# *CLE* genes regulating symbiotic nodule development in *Pisum sativum*

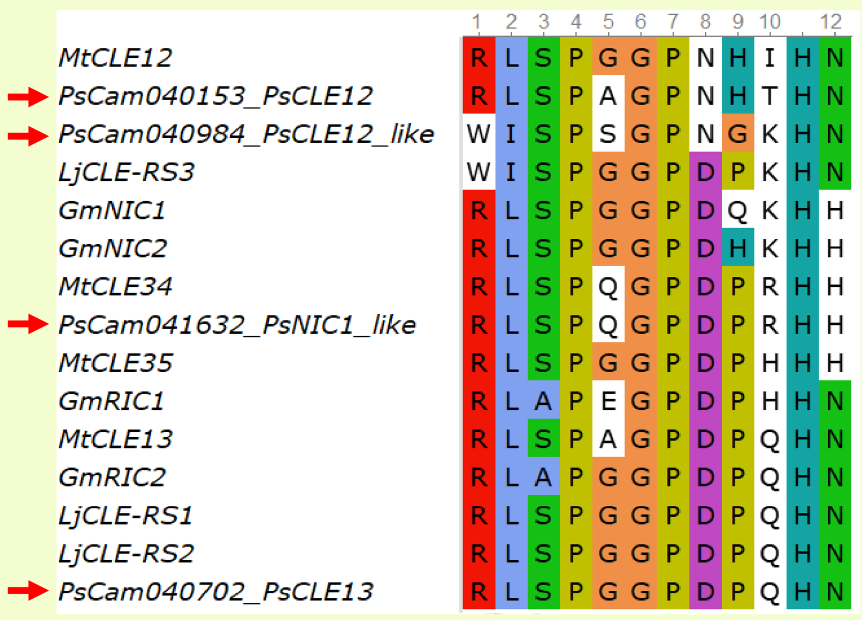
**Sadikova D.S.*, Lebedeva M.A., Lutova L.A.**

*Saint Petersburg State University, Saint Petersburg, Russia, *darinasadikova@yandex.ru*

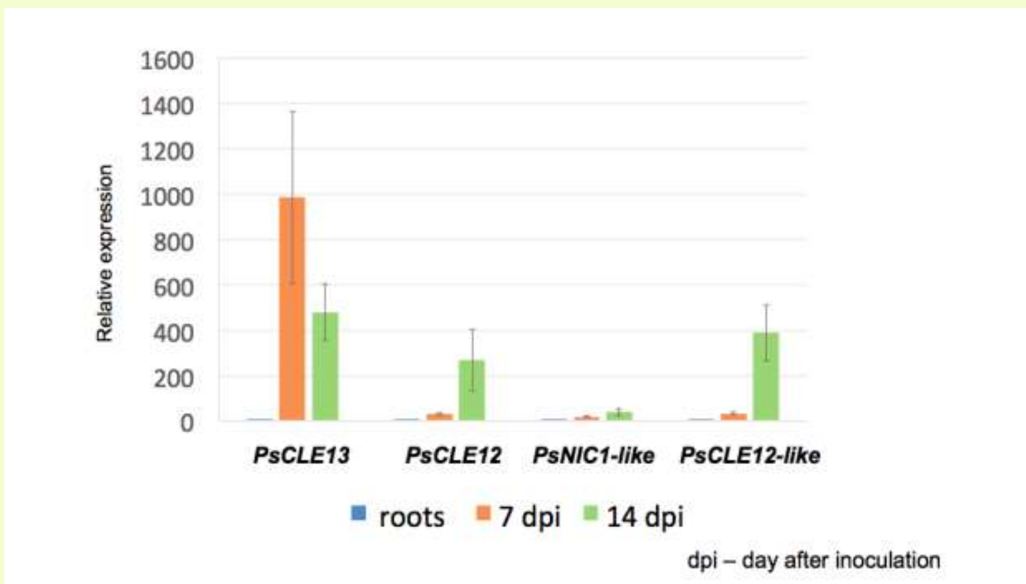
Legumes are able to enter into symbiosis with nitrogen-fixing bacteria rhizobia to build the root nodules. Symbiotic nodules provide transfer of nitrogen from rhizobia, necessary to support plant growth. This ability makes legumes key species for natural and agricultural ecosystems and allows to reduce the application of nitrogen-containing fertilizers. In different model legumes, supernodulating mutants have been described that form excessive number of symbiotic nodules even at a high nitrogen content in the soil. These mutants appeared to be defective in genes encoding CLV1-like kinase that acts in the shoot and systemically, via long-distance regulation, inhibits the initiation of symbiotic nodules on the root, thereby limiting the number of developing nodules. The ligands of CLV1-like kinases are mobile CLE peptides that are produced in the root in response to rhizobial inoculation and moves to the shoot to induce AON. In addition, nitrate-induced *CLE* genes have been identified in model legumes, which inhibited nodulation when overexpressed.

In our work we analyzed four homologues of *CLE* genes in *Pisum sativum*, which are closely related to nodulation-suppressing *CLE* in other legumes. We estimated the expression levels of these genes at different stages of nodulation, as well as in response to nitrate treatment. The effect of the *PsCLE12* and *PsCLE13* overexpression on nodulation has been analyzed. Our preliminary data show that overexpression of nodulation-specific *PsCLE12* gene did not suppress nodulation on transgenic roots, whereas *PsCLE13* overexpression decreased nodule number on transgenic roots. Therefore, these two close homologues might have diverse function in nodulation, and in our subsequent research we are going to study the mechanisms of their action in pea nodule development.

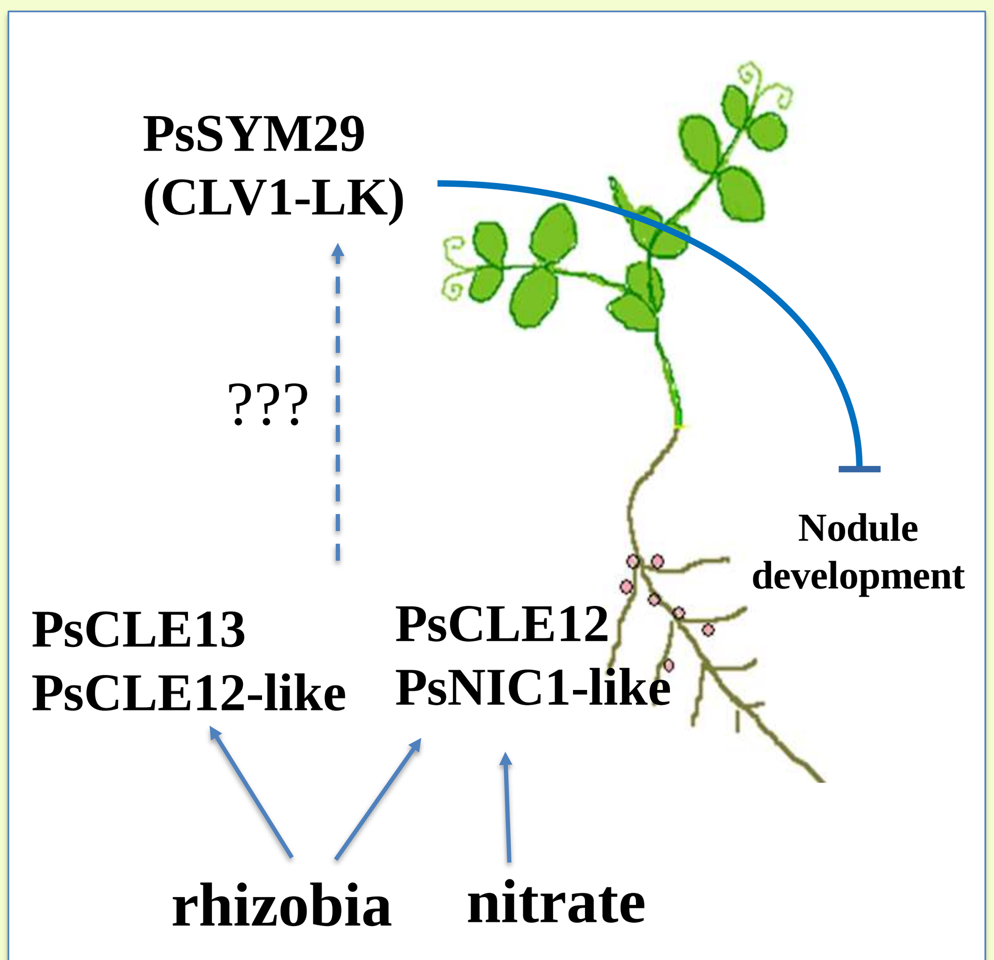
## Identification and expression analysis of *PsCLE* genes

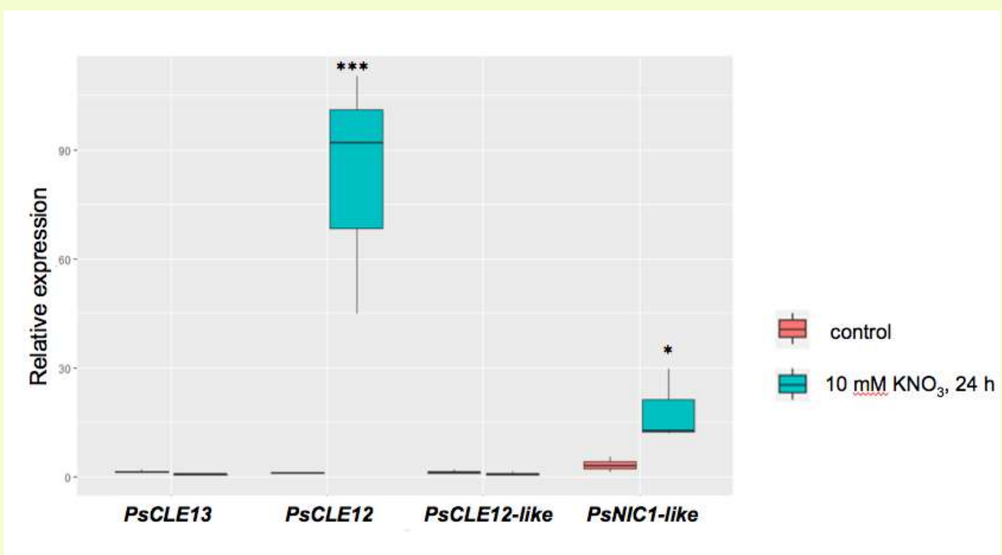



Sequence of CLE peptides in legumes



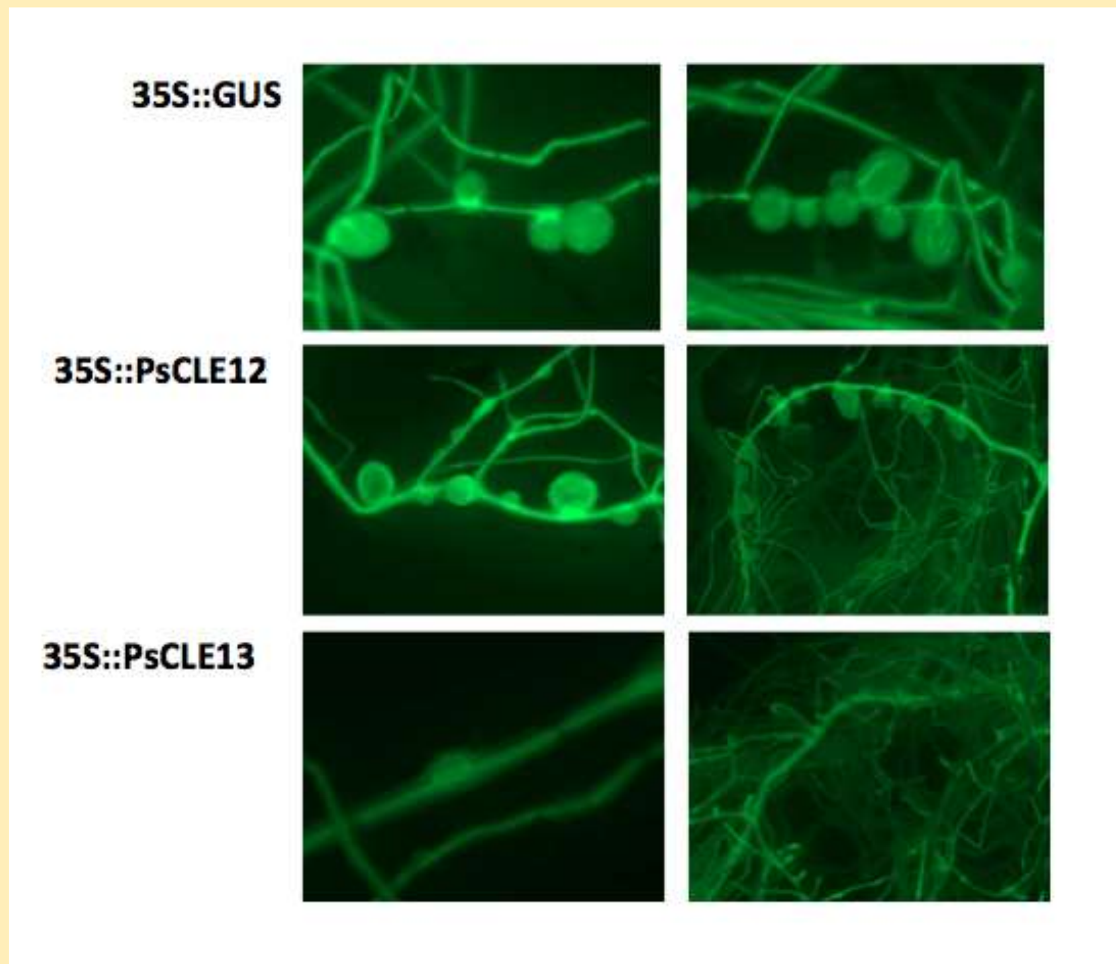

Expression of *PsCLE* genes during nodulation



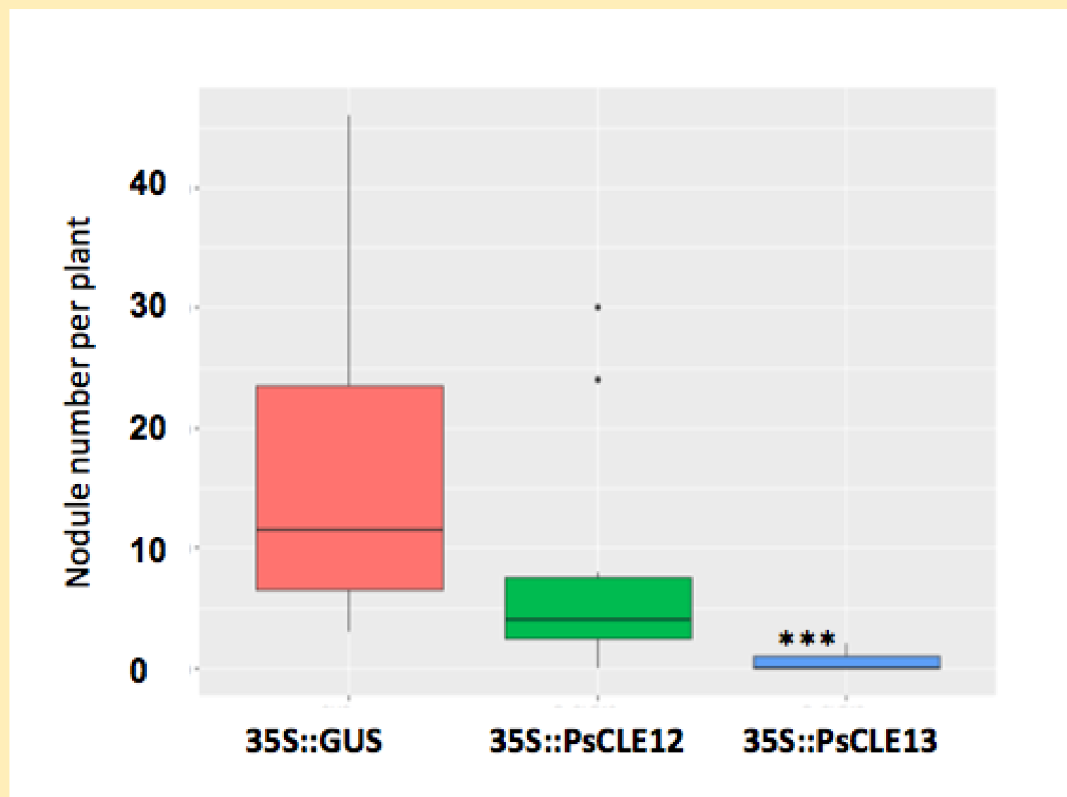




Expression of *PsCLE* genes in response to nitrate treatment

## Effect of *PsCLE12* and *PsCLE13* overexpression on nodulation

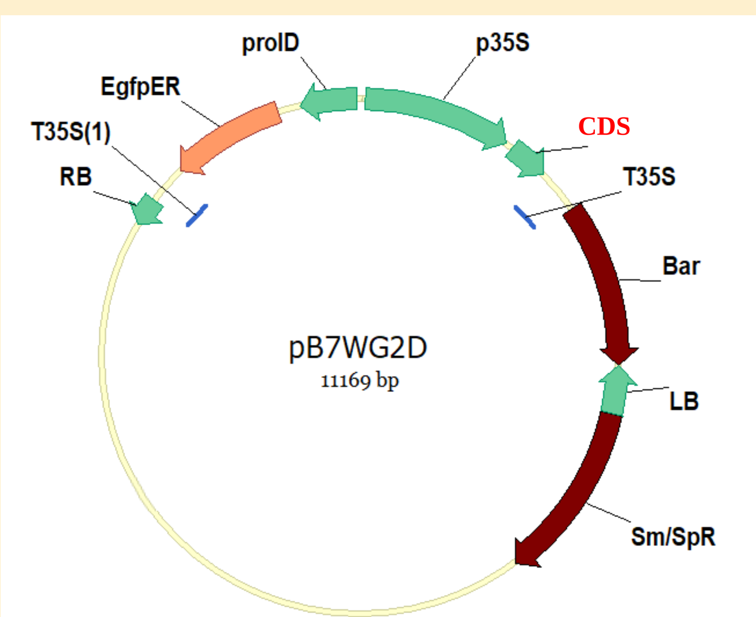



Transgenic roots and nodules




Number of nodules on transgenic roots

## Creation of vectors for *PsCLE* overexpression and obtaining composite plants with transgenic roots




Vector used for overexpression of *PsCLE* genes

Callus with emerging hairy roots formed on the site of inoculation with *Agrobacterim rhizogenes*

## Conclusions

- During nodulation, expression of *PsCLE13* gene increased earlier than other genes (7 days after inoculation). Expression of *PsCLE13* and *PsCLE12-like* genes increased by 14 day after inoculation.
- Expression of *PsCLE12* and *PsNIC1-like* genes significantly increased in response to nitrate treatment.
- The nodule number on transgenic roots was decreased by *PsCLE13* overexpression, whereas *PsCLE12* gene did not suppress nodulation.

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