doi 10.18699/PlantGen-2025-268

A hairy root path for genome editing in common buckwheat (*Fagopyrum esculentum*)

D.O. Omelchenko[1, 2]*, L.R. Abdulkina[1, 2], E.S. Glagoleva[1, 3], L.V. Omelchenko[1], M.D. Logacheva[1, 2]
[1] *Vavilov Institute of General Genetics of Russian Academy of Sciences, Moscow, Russia*
[2] *Skolkovo Institute of Science and Technology, Moscow, Russia*
[3] *M.V. Lomonosov Moscow State University, Moscow, Russia*
* *omdeno@yandex.com*

Motivation and aim: Buckwheat (*Fagopyrum esculentum* Moench) is a pseudo-cereal crop that belongs to the Polygonaceae family. The plant is valued for its high nutritional value and bioactive compounds, which have led to its extensive utilization in the food and pharmaceutical industries. CRISPR/Cas genome editing system provides a means to introduce precise changes in plant genome to create favorable traits in crops, thereby expediting the development of new, improved buckwheat varieties at a rate that exceeds the pace of classical breeding. However, to date, no reliable protocol have been developed for the production of gene-edited buckwheat plants. In this study, we propose a methodology for the genetic modification of buckwheat through the use of *Agrobacterium rhizogenes*-mediated transformation and the subsequent plant regeneration from hairy root culture.

Materials and methods: This study utilized two self-pollinating buckwheat varieties, KK8 и Shinano-SC, and a cross-pollinating Design variety to investigate the process of hairy root induction and the transfer of T-DNA bearing CRISPR/Cas9 system for genome editing. The method of insulin syringe injection of the *A. rhizogenes* strain R1000 suspension in young buckwheat seedlings was employed for plant transformation due to high infectivity of this strain, as evidenced by both studies conducted by the authors and existing published literature. The binary vectors used in this study carried GFP and BAR expression cassettes for selection of transformed plants and CRISPR/Cas9 expression cassettes with single sgRNA for knockout of DFR and LFY genes for easy detection of phenotypic changes. The sgRNAs were designed using the crisprVerse R package and a high-quality KK8 annotated genome as a reference. All vectors and expression cassettes used in the study were created using plasmid modules from the MoClo Toolkit and CRISPR Plants Kit (Addgene). Calli from hairy roots of *F. esculentum* were obtained with the classical composition of MS3 medium with 2 mg/L 2,4-Dichlorophenoxyacetic acid and 2 mg/L Kinetin. To induce regeneration from callus culture, the MS3 medium was supplemented with 6-benzylaminopurine (2 mg/mL), kinetin (1 mg/mL), and indole-3-acetic acid (0.2 mg/mL). Basta herbicide was added to the medium (6 μg/mL) for hairy root selection and when the first regenerant shoots appeared from the calli.

Results: The entire procedure, from transformation and hairy root culture isolation to callus induction and regeneration and subsequent acclimation of regenerated transgenic plants to soil, was initially established using a control vector carrying only GFP and BAR selectable markers. The procedure was repeated with vectors carrying CRISPR/Cas9 expression cassettes in addition to selectable markers up to the step of shoot regeneration induction from transgenic calli. The *A. rhizogenes* strain R1000 effectively transferred the T-DNA from the binary vectors into the plant genome, inducing hairy root culture growth in all three buckwheat varieties. The transgenic status of the hairy roots and regenerated plants was confirmed by Basta resistance and the presence of green fluorescent protein (GFP) fluorescence in hairy roots and calli induced from them. It is noteworthy that the fluorescence of the GFP marker gradually disappeared as the regenerants grew, even though the morphogenic calli from which they were derived was fully fluorescent and the regenerated plants still contained T-DNA based on PCR test. The success of the gene editing was confirmed in the fluorescent hairy roots by Sanger sequencing of the sgRNA target regions for the DFR and LFY genes in KK8 and Design varieties, and for the DFR gene only in Shinano SC. A notable observation is that two distinct single guide RNA (sgRNA) designed for the LFY gene each resulted in the generation of a substantial deletion (more than 250 bp) encompassing their target sites in the Design variety.

Conclusions: A protocol for genome editing of common buckwheat has been developed that involves transforming the plant with *A. rhizogenes* and producing transgenic hairy roots from which the edited plant can be regenerated.