

REVIEW PAPER

Molecular responses of legumes to abiotic stress: post-translational modifications of proteins and redox signaling

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Abstract

Legumes include several major crops that can fix atmospheric nitrogen in symbiotic root nodules, thus reducing the demand for nitrogen fertilizers and contributing to sustainable agriculture. Global change models predict increases in temperature and extreme weather conditions. This scenario might increase plant exposure to abiotic stresses and negatively affect crop production. Regulation of whole plant physiology and nitrogen fixation in legumes during abiotic stress is complex, and only a few mechanisms have been elucidated. Reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) are key players in the acclimation and stress tolerance mechanisms of plants. However, the specific redox-dependent signaling pathways are far from understood. One mechanism by which ROS, RNS, and RSS fulfil their signaling role is the post-translational modification (PTM) of proteins. Redox-based PTMs occur in the cysteine thiol group (oxidation, S-nitrosylation, S-glutathionylation, persulfidation), and also in methionine (oxidation), tyrosine (nitration), and lysine and arginine (carbonylation/glycation) residues. Unraveling PTM patterns under different types of stress and establishing the functional implications may give insight into the underlying mechanisms by which the plant and nodule respond to adverse conditions. Here, we review current knowledge on redox-based PTMs and their possible consequences in legume and nodule biology.

Keywords: Abiotic stress, legumes, nitric oxide, nitrogen fixation, post-translational modifications, reactive oxygen/nitrogen/sulfur species, redox signaling, symbiosis.

Introduction

Crops provide the vast majority of global food requirements. However, modern agriculture relies greatly on the supply of fertilizers, especially nitrogen, and the demand is expected to rise due to population growth. Industrially, nitrogen is reduced to ammonia by the Haber-Bosch reaction, yet this process has negative consequences for the environment because it depends on fossil fuel consumption. Also, the use of excess fertilizers in

the field often leads to water pollution, causing problems such as eutrophication or poisoning of drinking water (Erisman *et al.*, 2011). Legumes are the third largest family of angiosperms and the second most important crop worldwide. They have the capacity to fix atmospheric N₂ in root nodules, which are formed after infection of host plants by rhizobia present in the soil. Further details on the rhizobia-legume symbiosis

and on the structure of legume nodules can be found in the reviews by Oldroyd (2013) and Roy *et al.* (2020), and in Fig. 1. Bacterial nitrogenase activity injects around 40 million tons of nitrogen into agricultural systems every year, thus reducing the demand for nitrogen fertilizers and contributing to sustainable agriculture (Udvardi and Poole, 2013; Gresshoff *et al.*, 2015).

Mankind is confronted with environmental challenges that are predicted to worsen in the upcoming decades. Global change models predict higher temperatures and extreme weather conditions (Schmidhuber and Tubiello, 2007). This scenario might increase plant exposure to abiotic stresses and negatively affect crop production. In legumes, symbiotic nitrogen fixation

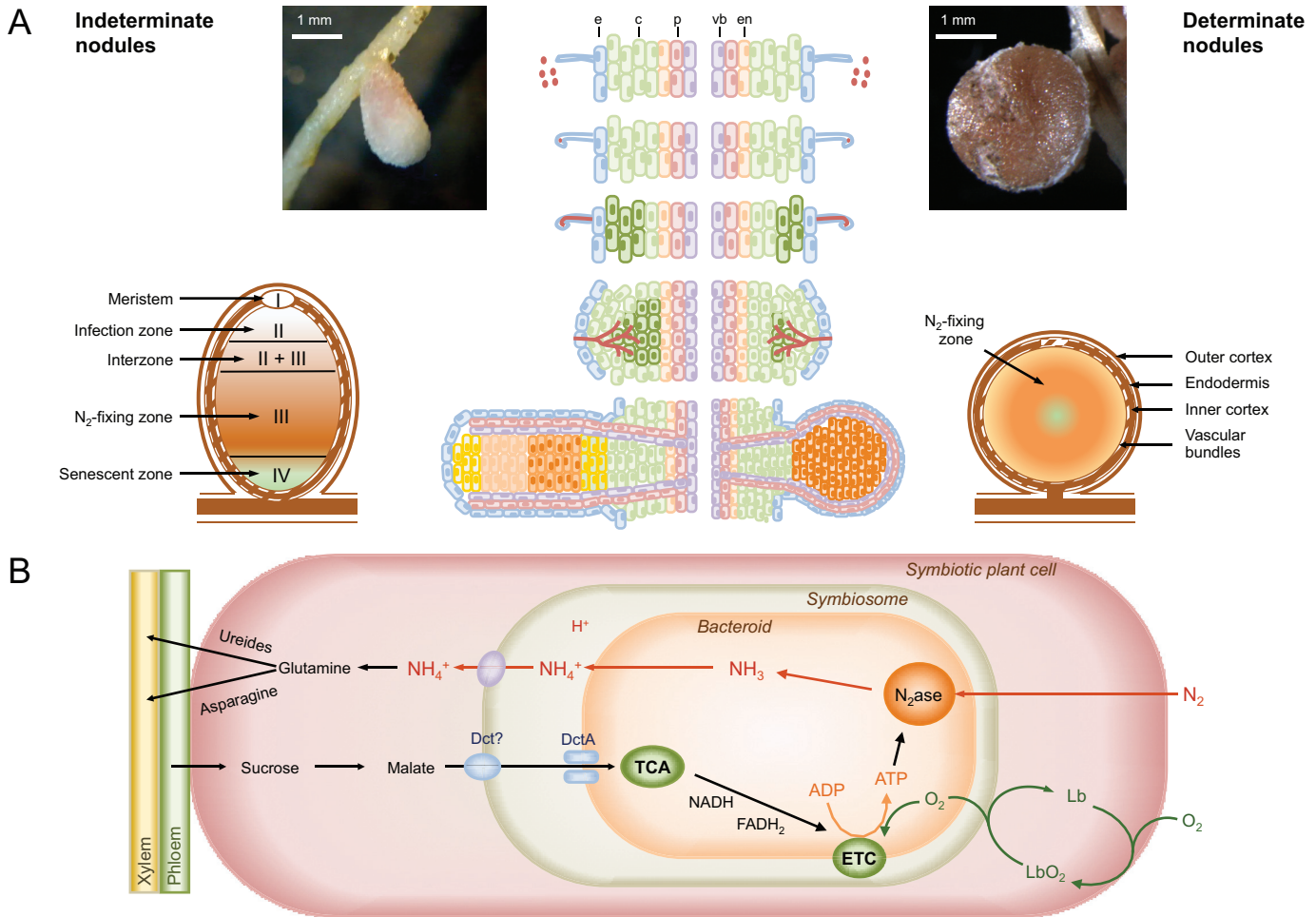


Fig. 1. Scheme showing the infection process, the major differences between indeterminate and determinate nodules, and some key metabolic pathways in the nodule cells. (A) Briefly, the infection process is as follows: legume roots release flavonoids to the rhizosphere which induce the production of nodulation (Nod) factors in compatible rhizobia (depicted as red dots next to root hairs); Nod factors are recognized by root receptors that activate the symbiotic signaling pathway; rhizobia enter the root through hairs that curve and trap the bacteria inside a curl; invaginations of the cell membrane form infection threads that permit the invasion of the root cortex by rhizobia; a new nodule meristem forms underneath the site of infection; as the nodule grows, the bacteria are released into membrane-bound compartments, the symbiosomes, inside the nodule cells, where the bacteria differentiate into bacteroids and start N_2 fixation. As a result, two major types of nodules are formed. Indeterminate nodules of *Medicago truncatula* and crops such as pea and alfalfa contain a persistent meristem and are generally elongated with a longitudinal gradient of age. Four zones can be distinguished from the apex (distal) to the base (proximal) regions: zone I (meristem), zone II (infection), zone III (N_2 -fixing), and zone IV (senescent). Determinate nodules of *Lotus japonicus* and of crops such as soybean and common bean lack permanent meristems and are usually spherical. In this case, N_2 fixation takes place in the central infected zone, which also contains uninfected or interstitial cells. Abbreviations of cell layers: c, cortex; e, epidermis; en, endodermis; p, pericycle; vb, vascular bundle. (B) Some key processes in the symbiotic nodule cells. Sucrose from the shoot is metabolized to malate that is transported into bacteroids through dicarboxylate transporters (Dct). In the bacteroids, malate is oxidized, providing energy through the tricarboxylic acid (TCA) cycle and the electron transport chain (ETC) for nitrogenase (N_2ase) activity. Fixed nitrogen in the form of ammonium is transported back to the plant where, with some exceptions, it is assimilated into the amides glutamine and asparagine in indeterminate nodules or ureides in determinate nodules. In the cytoplasm of infected cells, leghemoglobin (Lb) transports and delivers O_2 to the symbiosomes at a low steady concentration to avoid the inhibition of nitrogenase, but to simultaneously allow high rates of bacteroid respiration. After Oldroyd *et al.*, (2011), Popp and Ott (2011), and Hichri *et al.*, (2015).

(SNF) is particularly sensitive to adverse conditions, including drought (Ladrera *et al.*, 2007; Naya *et al.*, 2007; Aranjuelo *et al.*, 2014; Nasr Esfahani *et al.*, 2014; Dhanushkodi *et al.*, 2018), salinity (Jebara *et al.*, 2005; López *et al.*, 2008), and heavy metals (Marino *et al.*, 2013; Baig *et al.*, 2018). Plants under stress conditions produce enhanced amounts of reactive oxygen species (ROS), reactive nitrogen species (RNS), and reactive sulfur species (RSS) that need to be kept under control by antioxidant enzymes and metabolites, to avoid toxicity and allow their participation in signaling events (Becana *et al.*, 2010; Mittler, 2017; Begara-Morales *et al.*, 2019; Hancock, 2019). Hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), nitric oxide (NO), and hydrogen sulfide (H_2S) are key players in the acclimation of plants to abiotic stress (Choudhury *et al.*, 2017; Hancock, 2019). A substantial part of the signaling capacity and bioactivity of ROS, RNS, and RSS stems from their ability to cause post-translational modifications (PTMs), both reversible and irreversible, of amino acid side chains of proteins. PTMs may trigger changes in protein function, leading to increases or decreases in activity and, in certain cases, protein degradation (Friso and van Wijk, 2015; Gupta *et al.*, 2020). Here, we review current knowledge on redox-based PTMs of proteins in legumes. PTM-driven changes in the function of transcription factors and enzymes may be connected to alterations in gene expression, physiology, and metabolism that allow plant acclimation and tolerance to adverse environmental conditions. A deep understanding of how plant proteins are regulated by alterations in the redox state through PTMs can be of extraordinary significance in biotechnology and breeding programs.

Production of ROS, RNS, and RSS in legumes during abiotic stress

Plants perceive changes in environmental conditions through the activation of channels and/or sensors and the initiation of signaling cascades, which entail alterations in calcium and other ion fluxes, shifts in hormonal balance, protein phosphorylation, and ROS- and RNS-induced PTMs (Choudhury *et al.*, 2017; Kollist *et al.*, 2019; Lamers *et al.*, 2020). In the leaves, ROS are mostly produced in the apoplast by NADPH oxidases [also known as Respiratory Burst Oxidase Homologues (RBOHs)] and by other oxidases and peroxidases, but also in the chloroplasts, mitochondria, and peroxisomes (Choudhury *et al.*, 2017). Very recently, a sensor that detects H_2O_2 at the cell surface has been reported. This important discovery emphasizes the key role of ROS in the perception and response of plant cells to environmental stress (Wu *et al.*, 2020). As for NO, despite intensive research its production in plants is only partially understood. The best described enzymatic sources are cytosolic nitrate reductase, the mitochondrial electron transport chain, and the oxidation of aminated molecules (Astier *et al.*, 2018; Umbreen *et al.*, 2018). In plant cells, H_2S is produced in the plastids by sulfite reductase as an intermediate of assimilatory sulfate reduction, and then incorporated into O-acetylserine by

O-acetylserine thiol lyase (OASTL) to form cysteine (Cys) in the plastids, cytosol, and mitochondria. However, OASTL can also catalyze the reverse reaction, generating H_2S from Cys. In the cytoplasm, the main source of H_2S is L-Cys desulfhydrase, which decomposes Cys to H_2S , ammonia, and pyruvate. In the mitochondria, cyanoalanine synthase catalyzes the reaction between Cys and cyanide to produce β -cyanoalanine and H_2S (Aroca *et al.*, 2018).

The stress-induced senescence of legume nodules is characterized by a rapid decrease in SNF caused by the decline of leghemoglobin (Lb) content and the reduction of key activities such as sucrose synthase, nitrogenase, and nitrogen assimilatory enzymes (Aranjuelo *et al.*, 2014). In nodules, ROS are generated by NADPH oxidase activity in plant membranes, the oxidative metabolism of peroxisomes, the respiration of mitochondria, the autoxidation of oxyferrous Lb in the cytoplasm, and the oxidation of nitrogenase, ferredoxin, and hydrogenase in the bacteroids (Becana and Klucas, 1992; Santos *et al.*, 2001; Marino *et al.*, 2012; Puppo *et al.*, 2013; Arthikala *et al.*, 2017). Likewise, NO can be formed in nodules through the bacterial denitrification pathway, the reduction of NO_3^- by plant nitrate reductase, and the electron transport chain of mitochondria in the hypoxic tissue (Meakin *et al.*, 2007; Horchani *et al.*, 2011; Calvo-Begueria *et al.*, 2018). It has been recently reported that H_2S promotes SNF (Zou *et al.*, 2019). Although the synthesis of H_2S in nodules has not been investigated in detail yet, it is probably generated by both plant and bacterial enzymes. A recent study showed that deletion in *Sinorhizobium (Ensifer) fredii* of cystathionine γ -lyase, an enzyme that participates in the production of H_2S in some bacteria and mammals, caused a sharp decrease in H_2S content, inhibition of nitrogenase activity, and accumulation of H_2O_2 and malondialdehyde in soybean (*Glycine max*) nodules (Zou *et al.*, 2020).

Stress-induced alterations of ROS, RNS, and RSS amounts may also modify the ratios of the redox couples ascorbate/dehydroascorbate and reduced glutathione/glutathione disulfide, as well as the expression and activity of antioxidant enzymes, in the leaves (Corpas *et al.*, 2008; Noreen and Ashraf, 2009; Rodríguez-Serrano *et al.*, 2009; Rubio *et al.*, 2009; Navascués *et al.*, 2012b; Hancock, 2019) and nodules (Marino *et al.*, 2007; Naya *et al.*, 2007; Nasr Esfahani *et al.*, 2014; Marquez-García *et al.*, 2015). In general, stress-induced redox changes in cells and organelles entail an oxidative shift. However, the mechanisms by which variations in the cellular redox state are transduced into specific responses are unclear. Important factors are the concentration, exposure time, subcellular location, and type of ROS, RNS, and RSS. In each cell compartment, a consequence of the loss of homeostasis of these reactive molecules may be the modification of susceptible amino acids. These are mostly the sulfur-containing amino acids Cys and methionine (Met), but also tyrosine (Tyr), lysine (Lys), and arginine (Arg). These changes may potentially alter the activity of enzymes and transcription factors, thus regulating key metabolic processes, signaling pathways, and gene expression.

Proteomics provides insight into the mechanisms of stress adaptation of legumes

Over the last 15 years, the field of proteomics has revolutionized the identification and quantification of plant proteins involved in the responses to abiotic stress (Kosova *et al.*, 2018). These studies are invaluable as they provide a global perspective of plant adaptation to adverse conditions. Nevertheless, despite the extensive inventory of plant proteins involved in the stress response, many issues still need to be examined in detail. These include the functional characterization of proteins, the assessment of the effects of PTMs on the activity, location and interaction with other proteins, and the susceptibility of modified proteins to degradation under specific environmental conditions.

In legumes, a considerable number of proteomic studies have been carried out to unravel the mechanisms of stress tolerance (reviewed by Rathi *et al.* 2016). For example, comparison of dehydration-sensitive and dehydration-tolerant cultivars of chickpea (*Cicer arietinum*) revealed differential expression of many proteins. Tolerance was attributed, at least in part, to altered expression of proteins involved in ROS catabolism (Subba *et al.*, 2013). In the same species, up-regulation of expression of proteins with chaperone-like functions and of proteins involved in ROS homeostasis was associated with improved germination and early seedling growth under sub-optimal soil-water conditions (Vessal *et al.*, 2012). Other studies also suggested an important role of ROS in stress acclimation. Based on the proteomics of salt-tolerant and salt-sensitive genotypes of soybean, it was concluded that the tolerant genotype possessed a higher capacity to secure energy supply and to maintain ROS homeostasis, photosynthetic rate, and ethylene synthesis (Ma *et al.*, 2012). Salinity-induced changes in the root proteome of pea (*Pisum sativum*) pointed to the possible existence of a signal transduction pathway involving H₂O₂ and the antioxidant enzyme superoxide dismutase (Kav *et al.*, 2004). Overall, these studies highlight the importance of ROS homeostasis in stress tolerance.

The proteomic studies of nodules lag well behind those of leaves. Investigations in the model legumes *Lotus japonicus* and *Medicago truncatula* have provided insight into quantitative expression of proteins in plant organs, PTMs, and the mechanisms that regulate the symbiosis. The comparative analysis of *L. japonicus* root and nodule proteomes suggests that, in general, nodule proteins contain higher amounts of PTMs (Dam *et al.*, 2014). This might be related to a better capacity to face, and adapt to, stress conditions of metabolically active nodules, compared with the less active roots. Furthermore, to our knowledge, in the largest study to date on legume proteomics, >23 000 proteins, 20 000 phosphorylation sites, and 700 Lys acetylation sites were identified in *M. truncatula* and its symbiont *S. meliloti* (Marx *et al.*, 2016). This study established a core of *M. truncatula* proteins expressed in all organs, identified a subset of proteins that displayed organ-specific regulation, and

assigned putative functions to several uncharacterized proteins. Compared with roots, significant regulatory and phosphorylation events were identified during nodule development in proteins involved in oxygen transport, immune response, and senescence. This extensive resource might be useful for future studies aimed at identifying proteins and PTMs that participate in the stress response. Quantitative proteomics in *M. truncatula* revealed major changes in nodule proteins under drought conditions. This stress caused a decrease in the amount of proteins involved in SNF and carbon metabolism in the bacteroids, as well as of sucrose synthase and enzymes of the Met and ethylene biosynthetic pathways in the nodule host cells (Larrainzar *et al.*, 2009; 2014). Another study found that exposure of soybean nodules to toxic heavy metals, even at low concentrations, increased the expression of proteins involved in development, hormone signaling, and stress responses (Baig *et al.*, 2018).

Post-translational modifications of proteins and redox signaling under abiotic stress

Many proteins undergo one or more PTMs throughout their lifetime. These can be reversible, such as the formation of disulfide bonds, methionine sulfoxides (MetSO), S-nitrosylation, and S-glutathionylation, or irreversible, such as carbonylation and glycation (Friso and van Wijk, 2015). Redox modifications of proteins may affect their activity, stability, location, and interaction with other proteins. Protein PTMs constitute a fast and versatile mechanism by which plants can respond to the frequent environmental constraints faced in natural and agricultural systems. In the next sections, current knowledge of the role of redox-based protein PTMs in the response of legumes to abiotic stress is reviewed.

Methionine sulfoxidation

Under stress conditions, the redox state of the apoplast, cell cytoplasm and/or organelles may shift to a more oxidizing state and induce the oxidation of certain Met residues of proteins to a mixture of Met-S-sulfoxide and Met-R-sulfoxide (Fig. 2). To reverse this oxidation, most organisms, including plants, have evolved two monomeric methionine sulfoxide reductases (MsrA and MsrB) that reduce the S and R epimers, respectively (Tarrago *et al.*, 2009). Met oxidation may alter protein structure and function, making this modification of regulatory significance in redox signaling. In vertebrates, oxidation of Met residues of calmodulin disrupts downstream calcium-mediated signaling and targets the protein for proteasomal degradation (Snijder *et al.*, 2011). In *Arabidopsis thaliana*, the *in vivo* oxidation of Met-538 of nitrate reductase inhibits phosphorylation of serine-534 (Hardin *et al.*, 2009). Other studies indicate that aging is associated with the accumulation of oxidized Met in proteins, which increases their susceptibility to degradation by the proteasome (Stadtman *et al.*, 2005). In some cases, however,

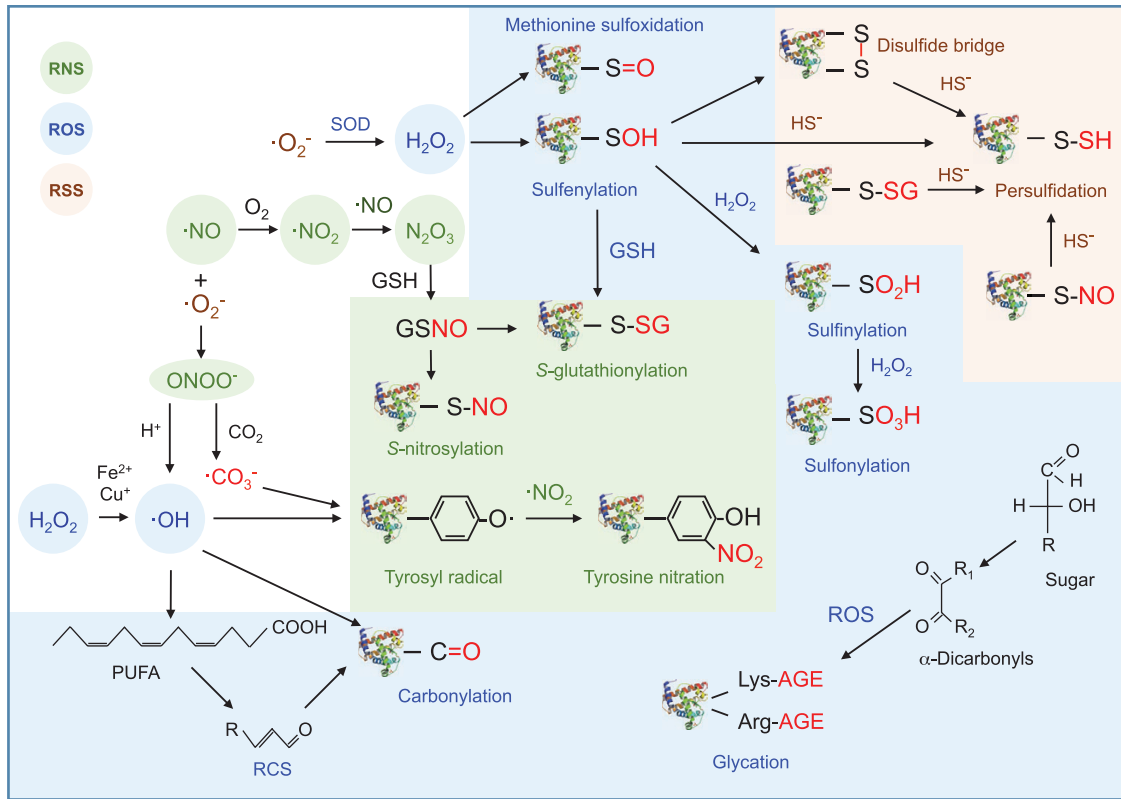


Fig. 2. Redox-dependent PTMs. Met residues can be oxidized by hydrogen peroxide (H_2O_2) to Met sulfoxides. The *S* and *R* stereoisomers are specifically reduced back to Met by methionine sulfoxide reductases A and B, respectively. Oxidation of deprotonated thiols of Cys residues ($-\text{S}^-$) by H_2O_2 leads to the formation of sulfenic acid ($-\text{SOH}$), which may react with another thiol to form disulfides ($-\text{S}-\text{S}-$). This modification can be reverted by thioredoxins and glutaredoxins. The $-\text{SOH}$ group can be an intermediate to other redox modifications (see below) or be further oxidized to sulfinic acid ($-\text{SO}_2\text{H}$) and sulfonic acid ($-\text{SO}_3\text{H}$). *S*-nitrosylation ($-\text{SNO}$) is mostly mediated by nitrogen oxides and *trans*-nitrosylating agents such as *S*-nitrosoglutathione (GSNO). *S*-glutathionylation ($-\text{SSG}$) occurs by two main mechanisms: reaction of the target protein with GSNO, and reaction of reduced glutathione (GSH) with $-\text{SOH}$. The reaction of hydrogen sulfide (HS^-) with $-\text{SOH}$, $-\text{SNO}$, $-\text{SSG}$, or disulfide bridges induces persulfidation ($-\text{SSH}$). Peroxynitrite (ONOO^-) is formed by the reaction of nitric oxide (NO) with superoxide (O_2^-) radicals. In turn, radicals derived from ONOO^- breakdown oxidize Tyr residues to tyrosyl radicals; these react with nitrogen dioxide (NO_2), produced from ONOO^- decomposition, to yield NO_2 -Tyr. The direct oxidation of Lys, Arg, Pro, and Thr by hydroxyl radicals ($\cdot\text{OH}$) incorporates the carbonyl moiety into proteins. Alternatively, oxidation of a polyunsaturated fatty acid (PUFA; a simplified representation is shown lacking part of the aliphatic chain) produces unstable lipid hydroperoxides that decompose to secondary products known as reactive carbonyl species (RCS). These react with amino acid side chains and generate carbonyl derivatives. Moreover, Arg and Lys residues may react with reducing sugars or α -dicarbonyls such as glyoxal and methylglyoxal, generating glycation products that are readily oxidized to form relatively stable advanced glycation end products (AGEs).

Met oxidation does not seem to affect protein function, and it has been hypothesized that Met residues could function as ultimate endogenous antioxidants in proteins, providing effective scavenging of oxidants before they can attack residues that are critical for structure or function (Stadtman et al., 2005).

Little is known about Met sulfoxidation in legumes. Genome-wide datasets of the two model legumes available at the Lotus Base and *M. truncatula* Gene Expression Atlas (He et al., 2009; Mun et al., 2016) reveal the expression of four *MsrA* and three *MsrB* genes in *L. japonicus*, and of four *MsrA* and five *MsrB* genes in *M. truncatula*. All genes are expressed in nodules, albeit some of them in very low levels. Transcriptional induction in response to drought and/or salt stress of one *MsrA* gene in *L. japonicus* shoots (Fig. 3; Díaz et al., 2010), and of two *MsrA* genes in *M. truncatula* roots and/or shoots (Fig. 4; Table 1;

Li et al., 2009), suggests a role of Msr enzymes in the regulation of Met redox homeostasis and in the tolerance to abiotic stress.

Sulfenylation

Together with Met, Cys is a principal target for redox-dependent PTMs in cells. This is due to the versatile chemistry of the sulfur atom that can display oxidation states ranging from -2 to $+6$, on account of the availability of empty *d*-orbitals for bonding. The sulfur of Cys is fully reduced but, because of its low redox potential, the thiol side chain of proteins can readily undergo a range of oxidative PTMs (Fig. 2). However, not all Cys residues are equally amenable to modification. Their reactivity depends on the residue accessibility, protein micro-environment, and pK_a value. Usually, only thiols with low pK_a

Gene	Gene ID	Salinity	Drought
ALDH7B4	Lj6g3v1177340		
2-AR	Lj3g3v0642510		
DR	Lj0g3v0326719		
GrxC9	Lj3g3v2825320		
GrxC9	Lj6g3v2006480		
GrxS2	Lj0g3v0132559		
GrxS6	Lj1g3v4917460		
Glb1-1	Lj3g3v3338170		
GSHS	Lj1g3v4955040		
GSTU13	Lj0g3v0288349		
GSTU23	Lj1g3v1526240		
MsrA	Lj0g3v0287649		

Fig. 3. Expression profile of genes involved in redox homeostasis in the shoots of *Lotus japonicus* plants exposed to drought or salt stress. Gene up-regulation (>two-fold) and down-regulation (<0.5-fold) are indicated in red and blue, respectively. Gene IDs are given according to the *L. japonicus* MG-20 genome v3.0 and data were retrieved from the *L. japonicus* Expression Atlas (Lotus Base; <https://lotus.au.dk>). Transcriptomic data under salt and drought stress were published, respectively, by Sanchez *et al.*, (2008) and Diaz *et al.*, (2010). Abbreviations: ALDH, aldehyde dehydrogenase; AR, alkenal reductase; DR, dehydroascorbate reductase; Glb, phytolegumin; Grx, glutaredoxin; GSHS, glutathione synthetase; GSTU, glutathione transferase tau family; Msr, methionine sulfoxide reductase.

play key roles in catalysis and serve as important sites for PTMs (Go *et al.*, 2015). Thus, deprotonated thiolates (R-S⁻) are reversibly oxidized by ROS to form sulfenic acids (R-SOH) and disulfide bridges (R-S-S-R'). Further oxidation of the former may lead to the formation of sulfinic (R-SO₂H) and sulfonic (R-SO₃H) acids (Fig. 2). Sulfonic acid formation seems to be irreversible but the other modifications can be reversed by thioredoxins (Trxs), glutaredoxins (Grxs), and sulfiredoxins (Meyer *et al.*, 2009; Sevilla *et al.*, 2015).

The identification of sulfenylated plant proteins, although challenging due to the instability of sulfenic acids, can be useful to discover redox sensors and as yet unknown components of ROS-mediated signaling cascades. It can also help establish the role of this PTM in the regulation of plant cell metabolism. Very recently, using a state-of-the-art chemoproteomics approach, Huang *et al.* (2019) mapped sulfenylated sites on more than 1000 *A. thaliana* proteins. Remarkably, the first extracellular sensor of H₂O₂, Hydrogen-Peroxide induced Calcium increases 1 (HPCA1), characterized in *A. thaliana* is composed of an intracellular kinase domain and an apoplastic extracellular domain. The latter has two pairs of Cys residues that are oxidized by H₂O₂ to form R-SOH and disulfide bonds. These PTMs cause a conformational change that triggers intracellular kinase activity and calcium influx into the cell, thus activating signaling pathways (Wu *et al.*, 2020). In *M. truncatula*, the use of chemical and genetic probes combined with mass spectrometry analyses allowed the identification of sulfenylated proteins in inoculated roots and mature nodules (Oger *et al.*, 2012). During the onset of symbiosis, most sulfenylated root proteins are involved in redox signaling and defense, whereas enzymes

involved in carbohydrate and amino acid metabolism are the predominant modified proteins in mature nodules. These results suggest that sulfenylation may regulate the activity of key proteins involved in nodule development and metabolism.

Little is known about the possible alterations of protein sulfenylation patterns during the perception and response to abiotic stress. Nevertheless, an important role can be anticipated because Cys oxidation is a highly sensitive and fast way to modify the function of enzymes and transcription factors in response to new conditions. Legumes contain large Trx and Grx families that keep the balance between reduced and oxidized thiols (Alkhalifi *et al.*, 2008; Tovar-Méndez *et al.*, 2011; Alloing *et al.*, 2018). The analysis of *M. truncatula* transcript profiles showed the up-regulation of several Trx and Grx genes in plants subjected to salt or drought stress. Interestingly, re-watering of plants for one day after 14 days of drought stress caused a fast increase in the amounts of some Trx transcripts both in shoots and roots (Fig. 4). In *L. japonicus*, salt stress induced the expression of two GrxS genes in the shoot (Fig. 3; Sanchez *et al.*, 2008). Recently, RNA-sequencing analysis of *M. truncatula* and *L. japonicus* nodules revealed a high number of differentially expressed genes in response to water stress (Saňko-Sawczenko *et al.*, 2019). Transcripts of some Trxh and Grx isoforms were induced in both legumes after four days of treatment (Fig. 5). An increase in mitochondrial Trxo activity in response to salt treatment was also observed in pea leaves (Martí *et al.*, 2011). These data suggest that restoration and maintenance of protein thiol homeostasis by various Trx and Grx isoforms are important features of the response of legumes to abiotic stress.

S-nitrosylation

The term S-nitrosylation is used by the scientific community to refer to the reversible covalent binding of NO to a reactive Cys thiol in a protein (Fig. 2), although some authors prefer to use S-nitrosation instead (for further clarification on these terms see Martínez-Ruiz and Lamas, 2004). This PTM has been recognized as a major mechanism by which NO conveys its bioactivity (Lamotte *et al.*, 2014; Gupta *et al.*, 2020). In *A. thaliana* and other species, S-nitrosylation of specific proteins is part of the immune and abiotic stress responses (Feechan *et al.*, 2005; Tada *et al.*, 2008; Begara-Morales *et al.*, 2019). One such response is the involvement of NO and S-nitrosylation as negative regulators in abscisic acid (ABA) signaling and stomatal closure under physiological and stress conditions. Under drought, ABA accumulates, which enhances the production of NO and the S-nitrosylation of the protein kinase OST1/SnRK2.6. This nitrosylation at a specific Cys residue adjacent to the catalytic site blocks the kinase activity of OST1/SnRK2.6, and results in feedback inhibition of ABA signaling in guard cells (Wang *et al.*, 2015). Similarly, diverse studies in legumes have shown that abiotic stress induces changes in the profiles of S-nitrosylated proteins.

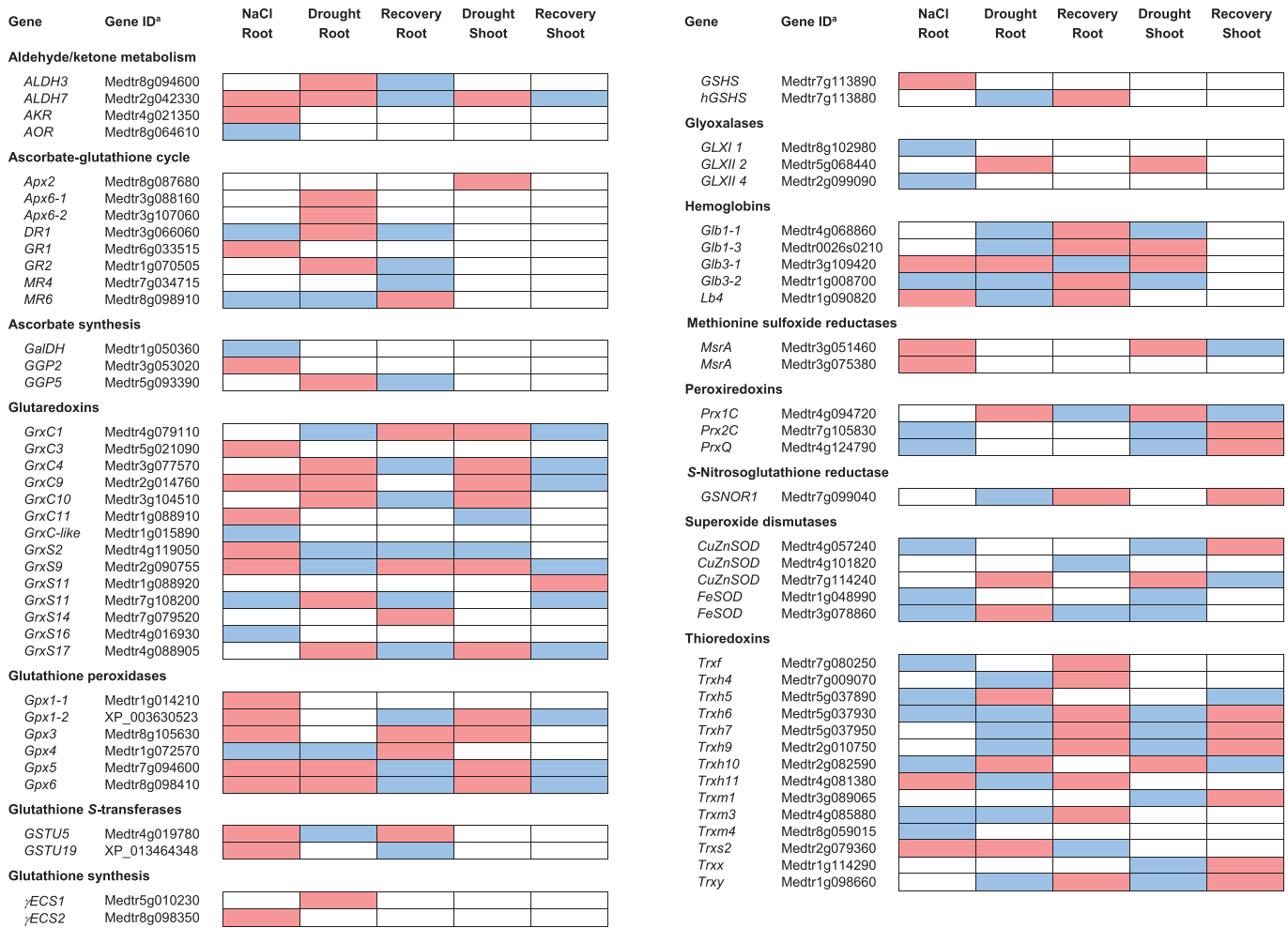


Fig. 4. Expression profile of genes involved in redox homeostasis in the roots and shoots of *Medicago truncatula* plants exposed to drought or salt stress. Gene up-regulation (>two-fold) and down-regulation (<0.5-fold) are indicated in red and blue, respectively. Gene IDs are given according to the *M. truncatula* genome v4.0. Transcriptomic data of roots and shoots under drought stress were retrieved from the Gene Expression Atlas (<https://mtgea.noble.org/v3/>) and those of salt stress in the roots were published by Li *et al.*, (2009). Gene sequences were obtained from Legume IP (<http://plantgrn.noble.org/LegumeIP/gdp/>), except for *Gpx1-2* and *GSTU19*, which were obtained from GenBank. The probes used to determine gene expression profiles are listed in Table 1. Abbreviations: AKR, aldo-keto reductase; ALDH, aldehyde dehydrogenase; Apx, ascorbate peroxidase; AOR, alkenal/one oxidoreductase; DR, dehydroascorbate reductase; γ ECS, γ -glutamylcysteine synthetase; GalDH, L-galactono-1,4-lactone dehydrogenase; GGP, GDP-L-galactose phosphorylase; Glb, phytohemoglobin; GLX, glyoxalase; Gpx, glutathione peroxidase; GR, glutathione reductase; Grx, glutaredoxin; GS, glutathione synthetase; GSNOR, S-nitrosoglutathione reductase; GSTU, glutathione S-transferase tau family; hGS, homoglutathione synthetase; Lb, leghemoglobin; MR, monodehydroascorbate reductase; Msr, methionine sulfoxide reductase; Prx, peroxiredoxin; SOD, superoxide dismutase; Trx, thioredoxin.

For example, in mitochondria of pea leaves, salt stress caused S-nitrosylation of peroxiredoxin IIF (PrxIIF), a key enzyme in ROS- and RNS-mediated redox signaling. Subsequent *in vitro* studies showed that this modification induced a conformational change in the protein that provoked the reduction of its peroxidase activity and the acquisition of a novel function as a *trans*-nitrosylase (Camejo *et al.*, 2013; 2015). In pea leaves, abiotic stress induced changes in S-nitrosylation of several enzymes that participate in H₂O₂ metabolism, such as catalase (Ortega-Galisteo *et al.*, 2012) and three enzymes of the ascorbate-glutathione cycle (Begara-Morales *et al.*, 2019).

Interestingly, this modification increased ascorbate peroxidase activity, but inhibited monodehydroascorbate reductase and dehydroascorbate reductase activities, suggesting a complex crosstalk between NO- and ROS-mediated signaling during the perception and response to abiotic stress.

S-nitrosylation was also observed in nodule proteins under optimal growth conditions (Puppo *et al.*, 2013). However, the effect of this modification on protein function, or its role in the response of nodules to stress, is virtually unknown. In one of the few studies addressing these issues, Melo *et al.* (2011) observed that two glutamine synthetase (GS) isoforms of

Table 1. Affimetrix *M. truncatula* Gene Chip probesets used for the gene expression analysis

Gene	Gene ID	Probeset ID	Gene	Gene ID	Probeset ID
Aldehyde/ketone metabolism					
ALDH3	Mecdr8g094600	Mtr.9402.1.S1_at	Glutathione synthesis	Mecdr5g010230	Mtr.5743.1.S1_at
ALDH7	Mecdr2g042330	Mtr.9030.1.S1_at	<i>γECS1</i>	Mecdr8g098350	Mtr.26628.1.S1_s_at
AKR	Mecdr4g021350	Mtr.46053.1.S1_at	<i>γECS2</i>	Mecdr7g113890	Mtr.40809.1.S1_at
AOR	Mecdr8g064610	Mtr.44591.1.S1_at	GSHS	Mecdr7g113880	Mtr.37763.1.S1_at
Ascorbate-glutathione cycle					
Apx2	Mecdr8g087680	Mtr.6533.1.S1_at	Glyoxalases	Mecdr8g102980	Mtr.43350.1.S1_at
Apx6-1	Mecdr3g088160	Mtr.12244.1.S1_s_at	GLX1	Mecdr5g068440	Mtr.43695.1.S1_at
Apx6-2	Mecdr3g107060	Mtr.2925.1.S1_at	GLXII	Mecdr2g099090	Mtr.38317.1.S1_at
DR1	Mecdr3g066060	Mtr.40092.1.S1_at	Hemoglobins		
GR1	Mecdr6g033515	Mtr.10610.1.S1_at	<i>Glb1-1</i>	Mecdr4g068860	Msa.878.1.S1_at
GR2	Mecdr1g070505	Mtr.38809.1.S1_at	<i>Glb1-3</i>	Mecdr0026s0210	Mtr.29072.1.S1_s_at
MR4	Mecdr7g034715	Mtr.38020.1.S1_at	<i>Glb3-1</i>	Mecdr3g109420	Mtr.47990.1.S1_at
MR6	Mecdr8g098910	Mtr.44027.1.S1_at	<i>Glb3-2</i>	Mecdr1g008700	Mtr.10341.1.S1_at
Ascorbate synthesis					
GalDH	Mecdr1g050360	Mtr.11321.1.S1_at	<i>Lb4</i>	Mecdr1g090820	Mtr.8550.1.S1_s_at
GGP2	Mecdr3g053020	Mtr.37321.1.S1_at	Methionine sulfoxide reductases		
GGP5	Mecdr5g093390	Mtr.37322.1.S1_at	<i>MsrA</i>	Mecdr3g051460	Mtr.11716.1.S1_at
Glutaredoxins					
GrxC1	Mecdr4g079110	Mtr.41411.1.S1_at	<i>MsrA</i>	Mecdr3g075380	Mtr.11204.1.S1_at
GrxC3	Mecdr5g021090	Mtr.40741.1.S1_at	Peroxioredoxins		
GrxC4	Mecdr3g077570	Mtr.50976.1.S1_at	<i>Prx1C</i>	Mecdr4g094720	Mtr.11099.1.S1_at
GrxC9	Mecdr2g014760	Mtr.44436.1.S1_at	<i>Prx2C</i>	Mecdr7g105830	Mtr.25633.1.S1_at
GrxC10	Mecdr3g104510	Mtr.22341.1.S1_at	<i>PrxQ</i>	Mecdr4g124790	Mtr.10844.1.S1_at
GrxC11	Mecdr1g088910	Mtr.42692.1.S1_at	S-Nitrosogluthathione reductase		
GrxC-like	Mecdr1g015890	Mtr.24815.1.S1_at	GSNOR1	Mecdr7g099040	Mtr.21158.1.S1_at
GrxS2	Mecdr4g119050	Mtr.34638.1.S1_at	Superoxide dismutases		
GrxS9	Mecdr2g090755	Mtr.35755.1.S1_at	<i>CuZnSOD</i>	Mecdr4g057240	Mtr.15585.1.S1_at
GrxS11	Mecdr1g088920	Mtr.32795.1.S1_x_at	<i>CuZnSOD</i>	Mecdr4g101820	Mtr.17367.1.S1_at
GrxS11	Mecdr7g108200	Mtr.34408.1.S1_s_at	<i>CuZnSOD</i>	Mecdr7g114240	Mtr.16601.1.S1_at
GrxS14	Mecdr7g079520	Mtr.14770.1.S1_at	<i>FeSOD</i>	Mecdr1g048990	Mtr.37849.1.S1_at
GrxS16	Mecdr4g016930	Mtr.19479.1.S1_at	<i>FeSOD</i>	Mecdr3g078860	Mtr.42746.1.S1_at
GrxS17	Mecdr4g088905	Mtr.12551.1.S1_at	Thioredoxins		
Glutathione peroxidases					
Gpx1-1	Mecdr1g014210	Mtr.48948.1.S1_at	<i>Trxf</i>	Mecdr7g080250	Mtr.43208.1.S1_at
Gpx1-2	XP_003630523	Mtr.12331.1.S1_at	<i>Trxh4</i>	Mecdr7g009070	Mtr.26137.1.S1_at
Gpx3	Mecdr8g105630	Msa.2641.1.S1_at	<i>Trxh5</i>	Mecdr5g037890	Mtr.9873.1.S1_at
Gpx4	Mecdr1g072570	Mtr.48820.1.S1_at	<i>Trxh6</i>	Mecdr5g037930	Mtr.12857.1.S1_at
Gpx5	Mecdr7g094600	Mtr.44501.1.S1_at	<i>Trxh7</i>	Mecdr5g037950	Mtr.9679.1.S1_at
Gpx6	Mecdr8g098410	Msa.1887.1.S1_at	<i>Trxh9</i>	Mecdr2g010750	Mtr.5202.1.S1_s_at
Glutathione S-transferases					
GSTU5	Mecdr4g019780	Mtr.40588.1.S1_at	<i>Trxh10</i>	Mecdr2g082590	Mtr.38559.1.S1_at
GSTU19	XP_013464348	Mtr.12287.1.S1_at	<i>Trxh11</i>	Mecdr4g081380	Mtr.18521.1.S1_at
			<i>Trxm1</i>	Mecdr3g089065	Mtr.42854.1.S1_at
			<i>Trxm3</i>	Mecdr4g085880	Mtr.43824.1.S1_at
			<i>Trxm4</i>	Mecdr8g059015	Mtr.13972.1.S1_at
			<i>Trxs2</i>	Mecdr2g079360	Mtr.40666.1.S1_at
			<i>Trxx</i>	Mecdr1g114290	Mtr.12884.1.S1_at
			<i>Trxy</i>	Mecdr1g098660	Mtr.15318.1.S1_at

Gene	Gene ID	Drought 2 days	Drought 4 days
ALDH7B4	Medtr2g042330		
FeSOD	Medtr1g048990		
Glb1-1	Lj3g3v3338170		
Glb2-1	Lj5g3v1699110		
Glb3-1	Medtr3g109420		
GSTU5	Medtr4g019780		
GrxC1	Lj1g3v0395760		
GrxC1	Medtr4g079110		
GrxC9	Lj6g3v2006480		
GrxC9	Medtr2g014760		
GrxC-like	Medtr1g015890		
GrxS2	Medtr4g119050		
Lb12	Medtr7g110180		
PrxQ	Lj4g3v2215040		
Trxf	Lj1g3v2838090		
Trxh9	Lj6g3v2168630		
Trxh11	Medtr4g081380		
Trxm1	Lj0g3v0095619		
Trxs1	Medtr2g079400		
Trxs2	Medtr2g079360		

Fig. 5. Expression profiles of genes involved in redox homeostasis in nodules of *Lotus japonicus* and *Medicago truncatula* following two or four days of drought stress. Gene up-regulation (>two-fold) and down-regulation (<0.5-fold) are indicated in red and blue, respectively. Expression data were retrieved from LegumelP v3 (<http://plantgrn.noble.org/LegumelP/gdp/>) and Sańko-Sawczenko *et al.*, (2019). Abbreviations: ALDH, aldehyde dehydrogenase; Glb, phytohemoglobin; Grx, glutaredoxin; GSTU, glutathione transferase tau family; Lb, leghemoglobin; Prx, peroxiredoxin; SOD, superoxide dismutase; Trx, thioredoxin.

M. truncatula are differentially regulated by NO. The activity of plastid MtGS2a was inhibited by S-nitrosylation, whereas cytosolic MtGS1a was unaffected by this PTM but inactivated by Tyr nitration (see below). Moreover, in *L. japonicus* and *M. truncatula*, glutathione peroxidases (Gpxs), an enzyme family closely related to Prxs, are S-nitrosylated *in vitro* and *in vivo*, which resulted in the partial inhibition of enzyme activities (Matamoros *et al.*, 2015; Castella *et al.*, 2017). These results strongly suggest an important role for NO signaling in the regulation of SNE, although a possible function under stress conditions awaits investigation.

In plant cells, S-nitrosoglutathione reductase (GSNOR) and Trxs are involved in S-nitrosothiol (SNO) homeostasis (Begara-Morales *et al.*, 2019; Fukudome *et al.*, 2019a; Matamoros *et al.*, 2020). GSNOR is essential for plant development and adaptation to abiotic and biotic stress (Kwon *et al.*, 2012; Lindermayr, 2018; Matamoros *et al.*, 2020). In *A. thaliana* the enzyme is required for acclimation to high temperatures and the null mutants have augmented SNO amounts (Lee *et al.*, 2008). Although a single-copy GSNOR gene is predominantly found in most species analysed so far, including *A. thaliana*, many legumes contain two GSNOR genes (Matamoros *et al.*, 2020). In *M. truncatula*, comparison of GSNOR1 and GSNOR2 expression between control and stress conditions

reveals up-regulation of the GSNOR1 transcript, which encodes the main GSNOR isoform, during the recovery from drought in roots and shoots (Fig. 4). Therefore, the restoration of SNO balance may be a key feature of legume tolerance to drought. Another mechanism for regulation of GSNOR activity is the PTM of the protein. Inhibition of *A. thaliana* GSNOR activity by S-nitrosylation led to transient accumulation of SNOs and transmission of the NO signal (Guerra *et al.*, 2016). It was subsequently demonstrated that under hypoxia, besides the inhibition of its enzymatic activity, S-nitrosylation induces the selective autophagy of GSNOR due to conformational changes that expose an interaction motif recognized by the autophagy machinery (Zhan *et al.*, 2018). Very recently, PTMs including S-glutathionylation, and probably Cys oxidation and persulfidation by H₂S, were detected in *L. japonicus* GSNOR1 and GSNOR2 recombinant proteins (Matamoros *et al.*, 2020). In addition, GSNOR seems to be central in the crosstalk between Ca²⁺- and NO-mediated signaling under stress conditions. A model was proposed in *A. thaliana* in which salt stress induces the entry of Ca²⁺ into the cell and the formation of the Ca²⁺/calmodulin complex. This interacts directly with GSNOR and inhibits its activity, thereby stimulating SNO accumulation and salt tolerance (Zhou *et al.*, 2016). All these results strongly suggest that plant GSNORs are regulatory hubs that integrate signals mediated by H₂O₂, NO, H₂S, and Ca²⁺, and that they are therefore critical for developmental and stress responses. In addition to GSNOR, mechanisms should exist in plants to keep SNO amounts of proteins under control by denitrosylation. Recently, it has been demonstrated in *A. thaliana* that Trxh5 catalyses denitrosylation by acting as a selective protein-SNO reductase (Kneeshaw *et al.*, 2014). Interestingly, Trxh5 (after reduction by NADPH-dependent Trx reductase) decreases excessive protein-SNO amounts, which reinstates signaling by salicylic acid and modulates plant immunity.

Phytoglobins (Glbs; formerly non-symbiotic hemoglobins) are another family of plant proteins that may regulate intracellular NO concentrations, and thereby influence the level of S-nitrosylation. Based on their structural characteristics and phylogeny, Glbs are categorized into three classes (for recent reviews see Mira *et al.*, 2016; Becana *et al.*, 2020). Class 1 Glbs display extremely high O₂ affinities, which makes them unsuitable for O₂ transport and delivery. They are involved in NO scavenging, probably through their NO dioxygenase (NO + O₂ → NO₃⁻) activity. Transgenic *A. thaliana* plants overexpressing *AtGlb1* are more tolerant to hypoxia (Hunt *et al.*, 2002), whereas salt, drought, and cold treatments up-regulate several *Glb* genes in rice (*Oryza sativa*; Shankar *et al.*, 2018). Class 1 Glbs may regulate programmed cell death in certain stress responses (Mira *et al.*, 2016). Plants of *A. thaliana* overexpressing rice *Glb1-2* show increased tolerance to potassium deficiency and lower ROS content, which led the authors to suggest that the corresponding protein might be involved in signaling responses to low nutrients stress (Shankar *et al.*, 2018). However, there is

less information on the other two classes of Glbs. Class 2 Glbs have O₂ affinities similar to those of symbiotic hemoglobins, and the transcript of the single class 2 Glb of *A. thaliana* accumulates in response to cold treatment (Trevaskis *et al.*, 1997). Class 3 Glbs have not been studied in detail in plants. However, their participation in the stress response is likely, based on the NO-related functions of their bacterial homologs (Vieweg *et al.*, 2005).

The functions of Glbs have also been studied in legumes. In *L. japonicus*, the expression of *LjGlb1-1* and the production of NO are concomitantly and transiently induced in roots after 4 h of inoculation with the compatible symbiotic rhizobial species *Mesorhizobium loti*, whereas pathogenic bacteria induced a sustained NO burst triggering the plant's defense response (Shimoda *et al.*, 2005; Nagata *et al.*, 2008). *LjGlb1-1* is mainly expressed in nodules and strongly induced by hypoxia, NO, and cold stress, whereas another class 1 Glb gene, *LjGlb1-2*, is mainly expressed in leaves and induced by sucrose (Shimoda *et al.*, 2005). Overexpression of *LjGlb1-1* in hairy roots increased nodule number and N₂ fixing activity, which was attributed to a higher capacity of nodules to scavenge NO and protect nitrogenase (Shimoda *et al.*, 2009). Stable lines overexpressing *LjGlb1-1* also had reduced NO amounts and enhanced N₂ fixation in mature and senescent nodules (Fukudome *et al.*, 2019a). These lines are more tolerant to flooding, which may be linked to lowered amounts of NO and ROS, compared with the wild-type under these stressful conditions (Fukudome *et al.*, 2019b). In *M. truncatula*, expression of *MtGlb1-1* is also transiently up-regulated after infection with its rhizobial partner, and this induction is thought to decrease NO concentrations in roots, thereby allowing the onset of symbiosis (Berger *et al.*, 2020). These authors also showed that *MtGlb1-1* modulates NO concentration throughout nodule development, controlling nodulation and N₂ fixation. Some Glbs are transcriptionally regulated in response to stress conditions in *L. japonicus* and *M. truncatula*. *LjGlb1-1* expression is down-regulated in response to salinity in the shoot (Fig. 3; Sanchez *et al.*, 2008), but up-regulated in water-stressed nodules (Fig. 5; Saňko-Sawczenko *et al.*, 2019). Also, *MtGlb1-1* expression is down-regulated by drought in roots and shoots, whereas *MtGlb3-1* is induced under salt or drought stress in roots, shoots, and nodules (Li *et al.*, 2009; Saňko-Sawczenko *et al.*, 2019). Remarkably, one *Lb* transcript was induced in the roots of *M. truncatula* in response to salt and re-irrigation following drought (Fig. 4). Further research is warranted to establish the role of Glbs in the stress responses of legumes and, in particular, of the legume-rhizobia symbiosis (for a recent review on this subject, see Larrainzar *et al.*, 2020).

S-glutathionylation

S-glutathionylation is the reversible addition of glutathione to a protein via the formation of a disulfide bond with a Cys thiol (Fig. 2). This PTM occurs in response to increases

in ROS and NO, and protects thiols from irreversible oxidation. It may also lead to structural and functional changes in the target protein that regulates signal transduction and metabolic pathways (Zaffagnini *et al.*, 2012). Whereas this modification may occur via non-enzymatic mechanisms, deglutathionylation is usually carried out by Grxs. In humans, deregulation of S-glutathionylation has been implicated in a number of pathologies, including cancer, cardiovascular disease, and diabetes (Zhang *et al.*, 2018). Much less information is available in plants. Dixon *et al.* (2005) demonstrated that S-glutathionylation occurs in response to oxidative conditions in suspension cultures of *A. thaliana*. In the same species, the modification of the catalytic Cys of glycolytic glyceraldehyde-3-phosphate dehydrogenase protects the enzyme from irreversible oxidation. However, the persistence of the glutathionylated state alters protein structure and causes the formation of insoluble aggregates (Zaffagnini *et al.*, 2019). A recent study has shown the effect of S-glutathionylation on the protein structure and activity of pea recombinant chloroplastic 2-cysteine Prx (Prx2C) and mitochondrial PrxIIF (Calderón *et al.*, 2017). The modification caused inhibition of the peroxidase activity of the two proteins. However, S-glutathionylation provoked specific alterations of the protein structures. It induced the conversion of Prx2C from decamer to dimer, whereas it did not change the oligomerization state of PrxIIF. Interestingly, sulfiredoxin was able to deglutathionylate Prx2C but not PrxIIF, suggesting a role for this protein in the deglutathionylation of specific enzymes. The physiological implications of these discoveries are not clear yet, but it is possible that S-glutathionylation of proteins increases under abiotic stresses that perturb the cellular redox state. This PTM may protect the protein from irreversible oxidative deactivation and/or regulate its activity as part of signaling events that control cell metabolism. Grx-mediated deglutathionylation might have a role in the stress response. As discussed above, in *M. truncatula*, several *Grx* transcripts are induced in response to salt or drought conditions (Figs. 4, 5). One of these transcripts (Medtr3g077570) is homologous to poplar (*Populus tremula* × *tremuloides*) *PtGrxC1*, which encodes a protein that shows deglutathionylating activity *in vitro* (Bedhomme *et al.*, 2012). S-glutathionylation might also act as a redox signal during the onset and functioning of the rhizobium-legume symbiosis. This is suggested by the observations that one Grx (*SmGrx1*) of *S. meliloti* displays deglutathionylation activity and that the corresponding bacterial mutant strain has an impaired symbiotic phenotype (Benyamina *et al.*, 2013). To our knowledge, there is no information about the occurrence of this PTM in nodules.

Persulfidation

The signaling molecule H₂S plays an important role in many physiological and pathological processes in plants and

animals (Aroca *et al.*, 2018). A mechanism of H₂S signaling is the conversion of the thiol group (R-SH) of reactive Cys residues into a perthiol (R-SSH, also called a persulfide), in a process known as persulfidation. In solution, H₂S may exist as deprotonated (H₂S), monoanion (HS⁻), and dianion (S²⁻) forms. Potential mechanisms for persulfidation include the nucleophilic attack of HS⁻ on oxidized protein thiols such as sulfenic acids, disulfide bridges, and S-glutathionylated and S-nitrosylated Cys residues (Fig. 2; Mishanina *et al.*, 2015). This PTM may alter protein structure and function because of the decrease in the pK_a and the increase in nucleophilicity of the persulfide group (Mishanina *et al.*, 2015). It may also prevent irreversible Cys over-oxidation and preserve protein function (Zivanovic *et al.*, 2019). There is ample evidence that H₂S mediates abiotic stress responses (reviewed by Hancock, 2019). In *A. thaliana*, a mutation of the H₂S-producing enzyme L-cysteine desulhydrase 1 (DES1) leads to drought sensitivity and premature leaf senescence (Jin *et al.*, 2018). Studies with broad bean (*Vicia faba*) indicate that H₂S functions downstream of H₂O₂ in salt stress-induced stomatal movements (Ma *et al.*, 2019), and very recent work with *A. thaliana* has demonstrated the involvement of H₂S in ABA-induced stomatal closure (Shen *et al.*, 2020). The latter authors proposed the following model: drought causes ABA accumulation that, in turn, induces the expression of *DES1* in guard cells by an unknown mechanism; H₂S generated by *DES1* activity may cause the persulfidation of many downstream proteins, including *DES1* itself, thus amplifying the H₂S-mediated signal; persulfidation of AtRBOHD provokes a ROS burst that triggers stomatal closure; ABA signaling ceases when the high accumulation of ROS triggers the oxidation of persulfides of AtRBOHD and *DES1*; and oxidized persulfides can be reduced back to thiols by Trxs (Shen *et al.*, 2020).

Information on protein persulfidation and its possible functions in legume physiology is virtually non-existent. A proteomic study is underway in our laboratory with bean nodules at different stages of development, which will provide insights into the possible roles of this PTM in nodule biology. Preliminary results suggest that protein persulfidation is involved in the plant and nodule responses to abiotic stress and in the aging process. This is consistent with interesting results reported recently by Zivanovic *et al.* (2019). These authors used different experimental models, including HeLa cells, *Caenorhabditis elegans*, yeast, and mammals, to show that persulfidation is evolutionarily conserved and plays an integral role in protecting proteins from excessive oxidation. Moreover, they also established a correlation between increased persulfidation, resistance to oxidative stress, and lifespan extension.

Tyrosine nitration

This PTM consists in the covalent addition of a nitro group (-NO₂) to one of the two equivalent ortho carbons in the

aromatic ring of Tyr residues, to form 3-nitrotyrosine (NO₂-Tyr; Kolbert *et al.*, 2017). Tyr nitration requires the presence of peroxyxynitrite (ONOO⁻) or nitrogen dioxide (NO₂) because NO itself is not reactive enough (Fig. 2). Tyr nitration causes a decrease of the residue pK_a, enhances its hydrophobicity, and provokes steric restrictions because NO₂-Tyr is larger than Tyr. In plant cells, Tyr nitration generally leads to loss-of-function of the protein, although there are a few exceptions (Corpas *et al.*, 2013; Kolbert *et al.*, 2017). In pea, this PTM occurs throughout plant development in roots, stems, and leaves, and is increased in aging roots but not in aging leaves (Corpas *et al.*, 2013). The contrasting results between roots and leaves could be related to obvious physiological peculiarities or to differences in the developmental stages of the two organs. Moreover, abiotic stress conditions such as salinity, high light intensity, or low and high temperature increase nitration levels of specific proteins in pea and other plant species (Corpas *et al.*, 2013).

In nodules, Tyr nitration may have an important regulatory role because two key proteins for nodule functioning, GS and Lb, are targets of this PTM. MtGS1a is inactivated by Tyr nitration, and the amount of nitrated protein increases under conditions in which SNF is impaired (Melo *et al.*, 2011). As for Lb, the protein is susceptible to nitration in both the heme and globin. Heme nitration was demonstrated in senescing soybean nodules containing green Lbs. The modified proteins have identical globins to the unaltered red Lbs, but their hemes are nitrated in a vinyl group (Navascués *et al.*, 2012a). In the globin moiety, a Tyr residue located in the distal heme pocket is the major target of nitration (Sainz *et al.*, 2015). Interestingly, the amount of nitrated globin decreased during senescence, suggesting that heme and globin nitration occurs through different mechanisms and/or that globin nitration, but not heme nitration, makes the protein prone to degradation by nodule proteases, as observed with other plant proteins (Castillo *et al.*, 2015).

The significance of protein nitration in redox signaling is still poorly defined. There is evidence that Tyr nitration may interfere with the phosphorylation of the residue and thus regulate signal transduction pathways. Although this stable PTM was categorized as irreversible, recent research in animals has identified denitrase mechanisms that could be also operative in plants (Kolbert *et al.*, 2017).

Carbonylation and glycation

In cells, metal-catalysed oxidation occurs when free Fe²⁺ or Cu⁺ reacts with H₂O₂ and generates hydroxyl radicals (·OH) through the Fenton reaction (Halliwell, 2006). These radicals can irreversibly oxidize amino acid side chains and introduce the carbonyl moiety in proteins (Møller *et al.*, 2011). Carbonyl groups may also be generated indirectly by Michael addition of lipid peroxidation decomposition products to Arg, Cys, histidine, and Lys residues (Møller *et al.*, 2011; Matamoros *et al.*, 2018; Fig. 2). Protein carbonylation contributes to cellular

damage caused by stress conditions and age-associated diseases in animals (Höhn *et al.*, 2013). In *A. thaliana* and other plant species, increases in carbonylated proteins were observed in response to abiotic stress (Tanou *et al.*, 2009; Mano *et al.*, 2014). As for legumes, the amount of carbonylated proteins was higher in the leaves of peas grown in the presence of heavy metals (Romero-Puertas *et al.*, 2002). Some of the oxidized proteins were identified as Rubisco and antioxidant enzymes such as glutathione reductase, manganese superoxide dismutase, and catalase. The oxidized proteins were more sensitive to proteolytic degradation. In soybean, protein carbonylation was induced in plants exposed to high CO₂ and was associated with loss of leaf chlorophyll and reduced photosynthesis (Qiu *et al.*, 2008). Natural senescence (aging) also entails protein carbonylation both in leaves and nodules (Evans *et al.*, 1999; Hernández-Jiménez *et al.*, 2002; Vanacker *et al.*, 2006; Matamoros *et al.*, 2013). It is not clear, however, if irreversible protein carbonylation is only a deleterious consequence of stress conditions or aging or, alternatively, contributes to redox signaling and plant acclimation to stress (Winger *et al.*, 2007; Tanou *et al.*, 2012).

Protein glycation occurs when Arg and Lys residues react with reducing sugars, generating Amadori and Heyns compounds. These glycation products are readily oxidized, yielding relatively stable advanced glycation end products (AGEs). Alternatively, AGEs can be formed by the reaction of Arg and Lys residues with α -dicarbonyls (mainly glyoxal and methylglyoxal), generated by monosaccharide autooxidation under oxidative conditions (Fig. 2). In humans, the cross-link products between proteins and sugars seem to be major contributors to age-related chronic diseases (Höhn *et al.*, 2013). However, very little is known about protein glycation in plants. The *A. thaliana* proteome modified by AGEs, as well as the age-dependent increase of glycation at specific sites, have been reported (Bilova *et al.*, 2017). In this species, osmotic stress also augmented protein glycation (Paudel *et al.*, 2016), and constitutively glycated proteins and age- and drought stress-specific targets have been recently identified (Chaplin *et al.*, 2019). *In vitro* assays showed that glycation inhibited two enzyme activities involved in carbohydrate metabolism, thus demonstrating the impact of this PTM on protein function.

To our knowledge, there is only one large scale study on protein oxidation in legumes (Matamoros *et al.*, 2018). The study revealed that, in bean nodules, carbonylation occurs under normal growth conditions and throughout development, and that this PTM has major effects on two key nodule proteins, malate dehydrogenase and Lb (Fig. 1). Malate dehydrogenase is essential for SNF because malate is the primary source of carbon transported to the bacteroids. Its activity is negatively correlated to the amount of carbonylation. Carbonylation also induces Lb aggregation, probably rendering the protein inactive and more susceptible to degradation by cell proteases. Moreover, numerous glycated proteins have been identified *in*

in vivo, including 10 plant and 18 bacterial proteins that were age-specifically glycated.

Reactive aldehydes and ketones arising from lipid peroxide degradation are major contributors to protein carbonylation (Mano, 2012; Matamoros *et al.*, 2018). Gpxs and Prxs catalyse the reduction of lipid hydroperoxides and might therefore decrease the rate of carbonylation. Gene expression profiling of *M. truncatula* roots and shoots showed that salt or drought stress up-regulates several Gpx and Prx genes (Fig. 4; Li *et al.*, 2009). It has also been observed that overexpression of reactive carbonyl-scavenging enzymes such as 2-alkenal reductase, alkenal/one oxidoreductase, aldehyde dehydrogenase, aldehyde reductase, and tau class glutathione S-transferase confers stress tolerance (Kotchoni *et al.*, 2006; Yin *et al.*, 2010; Yamauchi *et al.*, 2012; Mano *et al.*, 2019). In *L. japonicus*, genes encoding glutathione S-transferase, 2-alkenal reductase, and aldehyde dehydrogenase isoforms were up-regulated in response to drought or salt stress (Fig. 3; Sanchez *et al.*, 2008; Díaz *et al.*, 2010). Similarly, in *M. truncatula*, the transcripts of several enzymes known to scavenge reactive carbonyl species accumulated in response to abiotic stress (Fig. 4; Li *et al.*, 2009). Thus, aldehyde dehydrogenase 7B4 (ALDH7B4) plays an important antioxidative role by eliminating surplus aldehydes in plants exposed to high temperatures in combination with drought, wounding, or salinity stress (Zhao *et al.*, 2017). In *M. truncatula*, expression of *MtALDH7B4* is up-regulated in the nodules by drought (Fig. 5) and expression of its homolog in *L. japonicus*, *LjALDH7B4*, is up-regulated in the shoot by salt stress (Fig. 3). These studies highlight the importance of these enzymes in the defense response of legumes.

On the other hand, in eukaryotic cells the glyoxalase (GLX) system detoxifies glyoxal and methylglyoxal, and thus avoids the accumulation of AGEs. The system involves two consecutive reactions catalysed by GLXI and GLXII. *A. thaliana* contains three *GLXI* and three *GLXII* genes (Schmitz *et al.*, 2017). However, in *M. truncatula* only one *GLXII* gene was up-regulated in response to drought conditions (Fig. 4), suggesting that either the glyoxalase system is not regulated under stress conditions or that it relies on post-transcriptional control for its activation.

Conclusions and perspectives

In the last few decades, genomic and transcriptomic studies have provided a solid base to understand how plants tolerate and adapt to abiotic stress. As a next step, the thorough analysis of the stress-responsive proteome will allow further elucidation of the metabolic and signaling networks that determine the success of plants to thrive under adverse conditions. Nevertheless, a number of factors that define the proteome, including mRNA alternative splicing, protein subcellular location, protein-protein interactions, and PTMs,

add a further degree of complexity. As for PTMs, individual proteins may exist in several modified forms, each with different activity, stability, location, or capacity to interact with other proteins. For a given protein, the same PTM may have different effects depending on the modification site, and a specific PTM can have opposite effects on different proteins (activation or inhibition of enzyme activity). Moreover, several PTMs may coexist in the same protein, resulting in many possible combinations. To further complicate this picture, cellular redox homeostasis is probably regulated independently in each cell compartment, and PTMs may therefore vary with the subcellular location. From a technical viewpoint, redox PTMs are highly dynamic and labile and can be easily altered during sample manipulation if strict protocols are not followed. Although the sensitivity of analytical equipment has been greatly improved over the last few years, low abundant proteins whose modifications could be influential in the plant response, including transcription factors, key regulatory enzymes, and cell membrane receptors, are still generally overlooked.

To date, a large number of proteins are known to be post-translationally modified in response to different stimuli. But in most cases, the effect of these modifications on protein function has not been investigated. To better understand the role of ROS-, RNS- and RSS-mediated PTMs in the stress response, a number of issues need to be addressed. It will be necessary to understand the mechanisms that make PTMs selective toward a specific protein, quantify the percentage of protein molecules that contain a specific PTM, and establish the effect of individual PTMs, or the combination of several of them, on protein function, signaling pathways, and cell metabolism. It will also be required to determine organelle-specific PTMs and identify master proteins whose modification is highly influential. All this complexity often makes it necessary to carry out detailed case study approaches that can be integrated in a general picture of how redox-based protein regulation determines plant stress tolerance. In legumes, a deeper understanding of the molecular and physiological basis of their response to abiotic stress is crucial to improve plant growth and SNF, and thereby to secure food supply with a reduced input of fertilizers.

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Author contributions

MAM and MB wrote the manuscript. MAM performed the transcriptome database mining.

References

- Alkhalifioui F, Renard M, Frendo P, Keichinger C, Meyer Y, Gelhaye E, Hirasawa M, Knaff DB, Ritzenthaler C, Montrichard F.** 2008. A novel type of thioredoxin dedicated to symbiosis in legumes. *Plant Physiology* **148**, 424–435.
- Allouing G, Mandon K, Boncompagni E, Montrichard F, Frendo P.** 2018. Involvement of glutaredoxin and thioredoxin systems in the nitrogen-fixing symbiosis between legumes and rhizobia. *Antioxidants* **7**, article 182.
- Aranjuelo I, Arrese-Igor C, Molero G.** 2014. Nodule performance within a changing environmental context. *Journal of Plant Physiology* **171**, 1076–1090.
- Aroca A, Gotor C, Romero LC.** 2018. Hydrogen sulfide signaling in plants: emerging roles of protein persulfidation. *Frontiers in Plant Science* **9**, 1369.
- Arthikala MK, Montiel J, Sánchez-López R, Nava N, Cárdenas L, Quinto C.** 2017. Respiratory burst oxidase homolog gene A is crucial for *Rhizobium* infection and nodule maturation and function in common bean. *Frontiers in Plant Science* **8**, 2003.
- Astier J, Gross I, Durner J.** 2018. Nitric oxide production in plants: an update. *Journal of Experimental Botany* **69**, 3401–3411.
- Baig MA, Ahmad J, Bagheri R, Ali AA, Al-Huqail AA, Ibrahim MM, Qureshi MI.** 2018. Proteomic and ecophysiological responses of soybean (*Glycine max* L.) root nodules to Pb and Hg stress. *BMC Plant Biology* **18**, 283.
- Becana M, Klucas RV.** 1992. Oxidation and reduction of leghemoglobin in root nodules of leguminous plants. *Plant Physiology* **98**, 1217–1221.
- Becana M, Matamoros MA, Udvardi M, Dalton DA.** 2010. Recent insights into antioxidant defenses of legume root nodules. *New Phytologist* **188**, 960–976.
- Becana M, Yruela I, Sarath G, Catalán P, Hargrove MS.** 2020. Plant hemoglobins: a journey from unicellular green algae to vascular plants. *New Phytologist* **227**, 1618–1635.
- Bedhomme M, Adamo M, Marchand CH, Couturier J, Rouhier N, Lemaire SD, Zaffagnini M, Trost P.** 2012. Glutathionylation of cytosolic glyceraldehyde-3-phosphate dehydrogenase from the model plant *Arabidopsis thaliana* is reversed by both glutaredoxins and thioredoxins *in vitro*. *The Biochemical Journal* **445**, 337–347.
- Begara-Morales JC, Chaki M, Valderrama R, Mata-Pérez C, Padilla MN, Barroso JB.** 2019. The function of S-nitrosothiols during abiotic stress in plants. *Journal of Experimental Botany* **70**, 4429–4439.
- Benyamina SM, Baldacci-Cresp F, Couturier J, et al.** 2013. Two *Sinorhizobium meliloti* glutaredoxins regulate iron metabolism and symbiotic bacteroid differentiation. *Environmental Microbiology* **15**, 795–810.
- Berger A, Guinand S, Boscarri A, Puppo A, Brouquisse R.** 2020. *Medicago truncatula* phytohemoglobin 1.1 controls symbiotic nodulation and nitrogen fixation via the regulation of nitric oxide concentration. *New Phytologist* **227**, 84–98.
- Bilova T, Paudel G, Shilyaev N, et al.** 2017. Global proteomic analysis of advanced glycation end products in the *Arabidopsis* proteome provides evidence for age-related glycation hot spots. *The Journal of Biological Chemistry* **292**, 15758–15776.
- Calderón A, Lázaro-Payo A, Iglesias-Baena I, Camejo D, Lázaro JJ, Sevilla F, Jiménez A.** 2017. Glutathionylation of pea chloroplast 2-Cys Prx and mitochondrial Prx IIF affects their structure and peroxidase activity and sulfiredoxin deglutathionylates only the 2-Cys Prx. *Frontiers in Plant Science* **8**, 118.
- Calvo-Begueria L, Rubio MC, Martínez JI, Pérez-Rontomé C, Delgado MJ, Bedmar EJ, Becana M.** 2018. Redefining nitric oxide production in legume nodules through complementary insights from electron

- paramagnetic resonance spectroscopy and specific fluorescent probes. *Journal of Experimental Botany* **69**, 3703–3714.
- Camejo D, Ortiz-Espín A, Lázaro JJ, Romero-Puertas MC, Lázaro-Payo A, Sevilla F, Jiménez A.** 2015. Functional and structural changes in plant mitochondrial PrxII F caused by NO. *Journal of Proteomics* **119**, 112–125.
- Camejo D, Romero-Puertas MC, Rodríguez-Serrano M, Sandalio LM, Lázaro JJ, Jiménez A, Sevilla F.** 2013. Salinity-induced changes in S-nitrosylation of pea mitochondrial proteins. *Journal of Proteomics* **79**, 87–99.
- Castella C, Mirtziou I, Seassau A, Boscarì A, Montrichard F, Papadopoulou K, Rouhier N, Puppo A, Brouquisse R.** 2017. Post-translational modifications of *Medicago truncatula* glutathione peroxidase 1 induced by nitric oxide. *Nitric oxide* **68**, 125–136.
- Castillo MC, Lozano-Juste J, González-Guzmán M, Rodríguez L, Rodríguez PL, León J.** 2015. Inactivation of PYR/PYL/RCAR ABA receptors by tyrosine nitration may enable rapid inhibition of ABA signaling by nitric oxide in plants. *Science Signaling* **8**, ra89.
- Chaplin AK, Chernukhin I, Bechtold U.** 2019. Profiling of advanced glycation end products uncovers abiotic stress-specific target proteins in *Arabidopsis*. *Journal of Experimental Botany* **70**, 653–670.
- Choudhury FK, Rivero RM, Blumwald E, Mittler R.** 2017. Reactive oxygen species, abiotic stress and stress combination. *The Plant Journal* **90**, 856–867.
- Corpas FJ, Chaki M, Fernández-Ocaña A, Valderrama R, Palma JM, Carreras A, Begara-Morales JC, Airaki M, del Río LA, Barroso JB.** 2008. Metabolism of reactive nitrogen species in pea plants under abiotic stress conditions. *Plant and Cell Physiology* **49**, 1711–1722.
- Corpas FJ, Palma JM, Del Río LA, Barroso JB.** 2013. Protein tyrosine nitration in higher plants grown under natural and stress conditions. *Frontiers in Plant Science* **4**, 29.
- Dam S, Dyrland TF, Ussatjuk A, et al.** 2014. Proteome reference maps of the *Lotus japonicus* nodule and root. *Proteomics* **14**, 230–240.
- Dhanushkodi R, Matthew C, McManus MT, Dijkwel PP.** 2018. Drought-induced senescence of *Medicago truncatula* nodules involves serpin and ferritin to control proteolytic activity and iron levels. *New Phytologist* **220**, 196–208.
- Díaz P, Betti M, Sánchez DH, Udvardi MK, Monza J, Márquez AJ.** 2010. Deficiency in plastidic glutamine synthetase alters prolin metabolism and transcriptomic response in *Lotus japonicus* under drought stress. *New Phytologist* **188**, 1001–1013.
- Dixon DP, Skipsey M, Grundy NM, Edwards R.** 2005. Stress-induced protein S-glutathionylation in *Arabidopsis*. *Plant Physiology* **138**, 2233–2244.
- Erisman JW, Galloway J, Seitzinger S, Bleeker A, Butterbach-Bahl K.** 2011. Reactive nitrogen in the environment and its effect on climate change. *Current Opinion in Environmental Sustainability* **3**, 281–290.
- Evans PJ, Gallesi D, Mathieu C, Hernandez MJ, de Felipe M, Halliwell B, Puppo A.** 1999. Oxidative stress occurs during soybean nodule senescence. *Planta* **208**, 73–79.
- Feechan A, Kwon E, Yun BW, Wang Y, Pallas JA, Loake GJ.** 2005. A central role for S-nitrosothiols in plant disease resistance. *Proceedings of the National Academy of Sciences, USA* **102**, 8054–8059.
- Friso G, van Wijk KJ.** 2015. Posttranslational protein modifications in plant metabolism. *Plant Physiology* **169**, 1469–1487.
- Fukudome M, Watanabe E, Osuki KI, Imaizumi R, Aoki T, Becana M, Uchiumi T.** 2019a. Stably transformed *Lotus japonicus* plants overexpressing phytoalbumin LjGlb1-1 show decreased nitric oxide levels in roots and nodules as well as delayed nodule senescence. *Plant and Cell Physiology* **60**, 816–825.
- Fukudome M, Watanabe E, Osuki KI, Uchi N, Uchiumi T.** 2019b. Ectopic or over-expression of class 1 phytoalbumin genes confers flooding tolerance to the root nodules of *Lotus japonicus* by scavenging nitric oxide. *Antioxidants* **8**, 206.
- Go YM, Chandler JD, Jones DP.** 2015. The cysteine proteome. *Free Radical Biology and Medicine* **84**, 227–245.
- Gresshoff PM, Hayashi S, Biswas B, et al.** 2015. The value of biodiversity in legume symbiotic nitrogen fixation and nodulation for biofuel and food production. *Journal of Plant Physiology* **172**, 128–136.
- Guerra D, Ballard K, Truebridge I, Vierling E.** 2016. S-Nitrosation of conserved cysteines modulates activity and stability of S-nitrosogluthione reductase (GSNOR). *Biochemistry* **55**, 2452–2464.
- Gupta KJ, Kolbert Z, Durner J, et al.** 2020. Regulating the regulator: nitric oxide control of post-translational modifications. *New Phytologist* **227**, 1319–1325.
- Halliwell B.** 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiology* **141**, 312–322.
- Hancock JT.** 2019. Hydrogen sulfide and environmental stresses. *Environmental and Experimental Botany* **161**, 50–56.
- Hardin SC, Larue CT, Oh MH, Jain V, Huber SC.** 2009. Coupling oxidative signals to protein phosphorylation via methionine oxidation in *Arabidopsis*. *The Biochemical Journal* **422**, 305–312.
- He J, Benedito VA, Wang M, Murray JD, Zhao PX, Tang Y, Udvardi MK.** 2009. The *Medicago truncatula* gene expression atlas web server. *BMC Bioinformatics* **10**, 441.
- Hernández-Jiménez MJ, Lucas MM, de Felipe MR.** 2002. Antioxidant defence and damage in senescing lupin nodules. *Plant Physiology and Biochemistry* **40**, 645–657.
- Hichri I, Boscarì A, Castella C, Rovere M, Puppo A, Brouquisse R.** 2015. Nitric oxide: a multifaceted regulator of the nitrogen-fixing symbiosis. *Journal of Experimental Botany* **66**, 2877–2887.
- Höhn A, König J, Grune T.** 2013. Protein oxidation in aging and the removal of oxidized proteins. *Journal of Proteomics* **92**, 132–159.
- Horchani F, Prévot M, Boscarì A, et al.** 2011. Both plant and bacterial nitrate reductases contribute to nitric oxide production in *Medicago truncatula* nitrogen-fixing nodules. *Plant Physiology* **155**, 1023–1036.
- Huang J, Willems P, Wei B, et al.** 2019. Mining for protein S-sulfonylation in *Arabidopsis* uncovers redox-sensitive sites. *Proceedings of the National Academy of Sciences, USA* **116**, 21256–21261.
- 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, USA* **99**, 17197–17202.
- Jebara S, Jebara M, Limam F, Aouani ME.** 2005. Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. *Journal of Plant Physiology* **162**, 929–936.
- Jin Z, Sun L, Yang G, Pei Y.** 2018. Hydrogen sulfide regulates energy production to delay leaf senescence induced by drought stress in *Arabidopsis*. *Frontiers in Plant Science* **9**, 1722.
- Kav NNV, Srivastava S, Goonewardene L, Blade SF.** 2004. Proteome-level changes in the roots of *Pisum sativum* in response to salinity. *Annals of Applied Biology* **145**, 217–230.
- Kneeshaw S, Gelineau S, Tada Y, Loake GJ, Spoel SH.** 2014. Selective protein denitrosylation activity of thioredoxin-*h5* modulates plant immunity. *Molecular Cell* **56**, 153–162.
- Kolbert Z, Feigl G, Bordé Á, Molnár Á, Erdei L.** 2017. Protein tyrosine nitration in plants: Present knowledge, computational prediction and future perspectives. *Plant Physiology and Biochemistry* **113**, 56–63.
- Kollist H, Zandalinas SI, Sengupta S, Nuhkat M, Kangasjärvi J, Mittler R.** 2019. Rapid responses to abiotic stress: priming the landscape for the signal transduction network. *Trends in Plant Science* **24**, 25–37.
- Kosová K, Vítámvás P, Urban MO, Prášil IT, Renaut J.** 2018. Plant abiotic stress proteomics: the major factors determining alterations in cellular proteome. *Frontiers in Plant Science* **9**, 122.
- Kotchoni SO, Kuhns C, Ditzer A, Kirch HH, Bartels D.** 2006. Over-expression of different aldehyde dehydrogenase genes in *Arabidopsis thaliana* confers tolerance to abiotic stress and protects plants against lipid peroxidation and oxidative stress. *Plant, Cell and Environment* **29**, 1033–1048.

- Kwon E, Feechan A, Yun BW, Hwang BH, Pallas JA, Kang JG, Loake GJ.** 2012. *AtGSNOR1* function is required for multiple developmental programs in *Arabidopsis*. *Planta* **236**, 887–900.
- Ladrera R, Marino D, Larrainzar E, González EM, Arrese-Igor C.** 2007. Reduced carbon availability to bacteroids and elevated ureides in nodules, but not in shoots, are involved in the nitrogen fixation response to early drought in soybean. *Plant Physiology* **145**, 539–546.
- Lamers J, van der Meer T, Testerink C.** 2020. How plants sense and respond to stressful environments. *Plant Physiology* **182**, 1624–1635.
- Lamotte O, Bertoldo JB, Besson-Bard A, Rosnoblet C, Aimé S, Hichami S, Terenzi H, Wendehenne D.** 2014. Protein S-nitrosylation: specificity and identification strategies in plants. *Frontiers in Chemistry* **2**, 114.
- Larrainzar E, Molenaar JA, Wienkoop S, Gil-Quintana E, Alibert B, Limami AM, Arrese-Igor C, González EM.** 2014. Drought stress provokes the down-regulation of methionine and ethylene biosynthesis pathways in *Medicago truncatula* roots and nodules. *Plant, Cell and Environment* **37**, 2051–2063.
- Larrainzar E, Villar I, Rubio MC, Pérez-Rontomé C, Huertas R, Sato S, Mun JH, Becana M.** 2020. Hemoglobins in the legume-*Rhizobium* symbiosis. *New Phytologist* **228**, 472–484.
- Larrainzar E, Wienkoop S, Scherling C, Kempa S, Ladrera R, Arrese-Igor C, Weckwerth W, González EM.** 2009. Carbon metabolism and bacteroid functioning are involved in the regulation of nitrogen fixation in *Medicago truncatula* under drought and recovery. *Molecular Plant-Microbe Interactions* **22**, 1565–1576.
- Lee U, Wie C, Fernandez BO, Feelisch M, Vierling E.** 2008. Modulation of nitrosative stress by S-nitrosoglutathione reductase is critical for thermotolerance and plant growth in *Arabidopsis*. *The Plant Cell* **20**, 786–802.
- Li D, Su Z, Dong J, Wang T.** 2009. An expression database for roots of the model legume *Medicago truncatula* under salt stress. *BMC Genomics* **10**, 517.
- Lindermayr C.** 2018. Crosstalk between reactive oxygen species and nitric oxide in plants: Key role of S-nitrosoglutathione reductase. *Free Radical Biology and Medicine* **122**, 110–115.
- López M, Herrera-Cervera JA, Iribarne C, Tejera NA, Lluch C.** 2008. Growth and nitrogen fixation in *Lotus japonicus* and *Medicago truncatula* under NaCl stress: nodule carbon metabolism. *Journal of Plant Physiology* **165**, 641–650.
- Ma H, Song L, Shu Y, Wang S, Niu J, Wang Z, Yu T, Gu W, Ma H.** 2012. Comparative proteomic analysis of seedling leaves of different salt tolerant soybean genotypes. *Journal of Proteomics* **75**, 1529–1546.
- Ma Y, Zhang W, Niu J, Ren Y, Zhang F.** 2019. Hydrogen sulfide may function downstream of hydrogen peroxide in salt stress-induced stomatal closure in *Vicia faba*. *Functional Plant Biology* **46**, 136–145.
- Mano J.** 2012. Reactive carbonyl species: their production from lipid peroxides, action in environmental stress, and the detoxification mechanism. *Plant Physiology and Biochemistry* **59**, 90–97.
- Mano J, Kanameda S, Kuramitsu R, Matsuura N, Yamauchi Y.** 2019. Detoxification of reactive carbonyl species by glutathione transferase tau isozymes. *Frontiers in Plant Science* **10**, 487.
- Mano J, Nagata M, Okamura S, Shiraya T, Mitsui T.** 2014. Identification of oxidatively modified proteins in salt-stressed *Arabidopsis*: a carbonyl-targeted proteomics approach. *Plant and Cell Physiology* **55**, 1233–1244.
- Marino D, Damiani I, Gucciardo S, Mijangos I, Pauly N, Puppo A.** 2013. Inhibition of nitrogen fixation in symbiotic *Medicago truncatula* upon Cd exposure is a local process involving leghaemoglobin. *Journal of Experimental Botany* **64**, 5651–5660.
- Marino D, Dunand C, Puppo A, Pauly N.** 2012. A burst of plant NADPH oxidases. *Trends in Plant Science* **17**, 9–15.
- Marino D, Frendo P, Ladrera R, Zabalza A, Puppo A, Arrese-Igor C, González EM.** 2007. Nitrogen fixation control under drought stress. Localized or systemic? *Plant Physiology* **143**, 1968–1974.
- Marquez-García B, Shaw D, Cooper JW, Karpinska B, Quain MD, Makgopa EM, Kunert K, Foyer CH.** 2015. Redox markers for drought-induced nodule senescence, a process occurring after drought-induced senescence of the lowest leaves in soybean (*Glycine max*). *Annals of Botany* **116**, 497–510.
- Martí MC, Florez-Sarasa I, Camejo D, Ribas-Carbó M, Lázaro JJ, Sevilla F, Jiménez A.** 2011. Response of mitochondrial thioredoxin PsTrxo1, antioxidant enzymes, and respiration to salinity in pea (*Pisum sativum* L.) leaves. *Journal of Experimental Botany* **62**, 3863–3874.
- Martínez-Ruiz A, Lamas S.** 2004. S-nitrosylation: a potential new paradigm in signal transduction. *Cardiovascular Research* **62**, 43–52.
- Marx H, Minogue CE, Jayaraman D, et al.** 2016. A proteomic atlas of the legume *Medicago truncatula* and its nitrogen-fixing endosymbiont *Sinorhizobium meliloti*. *Nature Biotechnology* **34**, 1198–1205.
- Matamoros MA, Cutrona MC, Wienkoop S, Begara-Morales JC, Sandal N, Orera I, Barroso JB, Stougaard J, Becana M.** 2020. Altered plant and nodule development and protein S-nitrosylation in *Lotus japonicus* mutants deficient in S-nitrosoglutathione reductases. *Plant and Cell Physiology* **61**, 105–117.
- Matamoros MA, Fernández-García N, Wienkoop S, Loscos J, Saiz A, Becana M.** 2013. Mitochondria are an early target of oxidative modifications in senescing legume nodules. *New Phytologist* **197**, 873–885.
- Matamoros MA, Kim A, Peñuelas M, Ihling C, Griesser E, Hoffmann R, Fedorova M, Frolov A, Becana M.** 2018. Protein carbonylation and glycation in legume nodules. *Plant Physiology* **177**, 1510–1528.
- Matamoros MA, Saiz A, Peñuelas M, et al.** 2015. Function of glutathione peroxidases in legume root nodules. *Journal of Experimental Botany* **66**, 2979–2990.
- Meakin GE, Bueno E, Jepson B, Bedmar EJ, Richardson DJ, Delgado MJ.** 2007. The contribution of bacteroidal nitrate and nitrite reduction to the formation of nitrosylleghaemoglobin complexes in soybean root nodules. *Microbiology* **153**, 411–419.
- Melo PM, Silva LS, Ribeiro I, Seabra AR, Carvalho HG.** 2011. Glutamine synthetase is a molecular target of nitric oxide in root nodules of *Medicago truncatula* and is regulated by tyrosine nitration. *Plant Physiology* **157**, 1505–1517.
- Meyer Y, Buchanan BB, Vignols F, Reichheld JP.** 2009. Thioredoxins and glutaredoxins: unifying elements in redox biology. *Annual Review of Genetics* **43**, 335–367.
- Mira M, Hill RD, Stasolla C.** 2016. Regulation of programmed cell death by phytohemoglobins. *Journal of Experimental Botany* **67**, 5901–5908.
- Mishanina TV, Libiad M, Banerjee R.** 2015. Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways. *Nature Chemical Biology* **11**, 457–464.
- Mittler R.** 2017. ROS are good. *Trends in Plant Science* **22**, 11–19.
- Møller IM, Rogowska-Wrzęsinska A, Rao RS.** 2011. Protein carbonylation and metal-catalyzed protein oxidation in a cellular perspective. *Journal of Proteomics* **74**, 2228–2242.
- Mun T, Bachmann A, Gupta V, Stougaard J, Andersen SU.** 2016. Lotus Base: An integrated information portal for the model legume *Lotus japonicus*. *Scientific Reports* **6**, 39447.
- Nagata M, Murakami E, Shimoda Y, Shimoda-Sasakura F, Kucho K, Suzuki A, Abe M, Higashi S, Uchiyama T.** 2008. Expression of a class 1 hemoglobin gene and production of nitric oxide in response to symbiotic and pathogenic bacteria in *Lotus japonicus*. *Molecular Plant-Microbe Interactions* **21**, 1175–1183.
- Nasr Esfahani M, Sulieman S, Schulze J, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS.** 2014. Mechanisms of physiological adjustment of N₂ fixation in *Cicer arietinum* L. (chickpea) during early stages of water deficit: single or multi-factor controls. *The Plant Journal* **79**, 964–980.
- Navasqués J, Pérez-Rontomé C, Gay M, Marcos M, Yang F, Walker FA, Desbois A, Abián J, Becana M.** 2012a. Leghemoglobin green derivatives with nitrated hemes evidence production of highly reactive nitrogen species during aging of legume nodules. *Proceedings of the National Academy of Sciences, USA* **109**, 2660–2665.

- Navascués J, Pérez-Rontomé C, Sánchez DH, Staudinger C, Wienkoop S, Rellán-Álvarez R, Becana M. 2012b. Oxidative stress is a consequence, not a cause, of aluminum toxicity in the forage legume *Lotus corniculatus*. *New Phytologist* **193**, 625–636.
- Naya L, Ladrera R, Ramos J, González EM, Arrese-Igor C, Minchin FR, Becana M. 2007. The response of carbon metabolism and antioxidant defenses of alfalfa nodules to drought stress and to the subsequent recovery of plants. *Plant Physiology* **144**, 1104–1114.
- Noreen Z, Ashraf M. 2009. Assessment of variation in antioxidative defense system in salt-treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. *Journal of Plant Physiology* **166**, 1764–1774.
- Oger E, Marino D, Guignonis JM, Pauly N, Puppo A. 2012. Sulfenylated proteins in the *Medicago truncatula*-*Sinorhizobium melliloti* symbiosis. *Journal of Proteomics* **75**, 4102–4113.
- Oldroyd GE. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology* **11**, 252–263.
- Oldroyd GE, Murray JD, Poole PS, Downie JA. 2011. The rules of engagement in the legume-rhizobial symbiosis. *Annual Review of Genetics* **45**, 119–144.
- Ortega-Galisteo AP, Rodríguez-Serrano M, Pazmiño DM, Gupta DK, Sandalio LM, Romero-Puertas MC. 2012. S-Nitrosylated proteins in pea (*Pisum sativum* L.) leaf peroxisomes: changes under abiotic stress. *Journal of Experimental Botany* **63**, 2089–2103.
- Paudel G, Bilova T, Schmidt R, et al. 2016. Osmotic stress is accompanied by protein glycation in *Arabidopsis thaliana*. *Journal of Experimental Botany* **67**, 6283–6295.
- Popp C, Ott T. 2011. Regulation of signal transduction and bacterial infection during root nodule symbiosis. *Current Opinion in Plant Biology* **14**, 458–467.
- Puppo A, Pauly N, Boscari A, Mandon K, Brouquisse R. 2013. Hydrogen peroxide and nitric oxide: key regulators of the legume-*Rhizobium* and mycorrhizal symbioses. *Antioxidants and Redox Signaling* **18**, 2202–2219.
- Qiu QS, Huber JL, Booker FL, Jain V, Leakey AD, Fiscus EL, Yau PM, Ort DR, Huber SC. 2008. Increased protein carbonylation in leaves of *Arabidopsis* and soybean in response to elevated [CO₂]. *Photosynthesis Research* **97**, 155–166.
- Rathi D, Gayen D, Gayali S, Chakraborty S, Chakraborty N. 2016. Legume proteomics: Progress, prospects, and challenges. *Proteomics* **16**, 310–327.
- Rodríguez-Serrano M, Romero-Puertas MC, Pazmiño DM, Testillano PS, Risueño MC, del Río LA, Sandalio LM. 2009. Cellular response of pea plants to cadmium toxicity: cross talk between reactive oxygen species, nitric oxide, and calcium. *Plant Physiology* **150**, 229–243.
- Romero-Puertas MC, Palma JM, Gómez M, del Río LA, Sandalio LM. 2002. Cadmium causes the oxidative modification of proteins in pea plants. *Plant, Cell and Environment* **25**, 677–686.
- Roy S, Liu W, Nandety RS, Crook A, Mysore KS, Pislariu CI, Frugoli J, Dickstein R, Udvardi MK. 2020. Celebrating 20 years of genetic discoveries in legume nodulation and symbiotic nitrogen fixation. *The Plant Cell* **32**, 15–41.
- Rubio MC, Bustos-Sanmamed P, Clemente MR, Becana M. 2009. Effects of salt stress on the expression of antioxidant genes and proteins in the model legume *Lotus japonicus*. *New Phytologist* **181**, 851–859.
- Sainz M, Calvo-Begueria L, Pérez-Rontomé C, Wienkoop S, Abián J, Staudinger C, Bartsaghi S, Radi R, Becana M. 2015. Leghemoglobin is nitrated in functional legume nodules in a tyrosine residue within the heme cavity by a nitrite/peroxide-dependent mechanism. *The Plant Journal* **81**, 723–735.
- Sanchez DH, Lippold F, Redestig H, Hannah MA, Erban A, Krämer U, Kopka J, Udvardi MK. 2008. Integrative functional genomics of salt acclimatization in the model legume *Lotus japonicus*. *The Plant Journal* **53**, 973–987.
- Sańko-Sawczenko I, Łtocka B, Mielecki J, Rekosz-Burlaga H, Czarnocka W. 2019. Transcriptomic changes in *Medicago truncatula* and *Lotus japonicus* root nodules during drought stress. *International Journal of Molecular Sciences* **20**, 1204.
- Santos R, Hérouart D, Sigaud S, Touati D, Puppo A. 2001. Oxidative burst in alfalfa-*Sinorhizobium melliloti* symbiotic interaction. *Molecular Plant-Microbe Interactions* **14**, 86–89.
- Schmidhuber J, Tubiello FN. 2007. Global food security under climate change. *Proceedings of the National Academy of Sciences, USA* **104**, 19703–19708.
- Schmitz J, Dittmar IC, Brockmann JD, Schmidt M, Hüdig M, Rossoni AW, Maurino VG. 2017. Defense against reactive carbonyl species involves at least three subcellular compartments where individual components of the system respond to cellular sugar status. *The Plant Cell* **29**, 3234–3254.
- Sevilla F, Camejo D, Ortiz-Espín A, Calderón A, Lázaro JJ, Jiménez A. 2015. The thioredoxin/peroxiredoxin/sulfiredoxin system: current overview on its redox function in plants and regulation by reactive oxygen and nitrogen species. *Journal of Experimental Botany* **66**, 2945–2955.
- Shankar A, Fernandes JL, Kaur K, Sharma M, Kundu S, Pandey GK. 2018. Rice phytooglobins regulate responses under low mineral nutrients and abiotic stresses in *Arabidopsis thaliana*. *Plant, Cell and Environment* **41**, 215–230.
- Shen J, Zhang J, Zhou M, et al. 2020. Persulfidation-based modification of cysteine desulfhydrase and the NADPH oxidase RBOHD controls guard cell abscisic acid signaling. *The Plant Cell* **32**, 1000–1017.
- Shimoda Y, Nagata M, Suzuki A, Abe M, Sato S, Kato T, Tabata S, Higashi S, Uchiumi T. 2005. Symbiotic *Rhizobium* and nitric oxide induce gene expression of non-symbiotic hemoglobin in *Lotus japonicus*. *Plant and Cell Physiology* **46**, 99–107.
- Shimoda Y, Shimoda-Sasakura F, Kucho K, Kanamori N, Nagata M, Suzuki A, Abe M, Higashi S, Uchiumi T. 2009. Overexpression of class 1 plant hemoglobin genes enhances symbiotic nitrogen fixation activity between *Mesorhizobium loti* and *Lotus japonicus*. *The Plant Journal* **57**, 254–263.
- Snijder J, Rose RJ, Raijmakers R, Heck AJ. 2011. Site-specific methionine oxidation in calmodulin affects structural integrity and interaction with Ca²⁺/calmodulin-dependent protein kinase II. *Journal of Structural Biology* **174**, 187–195.
- Stadtman ER, Van Remmen H, Richardson A, Wehr NB, Levine RL. 2005. Methionine oxidation and aging. *Biochimica et Biophysica Acta* **1703**, 135–140.
- Subba P, Kumar R, Gayali S, Shekhar S, Parveen S, Pandey A, Datta A, Chakraborty S, Chakraborty N. 2013. Characterisation of the nuclear proteome of a dehydration-sensitive cultivar of chickpea and comparative proteomic analysis with a tolerant cultivar. *Proteomics* **13**, 1973–1992.
- Tada Y, Spoel SH, Pajeroska-Mukhtar K, Mou Z, Song J, Wang C, Zuo J, Dong X. 2008. Plant immunity requires conformational changes of NPR1 via S-nitrosylation and thioredoxins. *Science* **321**, 952–956.
- Tanou G, Filippou P, Belghazi M, Job D, Diamantidis G, Fotopoulos V, Molassiotis A. 2012. Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. *The Plant Journal* **72**, 585–599.
- Tanou G, Job C, Rajjou L, Arc E, Belghazi M, Diamantidis G, Molassiotis A, Job D. 2009. Proteomics reveals the overlapping roles of hydrogen peroxide and nitric oxide in the acclimation of citrus plants to salinity. *The Plant Journal* **60**, 795–804.
- Tarrago L, Laugier E, Rey P. 2009. Protein-repairing methionine sulfoxide reductases in photosynthetic organisms: gene organization, reduction mechanisms, and physiological roles. *Molecular Plant* **2**, 202–217.
- Tovar-Méndez A, Matamoros MA, Bustos-Sanmamed P, Dietz KJ, Cejudo FJ, Rouhier N, Sato S, Tabata S, Becana M. 2011. Peroxiredoxins and NADPH-dependent thioredoxin systems in the model legume *Lotus japonicus*. *Plant Physiology* **156**, 1535–1547.

- 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, USA* **94**, 12230–12234.
- Udvardi M, Poole PS.** 2013. Transport and metabolism in legume-rhizobia symbioses. *Annual Review of Plant Biology* **64**, 781–805.
- Umbreen S, Lubega J, Cui B, Pan Q, Jiang J, Loake GJ.** 2018. Specificity in nitric oxide signalling. *Journal of Experimental Botany* **69**, 3439–3448.
- Vanacker H, Sandalio L, Jiménez A, et al.** 2006. Roles for redox regulation in leaf senescence of pea plants grown on different sources of nitrogen nutrition. *Journal of Experimental Botany* **57**, 1735–1745.
- Vessal S, Siddique KH, Atkins CA.** 2012. Comparative proteomic analysis of genotypic variation in germination and early seedling growth of chickpea under suboptimal soil-water conditions. *Journal of Proteome Research* **11**, 4289–4307.
- Vieweg MF, Hohnjec N, Küster H.** 2005. Two genes encoding different truncated hemoglobins are regulated during root nodule and arbuscular mycorrhiza symbioses of *Medicago truncatula*. *Planta* **220**, 757–766.
- Wang P, Du Y, Hou YJ, Zhao Y, Hsu CC, Yuan F, Zhu X, Tao WA, Song CP, Zhu JK.** 2015. Nitric oxide negatively regulates abscisic acid signaling in guard cells by S-nitrosylation of OST1. *Proceedings of the National Academy of Sciences, USA* **112**, 613–618.
- Winger AM, Taylor NL, Heazlewood JL, Day DA, Millar AH.** 2007. The cytotoxic lipid peroxidation product 4-hydroxy-2-nonenal covalently modifies a selective range of proteins linked to respiratory function in plant mitochondria. *The Journal of Biological Chemistry* **282**, 37436–37447.
- Wu F, Chi Y, Jiang Z, et al.** 2020. Hydrogen peroxide sensor HPCA1 is an LRR receptor kinase in *Arabidopsis*. *Nature* **578**, 577–581.
- Yamauchi Y, Hasegawa A, Mizutani M, Sugimoto Y.** 2012. Chloroplastic NADPH-dependent alkenal/one oxidoreductase contributes to the detoxification of reactive carbonyls produced under oxidative stress. *FEBS Letters* **586**, 1208–1213.
- Yin L, Mano J, Wang S, Tsuji W, Tanaka K.** 2010. The involvement of lipid peroxide-derived aldehydes in aluminum toxicity of tobacco roots. *Plant Physiology* **152**, 1406–1417.
- Zaffagnini M, Bedhomme M, Lemaire SD, Trost P.** 2012. The emerging roles of protein glutathionylation in chloroplasts. *Plant Science* **185–186**, 86–96.
- Zaffagnini M, Marchand CH, Malferrari M, et al.** 2019. Glutathionylation primes soluble glyceraldehyde-3-phosphate dehydrogenase for late collapse into insoluble aggregates. *Proceedings of the National Academy of Sciences, USA* **116**, 26057–26065.
- 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.
- Zhang J, Ye ZW, Singh S, Townsend DM, Tew KD.** 2018. An evolving understanding of the S-glutathionylation cycle in pathways of redox regulation. *Free Radical Biology and Medicine* **120**, 204–216.
- Zhao J, Missihoun TD, Bartels D.** 2017. The role of *Arabidopsis* aldehyde dehydrogenase genes in response to high temperature and stress combinations. *Journal of Experimental Botany* **68**, 4295–4308.
- Zhou S, Jia L, Chu H, Wu D, Peng X, Liu X, Zhang J, Zhao J, Chen K, Zhao L.** 2016. *Arabidopsis* CaM1 and CaM4 promote nitric oxide production and salt resistance by inhibiting S-nitrosoglutathione reductase via direct binding. *PLoS Genetics* **12**, e1006255.
- Zivanovic J, Kouroussis E, Kohl JB, et al.** 2019. Selective persulfide detection reveals evolutionarily conserved antiaging effects of S-sulfhydration. *Cell Metabolism* **30**, 1152–1170.e13.
- Zou H, Zhang NN, Lin XY, Zhang WQ, Zhang JH, Chen J, Wei GH.** 2020. Hydrogen sulfide is a crucial element of the antioxidant defense system in *Glycine max*-*Sinorhizobium fredii* symbiotic root nodules. *Plant Soil* **449**, 209–231.
- Zou H, Zhang NN, Pan Q, Zhang JH, Chen J, Wei GH.** 2019. Hydrogen sulfide promotes nodulation and nitrogen fixation in soybean-rhizobia symbiotic system. *Molecular Plant-Microbe Interactions* **32**, 972–985.