We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



8

The Legume Root Nodule: From Symbiotic Nitrogen Fixation to Senescence

Laurence Dupont, Geneviève Alloing, Olivier Pierre,
Sarra El Msehli, Julie Hopkins, Didier Hérouart and Pierre Frendo
[1]*UMR "Biotic Interactions and Plant Health" INRA 1301-CNRS 6243*
University of Nice-Sophia Antipolis, F-06903 Sophia-Antipolis Cedex,
[2]*Laboratory of Plant Physiology, Science University, Tunis,*
[1]*France*
[2]*Tunisia*

1. Introduction

Biological nitrogen fixation (BNF) is the biological process by which the atmospheric nitrogen (N_2) is converted to ammonia by an enzyme called nitrogenase. It is the major source of the biosphere nitrogen and as such has an important ecological and agronomical role, accounting for 65 % of the nitrogen used in agriculture worldwide. The most important source of fixed nitrogen is the symbiotic association between rhizobia and legumes. The nitrogen fixation is achieved by bacteria inside the cells of *de novo* formed organs, the nodules, which usually develop on roots, and more occasionally on stems. This mutualistic relationship is beneficial for both partners, the plant supplying dicarboxylic acids as a carbon source to bacteria and receiving, in return, ammonium. Legume symbioses have an important role in environment-friendly agriculture. They allow plants to grow on nitrogen poor soils and reduce the need for nitrogen inputs for leguminous crops, and thus soil pollution. Nitrogen-fixing legumes also contribute to nitrogen enrichment of the soil and have been used from Antiquity as crop-rotation species to improve soil fertility. They produce high protein-containing leaves and seeds, and legumes such as soybeans, groundnuts, peas, beans, lentils, alfalfa and clover are a major source of protein for human and animal consumption. Most research concentrates on the two legume-rhizobium model systems *Lotus-Mesorhizobium loti* and *Medicago-Sinorhizobium meliloti,* with another focus on the economically-important *Glycine max* (soybean) -*Bradyrhizobium japonicum* association. The legume genetic models *Medicago truncatula* and *Lotus japonicus* have a small genome size of *ca*. 450 Mbp while *Glycine max* has a genome size of 1,115 Mbp, and all are currently targets of large-scale *genome sequencing* projects (He et al., 2009; Sato et al., 2008; Schmutz et al., 2010). The complete genome sequence of their bacterial partners has been established (Galibert et al., 2001; Kaneko et al., 2000; Kaneko et al., 2002; Schneiker-Bekel et al., 2011).

1.1 Early interaction and nodule development

Symbiotic interaction begins with the infection process, which is initiated by a reciprocal exchange of signals between plant and the compatible bacteria. Aromatic compounds -

mostly flavonoids - are secreted by the plants into the rhizosphere and activate the bacterial NodD proteins that are members of the LysR family of transcriptional activators, which in turn induce the expression of the *nod* genes (Long, 2001). This results in the secretion by the bacteria of lipo-chitin oligosaccharide molecules called Nod factors, which are recognized by epidermal cells via specific receptor kinases containing extracellular LysM domains. The spectrum of flavonoids exuded by a legume, as well as the strain-specific chemical structures of the Nod factors, are primary determinants of host specificity (Broughton et al., 2000). Additional bacterial components such as exopolysaccharides, type III and type IV secretion systems are also required for an effective infection (Perret et al., 2000; Saeki, 2011).

Nod factor perception initiates a complex signalling pathway essential for bacterial invasion of the host plant and formation of the nodule. Nod factor signal transduction requires a calcium signalling pathway, which includes the activation of a calcium and calmodulin dependent protein kinase in response to nuclear calcium oscillations. The ensuing induction of gene expression results in rearrangements of the root hair cytoskeleton and initiation of bacterial infection at the epidermis. The root hairs curl and trap the rhizobia which enter the root hair through tubular structures called infection threads. Simultaneously Nod factors induce root cortex cells dedifferentiation and division, leading to the formation of nodule primordia which then differentiate into N_2-fixing nodules (Crespi & Frugier, 2008; Oldroyd et al. 2011). The growing infection threads traverse the root epidermis and cortex, penetrate primordial cells, and then invading bacteria are released into the host cells by an endocytosis-like mechanism (Ivanov et al., 2010). Each bacterium is surrounded by a plant cell membrane, the peribacteroid membrane (PBM), the whole forming an organelle-like structure called the symbiosome where bacteria differentiate into nitrogen-fixing bacteroids. These symbiosomes ultimately completely fill the cytoplasm of infected cells. As the bacteria differentiate, infected cells undergo enlargement coupled to repeated endoreduplication cycles - genomic DNA replication without mitosis or cytokinesis - and become large polyploid cells housing thousands of bacteroids (Jones et al., 2007; Kondorosi et al., 2000). Mature nodules actively fix nitrogen until they enter senescence upon aging or stress.

Nodule organogenesis is accompanied by major changes in plant gene expression. Several hundred of genes were found to be strongly and specifically up- or -down regulated during the nodulation process (Benedito et al., 2008; El Yahyaoui et al., 2004). In *M. truncatula*, two distinct waves of gene expression reprogramming accompany the differentiation of both the plant infected cell and bacteroids (Maunoury et al., 2010). Genes exclusively expressed or strongly up-regulated in nodules have been termed “nodulins”. The early nodulins are involved in signal transduction and nodule development and the late nodulins are induced when N_2 fixation begins. Different expression profiling tools relying on genome and high-throughput EST-sequencing have been developed to identify nodulin genes on a large scale (Kuster et al., 2007; Schauser et al., 2008).

Nodules can be classified into two main groups according to their mode of development (Franssen et al., 1992; Maunoury et al., 2008) (Figure 1). Legumes such as *Phaseolus vulgaris* (bean), *Lotus japonicus* or *Glycine max* (soybean) form determinate nodules that have no permanent meristem and adopt a globular shape. The mature nodules contain a homogenous central tissue composed of infected cells fully packed with nitrogen-fixing bacteroids and some uninfected cells. Senescence in these nodules occurs radially, beginning at the center and extending to the periphery. Decaying nodules release bacteroids most of

which are able to revert to a free-living lifestyle. Conversely, legumes such as *Medicago truncatula*, *Pisum sativum* (pea) or *Trifolium* (clover) form indeterminate nodules that possess a permanent meristem and elongate, to become cylindrical. In mature nodule of this type, several histological zones of consecutive developmental states can be distinguished (Vasse et al., 1990). The apical meristem, free of bacteria, is the zone I. Zone II is the infection zone in which post-mitotic cells enter the nodule differentiation programme and where infection threads penetrate the plant cells and release rhizobia. In zone III, the bacteroids are able to fix N_2. A root proximal senescence zone (zone IV) can be observed in older nodules, where the bacteroids, together with the plant cells, are degraded. Upon aging this zone gradually extends to reach the apical part and the nodule degenerates. Proximal to the zone IV is a region (zone V) containing undifferentiated bacteria, which appear to proliferate in the decaying plant tissue (Timmers et al., 2000). In contrast to bacteroids housed in determinate nodules, those from undeterminate nodules have lost their capacity to reproduce. Thus at the end of symbiosis, essentially bacteria that are released from infection threads can return to a free-living lifestyle and recolonize the rhizosphere (Mergaert et al., 2006).

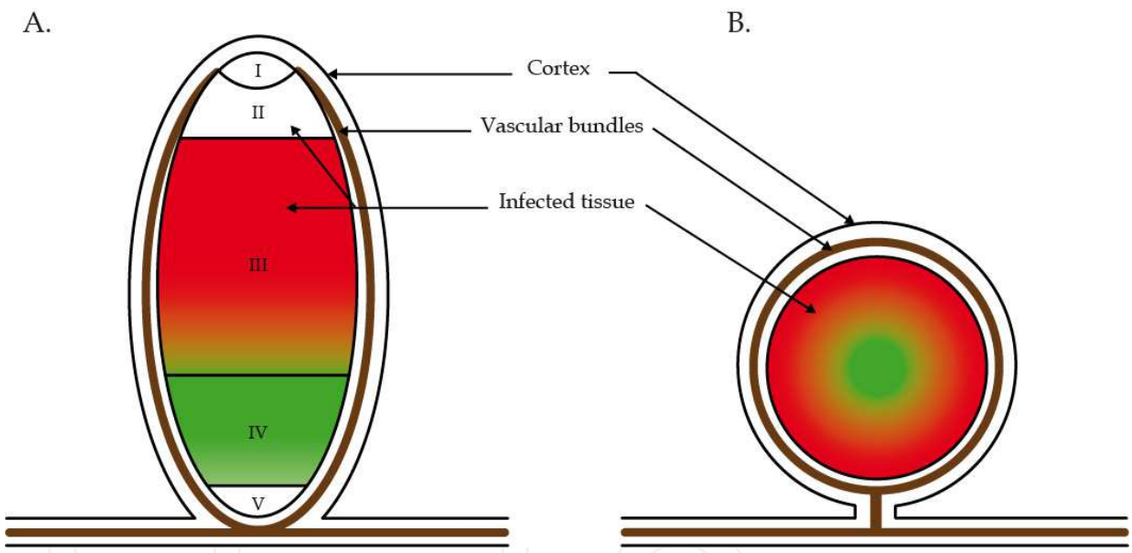


Fig. 1. Schematic representation of indeterminate (A) and determinate mature nodules (B) I, meristemic zone ; II, infection zone ; III, nitrogen fixing zone ; IV, senescence zone ; V, saprophytic zone.

Nitrogen fixing bacteroids in determinate and indeterminate nodules originate from distinct differentiation processes. Bacteroids in legume species forming determinate type nodules present the same cell size, genomic DNA content and reproductive capacity as the free-living bacteria. Conversely, differentiation of bacteroids in indeterminate nodules is linked to drastic morphological and cytological changes, such as cell elongation coupled to genome amplification, membrane permeabilisation and loss of reproductive capacity (Mergaert et al., 2006). This terminal differentiation is mediated by plant-host factors identified as the nodule-specific cysteine-rich (NCR) peptides (Kereszt et al., 2011; Van de Velde et al., 2010). In *M. truncatula* the NCR gene family encodes more than 300 different peptides, which resemble defensine-type antimicrobial peptides (AMPs) (Mergaert et al., 2003).

1.2 Nodule functioning

Bacteria that have completed the bacteroid differentiation program express the enzymes of the nitrogenase complex and begin to fix nitrogen. The reduction by nitrogenase of 1 molecule of N_2 to 2 molecules of NH_4^+ requires 16 molecules of ATP and 8 electrons (Jones et al., 2007). Thus, bacteroids require high rate flux of O_2 to enable high rates of ATP synthesis, but this must be achieved whilst maintaining a very low concentration of free O_2 to avoid inactivation of O_2-labile nitrogenase. These conditions exist due to the presence of an O_2 diffusion barrier and the synthesis of nodule-specific leghemoglobins, which accumulate to millimolar concentrations in the cytoplasm of infected cells prior to nitrogen fixation and buffer the free O_2 concentration at around 7-11 nM, while maintaining high O_2 flux for respiration (Appleby, 1984; Downie, 2005; Ott et al., 2005). The unique low-O_2 environment provided for the bacteroid is a key signal in bacteroid metabolism, inducing a regulatory cascade controlling gene expression of the nitrogenase complex and the microaerobic respiratory enzymes of the bacteroid. The O_2-sensing two-component regulatory system FixL-FixJ activates the transcription of the two intermediate regulators *nifA* and *fixK* genes, which induce the expression of *nif* and *fix* genes involved in nitrogen fixation and respiration (Reyrat et al., 1993). More generally, bacteroid differentiation is accompanied by a global change in gene expression compared with free-living bacteria. There is down-regulation of many genes such as most housekeeping genes and genes involved in synthesis of membrane proteins and peptidoglycan in favour of symbiosis specific processes (Becker et al., 2004; Bobik et al., 2006; Capela et al., 2006; Karunakaran et al., 2009; Pessi et al., 2007).

The reduction of N_2 to ammonium is accompanied, in bacteroids, by the switching-off of ammonium assimilation into amino acids. Ammonium is secreted to the plant cytosol, for assimilation into the amides glutamine and asparagine or into ureides. In return, the plant provides carbon and energy sources to bacteroids in the form of dicarboxylic acids, particularly malate and succinate, which are produced from sucrose via sucrose synthase and glycolytic enzymes. Their metabolization by the TCA cycle provides bacteroids with reducing equivalents, ATP and metabolites for amino acid synthesis and other biosynthetic pathways (White et al., 2007; Lodwig & Poole, 2003). Pea bacteroids also depend on plant for branched-chain amino-acid (LIV) supply, as the bacteroids become symbiotic auxotrophs for these amino-acids (Prell et al., 2009).

The nodule functioning has many peculiarities, involving a plant-microbe crosstalk associated to a metabolism which needs a high energy level under micro-oxic conditions. Nodule development and senescence also have specific features. Whereas multiple review articles have described the early steps of nodule formation and functioning, the rupture of the interaction has not been reviewed recently. In this context, this review focuses on the different characteristics of root nodule senescence.

2. Developmental senescence

For many years, the majority of the research concerning N-fixing symbioses focused on understanding the mechanisms leading to the establishment of this symbiotic relationship, from the invasion of plant cells to the N-fixing bacteroid state. In all nodule types, the N_2-fixation period is optimal between 4 and 5 weeks after infection. Beyond this period, first

reductions of N_2-fixing bacteroid capacity are detectable and a senescence process occurs in the N -fixing nodule zone. This phenomena is related to the onset of pod filling in grain legumes like soybean, pea and common bean (Bethlenfalvay & Phillips, 1977; Lawn & Brun, 1977). Thus, the lifespan of the rhizobia-plant symbiotic relationship is relatively short and the disruption of this symbiosis affects the yield of the culture.

2.1 Structural analysis of developmental senescence

The spatial dynamics of the senescence process in nodules is nodule type dependent. The pink N_2-fixing tissues of the zone III become green in color in zone IV due to leghemoglobin breakdown (Lehtovaara & Perttila, 1978). In determinate nodules, histological analyses of cross-sections using this simple visible change revealed that senescence develops radially, starting from the center and gradually spreading toward the outside (Puppo et al., 2005). In contrast, in undeterminate nodules, histological analyses of longitudinal sections of nodules based on pink-to-green color changes or based on the expression pattern of bacteroid genes involved in the N_2-fixing process using promoter-lacZ fusions (i.e. NifH) have led many authors to consider the front of senescence as a planar structure (Puppo et al., 2005). Recently, using toluidine blue staining to discriminate senescent from healthy cells and studying the gene expression pattern of a small family of plant cysteine proteases as early markers of nodule senescence on serial transversal sections of *M. truncatula* nodules, Pérez Guerra and collaborators proposed a conical organization of the developmental senescence zone: the earliest signs of senescence in a few infected cells in the center of the N-fixing zone occurred similar to determined nodules, and this phenomena progressively extended toward the nodule periphery in subsequent proximal cell layers of the nodule (Pérez Guerra et al., 2010).

Comparison between N_2-fixing and senescent cells in soybean nodule showed a decrease of density of plant cytoplasm, the apparition of vesicles associated with the deterioration of symbiosomes and modifications in organelles like peroxisomes, mitochondria and plastids (Lucas et al., 1998; Puppo et al., 2005). Ultrastructural analysis of *M. truncatula* mature nodule cells has revealed at least two stages during the developmental senescence of N_2-fixing cells: first, a disintegration of bacteroids and symbiosomes revealed by the presence of numerous membranes in the plant cytoplasm associated with the formation of lytic symbiosome compartments probably involved in reabsorption processes and second, the decay of plant infected cells associated with collapse phenomena and that of the plant uninfected cells (Perez Guerra et al., 2010; Timmers et al., 2000; Van de Velde et al., 2006; Vasse et al., 1990). The fusion of symbiosomes to form lytic compartments resembles vacuole formation. Analysis of the relation between the symbiosome formation and the endocytic pathway showed that the lifespan of bacteria in individual symbiosomes compartments during the N_2-fixing stage is achieved by delaying the acquisition of vacuolar identity such as vacuolar SYP22 and VTI11 SNAREs (Limpens et al., 2009). The acquisition of vacuolar identity by symbiosomes upon senescence likely allows the delivery of newly formed proteases to facilitate nutrient remobilization and a sink-to-source transition. Indeed, nodule senescence is accompanied by increased plant proteolytic activities that might cause large-scale protein degradation in soybean (Malik et al., 1981), French bean (Pladys et al., 1991) and alfalfa (Pladys & Vance, 1993).

2.2 Physiological and biochemical modifications during developmental senescence

Developmental nodule senescence is a complex and programmed process which induces a decrease of N_2-fixing activity and leghemoglobin content, modifications in the nodule redox state components and an increase of proteolytic activity, ultimately leading to the death of infected cells.

Leghemoglobin (Lb), which has a fundamental role in nodule functioning, is an important physiological marker for following the progression of nodule senescence (Figure 2). Lb content progressively decreases with the onset of senescence. This diminution of Lb impacts not only general metabolism of nodule by decreasing the O_2 availability to bacteroids with a low free O_2 content but also by potentially releasing free iron to produce reactive oxygen species (ROS) via the Fenton reaction. Indeed, the auto-oxidation of the active form of Lb, ferro-Lb-O_2 (Lb-Fe^{2+}-O_2), is associated with superoxide anion ($O_2^{\cdot-}$) production (Fridovich, 1986; Puppo et al., 1981) and the degradation of the heme group of Lb by H_2O_2 likely allows the release of the catalytic Fe which enhances the production of OH• through the Fenton and the Haberweiss reactions (Becana & Klucas, 1992; Puppo & Halliwell, 1988). The importance of Lb in nodule ROS production has been shown in a transgenic *Lotus japonicus* line in which diminution of the Lb content is correlated with diminution of the H_2O_2 production (Gunther et al., 2007).

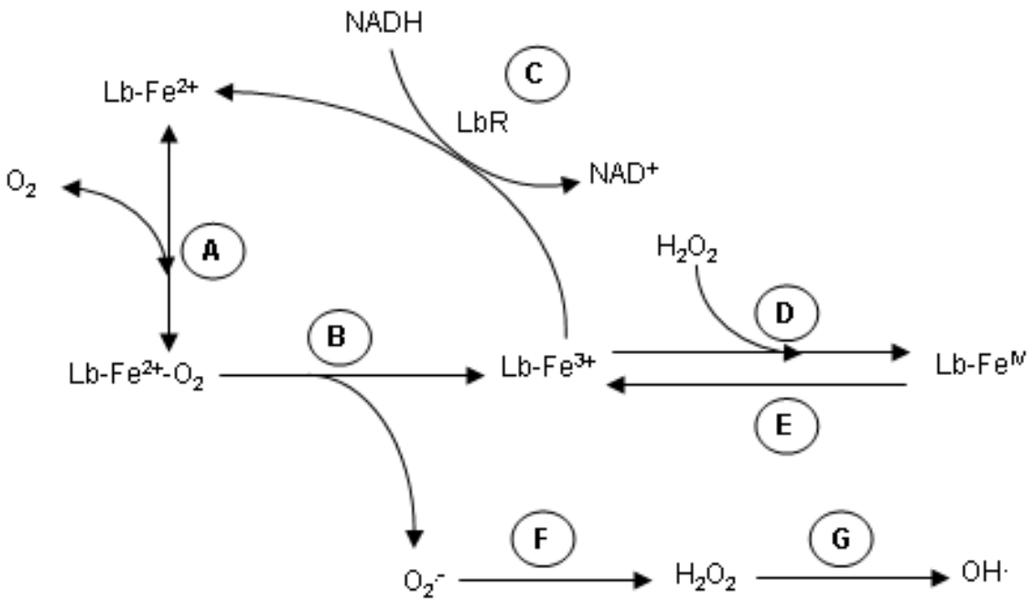


Fig. 2. Metabolic pathways involving Lb and formation of ROS.
A, Reversible oxygenation of Lb-Fe^{2+}; B, Autoxidation of Lb-Fe^{2+}-O_2 to Lb-Fe^{3+} with release of $O_2^{\cdot-}$; C, Lb-Fe^{3+} reduction by ferric Lb reductase (LbR); D, H_2O_2 reaction with Lb-Fe^{3+} to generate the inactive Lb-Fe^{IV} (ferryl) form; E, Lb-Fe^{IV} reduction to Lb-Fe^{3+} by ascorbate or thiols; other ROS can also be generated from $O_2^{\cdot-}$, by its dismutation to H_2O_2; F, and by H_2O_2 reduction to OH · through Fenton reaction (G).

Large modifications of the redox balance occur upon natural nodule senescence. Redox balance is defined by the equilibrium between the production of ROS and their degradation by the antioxidant defence system (Apel & Hirt, 2004). Ascorbate (Asc), homoglutathione (hGSH) and glutathione (GSH) are major antioxidants and redox buffers in plant nodule cells (Becana et al., 2010). The regulation of Asc and hGSH biosynthesis has been studied in common bean (*Phaseolus vulgaris*) nodules during aging (Loscos et al., 2008). The expression of five genes of the major Asc biosynthetic pathway was analyzed in nodules, and evidence was found that L-galactono-1,4-lactone dehydrogenase (GalLDH) , the last committed step of the pathway, is post-transcriptionally regulated. Large differences of Asc concentrations and redox states were observed in *P. vulgaris* nodules at different senescence stages suggesting that the lifespan of nodules is in part controlled by endogenous factors like Asc. Biochemical assays on alfalfa dissected nodules revealed that the senescent zone had lower GalLDH activity and ascorbate concentration compared to the infected zone (Matamoros et al., 2006). A strong positive correlation between N_2-fixing activity and nodule Asc and GSH contents was also observed during pea nodule development and senescence (Groten et al., 2005). Peroxiredoxins (Prx) have also been described in N_2-fixing nodules (Groten et al., 2006). Pea nodules contain at least two isoforms of Prx, located potentially in the cytosol (PrxIIB / C) and mitochondria (PrxIIF). The levels of PrxIIB /C declined with nodule senescence, but those of PrxIIF remained unaffected (Groten et al., 2006). The progressive decrease of antioxidant content during pea nodule senescence is not accompanied by an increase in ROS such as $O_2^{\cdot-}$ and H_2O_2 (Groten et al., 2005). In contrast, in aging soybean nodules, an oxidative stress has been detected including an increase of ROS, oxidized hGSH, catalytic Fe and oxidatively modified proteins and DNA bases, but no changes in Asc or tocopherol (Evans et al., 1999). The imbalance in redox state leading to oxidative stress induces the oxidation of lipids and proteins and the degradation of membranes. Lipid peroxidation was found to be elevated in senescent nodules of pigeon pea (*Cajanus cajan*) and bean (Loscos et al., 2008; Swaraj et al., 1995). In senescent soybean nodules, the presence of large amounts of H_2O_2 in the cytoplasmic and apoplastic compartments of the central infected tissue was detected and associated with a widespread expression of a cysteine protease gene (Alesandrini et al., 2003), suggesting a link between oxidative stress and proteolitic activities detected upon nodule senescence.

Various proteases, including those of the acid, serine, aspartic and cysteine types, have been isolated from senescing nodule tissue of soybean, alfalfa, French bean, and pea (Kardailsky & Brewin, 1996; Malik et al., 1981; Pfeiffer et al., 1983; Pladys & Vance, 1993; Pladys & Rigaud, 1985). The induction of cysteine protease genes during nodule senescence has been shown in soybean (Alesandrini et al., 2003), Chinese milk vetch (Naito et al., 2000), pea (Kardailsky & Brewin, 1996) and *M. truncatula* (Fedorova et al., 2002). The general transcriptomic analysis of senescent nodules in *M. truncatula* using cDNA-AFLP (Van de Velde et al., 2006) confirmed the predominant presence of genes encoding representatives of cysteine proteases that are highly homologous to one of the prominent markers of leaf senescence, *Sag12* (Lohman et al., 1994), indicating that these proteinases play an important role in the regulation of developmental nodule senescence. This hypothesis was confirmed in *Astragalus sinicus* since the silencing by RNA interference of the Asnodf32 gene, encoding a nodule-specific cysteine proteinase delayed root nodule senescence with a significant extention of the period of bacteroid active nitrogen fixation. Interestingly, elongated nodules were also observed on Asnodf32-silenced hairy roots (Li et al., 2008).

2.3 Transcriptomic analysis of developmental senescence

The onset of senescence involves the expression of genes whose products are required to carry out senescence-related processes (Gepstein, 2004). In order to isolate genes up- or down-regulated during nodule senescence, several genetic analyses including cDNA libraries and differential screening, mRNA differential display or cDNA-AFLP, have been performed in soybean (Alesandrini et al., 2003; Webb et al., 2008), and *M. truncatula* (Fedorova et al., 2002; Van de Velde et al., 2006). Using a mixture of effective nodules from 7 week-old plants, the first database specific to *M. truncatula* nodule senescence was obtained by isolating 140 000 Expressed Sequence Tags which are available in the J. Craig Venter Institute (http://www.jcvi.org/cms/research/groups/plant-genomics/resources/). To enrich plant material in senescent tissue, recent analyses in *M. truncatula* were performed from cross sections of nodules of 5 and 9 weeks by isolating the zones I, II and III from zones IV and V based on pink-to-green color changes (Van de Velde et al., 2006). This analysis using a modified cDNA-AFLP protocol has resulted in a collection of 508 gene tags that were expressed differentially. Functional clustering of these data has revealed a clear transition from carbon sink to nutriment source for the nodule by up-regulation of genes representative of several different proteases, genes involved in proteasome pathway and degradation of nucleic acids, membrane-derived lipids, and sugars. Moreover, this analysis suggests that three major hormones, ethylene, jasmonic acid and gibberellin, play an important role in nodule senescence (Van de Velde et al., 2006). From a more general point of view, it was been found that a significant overlap exists between genes expressed during leaf senescence in *Arabidopsis thaliana* and nodule senescence in *M. truncatula* (Van de Velde et al., 2006). However, more recent transcriptomic analysis of *M. truncatula* leaf senescence showed that only a minority of common genes are regulated during leaf and nodule senescence (De Michele et al., 2009).

2.4 Hormonal regulation of developmental senescence

Abscisic acid has been proposed to be an important signal in nodule senescence (Puppo et al., 2005), but no direct abscisic acid-responsive genes were present in the cDNA-AFLP dataset from *M. truncatula* nodules (Van de Velde et al., 2006). This analysis revealed that ethylene and jasmonic acid may play a positive role in nodule senescence, just as they do in the senescence of other plant tissues. The positive role of ethylene is illustrated by the up-regulation of ERF transcription factors and ethylene biosynthetic genes, such as S-adenosyl-Met (SAM) synthetase and 1-aminocyclopropane-1-carboxylate oxidase. Involvement of jasmonic acid is suggested by the induction of lipoxygenase genes during different stages of nodule senescence. Moreover, a strong induction of a gene coding for the GA 2-oxidase, that converts active gibberellins to inactive forms (Thomas et al., 1999), was observed in senescent nodule suggesting that gibberellins might repress the senescence process. Finally, the induction of genes encoding a SAM synthase and a spermidine synthethase suggests the involvement of polyamine biosynthetic pathways in nodule senescence. Concerning the potential implication of the two major hormones, auxin and cytokinin, in nodule senescence, only a small amount of data is available. In lupin (*Lupinus albus*), an elevated accumulation of the LaHK1 transcripts, encoding a cytokinin receptor homologue, was detected during nodule developmental senescence suggesting a putative role for this cytokinin receptor homologue in nodule senescence (Coba de la Pena et al., 2008).

3. Stress-induced senescence in legume root nodule

Legume BNF is particularly sensitive and perturbed by environmental stress conditions such as drought, salt stress, defoliation, continuous darkness and cold stress. Adverse environmental conditions affect nodule structure, impair nodule functioning and induce drastic metabolic and molecular modifications leading ultimately to a stress-induced senescence (SIS).

3.1 Stress induced senescence has typical features when compared to developmental senescence

As stated earlier, developmental induced senescence occurs typically in 5 to 11 week old nodules with a slow diminution of BNF during this time period (Evans et al., 1999; Puppo et al., 2005). In contrast, BNF declines drastically and quickly under environmental stress conditions. In less than a week, drought (Gonzalez et al., 1995; Larrainzar et al., 2007; Serraj et al., 1999), salt stress (Soussi et al., 1998; Swaraj and Bishnoi, 1999), dark stress (Matamoros et al., 1999; Gogorcena et al., 1997) and cold stress (van Heerden et al., 2008) decrease dramatically BNF. Thus, SIS is a much faster process than developmental senescence. Moreover, whereas developmental senescence is associated with the establishment of the nodule senescent zone which increases over time, SIS induces the degeneration of the whole nodule in a short time period (Matamoros et al., 1999; Perez Guerra et al., 2010; Vauclare et al., 2010). At the structural level, microscopic analyses also show that developmental senescence and dark stress-induced senescence present different features in *M. truncatula.* Dark-induced senescence leads to the condensation of the bacteroid content whereas the PBM remains intact even though most of the bacteroid content had disappeared (Perez Guerra et al., 2010). In contrast, developmental senescence induces a pronounced vesicle mobilisation in the host cytoplasm which is correlated with the degeneration of the PBM and the mixing of the symbiosome content with the cytoplasm (Perez Guerra et al., 2010; Van de Velde et al., 2006). However, structural analyses of the SIS have not been extensively performed on different nodule types for all the different environmental stress. Thus, it is possible that the different SIS do not develop similarly. Moreover, as for developmental senescence, SIS may process differently in determinate and indeterminate nodules. Indeed, nitrate induced senescence has been shown to induce bacteroid degradation in pea (indeterminate nodule) after two days of treatment in contrast to bean (determinate nodule), in which nitrate has little effect on the shape of bacteroids even after four days of 10mM nitrate treatment (Matamoros et al., 1999).

3.2 Stress induced senescence is characterized by modifications in nodule carbon metabolism and respiration

SIS is characterized by multiple early modifications of nodule physiology (Figure 2). Amongst them, modifications in carbon metabolism play a major role. As stated before, BNF is a highly energetic process which requires a constant energy supply. Modification of nodule sucrose content has been observed during drought stress (Galvez et al., 2005; Gordon et al., 1997), salt stress (Gordon et al., 1997; Lopez et al., 2008; Sanchez et al., 2011; Lopez et al., 2009; Ben Salah et al., 2009), dark stress (Gogorcena et al., 1997; Matamoros et al., 1999; Vauclare et al., 2010) and cold stress (Walsh & Layzell, 1986; van Heerden et al., 2008). However, whereas dark stress induces a diminution of sucrose concentration,

drought stress, salt stress and cold stress lead in general to its accumulation in nodules. The diminution of sucrose concentration linked to a shortage in photosynthate feeding result in a deficiency in energy production. In contrast, the accumulation of sucrose during stress suggests that nodule glycolytic enzymes are affected. Sucrose synthase, which is involved in the degradation of sucrose into glucose and fructose in the root nodule, is inhibited during drought stress and salt stress (Ben Salah et al., 2009; Gordon et al., 1997) and malate content, which is one of the preferred substrates for bacteroid respiration (Prell and Poole, 2006), decreases under these two stress conditions (Marino et al., 2007; Galvez et al., 2005; Ben Salah et al., 2009). A fine-tuning of O_2 concentration is also important for nodule functioning, since a good supply of O_2 is determinant for nodule respiration and energy requirement while a low O_2 pressure must be maintained in the nitrogen fixing zone to prevent nitrogenase inhibition. The availability of O_2 through the nodule diffusion barrier and the rate of nodule respiration are thus important parameters of the nodule fitness and they have been shown to be modified during various SIS such as drought stress (Del Castillo et al., 1994; Guerin et al., 1990; Naya et al., 2007; Serraj & Sinclair, 1996; Vessey et al., 1988), salt stress (Bekki et al., 1987; Serraj et al., 1994; Aydi et al., 2004; L'Taief et al., 2007), dark stress (Gogorcena et al., 1997) and cold stress (van Heerden et al., 2008; Kuzma et al., 1995).

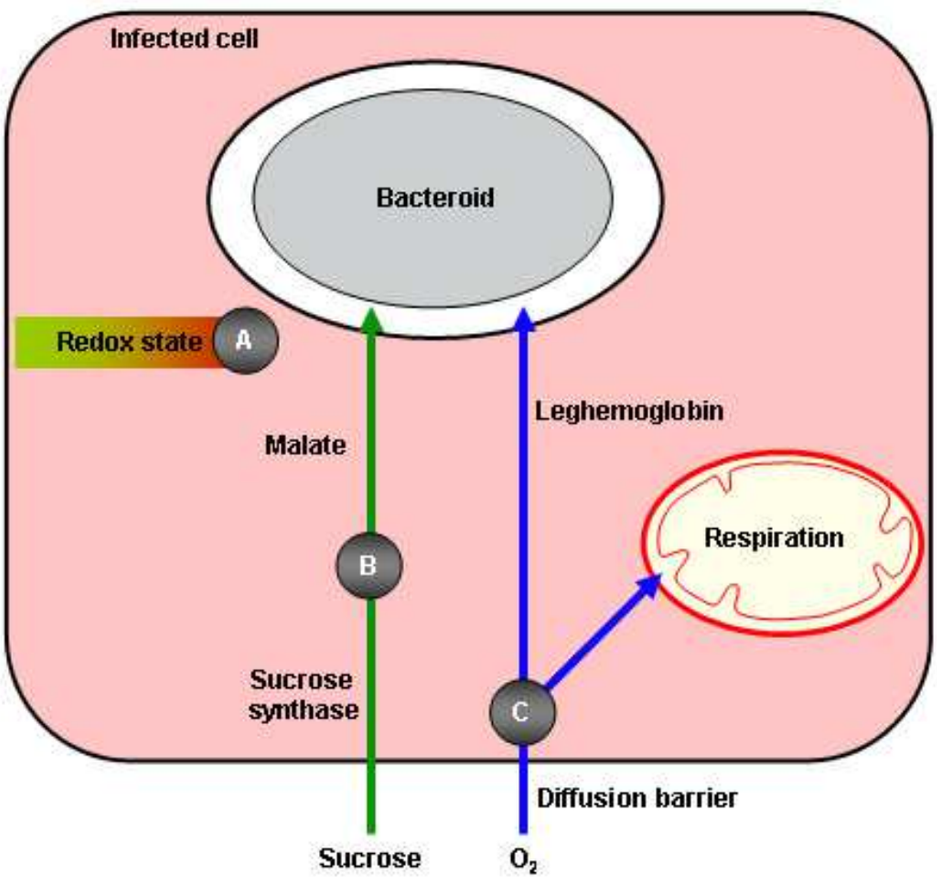


Fig. 3. Scheme showing the major processes modified during stress induced senescence. A, modification of the redox balance; B, alteration of the bacteroid nutrition; C, alteration of O_2 homeostasis.

However, nodule O_2 metabolic modifications are not always similar. Some types of stress decrease nodule permeability to O_2, lowering the O_2 availability to bacteroids, which in turn inhibits nitrogenase activity through a lower nodule respiration rate and lower energetic supply. In this context, a nitrate-nitric oxide respiration process has been identified in nodule which may play a role in the maintenance of energetic status under low oxygen conditions (Horchani et al., 2011). In contrast to stress which reduces oxygen availability, some stress increase nodule O_2 concentration (increased nodule permeability and/or lower respiration rate) which inhibits nitrogenase activity through a direct O_2-induced inactivation.

Leghemoglobin (Lb) is also a general physiological marker of SIS. As mentioned before, Lb has a crucial role in nodule functioning (Ott et al., 2009; Ott et al., 2005). Decrease of Lb content has been shown during drought stress (Gogorcena et al., 1995; Gordon et al., 1997; Guerin et al., 1990), salt stress (Gordon et al., 1997; Mhadhbi et al., 2011) and dark stress (Gogorcena et al., 1997; Matamoros et al., 1999). This diminution of Lb will impact the general metabolism of nodule by decreasing the O_2 availability to bacteroid and by potentially releasing free iron which may be a co-factor of the Fenton reaction to produce reactive oxygen species.

In conclusion, the general production of the high energy level needed for the efficient nitrogen fixation is generally altered at the onset of SIS.

3.3 Stress induced senescence is characterized by modifications in the nodule redox state components

As during developmental senescence, modifications of the redox balance are involved in nodule SIS. As discussed above, the high respiration rates, the important Lb concentration and the release of the catalytic Fe may be major ROS production systems. The accumulation of catalytic Fe has been detected during dark stress (Gogorcena et al., 1997; Becana & Klucas, 1992) and drought stress (Gogorcena et al., 1995) and participates in $OH^{\bullet}$ production during dark stress (Becana & Klucas, 1992). The modification of iron metabolism during SIS is also noticeable through the up regulation of ferritin and metallothionein during drought stress (Clement et al., 2008) and dark stress (Perez Guerra et al., 2010). These proteins sequester free Fe to decrease the Fenton reaction and protect the cellular primary components.

ROS accumulation is also regulated by antioxidant defence which participates in their degradation (Figure 4). Nodule antioxidant defence and the importance of the regulation of the redox balance has been extensively studied in root nodule (for review: Becana et al., 2010; Chang et al., 2009; Marino et al., 2009). Modifications of the antioxidant defence parameters have been used extensively as a marker for nodule SIS. Content and redox state of GSH and Asc, two antioxidant molecules, are modified during drought stress (Gogorcena et al., 1995; Marino et al., 2007), salt stress (Swaraj and Bishnoi, 1999) and dark stress (Matamoros et al., 1999; Gogorcena et al., 1997). Superoxide dismutase and catalase, two enzyme families involved in ROS degradation, are down regulated during dark stress (Matamoros et al., 1999; Gogorcena et al., 1997), salt stress (Jebara et al., 2005) and drought stress (Gogorcena et al., 1995; Rubio et al., 2002). Similarly, enzymes of the Asc-GSH cycle are modulated during nodule SIS (Gogorcena et al., 1995; Jebara et al., 2005; Matamoros et al., 1999; Mhadhbi et al., 2011). Nevertheless, whereas the majority of the reports suggest that SIS is associated with a decrease of the antioxidant defence, other articles have shown

that some elements of the antioxidant defence are stable or even up regulated during stress. This discrepancy may be linked to the stress intensity, the plant adaptation to the treatment and to the growth conditions which modify the responses of plant to stress.

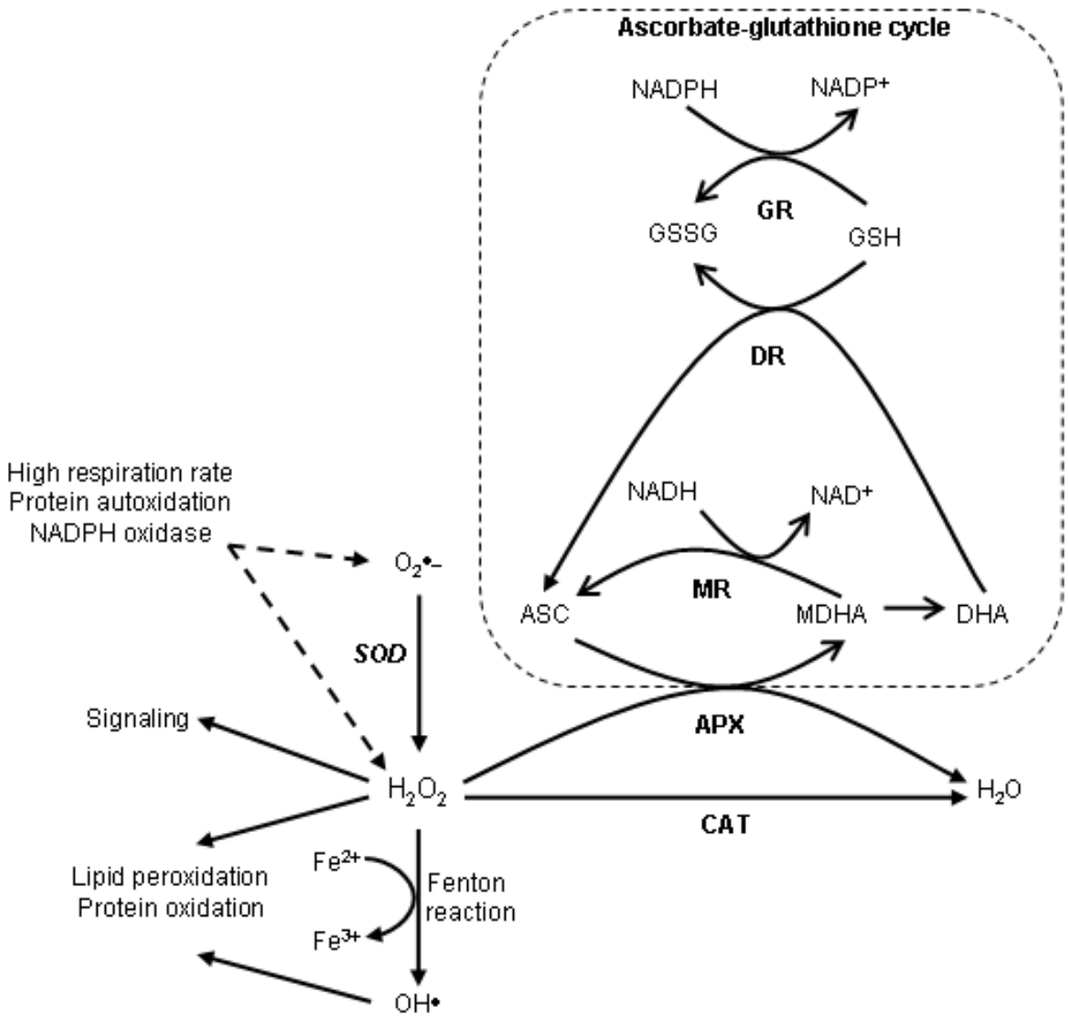


Fig. 4. Scheme showing processes for production and removal of Reactive Oxygen Species. CAT, catalase; SOD, superoxide dismutase; APX, ascorbate peroxidase; MR, monodehydroascorbate reductase; DR, dihydroascorbate reductase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; ASC, reduced ascorbate; MDHA, monodehydroascorbate; DHA, dehydroascorbate.

The imbalance in redox state leading to oxidative stress is characterized by the oxidation of major cellular components such as lipids and proteins. One of the major targets is the membrane. Lipid peroxidation, measured as the reaction of thiobarbituric acid with malondialdehyde, is significantly attenuated during dark stress (Matamoros et al., 1999; Gogorcena et al., 1997) in contrast to drought stress and salt stress during which lipid peroxidation increases (Gogorcena et al., 1995; Mhadhbi et al., 2011). Protein oxidation is

increased during dark stress (Matamoros et al., 1999; Gogorcena et al., 1997) and drought stress (Gogorcena et al., 1995). The differential regulation in lipid peroxidation suggests that different degradation mechanisms may partially occur during SIS. This raises the question of SIS regulation and the specificity of the plant response to the different stress.

3.4 Nodule senescence induced by nitrate

One of the specificities of root nodule is the rapid onset of senescence occurring under nitrate treatment. Nitrate concentrations above 2 to 3 mM have strong detrimental effects on the NFS as there is inhibition of several developmental steps ranging from the infection process to the nitrogen fixation in mature nodule (Mortier et al., 2011; Streeter & Wong, 1988). As for other SIS, the inhibition of nitrogenase is correlated with an increase in O_2 diffusion and supply (Escuredo et al., 1996; Matamoros et al., 1999; Minchin et al., 1986; Minchin et al., 1989). Nitrate application also reduces carbon supply from leaves to nodules as measured by plant treatment with ^{11}C and ^{14}C-labelled CO_2 (Fujikake et al., 2003). This reduction in carbon supply is sometimes correlated with the diminution of the sucrose pool (Matamoros et al., 1999) and the down expression of sucrose synthase (Gordon et al., 2002). Nitrate treatment also decreases the antioxidant defence of the nodule with a diminution of the ascorbate pool and of the activities of ascorbate peroxidases and catalases (Escuredo et al., 1996; Matamoros et al., 1999). At the ultrastructural level, the symbiosome membrane seems to be affected by the senescence process before the bacteroid (Matamoros et al., 1999).

Nitrate effect results in both local and systemic regulation of the nodulation process (Jeudy et al., 2009). Systemic regulation has been described in numerous leguminous plants. Indeed, shoot-determined supernodulators with a nitrate-tolerant nodulation process have been described in soybean (Searle et al., 2003), pea (Duc & Messager, 1989), *L. japonicus* (Krusell et al., 2002) and *M. truncatula* (Penmetsa et al., 2003). Gene analyses have led to the identification of orthologous Leucine Rich Repeat-Receptor Like Kinases (LRR-RLKs) which play a crucial role in the autoregulation of nodulation. The systemic regulation may occur via the induction of specific CLV3/ESR (CLE) peptides produced after nitrate treatment (Okamoto et al., 2009; Reid et al., 2011). In this context, NOD3 is involved in the production or in the transport of the root signal molecule involved in the systemic regulation (Novak, 2010).

Finally, nitrogen limitation regulates nodule growth and stimulates BNF activity via a LRR-RLKs independent response suggesting that a local nodule adaptation may also be involved in the nitrate regulation of the BNF (Jeudy et al., 2009).

3.5 Molecular modifications occurring during stress induced senescence

The last review analyzing root nodule senescence presented oxidative stress and hormones as potential key players of the senescence process (Puppo et al., 2005). The concentration of abscissic acid (ABA), a hormone involved in plant response to abiotic and biotic stress (Cutler et al., 2010; Raghavendra et al., 2010), is strongly increased in soybean nodules under drought stress (Clement et al., 2008). The five-fold accumulation of ABA in stressed nodules compared to stressed roots shows that ABA accumulation is much higher in nodules than in roots. The effect of ABA on nodule functioning has been shown by exogenous treatment of pea nodules (Gonzalez et al., 2001). ABA treatment decreases the BNF and Lb content

declines in parallel with the BNF. However, sucrose synthase activity, another parameter of drought stress effect, is not affected by this treatment suggesting that ABA is not the only player of nodule response to stress. Jasmonic acid (JA), another hormone involved in plant stress response (Reinbothe et al., 2009), has also been shown to be involved in the regulation of nodule functioning (Hause & Schaarschmidt, 2009). Exogenous JA treatment induces an accumulation of lipid peroxides and modifies ascorbate metabolism suggesting that JA could influence nodule senescence (Loscos et al., 2008).

Redox state modifications seem to be a regulatory element of the nodule SIS (Marino et al., 2006). Exogenous treatment with paraquat, which generates ROS, induces the alteration of the GSH and ASC pools toward a more oxidized state. This alteration of the redox state is associated with a diminution of BNF and decrease in Lb content. Moreover, an early decrease in sucrose synthase activity is also detected during the treatment. These results suggest that oxidative stress is involved in the signalling pathway leading to nodule SIS. Interestingly, sucrose synthase seems to be regulated at both the transcriptional and post-translational levels by oxidizing agents such as paraquat (Marino et al., 2008). Finally, genetic modifications allowing decrease and increase of GSH content in the nitrogen fixing zone has shown that BNF and Lb expression level are correlated with GSH content (El Msehli et al., 2011). These results strengthen the idea that cellular redox state plays a crucial role in the regulation of nodule functioning.

Developmental senescence and SIS present different structural and temporal features. At the transcriptomic level, analysis of the expression of 58 genes up regulated during developmental senescence has been performed during dark stress (Perez Guerra et al., 2010). 21 genes are induced during both types of senescence. Amongst these genes, some serine/threonine kinase and some genes involved in metal metabolism (metal transporters and metallothionein) have similar profiles of induction. Nine are up-regulated during both senescence types with different induction levels or transient induction during dark stress. Amongst these genes, cysteine and aspartic proteases are well represented. Finally, 28 genes are up regulated during developmental senescence and not by dark stress. Amongst these genes, proteins associated to proteasome function and vesicular trafficking are not induced during dark stress suggesting partial different regulatory processes between the two types of senescence. In soybean, a screen for genes involved in root nodule senescence has led to the isolation of the senescence-associated nodulin 1 (SAN1) multigene family showing a high homology with plant 2-oxoglutarate-dependent dioxygenases and including two functional genes *SAN1A* and *SAN1B* and a pseudogene *SAN1C* (Webb et al., 2008). Analyses of the steady-state mRNA levels of *SAN1A* and *SAN1B* during developmental senescence showed no significant differences for both genes. In contrast, during induced senescence by treatment with nitrate or darkness, *SAN1A* is down-regulated and *SAN1B* is up-regulated by both treatments.

Nevertheless, dark stress, drought stress and salt stress may induce specific senescence cascades and transcriptomic analyses will have to be realized to define the similarities and the differences in gene expression patterns in nodules subjected to the different stress.

4. Bacterial mutants and nodule senescence

The microsymbiont, differentiated into bacteroids inside the symbiosome, is not only dependent on "senescent" signals coming from the cytosolic environment of its host plant.

Mutations in some bacteroid genes have an incidence on its lifespan *in planta* and thus on the nodule integrity. However, it is difficult to assess the role of bacteroid genes on nodule senescence due to a lack of genetic tools to investigate this question.

Gene affected	**Species**	**Function**	**Symbiotic phenotype**	Reference
nifH, fixA, fixJ and fixK	*S. meliloti*	Nitrogen fixation	fix^{-}, elongated differentiated bacteroids	Maunoury et al., (2010)
lspB	*S. meliloti*	Lipopolysaccharide biosynthesis		
rpoH1	*S. meliloti*	σ^{23}-like protein , bacterial protection against environmental stresses	fix^{-}, reduced number of intracellular bacteria, mixture of normal elongated and abnormal bacteroids	Mitsui, (2004)
gltA	*S. meliloti*	Citrate synthase	fix$^{+/-}$ (20%/WT)	Grzemski et al., (2005)
aap bra	*R. leguminosarum*	Branched-chain amino acids transporters	fix$^{+/-}$ (30%/WT), fewer smaller bacteroids with a lower DNA content	Prell et al., (2009)
relA	*R. etli*	Stringent response	fix$^{+/-}$ (25%/WT), abnormal bacteroids, with an increased size	Moris et al., (2005)
sitA	*S. meliloti*	Manganese uptake	fix$^{+/-}$ (25%/WT), mixture of white, slightly pink and pink nodules	Davies et al., (2007)
katA katC	*S. meliloti*	Catalases	fix$^{+/-}$ (30%/WT), differentiated bacteroids irregular in shape	Jamet et al., (2003)
gshB	*S. meliloti*	Glutathione synthetase	fix$^{+/-}$ (25%/WT), senescence before or after differentiation into bacteroid	Harrison et al., (2005)
gshB	*R. tropici*	Glutathione synthetase	fix$^{+/-}$	Muglia et al., (2008)
lsrB1	*S. meliloti*	LysR-type transcriptional regulator	fix$^{+/-}$ (30%/WT), mixture of pink and white nodules	Luo et al., (2005)
hfq	*S. meliloti*	RNA chaperone	fix$^{+/-}$ (50%/WT), mixture of pink and white nodules	Torres-Quesada et al., (2010)

Table 1. Bacterial genes in which mutations cause early nodule senescence

Until now, there is no global transcriptomic study which could give information on the bacteroid gene expression profile when nitrogen fixing bacteroids turn off to become senescent. Moreover, due to the difficulty in finding a reliable and efficient screen to isolate bacteroid senescent mutants *in planta*, a library of such mutants is still not available. The data described below mostly come from the analysis of the symbiotic phenotype of rhizobial

strains affected in one specific gene. The characteristics associated to these analyses include: plant yield, nodule morphology, nitrogen fixation efficiency, and sometimes, ultrastructure of the nodule and of the bacteroid. More recently, some symbiotic phenotype studies also include bacterial genome endoreduplication and transcriptome analyses. This section will mainly focus on the impact of a mutation in the bacterial genome on early nodule senescence and on delayed nodule senescence (Table 1).

4.1 Bacterial mutants and early nodule senescence

The rhizobial mutants that present a symbiotic phenotype are divided into four groups: the nodule deficient mutants impaired in the first steps of infection (nod$^-$ fix$^-$), the bacterial mutants which induce nodules that present an early nodule senescence phenotype i) blocked in their bacteroid differentiation process (nod$^+$ fix$^-$), ii) fully differentiated but unable to reduce N_2 (nod$^+$ fix$^-$) and iii) differentiated into bacteroids less efficient in N_2 fixation compared to the wild-type strain (nod$^+$ fix$^{+/-}$). Bacterial mutants leading to nodule development abortion due to a defect in bacteroid differentiation such as *bacA* (Saeki, 2011) or *parA* (Liu et al., 2011) mutants will not be presented here. The rhizobial nod$^+$ fix$^-$ mutants that could differentiate into bacteroids and pass the two transcriptome switch-points encountered during the differentiation of the wild-type bacteroids have been described recently (Maunoury et al., 2010). They are affected in genes encoding for symbiotic function and nitrogen fixation machinery (*nifH*, *fixA*, *fixJ* and *fixK*) or in lipopolysaccharide biosynthesis (*lspB*). The *rpoH1* mutant of *S. meliloti*, impaired in the synthesis of the σ^{32}-like protein, involved in bacterial protection against environmental stresses has also a nod$^+$ fix$^-$ symbiotic phenotype in interaction with alfalfa (Mitsui et al., 2004). The bacterial *rpoH1* mutant is still able to elicit nodule formation, efficient plant cell invasion and differentiation into bacteroids. But, the degeneration of bacteroids rapidly occurred in the proximal zone, adjacent to the infection zone, leading to ineffective white nodules associated to an early nodule senescence phenotype. Only the latter group of early nodule senescence mutants (nod$^+$ fix$^{+/-}$) will be described below. The genes affected in these mutants fall mainly into two categories: genes encoding function involved in carbon and nitrogen metabolism, and genes important for stress adaptation. Genes with other function will also be presented.

4.1.1 Genes encoding function involved in carbon and nitrogen metabolism of the bacteroid and in the nutriment stress response

The host plant supplies bacteroids with metabolites, including dicarboxylic and amino acids, used by the bacteroids to support the reduction of N_2 into ammonia in amounts sufficient for plant growth. To better understand the role of the decarboxylating part of the *S. meliloti* TCA cycle in a nitrogen fixing nodule, Gremski and collaborators (2005) have used an elegant approach (Grzemski et al., 2005). In *S. meliloti*, a mutant in the TCA cycle *gltA* gene encoding citrate synthase forms empty nodules devoid of intracellular bacteria. The *gltA* mutants are clearly unsuitable for experiments to determine whether citrate synthase (CS) is essential during nitrogen fixation in a mature nodule because they have a defect in development that prevents them from forming normal bacteroids. So, the authors constructed temperature-sensitive (ts) mutants in the *S. meliloti* citrate synthase (*gltA*) gene. This allows the formation of nitrogen-fixing nodules at the permissive temperature but, once nodule development was complete, an elevation of root temperature prevents CS

expression. When alfalfa plants infected with the ts mutants were transferred to 30°C, the nodules lost the ability to fix nitrogen. Microscopic examination of the nodules revealed the loss of bacteroids in infected cells and morphological changes that resembled changes seen during nodule senescence.

These experiments with CS ts mutants showed that CS activity is needed in mature nodules to maintain bacteroid integrity and that removing CS activity via a temperature shift converts an effective nodule into an empty nodule. This implies that CS is essential for nodule maintenance as well as in the early stages of plant cell invasion.

Another example of early nodule senescence associated to a nutrient defect in *Rhizobium* has been described recently. Prell and collaborators (2009) have shown that *Rhizobium leguminosarum*, the bacterial partner of peas and broad beans (biovar *viciae*), becomes symbiotic auxotroph for the branched-chain amino acids Leucine, Isoleucine, Valine (LIV) when differentiated into bacteroids in root nodules (Prell et al., 2009). While these bacteria are prototrophs for LIV amino acids as free-living bacteria, they become dependent on the plant as nitrogen-fixing bacteroids, due to a major reduction in gene expression and activity of LIV biosynthetic enzymes. Peas inoculated with bacterial mutants impaired in their capacity to transport LIV, have an early senescent phenotype (nod^+, $fix^{+/-}$). Peas are yellow, have small, pale pink nodules and a dry weight similar to un-inoculated plants. This is correlated with a 70% decrease of the nitrogen fixation capacity for plants inoculated with these mutants compared to the plants inoculated with the *R. leguminosarum* wild-type strain. Thus, plants not only provide a carbon source (dicarboxylic acids) to the bacteroid but also precursors of proteins. The authors have shown that a defect in bacteroid LIV nutrition leads to a reduction in its persistence in plant infected cells, which in turn induces senescence. This means that the plant cell might receive information from the bacteroid in order to sense the fitness of the microsymbiont to maintain or interrupt the symbiotic interaction.

Concerning nutriment stress perceived by the bacteria *in planta*, a mutation in the *relA* gene of *Rhizobium etli*, induces symbiotic defects at the intermediate and/or late stages of the interaction with *Phaseolus vulgaris* (Moris et al., 2005). RelA allows the production of the alarmone (p)ppGpp, which mediates the stringent response in bacteria. This response results in transcriptional down regulation of ribosomal and tRNA genes, upon conditions of amino acid starvation. Despite this role, RelA has been reported to be important for biofilm formation and for interaction of bacteria, pathogenic or beneficial, with their eukaryotic host. Interaction of a *relA* mutant with common bean plants strongly reduces the nitrogen fixation efficiency by 75% and the plant yield. Microscopic studies showed that bacteria differentiated into bacteroids in the symbiosomes were larger in size than the wild-type ones. Thus, in the *R. etli* bacteroids, *relA* plays a role in physiology adaptation and regulation of gene expression. However, the step impaired in the nitrogen fixation defect of a *relA* mutant has not been investigated.

4.1.2 Genes encoding function involved in oxidative stress response

It is generally accepted that symbiotic bacteria are submitted to an oxidative burst released by the host plant during the first steps of infection (Pauly et al., 2006). In addition to this role as a general plant defence mechanism against bacterial invasion, oxidative burst might also

play a role in the lifespan of the bacteroid. The high rate of bacteroid respiration necessary to supply energy required for the nitrogen reduction process generates high levels of ROS in the nodule. In legume root nodules, a large amount of H_2O_2 surrounding disintegrating bacteroids in senescent zone IV is detected (Rubio et al, 2004). This reflects the close relationship between oxidative stress and nodule senescence. Indeed, most of the bacterial mutant strains that show a symbiotic nod$^+$ fix$^{+/-}$ phenotype (early senescence) are affected in their antioxidant defence. To escape the stress generated by H_2O_2 and $O_2^{\cdot-}$, bacteria encode a set of enzymes such as superoxide dismutases, catalases and alkylhydroperoxidases, and also antioxidant molecules such as GSH.

In *S. meliloti*, disruption of the *sitA* gene induces a decrease in Mn/Fe SodB activity and a higher sensitivity to ROS (Davies and Walker, 2007). The *sitA* gene encodes a periplasmic protein involved in manganese uptake. During alfalfa interaction, a *sitA* mutant is either affected in its infection efficiency leading to small white nodules or is possibly altered in the survival of the differentiated form leading to intermediate nodules, with a slight pink fixing zone, smaller in size than the wild-type nodules. As a consequence, the nitrogenase activity and the plant yield are greatly reduced in these mutant infected plants compared to the plants inoculated with the wild-type bacteria. It is difficult to assess the role of the other superoxide dismutase of *S. meliloti (*SodA) in the natural senescence process since a *sodA* mutant failed to differentiate into bacteroids after release into plant cells (Santos et al., 2000).

To cope with H_2O_2 production, *S. meliloti* possesses three catalases. In free living bacteria, KatA and KatC are encoded by genes mainly transcribed in oxidative stress conditions while the *katB* gene is constitutively expressed. In 6 week-old nodules of M*edicago sativa*, KatA is the predominant catalase present in the bacteroids. The *katB* gene is also expressed in the nitrogen fixation zone III while *katC* is only transcribed in the infection zone II (Jamet et al., 2003). Single mutant strains of *katA*, *katB* or *katC* genes have no significant impact on nitrogen fixation efficiency of alfalfa nodules containing these mutants compared to those infected with wild-type bacteria. However a *katA katC* double mutant presents a dramatic decrease of nitrogen fixation capacity (Sigaud et al., 1999), associated with an early senescence of the nodule. These nodules were devoid of a clear zone III, instead the senescent zone IV was adjacent to interzone II-III (Jamet et al., 2003). In most plant cells, bacteria were correctly released from infection threads and were able to differentiate into bacteroids. This shows that efficient detoxification of H_2O_2 by the microsymbiont is essential in the latter steps of bacteroid differentiation leading to nitrogen fixing bacteria.

The antioxidant GSH plays an important role during symbiosis and nodule senescence. This tripeptide is synthesized by a two-step process. In bacteria, GshA catalyses the conjugation of glutamate and cysteine to form γEC and, in a second enzymatic step, GshB completes GSH synthesis by addition of glycine. In *S. meliloti*, while a *gshA* mutant is unable to form nodules (nod$^-$ fix$^-$ phenotype), a *gshB* mutant has a nod$^+$ phenotype coupled to a 75% reduction in the nitrogen fixation capacity (Harrison et al., 2005). In these nodules, bacteria are correctly released from the infection thread into host plant cells and enter into early senescence after differentiation into bacteroids. These data show that, in *S. meliloti*, GSH is important to maintain bacteroid during symbiotic interaction with alfalfa. This is also true in some determinate-type nodules as the survival of the common bean (*P. vulgaris)* microsymbiont, *Rhizobium tropicii*, is dependent on GSH production (Muglia et al., 2008). A *gshB* mutant has an early senescent pattern associated with increased levels of superoxide

accumulation. Expression of this gene in a wild-type background is enhanced at late stage of nodule development, suggesting its antioxidant role against ROS accumulation during nodule senescence. In these species, GSH is important to keep nodules functional over time. In contrast, this does not hold true for *Bradyrhizobium* sp. where disruption of the *gshA* gene does not affect the ability to form effective nodules (Sobrevals et al., 2006). In this latter case, it is possible that the defect in intracellular GSH was compensated for by other compounds acting as antioxidants.

4.1.3 Genes encoding bacterial function involved in the regulation of gene expression

Ninety putative genes encoding LysR-type transcriptional regulators were identified in the *S. meliloti* genome. These regulators are typically 300 amino acids long with an N-terminal DNA binding domain and a C-terminal sensing domain for signal molecules and function as activators or repressors. LysR regulated genes have promoters which contain at least one TN11A motif and are usually divergently transcribed from the LysR regulator (Schell, 1993). To determine the role of LysR regulators in symbiosis, a mutagenesis analysis of all 90 putative *lysR* genes was realized (Luo et al., 2005). This allowed the isolation of the *lsrB1* mutant that presents a symbiotic phenotype. An *lsrB1* mutant was deficient in symbiosis and elicited a mixture of pink (45%) and white (55%) nodules on alfalfa plants. These plants exhibited lower overall nitrogenase activity (30%) than plants inoculated with the wild-type strain. This is consistent with the fact that most of the alfalfa plants inoculated with the *lsrB1* mutant were short (50 to 80% shorter than the plants inoculated with the WT strain) and light green. Cells of the *lsrB1* mutant were recovered from both pink and white nodules, suggesting that *lsrB1* mutants could be blocked either early or late during nodulation. Similar numbers of bacterial cells were recovered from the pink nodules of plants inoculated with the wild-type strain Rm1021 and pink and white nodules from the plants inoculated with the mutants. These findings suggest that the *lsrB1* mutants were able to invade plant cells. The *lsrB* gene is located downstream from the *trxB* gene for thioredoxin reductase, which also participates in the bacterial antioxidant defence. The *trxB* gene is transcribed from its own promoter in the same direction as the *lsrB* gene. The *trxB* promoter contains a nearly perfect recognition site (TN11A) for a LysR regulator so it is possible that LsrB regulates the expression of both the *trxB* and *lsrB* genes. The authors suggest that the early senescence phenotype observed *in planta* could be linked to a defect in detoxification of ROS in *S. meliloti*. However, this has not been demonstrated.

It has been shown recently that the *S. meliloti* RNA chaperone Hfq plays a role in the survival of the microsymbiont within the alfalfa nodule cells (Torres-Quesada et al., 2010). Hfq is considered to act as a global post-transcriptional regulator of gene expression since it interacts with diverse RNA molecules and small non-coding RNAs (sRNA). In free living bacteria, an *hfq* mutant down-regulates 91 genes mostly involved in central carbon metabolism (uptake and utilization of carbon substrates) and up-regulates genes involved in the uptake and catabolism of diverse N compounds. During late interaction with alfalfa (30 days post-infection), plants inoculated with the *hfq* mutant strain are composed of 60% of white non fixing nodules and 40% of pink fixing nodules. Thus, the plant yield was 64% of that of the wild-type-inoculated plants. Histological analysis of the white nodules revealed that the bacteroid differentiation was efficient but the bacteroid-infected tissues were restricted to the interzone II-III since the zone III was replaced by a large senescent zone IV. Indeed, an Hfq impaired mutant showed a premature senescent phenotype. The authors

proposed that this phenotype could be linked to a defect in intracellular survival under prolonged stress present in the plant cell environment.

4.2 Bacterial mutants and delayed senescence

The delayed senescent bacterial mutants might have a nod^+ phenotype associated with a fix^+ phenotype for a period longer than the natural fixing period associated with the wild type bacteria in interaction with its host plant. Thus, such mutants are obligate differentiated bacteroids.

Compared to the data connected with the consequences of bacterial gene inactivation on early nodule senescence, little information on the role of bacterial mutants on delayed nodule senescence are available. Knowing that most of the mutations that induce an acceleration of senescence affect genes involved in ROS detoxification, in bacterial fitness, in import and/or processing of carbon skeletons, amino acids and antioxidants, we might suspect that a delayed senescence bacterial mutant should have a gain of function rather than an invalidated one. In that sense, it is possible that an increase in synthesis and/or activity of molecules involved in stress resistance, especially to ROS, should improve the bacteroid lifetime in the symbiosome and thus should enhance the nitrogen fixing period. This aspect of the role of bacterial genes in the functional life of symbiotic fixing nodules remains to be explored. However, one encouraging study sustains this postulate (Redondo et al., 2009). In fact, the authors of this work have overexpressed a cyanobacteria *Anabaena variabilis* gene encoding flavodoxin in *S. meliloti*. Knowing that natural senescence-inducing signals from the plant leads to a decrease in antioxidant content and thus an increase in ROS accumulation in an irreversible manner, they analyse the consequences of the over-expression of this flavodoxin protein involved in the response to oxidative stress. They have shown that the decline of nitrogenase activity was delayed and that the structural and ultrastructural modifications associated with nodule senescence had a later onset in flavodoxin-expressing nodules. Lipid peroxidation, a marker of senescence, was significantly reduced and the oxidative balance was improved in comparison to the control nodules. In conclusion, flavodoxin over-expression had an impact on bacteroid antioxidant metabolism, leading to delayed senescence.

In conclusion, we can propose that bacteroids inside the nodule infected cells are not only tributary from the plant to initiate nodule senescence. Genes encoding proteins implicated in bacterial nutrition and stress response are also essential since mutations in these genes alter the fitness of the differentiated bacteroids. In turn, this leads to the death of the microsymbiont followed by the senescence of the plant cells and nodule. Future aspects on the role of bacteroid genes on senescence should include the development of bacteroid genetic tools. The over-expression of pertinent genes specifically in the bacteroid or the conditional invalidation of rhizobial genes after bacteroid differentiation will be important to define senescence-related genes.

5. Perspectives

The data summarized in this review indicate that one of the general physiological features of nodule senescence is the decrease in nitrogen fixation efficiency. This diminution may be related to plant and/or bacterial-dependent factors. However whereas this diminution of the nitrogen fixation efficiency is observed during both developmental and stress induced-

nodule senescence, the progression of senescence symptoms seems to be differentially controlled. Indeed, even if common general features have been described during nodule senescence, the few available microscopic and transcriptomic analyses show that nodule senescence may occur differently in developmental senescence and SIS. Moreover, the senescence occurring under different environmental stress conditions such as dark stress, drought stress or nitrate treatment may also involve different genetic and physiological programs. In this context, more detailed spatiotemporal analysis of the multiple senescence processes will have to be performed to determine the similarities and the differences between the various senescence processes. Microarray analysis or "Whole Transcriptome Shotgun Sequencing" will be valuable tools to analyse the transcriptome modifications occurring under the different senescence processes (Lister et al., 2009). In conditions in which the senescence process does not appear to be a homogenous process such as in developmental senescence, laser capture microdissection (Barcala et al., 2009) will allow the analysis of transcriptomic patterns of senescent infected cells. The development of two legume model systems, *M. truncatula* (http://www.medicago.org/) and *L. japonicus* (http://www.lotusjaponicus.org/) will facilitate an efficient analysis of the senescence process by developing tools dedicated to cell biology, genetic and transcriptomic analyses.

The role of the bacterial partner needs also to be clarified. Indeed, whereas senescence phenotypes are observed in nodules obtained with bacterial mutants affected in their nitrogen fixation efficiency, the regulation of this bacterial-induced senescence has not yet been studied. The abortion of nodule development when using such bacterial mutants suggests that the interaction may switch from a compatible to an incompatible interaction. In this context, the molecular events which trigger this switch still need to be defined. Nevertheless, the work with the flavodoxin overexpressing-bacterial strain showed that the symbiotic interaction may also be improved to resist to the various endogenous and environmental stress conditions. The construction of plant and bacteria with higher resistance to environmental stress (Zurbriggen et al., 2008) may be an interesting opportunity to increase the benefit from an efficient BNF in agronomy.

6. References

Alesandrini, F., Mathis, R., Van de Sype, G., Herouart, D. & Puppo, A. (2003). Possible roles for a cysteine protease and hydrogen peroxide in soybean nodule development and senescence. *New Phytol,* Vol. 158, No. 1, pp. 131-138

Apel, K. & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol,* Vol. 55, pp. 373-399

Appleby, C. A. (1984). Leghemoglobin and Rhizobium respiration. *Annu Rev Plant Physiol Plant Mol Biol.,* Vol. 35, pp. 443-478

Aydi, S., Drevon, J. J. & Abdelly, C. (2004). Effect of salinity on root-nodule conductance to the oxygen diffusion in the *Medicago truncatula-Sinorhizobium meliloti* symbiosis. *Plant Physiol Biochem,* Vol. 42, No. 10, pp. 833-840

Barcala, M., Garcia, A., Cabrera, J., Casson, S., Lindsey, K., Favery, B., Garcia-Casado, G., Solano, R., Fenoll, C. & Escobar, C. (2009). Early transcriptomic events in microdissected Arabidopsis nematode-induced giant cells. *Plant J,* Vol. 61, No. 4, pp. 698-712

Becana, M. & Klucas, R. V. (1992). Transition metals in legume root nodules: iron-dependent free radical production increases during nodule senescence. *Proc Natl Acad Sci U S A,* Vol. 89, No. 19, pp. 8958-8962

Becana, M., Matamoros, M. A., Udvardi, M. & Dalton, D. A. (2010). Recent insights into antioxidant defenses of legume root nodules. *New Phytol,* Vol. 188, No. 4, pp. 960-976

Becker, A., Berges, H., Krol, E., Bruand, C., Ruberg, S., Capela, D., Lauber, E., Meilhoc, E., Ampe, F., de Bruijn, F. J., Fourment, J., Francez-Charlot, A., Kahn, D., Kuster, H., Liebe, C., Puhler, A., Weidner, S. & Batut, J. (2004). Global changes in gene expression in Sinorhizobium meliloti 1021 under microoxic and symbiotic conditions. *Mol Plant Microbe Interact,* Vol. 17, No. 3, pp. 292-303

Bekki, A., Trinchant, J.-C. & Rigaud, J. (1987). Nitrogen fixation (C_2H_2 reduction) by Medicago nodules and bacteroids under sodium chloride stress. *Physiologia Plantarum,* Vol. 71, No. 1, pp. 61-67

Ben Salah, I., Albacete, A., Martinez Andujar, C., Haouala, R., Labidi, N., Zribi, F., Martinez, V., Perez-Alfocea, F. & Abdelly, C. (2009). Response of nitrogen fixation in relation to nodule carbohydrate metabolism in Medicago ciliaris lines subjected to salt stress. *J Plant Physiol,* Vol. 166, No. 5, pp. 477-488

Benedito, V. A., Torres-Jerez, I., Murray, J. D., Andriankaja, A., Allen, S., Kakar, K., Wandrey, M., Verdier, J., Zuber, H., Ott, T., Moreau, S., Niebel, A., Frickey, T., Weiller, G., He, J., Dai, X., Zhao, P. X., Tang, Y. & Udvardi, M. K. (2008). A gene expression atlas of the model legume Medicago truncatula. *Plant J,* Vol. 55, No. 3, pp. 504-513

Bethlenfalvay, G. J. & Phillips, D. A. (1977). Ontogenetic Interactions between Photosynthesis and Symbiotic Nitrogen Fixation in Legumes. *Plant Physiol,* Vol. 60, No. 3, pp. 419-421

Bobik, C., Meilhoc, E. & Batut, J. (2006). FixJ: a major regulator of the oxygen limitation response and late symbiotic functions of Sinorhizobium meliloti. *J Bacteriol,* Vol. 188, No. 13, pp. 4890-4902

Broughton, W. J., Jabbouri, S. & Perret, X. (2000). Keys to symbiotic harmony. *J Bacteriol* Vol. 182, No. 20, pp. 5641-5652

Capela, D., Filipe, C., Bobik, C., Batut, J. & Bruand, C. (2006). Sinorhizobium meliloti differentiation during symbiosis with alfalfa: a transcriptomic dissection. *Mol Plant Microbe Interact,* Vol. 19, No. 4, pp. 363-372

Chang, C., Damiani, I., Puppo, A. & Frendo, P. (2009). Redox changes during the legume-rhizobium symbiosis. *Mol Plant,* Vol. 2, No. 3, pp. 370-377

Clement, M., Lambert, A., Herouart, D. & Boncompagni, E. (2008). Identification of new up-regulated genes under drought stress in soybean nodules. *Gene,* Vol. 426, No. 1, pp. 15-22

Coba de la Pena, T., Carcamo, C. B., Almonacid, L., Zaballos, A., Lucas, M. M., Balomenos, D. & Pueyo, J. J. (2008). A cytokinin receptor homologue is induced during root nodule organogenesis and senescence in Lupinus albus L. *Plant Physiol Biochem,* Vol. 46, No. 2, pp. 219-225

Crespi, M. & Frugier, F. (2008). De novo organ formation from differentiated cells: root nodule organogenesis. *Sci Signal,* Vol. 1, No. 49, pp. re11

Cutler, S. R., Rodriguez, P. L., Finkelstein, R. R. & Abrams, S. R. (2010). Abscisic acid: emergence of a core signaling network. *Annu Rev Plant Biol,* Vol. 61, pp. 651-679

Davies, B. W. & Walker, G. C. (2007). Disruption of sitA compromises Sinorhizobium meliloti for manganese uptake required for protection against oxidative stress. *J Bacteriol,* Vol. 189, No. 5, pp. 2101-2109

De Michele, R., Formentin, E., Todesco, M., Toppo, S., Carimi, F., Zottini, M., Barizza, E., Ferrarini, A., Delledonne, M., Fontana, P. & Lo Schiavo, F. (2009). Transcriptome analysis of Medicago truncatula leaf senescence: similarities and differences in metabolic and transcriptional regulations as compared with Arabidopsis, nodule senescence and nitric oxide signalling. *New Phytol,* Vol. 181, No. 3, pp. 563-575

Del Castillo, L. D., Hunt, S. & Layzell, D. B. (1994). The role of oxygen in the regulation of nitrogenase activity in drought-stressed soybean nodules. *Plant Physiol,* Vol. 106, No. 3, pp. 949-955

Downie, J. A. (2005). Legume haemoglobins: symbiotic nitrogen fixation needs bloody nodules. *Curr Biol,* Vol. 15, No. 6, pp. R196-198

Duc, G. & Messager, A. (1989). Mutagenesis of pea (Pisum sativum L.) and the isolation of mutants for nodulation and nitrogen fixation. *Plant Science,* Vol. 60, No. 2, pp. 207-213

El Msehli, S., Lambert, A., Baldacci-Cresp, F., Hopkins, J., Boncompagni, E., Smiti, S. A., Herouart, D. & Frendo, P. (2011). Crucial role of (homo)glutathione in nitrogen fixation in Medicago truncatula nodules. *New Phytol,* in press

El Yahyaoui, F., Kuster, H., Ben Amor, B., Hohnjec, N., Puhler, A., Becker, A., Gouzy, J., Vernie, T., Gough, C., Niebel, A., Godiard, L. & Gamas, P. (2004). Expression profiling in Medicago truncatula identifies more than 750 genes differentially expressed during nodulation, including many potential regulators of the symbiotic program. *Plant Physiol,* Vol. 136, No. 2, pp. 3159-3176

Escuredo, P. R., Minchin, F. R., Gogorcena, Y., Iturbe-Ormaetxe, I., Klucas, R. V. & Becana, M. (1996). Involvement of Activated Oxygen in Nitrate-Induced Senescence of Pea Root Nodules. *Plant Physiol,* Vol. 110, No. 4, pp. 1187-1195

Evans, P. J., Gallesi, D., Mathieu, C., Hernandez, M. J., de Felipe, M., Halliwell, B. & Puppo, A. (1999). Oxidative stress occurs during soybean nodule senescence. *Planta,* Vol. 208, No.1, pp. 73-79

Fedorova, M., van de Mortel, J., Matsumoto, P. A., Cho, J., Town, C. D., VandenBosch, K. A., Gantt, J. S. & Vance, C. P. (2002). Genome-wide identification of nodule-specific transcripts in the model legume Medicago truncatula. *Plant Physiol,* Vol. 130, No. 2, pp. 519-537

Franssen, H. J., Vijn, I., Yang, W. C. & Bisseling, T. (1992). Developmental aspects of the Rhizobium-legume symbiosis. *Plant Mol Biol,* Vol. 19, No. 1, pp. 89-107

Fridovich, I. (1986). Biological effects of the superoxide radical. *Arch Biochem Biophys,* Vol. 247, No. 1, pp. 1-11

Fujikake, H. - Yamazaki, A., Ohtake, N., Sueyoshi, K., Matsuhashi, S., Ito, T., Mizuniwa, C., Kume, T., Hashimoto, S., Ishioka, N.-S., Watanabe, S., Osa, A., Sekine, T., Uchida, H., Tsuji, A. & Ohyama, T. (2003). Quick and reversible inhibition of soybean root nodule growth by nitrate involves a decrease in sucrose supply to nodules. *J Exp Bot,* Vol. 54, No. 386, pp. 1379-1388

Galibert, F., Finan, T. M., Long, S. R., Puhler, A., Abola, P., Ampe, F., Barloy-Hubler, F., Barnett, M. J., Becker, A., Boistard, P., Bothe, G., Boutry, M., Bowser, L., Buhrmester, J., Cadieu, E., Capela, D., Chain, P., Cowie, A., Davis, R. W., Dreano, S., Federspiel, N. A., Fisher, R. F., Gloux, S., Godrie, T., Goffeau, A., Golding, B., Gouzy, J., Gurjal, M., Hernandez-Lucas, I., Hong, A., Huizar, L., Hyman, R. W., Jones, T., Kahn, D., Kahn, M. L., Kalman, S., Keating, D. H., Kiss, E., Komp, C., Lelaure, V., Masuy, D., Palm, C., Peck, M. C., Pohl, T. M., Portetelle, D., Purnelle, B., Ramsperger, U., Surzycki, R., Thebault, P., Vandenbol, M., Vorholter, F. J., Weidner, S., Wells, D. H., Wong, K., Yeh, K. C. & Batut, J. (2001). The composite genome of the legume symbiont Sinorhizobium meliloti. *Science,* Vol. 293, No. 5530, pp. 668-672

Galvez, L., Gonzalez, E. M. & Arrese-Igor, C. (2005). Evidence for carbon flux shortage and strong carbon/nitrogen interactions in pea nodules at early stages of water stress. *J Exp Bot,* Vol. 56, No. 419, pp. 2551-2561

Gepstein, S. (2004). Leaf senescence--not just a 'wear and tear' phenomenon. *Genome Biol,* Vol. 5, No. 3, pp. 212

Gogorcena, Y., Iturbe-Ormaetxe, I., Escuredo, P. R. & Becana, M. (1995). Antioxidant defenses against activated oxygen in pea nodules subjected to water stress. *Plant Physiol,* Vol. 108, No. 2, pp. 753-759

Gogorcena, Y., Gordon, A. J., Escuredo, P. R., Minchin, F. R., Witty, J. F., Moran, J. F. & Becana, M. (1997). N_2 fixation, carbon metabolism and oxidative damage in nodules of dark stressed Common Bean plants. *Plant Physiol,* Vol. 113, No.4, pp. 1193-1201

Gonzalez, E. M., Gordon, A. J., James, C. L. & Arrese-lgor, C. (1995). The role of sucrose synthase in the response of soybean nodules to drought. *J Ex Bot,* Vol. 46, No. 10, pp. 1515-1523

Gonzalez, E. M., Galvez, L. & Arrese-Igor, C. (2001). Abscisic acid induces a decline in nitrogen fixation that involves leghaemoglobin, but is independent of sucrose synthase activity. *J Exp Bot,* Vol. 52, No. 355, pp. 285-293

Gordon, A. J., Minchin, F. R., Skot, L. & James, C. L. (1997). Stress-induced declines in soybean N_2 fixation are related to nodule sucrose synthase activity. *Plant Physiol,* Vol. 114, No. 3, pp. 937-946

Gordon, A. J., Skot, L., James, C. L. & Minchin, F. R. (2002). Short-term metabolic responses of soybean root nodules to nitrate. *J Exp Bot,* Vol. 53, No. 368, pp. 423-428

Groten, K., Vanacker, H., Dutilleul, C., Bastian, F., Bernard, S., Carzaniga, R. & Foyer, C. H. (2005). The roles of redox processes in pea nodule development and senescence. *Plant, Cell & Environment,* Vol. 28, No. 10, pp. 1293-1304

Groten, K., Dutilleul, C., van Heerden, P. D., Vanacker, H., Bernard, S., Finkemeier, I., Dietz, K. J. & Foyer, C. H. (2006). Redox regulation of peroxiredoxin and proteinases by ascorbate and thiols during pea root nodule senescence. *FEBS Lett,* Vol. 580, No. 5, pp. 1269-1276

Grzemski, W., Akowski, J. P. & Kahn, M. L. (2005). Probing the Sinorhizobium meliloti-alfalfa symbiosis using temperature-sensitive and impaired-function citrate synthase mutants. *Mol Plant Microbe Interact,* Vol. 18, No. 2, pp. 134-141

Guerin, V., Trinchant, J. C. & Rigaud, J. (1990). Nitrogen Fixation (C(2)H(2) Reduction) by Broad Bean (Vicia faba L.) Nodules and Bacteroids under Water-Restricted Conditions. *Plant Physiol,* Vol. 92, No. 3, pp. 595-601

Gunther, C., Schlereth, A., Udvardi, M. & Ott, T. (2007). Metabolism of reactive oxygen species is attenuated in leghemoglobin-deficient nodules of Lotus japonicus. *Mol Plant Microbe Interact,* Vol. 20, No. 12, pp. 1596-1603

Harrison, J., Jamet, A., Muglia, C. I., Van de Sype, G., Aguilar, O. M., Puppo, A. & Frendo, P. (2005). Glutathione plays a fundamental role in growth and symbiotic capacity of Sinorhizobium meliloti. *J Bacteriol,* Vol. 187, No. 1, pp. 168-174

Hause, B. & Schaarschmidt, S. (2009). The role of jasmonates in mutualistic symbioses between plants and soil-born microorganisms. *Phytochemistry,* Vol. 70, No. 13-14, pp. 1589-1599

He, J., Benedito, V. A., Wang, M., Murray, J. D., Zhao, P. X., Tang, Y. & Udvardi, M. K. (2009). The Medicago truncatula gene expression atlas web server. *BMC Bioinformatics,* Vol. 10, pp. 441

Horchani, F., Prevot, M., Boscari, A., Evangelisti, E., Meilhoc, E., Bruand, C., Raymond, P., Boncompagni, E., Aschi-Smiti, S., Puppo, A. & Brouquisse, R. (2011). Both plant and bacterial nitrate reductases contribute to nitric oxide production in Medicago truncatula nitrogen-fixing nodules. *Plant Physiol,* Vol. 155, No. 2, pp. 1023-1036

Ivanov, S., Fedorova, E. & Bisseling, T. (2010). Intracellular plant microbe associations: secretory pathways and the formation of perimicrobial compartments. *Curr Opin Plant Biol,* Vol. 13, No. 4, pp. 372-377

Jamet, A., Sigaud, S., Van de Sype, G., Puppo, A. & Herouart, D. (2003). Expression of the bacterial catalase genes during Sinorhizobium meliloti-Medicago sativa symbiosis and their crucial role during the infection process. *Mol Plant Microbe Interact,* Vol. 16, No. 3, pp. 217-225

Jebara, S., Jebara, M., Limam, F. & Aouani, M. E. (2005). Changes in ascorbate peroxidase, catalase, guaiacol peroxidase and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress. *J Plant Physiol,* Vol. 162, No. 8, pp. 929-936

Jeudy, C., Ruffel, S., Freixes, S., Tillard, P., Santoni, A. L., Morel, S., Journet, E. P., Duc, G., Gojon, A., Lepetit, M. & Salon, C. (2009). Adaptation of Medicago truncatula to nitrogen limitation is modulated via local and systemic nodule developmental responses. *New Phytol,* Vol. 185, No. 3, pp. 817-828

Jones, K. M., Kobayashi, H., Davies, B. W., Taga, M. E. & Walker, G. C. (2007). How rhizobial symbionts invade plants: the Sinorhizobium-Medicago model. *Nat Rev Microbiol,* Vol. 5, No. 8, pp. 619-633

Kaneko, T., Nakamura, Y., Sato, S., Asamizu, E., Kato, T., Sasamoto, S., Watanabe, A., Idesawa, K., Ishikawa, A., Kawashima, K., Kimura, T., Kishida, Y., Kiyokawa, C., Kohara, M., Matsumoto, M., Matsuno, A., Mochizuki, Y., Nakayama, S., Nakazaki, N., Shimpo, S., Sugimoto, M., Takeuchi, C., Yamada, M. & Tabata, S. (2000). Complete genome structure of the nitrogen-fixing symbiotic bacterium Mesorhizobium loti. *DNA Res,* Vol. 7, No. 6, pp. 331-338

Kaneko, T., Nakamura, Y., Sato, S., Minamisawa, K., Uchiumi, T., Sasamoto, S., Watanabe, A., Idesawa, K., Iriguchi, M., Kawashima, K., Kohara, M., Matsumoto, M., Shimpo, S., Tsuruoka, H., Wada, T., Yamada, M. & Tabata, S. (2002). Complete genomic sequence of nitrogen-fixing symbiotic bacterium Bradyrhizobium japonicum USDA110. *DNA Res,* Vol. 9, No. 6, pp. 189-197

Kardailsky, I. V. & Brewin, N. J. (1996). Expression of cysteine protease genes in pea nodule development and senescence. *Mol Plant Microbe Interact,* Vol. 9, No. 8, pp. 689-695

Karunakaran, R., Ramachandran, V. K., Seaman, J. C., East, A. K., Mouhsine, B., Mauchline, T. H., Prell, J., Skeffington, A. & Poole, P. S. (2009). Transcriptomic analysis of Rhizobium leguminosarum biovar viciae in symbiosis with host plants Pisum sativum and Vicia cracca. *J Bacteriol,* Vol. 191, No. 12, pp. 4002-4014

Kereszt, A., Mergaert, P., Maroti, G. & Kondorosi, E. (2011). Innate immunity effectors and virulence factors in symbiosis. *Current opinion in microbiology,* Vol. 14, No. 1, pp. 76-81

Kondorosi, E., Roudier, F. & Gendreau, E. (2000). Plant cell-size control: growing by ploidy? *Curr Opin Plant Biol,* Vol. 3, No. 6, pp. 488-492

Krusell, L., Madsen, L. H., Sato, S., Aubert, G., Genua, A., Szczyglowski, K., Duc, G., Kaneko, T., Tabata, S., de Bruijn, F., Pajuelo, E., Sandal, N. & Stougaard, J. (2002). Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature,* Vol. 420, No. 6914, pp. 422-426

Kuster, H., Vieweg, M. F., Manthey, K., Baier, M. C., Hohnjec, N. & Perlick, A. M. (2007). Identification and expression regulation of symbiotically activated legume genes. *Phytochemistry,* Vol. 68, No. 1, pp. 8-18

Kuzma, M. M., Topunov, A. F. & Layzell, D. B. (1995). Effects of Temperature on Infected Cell O2 Concentration and Adenylate Levels in Attached Soybean Nodules. *Plant Physiol,* Vol. 107, No. 4, pp. 1209-1216

Larrainzar, E., Wienkoop, S., Weckwerth, W., Ladrera, R., Arrese-Igor, C. & Gonzalez, E. M. (2007). *Medicago truncatula* root nodule proteome analysis reveals differential plant and bacteroid responses to drought stress. *Plant Physiol,* Vol. 144, No. 3, pp. 1495-1507

Lawn, R. J. & Brun, W. A. (1977). Symbiotic Nitrogen Fixation in Soybeans. I. Effect of Photosynthetic Source-Sink Manipulations1. *Crop Sci.,* Vol. 14, No. 1, pp. 11

Lehtovaara, P. & Perttila, U. (1978). Bile-pigment formation from different leghaemoglobins. Methine-bridge specificity of coupled oxidation. *Biochem J,* Vol. 176, No. 2, pp. 359-364

Li, Y., Zhou, L., Li, Y., Chen, D., Tan, X., Lei, L. & Zhou, J. (2008). A nodule-specific plant cysteine proteinase, AsNODF32, is involved in nodule senescence and nitrogen fixation activity of the green manure legume Astragalus sinicus. *New Phytol,* Vol. 180, No. 1, pp. 185-192

Limpens, E., Ivanov, S., van Esse, W., Voets, G., Fedorova, E. & Bisseling, T. (2009). Medicago N2-fixing symbiosomes acquire the endocytic identity marker Rab7 but delay the acquisition of vacuolar identity. *Plant Cell,* Vol. 21, No. 9, pp. 2811-2828

Lister, R., Gregory, B. D. & Ecker, J. R. (2009). Next is now: new technologies for sequencing of genomes, transcriptomes, and beyond. *Curr Opin Plant Biol,* Vol. 12, No. 2, pp. 107-118

Liu, C. T., Lee, K. B., Wang, Y. S., Peng, M. H., Lee, K. T., Suzuki, S., Suzuki, T. & Oyaizu, H. (2011). Involvement of the azorhizobial chromosome partition gene (parA) in the onset of bacteroid differentiation during Sesbania rostrata stem nodule development. *Applied and environmental microbiology,* Vol. 77, No. 13, pp. 4371-4382

Lodwig, E. & Poole, P. S. (2003). Metabolism of Rhizobium bacteroids. *Crit Rev Plant Sci*, Vol. 22, No.1, pp. 37-78

Lohman, K. N., Gan, S., John, M. C. & Amasino, R. M. (1994). Molecular analysis of natural leaf senescence in Arabidopsis thaliana. *Physiologia Plantarum,* Vol. 92, No. 2, pp. 322

Long, S. R. (2001). Genes and signals in the rhizobium-legume symbiosis. *Plant Physiol,* Vol. 125, No. 1, pp. 69-72

Lopez, M., Herrera-Cervera, J. A., Iribarne, C., Tejera, N. A. & Lluch, C. (2008). Growth and nitrogen fixation in *Lotus japonicus* and *Medicago truncatula* under NaCl stress: Nodule carbon metabolism. *J Plant Physiol,* Vol. 165, No. 6, pp. 641-650

Lopez, M., Tejera, N. A. & Lluch, C. (2009). Validamycin A improves the response of Medicago truncatula plants to salt stress by inducing trehalose accumulation in the root nodules. *J Plant Physiol,* Vol. 166, No. 11, pp. 1218-1222

Loscos, J., Matamoros, M. A. & Becana, M. (2008). Ascorbate and homoglutathione metabolism in common bean nodules under stress conditions and during natural senescence. *Plant Physiol,* Vol. 146, No. 3, pp. 1282-1292

Lucas, M. M., Van de Sype, G., Hérouart, D., Hernandez, M. J., Puppo, A. & de Felipe, M. R. (1998). Immunolocalization of ferritin in determinate and indeterminate legume root nodules. *Protoplasma,* Vol. 204, No. 1, pp. 61-70

Luo, L., Yao, S. Y., Becker, A., Ruberg, S., Yu, G. Q., Zhu, J. B. & Cheng, H. P. (2005). Two new Sinorhizobium meliloti LysR-type transcriptional regulators required for nodulation. *J Bacteriol,* Vol. 187, No. 13, pp. 4562-4572

L'Taief, B., Sifi, B., Zaman-Allah, M., Drevon, J. J. & Lachaal, M. (2007). Effect of salinity on root-nodule conductance to the oxygen diffusion in the *Cicer arietinum-Mesorhizobium ciceri* symbiosis. *J Plant Physiol,* Vol. 164, No. 8, pp. 1028-1036

Malik, N. S., Pfeiffer, N. E., Williams, D. R. & Wagner, F. W. (1981). Peptidohydrolases of Soybean Root Nodules: Identification, separation and partial characterisation of enzymes from bacteroid-free extracts. *Plant Physiol,* Vol. 68, No. 2, pp. 386-392

Marino, D., Gonzalez, E. M. & Arrese-Igor, C. (2006). Drought effects on carbon and nitrogen metabolism of pea nodules can be mimicked by paraquat: evidence for the occurrence of two regulation pathways under oxidative stresses. *J Exp Bot,* Vol. 57, No. 3, pp. 665-673

Marino, D., Frendo, P., Ladrera, R., Zabalza, A., Puppo, A., Arrese-Igor, C. & Gonzalez, E. M. (2007). Nitrogen fixation control under drought stress. Localized or systemic? *Plant Physiol,* Vol. 143, No. 4, pp. 1968-1974

Marino, D., Hohnjec, N., Kuster, H., Moran, J. F., Gonzalez, E. M. & Arrese-Igor, C. (2008). Evidence for transcriptional and post-translational regulation of sucrose synthase in pea nodules by the cellular redox state. *Mol Plant Microbe Interact,* Vol. 21, No. 5, pp. 622-630

Marino, D., Pucciariello, C., Puppo, A. & Frendo, P. (2009).The Redox State, a Referee of the Legume-Rhizobia Symbiotic Game. In *Advances in Botanical Research: Oxidative Stress and Redox Regulation in Plants*, J. P. Jacquot, pp. 115-151, Burlington: Academic Press

Matamoros, M. A., Baird, L. M., Escuredo, P. R., Dalton, D. A., Minchin, F. R., Iturbe-Ormaetxe, I., Rubio, M. C., Moran, J. F., Gordon, A. J. & Becana, M. (1999). Stress induced legume root nodule senescence. Physiological, biochemical and structural alterations. *Plant Physiol,* Vol. 121, No.1, pp. 97-112

Maunoury, N., Kondorosi, A., Kondorosi, E. & Mergaert, P. (2008).Cell biology of nodule infection and development. In *Nitrogen-fixing Leguminous Symbioses* E. K. James, J. I. Sprent, W. E. Dilworth and N. W.E., pp. 153–189, Springer, the Netherlands

Maunoury, N., Redondo-Nieto, M., Bourcy, M., Van de Velde, W., Alunni, B., Laporte, P., Durand, P., Agier, N., Marisa, L., Vaubert, D., Delacroix, H., Duc, G., Ratet, P., Aggerbeck, L., Kondorosi, E. & Mergaert, P. (2010). Differentiation of symbiotic cells and endosymbionts in Medicago truncatula nodulation are coupled to two transcriptome-switches. *PLoS One,* Vol. 5, No. 3, pp. e9519

Mergaert, P., Nikovics, K., Kelemen, Z., Maunoury, N., Vaubert, D., Kondorosi, A. & Kondorosi, E. (2003). A novel family in Medicago truncatula consisting of more than 300 nodule-specific genes coding for small, secreted polypeptides with conserved cysteine motifs. *Plant Physiol,* Vol. 132, No. 1, pp. 161-173

Mergaert, P., Uchiumi, T., Alunni, B., Evanno, G., Cheron, A., Catrice, O., Mausset, A. E., Barloy-Hubler, F., Galibert, F., Kondorosi, A. & Kondorosi, E. (2006). Eukaryotic control on bacterial cell cycle and differentiation in the Rhizobium-legume symbiosis. *Proc Natl Acad Sci U S A,* Vol. 103, No. 13, pp. 5230-5235

Mhadhbi, H., Fotopoulos, V., Mylona, P. V., Jebara, M., Elarbi Aouani, M. & Polidoros, A. N. (2011). Antioxidant gene-enzyme responses in Medicago truncatula genotypes with different degree of sensitivity to salinity. *Physiol Plant,* Vol. 141, No. 3, pp. 201-214

Minchin, F. R., Minguez, M. I., Sheehy, J. E., Witty, J. F. & SkÃ˜T, L. (1986). Relationships Between Nitrate and Oxygen Supply in Symbiotic Nitrogen Fixation by White Clover. *J Ex Bot,* Vol. 37, No. 8, pp. 1103-1113

Minchin, F. R., Becana, M. & Sprent, J. I. (1989). Short-term inhibition of legume N_2 fixation by nitrate. II. Nitrate effects on nodule oxygen diffusion. *Planta,* Vol. 180, No.1, pp. 46-52

Mitsui, H., Sato, T., Sato, Y., Ito, N. & Minamisawa, K. (2004). Sinorhizobium meliloti RpoH1 is required for effective nitrogen-fixing symbiosis with alfalfa. *Mol Genet Genomics,* Vol. 271, No. 4, pp. 416-425

Moris, M., Braeken, K., Schoeters, E., Verreth, C., Beullens, S., Vanderleyden, J. & Michiels, J. (2005). Effective symbiosis between Rhizobium etli and Phaseolus vulgaris requires the alarmone ppGpp. *J Bacteriol,* Vol. 187, No. 15, pp. 5460-5469

Mortier, V., Holsters, M. & Goormachtig, S. (2011). Never too many? How legumes control nodule numbers. *Plant Cell Environ,* in press

Muglia, C., Comai, G., Spegazzini, E., Riccillo, P. M. & Aguilar, O. M. (2008). Glutathione produced by Rhizobium tropici is important to prevent early senescence in common bean nodules. *FEMS microbiology letters,* Vol. 286, No. 2, pp. 191-198

Naito, Y., Fujie, M., Usami, S., Murooka, Y. & Yamada, T. (2000). The involvement of a cysteine proteinase in the nodule development in Chinese milk vetch infected with Mesorhizobium huakuii subsp. rengei. *Plant Physiol,* Vol. 124, No. 3, pp. 1087-1096

Naya, L., Ladrera, R., Ramos, J., Gonzalez, E. M., Arrese-Igor, C., Minchin, F. R. & 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 Physiol,* Vol. 144, No. 2, pp. 1104-1114

Novak, K. (2010). Early action of pea symbiotic gene NOD3 is confirmed by adventitious root phenotype. *Plant Sci,* Vol. 179, No. 5, pp. 472-478

Oldroyd, G. E., Murray, J. D., Poole, P. S. & Downie, J. A. (2011). The Rules of Engagement in the Legume-Rhizobial Symbiosis. *Annu Rev Genet,* Vol.45, in press

Ott, T., van Dongen, J. T., Gunther, C., Krusell, L., Desbrosses, G., Vigeolas, H., Bock, V., Czechowski, T., Geigenberger, P. & Udvardi, M. K. (2005). Symbiotic leghemoglobins are crucial for nitrogen fixation in legume root nodules but not for general plant growth and development. *Curr Biol*,Vol. 15, No. 6, pp. 531-535

Ott, T., Sullivan, J., James, E. K., Flemetakis, E., Gunther, C., Gibon, Y., Ronson, C. & Udvardi, M. (2009). Absence of symbiotic leghemoglobins alters bacteroid and plant cell differentiation during development of Lotus japonicus root nodules. *Mol Plant Microbe Interact,* Vol. 22, No. 7, pp. 800-808

Pauly, N., Pucciariello, C., Mandon, K., Innocenti, G., Jamet, A., Baudouin, E., Herouart, D., Frendo, P. & Puppo, A. (2006). Reactive oxygen and nitrogen species and glutathione: key players in the legume-Rhizobium symbiosis. *J Exp Bot,* Vol. 57, No. 8, pp. 1769-1776

Penmetsa, R. V., Frugoli, J. A., Smith, L. S., Long, S. R. & Cook, D. R. (2003). Dual genetic pathways controlling nodule number in Medicago truncatula. *Plant Physiol,* Vol. 131, No. 3, pp. 998-1008

Perez Guerra, J. C., Coussens, G., De Keyser, A., De Rycke, R., De Bodt, S., Van De Velde, W., Goormachtig, S. & Holsters, M. (2010). Comparison of developmental and stress-induced nodule senescence in Medicago truncatula. *Plant Physiol,* Vol. 152, No. 3, pp. 1574-1584

Perret, X., Staehelin, C. & Broughton, W. J. (2000). Molecular basis of symbiotic promiscuity. *Microbiol Mol Biol Rev,* Vol. 64, No. 1, pp. 180-201

Pessi, G., Ahrens, C. H., Rehrauer, H., Lindemann, A., Hauser, F., Fischer, H. M. & Hennecke, H. (2007). Genome-wide transcript analysis of Bradyrhizobium japonicum bacteroids in soybean root nodules. *Mol Plant Microbe Interact,* Vol. 20, No. 11, pp. 1353-1363

Pfeiffer, N. E., Torres, C. M. & Wagner, F. W. (1983). Proteolytic Activity in Soybean Root Nodules : Activity in Host Cell Cytosol and Bacteroids throughout Physiological Development and Senescence. *Plant Physiol,* Vol. 71, No. 4, pp. 797-802

Pladys, D. & Rigaud, J. (1985). Senescence in French-bean nodules: Occurrence of different proteolytic activities. *Physiologia Plantarum,* Vol. 63, No. 1, pp. 43

Pladys, D., Dimitrijevic, L. & Rigaud, J. (1991). Localization of a protease in protoplast preparations in infected cells of French bean nodules. *Plant Physiol,* Vol. 97, No. 3, pp. 1174-1180

Pladys, D. & Vance, C. P. (1993). Proteolysis during Development and Senescence of Effective and Plant Gene-Controlled Ineffective Alfalfa Nodules. *Plant Physiol,* Vol. 103, No. 2, pp. 379-384

Prell, J. & Poole, P. (2006). Metabolic changes of rhizobia in legume nodules. *Trends Microbiol* Vol. 14, No. 4, pp. 161-168

Prell, J., White, J. P., Bourdes, A., Bunnewell, S., Bongaerts, R. J. & Poole, P. S. (2009). Legumes regulate Rhizobium bacteroid development and persistence by the supply of branched-chain amino acids. *Proc Natl Acad Sci USA*,Vol. 106, No. 30, pp. 12477-12482

Puppo, A., Rigaud, J. & Job, D. (1981). Role of superoxide anion in leghemoglobin autoxidation. *Plant Science Letters,* Vol. 22, No. 4, pp. 353

Puppo, A. & Halliwell, B. (1988). Formation of hydroxyl radicals from hydrogen peroxide in the presence of iron. Is haemoglobin a biological Fenton reagent? *Biochem J,* Vol. 249, No. 1, pp. 185-190

Puppo, A., Groten, K., Bastian, F., Carzaniga, R., Soussi, M., Lucas, M. M., de Felipe, M. R., Harrison, J., Vanacker, H. & Foyer, C. H. (2005). Legume nodule senescence: roles for redox and hormone signalling in the orchestration of the natural aging process. *New Phytol,* Vol. 165, No. 3, pp. 683-701

Raghavendra, A. S., Gonugunta, V. K., Christmann, A. & Grill, E. (2010). ABA perception and signalling. *Trends Plant Sci,* Vol. 15, No. 7, pp. 395-401

Redondo, F. J., de la Pena, T. C., Morcillo, C. N., Lucas, M. M. & Pueyo, J. J. (2009). Overexpression of flavodoxin in bacteroids induces changes in antioxidant metabolism leading to delayed senescence and starch accumulation in alfalfa root nodules. *Plant Physiol*,Vol. 149, No. 2, pp. 1166-1178

Reinbothe, C., Springer, A., Samol, I. & Reinbothe, S. (2009). Plant oxylipins: role of jasmonic acid during programmed cell death, defence and leaf senescence. *Febs J,* Vol. 276, No. 17, pp. 4666-4681

Reyrat, J. M., David, M., Blonski, C., Boistard, P. & Batut, J. (1993). Oxygen-regulated in vitro transcription of Rhizobium meliloti nifA and fixK genes. *J Bacteriol,* Vol. 175, No. 21, pp. 6867-6872

Rubio, M. C., Gonzalez, E. M., Minchin, F. R., Webb, K. J., Arrese-Igor, C., Ramos, J. & Becana, M. (2002). Effects of water stress on antioxidant enzymes of leaves and nodules of transgenic alfalfa overexpressing superoxide dismutases. *Physiol Plant,* Vol. 115, No. 4, pp. 531-540

Saeki, K. (2011). Rhizobial measures to evade host defense strategies and endogenous threats to persistent symbiotic nitrogen fixation: a focus on two legume-rhizobium model systems. *Cell Mol Life Sci,* Vol. 68, No. 8, pp. 1327-1339

Sanchez, D. H., Pieckenstain, F. L., Escaray, F., Erban, A., Kraemer, U., Udvardi, M. K. & Kopka, J. (2011). Comparative ionomics and metabolomics in extremophile and glycophytic Lotus species under salt stress challenge the metabolic pre-adaptation hypothesis. *Plant Cell Environ,* Vol. 34, No. 4, pp. 605-617

Santos, R., Herouart, D., Puppo, A. & Touati, D. (2000). Critical protective role of bacterial superoxide dismutase in rhizobium-legume symbiosis. *Molecular microbiology,* Vol. 38, No. 4, pp. 750-759

Sato, S., Nakamura, Y., Kaneko, T., Asamizu, E., Kato, T., Nakao, M., Sasamoto, S., Watanabe, A., Ono, A., Kawashima, K., Fujishiro, T., Katoh, M., Kohara, M., Kishida, Y., Minami, C., Nakayama, S., Nakazaki, N., Shimizu, Y., Shinpo, S., Takahashi, C., Wada, T., Yamada, M., Ohmido, N., Hayashi, M., Fukui, K., Baba, T., Nakamichi, T., Mori, H. & Tabata, S. (2008). Genome structure of the legume, Lotus japonicus. *DNA Res*,Vol. 15, No. 4, pp. 227-239

Schauser, L., Udvardi, M., Tabata, S. & Stougaard, J. (2008).Legume genomics relevant to N_2 fixation. In *Nitrogen-fixing Leguminous Symbioses*, S. J. James EK, Dilworth WE, Newton WE, pp. 211–233, Springer, the Netherlands

Schell, M. A. (1993). Molecular biology of the LysR family of transcriptional regulators. *Annu Rev Microbiol,* Vol. 47, pp. 597-626

Schmutz, J., Cannon, S. B., Schlueter, J., Ma, J., Mitros, T., Nelson, W., Hyten, D. L., Song, Q., Thelen, J. J., Cheng, J., Xu, D., Hellsten, U., May, G. D., Yu, Y., Sakurai, T.,

Umezawa, T., Bhattacharyya, M. K., Sandhu, D., Valliyodan, B., Lindquist, E., Peto, M., Grant, D., Shu, S., Goodstein, D., Barry, K., Futrell-Griggs, M., Abernathy, B., Du, J., Tian, Z., Zhu, L., Gill, N., Joshi, T., Libault, M., Sethuraman, A., Zhang, X. C., Shinozaki, K., Nguyen, H. T., Wing, R. A., Cregan, P., Specht, J., Grimwood, J., Rokhsar, D., Stacey, G., Shoemaker, R. C. & Jackson, S. A. (2010). Genome sequence of the palaeopolyploid soybean. *Nature,* Vol. 463, No. 7278, pp. 178-183

Schneiker-Bekel, S., Wibberg, D., Bekel, T., Blom, J., Linke, B., Neuweger, H., Stiens, M., Vorholter, F. J., Weidner, S., Goesmann, A., Puhler, A. & Schluter, A. (2011). The complete genome sequence of the dominant Sinorhizobium meliloti field isolate SM11 extends the S. meliloti pan-genome. *J Biotechnol,* Vol. 155, No. 1, pp. 20-33

Searle, I. R., Men, A. E., Laniya, T. S., Buzas, D. M., Iturbe-Ormaetxe, I., Carroll, B. J. & Gresshoff, P. M. (2003). Long-distance signaling in nodulation directed by a CLAVATA1-like receptor kinase. *Science,* Vol. 299, No. 5603, pp. 109-112

Serraj, R. & Sinclair, T. R. (1996). Inhibition of nitrogenase activity and oxygen permeability by water deficit. *J Exp Bot,* Vol. 47, No., pp. 1067-1073

Serraj, R., Vadez, V. V., Denison, R. F. & Sinclair, T. R. (1999). Involvement of ureides in nitrogen fixation inhibition in soybean. *Plant Physiol,* Vol. 119, No. 1, pp. 289-296

Sigaud, S., Becquet, V., Frendo, P., Puppo, A. & Herouart, D. (1999). Differential regulation of two divergent Sinorhizobium meliloti genes for HPII-like catalases during free-living growth and protective role of both catalases during symbiosis. *J Bacteriol,* Vol. 181, No. 8, pp. 2634-2639

Sobrevals, L., Muller, P., Fabra, A. & Castro, S. (2006). Role of glutathione in the growth of Bradyrhizobium sp. (peanut microsymbiont) under different environmental stresses and in symbiosis with the host plant. *Canadian journal of microbiology,* Vol. 52, No. 7, pp. 609-616

Soussi, M., Ocana, A. & Lluch, C. (1998). Effects of salt stress on growth, photosynthesis and nitrogen fixation in chick-pea (Cicer arietinum L.). *J Ex Bot,* Vol. 49, No. 325, pp. 1329-1337

Streeter, J. & Wong, P. P. (1988). Inhibition of legume nodule formation and N2 fixation by nitrate. *Critical Reviews in Plant Sciences,* Vol. 7, No. 1, pp. 1-23

Swaraj, K., Dhandi, S. & Sheokand, S. (1995). Relationship between defense mechanism against activated oxygen species and nodule functioning with progress in plant and nodule development in Cajanus cajan L. Millsp. *Plant Sci,* Vol. 112, No. 1, pp. 65

Swaraj, K. & Bishnoi, N. R. (1999). Effect of salt stress on nodulation and nitrogen fixation in legumes. *Indian J Exp Biol,* Vol. 37, No. 9, pp. 843-848

Thomas, S. G., Phillips, A. L. & Hedden, P. (1999). Molecular cloning and functional expression of gibberellin 2- oxidases, multifunctional enzymes involved in gibberellin deactivation. *Proc Natl Acad Sci U S A*,Vol. 96, No. 8, pp. 4698-4703

Timmers, A. C., Soupene, E., Auriac, M. C., de Billy, F., Vasse, J., Boistard, P. & Truchet, G. (2000). Saprophytic intracellular rhizobia in alfalfa nodules. *Mol Plant Microbe Interact,* Vol. 13, No. 11, pp. 1204-1213

Torres-Quesada, O., Oruezabal, R. I., Peregrina, A., Jofre, E., Lloret, J., Rivilla, R., Toro, N. & Jimenez-Zurdo, J. I. (2010). The Sinorhizobium meliloti RNA chaperone Hfq influences central carbon metabolism and the symbiotic interaction with alfalfa. *BMC microbiology,* Vol. 10, pp. 71

Van de Velde, W., Guerra, J. C., De Keyser, A., De Rycke, R., Rombauts, S., Maunoury, N., Mergaert, P., Kondorosi, E., Holsters, M. & Goormachtig, S. (2006). Aging in legume symbiosis. A molecular view on nodule senescence in Medicago truncatula. *Plant Physiol,* Vol. 141, No. 2, pp. 711-720

Van de Velde, W., Zehirov, G., Szatmari, A., Debreczeny, M., Ishihara, H., Kevei, Z., Farkas, A., Mikulass, K., Nagy, A., Tiricz, H., Satiat-Jeunemaitre, B., Alunni, B., Bourge, M., Kucho, K., Abe, M., Kereszt, A., Maroti, G., Uchiumi, T., Kondorosi, E. & Mergaert, P. (2010). Plant peptides govern terminal differentiation of bacteria in symbiosis. *Science,* Vol. 327, No. 5969, pp. 1122-1126

van Heerden, P. D., Kiddle, G., Pellny, T. K., Mokwala, P. W., Jordaan, A., Strauss, A. J., de Beer, M., Schluter, U., Kunert, K. J. & Foyer, C. H. (2008). Roles for the regulation of respiration and the oxygen diffusion barrier in soybean in the protection of symbiotic nitrogen fixation from chilling -induced inhibition and shoots from premature senescence. *Plant Physiol,* Vol. 148, No. 1, pp. 316-327

Vasse, J., de Billy, F., Camut, S. & Truchet, G. (1990). Correlation between ultrastructural differentiation of bacteroids and nitrogen fixation in alfalfa nodules. *J Bacteriol,* Vol. 172, No. 8, pp. 4295-4306

Vauclare, P., Bligny, R., Gout, E., De Meuron, V. & Widmer, F. (2010). Metabolic and structural rearrangement during dark-induced autophagy in soybean (Glycine max L.) nodules: an electron microscopy and 31P and 13C nuclear magnetic resonance study. *Planta,* Vol. 231, No. 6, pp. 1495-1504

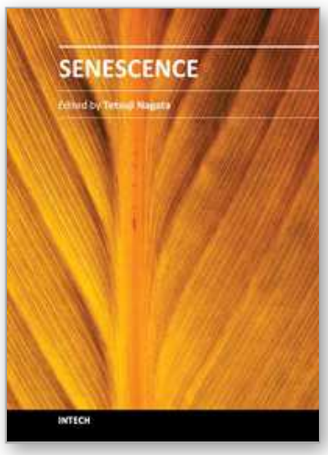
Vessey, J. K., Walsh, K. B. & Layzell, D. B. (1988). Oxygen limitation of nitrogen fixation in stem-girdled and nitrate-treated soybean. *Physiol Plant,* Vol. 73, No., pp. 113-121

Walsh, K. B. & Layzell, D. B. (1986). Carbon and nitrogen assimilation and partitioning in soybeans exposed to low root temperatures. *Plant Physiol,* Vol. 80, No. 1, pp. 249-255

Webb, C. J., Chan-Weiher, C. & Johnson, D. A. (2008). Isolation of a novel family of genes related to 2-oxoglutarate-dependent dioxygenases from soybean and analysis of their expression during root nodule senescence. *J Plant Physiol,* Vol. 165, No. 16, pp. 1736-1744

White, J., Prell, J., James, E. K. & Poole, P. (2007). Nutrient sharing between symbionts. *Plant Physiol,* Vol. 144, No. 2, pp. 604-614

Zurbriggen, M. D., Tognetti, V. B., Fillat, M. F., Hajirezaei, M. R., Valle, E. M. & Carrillo, N. (2008). Combating stress with flavodoxin: a promising route for crop improvement. *Trends Biotechnol,* Vol. 26, No. 10, pp. 531-537



Senescence
Edited by Dr. Tetsuji Nagata

ISBN 978-953-51-0144-4
Hard cover, 850 pages
Publisher InTech
Published online 29, February, 2012
Published in print edition February, 2012

The book "Senescence" is aimed to describe all the phenomena related to aging and senescence of all forms of life on Earth, i.e. plants, animals and the human beings. The book contains 36 carefully reviewed chapters written by different authors, aiming to describe the aging and senescent changes of living creatures, i.e. plants and animals.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Laurence Dupont, Geneviève Alloing, Olivier Pierre, Sarra El Msehli, Julie Hopkins, Didier Hérouart and Pierre Frendo (2012). The Legume Root Nodule: From Symbiotic Nitrogen Fixation to Senescence, Senescence, Dr. Tetsuji Nagata (Ed.), ISBN: 978-953-51-0144-4, InTech, Available from: http://www.intechopen.com/books/senescence/the-legume-root-nodule-from-symbiotic-nitrogen-fixation-to-senescence

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen