Molecular identification of nodule-specific and nodule-induced monosaccharide transporters (MSTs) in *Medicago truncatula.*

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Abstract

Legumes are cornerstones of sustainable agriculture, as the symbiotic relation they form with soil bacteria, called rhizobia, results in nitrogen fixation (symbiotic nitrogen fixation – SNF). Plant cell membrane transporters are essential for nutrient exchange between legumes and rhizobia, facilitating the appropriate conditions for nodule metabolism and being potential sites of SNF regulation. *M. truncatula* is an excellent candidate for studies of nodule transporters, due to the already existing gene expression data, the fact that it is a model organism for biological studies, and the symbiotic relations it forms with the rhizobium *Sinorhizobium meliloti*. Phylogenetic taxonomy and identification of nodule-induced and nodule-specific MSTs in *M.truncatula* was conducted *in silico,* using the MtGEA (Noble foundation) and JCVI: Medicago public databases. Gene structure, relative expression levels of MSTs in different organs and nodule developmental stages were obtained in order to identify nodule - specific and nodule - induced MSTs. Furthermore, amino acid sequence of the putative MSTs and topology-secondary structure of the encoded protein were predicted. Total RNA was extracted from different organs and nodule developmental stages of *M. truncatula,* gene-specific primers were designed and RT-qPCR analysis was performed to verify the expression patterns of putative transporters. This work represents the starting point for the elucidation of the MSTs exact physiological and biochemical role during SNF using the available reverse genetic resources for *M. truncatula*.

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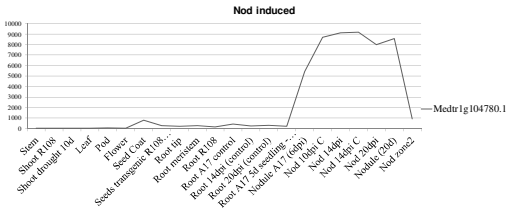
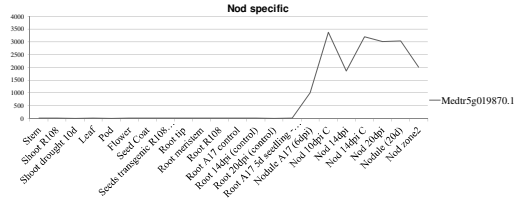
Materials and Methods

- *In silico* analysis in Noble Foundation's *Medicago truncatula* Expression Atlas (MtGEA) and JCVI: Medicago public databases.
- Bioedit and Mega 5.2 programs were used for the construction of the MSTs dendrogram.
- TMHMM Server v. 2.0 for the prediction of transmembrane helices in proteins.
- RNA isolation and cDNA synthesis.
- Design of gene-specific primers.
- qRT-PCR to estimate gene expression levels.



Graph 1: Relative expression levels of Medtr 1g104780.1 according to MtGEA.

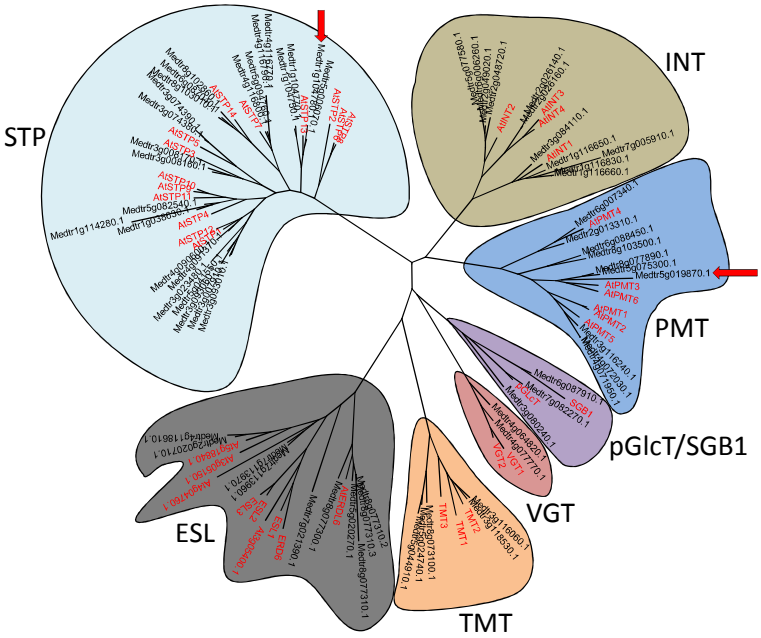


Graph 2: Relative expression levels of Medtr 5g019870.1 according to MtGEA.

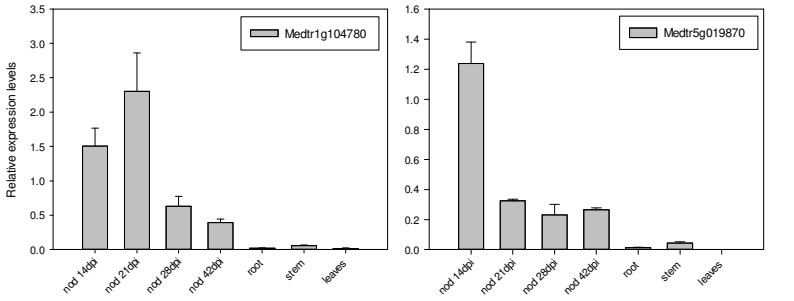
Conclusions

- Phylogenetic taxonomy of the MSTs was generated. Medtr1g104780.1 (STP) and Medtr5g019870.1 (PMT) were selected for further analysis due to their expression levels in nodules.
- Nucleotide and protein sequences are presented in addition to transporter putative secondary structure.
- The results from the qRT-PCR verified the specificity in the expression of the two sugar transporters during nodulation, as found in MtGEA.
- First indication of the MSTs' physiological and biochemical role during SNF.
- Future goals:
  a) topology studies concerning the localization of the mRNAs of Medtr1g104780.1 and Medtr5g019870.1 and their corresponding proteins through expression studies of transporter-YFP hybrids in the nodules.
  b) biochemical characterization of the transport activity after their expression in the *Saccharomyces cerevisiae* system.
  c) phenotypic characterization of *M. truncatula Tnt1* mutant lines for the target genes.

Results



Dendrogram 1: Phylogenetic tree of the MST family in *M. truncatula* and *Arabidopsis thaliana* (STP: sugar transport protein, PMT: polyol/monosaccharide transporter, INT: inositol transporter, TMT: tonoplast membrane transporter, VGT: vacuolar glucose transporter, pGlcT/SGB1: plastidic glucose transporter/suppressor of G Protein Beta 1, ESL: early-responsive to dehydration six-like).



Graph 3: Relative expression levels of Medtr 1g104780.1 and Medtr5g019870.1 in different nodule developmental stages and in different non-symbiotic organs.

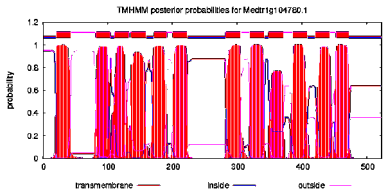


Figure 1: Transmembrane helices of Medtr 1g104780.1

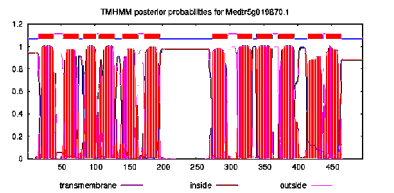


Figure 2: Transmembrane helices of Medtr 5g019870.1

Figure 3: Gene structure of Medtr 1g104780.1

Figure 4: Gene structure of Medtr 5g019870.1

Medtr1g104780
CDS sequence:

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Proteinsequence:

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Medtr5g019870
CDS sequence:

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Protein sequence:

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Figure 5: cds and protein sequence of Medtr1g104780.1 and Medtr5g019870.1 genes.