

Overexpression of the *MtCLE35* gene in transgenic *Medicago truncatula* plants inhibits nodulation at early stages of symbiosis development



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CLE (CLAVATA3/ENDOSPERM SURROUNDING REGION-related) peptides are known as systemic regulators of legume-rhizobium symbiosis that negatively control the number of nitrogen-fixing nodules. These regulatory peptides are produced in the root in response to inoculation with rhizobia, and are transported through the xylem to the shoot, where they are recognized by their receptor, CLV1-like (CLAVATA1-like) kinase, active in leaf phloem cells. After that, a shoot-derived signaling pathway is activated that inhibits subsequent nodule development in the root. Previously, we found that in *Medicago truncatula*, the expression of the *MtCLE35* gene is activated in response to rhizobia and nitrate treatment, and its overexpression systemically inhibits nodulation. However, little is known about the downstream target genes regulated by a *MtCLE35* signaling pathway in the root. Moreover, it is not completely clear which stage of symbiosis development is affected by *MtCLE35*-activated pathway. In order to identify genes regulated by the *MtCLE35*-induced signaling pathway, we performed a transcriptomic analysis of the roots overexpressing the *MtCLE35* gene. Totally, 1122 genes were found to be differentially expressed between *MtCLE35*-overexpressing and control roots after rhizobial inoculation, among them 185 genes were upregulated and 937 genes were downregulated. Among downregulated genes, many known regulators of legume-rhizobia symbiosis were found. In addition to this, we analyze early steps of interaction between *M. truncatula* overexpressing the *MtCLE35* gene and *Sinorhizobium meliloti* labeled with fluorescent reporter. We did not observe penetration of *S. meliloti* into host plant roots with *MtCLE35* overexpression. Our data suggest that overexpression of the *MtCLE35* gene inhibits nodulation at the very early stages of symbiosis development.

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