

CRISPR-CAS9-mediated editing of *the too much love* genes – the negative regulators of symbiotic nodule development in *Medicago truncatula*

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CRISPR-CAS9-опосредованное редактирование генов *too much love* – негативных регуляторов развития симбиотических клубеньков у люцерны

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The formation of symbiotic nodules in legume plants is regulated by the autoregulation of nodulation (AON) system. Key components of this system are CLE peptides, synthesized in the roots, and their receptors, CLV1-like kinases, operating in the shoots. The binding of CLE peptides to their receptors suppresses nodule formation. In *Medicago truncatula*, the activation of CLE receptors leads to the increased expression of the *MtTML1* and *MtTML2* (*TOO MUCH LOVE 1* and *2*) genes, which also suppresses nodule development [Gautrat et al., 2019; Lebedeva et al., 2023]. Mutations in the *TML* gene in the *Lotus japonicus* result in excessive nodulation [Gautrat et al., 2019; Lebedeva et al., 2023]. The *TML1* and *TML2* genes encode F-box proteins with Kelch repeats, which function as E3 ubiquitin ligases. These enzymes are involved in the ubiquitin-dependent degradation of proteins that are key regulators of symbiosis development [Takahara et al., 2013]. However, the specific target proteins of TML and the mechanisms of their action remain unstudied.

To investigate the role of the *TML* genes, CRISPR-Cas9 genome editing constructs were designed. This allowed for the generation of plants harboring a single-nucleotide deletion (del-1) and a 72-nucleotide deletion (del-72) in the *MtTML2* gene. Loss-of-function of the *MtTML2* gene (*tml2-1*) results in a significant increase in symbiotic nodule number. Moreover, a heterozygous plant with 5-nucleotide deletion (del-5) in the *MtTML1* gene was obtained. Furthermore, work is currently ongoing to obtain regenerant plants with simultaneous deletions in both the *MtTML1* and *MtTML2* genes. As part of this study, constructs for the overexpression of the *MtTML1* and *MtTML2* genes under the control of the CaMV 35S promoter were also developed. Additionally, vectors for site-directed PCR mutagenesis of the *MtTML1* and *MtTML2* genes within the microRNA miR2111 recognition site have been created. The cloning of genes encoding proteins that are putative targets of the TML protein is also actively underway. Studying plants with modified *MtTML1* and *MtTML2* gene activity will help elucidate their role in suppressing the symbiosis program.

Keywords: symbiotic nodule, Autoregulation of nodulation, TOO MUCH LOVE, miR2111.