Journal of Plant Biology, April 2007, 50(2) : 206-212

Selection of *nptII* Transgenic Sweetpotato Plants Using G_{418} and Paromomycin

Young-Mi Shin[1], Goh Choe[1], Byoungchul Shin[1], Gibum Yi[1], Pil-Yong Yun[2], Kiyoung Yang[1], Joon Seol Lee[3], Sang-Soo Kwak[4], and Kyung-Moon Kim[1, 5, 6]*

[1]Kumho Life and Environmental Science Laboratory, Chonnam National University, Gwangju 500-712, Korea
[2]Jeju-Regional Innovation Agency, Jeju Hi-Tech Industry Development Institute, Jeju 690-121, Korea
[3]Mokpo Experiment Station, National Institute of Crop Science, RDA, Mokpo 534-833, Korea
[4]Laboratory of Environmental Biotechnology, KRIBB, Daejeon 305-806, Korea
[5]Agricultural Plant Stress Research Center, Chonnam National University, Gwangju 500-757, Korea
[6]Subtropical Horticulture Research Center, Cheju National University, Jeju 690-756, Korea

We have used two aminoglycosides, G_{418} and paromomycin, to develop a reliable selection system for *nptII* transgenic sweetpotato (*Ipomoea batatas* (L.) Lam.). Embryogenic calli derived from shoot apical meristems were bombarded with gold particles coated with pCAMBIA2301, which contained the *nptII* and *gusA* genes. When compared on a kill curve that was based on calli proliferation and cell viability, G_{418}-selection proved to be more efficient and had fewer escapes than kanamycin. These bombarded explants were then selected on G_{418}-containing media. The total time required from bombardment to plant establishment in soil was seven to nine months. Multiple copies of the transgene were integrated into the sweetpotato genome. Northern analysis confirmed transgene expression in the regenerated plants, and a paromomycin assay demonstrated that the *nptII* gene was functionally expressed in transformed sweetpotato. These molecular analyses and assays all showed that selection with G_{418} and paromomycin is reliable. So far, we have produced 69 transgenic events with this system, at a transformation frequency of approx. 1.1%. That efficiency is based on the number of transgenic plants obtained and the amount of calli bombarded. Thus, this selection method that combines G_{418} with paromomycin is now available for selecting *nptII* transgenic sweetpotato.

Keywords: biolistics, genetic engineering, GUS, *Ipomoea batatas* (L.) Lam., shoot apical meristem

Sweetpotato [*Ipomoea batatas* (L.) Lam.] is an important food crop that, because of its tolerance to drought, infertile soils, and limited management (Woolfe, 1992), can be grown on marginal lands where economically important cereal crops are vulnerable to those abiotic stresses.

Improvements to sweetpotato are highly dependent upon conventional breeding programs that are based on sexual hybridization. However, their success is hindered by the low number of genetic resources, many of which are cross-incompatible, with their progeny often being male-sterile (Jones, 1980). To overcome such limitations, biotechnological techniques, e.g., tissue culture and genetic transformation, have been introduced.

Although sweetpotato has already been transformed (Prakash and Varadarajan, 1992; Newell et al., 1995; Okada et al., 1995, 2001; Gama et al., 1996; Min et al., 1998; Morán et al., 1998; Otani et al., 1998; Kimura et al., 2001; Wakita et al., 2001; Yamaguchi et al., 2004), a more efficient system is needed. The choice of selectable marker and selection agent is critical because of currently low transformation frequencies. For sweetpotato, two genes for antibiotic resistance -- neomycin phosphotransferase (*nptII*) and hygromycin phosphotransferase (*hpt*) -- are the most commonly used selectable markers for confirming transformation events. The combination of hygromycin and *hpt* is more effective than kanamycin/*nptII* in screening for antibiotic-resistant calli (Okada et al., 1995, 2001; Kimura et al., 2001; Wakita et al., 2001). However, sweetpotato species show endogenous resistance to kanamycin (Okada et al., 1995, 2001; Wakita et al., 2001), making it ineffective for the selection of *nptII* transgenic sweetpotato and resulting in extremely low final efficiencies. As a solution to this problem, the aminoglycosides G_{418} and paromomycin are now more widely used in crops that exhibit intrinsic kanamycin resistance (Dekeyser et al., 1989; Nehra et al., 1994; Cheng et al., 1997; Rajasekaran et al., 2000; Zhang and Puonti-Kaerlas, 2000; Howe et al., 2006).

An improved selection strategy for efficient sweetpotato transformation would retain *nptII* as a selectable marker. Moreover, an effective marker selection strategy would save time and labor when monitoring for transformation events among the proliferating populations of non-transformed cells or escapes. The study presented here is the first to report reliable selection of *nptII* transgenic sweetpotato that utilizes G_{418} and paromomycin. Its reproducibility, efficiency, and relative rapidity make this system a useful tool for genetically engineering improvements in that crop.

MATERIALS AND METHODS

Plant Material and Callus Induction

We used an elite sweetpotato [*I. batatas* (L.) Lam.] cv. Yulmi that was kindly supplied by the Mokpo Experiment Station, National Institute of Crop Science, Rural Development Administration (Mokpo, Korea). Plants were maintained in pots in the greenhouse at 26°C and under a 16-h

*Corresponding author; fax +82-64-725-0989
e-mail kmkimus@hanmail.net

photoperiod from natural light augmented by artificial lamps. Stems containing three to five buds were surface-sterilized in 70% ethanol for 1 min, then in 50% (v/v) commercial bleach (Yuhanrox, Korea) for 20 min with periodic agitation, followed by three rinses with sterile distilled water. The stems were then placed on sterile filter paper to remove excess moisture before the meristems were isolated from the buds under a stereomicroscope. These excised meristems were plated on an MS (Murashige and Skoog, 1962) basal medium containing 1.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and were cultured at 26°C in the dark to promote embryogenesis (Min et al., 1998). The induced calli were transferred and maintained on an N6 medium (Chu et al., 1975) containing 2 mg L^{-1} 2,4-D (2N6) at 26°C under a 16-h photoperiod (90 to 100 μmol m^{-2} s^{-1}).

Sensitivity of Explants to Selectable Agents

Preliminary sensitivity of the embryogenic calli was evaluated with various concentrations of kanamycin or G_{418}. Twenty wild-type calli (1 to 2 mm) were cultured on 2N6 containing either kanamycin at 0, 25, 50, 100, or 200 mg L^{-1}; or G_{418} (Duchefa Biochemie B.V., Netherlands) at 0, 5, 10, 20, 50, or 100 mg L^{-1}. After four weeks of culture, the resistance threshold of these non-transformed calli was evaluated on the basis of fresh weights measured from three-calli clumps. Cell viability also was assayed according to the conversion of 2,3,5-triphenyltetrazolium salt (TTC) into red formazan by the dehydrogenase activity of viable cells (Towill and Mazur, 1975), measuring absorbance at 485 nm. Each treatment consisted of three replicates. After four weeks, the calli were sub-cultured on a regeneration medium that consisted of an MS basal medium supplemented with 0.1 mg L^{-1} 6-benzylaminopurine (BAP). Shoot formation was scored.

Plasmid DNA, Transformation, and Regeneration

Plasmid pCAMBIA2301 (CAMBIA, Australia) was used for transformation via microprojectile bombardment. Its vector carries a chimeric neomycin phosphotransferase II gene (*nptII*) and a β-glucuronidase gene (*gusA*), both under the control of the CaMV35S promoter (Fig. 1). The *gusA* reporter gene contains a catalase intron within the coding sequence to ensure that expression of glucuronidase activity is derived from eukaryotic cells (www.cambia.org). Gold particles were coated with plasmid DNA according to the procedure of Weeks et al. (1993), with the following modifications. A stock suspension comprised 1-μm-diameter gold particles suspended at 60 mg mL^{-1} in 50% glycerol. Fifty μL of this suspension was transferred into a 1.5-mL microfuge tube and the following chilled sterile solutions were added in order: 5 μL plasmid DNA (1 μg μL^{-1}), 50 μL 2.5M $CaCl_2$, and 20 μL 0.1 M spermidine. The tube was vortexed for 2 to 3 min and centrifuged at 14000*g* for 30 s. The pellet was washed in 140 μL of 70% ethanol and the DNA-coated gold particles were suspended in 48 μL of 100% ethanol. Approximately 50 embryogenic calli (each 1 to 2 mm) were placed in a circle (2.2-cm diam.) in the center of a 15 X 90 mm Petri dish containing 2N6, which was then placed in the Biolistic Delivery System (Bio-Rad Laboratories, USA). Next, 6 μL of the DNA-gold suspension was loaded onto the center of a macroprojectile. After the distance between the stopping plate and the target tissues was adjusted to 6 cm, the calli were bombarded under vacuum with the strength of the rupture disk set at 1100 psi. After bombardment, the explants were kept on the same medium for 5 d before being transferred to an 2N6 medium containing 20 mg L^{-1} G_{418} for selection. The explants were sub-cultured onto fresh media at three-week intervals. Selected explants were then transferred onto a plant regeneration medium that consisted of an MS medium supplemented with 0.1 mg L^{-1} BAP and 10 mg L^{-1} G_{418} for three to six weeks. In addition, G_{418}-resistant shoots were maintained on a plant regeneration medium without G_{418}. The regenerated plantlets were then transferred onto a rooting medium containing a half-strength MS basal medium. All tissues were sub-cultured onto fresh media every three weeks. These cultures were maintained at 26°C under a 16-h photoperiod (at 90 to 100 μmol m^{-2} s^{-1}). Plants with well-developed roots were transplanted into 15-cm standard pots containing a soil mixture for growth in a greenhouse.

Molecular Analysis of Transgenic Plants

DNA isolation and PCR: Genomic DNA was isolated from transgenic and wild-type sweetpotato plants (Dellaporta et al., 1983). PCR was performed with *Taq* DNA polymerase (TaKaRa Bio, Japan) in a 50-μL volume in a thermal cycler (Gene Amp® PCR System) (Applied Biosystems, USA). The PCR program consisted of an initial denaturation at 94°C for 30 s; followed by 30 cycles of 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 60 s; followed by a final extension at 72°C for 5 min. DNA samples were used as template to amplify the *nptII* and *gusA* genes. The primer sequences, designed for 715 bp of *nptII* and 1 kbp of *gusA*, were as follows: *nptII* forward, 5′-gatgcgctgcgaatcgggagcg-3′; *nptII* reverse, 5′-ggagaggctattcggctatgac-3′; *gusA* forward, 5′-tggtgacgcatgtcgcgcaagac-3′; and *gusA* reverse, 5′-ggtgat-gataatcggctgatgcag-3′. PCR products were electrophoresed in a 0.7% (w/v) agarose gel and then visualized.

Southern hybridization: Ten μg of each DNA sample was digested overnight with the restriction endonuclease *Eco*RI

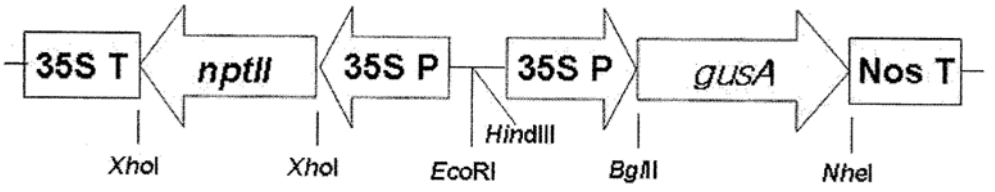

Figure 1. Map of vector pCAMBIA2301. 35S P, CaMV35S promoter; Nos T, Nos poly A; 35S T, CAMV35S poly A; *nptII*, neomycin phosphotransferase II; *gusA*, β-glucuronidase.

in 50 μL of the manufacturer's buffer (New England Biolabs, USA), then separated on a 0.8% (w/v) agarose gel. Southern blotting and hybridization were carried out according to the Amersham Biosciences (USA) protocol for "Hybond-N+" charged nylon membranes. The DNA was hybridized with a 1.0-kb fragment of *gusA* that was PCR-amplified according to the manufacturer's instructions.

Northern hybridization: Total RNA was isolated from leaves of transgenic and wild-type sweetpotato plants by using TRIzol® Reagent (Invitrogen Life Technologies, USA). A 30-μg RNA sample was separated by electrophoresis on 1% (w/v) agarose gels containing 1×MOPS solution and 5% formaldehyde, and then transferred to positively charged nylon membranes. Hybridization was carried out according to the manufacturer's protocol, using *gusA*- and *nptII*-specific probes.

Histochemical β-Glucuronidase Assay

Transformed calli and shoots were analyzed for GUS expression, following the histochemical assay protocol of Jefferson et al. (1987). Explants were incubated at 37°C for 24 h in 100 mM sodium phosphate (pH 7.0), 10 mM Na-EDTA, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 0.1% (w/v) X-gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid, cyclohexylammonium salt; Duchefa Biochemie B.V., the Netherlands), and 0.1% (v/v) Triton X-100.

Application of Paromomycin

Transgenic plants were assayed for their expression of *nptII* to distinguish them from the non-transformed or transgene-silenced plants. This was accomplished by applying paromomycin (Sigma, USA), as described by Cheng et al. (1997), but with some modifications. Three leaf disks were punched from each transgenic or wild-type (negative control) plant and placed in wells containing 50 ppm paromomycin solution. The samples were placed under continuous light (90 to 100 μmol m^{-2} s^{-1}) at 26°C for 5 d. Before transplanting, 500 ppm paromomycin was sprayed onto plants growing in magenta boxes containing soil mix. These plants were observed for a week to determine their resistance or susceptibility to the antibiotic. Undamaged plants were then transplanted to 15-cm standard pots for further tests in the greenhouse.

RESULTS AND DISCUSSION

Sensitivity of Explants to Kanamycin and G_{418}

Callus fresh weights gradually decreased as the concentration of kanamycin increased from 0 to 200 mg L^{-1}, but were dramatically lower when the G_{418} concentration was elevated from 0 to 100 mg L^{-1}. A particularly sharp decline in weight was noted at 10 mg L^{-1} G_{418} (Fig. 2A). Cell viability was somewhat reduced at increasingly higher concentrations of kanamycin, but was markedly lower at greater concentrations of G_{418} (Fig. 2B). Here, TTC solutions extracted from the calli grown in a kanamycin medium or a G418 medium were diluted with 95% alcohol to 1:16 and 1:8, respectively. To test whether the calli cultured on a medium containing either kanamycin or G_{418} were regenerable, they were then transferred to a regeneration medium without selection agents. All calli cultured on the kanamycin medium produced green spots, whereas those placed on a medium containing 5 mg L^{-1} G_{418} formed only a few spots while none arose from calli cultured with at least 10 mg L^{-1} G_{418} (data not shown). In previous sweetpotato transformations, 25 to 100 mg L^{-1} kanamycin was used to select for *nptII* transgenic plants (Prakash and Varadarajan, 1992; Newell et al., 1995; Gama et al., 1996; Min et al., 1998; Morán et al., 1998; Yamaguchi et al., 2004). Here, however, our tissues showed a higher intrinsic resistance to kanamycin, even at 200 mg L^{-1}. Therefore, we considered it less effective for screening than G_{418}. The use of the latter for selection of transformed sweetpotato with *nptII* also resulted in fewer escapes. Based on these current results, we propose that 20 mg L^{-1} G_{418} be applied at the callus-proliferating stage and that 10 mg L^{-1} be used during the early regeneration (three to six weeks) of transgenic plants.

Many crops are resistant to kanamycin, making that antibiotic inefficient for the selection of *nptII* transgenic plants because it results in many escapes. Thus, G_{418} has been used to produce *nptII* transgenic plants in broccoli (Kim and Botella, 2002), cassava (Zhang and Puonti-Kaerlas, 2000), cotton (Rajasekaran et al., 2000), rice (Dekeyser et al., 1989), sorghum (Howe et al., 2006), and wheat (Nehra et al., 1994; Cheng et al., 1997). Hygromycin phosphotransferase (*hpt*) has been utilized in crop transformations, and its use as a selectable marker allows for stringent selection of those events. Kimura et al. (2001), Okada et al. (2001), and Wakita et al. (2001) have reported that hygromycin is suitable for selecting sweetpotato transformed with the *hpt* selectable marker. Yamaguchi et al. (2004) also have adopted a two-step selection system that relies on kanamycin/*nptII* and hygromycin/*hpt* for sweetpotato transformation.

Transformation, Selection, and Regeneration of Sweetpotato

Bombarded explants (Fig. 3C) were transferred to a 2N6 medium containing 20 mg L^{-1} G_{418}. This concentration was used throughout our study because it was previously determined to be the optimal level based on primary kill-curve experiments (Fig. 2). The selection pressure applied by G_{418} during the callus-proliferating stage was very effective in early identification of putative transgenic embryogenic clusters, enabling us to discard a significant amount of untransformed tissue. This, in turn, markedly reduced the effort associated with selecting transformed calli. Howe et al. (2006) has also emphasized the importance of early selection pressure using G_{418} or paromomycin in sorghum.

After three to four sub-culture periods (9 to 12 weeks), our G_{418}-resistant sweetpotato calli were compact and yellowish-white, but were proliferating and developing green sectors. They were easily distinguished from susceptible calli. Clusters of resistant calli showing green sectors were then transferred to a plant regeneration medium containing 10 mg L^{-1} G_{418}. At this stage, callus proliferation decreased while shoot differentiation from the green sec-

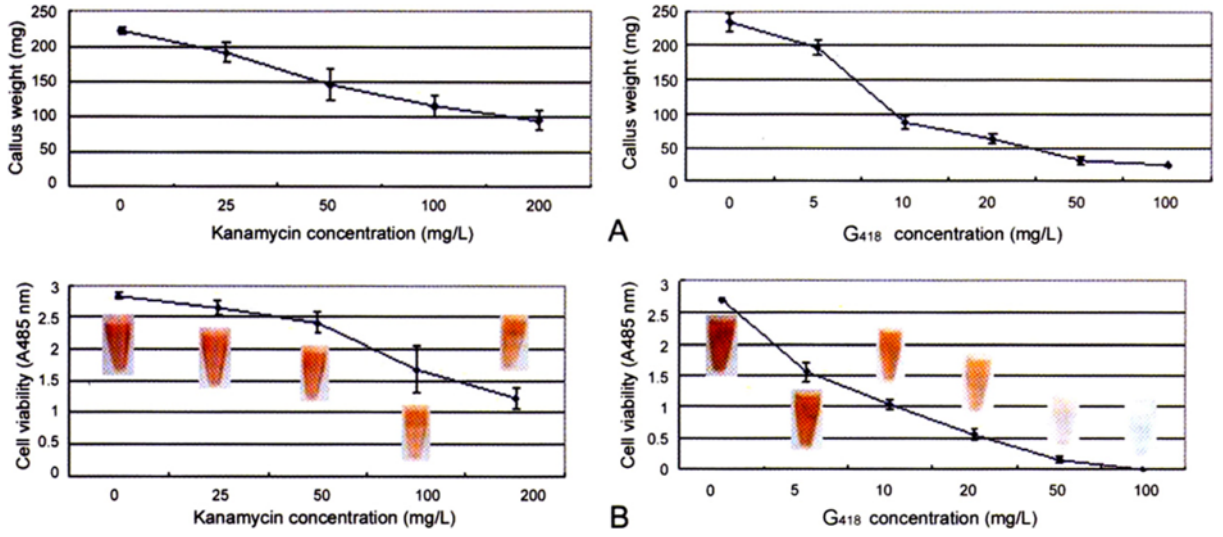

Figure 2. Kill-curve for calli cultured on kanamycin and G_{418} media for 4 weeks. (**A**) Calli fresh weight, (**B**) cell viability (kanamycin 1:16 dilution; G_{418} 1:8 dilution; control (0) 1:16 dilution).

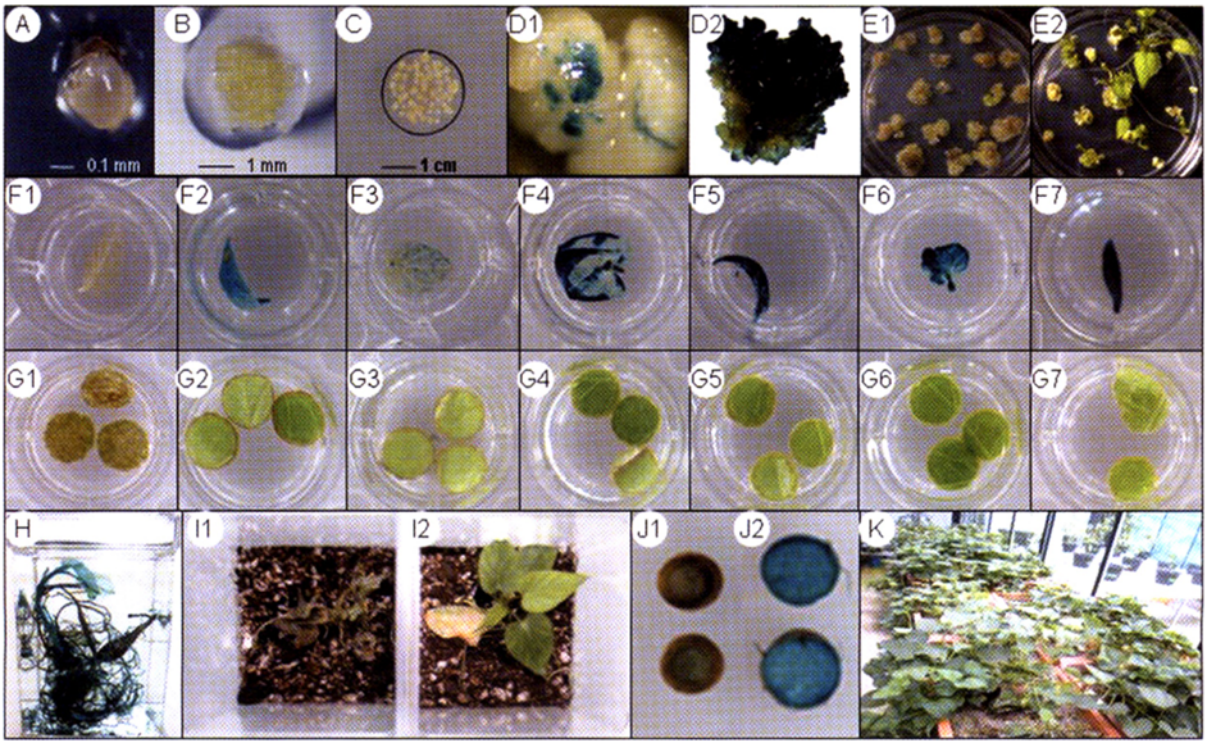

Figure 3. Sweetpotato regeneration, transformation, confirmation of transgenic events, and transgenic plants growing in greenhouse. (**A**) Bombarded calli. (**B**) GUS expression in bombarded callus. (**C**) Selection of G_{418}-resistant calli developing shoots. (**D**) GUS expression in calli developing shoots. (**E**) Plant regeneration. (**F**) GUS expression in leaf tissues: **F1**, wild type; **F2** to **F7**, independent transgenic lines S23, S28, S31, S32, S34, and S35. (**G**) Determination of transgenic plants using paromomycin solution (50 ppm) after 5 d: **G1**, wild type; **G2** to **G7**, S23, S28, S31, S32, S34, and S35. (**H**) GUS expression in whole plant. (**I**) Determination of functional expression of *nptII* gene in transgenic plant by paromomycin spray (500 ppm): **I1**, wild type; **I2**, transgenic. (**J**) GUS expression in sweetpotato roots: **J1**, wild type; **J2**, transgenic. (**K**) Transgenic sweetpotato plants in greenhouse.

tors increased. This selection step was critical because our selection of transgenic sweetpotato plants occurred at the first regeneration step of this system. Our three- to six-week regeneration selection in the G_{418}-containing medium provided a stringent screen for transgenic events because the frequency of recovering non-transgenic sweetpotato was very low. Thus, calli that appeared healthy and which were developing shoots were transferred to the regeneration medium without a selection agent and were allowed to proliferate. After three to five subcultures on this medium, plantlets formed (Fig. 3E) and were transferred to a rooting medium without selection. These plantlets produced roots

Table 1. Sweetpotato transformation frequencies.

Experiment	No. of bombardments (No. of explants)[a]	No. of events[b]	Transformation frequency (%)
1	13 (~650)	12	1.9
2	50 (~2500)	24	1.0
3	22 (~2700)	11	0.9
4	39 (~2100)	21	1.1
Total	124 (~6200)	69	1.1

[a]Each bombardment involved approximately 50 embryogenic calli.
[b]Total number of independent transgenic sweetpotato events confirmed by GUS assay and paromomycin assay.

within four to six weeks, and were then transferred to magenta boxes containing soil mix. They were placed in a growth room under high humidity, where they were allowed to acclimate for four to six weeks before they were transferred to a greenhouse for further growth and visual characterization (Fig. 3K)

In all, we produced 69 plants from 124 bombardments (with each concerning approximately 50 embryogenic calli). Our transformation frequency here was 1.1%, which was calculated by dividing the number of independent transgenic events that produced at least one soil-established transgenic plant by the number of bombarded calli (Table 1). These events were confirmed by histochemical GUS assay and the application of paromomycin. The total time from bombarding the explants to having a viable plant in the greenhouse was approximately seven to nine months.

Histochemical GUS Assay

To distinguish transgenic from non-transgenic events post-bombardment, GUS expression in the calli (Fig. 3B), developing shoots (Fig. 3D), or leaf tissues from regenerated plantlets (Fig. 3F) was histochemically assayed with X-gluc solution. All GUS-positive explants developed into plantlets. Some explants that were GUS-negative at the calli selection stage but GUS-positive at the plant regeneration stage, or vice versa, also produced well-developed plantlets. Explants that were GUS-negative at both stages did not form plantlets. Histochemical GUS activity showed *gusA* expression in the roots of transgenic plants harvested from the greenhouse (Fig. 3J2), but not in the roots of non-transformed control plants (Fig. 3J1). Figure 3H illustrates GUS activity in the whole plant, including the roots. Such stable expression clearly demonstrated the integration of this introduced transgene into the sweetpotato genome.

Characterization of Transgenic Sweetpotato

Genomic DNA isolated from six putative transformed plants (S23, S28, S31, S32, S34, and S35) and one non-transformed plant (negative control), along with the plasmid pCAMBIA2301 (positive control), were used as template DNA for PCR amplification of *gusA* and *nptII*. The presence of 715-bp and 1-kbp bands in samples from all six putative transformants confirmed the integration of *gusA* (Fig. 4A) and *nptII* (Fig. 4B), respectively, into the sweetpotato genome. No amplification of either fragment was detected in the non-transformed controls (Fig. 4).

To confirm that the introduced transgenes were stably integrated, we performed Southern blot analysis with the six transgenics and the single wild-type plant. All of the transformed plants had different band patterns with multiple transgene copy numbers, whereas no signal was detected in the wild type (Fig. 5). The number of hybridized bands directly corresponded to the copy number of that integrated transgene, owing to the absence of the *Eco*RI site within the probe transgene. This indicated that these six transgenic lines were derived from independent events. Multiple copies of introduced transgenes are commonly found among transgenic plants produced by microprojectile bombardment-mediated transformation (Gordon-Kamm et al., 1990; Weeks et al., 1993; Min et al., 1998). Thus, PCR and Southern blot analyses demonstrated stable integration.

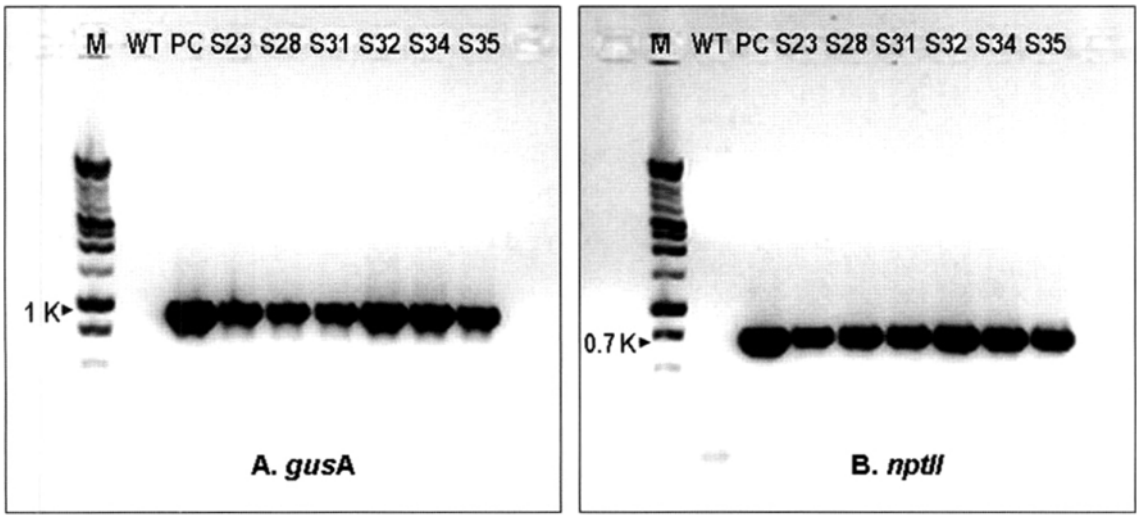

Figure 4. PCR analysis of six putative transgenic plants with pCAMBIA2301 on *gusA* (**A**) and *nptII* (**B**) genes. M, DNA size marker; WT, wild type; PC, positive control (pCAMBIA2301).

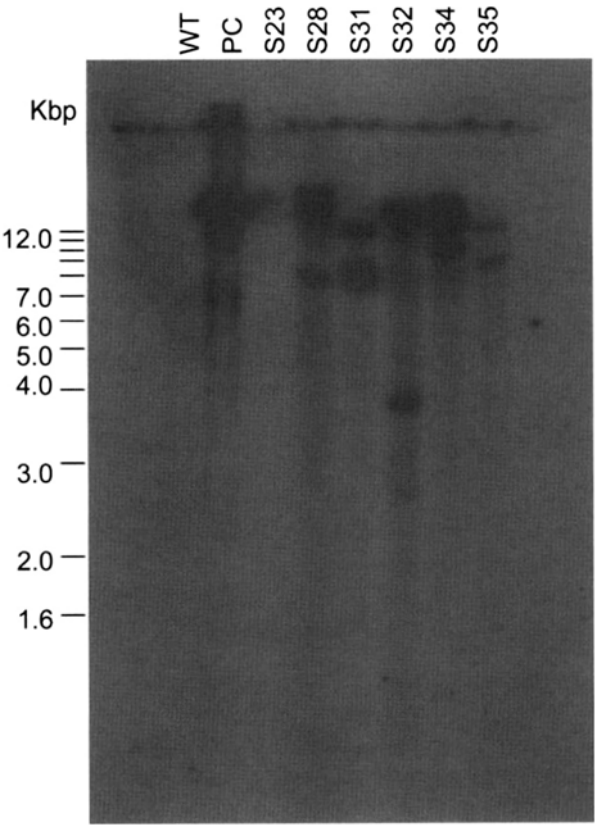

Figure 5. Southern blot analysis of transgenic sweetpotato. Genomic DNA from each event; wild type (WT) and positive control (pCAMBIA2301) were digested with *Eco*RI and hybridized with 1.0-kbp probe of *gusA* gene fragment. Positions and lengths (kbp) of molecular size markers are indicated.

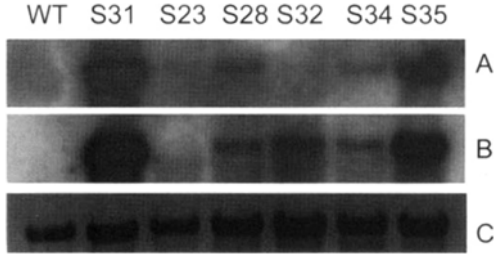

Figure 6. Northern hybridization of transgenic plants. 30 μg of total RNA was extracted from six independent transgenic events (S31, S23, S28, S32, S34, and S35), with one non-transformed sweetpotato plant (WT) used as negative control. RNAs were electrophoresed and hybridized with ^{32}P-labeled DNA probes corresponding to *nptII* (**A**) and *gusA* (**B**) genes. (**C**) *Actin* served as positive control.

We also performed northern hybridization to investigate the expression of transgenes at the mRNA transcription level, and found that both *nptII* and *gusA* were stably expressed at various levels in the six transgenics but not in the wild-type control plant (Fig. 6).

To evaluate plant resistance to paromomycin, leaf-disk samples were collected from the transgenic and wild-type plants. These were placed in a 24-well plate containing 50 ppm paromomycin. One week later, their resistance or susceptibility was evaluated. All leaf disks from transgenic plants remained green except at the margins, proving their resistance, whereas the wild-type samples were bleached or necrotized by the paromomycin (Fig. 3G). In addition, before their transfer to the greenhouse, plants grown in magenta boxes containing soil were sprayed with 500 ppm paromomycin. All the transgenic plants survived and showed no antibiotic damage (Fig. 3I), while the wild types died. This indicates that the introduced *nptII* gene was functionally expressed in transgenic sweetpotato.

To summarize, our proposed selection strategy resulted in 72 transgenic events produced from 124 bombardments. These were confirmed by histochemical GUS assay and PCR amplification of both transgenes, *nptII* and *gusA* (data not shown). In all, 72 transformed plants and 10 non-transformed controls of the same age were sprayed with 500 ppm paromomycin; 69 of the 72 resistant transgenics survived and showed no antibiotic damage. These plants are now growing in the greenhouse (Fig. 3K). In contrast, all 10 non-transformed control plants, plus 3 showing low expression, died within a week after such treatment. Therefore, we have now demonstrated that sweetpotato plants transformed with *nptII* as the selection marker can be successfully generated via the method described here.

In conclusion, we report the development of a reliable protocol for selecting transgenic sweetpotato plants with *nptII*. Optimization of a selection strategy is critical for improving transformation efficiency of that species. Successful implementation of *nptII* for sweetpotato has previously been reported in a biolistics transformation system (Prakash and Varadarajan, 1992; Min et al., 1998) as well as an *Agrobacterium*-mediated gene transfer system (Newell et al., 1995; Gama et al., 1996; Morán et al., 1998; Yamaguchi et al., 2004). However, those selections of *nptII* transgenic sweetpotato depended upon the use of kanamycin, and that process requires extended *in vitro* culture time and labor and is accompanied by a high proportion of escapes. The number of transgenic events characterized here confirms the utility and enhanced reliability of this new strategy when the aminoglycosides G_{418} and paromomycin are used instead of kanamycin. These two components allow for stringent selection of *nptII* transgenic plants that also eliminates untransformed or silenced transgenic plants and reduces escapes. This system will improve research efforts to transfer agronomically useful genes into sweetpotato.

ACKNOWLEDGEMENT

This work was supported by a grant (code #20050401-034-750-142-03-00) from the BioGreen 21 Program, Rural Development Administration, Korea.

Received November 1, 2006; accepted March 20, 2007.

LITERATURE CITED

Cheng M, Fry JE, Pang S, Zhou H, Hironaka CM, Duncan DR, Conner TW, Wan Y (1997) Genetic transformation of wheat

mediated by *Agrobacterium tumefaciens*. Plant Physiol 115: 971-980

Chu CC, Wang CC, Sun CS, Hsu C, Yin KC, Chu CY (1975) Establishment of an efficient medium for anther culture in rice through comparative experiments on the nitrogen sources. Sci Sin 18: 659-668

Dekeyser R, Claes B, Marichal M, van Montagu M, Caplan A (1989) Evaluation of selectable markers for rice transformation. Plant Physiol 90: 217-223

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: Version II. Plant Mol Biol Rep 1: 19-21

Gama MIC, Leite JPR, Cordeiro AR, Cantliffe DJ (1996) Transgenic sweetpotato plants obtained by *Agrobacterium tumefaciens*-mediated transformation. Plant Cell Tiss Organ Cult 43: 237-244

Gordon-Kamm WJ, Spencer TM, Mangano ML, Adams TR, Daines RJ, Start WG, O'Brien JV, Chambers SA, Adams JWR, Willets NG, Rice TB, Mackey CJ, Krueger RW, Kausch AP, Lemaux PG (1990) Transformation of maize cells and regeneration of fertile transgenic plants. Plant Cell 2: 603-618

Howe A, Sato S, Dweikat I, Fromm M, Clemente T (2006) Rapid and reproducible *Agrobacterium*-mediated transformation of sorghum. Plant Cell Rep 25: 784-791

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β-Glucuronidase as a versatile gene fusion marker in higher plants. EMBO J 6: 3901-3907

Jones A (1980) Sweet potato, *In* Fehr W, Hadley HH, eds, Hybridization of Crop Plants. American Society of Agronomy, Madison, pp 645-655

Kim JH, Botella JR (2002) Callus induction and plant regeneration from broccoli (*Brassiaca oleracea* var. italica) for transformation. J Plant Biol 45: 177-181

Kimura T, Otani M, Noda T, Ideta O, Shimada T, Saito A (2001) Absence of amylose in sweetpotato [*Ipomoea batatas* (L.) Lam.] following the introduction of granule-bound starch synthase I cDNA. Plant Cell Rep 20: 663-666

Min SR, Jeong WJ, Lee YB, Liu JR (1998) Genetic transformation of sweetpotato by particle bombardment. Kor J Plant Tiss Cult 25: 329-333

Morán R, García R, López A, Zaldúa Z, Mena J, García M, Armas R, Somonte D, Rodríguez J, Gómez M, Pimentel E (1998) Transgenic sweetpotato plants carrying the delta-endotoxin gene from *Bacillus thuringiensis* var. *tenebrionis*. Plant Sci 139: 175-184

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-497

Nehra ND, Chibbar RN, Leung N, Caswell K, Mallard C, Steinhauer L, Baga M, Kartha KK (1994) Self-fertile transgenic wheat plants regenerated from isolated scutellar tissues following microprojectile bombardment with two distinct gene constructs. Plant J 5: 285-297

Newell CA, Lowe JM, Merrywether A, Rooke LM, Hamilton WDO (1995) Transformation of sweetpotato [*Ipomoea batatas* (L.) Lam.] with *Agrobacterium tumefaciens* and regeneration of plants expressing cowpea trypsin inhibitor and snowdrop lectin. Plant Sci 107: 215-227

Okada Y, Kimura T, Mori M, Nishiguchi M, Saito A, Murata T, Fukuoka H (1995) Introduction of sweetpotato feathery mottle virus severe strain (SPFMV-S) coat protein to sweetpotato and regeneration of transgenic plants by electroporation. Jpn J Breed 45: 116

Okada Y, Saito A, Nishiguchi M, Kimaru T, Mori M, Hanada K, Sakai J, Miyazaki C, Matsuda Y, Murata T (2001) Virus resistance in transgenic sweetpotato [*Ipomoea batatas* (L.) Lam] expressing the coat protein gene of sweetpotato feathery mottle virus. Theor Appl Genet 103: 743-751

Otani M, Shimada T, Kimura T, Saito A (1998) Transgenic plant production from embryogenic callus of sweetpotato [*Ipomoea batatas* (L.) Lam.] using *Agrobacterium tumefaciens*. Plant Biotechnol 15: 11-16

Prakash CS, Varadarajan U (1992) Genetic transformation of sweetpotato by particle bombardment. Plant Cell Rep 11: 53-57

Rajasekaran K, Hudspeth RL, Cary JW, Anderson DM, Cleveland TE (2000) High-frequency stable transformation of cotton (*Gossypium hirsutum* L.) by particle bombardment of embryogenic cell suspension cultures. Plant Cell Rep 19: 539-545

Towill LE, Mazur P (1975) Studies on the reduction of 2,3,5-triphenyltetrazolium chloride as a viability assay for plant tissue cultures. Can J Bot 53: 1097-1102

Wakita Y, Otani M, Hamada T, Mori M, Iba K, Shimada T (2001) A tobacco microsomal ω-3-fatty acid desaturase gene increases the linolenic acid content in transgenic sweetpotato [*Ipomoea batatas*]. Plant Cell Rep 20: 244-249

Weeks JT, Anderson OD, Blechl AE (1993) Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum*). Plant Physiol 102: 1077-1084

Woolfe JA (1992) Sweetpotato, An Untapped Food Resource. Cambridge University Press, New York

Yamaguchi KI, Song GQ, Honda H (2004) Efficient *Agrobacterium tumefaciens*-mediated transformation of sweetpotato [*Ipomoea batatas* (L.) Lam.] from stem explants using a two-step kanamycin-hygromycin selection method. In Vitro Cell Dev Biol Plant 40: 359-365

Zhang P, Puonti-Kaerlas J (2000) PIG-mediated cassava transformation using positive and negative selection. Plant Cell Rep 19: 1041-1048