Biolistic transformation of plants using CRISPR/Cas9 technology

Gumerova G., Chemeris A., Kuluev B.

Institute of Biochemistry and Genetics - Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences,
Ufa, Russia

E-mail: gulnar.yas@mail.ru

Key message. *Experiments on CRISPR/Cas9 mediated knock-in approaches in the PDS gene of various genes of interest were planned. Biolistic bombardment mediated delivery of target vectors to plant explants was suggested.*

Keywords: *genetic transformation of plants, CRISPR/Cas9 system, biolistic bombardment, tobacco*

Constantly new methods of gene editing are being created in modern genetic engineering. One of the most promising among them is CRISPR/Cas9 system. This technique is based on microbial adaptive immune system and allowed to modify a specific genome in precise and predictable manner. Researches on this area have shown that CRISPR/Cas9 is a great tool to edit many genes in a variety of plant species, including the model plant species as well as agriculturally important crops. The purpose of our research is silencing of tobacco phytoendosaturase (*PDS*) gene using CRISPR/Cas9 technology and obtaining tobacco *PDS* - knockout plants. This work is the first step for further researches on plant genome editing in our laboratory. Subsequently, experiments on CRISPR/Cas9 mediated knock-in approaches in the *PDS* gene of various genes of interest were planned. Tobacco leaf explants was chosen as a model object in our work. CRISPR vectors containing gene of Cas9 protein (pKIR1.1 and pK7WGF2::hCas9) and corresponding gRNA (pICH86966::AtU6p::sgRNA_PDS) were bought in «AddGene». Phytoendosaturase gene of tobacco was selected as the site for gene knockout experiments. This protein is a key enzyme in carotenoid biosynthesis. Knockout of *PDS* leads to inhibition of carotene synthesis resulting in the formation of decolorized plants. Biolistic bombardment mediated delivery of CRISPR vectors to plant explants on PDS-1000/He System (BioRad) was planned. The following gen gun parameters will be used: gold particles an average size of 0.6 microns, target distance - 6 cm, rupture disk 650 or 900 PSI, vacuum 0.9 bar, the amount of DNA per one shot - 1 µL (1 µg/µL). The gold particles will be coated with target vectors mixed with 0.1 µM spermidine and 2.5 M CaCl₂. Leaf discs will be pre-incubated on MS medium with 90 g/L sucrose for 48 hours before transformation (cultivating with increased osmotic pressure) to improve explants survival.