Testing *Agrobacterium* binary vectors with kanamycin resistance and β-glucuronidase (*gus*) genes for plant transformation

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Abstract

Agrobacterium tumefaciens **is a plant pathogen which can transfer its DNA (transferred DNA, T-DNA) to the plant nucleus. Many *Agrobacterium* virulence (Vir) proteins play roles in the transformation process. In a binary vector system, the T-DNA and the *vir* genes are located in the binary vector and the *vir* plasmid, respectively. This system is commonly used to obtain transgenic plants. During the plant transformation process, T-DNA is delivered by linkage to the VirD2 protein, which guides the T-DNA through the Type IV secretion system. Subsequently, the T-DNA is integrated into the plant nuclear genome. Our goal is to test the functionality of a newly constructed binary vector, pSSK6A, carrying a kanamycin resistance gene as a selection marker and the β-glucuronidase (*gus*) reporter gene. We transformed tobacco leaves with pSSK6A and pCAMBIA2301 as positive control and selected for kanamycin resistance. Newly emerged shoots and calli were used for histochemical GUS detection. We observed blue staining from transformed leaves. In conclusion, our newly constructed pSSK6A is functional. Transformation efficiently will be quantified by comparing with pCAMBIA2301 in the future.**

Introduction

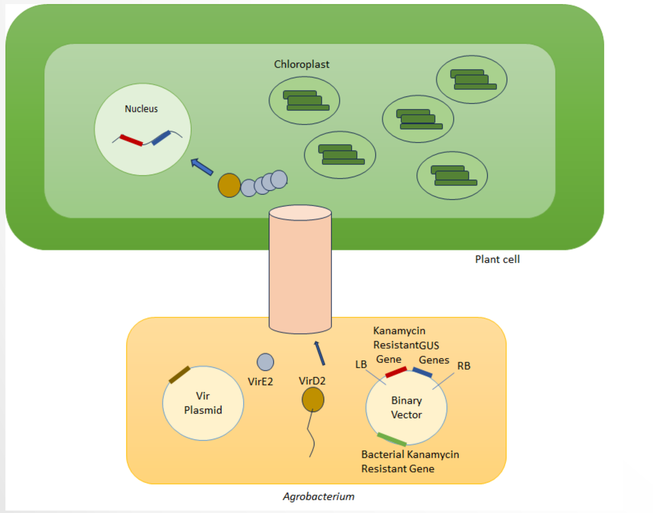

Fig 1. *Agrobacterium*-mediated plant nuclear transformation using a binary vector system. VirD2 nicks the left and right borders (LB and RB) and then attaches at the 5' end of single-stranded T-DNA (T-strand), forming the T-complex. The complex is exported to the plant cell through the type IV secretion system. VirE2 proteins coat the T-strand in the plant cell to protect from degradation. The complex is directed to the plant nucleus due to the nuclear localization signals (NLSs) in VirD2. The T-strand is integrated into the plant nuclear genome at random locations (add two references).

Materials and Methods

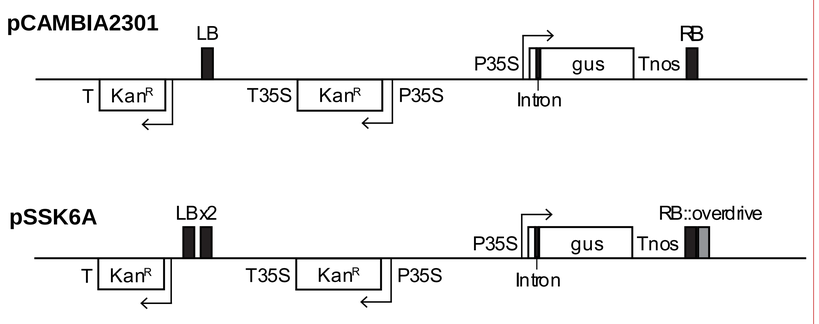

Fig 2. Binary vectors used for tobacco leaf transformation. pCAMBIA2301 has a kanamycin resistance gene and a *gus* gene in the T-DNA region. pSSK6A binary contains also a kanamycin resistance gene and a *gus* gene in the T-DNA region, and it also has two LB and RB with overdrive sequence. P35S, the CaMV 35S promoter; T35S, the CaMV 35S terminator; Tnos, the nopaline synthase terminator.

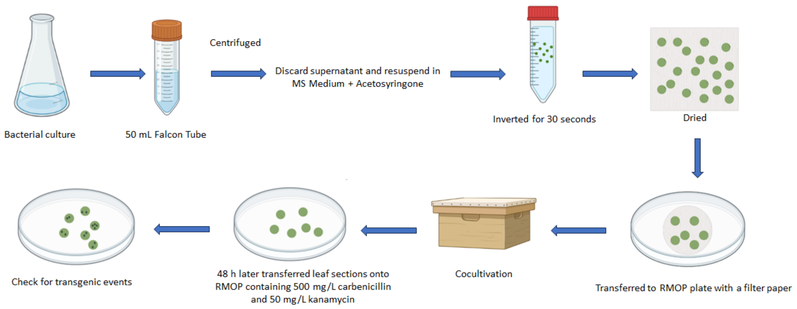

Fig 3. A schematics of *Agrobacterium*-mediated tobacco leaf transformation. *Agrobacterium* strain XYA105*(pCAMBIA2310 or pSSK6A) was used to transform tobacco leaves. *Agrobacterium* was inoculated in 50 mL LB containing 50 mg/L kanamycin and incubated at 28^{O}C with shaking overnight. Cultures were centrifuged. The supernantant was discarded, and bacterial cells were resuspended in the MS medium with acetosyringone. Tobacco leaf sections were placed in the bacterial suspension and then inverted gently for 30 sec. After leaf sections were dried on the sterilized paper towels, they were placed on a filter paper on a RMOP plate. Plates were placed in the dark for 48 hours. Leaf sections were transferred onto RMOP with 500 mg/L carbenicillin and 50 mg/L kanamycin. Carbenicillin was used to prevent *Agrobacterium* overgrowth. Kanamycin was used for selection. A few days later, shoots/calli were observed. They were used for GUS histochemical analysis for transgenic events.

Results

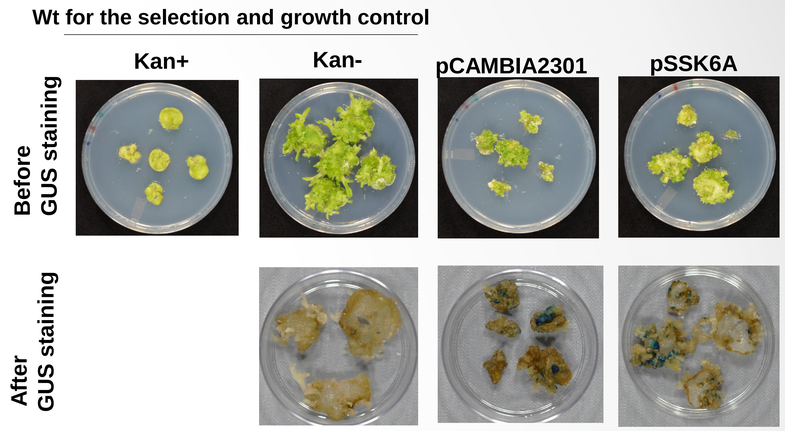

Fig 4. 13 days post *Agrobacterium*-mediated tobacco leaf transformation. Non-transformed leaves were plated on RMOP without kanamycin (Kan-) as a growth control and with kanamycin (Kan+) as a selection control. GUS histochemical analysis was done using X-gluc solution. Chlorophyll was removed by incubating several times in 70% ethanol. Blue staining was observed from newly formed shoots/calli transformed with pCAMBIA2301 and pSSK6A.

Conclusion

Results indicated that newly constructed pSSK6A was functional to select transgenic events for kanamycin resistance and the *gus* expression. This binary vector will be used for further research.

References

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