Analysis of Gene Expression Pattern Induced by Rhizobia in Early Nodulation Mutant *nsp1* of Legume Genetic Model *Medicago truncatula*

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The symbiotic interactions between the soil bacteria, called rhizobia, and legume plants induce a complex developmental program resulted in formation on the legume roots of novel organs, nodules. Nodule is a symbiotic organ where atmospheric molecular dinitrogen reduced (fixed) by rhizobia into ammonia easily assimilated by plants. This symbiotic nitrogen fixation (SNF) allows legume plants to grow well on nitrogen-starved soils without adding nitrogen fertilizer. Therefore, the rhizobia-legume symbiosis is very important for sustainable agriculture and contributes significantly to the global nitrogen cycle. However, most of economically important legume species like soybean and alfalfa have relatively large genomes with complex organization which slows down the dissection of symbiotic mechanisms to better adopt SNF to human needs through the improvement of legume crops. In this situation, the adoption of *Medicago truncatula*, a species better characterized genetically, as one of the legume genetic model system has significantly accelerated discoveries related to the nodulation process. A considerable contribution has become the development and analysis of a number of nodulation-defective *M. truncatula* mutants which resulted in discovery of some major participants of the Nodulation signaling pathway, a cascade of genes activating and maintaining the nodulation machinery. One of key players at the early stage of this process was found to be the *NSP*1 gene which is important both for nodule organogenesis and for infection penetration. The *nsp1* mutant demonstrates only very early responses to rhizobia which are blocked after hair tip branching and doesn't form the nodule primordia and nodules. *NSP*1, a SCARECROW-like transcription factor, has been cloned recently (Smit et al. *Nature* 2005). The major objectives of this research were a) characterization of expression of the *NSP*1 gene; b) studying the effect of mutation at this locus on the expression of the other genes responding to inoculation with rhizobia. The expression pattern of *NSP*1 was analyzed in inoculated and uninoculated roots in wild-type (A17) and nodulation-deficient *nsp1* mutant (F4MtapC108; allele *nsp1-1*) plants using Real-Time PCR. The expression profiles of some of transcriptional markers of early nodulation (ENOD40, ENOD20, MtN6) also were studied. The analysis of transcript abundance for the genes downstream of the *NSP1* was based on microarrays included 6,000 well-annotated *M.truncatula* cDNA clones. Both Real-Time PCR and microarray research were conducted for series of time-course experiments included time-points of 0, 6, 24, and 48 hours post-inoculation with the *Sinorhizobium meliloti* (strain ABS7) and the corresponding developmental stages of uninoculated plants. The differences in gene expression between wild-type and the *nsp1* mutant roots were found for both responses to inoculation and between uninoculated tissues. We identified groups of co-regulated genes acting in *NSP*1-dependent as well as in *NSP*1-independent manner. Based on microarray experiments the putative regulatory candidate genes were selected for detailed analysis through Real-time PCR as well as via reverse genetic approach (RNAi). The expression profiling of such genes is underway. The results obtained from both Real-Time PCR and microarray experiments will be discussed.