Forschungsverbund:
Gezielte Übertragung minimierter Transgensequenzen mit optimierter Funktion

Marker gene elimination mediated by transient expression of bacteriophage P1 Cre recombinase in plants

Kopertekh, L., Schiemann, J., Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Virology, Microbiology and Biosafety, Braunschweig

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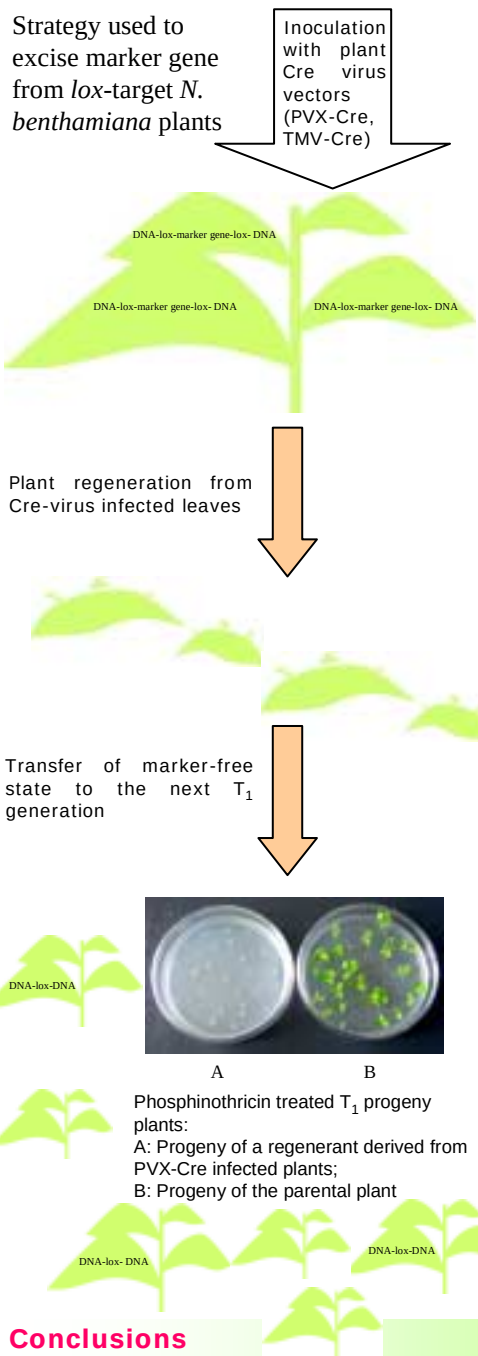
Strategy used to excise marker gene from *lox*-target *N. benthamiana* plants



Phosphinothricin treated T_1 progeny plants:
A: Progeny of a regenerant derived from PVX-Cre infected plants;
B: Progeny of the parental plant

Introduction

Incorporation of a selectable marker is essential to identify transgenic cells and regenerate transgenic plants. However, once transformation is accomplished, the marker gene becomes superfluous. The Cre-*lox* recombination system from bacteriophage P1 was shown to be useful for marker gene elimination in higher plants. Cre-*lox* recombination system includes two *lox* sites and a 38 kDa Cre recombinase protein that mediates recombination reaction. When two *lox* sites are directly repeated the flanked marker gene is deleted. The future employment of the Cre-*lox* recombination system for practice will need a greater control of the recombinase activity. Limiting recombinase activity can help to minimise possible negative effects from constitutive recombinase expression.

Objective

The aim of this project is developing an alternative strategy to excise selectable markers from transgenic plants by site-specific recombination via transient expression of the *cre* recombinase gene.

Results

We utilised plant Cre virus vectors (TMV-Cre and PVX-Cre) for transient expression of *cre* recombinase in *lox*-target *N. benthamiana* plants. These plants contain the *bar* gene which confirms resistance to phosphinothricin flanked by two directly oriented *lox* sites. Cre-mediated site-specific recombination results in deletion of the *bar* sequence and phosphinothricin sensitivity. Our strategy includes several steps:

1. Efficient *cre* transient expression in *lox*-target *N. benthamiana* plants.
2. Selection of plants with precise marker gene excision. PVX-Cre and TMV-Cre systemically infected leaves were taken as explants for subsequent plant regeneration.
3. Transfer of the recombined state to the next T_1 generation.

Transgenic plants containing *lox* sites and *bar* gene were inoculated with PVX-Cre and TMV-Cre recombinant viruses. PVX-Cre and TMV-Cre systemically infected leaves were allowed to regenerate without selection pressure. Western blot and PCR analysis showed that selectable marker gene (*bar*) was eliminated in PVX-Cre systemically infected leaf tissue. To remove the virus from plant tissue the nucleoside analogue ribavirin was used in the regeneration medium. The frequency of Cre-mediated *bar* gene excision was evaluated by PCR analysis. This approach allowed us to separate regenerants with complete *bar* gene excision from chimeric plants. 50-82% of the regenerants from TMV-Cre and PVX-Cre infected explants were marker free. Self progeny of virus free and marker free plants was examined for the inheritance of the recombined state. Seeds were grown on medium with 6 mg/l phosphinothricin. No phosphinothricin-resistant seedlings for all examined lines were observed on the selective medium, confirming that excision of the *bar* sequence has been transmitted to the next generation. Molecular analysis proved that T_1 progeny plants did not contain selectable marker gene (*bar*) in their genome.

Conclusions

An alternative method for the production of marker-free transgenic *N. benthamiana* plants has been developed. We used plant Cre virus vectors to express *cre* recombinase transiently. The frequency of recombination expressed as a percentage of regenerated plants without marker gene varied from 50 to 82 % for TMV-Cre and PVX-Cre virus vectors, respectively. The T_1 progeny of these regenerants did not contain the marker gene (*bar*) in their genome. The strategy can be applied to plant species that depend on organogenesis or somatic embryogenesis for regeneration, particularly, potato and woody plants.