

## Obtaining of transgenic barrelclover plants (*Medicago truncatula*) producing chicken interferon gamma for veterinary use



Elena S. Okulova, Mikhail S. Burlakovskiy,  
Marina V. Padkina, Ludmila A. Lutova

Saint Petersburg State University, Saint Petersburg, Russia

At the Laboratory of Plant Genetic and Cellular Engineering, Department of Genetics and Biotechnology, St. Petersburg State University, five transgenic *Medicago truncatula* plants were obtained through Agrobacterium-mediated transformation, carrying one of the variants of the heterologous chicken interferon-gamma gene under the control of the constitutive 35S CaMV promoter. Among these, one plant harbored an unmodified gene insertion, while four had a modified gene with a deletion at the protease recognition site, providing resistance to proteolytic degradation.

We demonstrate the application of the SWPOP-PCR “genome walking” method to determine the integration sites of T-DNA into the plant genome, identify the number of insertion copies and their orientation. Analysis of the obtained sequences revealed that only one plant exhibited a single T-DNA insertion, which represents the most optimal structure for stable expression.

Upon self-pollination of T<sub>0</sub> plants, 39 offspring were obtained and subjected to testing for the presence and expression of the transgene. Among them, six homozygous plants were identified using molecular methods. Quantitative assessment of transgene expression levels showed significant differences among representatives of different lines and among the offspring derived from a single transformed plant. Among the T<sub>1</sub> and T<sub>2</sub> progeny, the presence of the heterologous interferon protein in plant tissues was confirmed through Western blot analysis.

The engineered barrelclover plants hold potential as bioreactors for the production of chicken interferon-gamma for veterinary applications. The use of an edible plant allows eliminating protein extraction and purification procedures, thereby resulting in a noteworthy reduction in production expenses of up to 80%.

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**AUTHORS' INFO**

**Elena S. Okulova**, Master of Science, Research Associate, Department of Genetics and Biotechnology; Saint Petersburg State University, Saint Petersburg, Russia; e-mail: elenaok.advert@gmail.com

**Mikhail S. Burlakovskiy**, PhD, Junior Researcher, Department of Genetics and Biotechnology; Saint Petersburg State University, Saint Petersburg, Russia; ORCID: 0000-0001-6694-0423; SPIN: 3679-0860; e-mail: burmish@yandex.ru

**Marina V. Padkina**, Dr. Sci., Professor of the Department of Genetics and Biotechnology; Saint Petersburg State University, Saint Petersburg, Russia; ORCID: 0000-0002-4051-4837; eLibrary SPIN: 7709-0449; e-mail: m.padkina@spbu.ru

**Ludmila A. Lutova**, Dr. Sci. (Biol.), Professor; Saint Petersburg State University, Saint Petersburg, Russia; ORCID: 0000-0001-6125-0757; eLibrary SPIN: 3685-7136; e-mail: la.lutova@gmail.com