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Failure of self-control

Defense-like reactions during legume/rhizobia symbiosis

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Medicago truncatula and *Sinorhizobium meliloti* form a symbiotic association resulting in the formation of nitrogen-fixing nodules. In this organ, symbiotic cells contain large numbers of bacteroids. Remarkably, this chronic infection does not trigger visible defense reactions. Despite the importance of this phenomenon for potential transfer of the symbiotic capacity to non-legume plants, the molecular mechanisms underlying this tolerance are not understood.

We have characterized the *dnf2* *M. truncatula* mutant blocked in the symbiotic process after bacterial infection of the symbiotic cells. Nodules formed by the mutant contain only few layers of infected cells. Furthermore, they exhibit defense-like reactions which clearly contrast with premature senescence frequently observed during inefficient symbioses. This atypical phenotype raises *DNF2* as an exciting starting point to investigate the molecular basis of symbiotic repression of plant defenses.

Most legume species are able to form symbiotic interactions with soil bacteria referred to as rhizobia. During these associations, legumes differentiate a new organ dedicated to house bacteria: the nodule. Rhizobia often invade this organ using specialized symbiotic structures called infection threads (IT). These symbioses involve intimate contact between the two partners. The rhizobia reside in symbiotic nodule cells within organelle-like structures, separated from the host cell cytoplasm by a plant membrane. This structure is called symbiosome. In contrast to what

is generally observed during microbial invasion, legumes do not elicit defense reactions during symbiosis despite that the bacterial population reaches massive densities in nodules. It has been suggested that the innate plant immune system might be suppressed in nodules¹ and that this mechanisms is reversed as the nodules senesce.^{2,3} Not harming the rhizobium bacteria in the nodules is in the interest of the plants because rhizobia promote their growth by providing reduced nitrogen under the form of ammonium directly available for the plant. In exchange, bacteria receive carbon and energy supplies from the plants.

We recently characterized the *DNF2* gene. *dnf2* plant mutants develop nodules which are correctly invaded during the first stages of the symbiosis but in which the symbiotic process aborts once bacteria are released into the plant cell.⁴ This arrest of the symbiotic process is accompanied with symbiosome degeneration, induction of a Pathogenesis Related 10 gene (PR10) and a senescence marker, necrosis due to phenolics accumulation as well as bacterial death.⁴ These features are reminiscent of defense reactions and together clearly differ from early senescence which is commonly observed in non-fixing mutants (for instance nodules induced by a fix-minus rhizobium, Fig. 1).

Defense reactions in the frame of the rhizobium/legume symbiotic process have been discussed before.⁵ Essentially all the previous studies related to defense reactions focused on early steps of the symbiotic process or on artificial cell culture system.⁵

Keywords: symbiosis, defense reaction, immune status, *Medicago truncatula*, *Sinorhizobium*

Abbreviations: IT, infection threads; PR10, Pathogenesis Related 10 gene; PRR, Pattern-Recognition Receptors; MAMP, Microbial Associated Molecular Pattern

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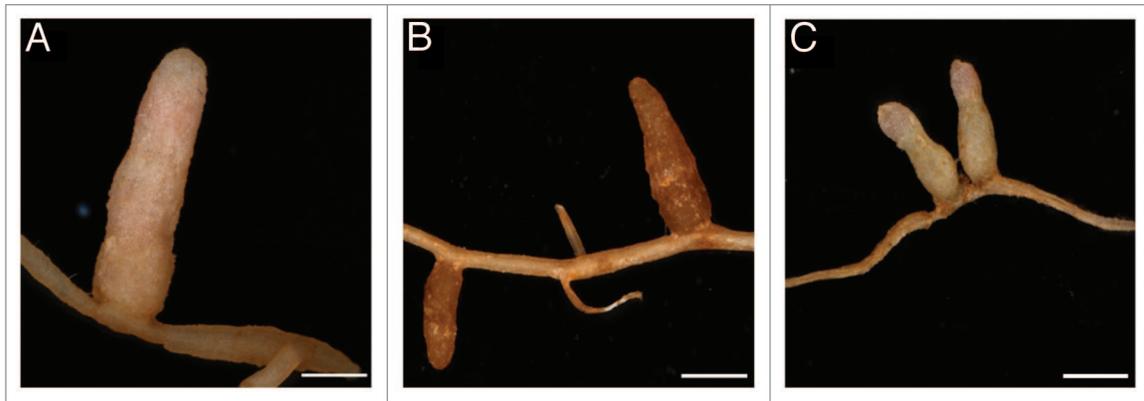


Figure 1. *dnf2-4* displays typical features of defense reactions. Pictures show Wild Type (WT) R108 (A and C) and *dnf2-4* (B) nodules 4 weeks after inoculation with *Sinorhizobium meliloti* 2011 (A and B) or its $\Delta nifH$ derivative (C). WT R108 shows big, pink nodules with WT strain and small, white nodules with Sm2011 $\Delta nifH$ strain. In contrast to the fix⁺ nodule shown in (C), *dnf2* nodules show necrotic aspect. Scale bars: 1 cm.

Here we highlight the interest to study the later steps of the symbiosis and particularly to understand how the plant cell can support the presence of so many bacteria into its cytoplasm without triggering defense reactions. Indeed, as the transfer of nitrogen-fixing symbiotic capacity to non-legume plants is an emerging goal,⁶ studying the immune status of nodules should be one of the future priorities. In other words, it will be essential to understand how the plant cells and the thousands of bacteria within them stay alive and metabolically active during the symbiotic interaction.

The phenotype of the *dnf2* mutants⁴ suggests an active mechanism responsible for the repression of plant defense within the symbiotic nodule cells. The requirement of such a specific mechanism taking place at the stage of cell invasion, by opposition to the one avoiding plant defenses in the IT, could be explained by the closer contact existing in symbiosomes between the bacterial cell envelope and the plant peribacteroid membrane. Indeed, the matrix that embedded bacteria in the IT⁷ is drastically reduced in the symbiosomes leaving the bacterial cell surface and Pattern-Recognition Receptors (PRR) located on the plant plasma membrane in dangerous vicinity.

At least three processes can be envisaged to prevent defense reactions in symbiotic cells and to maintain bacteria alive: (1) Bacteria are not detectable, (co)evolution might have led rhizobia to stop producing molecules eliciting plant defenses; (2)

Detection systems are off; one can imagine that all PRRs, the receptors dedicated to detect the microbial presence, are not expressed in the symbiotic cells; (3) signal transduction might be compromised downstream of active PRRs either by the rhizobia or by the plant itself via a nodule specific system. Examples of each of these possibilities are described in the literature: (1) Defense reactions are elicited in *Lotus japonicus* root systems in response to flg22 the active epitope of flagellin. In contrast, flagellin purified from *Mesorhizobium loti* does not elicit such defenses.⁸ Modification of this Microbial Associated Molecular Pattern (MAMP) making it undetectable by the plant could be a general feature of rhizobia;⁹ (2) The Pathogenesis Related Receptor *FLS2* is drastically downregulated in *L. japonicus* nodules.⁸ This might reflect a general downregulation of PRRs in the symbiotic organ (note that it seems futile to repress the gene required for flagellin repression if rhizobial flagellin cannot elicit defense reactions); (3) Bacterial exopolysaccharides are negatively charged polymers that chelate divalent cations and by this property they can inhibit Ca²⁺ influx required to develop MAMP triggered immunity.¹⁰ It will be a priority for future research to determine whether the role of DNF2 fits in one of these scenarios. DNF2 encodes a PhosphoInositol-PhosphoLipaseC-XD-containing protein. It could function by masking or removing a bacterial surface MAMP (scenario 1), by interfering with PRR expression on symbiosome membranes (scenario 2)

or by interfering with signal transduction (scenario 3).

To investigate further defense-like reactions development, studies should be conducted on both “normal” and altered rhizobia/legume interactions. Beyond the “normal” wild type situation, these studies should include: (1) Mutant plants developing nodule necrosis, such as *dnf2*,⁴ TRV36,¹ NF4608, NF1320¹¹ and *latD/NIP*;¹² (2) Bacterial mutants triggering similar phenotype such as mutants altered in the general stress response regulator *phyR/lecG*¹³ as well as auxotrophic mutants;¹⁴ (3) Environmental conditions that trigger necrotic nodules such as boron deficiency which also results in induction of pathogenesis-related proteins from the PR10 family.¹⁵ Characterization of the defense-like reactions developed in these different situations should be very informative and indicate if each situation requires a different signaling pathway. Are Rhizobia the eliciting agents? Are the defense-like reactions identical in all these situations? Do they induce priming of the defense reactions at distance? Are they primed by defenses related phytohormones? Under efficient symbiotic situation, exciting questions should also be addressed such as: is the immune system totally KO in the symbiotic cells and how can the plant do this? Are nodules responsive to priming by Induced Systemic Resistance or Systemic Acquired Resistance mechanisms?

The characterization of the *dnf2* mutant and the corresponding *DNF2*

gene unravel a new aspect of the symbiotic interaction and the further unraveling of its mode of action will allow a better understanding of how plants can accommodate thousands of bacteria in a single cell without triggering defense reactions.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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