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Systemic control of symbiotic nodulation in legume plants: genetic engineering in functional studies of key regulators

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Legumes are important suppliers of vegetable protein. In symbiotic interactions with soil bacteria rhizobia, legume plants form nitrogen-fixing nodules on their roots, where molecular nitrogen is fixed and incorporated into organic compounds. A host plant controls the number of symbiotic nodules to meet its nitrogen demands. The presence of high amount of nitrate in the soil suppresses the formation of nodules. CLE (CLAVATA3/EMBRYO SURROUNDING REGION) peptides produced in the root in response to rhizobial inoculation and/or nitrate were shown to control the number of symbiotic nodules. Previously, we have identified the *MtCLE35* gene upregulated by the rhizobia and the nitrate treatment in *Medicago truncatula*, which systemically inhibited nodulation when overexpressed. Using genetic engineering approaches we increased its transcriptional activity in transgenic roots, which almost completely prevented the formation of symbiotic nodules. Moreover, we obtained stable transgenic lines overproducing the MtCLE35 peptide, and found that their had lower shoot and root biomass in comparison to the wild-type plants. The study of metabolome of MtCLE35-overproducing plants revealed the increased level of amino acids in the roots, suggesting the stimulating effect of MtCLE35 on amino acid synthetic pathways.

In addition, we obtained several knock-out lines, where the *MtCLE35* gene was edited using the CRISPR/Cas9-mediated system. The detailed analysis of these plants will allow us to understand the mechanisms of MtCLE35 action in the regulation of plant nitrogen status and symbiotic interaction with rhizobia.

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