

Putatively Hypoxia-regulated genes that control the Carbon allocation and metabolism in the Nodule of *Medicago truncatula*

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Although N₂ is extremely abundant, comprising about 79% of the atmosphere, plants cannot convert it to useful organic forms and mineral nitrogen is limited in soils. Legumes are unique among crop plants in the ability to fix N₂ in symbiotic association with bacteria called rhizobia. Symbiotic nitrogen fixation (SNF) takes place in legume root nodules, organs of tumor-like structure, and is accomplished by the bacterial nitrogenase, an enzyme that requires hypoxic microenvironment (10 to 40 nM of O₂) and high ATP levels for its activity. Hypoxia has been shown to alter the expression of genes involved in several metabolic pathways with the exact response being plant organ-specific. Here, we have used the model symbiotic system of *Medicago truncatula*–*Sinorhizobium meliloti* to identify plant genes involved in carbon allocation and metabolism in the nodule, that their expression is putatively under hypoxia-related regulation. *M. truncatula* is an excellent candidate for such studies, due to the available databases concerning the sequencing of the genome (<http://mtgea.noble.org/v2>, <http://www.medicago.org/genome>, NCBI), the expression of genes (<http://mtgea.noble.org/v2>), and the active metabolic pathways (<http://www.genome.jp/kegg/pathway.html>), as well as, the existence of established Tnt1-insertion mutant lines. *In silico* analysis was conducted to identify *M. truncatula* genes encoding for sugar transporters and glycolysis enzymes isoforms that are nodule-specifically expressed or nodule-highly induced. A small number of such genes were identified; however all the corresponding encoded proteins control significant regulatory steps of carbon allocation and metabolism in the plant cell. To verify the *in silico* analysis, total RNA was extracted from different organs and nodule developmental stages of *M. truncatula*, and the expression of these genes (RT-qPCR) is depicted. Furthermore, we present data concerning the structure of these genes, the amino acid sequence, and the prediction of the secondary structure together with the annotated functions of the encoded proteins. The corresponding cDNAs were obtained and the coded sequences of these genes were cloned. Moreover, we present data concerning the spatial expression and the subcellular localization of the products of these genes in the nodule of *M. truncatula* and results concerning their physiological role during SNF.

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