

How does *Medicago truncatula* select against *Sinorhizobium meliloti* inefficient strains in its nodules?

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The symbiosis between the legume *Medicago truncatula* and the prokaryotic rhizobia *Sinorhizobium meliloti* is one of the main studied examples of mutualistic symbiosis. Rhizobia has the ability to fix nitrogen from the atmosphere with reduction of N₂ into ammonium with nitrogenase enzyme. The ammonium is transferred to the host plants in exchange for dicarboxylic acids and essential amino acids. This exchange of nutrients takes place in special root organs, called nodules. Nitrogen is essential for the normal growth of a plant however its amount in the soil is limited. The symbiotic relationship between the host plant and rhizobia allows the plant to be auto-fertilized. The excessive use of fertilizers can cause soil acidification and eutrophication of lakes rivers and even oceans. The study of this association could allow us to find more sustainable ways to increase crop production and reduce the side effects of excessive use of fertilizers. In this project, we aimed to understand better the symbiotic association between *M. truncatula* and the rhizobia *S. meliloti*. Using a mutant strain, *nifH*, with failure to fix nitrogen due to a transposon insertion in the *nifH* gene, which encodes a subunit of nitrogenase, we want to figure out how the host plants can select against inefficient nitrogen-fixing bacteria in its nodules. Our investigation of senescence markers inside nodules colonized with *nifH* bacteria compared to WT bacteria allows us to conclude that the plant induces early senescence inside *nifH* nodules. The defense reactions inside nodules were analyzed by using two different staining methods, nitroblue tetrazolium, and lignin staining. The defense reactions are localized mainly in single symbiotic cells of infected and fixation zone of *nifH* nodules. The proteomic analysis of nodules confirmed the defense reactions revealed by the staining methods and help us to understand more about the immune response of host plants against inefficient strains. Our last investigation was to observe if the plant could select for restored bacteria where the transposon has jumped out of the loci in the *nifH* mutant. This phenomenon would lead to recovered ability of *nifH* strains to fix nitrogen. In this research, we were able to confirm that the plants can recognize inefficient endosymbionts and trigger defense mechanisms used to counter the inefficient *nifH* bacteria.

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