Note

Construction of a Binary Vector for Transformation of Arabidopsis thaliana with a New Selection Marker

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A new binary vector, pZT4B, containing the UDP-N-acetylglucosamine-1-P transferase (GPT) gene as a selection marker, was constructed. The green fluorescent protein (GFP) gene was inserted into pZT4B, and the resulting plasmid was used in the transformation of Arabidopsis. All of six independent transformants obtained after selection with 0.3 mg/l tunicamycin contained the transgene and showed GFP fluorescence.

Key words: Arabidopsis; binary vector; selection marker; tunicamycin

Transgenic plants are routinely generated in the laboratory for both basic and applied research purposes. Two approaches can be used to transform plants: direct gene transfer and Agrobacterium tumefaciens-mediated gene transfer.1,2 In most cases, one or more selection marker genes are co-integrated with the genes of interest in order to identify plants with the desired genes in their genome. In general, selection marker genes encode enzymes that detoxify antibiotics that are toxic to plants. Genes encoding neomycin phosphotransferase II3,4 and hygromycin phosphotransferase,5 which detoxify kanamycin and hygromycin respectively, are widely used in the transformation of plants. Genes encoding enzymes that detoxify herbicides are also used.6

It is often necessary to introduce multiple genes into a plant genome. For this purpose, different selection markers are necessary, because transgenic plants already contain one or more selection marker genes. In Arabidopsis, for example, T-DNA insertion mutants are powerful tools to investigate the functions of genes.7 Most T-DNA insertion mutants contain at least one selection marker gene, and an additional selection marker gene is then necessary to re-introduce the genes. In many cases, it is possible to deal with this by using currently available multiple selection markers.8 However, it would be advantageous for researchers if more selection markers that are convenient for use were available. Previously we found that the GPT gene could be used as a selection marker when it is overexpressed in Arabidopsis grown in the presence of tunicamycin.9 Present study was conducted to construct a new binary plasmid using this selection system.

The starting plasmid, pPZP2028 (Kuroda et al., unpublished) was constructed by modification of pPZP202,[10] two eight-base-cutter restriction sites, Asc I and Pac I, were introduced in accordance with the method of van Engelen et al.11 A gene cassette comprising the cauliflower mosaic virus 35S (CaMV35S) promoter, GPT cDNA, and the nopaline synthase (Nos) terminator was amplified by PCR from a binary plasmid described previously,12,13 using primers containing the Mlu I site on the 5′ end and the Asc I site on the 3′ end. The PCR product was digested with Mlu I and Asc I, then ligated with pPZP2028 that had been digested with Asc I and Asc I, and treated with phosphatase, yielding pZT4B.

Subsequently, another gene cassette, comprising the CaMV35S promoter, sGFP and the Nos terminator, was amplified by PCR from CaMV35S-sGFP(S65T)-NOS2113 using primers with the Xho I or Eco R I site, and digested with Xho I and Eco R I. The resulting fragment was ligated with pZT4B that had been digested with Sal I and Eco R I to obtain pZT4B-GFP. The method used to construct the vectors is summarized in Fig. 1.

pZT4B-GFP was used for in planta A. tumefaciens-mediated transformation of Arabidopsis (ecotype Columbia) in accordance with the method of Clough and Bent.14 T0 seeds were sown on agar plates (1 × Murashige and Skoog salt, 1 × Gamborg’s B5 vitamins, 1% w/v sucrose, and 0.8% w/v agar, pH 5.6) supplemented with 0.3 mg/l tunicamycin, and incubated at 22°C. Most seedlings died just after germination, but some continued to grow and developed true leaves.

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Abbreviations: CaMV35S, cauliflower mosaic virus 35S; GPT, UDP-N-acetylglucosamine-1-P transferase; Nos, nopaline synthase; sGFP, synthetic green fluorescent protein
These seedlings were transferred to soil and grown to harvest the seeds.

Seeds from six independent T\textsubscript{1} plants were sown on plates containing tunicamycin (0.3 mg/l), and the resulting T\textsubscript{2} seedlings from all lines showed resistance to tunicamycin at different segregation rates (data not shown), suggesting that all six T\textsubscript{1} plants contained the transgene. To confirm the transformation event, genomic DNA was isolated from these six lines and subjected to PCR using primers amplifying the GPT genes. As shown in Fig. 2, a signal for the authentic GPT gene and an extra signal derived from the transgene were detected in all transgenic lines, but not in the wild-type plants. In addition, all six lines showed clear fluorescence under fluorescent microscopy (data not shown). These observations confirmed that all lines were transformants.

Seeds from wild-type and transgenic plants (one line only) were sown on plates with (+Tm) and without (−Tm) tunicamycin (0.3 mg/l) on a 16-h light/8-h dark cycle at 22°C, and 6-d-old seedlings were photographed. The presence of GFP fluorescence was assessed in the same seedlings using an LAS-1000plus CCD camera (Fujifilm, Tokyo). Seeds of wild-type (upper half of each plate) and transgenic plants (lower half of each plate) were sown on plates with (C, D) and without (A, B) tunicamycin. Photos were taken under normal light (A, C) and fluorescent light (B, D).

The selection procedure is basically the same as the method used with other drugs, such as kanamycin and hygromycin, but clearer selection is possible, because selection by tunicamycin is quite distinctive, as described previously.\textsuperscript{9}
In the present study we generated a new binary vector pZT4B that contains the GPT gene as a selection marker. This vector was confirmed to function at least in Arabidopsis, and should be useful to Arabidopsis researchers. Since pZT4B contains two eight-base-cutter restriction sites, Asc I and Pac I, introduction of multiple genes into pZT4B can easily be achieved (Kuroda, personal communication). The CaMV35S promoter driving the GPT gene might affect the expression of desired genes cloned into the multicloning site, since the CaMV35S promoter was recently reported to affect the expression of adjacent genes.15) Hence another promoter such as Nos promoter may be more suitable for driving the GPT gene but it is currently unclear whether the Nos promoter is sufficiently strong to overexpress the GPT gene to achieve tunicamycin resistance.

Use of the plant gene as a selection marker might be advantageous if this system is used in the generation of transgenic crops, since the Food and Agriculture Organization of the United Nations (FAO) has recommended that selection markers derived from bacteria not be used. Another endogenous gene of Arabidopsis has also been reported to be effective as a selection marker.16) Application of this system to crop plants is the next challenge.

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