Characterization of pea (*Pisum sativum* L.) microRNAs

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Key message. Pea microRNAs and their targets were identified, and their differential expression was analyzed during the development of symbiosis with rhizobia and mycorrhizal fungi, and under conditions of abiotic stress caused by cadmium.

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MicroRNAs play a key role in regulation of gene expression in processes of plant development, biotic and abiotic interactions. Development of high throughput sequencing technologies provides the opportunity to study the full set of small regulatory RNAs even in non-model objects. Pea (*Pisum sativum* L.) is one of the oldest objects of plant genetics and a valuable crop. To date, small regulatory RNAs of pea remain undetected.

The aim of the study was to sequence and to characterize pea microRNAs and their targets, and to analyze changes in their expression level in different conditions.

Libraries of small RNAs isolated from the roots, nodules and shoots of pea plants were prepared and sequenced in GenXPro (Germany). Target genes of microRNAs were analyzed by "degradome" sequencing, which made it possible to identify cleavage sites in transcripts. Different pea lines were used for the analysis of differential expression: wild-type lines Frisson and SGE; supernodulating mutants P88 and P64, obtained after mutagenesis of line Frisson; cadmium-tolerant mutant SGECdt, obtained after mutagenesis of line SGE.

The analysis revealed 281 microRNAs, of which 140 have conserved sequences. Analysis of degradome revealed 1238 probable target for the identified microRNAs. For a number of transcripts, the regulation by more than one miRNA is shown. It was shown that some pea microRNAs are involved in the regulation of several transcripts.

Constitutive expression of some microRNAs was detected in pea roots and nodules, collected at different stages of development. These microRNAs can be considered as reference sequences for the further study of expression by real-time PCR. A number of microRNAs involved in response to inoculation with rhizobia and mycorrhizal fungi have been identified that are presumably involved in nodule autoregulation processes controlled by the CLAVATA system. Without inoculation, expression of Ps169 and Ps393 microRNA was significantly reduced in the mutant P88, while Ps160 miRNA expression was enhanced in the mutant P64. Rhizobia inoculation reduced the level of expression of a large number of microRNAs in the mutant P88, while during mycorrhization, the level of corresponding microRNAs was reduced in both mutant lines compared to the Frisson genotype. The target genes of the identified microRNAs are associated with the regulation of gene expression, including the Auxin Response Factors family; homologues of NAM (No apical meristem) proteins associated with the meristem formation; components of ribosome; peroxidases and proteins associated with the transmission of intracellular signals. A comparative analysis of gene expression in Frisson, P64, P88 revealed multidirectional expression in response to inoculation with rhizobia and mycorrhizal fungi of a sequence homologous to the MtNF-YA1 - a key transcriptional regulator of nodule development in *Medicago truncatula*. The P64 line showed a reduced level of expression of this gene compared to the P88 line.

We also studied the change in the expression of microRNAs and their targets under abiotic stress caused by heavy metals, in particular cadmium. It was found that cadmium causes a change in the expression of a number of microRNAs, while no difference between the cadmium-sensitive and cadmium-tolerant genotypes was detected. Generally, cadmium caused increase of microRNA expression. Analysis of the targets of the identified microRNAs showed that the processes of response to abiotic stress were the most affected ones.

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