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Commentary

It's Not You, It's Me: *Medicago truncatula efd-1* Mutant Phenotype Depends on *Rhizobium* Symbiont

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Legume plants are essential components of sustainable agriculture, thanks to their ability to establish nitrogen (N)-fixing symbiosis with soil bacteria of the genus *Rhizobium*. These positive interactions lead to a developmental reprogramming of plant and bacterial cells, culminating in the formation of an N-fixing organ in roots termed the nodule. This symbiotic interaction requires a finely tuned molecular communication between both symbionts occurring via multiple mechanisms. The model legume *Medicago truncatula* forms indeterminate, elongated nodules with a persistent apical meristem and a gradient of bacterial infection and N fixation zones. Zone I corresponds to the meristematic nodule zone, where no infection occurs; zone II is associated with the nodule infection region; zone III is the region where *rhizobium* bacteria are differentiated into bacteroids and actively fix N and eventually, senescence-related processes will occur in zone IV (Vasse et al. 1990, **Fig. 1**). In this type of nodules, infecting bacteria undergo terminal differentiation through the activity of various plant peptides, of which the NODULE-SPECIFIC CYSTEINE-RICH (NCR) peptide family (Van de Velde et al. 2010, Wang et al. 2010) is the best characterized.

Additional regulators of nodule differentiation include the *M. truncatula* transcription factor MtEFD (for ethylene response factor required for nodule differentiation), which was first identified by transcriptomics as differentially expressed during nodule development (El Yahyaoui et al. 2004). A deletion mutant of *MtEFD*, *Mtefd-1*, showed increased infections and higher nodule numbers at early stages. However, these nodules failed to fix N (Fix$^-$ phenotype) and presented alterations in the nodule infection (zone II) and N fixation fraction (zone III) at later stages (Vernié et al. 2008). Transcriptional analysis followed by transactivation assays showed that MtEFD activates the expression of a negative regulator of cytokinin signaling, *MtRR4* (Vernié et al. 2008). The involvement of the cytokinin pathway was able to explain the early hypernodulation phenotype observed, as later experimentally demonstrated by complementation analysis (Jardinaud et al. 2022b), yet its connection to nodule differentiation remained unclear.

In the current issue, Jardinaud et al. (2022a) add one extra layer of complexity to these regulatory mechanisms by showing that the Fix$^-$ and altered nodule differentiation phenotype of *Mtefd-1* depends on the rhizobial strain employed as the microsymbiont. Initial work was carried out with one of the most widespread symbionts for *M. truncatula*, the strain *Sinorhizobium (Ensifer) meliloti* 2011. However, when *Mtefd-1* plants were inoculated with more efficient *rhizobium* strains such as *S. medicae* WSM419, *S. meliloti* BL225C or *S. meliloti* BO21CC, the Fix$^-$ aberrant differentiation phenotype was less obvious. It turned out that a significant fraction of nodules was elongated and contained differentiated bacteroids comparable to those found in the *M. truncatula* A17 wild-type genotype. Comparison with nodules from other poor N-fixers such as *S. meliloti* AK58 or AK83 (Biondi et al. 2009) again showed the formation of small white Fix$^-$ nodules in *Mtefd-1*, as originally observed for *S. meliloti* 2011. This suggests that the symbiotic outcome of this mutant is highly dependent on the *rhizobium* strain employed for inoculation.

The authors next analyzed the level of ploidy of both plant and bacterial cells and found that the amount of 16C plant nuclei (i.e. plant nuclei undergoing four cycles of endoreduplication), as well as the level of bacteroid terminal differentiation, was positively associated with symbiotic efficiency in the *Mtefd-1* mutant.

As a first step toward the identification of additional transcriptional targets of MtEFD (other than *MtRR4*), Jardinaud and collaborators also carried out an RNA-sequencing analysis in A17 and *Mtefd-1* plants inoculated with the efficient strain *S. meliloti* BL225C and the less efficient strain *S. meliloti* 2011 at two stages of nodulation (4 and 10 d post-inoculation). A summary of the main outcomes of this analysis is schematized in **Fig. 1**. Inoculation of A17 wild-type plants with *S. meliloti* BL225C led to faster nodule development and an increased expression of genes related to metabolic and sugar transport functions, while nodule development was both slower in *Mtefd-1* plants and accompanied by a mild induction of senescence-related genes (**Fig. 1**). In contrast, inoculation with *S. meliloti* 2011 induced a strong increase in the expression of senescence genes in A17 plants, which was exacerbated in the mutant *Mtefd-1*. Among the set of differentially expressed genes in the *Mtefd-1* mutant regardless of the *rhizobium* strain used, there

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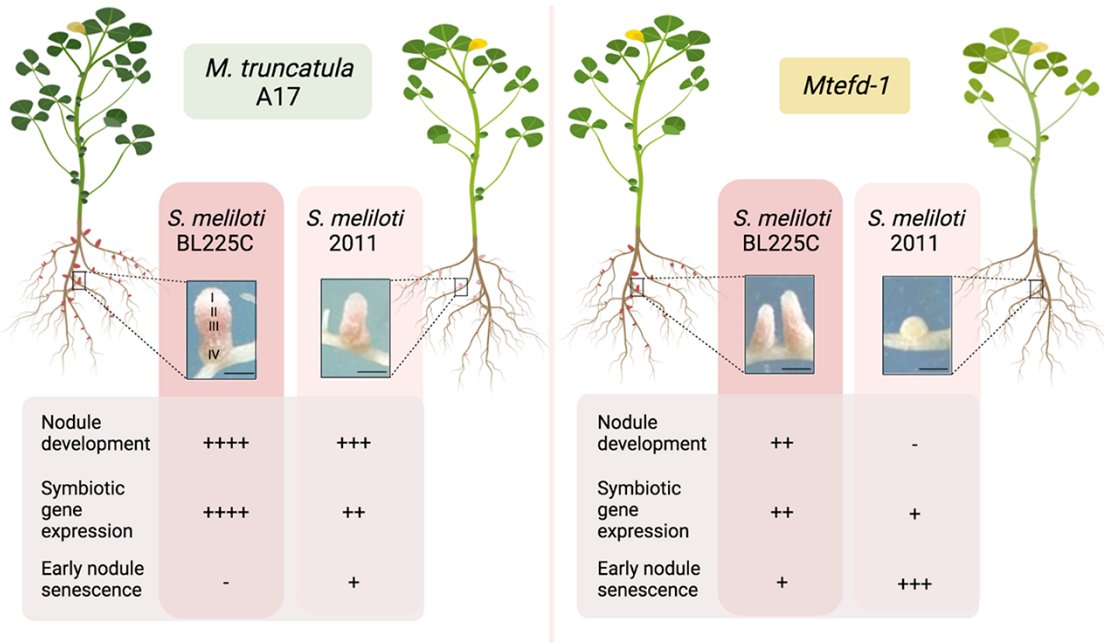

Fig. 1 Symbiotic responses of the *Mtefd-1* mutant to an efficient or a non-efficient N-fixer *rhizobium* strain. The *Mtefd-1* mutant shows altered bacteroid differentiation and nodule development (reduced nodule elongation) when inoculated with *S. meliloti* 2011. However, these alterations in the symbiotic phenotype are much less severe when using a more efficient N-fixer such as *S. meliloti* BL225C. The more efficient the symbiotic interaction the darker the color of leaves and nodules. Nodule images (at 21 d post-inoculation) are extracted from Jardinaud et al. (2022a) with permission; scale bars, 1 mm; I, meristematic nodule zone; II, infection zone; III, N fixation zone; IV, senescence zone. Image created with BioRender.com.

were promising target candidates including several early NCR peptide genes, not functionally characterized yet, and a nodule-specific thioredoxin, *MtTrx s1*. Interestingly, *MtTrx s1* and *MtTrx s3* have been found to localize at the symbiosome membrane and to interact with NCR247 and NCR335, regulating bacteroid differentiation (Ribeiro et al. 2017). It is, thus, tempting to hypothesize that the transcription factor MtEFD may indeed be involved in regulating the expression of these early NCR peptide genes and *MtTrx s1*. In this scenario, MtEFD would induce early nodule senescence in *M. truncatula* plants inoculated with a less efficient N-fixer, while allowing faster nodule growth, full bacteroid differentiation and N fixation with more efficient strains.

The work by Jardinaud et al. (2022a) illustrates the need to reconsider the identification of genes with a functional role in symbiosis when using a model system in which nodule development and plant growth are constrained due to a less efficient N-fixer. The influence of genotype–genotype interactions on the outcome of the legume–*rhizobium* symbiosis is a growing field of research (Cangioli et al. 2022). It is worth noting that this regulation is clearly bidirectional: not only the *rhizobium* strain influences the plant phenotype but also the other way around. One fine example of the latter is the host-dependent regulation of symbiotic hydrogenase expression in *Rhizobium leguminosarum* bv. *viciae* (Brito et al. 2008). Identifying the plant and bacterial factors that control the efficiency of these symbiotic systems is an exciting challenge that lies ahead.

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Disclosures

The author has no conflicts of interest to declare.

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