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BSMV-Mediated Genome Editing Exhibits Host-Specific Heritability: Germline Transmission in Barley and Somatic Edits in *Nicotiana benthamiana*

Background/Objective

Plant RNA virus–mediated guide RNA (gRNA) delivery represents a transformative advance in genome editing technologies. Unlike conventional transformation methods that rely on labor-intensive tissue culture and regeneration for each gRNA delivery, viral vectors can rapidly and systemically transmit gRNAs into pre-established Cas-expressing plants, providing an accelerated route for functional genomics and trait discovery directly *in planta*. However, key design parameters, including subgenomic promoter choice, transcript architecture, and their effects on viral fitness and editing outcomes, remain to be elucidated for most viral platforms.

Approach

We developed five Barley stripe mosaic virus (BSMV) vectors, each with distinct subgenomic promoter elements to drive single gRNA expression. These were initially evaluated in Cas9-expressing transgenic *Nicotiana benthamiana* plants targeting the *Phytoene desaturase* (*PDS*) gene to compare their editing efficiencies.

Results

Single gRNAs expressed under the duplicated γb subgenomic promoter or when fused directly to the γb genome achieved the highest mutation frequencies, whereas β1- and β2-driven sgRNAs produced delayed and reduced editing. Thus, promoter selection critically determines gRNA accumulation and the efficacy of BSMV-mediated genome editing. The top-performing design was then applied to Cas9-expressing barley (*Hordeum vulgare*) targeting *HvCMF7* (conferring green-white variegation) and *HvGW2.1* (impacts grain width and weight). BSMV spread systemically throughout barley, inducing somatic and heritable mutations at frequencies up to 100%, with virus-free edited progeny.

M_1 generation: Albino phenotype of Plant-14 caused by BSMV-mediated, heritable biallelic mutations in *HvCMF7*.

Significance/Impacts

Our systematic comparison of subgenomic promoter architectures establishes clear design principles for optimizing viral vector–mediated delivery. Promoter choice and transcript structure critically shape editing efficiency and viral stability. The host-specific boundary for germline editing, defined by efficient heritable editing in barley but not *N. benthamiana*, highlights where BSMV offers advantages and where alternative vectors or hybrid strategies are required, guiding rational platform selection for diverse crop species and applications. Collectively, these findings establish BSMV as a promising next-generation vector for rapid, tissue culture–free, and transformation-independent genome editing in cereals and other recalcitrant monocots.

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