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Viral Delivery of Recombinases to Activate Heritable Genetic Switches in Plants

Background/Objective

- Genome engineering in plants is limited by challenges in reagent delivery. Viral vectors provide an increasingly versatile platform for transformation-free reagent delivery to plants. RNA viral vectors can be used to induce gene silencing, overexpress proteins, or introduce gene editing reagents; however, they are often constrained by carrying capacity or restricted tropism in germline cells.
- Site-specific recombinases that catalyze precise genetic rearrangements are powerful tools for genome engineering that vary in size and, potentially, efficacy in plants.

Approach

In this study, we developed *Nicotiana benthamiana* target lines with a recombination-activated Ruby reporter for four different recombinases and infected T1 plants from each line with a corresponding TRV recombinase vector.

Results

Viral vectors based on tobacco rattle virus (TRV) deliver and stably express four recombinases ranging in size from ~0.6kb to ~1.5kb and achieve simultaneous marker removal and reporter activation through targeted excision in transgenic *N. benthamiana* lines. TRV vectors with Cre, FLP, CinH, and Integrase13 efficiently mediated recombination in infected somatic tissue and led to heritable modifications at high frequency. An excision-activated Ruby reporter enabled simple and high-resolution tracing of infected cell lineages without the need for molecular genotyping.

NptII/Ruby switch actuated by recombinase delivery via TRV. Timelapse of emerging Ruby phenotype from 6 to 14 days after infection.

Significance/Impacts

We have demonstrated an effective approach for viral-mediated recombinase delivery and developed a reporter platform for viral infection that may be applied for rapid optimization of vector architecture and delivery conditions in *N. benthamiana* and potentially other species, such as C_4 grasses and bioenergy crops. This study opens the door to many opportunities afforded by viral delivery of recombinases to activate genetic switches for both biological discovery and production of high-value metabolites such as biofuels.

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