doi 10.18699/PlantGen-2025-234

Agrobacterium tumefaciens GV3101-mediated *Fagopyrum esculentum* genome transformation and DFR, LFY genes editing

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Motivation and aim: Previously, it could take tens or even hundreds of years to improve the properties of agricultural crops. However, methods have now been developed for editing almost any part of the genome in a year or even less for model plants. Buckwheat (*Fagopyrum esculentum*) is a valuable agricultural crop whose nutritional value increases with the study of its biochemistry. Nevertheless, publications on the successful transformation and editing of the *F. esculentum* genome are practically absent. This paper describes a method for transforming *F. esculentum* using *A. tumefaciens* strain GV3101.

Materials and methods: Three varieties of *F. esculentum* (KK8, Shinano SC, and Design) were selected for plant transformation. *A. tumefaciens* strain GV3101 was used for agrobacterial infection. This strain is characterized by the presence of the trans-zeatin gene in the vir region, which improves the regenerative abilities of plants (Han et al., 2013). In preliminary experiments, this strain demonstrated the highest efficiency for plant infection. DFR and LFY were chosen as target genes for editing. DFR (dihydroflavonol 4-reductase) is one of the key enzymes in the biosynthesis of anthocyanins, proanthocyanidins, and other flavonoids. It participates in the regulation of phenolic metabolism and affects plant resistance to stress (Jiang et al., 2023; Li et al., 2023). The LFY gene is involved in the regulation of cell proliferation and flower development. Its functions may vary depending on the plant species: it can regulate the timing of flowering, flower formation, apical meristem development, and lateral shoot formation (Jue et al., 2022; Rodriguez-Pelayo et al., 2022; Thekkeveedu, Hegde, 2022). GFP and the Basta herbicide resistance were used as reporter genes. In our work, we used several variants of plant transformation:

1. Floral dip transformation
2. Transformation of callus tissue without vacuum infiltration of agrobacteria
3. Transformation of callus tissue with vacuum infiltration of agrobacteria
4. Transformation of etiolated sprouts without vacuum infiltration of agrobacteria
5. Transformation of etiolated sprouts with vacuum infiltration of agrobacteria

For bacterial preparation, we used a modified “starvation” protocol (Gelvin, 2006). If the seeds were heavily contaminated with bacteria, we performed preliminary sterilization with 3% hydrogen peroxide, followed by washing with distilled water. The selection of transformants and their regeneration were carried out on specific media for direct regeneration, SIM (Omelchenko et al., 2024) or MSH (MS with a sugar content of 3%, Kin 1 μg/ml, BAP 2 μg/ml, IAA 0.2 μg/ml). All nutrient media following transformation and washing contained the selective agent Basta (6 μg/ml).

Results: Floral dip transformation of buckwheat and the transformation of calli were extremely ineffective, despite using various detergents to improve tissue wettability and regardless of vacuum infiltration. The most effective infection was achieved by vacuum infiltration of agrobacteria into etiolated seedlings. To date, we have successfully performed the infiltration of agrobacteria into buckwheat seedlings, selected transformed plants using Basta, regenerated herbicide-resistant, luminescent GFP explants, rooted the regenerants, and planted them in soil. In *dfr* mutant plants, a decrease in anthocyanin synthesis was noted compared to the control, and in *lfy* mutants, flower bud formation was impaired. Despite the successful phenotypic signs of editing, we have not yet been able to confirm editing using Sanger sequencing, possibly due to the chimerism of regenerants.

Conclusions: We have developed a method for the agrobacterial (*A. tumefaciens* GV3101) transformation of *F. esculentum*.