

Bioinformatic and Transcriptomic Analysis Identifies Small Signaling Peptides with Nodule- and Macronutrient-Responsive Expression in *Medicago truncatula*

Peter K Lundquist, Thomas C de Bang, Xinbin Dai, Patrick Zhao, Michael K Udvardi, Wolf R Scheible

Legumes represent a significant source of global food and feed, and are a staple in many crop rotation programs. Due to their ability to fix nitrogen in cooperation with bacterial rhizobia, legumes play a crucial role in the agricultural ecosystem. *Medicago truncatula* has emerged as an excellent model for studies of legume physiology and nodulation. Growing evidence indicates that small signaling peptides (SSPs) play a critical role in legume growth, development and productivity, yet the magnitude of SSP-coding potential within *M. truncatula* remains unexplored. Moreover, very few of the known SSP-coding genes have been connected to specific functions. We have employed a systematic re-annotation of the *M. truncatula* genome to identify a comprehensive dataset of all SSP gene families from *M. truncatula*. With this dataset in hand, we investigated the genome-wide expression analysis of known and previously unidentified SSP genes during macronutrient deficiencies, salt stress, nodule development, and symbiotic interactions based on a genome-wide RNAseq study. We identified a number of nutrient-responsive SSPs, greatly expanding the number of SSP gene families involved in adaptation to nutrient deficiencies. Likewise, we identified more than 50 SSP genes responding to inoculation with rhizobia, a more than ten-fold increase over previously known SSP genes. Finally, we introduce novel SSP genes with nodule-specific expression, opening up the possibility for a much greater number of SSPs involved in establishment and regulation of nodulation. Our bioinformatic and transcriptomic results open the door to investigate the function of specific SSPs via peptide treatment and gene overexpression.