Genetic characterization of pea (*Pisum sativum* L.) mutants P59 and P60, defective in nitrogen fixationKlimenko O.P.¹, Kulaeva O.A.¹, Shtark O.Y.¹, Zhernakov A.I.¹, Tikhonovich I.A.^{1,2}, Zhukov V.A.¹¹All-Russia Research Institute for Agricultural Microbiology, St. Petersburg, Russia; ² St. Petersburg State University, St. Petersburg, Russia

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Key message. Several genes involved in development of symbiosis between pea and rhizobia haven't yet been characterized in detail. Here, the first results of genetic analysis of pea mutants in the symbiotic genes *Sym23* and *Sym24* are presented.

Keywords: nitrogen fixation, symbiotic genes, nodulation

An important factor of soil productivity is the ability of legumes to fix nitrogen in symbiosis with nodule bacteria. Identification of genes that are important for these symbiotic relationships is the key to understanding the functioning of nitrogen-fixing systems, and thus is an important task for both applied and fundamental science. A large collection of mutants with defects in nodulation is available for pea, and for several mutants, comprehensive phenotypic characterization and genetic analysis is needed.

Aim: To perform the phenotypic and genetic characterization of pea (*Pisum sativum* L.) mutants P59 and P60, defective in nitrogen fixation.

In this work different lines of pea were used: Frisson, NGB1238 and SGE, characterized by pink effective nodules; two mutant lines obtained on the Frisson line: P59 (mutation in the *Sym23* gene), P60 (mutation in the *Sym24* gene), characterized by the formation of white, ineffective nodules. The microorganisms that were used are *Rhizobium leguminosarum* bv. *viciae* RCAM1026 strain.

The nitrogen-fixing activity of nodules was assessed by the acetylene reduction method. Localization of mutant loci was carried out using bulk segregant analysis (Galvão et al., 2012) in combination with RNA-seq.

Comparative analysis of the vegetative mass of plants showed that the P59 line had the lowest mass (1,38 times lower compare to Frisson, $p=0,021$), while the P60 line did not differ in this parameter from wild-type plants. However, the difference in shoot mass between the three analyzed lines was not statistically significant.

Analysis of acetylene reduction showed that mutant plants had a greatly reduced nitrogen-fixing ability, especially plants of the P59 line – among two mutants, this line has the lowest ability to reduce acetylene (17, 5 times lower compare to Frisson, $p=0,00023$). Nitrogen fixing ability of P60 plants was 9,7 times lower compare to Frisson plants ($p=0,0007$).

For localization of genes *Sym23*, *Sym24* in pea genome, the F2 population from the cross of P59 and NGB1238 and the F2 population from the cross of P60 and SGE were analyzed for mutant phenotypes. Two separate bulks consisting of fix+ and fix- plants were pooled for future next generation sequencing of transcriptomes via MACE and subsequent search for the genes which, according to segregation analysis, are linked to the mutant genes. So, we plan to determine region of localization of mutations.

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