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Enhancing transformation and genome editing efficiency of commercial barley cultivars using the *GRF4-GIF1* chimera

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Plant genome editing using CRISPR/Cas9 technology has emerged as a promising tool for crop improvement and gene function research. Despite significant advances in barley gene editing, modification of commercial cultivars remains challenging, as traits such as *in vitro* regeneration capacity and *Agrobacterium*-mediated transformation efficiency are highly genotype-dependent, with few cultivars amenable to transformation. Consequently, the majority of barley genome editing studies have been conducted on the model *cv.* Golden Promise, known for its high regeneration and transformation capacity.

Recently, developmental regulators genes have demonstrated the ability to promote somatic embryogenesis, thereby facilitating much more effective transformation and genome editing of recalcitrant genotypes. In our study, we tested the capacity of wheat morphogenic regulators *GRF4-GIF1* to influence transformation efficiency and genome editing in three commercial barley cultivars: Tselinny 5, Aley, and G-23035. We used particle bombardment of immature embryos to deliver the JD633 vector containing the *TaGRF4-GIF1* chimera and compared outcomes with the control vector L2_41780, which lacked morphogenic regulators.

Our results demonstrated that the *GRF4-GIF1* gene combination significantly enhances the efficiency of transformation and editing in barley. Using vectors JD633 and L2_41780, we obtained two populations of 27 and 60 T_0 plants, respectively. Notably, stable transformants were exclusively generated in experiments utilizing the JD633 vector (14 out of 27 plants contained vector integration). The efficiency of stable transgenic lines production was approximately 2.8% across all three cultivars, in contrast to 0% for the control vector without *GRF4-GIF1*.

The efficiency of CRISPR/Cas9 editing was verified by targeting the *Nud* gene, which controls naked/hulled grains phenotype. Sequencing analysis identified 5 plants of the Aley, 5 plants of the Tselinny 5 and 4 plants of the G-23035 cultivars exhibiting various mono- and biallelic mutations. Our research thus presents a promising tool for future application of CRISPR technologies for precise improvement directly in commercial barley cultivars.

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