or mutant)	Time	Tissues	NR activity	References
Chinese cabbage pakchoi (*Brassica chinensis* L.)	1 mM KNO_3 and 0.25 mM $(NH_4)_2SO_4$	Up to 100 µM of SNP and DEA/NONOate (in the nutrient solution)	3 h	Roots	↑ 205% at 40 µM SNP ↑ 282% at 80 µM DEA/NONOate	Du *et al*., 2008
Tomato (*Solanum lycocarpum*)	0.250 mM $(NH_4)_2SO_4$ and two different levels of NO_3^-: 0.5 mM (low NO_3^- supply) and 5 mM (high NO_3^- supply)	SNP and DEA/NONOate, up to 10 µM (in the nutrient solution)	3 h	Roots	↑ at low NO_3^- supply up to 2 µM SNP and 10 µM DEA/NONOate ↓ at high NO_3^- supply up to 10 µM of both NO donors	Jin *et al*., 2009
Wheat (*Triticum aestivum* L.)	Plants grown in Hoagland solution (5mM KNO_3 and 1mM NH_4NO_3)	Up to 500 µM SNP or GSNO (in distilled water) Leaf segments in flasks with the NO donors under continuous light	3 or 21 h	Leaf segments	↓45% and ↓90% on average, at all incubation times with 10 and 500 µM SNP respectively ↓ 18% with 10 or 100 µM GSNO, and 26% with 500 µM for 3h ↑27% after 21 h with 500 µM GSNO	Rosales *et al*., 2011
Wheat *(Triticum aestivum*, cvs Spitfire and Westonia [1]	Nutrient solution similar to Hoagland and irrigated with N-free nutrient solution for one week	Up to 100 µM of SNP under different low (4mM) or high (40 mM) N supply as: KNO_3, NH_4Cl or NH_4NO_3	3 days	Leaf tissues	↑ Under low NO_3^- or NH_4^+ in both cultivars, and in low NH_4NO_3 in Spitfire cultivar ↓ Under high NO_3^-, but ↑ in high NH_4NO_3 in Spitfire cultivar	Balotf *et al*., 2018
Chlamydomonas reinhardtii	Cells grown in 8 mM amonium medium and then induced in 4 mM NO_3^- medium for 3h	20 µM DEA/NONOate, or 50 and 100 µM GSNO (continuous ligth)	Up to 60 min	Cells	↓ 60% after 10 and 20 min with DEA-NONOate and ↓ about 60% after 40 min with GSNO	Sanz-Luque *et al*., 2013
Macroalga *Gracilaria chilensis*	Seawater enriched with 100 % von Stosch medium (0.5 mM $NaNO_3$)	1 mM SNP	2 h	Unbranched tips (2 cm)	↓99.98 %	Chow *et al*., 2013
Arabidopsis thaliana	Modified Murashige–Skoog nutrient solution Nitrate (about 39,3 mM) composed of half KNO_3 and half NH_4NO_3	Columbia-0 WT and the mutants *gsnor1* and *nox1*[2]	-	Leaf extracts	↓ in *gsnor1* mutant *nox1* mutant did not exhibit altered NR activity	Frungillo *et al*., 2014

[1] Spitfire (high nitrogen use efficiency, NUE) and Westonia (normal NUE)

[2] *nox1* plants overproduce free NO (30-40 % more SNO than WT under basal conditions), and *gsnor1* plants accumulate high levels of GSNO.

DEA/NONOate, diethylamine NONOate; SNP, sodium nitroprusside; GSNO, S-nitrosoglutathion

Figure legends

Fig. 1. NO mediated responses affecting nitrogen uptake and assimilation.
Changes in nitrogen supply may enhance NR activity (or expression) leading to NO accumulation that triggers root growth and the modulation of NH_4^+ and NO_3^- transporters. However, other sources of NO generation may enhance NO levels. NO may control its bioavailability through the modulation of NR activity, and S-nitrosoglutathione (GSNO) levels due to GSNO reductase 1 (GSNOR1) inhibition and GSNO synthesis. Non-symbiotic haemoglobins (nsHb) may also regulate NO steady state. **1**- Sun *et al*., 2015; **2**- Manoli *et al*., 2014; **3**- See data in Table 1; **4**- Frungillo *et al*., 2014; **5**- Perazzolli *et al*., 2004; **6**- Sanz-Luque *et al*., 2013; **7**- Lindermayr *et al*., 2018. Arrows show induction and blocked lines show inhibition.

Fig. 2. Low P-induced responses in plants mediated by NO and its interaction with hormones.
Low P in soils induces NO synthesis in plants (for details see the text). NO accumulation was related to enhanced acid phosphatase (AP) release and P-uptake in roots, increased organic acid (OA) exudation and H^+ release to the rhizosphere. NO interaction with hormones participates in root morphological changes. NO acts upstream of ethylene leading to cell wall composition changes and enhanced P remobilization and translocation to shoots through increased P transporter expression. Ethylene is also involved in the modulation of AP activity, P-uptake and OA exudation in response to low P levels. Low P and NO elicit auxin accumulation that was also associated with H^+ release. NO also inducts alternative oxidase (AOX) activity that allows metabolic flexibility. **1**- Wang *et al*., 2010; **2**- Zhu CQ *et al*., 2016; **3**- Zhu *et al*., 2017; **4**- Ramos-Artuso *et al*., 2018; **5**- Zandonadi *et al*., 2010; **6**- Shen *et al*., 2006; **7**- Correa-Aragunde *et al*., 2015; **8**- Royo *et al*., 2015; **9**- Roldan *et al*., 2013; **10**- Li *et al*., 2011. Arrows show induction and blocked lines inhibition.

Fig. 3. Low K^+ induced responses in plants mediated by NO.
It was reported that in tobacco plants susceptible to K^+ restriction, NO increased in roots under K^+-starvation, leading to root morphological changes. In plants exposed to excess Fe, NO induce K^+ loss via nonselective cation channels (NSCCs). NO also mediates K^+ homeostasis by the negative regulation of K^+ uptake and inactivates the K^+ inward rectifying channel ($I_{K,\ in}$). Outward rectifying channel ($I_{K,\ out}$) is inhibited through S-nitrosation. In guard cells, the production of NO is required for ABA-induced stomatal closure. In addition, there is an attenuating effect of NO breaking the ABA stimulus by the inhibition and degradation of the ABA receptor through the nitration of Tyr residues. **1**- Song *et al*., 2018; **2**- Xia *et al*., 2014; **3**- Garcia-Mata and Lamattina, 2003; **4**- Garcia-Mata et al., 2003; **5**- Zhang et al., 2018; **6**- Laxalt et al., 2016; **7**- Chen et al., 2016; **8**- Sokolovski and Blatt, 2004. Arrows show induction and blocked lines inhibition.

Fig. 4. Low Fe-induced responses mediated by NO and proposed model for low Fe-induced responses in strategy I plants mediated by NO-phytohormone interaction. **Panel A.** Low Fe in soil promotes NO synthesis through NR and Arg-dependent via. NO accumulation elicits Fe-acquisition responses. Strategy I consists in FRO and IRT expression and H^+ release to rhizosphere through H^+-ATPase pump expression. An unknown signal from Fe-sufficient shoots is sent to roots and inhibits Strategy I responses, this signal is absent in low Fe conditions. It was also reported that NO triggers the expression of YSL-like Fe-transporter in rice, a plant that employs Strategy II. **Panel B.** In shoots, low Fe impairs chlorophyll synthesis leading to leaf chlorosis which is reverted by NO, possibly through the formation of mononitrosyl- (MNIC) and dinitrosyl-Fe complexes (DNIC) which contribute to labile iron pool (LIP). More studies are required to confirm this hypothesis. **Panel C.** Proposed model for NO-phytohormone interaction in low Fe-induced responses in Strategy I plants. Low Fe promotes auxins increase that acts upstream to NO. Auxins are modulated through NO and NO-triggered ethylene accumulation. In turn, ethylene elicits FER/FIT transcription factor which, in turn, elicits Strategy I Fe-acquisition responses. Ethylene is also able to induct NO accumulation. NO interaction with auxins participates in root morphological changes and Fe-acquisition induction. **1-** Graziano and Lamattina, 2007; **2-** Chen *et al*., 2010; **3-** Jin *et al*., 2011; **4-** García *et al*., 2018; **5-** Sun *et al*., 2017; **6-** Zhu *et al*., 2018; **7-** Simontacchi *et al*., 2012; **8-** García *et al*., 2011; **9-** Terrile *et al*., 2012; **10-** Stepanova *et al*., 2007; **11-** García *et al*., 2010. Arrows show induction and blocked lines inhibition, dotted line stands for proposed mechanisms.

Fig. 5. Common mechanisms involving NO participation under micro and macronutrient scarcity. Low nutrient availability promotes NO accumulation. NO leads to plant acclimation to nutrient scarcity through PTM to proteins. For some elements, it was reported that NO leads to auxins and ethylene accumulation that elicits nutrient acquisition and reutilization responses. Ethylene and auxins are also able to induct NO accumulation. NO interaction with auxins and ethylene participates in root morphological changes and nutrient acquisition induction. Arrows show induction and blocked lines inhibition.

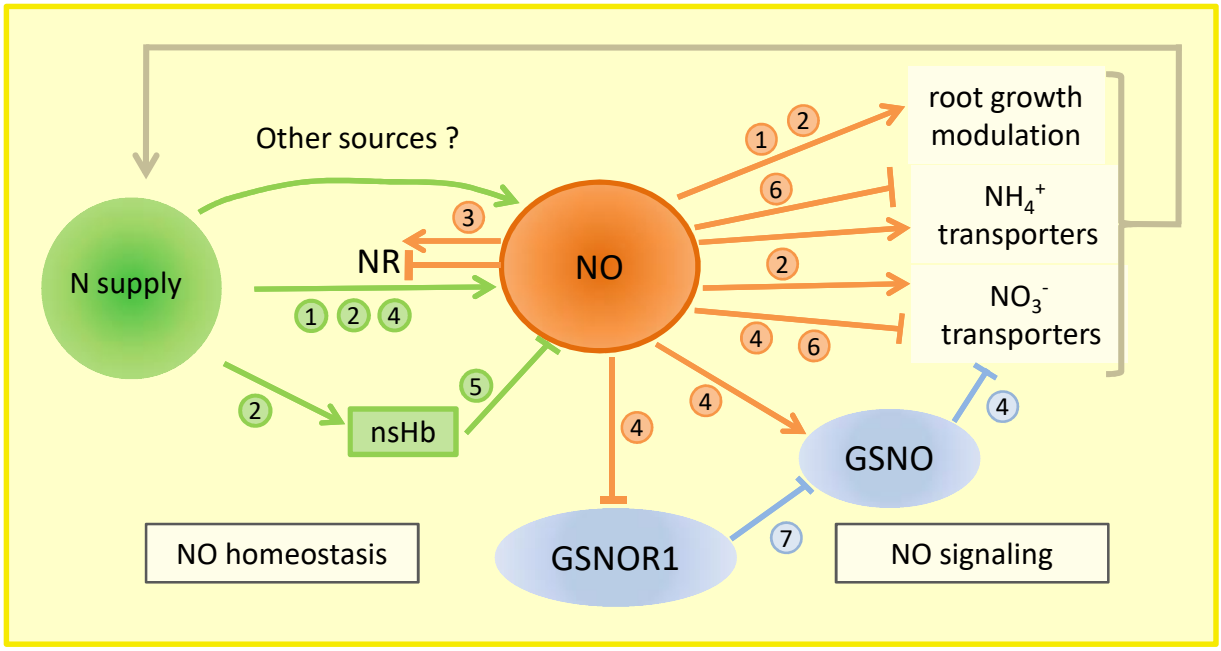

Figure 1

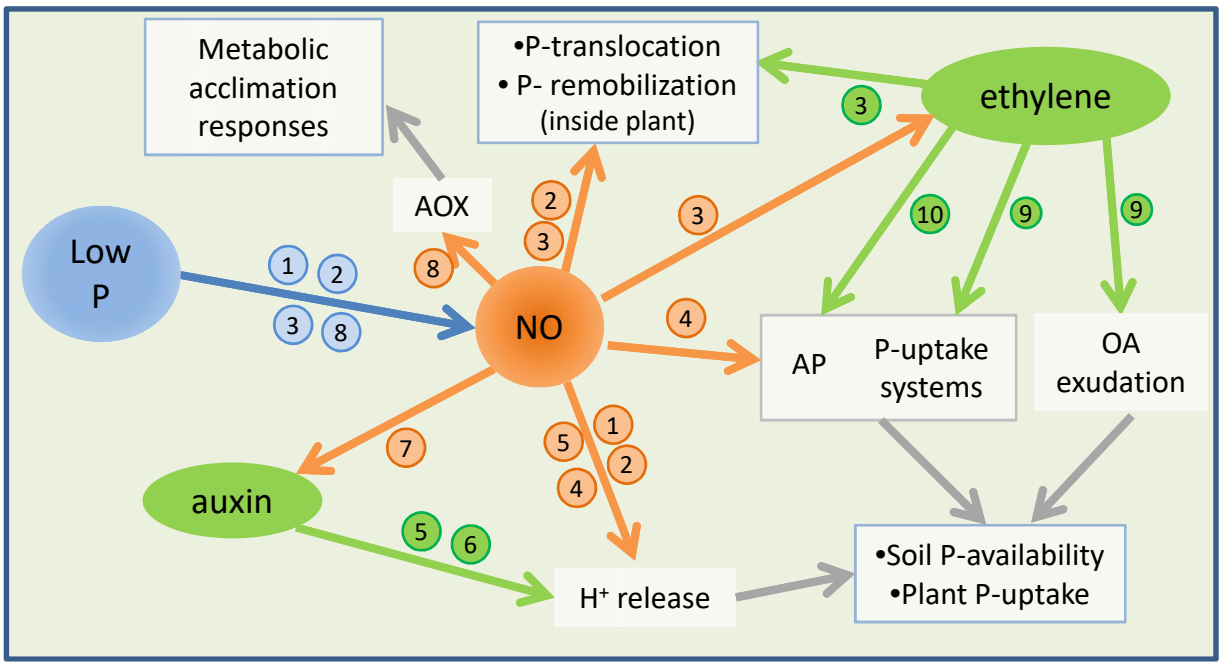

Figure 2

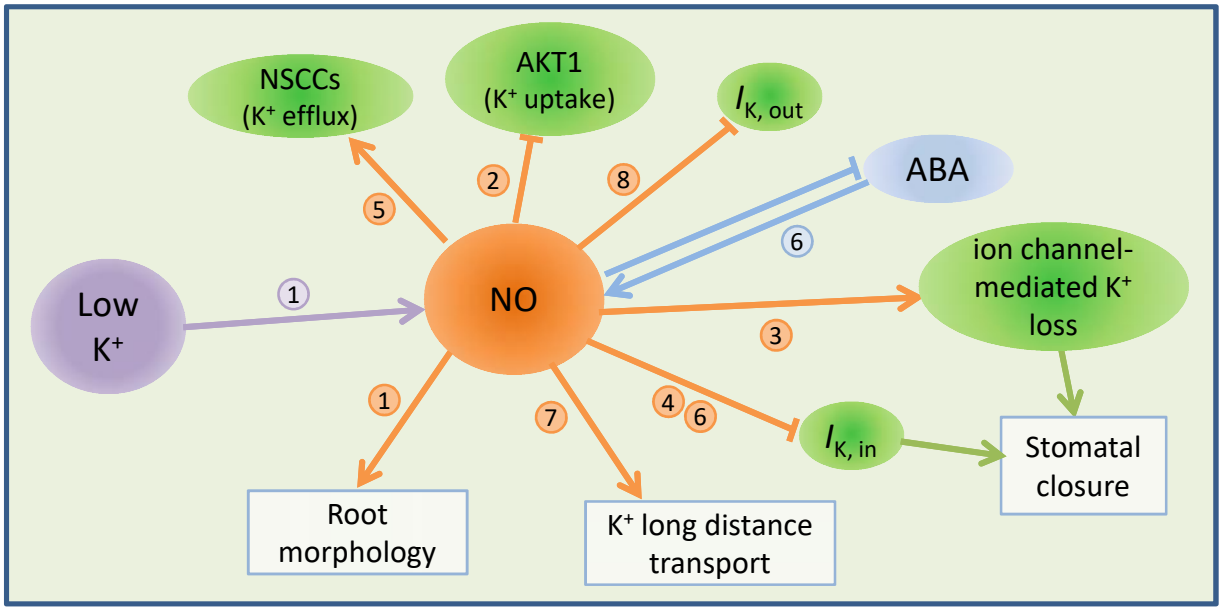

Figure 3

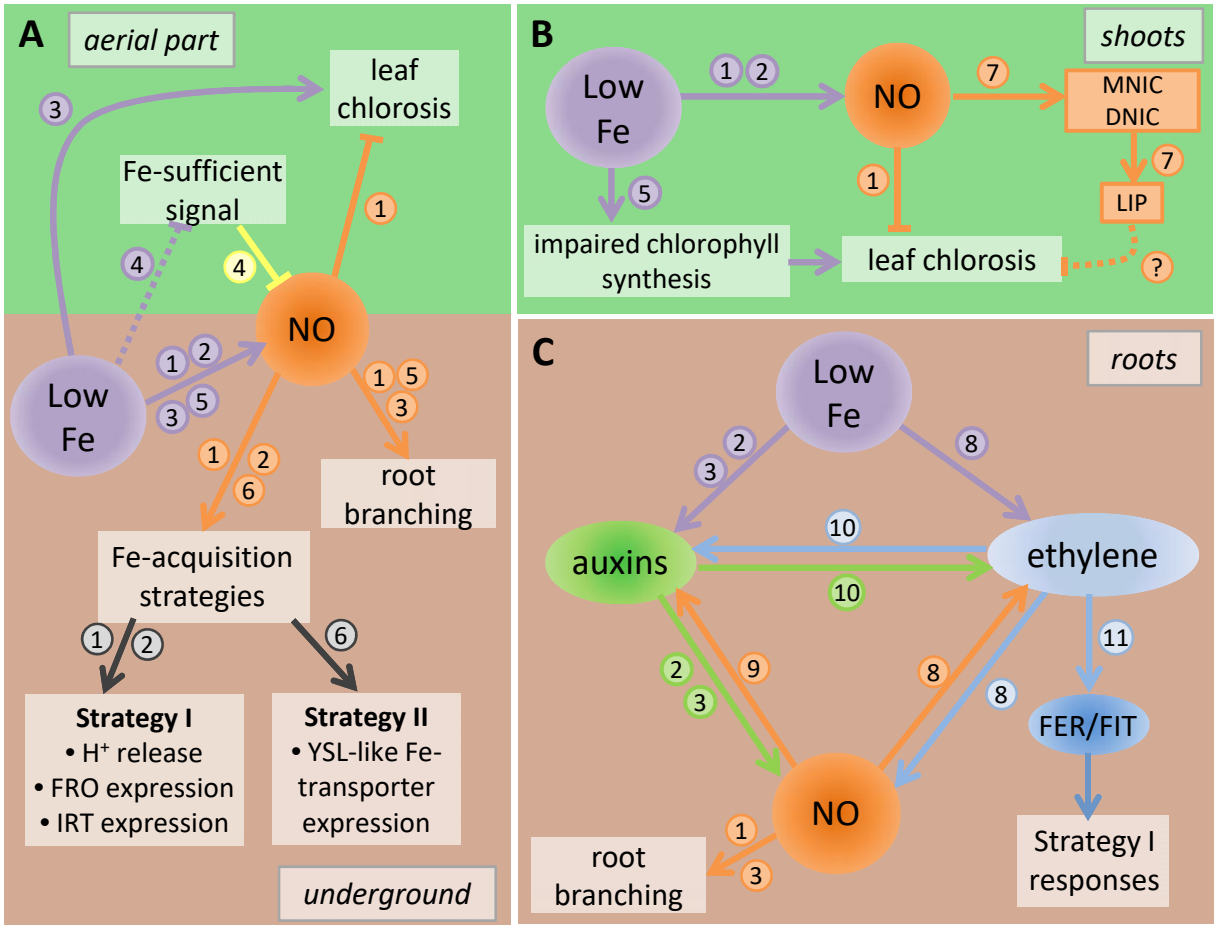

Figure 4

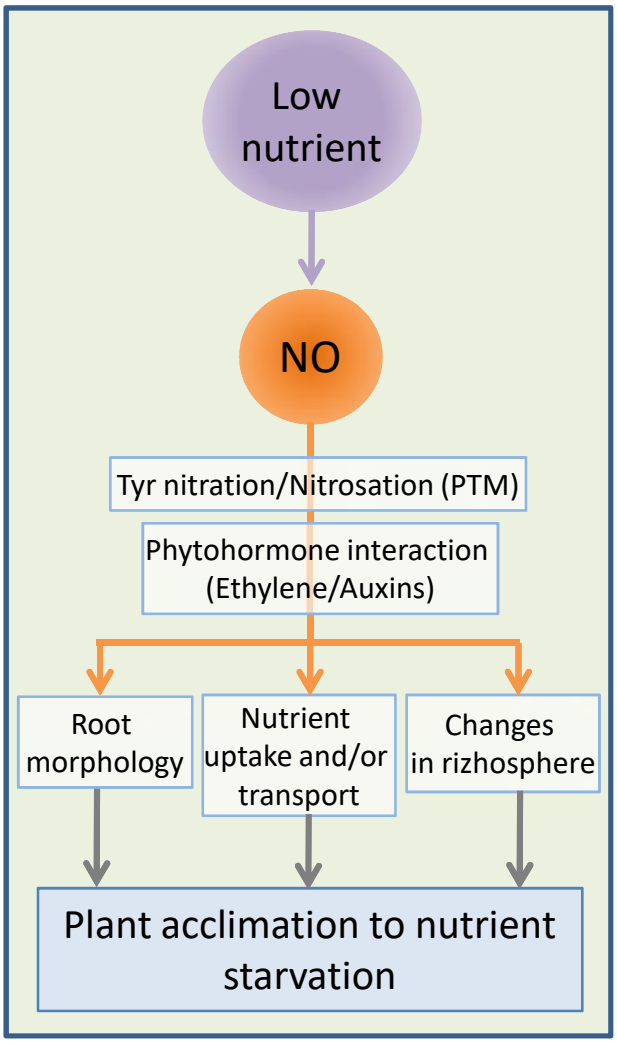

Figure 5