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Defense and senescence interplay in legume nodules

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ABSTRACT

Immunity and senescence play a crucial role in the functioning of the legume symbiotic nodules. The misregulation of one of these processes compromises the symbiosis leading to death of the endosymbiont and the arrest of the nodule functioning. The relationship between immunity and senescence has been extensively studied in plant organs where a synergistic response can be observed. However, the interplay between immunity and senescence in the symbiotic organ is poorly discussed in the literature and these phenomena are often mixed up. Recent studies revealed that the cooperation between immunity and senescence is not always observed in the nodule, suggesting complex interactions between these two processes within the symbiotic organ. Here, we discuss recent results on the interplay between immunity and senescence in the nodule and the specificities of this relationship during legume-rhizobium symbiosis.

Key words: immunity, senescence, symbiosis, nodules, legumes, rhizobia

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INTRODUCTION

When assimilable nitrogen is limited in the environment, leguminous plants have the ability to establish a symbiosis with nitrogen-fixing soil bacteria, called rhizobia, and to form dedicated symbiotic root organs, the nodules, which are chronically infected by thousands of bacteroids (the intracellular forms of the rhizobium embedded into the symbiosome). Inside the host cell, bacteroids convert atmospheric nitrogen (N₂) into NH₄⁺ thanks to the activity of the nitrogenase enzyme (Yang et al., 2022). Due to the high oxygen (O₂) sensitivity of the nitrogenase, the nitrogen fixation occurs under low concentration of O₂ controlled by a legume protein, the leghemoglobin (Larrainzar et al., 2020). Leghemoglobin is a marker of functional nodules, giving to this organ its characteristic pink color (Pislariu et al., 2012). Two major morphotypes of nodules exist: (1) the determinate nodules as those formed by *Lotus japonicus*, *Phaseolus vulgaris*, and *Glycine max* showing a spherical shape due to the non-persistent nature of the meristem and (2) the indeterminate nodules formed by plants such as *Medicago* and *Pisum* species that have an elongated shape due to a persistent meristem located in the nodule apex (Hirsch, 1992).

Legume species belonging to the Inverted Repeat Lacking Clade (IRLC) including *Medicago* species produce NODULE-SPECIFIC CYSTEINE-RICH (NCR) peptides specifically expressed in the nodule. Prior to nitrogen-fixation, NCRs trigger the Terminal Differentiation of the Bacteroids (TDB) by inducing genome endoreduplication together with an increased size of the endosymbiont and the inhibition of bacterial cell division (Montiel et al., 2017). In addition, NCR247 can also promote symbiosis through the control of iron homeostasis of the symbiotic bacteria (Sankari et al., 2022). Due to the lack of the NCR genes, most leguminous plants outside the IRLC including *L. japonicus* and *G. max*, produce nodules containing fixing-nitrogen bacteroids, which are non-terminally differentiated (Czernic et al., 2015; Alunni and Gourion, 2016). This absence of the TDB is associated to a reduction of the symbiont efficiency in these species (Oono and Denison, 2010; Alunni and Gourion, 2016). The level of bacteroid differentiation can reflect the symbiotic

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Plant Communications

process achievement in the IRLC clade. As an example, the absence of bacteroid differentiation is associated with an early symbiotic arrest in legume inducing TDB (Berrabah et al., 2018a).

Inside the host cell, rhizobium survival is linked to the activity of nodule-specific genes, namely *NODULINS*, some of which are involved in the control of nodule immunity. Several genes controlling nodule defense and/or senescence have been characterized in *Medicago littoralis* (formerly called *M. truncatula* R108 ecotype) (Choi et al., 2022) such as *DEFECTIVE IN NITROGEN FIXATION 2 (DNF2)* (Bourcy et al., 2013), *Symbiotic CYSTEINE-RICH RECEPTOR-LIKE KINASE (SymCRK)* (Berrabah et al., 2014a), *REGULATOR OF SYMBIOSOME DIFFERENTIATION (RSD)* (Sinharoy et al., 2013), *NODULES WITH ACTIVATED DEFENSE 1 (NAD1)* (Wang et al., 2016), and *NODULE INCEPTION (NIN)* (Liu et al., 2021). They correspond respectively to a phospholipase-C phosphatidyl-dependent protein-like, a cysteine-rich receptor kinase, a C₂H₂ transcription factor (TF), a transmembrane protein, and a symbiotic TF. These genes coordinate their action to control nodule immunity, as an example *DNF2*, *SymCRK*, and *RSD* act sequentially during nodule development to repress defense (Berrabah et al., 2015). Studies of the bacteroid differentiation process revealed that plants lacking *NAD1* (Wang et al., 2016; Domonkos et al., 2017), *DNF2* (Bourcy et al., 2013), *SymCRK* (Berrabah et al., 2014a), and *RSD* (Sinharoy et al., 2013) display symbiotic arrest early in the TDB process. Interestingly, identification of the signal involved in the activation of defense in *dnf2*, *symCRK*, and *rsd* nodules revealed that *DNF2* prevents defenses in response to environmental elicitors (Berrabah et al., 2014b), whereas *SymCRK* and *RSD* repress the immunity in response to bacterial infection and/or bacteroid differentiation (Berrabah et al., 2015).

The legume-rhizobium interaction is energy-consuming and the host can tightly control malfunctioning nodules by activating senescence (Booth et al., 2021; Zhou et al., 2021). The senescence can be defined as a phenomenon of decline leading to the end of cell functions with progressive destruction and nutrient recycling from senescing plant tissues (Gan, 2018). The senescence naturally occurs when nodule efficiency decreases with aging (developmental senescence) but can also be induced in response to stress or in the presence of nitrogen in the environment (induced senescence) (Berrabah et al., 2018a; Zhou et al., 2021). Inactivation of genes required for nodule metabolism and functioning such as iron (Walton et al., 2020) and sulfate transport (Ma et al., 2023) or involved in the TDB (Horvath et al., 2015, 2023; Kim et al., 2015) also results in the formation of non-fixing nitrogen (fix-) nodules with early senescence features. The senescence in the nodule aims to adjust the metabolic balance between the carbon (C) provided by the host to the symbiont and the nitrogen (N) fixed by the bacteroid (Laguerre et al., 2012; Staudinger et al., 2016; Lambert et al., 2020) and can occur differentially according to the nodule and the senescence types (developmental or induced). In determinate nodules the senescence starts from the central zone and extends outwards, whereas in indeterminate nodules the senescent zone develops at the nodule base, harbors a cylindrical shape in the center of the nodule, and spreads to the periphery (Puppo et al., 2005; Van de Velde et al., 2006; Pérez Guerra et al., 2010). The senescence is

Defense and senescence interplay in legume nodules

associated with the oxidation of the leghemoglobin resulting in a greenish coloration of senescent nodules (Xia, 2018). In addition, during this process, the induction of specific genes, the *CYSTEINE PROTEASES (CPs)*, occurs. These genes are involved in cell content recycling and potentially in bacteroid degradation (Pierre et al., 2014).

Beside senescence, immunity is also an essential process controlling the symbiotic interaction (Gourion et al., 2015; Berrabah et al., 2019). Immune responses arise in nodules upon some treatments such as wounding (Berrabah et al., 2023), boron deficiency (Reguera et al., 2010), or in mutants defective in genes that control immunity and chronic infection (Bourcy et al., 2013; Sinharoy et al., 2013; Berrabah et al., 2014a; Wang et al., 2016; Liu et al., 2021). Suppression of defenses is therefore required for nodule functioning. Interestingly, senescence and immunity start from the host infected cells and spread to uninfected cells (Van de Velde et al., 2006; Berrabah et al., 2018b); however, unlike the senescence that leads to a progressive destruction and recycling of cell components, the immune response is generally a rapid reaction leading to an increased expression of defense and stress-response genes like *PATHOGENESIS-RELATED PROTEINS (PRs)* and the secretion of defense compounds like phenols or phytoalexins (Naoumkina et al., 2007; Miller et al., 2017).

Immunity and senescence play a crucial role in nodule functioning and deregulation of these processes can lead to the suppression of the bacteroid and the arrest of the nodule development or functioning. However, the relationship between immunity and senescence is poorly discussed in the literature. In this review, we discuss the types of interactions occurring between immunity and senescence in the nodule in association with genetic and physiological factors that control or influence this relationship.

SENESCENCE AND IMMUNITY INDUCE DISTINCT PHYSIOLOGICAL RESPONSES IN THE NODULE

Due to the difficulty to distinguish between senescence or defense responses in the nodule, authors often confuse them in the literature. Defense and senescence markers are generally used to assess these responses in the symbiotic organ. Senescence is evaluated by analyzing the expression of genes involved in cell recycling such as the *CPs* (Wyk et al., 2014; Vorster et al., 2019), such as *CP2* to *CP6* genes, which are specific of the nodule senescence in *M. truncatula* (Pérez Guerra et al., 2010; Zhou et al., 2021; Sauviac et al., 2022). By contrast, the expression of various defense genes (Table 1) like *PRs* is used to evaluate the nodule defense status. However, defense genes used in these analyses can be different from one publication to another due to the fluctuation of gene expression depending on culture conditions and nodule age (Bourcy et al., 2013; Berrabah et al., 2023). Moreover, the expression level of certain defense genes like *PRs* is frequently higher than that of *CPs* in the nodule (Berrabah et al., 2023; Nguyen et al., 2023), which sometimes leads to confusion about the activated process. It is thus important to select multiple and strongly expressed defense markers or to perform transcriptomic analyses to

Gene ID	Genotype	Abbreviation	Full name	Reference
Medtr4g076470	Medicago sp.	BGL	β 1-3 glucanase	Berrabah et al. (2014a)
Medtr3g080050	Medicago sp.	CERK1-like	Chitin Elicitor Receptor Kinase 1	Wang et al. (2016)
Medtr3g118390	Medicago sp.	Chitinase	Chitinase Class I	Berrabah et al. (2014a)
Medtr6g007763	Medicago sp.	DOX	Pathogen-inducible α -dioxxygenase	Wang et al. (2016)
Medtr2g084010	Medicago sp.	Lip	Lignin Biosynthetic Peroxidase	Wang et al. (2016)
Medtr2g008360	Medicago sp.	MtBAK1	BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1	Wang et al. (2016)
Medtr4g094610	Medicago sp.	MtFLS2	Flageline Sensing 2	Wang et al. (2016)
Medtr5g076170	Medicago sp.	NDR1	NONRACE-SPECIFIC DISEASE RESISTANCE1	Berrabah et al. (2014a)
Medtr1g064090	Medicago sp.	PAL	Phenlyalanine Amonia-Lyase	Berrabah et al. (2014a)
Medtr4g128750	Medicago sp.	PR1	Pathogenesis-related protein 1	Wang et al. (2016)
Medtr2g035150	Medicago sp.	PR10	Pathogenesis-related protein 10	Bourcy et al. (2013)
Medtr6g033450	Medicago sp.	PR10	Pathogenesis-related protein 10	Berrabah et al. (2023)
Medtr4g120970	Medicago sp.	PR10	Pathogenesis-related protein 10	Berrabah et al. (2023)
Medtr5g010640	Medicago sp.	PR5	Pathogenesis-related protein 5	Berrabah et al. (2023)
Medtr8g096910	Medicago sp.	PR5	Pathogenesis-related protein 5	Berrabah et al. (2023)
Medtr1g099310	Medicago sp.	PR8	Pathogenesis-related protein 8	Berrabah et al. (2023)
Medtr5g027860	Medicago sp.	R gene	CC-NBS-LRR	Wang et al. (2016)
Medtr4g091380	Medicago sp.	R gene	LRR - NBS	Wang et al. (2016)
Medtr8g470390	Medicago sp.	R gene	TIR-NBS-LRR	Wang et al. (2016)
Medtr3g098350	Medicago sp.	RbohD	Respiratory Burst Oxidase-like Protein D	Wang et al. (2016)
Medtr1g016780	Medicago sp.	VPE	Vacuolar Processing Enzyme	Wang et al. (2016)
Medtr8g014650	Medicago sp.	VSP	Vesicles Storage Protein	Berrabah et al. (2014a)
Medtr8g098945	Medicago sp.	WRKY29	WRKY 29	Wang et al. (2016)
Medtr3g031220	Medicago sp.	WRKY33	WRKY 33	Wang et al. (2016)
Glyma.15G062500	G. max	PR1	Pathogenesis-related protein 1	Krishnan et al. (2016)
Glyma.03G132700	G. max	PR2	Pathogenesis-related protein 2	Nguyen et al. (2023)
Glyma.01G165800	G. max	Rj4	Pathogenesis-related protein 5	Yasuda et al. (2016)
Glyma.15G062400	G. max	PR1	Pathogenesis-related protein 1	Yasuda et al. (2016)
Glyma.13G278000	G. max	PDF1.2	Plant Defensin 1.2	Yasuda et al. (2016)
Glyma.16G128000	G. max	Rj2	TIR-NBS-LRR	Sugawara et al. (2018)
Glyma.05G245800	G. max	PR5	Pathogenesis-related protein 5	Hashami et al. (2019)
Lj6g0000876	L. japonicus	PR10	Pathogenesis-related protein 10	Nakatsukasa-Akune et al. (2005)
Lj1g0008690	L. japonicus	Chitinase	Chitinase class I	Nakatsukasa-Akune et al. (2005)
Lj3g0020393	L. japonicus	BGL	β 1-3 glucanase	Nakatsukasa-Akune et al. (2005)
Lj2g0017950	L. japonicus	DOX	Pathogen-inducible α -dioxxygenase	Nakatsukasa-Akune et al. (2005)
Lj3g0025759	L. japonicus	MPK3	Mitogen-associated Protein Kinase 3	Lopez-Gomez et al. (2012)
Lj4g0021901	L. japonicus	CP450	Cytochrome P450	Lopez-Gomez et al. (2012)

Table 1. Defense markers validated by reverse transcription qPCR for the nodule immune response analysis.

validate defense responses in nodules (Wang et al., 2016; Liu et al., 2021; Zhang et al., 2023).

To avoid any potential confusion between senescent nodules and those with exacerbated defenses, gene expression needs to be associated with analysis of the nodule phenotype. In addition to

the induction of CPs, senescent nodules display a white or greenish color due to the absence or the degradation of the leghemoglobin, respectively (Luo et al., 2005; Xia, 2018). By contrast, necrotic nodules with exacerbated defenses display a brownish color, accumulate defense compounds such as phenols and often overexpress defense markers (Bourcy et al.,

Plant Communications

2013; Sinharoy et al., 2013; Berrabah et al., 2014a; Wang et al., 2016; Liu et al., 2021).

It is noteworthy that, in the literature, senescence is less studied in the nodule than in other plant organs. Similarly, the expression of defense and senescence markers, or the accumulation of phenolic compounds, are not always reported or described. In this review, the immunity is considered as stimulated when at least an overexpression of defense genes is observed compared with senescence markers. In contrast, senescence is considered to be stimulated when senescence markers are more induced than defense genes.

SENESCENCE AND IMMUNITY COOPERATE IN NON-SYMBIOTIC PLANT ORGANS

The relationship between immunity and senescence is poorly understood within the nodule, whereas outside this organ, this relationship has been studied in depth and provides valuable information for understanding the interplay between immunity and senescence.

It is largely accepted that senescence and immunity cooperate during senescence or defense responses in plant organs other than nodules. As an example, the treatment of *Arabidopsis thaliana* seedlings with the FLAGELLIN 22 (FLG22) peptide, a bacterial Microbial/Pathogen-Associated Molecular Pattern (M/PAMP), leads to the activation of an M/PAMP-Triggered Immunity (MTI or PTI) (Sanguankiatichai et al., 2022). This response is accompanied by the stimulation of senescence markers, indicating that PTI activates senescence (Denoux et al., 2008). Moreover, in *A. thaliana* the proliferation of the pathogenic bacteria *Pseudomonas syringae in planta* can be suppressed by an induced senescence through the abscission of cauline leaves. This induced senescence is only observed with the *P. syringae* strains carrying a functional TYPE III SECRETION SYSTEM (T3SS) (Patharkar et al., 2017), a molecular complex used by the bacteria to inject effectors into the host cell to shut down the PTI signaling and hijack plant metabolism (Ngou et al., 2022). Plants can perceive effectors through intracellular NUCLEOTIDE-BINDING SITE LEUCINE-RICH REPEAT receptors (NBS-LRRs) and induce effector-triggered immunity (ETI). This response represents a second layer of the plant immunity after the PTI (Wu et al., 2014). The *A. thaliana* mutant impaired in *SENESCENCE-ASSOCIATED GENE 101* (*SAG101*) fails to induce leaf senescence (He and Gan, 2002). *SAG101* encodes an acyl hydrolase forming a complex with the NBS-LRR, ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1), and controls plant innate immunity. Together these data indicate that beyond PTI, ETI can also trigger senescence responses (Feys et al., 2005; Dongus and Parker, 2021).

In addition to PTI and ETI, phytohormones such as jasmonic acid (JA) and salicylic acid (SA) also regulate plant immunity (Pieterse et al., 2012). The analysis of natural leaf senescence in the non-legume *A. thaliana* or the leguminous plant *G. max*, revealed the activation of genes associated with JA and the stimulation of defense-related genes such as *PATHOGENESIS-RELATED*

Defense and senescence interplay in legume nodules

PROTEIN 1 (*PR1*, Gupta et al., 2016; Borniego et al., 2020). Moreover, silencing of the *AUTOPHAGY-RELATED GENE 2* (*ATG2*) in soybean led to acceleration of the senescence (Hashimi et al., 2021). These fast senescence responses co-occur with the stimulation of immunity through accumulation of the SA hormone and reactive oxygen species (ROS) as well as induction of *PR1* (Hashimi et al., 2021). Other studies revealed a tight cooperation between immunity and senescence in plants for the suppression of inefficient and pathogen-infected organs (Zhang et al., 2020).

It has been proposed that pathogen-triggered senescence in plants is a part of the immune response and that senescence increases the plasticity of immunity as plants are able to eliminate infected organs to withstand the infection (Patharkar et al., 2017). In addition, the activation of the senescence can also be the result of metabolic depletion induced by pathogens (Häffner et al., 2015). In agreement with that, the inoculation of *Oryza sativa* with the pathogen *Cochliobolus miyabeanus* decreases the photosynthesis and the nitrogen contents, which are associated with the induction of the senescence process (Bockhaven et al., 2015). In contrast, the role of the defense genes whose expression is stimulated during the developmental senescence remains to be clarified. Different non-exclusive hypotheses can explain this observation: (1) defenses could help plants to destroy senescent tissues. Indeed, the defense gene *PATHOGENESIS-RELATED PROTEIN 10* (*PR10*) encoding an endonuclease involved in programmed cell death, is induced in different senescent organs and could participate in the cell death establishment observed during senescence (Wu et al., 2017; Lee et al., 2018; Morris et al., 2021). (2) Immunity could also protect the senescent organs from potential invaders as senescent organs display a drastic reduction of the cell integrity, which can represent an entrance for microbes. (3) The defense response can potentially result from a stress generated during the senescence. In agreement with this, the treatment of nodulated plants with phosphinothricin, a herbicide inhibiting the glutamine synthetase (Takano and Dayan, 2020), leads to a stress-induced senescence in the nodule. This induced senescence displays defense stimulation higher than those observed during developmental senescence, indicating that the stress is an important factor affecting the recruitment of defense during the senescence (Van de Velde et al., 2006; Seabra et al., 2012). (4) The stimulation of immunity observed during senescence could also result from the perception of Damage-Associated Molecular Pattern (DAMP) molecules by the host, which are plant compounds released during pathogen infection, cell damage, or in response to cell death signaling (Li et al., 2020). According to that, the treatment of *A. thaliana* seedlings with the DAMP oligogalacturonides stimulates defense and senescence genes (Davidsson et al., 2017).

SENESCENCE OF NITROGEN-FIXING NODULES CO-OCCURS WITH IMMUNE STIMULATION

Senescence of the nitrogen-fixing (fix+) nodules is different from that of other plant organs since, in addition to the recycling of the host cell, it also allows the recycling of the bacteroids (Gourion et al., 2015). Despite nodule specificities, the comparison of

M. truncatula transcriptomes from senescent leaves and nodules revealed an overlap of physiological responses (Pérez Guerra et al., 2010). Similarly, treatment of nodulated plants with phosphinothricin induces nodule senescence and a massive induction of CPs and defense genes (Seabra et al., 2012). In addition, RNA-seq analyses of *G. max* nodules undergoing developmental senescence revealed an accumulation of PR transcripts (Yuan et al., 2017). Together these observations indicate an activation of the immunity in senescent nodules as reported for leaves.

Unlike some CPs specifically expressed during nodule senescence, activated defense genes do not appear to be exclusively related to symbiosis. For example, in *M. truncatula*, several PRs that are upregulated during nodule senescence, are also overexpressed in roots exposed to pathogens such as *Phymatotrichopsis omnivora* (Benedito et al., 2008; Uppalapati et al., 2009; Seabra et al., 2012). In addition, it has recently been shown that the root pathogen *Ralstonia solanacearum* is able to infect *M. truncatula* nodules and that defense genes induced in senescent nodules are also induced in nodules infected by this pathogen without apparent senescence features (e.g., formation of senescent zone or overexpression of CPs) (Benezech et al., 2020). It indicates that defense activation is not systematically associated with senescence response in fix+ nodules.

IMMUNITY INFLUENCES SENESCENCE ACTIVATION IN THE NODULE

In certain circumstances the stimulation of the immune response in the nodule also leads to the induction of the senescence. For example, the accumulation of necrotic tissues that are characteristic of defense responses, can also be associated with senescence when CPs are stimulated (Horvath et al., 2015; Liu et al., 2021). Moreover, induction of defense and senescence markers combined with bacteroid death is observed in *M. littoralis* (R108) nodules exposed to wounding, suggesting that the loss of the symbiotic organ integrity (due to mechanical stress or the rupture of the oxygen barrier) compromises the symbiont survival through stimulation of both immunity and senescence (Berrabah et al., 2023). A transcriptome analysis of *Bradyrhizobium diazoefficiens* 113-2, the endosymbiont of *G. max*, reveals the accumulation of core T3SS transcripts during nodule senescence (Yuan et al., 2021). Similarly, the inoculation of *L. japonicus* ecotype MG-20 or *Lotus burtii* with the *Bradyrhizobium elkanii* incompatible strain leads to the formation of senescent nodules. The use of the *B. elkanii* strain mutated in the T3SS or in the symbiotic effector NopM delays the senescence response (Kusakabe et al., 2020). NopM was shown to prevent defense signaling through ubiquitin-ligase activity resulting in ROS accumulation (Xin et al., 2012). Together these observations indicate that a default in immune suppression can lead to senescence responses. NopL is another effector preventing nodule senescence during the interaction between *Sinorhizobium* sp. strain NGR234 and its host *P. vulgaris* (Zhang et al., 2011). NopL prevents early senescence through the phosphorylation of a MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) involved in defense signaling activation (Ge et al., 2016). Taken together these data indicate that default in PTI inhibition can trigger a senescence process in the nodule.

IMMUNITY AND SENESCENCE SHOW CONTRASTED RESPONSES DURING NODULE DEVELOPMENT

Nodule development involves hundreds of plant and rhizobia genes synchronized during symbiosis. Some of these genes act sequentially during the nodule development and their inactivation can abort symbiosis at different nodule developmental stages, which can be correlated with the arrest of the bacteroid differentiation (Oke and Long, 1999; Mitra and Long, 2004; Maunoury et al., 2010). Accordingly numerous legume mutants forming fix-nodules have been identified with the largest collection described in *M. truncatula* and *M. littoralis* (R108) (Starker et al., 2006; Pislariu et al., 2012; Kang et al., 2016; Roy et al., 2020). The nodule development of fix- mutants is generally arrested during the bacteroid differentiation process. In *M. littoralis* (R108)-producing indeterminate nodules with TDB, fix- mutants can be classified into three groups depending on the symbiotic phenotype and CP/PR expression (Figure 1): the first group (G1) develops small brownish nodules with exacerbated defenses characterized by phenol accumulation and premature abortion of the differentiation process (non-elongated bacteroids). This group almost displays higher stimulation of defense genes than senescence markers, as observed in *dnf2* (Bourcy et al., 2013; Berrabah et al., 2014a, 2023). The second group (G2) includes mutants producing senescent nodules with dead elongated bacteroids as for *sultr3.5* and a higher induction of CPs than defense genes (Berrabah et al., 2023). The third group (G3) includes mutants producing necrotic nodules with elongated bacteroids and phenol accumulation and a high expression level of both CPs and PRs as observed in the fix- mutant *nf2100* (Berrabah et al., 2023). These different behaviors reflect a complex relationship between defense and senescence responses, which are not always stimulated with similar amplitude in fix- nodules. In addition, the differences in bacteroid differentiation observed between the fix- mutants seem to indicate that the variation in PRs and CPs stimulation can change according to the nodule developmental stage (Berrabah et al., 2023).

Leguminous plants producing determinate nodules without TDB, such as *G. max*, show co-activation of the immunity and the senescence in senescent wild-type (WT) fix+ nodules (Yuan et al., 2017). Unlike *M. littoralis* (R108), in mutants producing determinate nodules impaired in nitrogen fixation, the contrasted responses observed between immunity and senescence stimulation are not clear. As an example, mutants of *G. max* URICASE (*UOX*) and XANTHINE DEHYDROGENASE (*XDH*) genes form necrotic nodules with a low nitrogen-fixation activity. Expression analyses revealed an overexpression of both defense (*PR1*) and senescence (*CP1*) markers in *uox* compared with the WT, although *PR1* shows a higher expression than *CP1* (Nguyen et al., 2023). These observations suggest that *uox* and potentially *xdh* mutants develop nodules with a phenotype similar to that observed in the G1 mutant group of *M. littoralis* (R108). Like for plant producing indeterminate nodules (*Medicago* species), this necrotic phenotype is not always observed in *G. max* mutants producing fix- nodules since several mutants developing non-necrotic nodules have been isolated (So et al., 1987; Thöny-Meyer et al., 1989; Appunu and Dhar, 2008); however, the

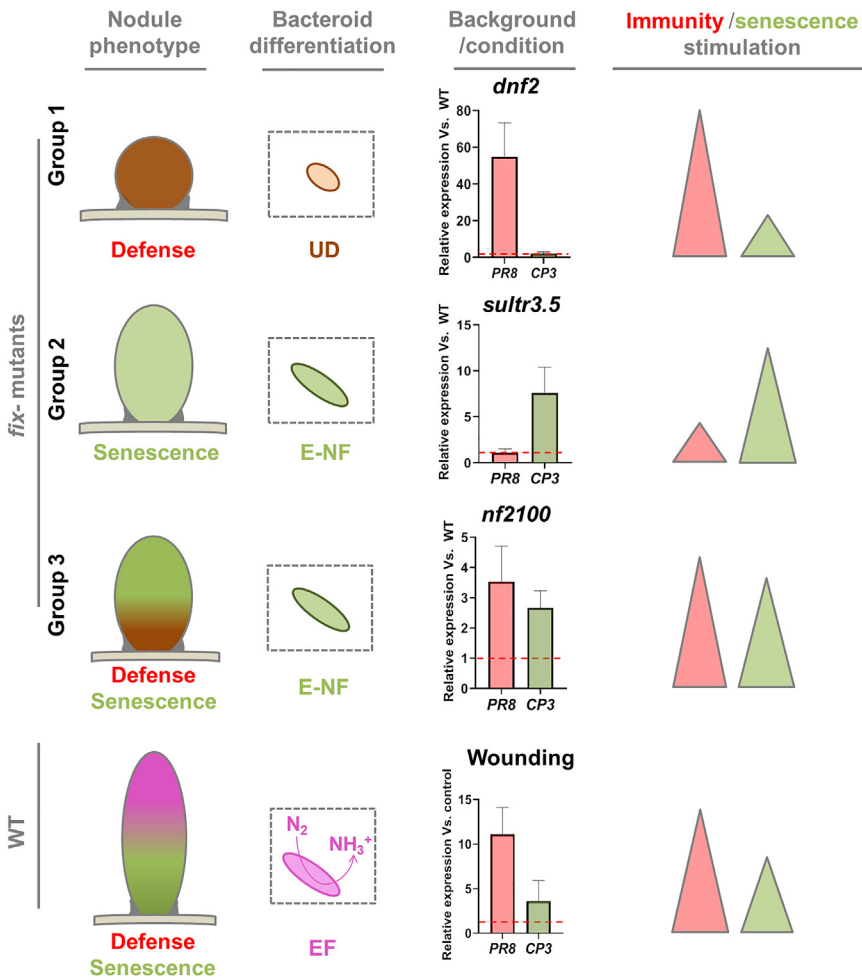


Figure 1. Immunity/senescence stimulation in indeterminate nodules.

In *M. littoralis* (formerly *M. truncatula* R108), three groups of *fix-* mutants can be distinguished based on nodule phenotype and stimulation of immunity/senescence markers. Group 1 (G1) mutants like *dnf2* (Bourcy et al., 2013) produce small necrotic nodules showing UnDifferentiated (UD, non-elongated) bacteroids and exacerbated defense reactions (Bourcy et al., 2013; Sinharoy et al., 2013; Berrabah et al., 2014a). G1 mutants show higher stimulation of defense genes (example *PR8*) than senescence markers (example *CP3*) (Berrabah et al., 2023). Group 2 (G2) mutants develop elongated fix- nodules with senescent behaviors and Elongated Non-Fixing (E-NF) nitrogen bacteroids, such as *sultr3.5* (Berrabah et al., 2023). This group shows a higher expression of senescence genes than defense genes. Group 3 (G3) mutants such as *nf2100* develop elongated nodules with E-NF bacteroids and nodule necrosis. These mutants present high stimulation of both defense and senescence genes (Berrabah et al., 2023). WT plants produce nodules hosting fully differentiated (Elongated Fixing [EF] nitrogen) bacteroids. Induction of senescence response in WT nodules leads to the degradation of the leghemoglobin (conferring a greenish color to the nodule) and to the stimulation of defense and senescence markers, as observed during nodule response to wounding (Berrabah et al., 2023). The graphs show the mean relative gene expression in the mutants (*dnf2*, *sultr3.5*, *nf2100*) compared with the WT and in the wounding nodules compared with the control. Error bars represent the standard errors. Triangles represent the amplitudes of defense (red) and senescence (green) stimulation in the nodules.

evaluation of the defense stimulation is not yet compared in these nodules. Surprisingly, in *L. japonicus*, the inactivation of *SymCRK* and *NAD1*, two genes involved in the suppression of nodule immunity, does not affect nodule functioning (Yu et al., 2019), whereas the mutated alleles of this genes in *M. littoralis* (R108) develop nodules with exacerbated defense responses (Berrabah et al., 2014a; Wang et al., 2016). The different behaviors between nodules producing or not TDB, point to the presence of a divergent control of immunity and senescence.

THE IMMUNITY AND SENESCENCE BALANCE IN NODULES

The expression patterns of defense and senescence markers in *M. littoralis* (R108) nodules suggest a balance between these processes rather than an opposition (Figure 1). As mentioned earlier, a slight stimulation of *CP* and *PR* genes can be observed in G1 and G2 mutants, respectively, indicating that senescence and defense processes are not totally suppressed in nodules showing exacerbated defense or senescence (Sinharoy et al., 2013; Wang et al., 2016; Berrabah et al., 2023). This hypothesis is supported by the observation demonstrating that cultivation of G2 mutants in sand/perlite media which can contain

environmental elicitors such as microbes or polysaccharides (Elieh-Ali-Komi and Hamblin, 2016; Chialva et al., 2018; Semchenko et al., 2022), reduces senescence and enhances immune responses compared with axenic culture conditions (Berrabah et al., 2023). This suggests that environmental elicitors can reverse the immunity/senescence stimulation in G2 mutants. In line with this, it was previously demonstrated that defenses observed in the *M. littoralis* (R108) *dnf2* mutant are dependent of environmental conditions. Indeed, the cultivation of this mutant on a medium containing a low concentration of elicitor (e.g., agarose) restores the WT nitrogen-fixation capacity, whereas growing it on a medium enriched in a defense elicitor such as ulvan (Jaulneau et al., 2010) leads to a *fix-* phenotype (Berrabah et al., 2014b). Based on these observations, we speculate that specific genes such as *DNF2* may influence the immunity/senescence relationship in the nodule in response to environmental elicitors.

PHYTOHORMONES MODULATE THE IMMUNITY/SENESCENCE INTERPLAY IN THE NODULE

Ethylene is a central hormone controlling senescence and immunity in plants. Ethylene impacts the gene expression through the

de-repression of the TF ETHYLENE-INSENSITIVE 2 (EIN2) (Guinel, 2015; Binder, 2020). Inoculation of legumes with rhizobia strains overexpressing the 1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID (ACC) DEAMINASE, an enzyme degrading the ethylene precursor ACC, leads to the formation of nodules exhibiting delayed senescence (Tittabutr et al., 2015; López et al., 2018). Moreover, the application of ethylene on *M. littoralis* (R108) fix+ nodules results in an upregulation of defense and senescence markers (Berrabah et al., 2018b). Similarly, reduction of the ethylene biosynthesis in nodules of *dnf2* and *symCRK* (G1 mutants) treated with an ethylene biosynthesis inhibitor, the aminoethoxyvinylglycine, decreases defense responses (van Loon et al., 2006; Berrabah et al., 2018b). These findings indicate that ethylene signaling affects defense and senescence in fix- and fix+ nodules, playing a key role in controlling the immunity/senescence interplay. Interestingly, ethylene seems to affect the senescence/immunity balance differentially depending on the legume species. *L. japonicus* and *M. truncatula* nodules treated with ethylene develop a typical senescence zone, whereas *M. littoralis* (R108)-treated nodules display necrosis (Berrabah et al., 2018b). Similarly, fix- mutants of *P. sativum* exhibiting immune or senescence responses in nodules show, respectively, low or high expression of ETHYLENE BIOSYNTHESIS GENES (EBGs), suggesting that ethylene stimulates nodule senescence rather than immunity in *P. sativum* (Serova et al., 2018). In addition, nitrogen supply on *M. truncatula* nodulated plants cultivated in a split-root system leads to nodule senescence stimulation of treated and non-treated roots (Kosslak and Bohlool, 1984; Lambert et al., 2020). These senescent nodules showed expression stimulation of defense genes, senescence genes, and EBGs, suggesting that the ethylene could control immunity and senescence at a systemic level (Lambert et al., 2020).

Other hormones interplay with the ethylene signaling pathway during plant senescence. The JA acts synergistically with the ethylene to control plant defense and senescence stimulation (Guinel, 2015). JA promotes ethylene responses through the degradation of JASMONATE-ZIM DOMAIN 2 (JAZ2), leading to the release of MYC2 involved in the stimulation of numerous genes including EBGs and ETHYLENE RESPONSE FACTORS (ERFs) (Wasternack, 2007; Ju and Chang, 2015). Senescent nodules of *G. max* display an accumulation of JA (Costanzo et al., 2012) and some LIPOXYGENASE (LOX) genes involved in JA biosynthesis are upregulated in *M. littoralis* (R108) fix- mutants such as *symCRK* (Berrabah et al., 2018b). LOX transcripts also accumulate in senescent WT fix+ nodules and induction of LOX genes is often associated with the accumulation of the stress hormone, the abscisic acid (ABA) (Ferguson and Mathesius, 2003). These findings indicate that JA and ABA also participate in the control of defense and senescence responses in the nodule.

Beside the JA, SA effects are also extensively studied in plants since this hormone contributes to the regulation of defense and senescence responses (Zhang et al., 2020). The SA acts antagonistically to the JA and prevents JA-induced leaf senescence (Ji et al., 2016). During early symbiotic interactions (e.g., nodule formation), SA hinders root infection by the rhizobia. For example, expression of the *NahG* gene encoding salicylate hydroxylase (SA degrading enzyme) in *M. truncatula* A17 or in

L. japonicus reduces the SA content and increases root infection and nodule numbers (Stacey et al., 2006). In the nodules, the role of the SA is not yet understood, but regarding to its essential function in other plant organs, SA probably plays a key role in the regulation of the senescence and the defense in the nodules.

Gibberellins (GAs) are also antagonist actors of the ethylene-JA and ABA pathways (Pieterse et al., 2012). The *P. sativum* mutants, *sym26*, *sym33*, *sym27*, and *sym40* exhibiting senescent nodules display low concentration of GAs and high EBGs expression, whereas an application of GAs on WT fix+ nodules leads to a delayed senescence and a decrease of EBG expression (Serova et al., 2019). Therefore, GAs likely act in opposition with other phytohormones for the adjustment of the immunity/senescence stimulation in the nodules. Strigolactones are other important phytohormones that regulate nodulation (McAdam et al., 2017). Van Dingenen and colleagues showed that *ramosus* (*rms*) mutants of *P. sativum* impaired in strigolactone synthesis (*rms1* and *rms5*) and signaling (*rms3* and *rms4*) display a delay of the nodule senescence. This behavior correlates with a slight reduction of malic acid level during nodule organogenesis and a reduction of sugars in the senescent nodules (Van Dingenen et al., 2023). These observations suggest that strigolactones also participate in nodule senescence, potentially by affecting sugar levels.

THE REDOX STATE CONTRIBUTES TO THE CONTROL OF THE BALANCE BETWEEN IMMUNITY AND SENESCENCE

The redox state is the result of homeostasis between the presence on one hand of oxidative molecules such as ROS and reactive nitrogen species (RNS), and on the other hand antioxidants able to buffer the oxidative molecules (Mandon et al., 2021). Several reviews discussing the role of ROS, RNS, and antioxidant molecules during symbiosis have been published (Montiel et al., 2016; Bruand and Meilhoc, 2019; Fernandez-Göbel et al., 2019; Minguiñón et al., 2022). It was shown that both ROS and RNS trigger defenses during the root infection by the rhizobia, stimulate symbiotic gene expression and control nodule senescence (Berger et al., 2021; Minguiñón et al., 2022). However, the precise role of these reactive species in the immunity/senescence interplay in the nodule needs further investigations. *Sinorhizobium meliloti* mutants *IsrB* altered in the LysR regulator show an increased ROS production and induce senescent nodules (Luo et al., 2005; Tang et al., 2013; Syska et al., 2019). Furthermore, in *M. littoralis* (R108), *NAD1* represses the symbiotic immunity through the prevention of ROS production by inhibiting the RESPIRATORY BURST OXIDASE HOMOLOGS (*RBOHB* and *RBOHD*) (Yu et al., 2018), suggesting that ROS contribute to the symbiotic defense signaling. In addition, the role of ROS in immunity and senescence stimulation seems to be divergent between legume species as, unlike in *M. littoralis* (R108), the knockout mutant of *NAD1* in *L. japonicus* does not alter the symbiotic nodule functioning (Yu et al., 2019). Moreover overexpression of *PvRBOHB* in *P. vulgaris* leads to ROS accumulation and delays nodule senescence, indicating that ROS likely act as anti-senescence molecules in *P. vulgaris* (Arthikala et al., 2014). More generally, the production of ROS

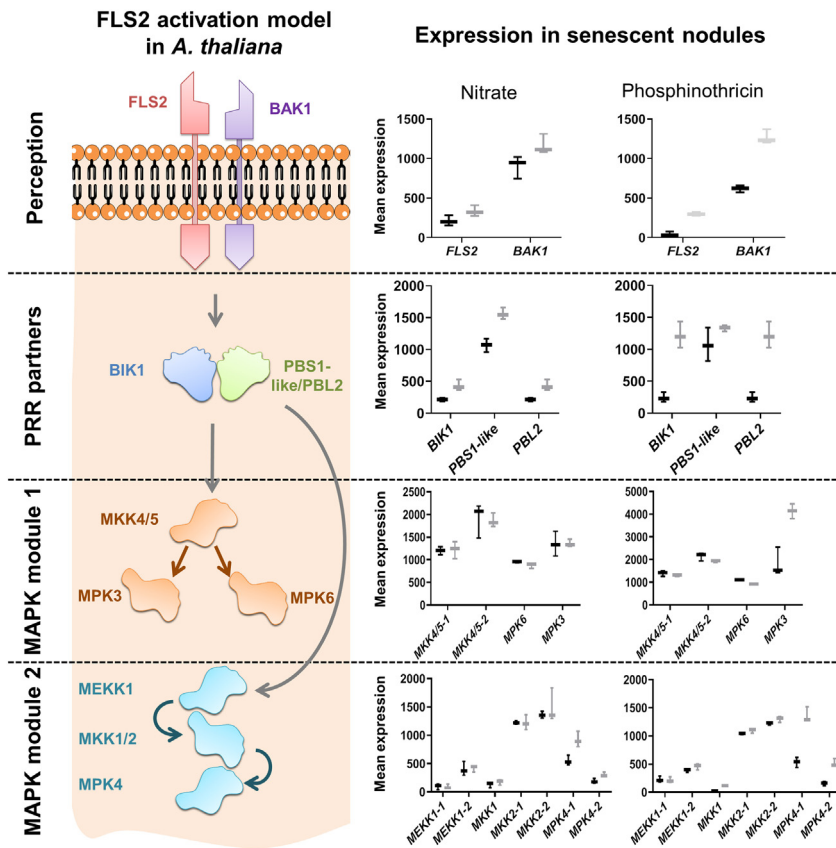


Figure 2. PTI-associated genes are expressed during the nodule senescence in *M. truncatula*.

Senescent nodules overexpress some PTI-associated genes such as the *FLS2*-*BAK1* signaling components. On the left, the molecular model of *FLS2*-*BAK1* signaling described in *A. thaliana* by Bigeard et al. (2015): *FLS2* perceives the *FLG22* peptide, a component of the bacterial flagellin. The binding of *FLG22* to *FLS2* induces its heterodimerization with *BAK1*. After a trans-phosphorylation, the activated *FLS2*-*BAK1* complex phosphorylates *BOTRYTIS-INDUCED KINASE 1* (*BIK1*), an intracellular protein kinase. *BIK1* phosphorylates *PBS1/PBLs* and activates two Mitogen-Associated Protein Kinase (*MAPK*) cascades: *MKK4-5/MPK6-3* and *MEKK1/MKK1-2/MPK4*. The *MAPKs* stimulate transcriptional factors involved in the induction of defense genes (Bigeard et al., 2015). On the right panel is shown the expression in nodules, of *M. truncatula* orthologs of *A. thaliana* genes associated to the *FLS2* signaling, in response to nitrate (Benedito et al., 2008) and the herbicide phosphinothricin (Seabra et al., 2012). These analyses reveal that the orthologs of *FLS2* (*Medtr4g094610*), *BAK1* (*Medt2g008360*), *BOTRYTIS-INDUCED KINASE 1* (*BIK1*; *Medtr5g079840*), *PBS1-LIKE* (*Medtr5g038870*), and *PBL2* (*Medtr5g079840*) are overexpressed in senescent nodules compared with the controls. Expression analyses of *A. thaliana* orthologs of the two *Medicago* *MAPK* modules (module 1: *MKK4*: *Medtr4g005830*, *MKK5*: *Medtr4g005830/MPK6*: *Medtr4g087620*, *MPK3*: *Medtr4g061130*, and module 2: *MEKK1*: *Medtr5g060350/MKK1*:

Medtr2g040510, *MKK2*: *Medtr4g125800/MPK4*: *Medtr7g078690*) show similar expression in non-senescent and senescent nodules except for *MPK3* and *MPK4*, which are more expressed in senescent nodules. The graphs show the mean gene expression and the error bars represent the standard errors. The black colors represent the expression values for control treatments. The gray colors represent the expression values in nodules after treatments with nitrate or phosphinothricin. Orthologous genes were identified using the Phytozome database (<https://phytozome.jgi.doe.gov/pz/portal.html>) and the expression data were obtained from the *M. truncatula* Gene Expression Atlas (<https://medicago.toulouse.inrae.fr/GEA>). The genes detected by two probsets (*MKK4/5*, *MEKK1*, *MKK2*, and *MPK4*) are distinguished in the graphics and annotated with numbers 1 and 2.

stimulates the senescence in indeterminate nodules and prevents it in determinate nodules (Puppo et al., 2005).

Nitric oxide (NO) is the most studied RNS produced during symbiosis. In *M. truncatula* and *M. littoralis* nodules, NO is highly produced in the fixation zone and close to the senescent zone (Berger et al., 2021). Inoculation of *M. truncatula* with *S. meliloti* strain overexpressing the *Flavo-hemoglobin* (*HMP*) encoding NO-detoxifying enzyme, delays nodule senescence (Cam et al., 2012). The role of NO in nodule defense and senescence activation requires further studies, but it has been proposed that NO acts as a signal molecule controlling protein activities through S-nitrosylation, tyrosine nitration, and metal nitrosylation (Berger et al., 2021). In agreement with that, different symbiotic proteins are able to complex with NO, such as the rhizobial TF NnrR regulating denitrification (Mesa et al., 2003; De Bruijn et al., 2006; Meilhoc et al., 2010). NO could also interplay with the ethylene signaling as it was shown that the group VII ethylene TFs of *A. thaliana* participate in the NO signaling (Gibbs et al., 2015). Interestingly, the overexpression of the *HMP* gene in *Mesorhizobium loti* reduces NO without affecting the senescence in *L. japonicus* nodules, suggesting that, in addition to ROS, NO also shows differential effects in nodules depending on the legumes species (Fukudome et al., 2021).

DEFENSE SIGNALING EVENTS ASSOCIATED WITH THE NODULE SENESCENCE

The expression of a wide range of genes controlling the immune signaling pathways is stimulated in the nodule during senescence and defense responses. Several *LRR-RLK* genes are upregulated in *M. littoralis* (R108) mutants showing nodules with exacerbated defense or in fix+ senescent nodules. These genes include the *FLAGELLIN SENSING 2* (*FLS2*) and *BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED RECEPTOR KINASE 1* (*BAK1*) receptors whose expression are induced in G1 mutants *nad1*, *rsd*, and in fix+ senescent nodules (Benedito et al., 2008; Seabra et al., 2012; Sinharoy et al., 2013; Wang et al., 2016). Interestingly, expression analyses of the *M. truncatula* orthologs of *A. thaliana* genes associated to the *FLS2*-*BAK1* signaling cascade (Bigeard et al., 2015) reveals that these orthologs are expressed in the nodules and that the expression of several of them (8 out of 13 genes) is increased upon nitrate- and phosphinothricin-triggered senescence (<https://lipm-browsers.toulouse.inra.fr/expression-atlas/mtgeav3/>, Figure 2). The expression of genes downstream to *FLS2*-*BAK1* signaling during senescence suggests that components necessary for the activation of the PTI signaling are induced during nodule senescence and that senescent nodules

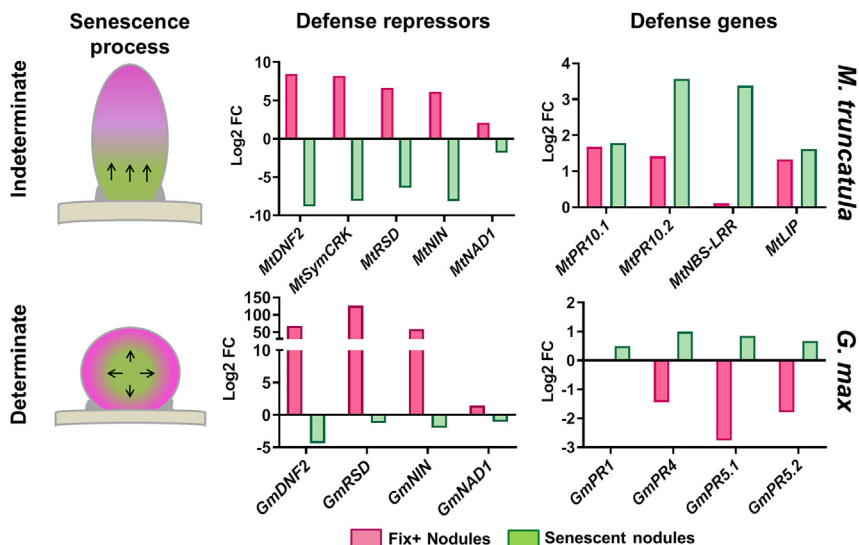


Figure 3. Upregulation of defense genes during nodule senescence correlates with a decrease of the symbiotic defense repressor expression.

Medicago produces indeterminate nodules in which a senescent zone is formed at the base of the nodule and progresses toward the apex. During the senescence of this organ, a downregulation of symbiotic defense repressors (*MtSymCRK*, *MtDNF2*, *MtRSD*, *MtNAD1*, and *MtNIN*) is observed and correlates with an upregulation of defense genes (*MtPR10*, *MtNBS-LRR*, and *MtLYST-INTERACTING PROTEIN: MtLIP*). In *G. max* determinate nodules, senescence starts from the central zone and spreads outwards. Similarly to *Medicago*, homologs of the symbiotic defense repressors (*GmDNF2*, *GmRSD*, *GmNAD1*, and *GmNIN*) (Roy et al., 2020) are suppressed, whereas an induction of defense markers (*GmPR1*, *GmPR4*, *GmPR5.1*, and *GmPR5.2*) is observed. Transcriptomic data for *M. truncatula* and *G. max* are here analyzed using GENEVESTIGATOR (Hruz et al., 2008). Log2 FC corresponds to the log2 fold change test versus control.

are potentially more sensitive to M/PAMPs than non-senescent nodules. Metagenomic analyses reveal that, in nature, the nodule can contain several endophytic microbes in addition to the symbiont (Brown et al., 2020; Zheng et al., 2020). The higher M/PAMP sensitivity of the senescent nodule can potentially prevent uncontrolled endophyte proliferation during senescence.

In addition to the PTI signaling genes, the expression of the *NBS-LRRs* and *NON-RACE-SPECIFIC DISEASE RESISTANCE-1 LIKE (NDR1-LIKE)* genes controlling PTI and ETI (DeYoung and Innes, 2006; Knepper et al., 2011; Yang et al., 2021) is also induced during nodule defense and senescence (Benedito et al., 2008; Seabra et al., 2012; Wang et al., 2016; Liu et al., 2021). These observations suggest a high potential of M/PAMPs and effector perception of the nodule symbiotic organ during immune and senescence responses. Furthermore, other proteins such as the CALCIUM-DEPENDENT PROTEIN KINASE (CDPK) and RBOH enzymes also participate in nodule immunity signaling (Yu et al., 2018). Interestingly, the analysis of the *symCRK* proteome reveals the accumulation of RBOHG and calcium binding proteins CDPK-RELATED KINASE 1 (CRK1), CDPK6, 15, and 23 proteins, suggesting the conservation of similar defense signaling pathways in G1 mutants (Berrabah et al., 2018b; Zhao et al., 2021). These observations indicate that various members of the CDPK and RBOH families are recruited in the nodules and their activities appear essential for the control of defense signaling during the development and the senescence of the symbiotic organ.

In *M. littoralis* (R108), the immune response observed during the senescence process in fix+ nodules correlates with the downregulation of *SymCRK*, *RSD*, *DNF2*, *NAD1*, and *NIN* (Figure 3). This suggests that defenses produced in fix+ nodules during senescence result from a reduced expression of genes suppressing nodule immunity and consequently activation of defense signaling (Berrabah et al., 2018b, 2023). Interestingly, expression analyses in senescent nodules of *G. max* orthologs of *RSD*, *DNF2*, *NAD1*, and *NIN* reveal that downregulation of these genes co-occurs with an upregulation of defense markers

(Figure 3). These data suggest that the reduced expression of the defense suppressors is a common feature of indeterminate and determinate nodules during the senescence.

TFs CONTROLLING IMMUNITY AND SENESCENCE IN THE NODULE

Different TFs regulating the immunity and the senescence in nodules have been identified. In addition to *RSD*, which is required to prevent defenses in *M. littoralis* (R108) nodules, TFs belonging to the NAC family control the nodule senescence (De Zélicourt et al., 2012; Zhou et al., 2021; Sauviac et al., 2022). Several members of this family are associated with plant responses to biotic and abiotic stresses (Bian et al., 2020; Meraj et al., 2020). In *G. max*, *NAC018* and *NAC039* are two master regulators of the nodule senescence (Yu et al., 2023). Interestingly these NAC genes are upregulated during the response of both *G. max*-resistant and -susceptible genotypes to the pathogenic fungus *Sclerotinia sclerotiorum*, indicating that *NAC018* and *NAC039* might be recruited during *G. max* in response to pathogens (Ranjan et al., 2019). Another well-characterized NAC TF is *NAC969*, involved in *M. truncatula* response to salt stress. The silencing of *NAC969* leads to early nodule senescence (De Zélicourt et al., 2012). Moreover, the expression profile of *NAC969* in the *MtGEA* database reveals a stimulation of this gene in response to the root pathogen *P. omnivora* in *M. truncatula* (<https://lipm-browsers.toulouse.inra.fr/pub/expressionAtlas/app>) (Uppalapati et al., 2009). These observations indicate that NAC TFs represent promising candidates for the control of plant defense during nodule senescence. In agreement with this, the NAC TFs SOYBEAN NITROGEN-ASSOCIATED NAPs (SNAPs) 1, 2, 3, and 4 constitute a transcriptional regulation hub of nodule senescence induced by nitrate. In *G. max* SNAP1, 2, 3, and 4 activate the expression of *WRKY6*, 22, 33, and 7 *ERF* during the nodule senescence (Wang et al., 2023). The WRKY family is involved in the plant immunity in *Medicago* species as shown through transcriptomic

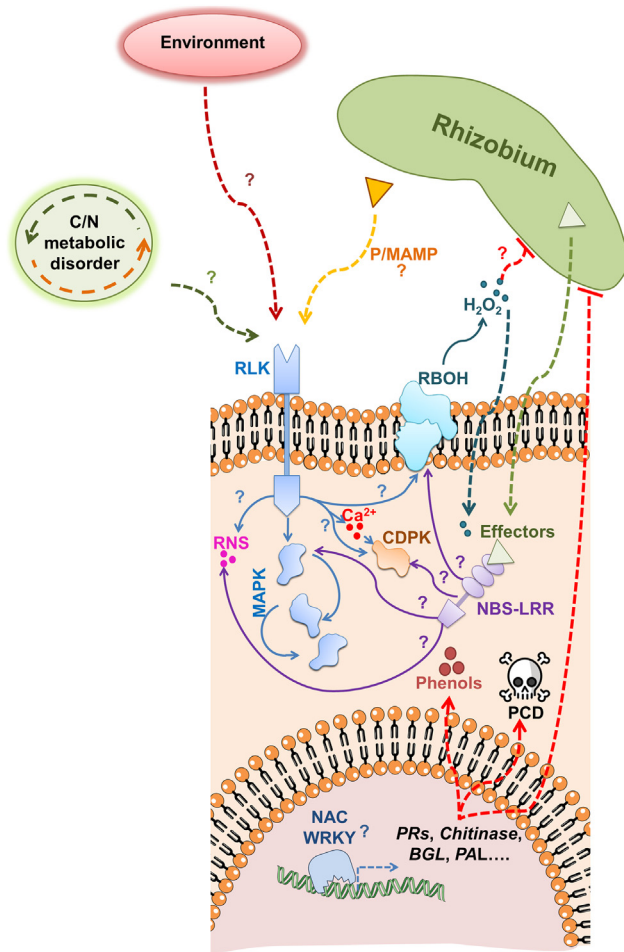


Figure 4. Defense events associated with the nodule senescence or loss of the immune repression.

The immune genetic network is recruited during the nodule senescence and defense, and includes membrane-anchored (Receptor-like Kinase [RLK]) and cytoplasmic receptors (NBS-LRR). These receptors activate the defense signaling cascade, which can occur in response to various signals including disorder between C and N metabolisms, environment (including Microbial/Pathogen-Associated Molecular Patterns [M/PAMP], biotic and abiotic stresses), and effectors injected by the rhizobium and recognized by NBS-LRR. Activated receptors lead to the stimulation of ROS production (generated by RBOH enzymes), the production of RNS (like NO), the activation of Ca²⁺ binding proteins such as CDPK, and the stimulation of defense signaling proteins like MAPK. Defense signaling activates TFs such as NAC and WRKY transcription factors that can potentially induce defense genes like PRs, CHITINASES, BETA-ENDO-GLUCANASE (BGL), and PHENYLALANINE AMMONIA LYASE (PAL). Defense signaling together with induced defense genes leads to the accumulation of defense compounds (phenol), activation of programmed cell death (PCD) and bacteroid death.

analyses of resistant versus susceptible alfalfa (*Medicago sativa* L.) plants to *Pseudomonas syringae* pv. *syringae* or *Fusarium* root rot (Nemchinov et al., 2017). ERF genes are associated with ethylene signaling and some of them are required in the defense response against the spotted alfalfa aphid of resistant versus susceptible *M. truncatula* lines (Jacques et al., 2020). Two ERF genes from the B-3 subgroup, ERF1-3 and ERF1-4, are similarly upregulated in WT and *ein2* mutant insensitive to ethylene (also called *sickle*) of *M. truncatula* in response to

Defense and senescence interplay in legume nodules

the fungal necrotrophic pathogen *Rhizoctonia solani*, indicating that an EIN2-independent mechanism may contribute to the pathogen-mediated regulation of these genes (Varma Penmetsa et al., 2008; Anderson et al., 2010).

In contrast with NAC behaviors, the TFs *bHLH2* and *ATB2*, which are required to prevent *M. truncatula* nodule senescence, are repressed during *M. truncatula* response to *P. omnivorum* (<https://lipm-browsers.toulouse.inra.fr/pub/expressionAtlas/app/>) (Uppalapati et al., 2009; D’Haeseleer et al., 2010; Deng et al., 2019). Together these observations suggest the presence of common and specific TFs regulating immunity and senescence in the nodule.

IMMUNITY LEADS TO THE INDUCTION OF GENES AFFECTING BACTERIODS AND HOST CELL SURVIVAL

Defense genes with different biochemical activities are associated with the nodule defense. As described earlier, some members of the PR10 family are induced during senescence and immunity in different organs including necrotic or senescent nodules (Liu et al., 2006; He et al., 2013). The PR10 family is involved in the activation of programmed cell death in response to pathogens and likely during nodule senescence (Reguera et al., 2010; Ma et al., 2018). Moreover, PR10 can bind phenols and potentially regulate the accumulation of these antimicrobial compounds during nodule defense (Morris et al., 2021).

Other genes may participate in the death of bacteroids, including PR5 (Berrabah et al., 2023), a homolog of thaumatin-like proteins showing antimicrobial activity (Hu and Reddy, 1997). The thaumatin-like *Rj4* soybean gene controls host selection of the rhizobium by activation of defense signaling (Tang et al., 2016; Yasuda et al., 2016; Nguyen et al., 2017). PR8 is another upregulated gene during nodule defense and senescence in G3 mutant and WT senescent nodules (Benedito et al., 2008; Seabra et al., 2012; Berrabah et al., 2023) encoding a chitinase class III protein (Ali et al., 2018) with homology to antibacterial lysozyme enzyme (Scheltinga et al., 1994). In *Malus pumila* Mill. (apple tree), PR8 is induced during response to the bacterial pathogen *Erwinia amylovora* (Santander et al., 2022). In *symCRK* nodules, PR8 is detected in the symbiosome (Berrabah et al., 2018b), suggesting a potential role of this protein in the alteration of the bacteroid survival.

CONCLUDING REMARKS

Immunity and senescence show a versatile and complex interplay in legume nodules, challenging the model of cooperation observed in other organs. In indeterminate nodules of *Medicago* species, the stimulation of these processes can change during nodule development. Mutants arrested early in nodule formation overproduce defense or senescence responses, whereas these processes can be co-stimulated during late nodule developmental stages or in senescent WT nodules. A genetic network including various immunity-related genes is required during the symbiotic defense and senescence responses (Figure 4), highlighting the complexity of the defense response in the nodule. This review suggests that immunity and senescence are in equilibrium and

are influenced by many factors such as development, environment, and stresses. The immunity/senescence balance allows plants to tightly regulate and protect the nodule functioning. The immune and senescence responses can diverge depending on legume species and hormones including ethylene, JA, SA, ABA, GAs, and strigolactones together with the redox state, which represent key components adjusting the immunity/senescence balance locally and systemically. These differences could represent key elements explaining the variation of immunity and senescence management in the nodule between legume species.

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Defense and senescence interplay in legume nodules

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