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Update section

Short communication

New plant binary vectors with selectable markers located proximal to the left T-DNA border

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Abstract

Five new binary vectors have been constructed which have the following features: (1) different plant selectable markers including neomycin phosphotransferase (*nptII*), hygromycin phosphotransferase (*hpt*), dihydrofolate reductase (*dhfr*), phosphinothricin acetyl transferase (*bar*), and bleomycin resistance (*ble*); (2) selectable markers are located near the T-DNA left border and; (3) selectable marker and β-glucuronidase (*uidA*) reporter genes are divergently organized for efficient expression, and can easily be removed or replaced as needed.

In the course of plant molecular biology experiments it is often advantageous to be able to choose between various plant transformation vectors which contain different selectable markers, reporter genes and unique restriction sites. In this short communication we describe five plant transformation vectors which contain different selectable markers located near the T-DNA left border; a β-glucuronidase (*uidA*) reporter gene [5] is located near the right border. The transfer of T-DNA to plant cells by *Agrobacterium*-mediated transformation is thought to occur in a polar fashion starting at the right border [10]. Therefore, the identification of drug-resistant, transformed plant cells containing complete T-DNA insertions is facilitated by using constructs which contain selectable markers located near the left border. Plant cells harbouring truncated T-DNA insertions, lacking part or all of the selectable marker gene, are counter-selected.

Schematic diagrams of pGPTV (glucuronidase plant transformation vector) binary vectors indicate the region between the right and the left T-DNA borders (Fig. 1). The glucuronidase (*uidA*) reporter gene can be used for promoter studies, or may be exchanged by any other gene of interest as desired. In order to reduce antisense effects, the reporter and selectable marker genes have been arranged in divergent orientations. The plant selectable marker genes *nptII* [7], *hpt* [1], *dhfr* [6], and *bar* [8] are driven by the nopaline synthase (*nos*) promoter, while the *ble* marker [4] is expressed under the control of the CaMV 35S promoter. An *nptII* gene derived from pRT100neo [7], which lacks a specific point mutation known to reduce NPT II enzyme activity

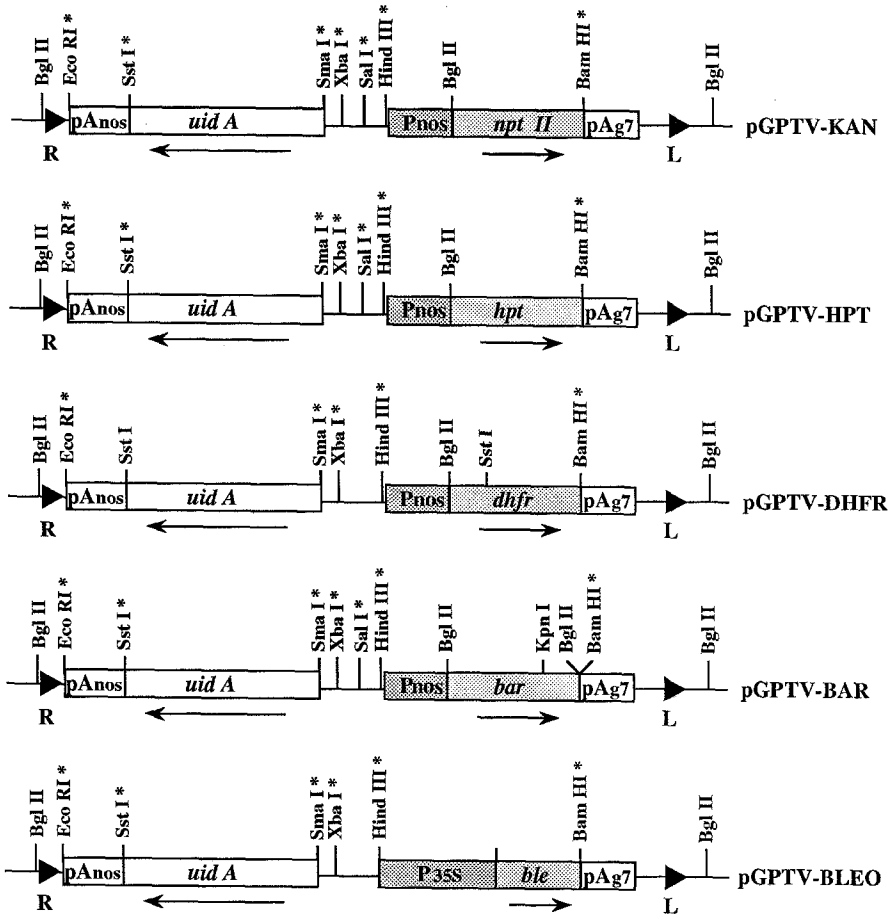

Fig. 1. Schematic diagrams of the pGPTV binary vectors (not drawn to scale). Each construct contains unique cloning sites (*) upstream of the β-glucuronidase (*uidA*) gene which allow the construction of promoter fusions. The T-DNA nopaline synthase (pAnos) and gene 7 (pAg7) poly(A) signals follow the *uidA* gene and each of the different selectable marker genes, respectively. Arrows indicate direction of transcription; R, right T-DNA border; L, left T-DNA border.

[9], was used in the pGPTV-KAN construct. While all of the markers have previously been tested in plants [1, 4, 6, 8], we nevertheless transformed three of the constructs, including pGPTV-KAN, pGPTV-HPT, and pGPTV-DHFR, into *Arabidopsis thaliana* using an *Agrobacterium*-mediated root transformation protocol [6]. The transformation efficiency of the pGPTV vectors in *Arabidopsis* was similar to results previously obtained with four independent transformations in which binary vectors were used, including pBIB-HYG, the progenitor of the pGPTV vectors [6].

Calli were selected on 50 mg/l kanamycin, 20 mg/l hygromycin B, and 0.1 mg/l methotrexate, respectively; these concentrations are effective with *Arabidopsis*, and may vary with other plant species. Hille *et al.* [4] have reported using 10 mg/l bleomycin for the selection of transformed *Nicotiana plumbaginifolia* protoplasts, and De Block *et al.* [3] have found 500 mg/l phosphinothricin (PPT) effective with *N. tabacum* leaf disc infection.

The pGPTV vectors are derivatives of the plant binary vectors pBIN19 [2], pBI101 [5], and pBIB-HYG [1]. They contain the broad host range RK2 origin of replication and a bacterial kanamycin resistance marker for growth in

Escherichia coli and *A. tumefaciens*. Further details concerning the construction of the vectors can be provided by corresponding to the authors.

References

1. Becker D: Binary vectors which allow the exchange of plant selectable markers and reporter genes. Nucl Acids Res 18: 203 (1990).
2. Bevan M: Binary *Agrobacterium* vectors for plant transformation. Nucl Acids Res 12: 8711–8721 (1984).
3. De Block M, Botterman J, Vandewiele M, Dockx J, Thoen C, Gossele V, Movva NR, Thompson C, Van Montagu M, Leemans J: Engineering herbicide resistance in plants by expression of a detoxifying enzyme. EMBO J 6: 2513–2518 (1987).
4. Hille J, Verheggen F, Roelvink P, Franssen H, Kammen AV, Zabel P: Bleomycin resistance: a new dominant selectable marker for plant cell transformation. Plant Mol Biol 7: 171–176 (1986).
5. Jefferson RA, Kavanagh TA, Bevan MW: GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6: 3901–3907 (1987).
6. Kemper E, Grevelding C, Schell J, Masterson R: Improved method for the transformation of *Arabidopsis thaliana* with chimeric dihydrofolate reductase constructs which confer methotrexate resistance. Plant Cell Rep 11: 118–121 (1992).
7. Töpfer R, Gronenborn B, Schell J, Steinbiss H-H: Uptake and transient expression of chimeric genes in seed-derived embryos. Plant Cell 1: 133–139 (1980).
8. White J, Chang S-YP, Bibb MJ, Bibb MJ: A cassette containing the *bar* gene of *S. hygroscopicus*: a selectable marker for plant transformation. Nucl Acids Res 18: 1062 (1990).
9. Yenofsky RL, Fine M, Pellows JW: A mutant neomycin phosphotransferase II gene reduces the resistance of transformants to antibiotic selection pressure. Proc Natl Acad Sci USA 87: 3435–3439 (1990).
10. Zambryski P: Basic processes underlying *Agrobacterium*-mediated DNA transfer to plant cells. Annu Rev Genet 22: 1–30 (1988).