

Transcriptomic and metabolomic insights into the action of *MtCLE35* gene — nitrate-regulated inhibitor of symbiosis

*Petrenko V. A. *, Rubtsova D. N., Berdigan R. D., Lebedeva M. L., Lutova L. A.*

St. Petersburg State University, St. Petersburg, Russia

**st106188@student.spbu.ru*

Изучение механизмов действия гена *MtCLE35*, нитрат-регулируемого ингибитора развития симбиоза, на транскриптомном и метаболомном уровне

*Петренко В. А. *, Рубцова Д. Н., Бердиган Р. Д., Лебедева М. Л., Лутова Л. А.*

Санкт-Петербургский государственный университет, Санкт-Петербург, Россия

The symbiotic interaction between legume plants and nitrogen-fixing rhizobia bacteria is a unique phenomenon that enables the plants to utilize atmospheric nitrogen. Since this symbiosis is energetically costly, the plant controls number of nodules through a process known as Autoregulation of nodulation (AON). In this system CLE peptides act as key negative regulators through a negative feedback mechanism.

One member of this gene family, *MtCLE35*, acts as a systemic inhibitor of nodulation. This was demonstrated by the suppression of 1122 genes in the roots of plants overexpressing *MtCLE35*. Interestingly, transcriptomic analysis revealed that the overexpression of *MtCLE35* also led to the upregulation of other genes. Gene Ontology (GO) enrichment analysis indicated that these differentially expressed genes (DEGs) were significantly associated with terms such as «oxidoreductase activity,» «peroxidase activity,» «antioxidant activity,» «heme binding,» and «tetrapyrrole binding.»

Transcriptomic analysis of *MtCLE35*-oe roots revealed the upregulation of defense-related genes compared to the control (roots of R108 line). These genes encode peroxidases, ethylene biosynthesis enzymes, thioredoxin, chitinases, chalcone O-methyltransferase-1, and cysteine-rich peptides. The significant induction of these genes was confirmed by qRT-PCR. These findings suggest that *MtCLE35* overexpression may suppress symbiosis by activating plant defense responses. This potentially causes the plant to perceive rhizobia as pathogens, thereby preventing the establishment of mutualism.

Under controlled phytotron conditions, the *MtCLE35*-oe line exhibited reddening of shoots and leaf petioles, likely due to anthocyanin accumulation. Biochemical analysis confirmed a statistically significant increase in the total content of flavonoids and anthocyanins in both roots and shoots of the *MtCLE35*-oe line compared to the R108 control line.

Metabolomic profiling further revealed that *MtCLE35*-oe plants had increased amount of phenolic compounds, glycosides, and free saturated fatty acids. Conversely, the content of nitrogen-containing compounds was significantly lower in the shoots and roots of the *MtCLE35*-oe line.

Therefore, our multi-omics analysis reveals that the inhibition of nodule development caused by *MtCLE35* overexpression is linked to the activation of defense processes.

Keywords: legume-rhizobium symbiosis, CLE peptides, autoregulation of nodulation.