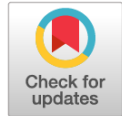


# Putative molecular pathways of autoregulation of nodulation activated by CLE peptides in pea

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Legume plants are important for ecosystems due to their ability to form root nodules in symbiosis with rhizobia, where nitrogen fixation takes place. The number of symbiotic nodules is regulated by the CLE peptides inhibiting excessive nodule formation. Previously, we have identified four genes encoding CLE peptides, activated in response to rhizobia inoculation in pea. Three of them, *PsCLE13*, *PsCLE12* and *PsNIC-like*, were also activated by nitrate, and, therefore, they could mediate nitrate-dependent inhibition of nodulation [1]. Overexpression of *PsCLE13* and *PsCLE12* inhibited nodulation on transgenic roots: however, the role of *PsNIC-like* and *PsCLE12-like* have not been investigated.

In this study, we constructed vectors for overexpression of the *PsCLE12-like* and *PsNIC-like* genes to study their possible role in nodulation, and also analyzed the expression levels of nodulation-related genes in transgenic roots overexpressing four *PsCLEs* genes. Moreover, vectors for CRISPR-Cas9-mediated gene editing of the *PsCLE12* and *PsCLE13* genes were constructed to further explore the role of these genes in nodulation. Overexpression of *PsCLE12-like*, *PsCLE13* and *PsCLE12* resulted in increased expression levels of *TOO MUCH LOVE* (*PsTMLs*) genes known as root-acting regulators of nodule number. In addition, in the roots overexpressing four *PsCLEs* genes, down regulation of the *PsSYM37* gene (encoding the receptor for Nod-factors) was observed, suggesting that the CLE peptides might inhibit the development of symbiotic nodules at the earliest stages of symbiosis development upon Nod-factor perception.

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**Keywords:** CLE peptides; pea; nodulation.

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