Journal of Plant Biology, December 1999, 42(4) : 310-316

Binary Vectors for Efficient Transformation of Rice

Sichul Lee, Jong-Seong Jeon, Ki-Hong Jung, and Gynheung An*
Department of Life Science and National Research Laboratory of Plant Functional Genomics, Pohang University of Science and Technology (POSTECH), Pohang 790-784, Korea

We constructed binary vectors that were designed for transfer and expression of a gene into rice chromosomes. The binary vectors contained the hygromycin-resistance gene for selection of transformants and multiple- cloning sites within the transfer DNA. In addition, vectors were designed to express foreign genes using four kinds of promoters. We also report a procedure for efficient transformation of rice plants using scutellum-derived calli and the *Agrobacterium* strain LBA4404.

Keywords: *Agrobacterium*, binary vector, LBA4404, monocots, rice, transformation

Recent progress in rice research has included the construction of a fine genetic and physical map, the large-scale analysis of expressed sequence tags (EST), international efforts in genome sequencing, and efficient tools for genetic transformation (Sasaki, 1999). Plants in the grass family have similar genome structure and gene organization (Bennetzen, 1999). These factors, therefore, have made rice an ideal model system for monocot species.

Agrobacterium tumefaciens can genetically transform a large number of plant species by transferring its T-DNA into the plant genome (An et al., 1988). This process requires the *trans*-acting virulence (*vir*) function encoded by the Ti plasmid and the *Agrobacterium* chromosome, and the *cis*-acting T-DNA border sequences. The genes responsible for the T-DNA transfer are clustered in the *vir* region (Klee et al., 1983) of the Ti plasmid. The T-DNA region, however, does not have to be physically linked to the *vir* genes (Hoekema et al., 1983). Based on these facts, we and others have developed the binary vector system, in which T-DNA and the *vir* genes are located in two different plasmids (An et al., 1985; reviewed in Lichtenstein and Fuller, 1987; reviewed in Guerineau and Mullineaux, 1993). This system has been designed so that T-DNA contains a plant selectable marker and cloning sites within the border sequences. The plasmids carrying T-DNA replicate in both *Agrobacterium* and *Escherichia coli*, and are relatively small.

Agrobacterium-mediated transformation usually results in a discrete, unrearranged segment of DNA being inserted in a plant genome at fairly low copy numbers. Transformation procedures for most dicot species have been well-developed using *Agrobacterium* and binary vectors. In contrast, cereal monocot species have been recalcitrant because they are outside the host range of *Agrobacterium* in nature. *Agrobacterium*-mediated transformation has been reported in maize (Graves and Goldman, 1986; Gould et al., 1991) and rice (Raineri and Goldman, 1990; Chan et al., 1992). However, the early results from *Agrobacterium*-mediated transformation of monocot plants were equivocal because of low efficiency and lack of progeny analysis.

Previously, immature embryos were utilized as starting material for rice transformation via the *Agrobacterium*-mediated co-cultivation method (Chan et al., 1993; Aldemita and Hodges, 1996). However, scutellum-derived calli from mature seeds have turned out to be more efficient for the transformation procedure (Hiei et al., 1994). Since then, *Agrobacterium*-mediated transformation procedures have been developed for javanica rice (Dong et al., 1996) and indica rice (Rachid et al., 1996). An improved protocol for a rapid transformation procedure was reported by Toki (1997). However, because these procedures are largely dependent on use of the supervirulent *Agrobacterium* strains or superbinary vectors (reviewed in Hiei et al., 1997: Cho et al., 1998), they are not suitable for general use. Here we report development of general binary vectors that can be used for efficient transformation of rice.

MATERIALS AND METHODS

Bacterial Strains and Vector Construction

The *E. coli* strain JM83 (*ara, leu, lac, gal, str*) was used as the recipient in routine cloning experiments.

*Corresponding author; fax +82-562-279-2199
e-mail genean@postech.ac.kr

All DNA cloning procedures were carried out as previously described (Sambrook et al., 1989). *A. tumefaciens* LBA4404, containing the Ach5 chromosomal background and a disarmed helper-Ti plasmid pAL4404, was used for rice transformation (Hoekema et al., 1983). All plasmids for this study were transferred to *A. tumafaciens* LBA4404 using the freeze-thaw method (An et al., 1988).

Plant Materials and Culture Media

Ten japonica rice (*Oryza sativa* L.) varieties - Daisan, Dongjin, Hwayoung, Milyang 99, Milyang 109, Milyang 151, Nakdong, Palgong, Shingeumobyeo, and YR 17104 Acp5- were obtained from Yeongnam Agriculture Experiment Station, Korea. All of the media for rice tissue culture are listed in Table 1.

Callus Induction

Mature seeds were manually dehusked, then sterilized, first with 70% ethanol for 1 min, next with 50% commercial bleach for 1 h with vigorous shaking (120 rpm). Seeds were rinsed thoroughly with sterilized water and cultured for four weeks on the 2N6 medium at 25°C in darkness. Actively proliferating calli derived from scutellum were selected and subcultured on fresh 2N6 media for four to five days. Rapidly growing embryogenic calli (1 to 2 mm in diameter, yellowish and nonfriable) were used for subsequent experiments. Although old calli also provide efficient transformation, transgenic plants from those calli frequently exhibit severe somaclonal variations.

Co-Cultivation

Agrobacterium cells were cultured in an AB liquid medium with appropriate antibiotics (10 mg L^{-1} hygromycin and 3 mg L^{-1} tetracycline) for three days at 28 to 30°C with vigorous rotary shaking (125 rpm). The bacteria were collected by centrifugation. Cell pellets were washed with 1 mL of AAM medium and then suspended in the AAM medium containing 100 µM acetosyringone (3',5'-dimethoxy-4'hydroxy-acetophenone; Aldrich Chem. Co.) at a density of 3 to 5×10^9 cells mL^{-1}. A 3-mL aliquot of each bacterial suspension was mixed with rice calli and incubated for 10 min. The calli then were transferred onto a 2N6-ASB medium with removing the *Agrobacterium* cells. The calli were co-cultured in darkness three days at 25°C.

Selection of Transgenic Calli

After co-cultivation, *Agrobacteria* were thoroughly removed by washing them several times with sterile water containing 250 mg L^{-1} cefotaxime (Claforan). The cells were then placed on a 2N6-CH30 medium. After three to four weeks of culture in darkness at 25°C, the proliferating hygromycin-resistant calli were excised, transferred onto a 2N6-BA medium, and

Table 1. Media for *Agrobacterium*-mediated rice transformation.

Medium	Components
AAM	AA salts and amino acids[a], MS vitamins[b], 500 mg L^{-1} casamino acids, 68.5 g L^{-1} sucrose, 36 g L^{-1} glucose, 100 µM acetosyringone, pH 5.2 (filter-sterilized)
AB	3 g L^{-1} K_2HPO_4, 1 g L^{-1} NaH_2PO_4, 1 g L^{-1} NH_4Cl, 0.3 g L^{-1} $MgSO_47H_2O$, 0.15 g L^{-1} KCl, 0.10 gL^{-1} $CaCl_2$, 0.0025 g L^{-1} $FeSO_47H_2O$, 5 g L^{-1} glucose, pH 7.0-7.2
2N6	N6 medium[c], 2 mg L^{-1} 2,4-D, 30 g L^{-1} sucrose, 1 g L^{-1} casamino acids, 2 g L^{-1} phytagel (Sigma, catalog no. P8169), pH 5.6-5.7
2N6-ASB	N6 medium[c], 2 mg L^{-1} 2,4-D, 30 g L^{-1} sucrose, 1 g L^{-1} casamino acids, 2 g L^{-1} phytagel, 10 g L^{-1} glucose, 100 µM acetosyringone, 1 mM betaine, pH 5.2
2N6-CH30	2N6 medium, 250 mg L^{-1} cefotaxime, 30 mg L^{-1} hygromycin B, 2 g L^{-1} phytagel, pH 5.6-5.7
2N6-BA	N6 medium[c], 1 mg L^{-1} 2,4-D, 0.5 mg L^{-1} BAP, 2 g L^{-1} casamino acids, 20 g L^{-1} sucrose, 30 g L^{-1} sorbitol, 250 mg L^{-1} cefotaxime, 40 mg L^{-1} hygromycin B, 2 g L^{-1} phytagel, pH 5.6-5.7
MSR16	MS medium[d], 100 mg L^{-1} myo-inositol, 50 g L^{-1} sucrose, 20 g L^{-1} sorbitol, 0.1 mg L^{-1} NAA, 1 mg L^{-1} kinetin, 16 g L^{-1} agar (Sigma, catalog no. P7921), pH 5.6-5.7
MS	MS medium[d], 100 mg L^{-1} myo-inositol, 2 g L^{-1} phytagel, pH 5.6-5.7

[a]AA salts and amino acids (Toriyama and Hinata, 1985)
[b]MS vitamins (Murashige and Skoog, 1962)
[c]N6 medium (Chu et al., 1975)
[d]MS medium (Murashige and Skoog, 1962)

cultured for two additional weeks in darkness at 25°C.

Plant Regeneration

Actively growing calli were transferred to an MSR16 medium and incubated at 26 to 30°C under continuous light (40 μmol m^{-2} s^{-1}). Plant regeneration was accomplished in 2 to 10 weeks. Regenerated plantlets were transferred to a hormone-free phytagel-solidified MS medium without selective chemicals (hygromycin and cefotaxime) to allow further root development. After two to three weeks in culture, transgenic plants with healthy roots were transferred to soil and grown to maturity.

RESULTS AND DISCUSSION

Construction of a Binary Vector pGA1605

The majority of binary vectors contain the neomycin phosphotransferase (*npt*) gene as a plant selectable marker. However, rice is highly resistant to kanamycin. Although G-418 can be used as a chemical to screen for transformants, the efficiency is low. We, therefore, developed binary vectors that contain the hygromycin phosphotransferase (*hph*) gene instead of the *npt* gene. The plasmid pGA748 was used as a starting material (An, 1995). Vector construction is outlined in Figure 1. The BamHI-HindIII fragment including *nopaline synthase* (*nos*)-*npt* and the CaMV 35S promoter was replaced with the 1.4-kb BamHI-HindIII fragment carrying the 35S promoter. This created pGA 1178. A one-kb fragment containing the *hph* gene was inserted downstream of the 35S promoter in the sense orientation, which resulted in pGA1179. Multi-cloning sites between the 35S promoter and the *hph* gene were removed by digestion with HindIII and ClaI, followed by a T4 DNA polymerase treatment.

The resulting plasmid, pGA1180, can be used for a rice transformation vector. It contains a unique enzyme site, BamHI, where a foreign DNA fragment can be inserted for delivery to plant chromosomes. To create multiple-cloning sites, the KpnI and SacI sites in the terminatior region of the *T5* gene of the TiA6 plasmid were removed by deleting the SacI fragments. After S1 nuclease treatment, pGA 1181 was the result. Multiple-cloning sites were inserted at the BamHI site between the right border and the 35S promoter. The plasmid, pGA1182, contained a portion of the *nos* promotoer before the multi-cloning sites and the human *thymidine kinase* terminator after the sites. The *nos* promoter was then removed, thereby generating the binary vector pGA1605.

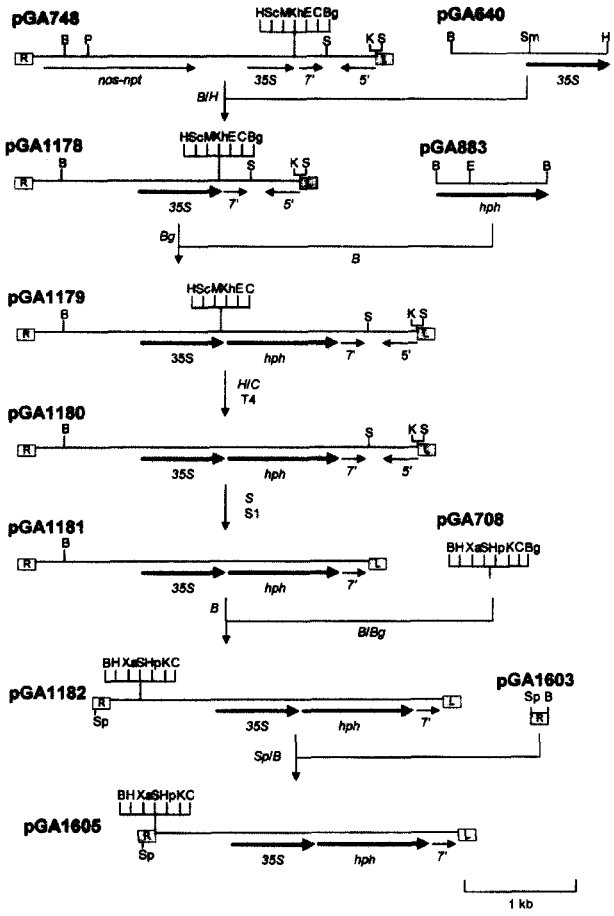

Figure 1. The schematic diagram for construction of binary vectors pGA1182 and pGA1605. R, right border; L, left border; *nos-npt*, a chimaeric gene fusion between the *nos* promoter and *npt* gene; *35S*, the CaMV 35S promoter; *5'* and *7'*, termination regions of the transcripts 5 and 7, respectively, of pTiA6; *hph*, the *hygromycin phosphotransferase* gene; T4, T4 DNA polymerase treatment; S1, S1 nuclease treatment. Restriction enzyme sites: B, BamHI; Bg, BglII; C, ClaI; E, EcoRI; H, HindIII; Hp, HpaI; K, KpnI; M, MluI; P, PstI; S, SacI; Sc, ScaI; Sm, SmaI; Sp, SphI; Xa, XbaI; Xh, XhoI.

This plasmid, binary vector pGA1605, can be used for transferring a DNA fragment to rice plants using the multi-cloning sites. Using this plasmid, we have introduced a chimaeric fusion molecule between the *RA8* promoter and β-*glucuronidase* (*gus*) coding region, and observed an anther-specific expression pattern of the reporter gene (Jeon et al., 1999).

Because the cloning sites are located immediately next to the right border without any translation initiation or stop codons, the vector can be used for constructing gene-trap or promoter-trap vectors. Detailed descriptions for construction and utilization of such vectors will be reported elsewhere.

T-DNA transfer starts from the right border and generally ends at the left border (An et al., 1988). However, the transfer often terminates in the preceding region of the left border, resulting in a partial transfer of T-DNA. In our binary vectors, the selectable marker was located next to the left border to increase the probability that transformants will carry the introduced gene.

Construction of an Expression Vector, pGA1611, for Strong Expression of a Foreign Gene

We constructed the binary vector pGA1611 (Fig. 2) that contains the maize ubiquitin (*Ubi*) promoter and the *nos* terminator (An et al., 1988). The 1.98-kb BamHI-HindIII fragment carrying the *ubi* promoter and the first intron of the gene (Christensen et al., 1992) was isolated form the plasmid pUBA (Toki et al., 1992) and inserted to the BamHI and HindIII sites of pGA 1605. The 0.3 kb *nos* terminator region was isolated from pGA 1596-1 (unpublished data) and inserted into the KpnI and ClaI sites. The resulting pGA1611 vector contained the unique HindIII, SacI, HpaI, and KpnI sites for cloning of a gene between the maize *Ubi* promoter and the *nos* terminator. The maize *ubi* promoter provided significantly high levels of gene expression in rice (Cornejo et al., 1993; McElory and Brettell, 1994). The pGA1611 vector has been used to study the function of rice *MADS* box genes by antisense expression of *OsMADS3* and *OsMADS4* (Kang et al., 1998).

Construction of Binary Vectors for Moderate Expression of a Foreign Gene

The rice α-tubulin gene *Os-TubA4* is strongly

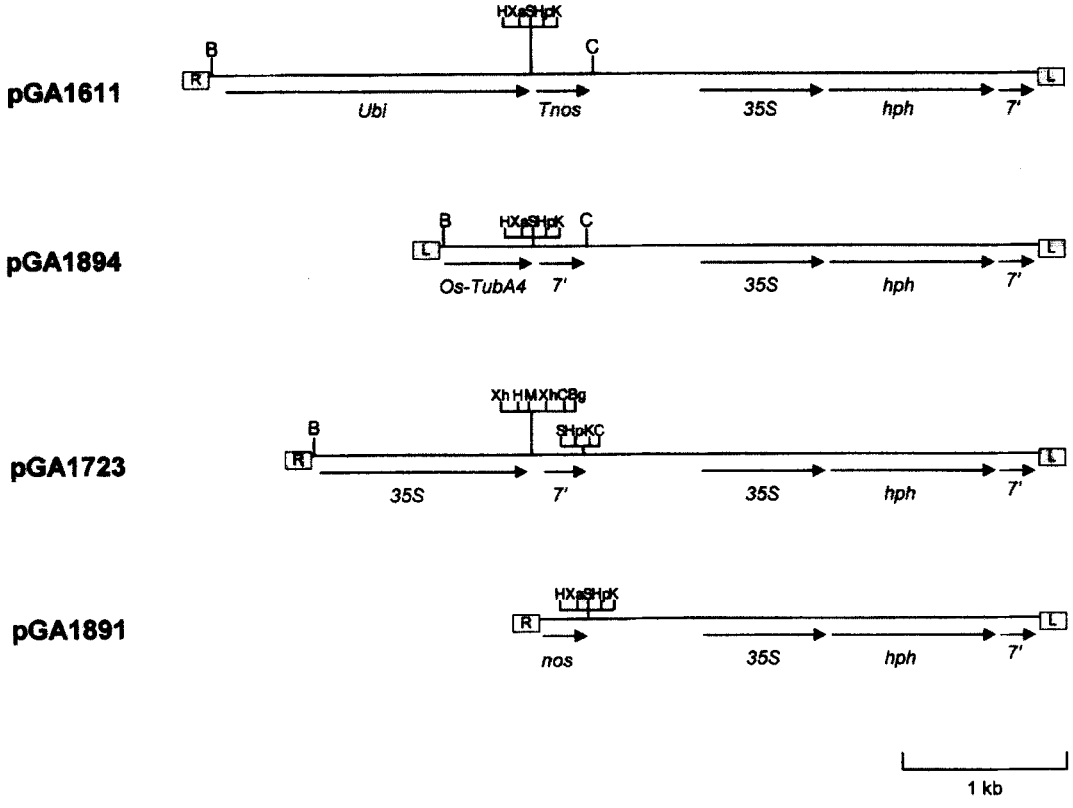

Figure 2. Construction of expression vectors. Linear maps of T-DNA regions between the right and left borders are shown. *Ubi*, the maize ubiquitin promoter; *35S*, the CaMV 35S promoter; *nos*, the *nos* promoter; *Os-TubA4*, the rice α-tubulin4 promoter; *hph*, the hygromycin phosphotransferase gene; *7'*, transcription region of the transcript 7 of pTiA6. All restriction sites shown are unique in each vector, and can be used for expression of a gene in plant cells. Restriction enzyme sites: B, BamHI; Bg, BglII; C, ClaI; H, HindIII; Hp, HpaI; K, KpnI; M, MluI; S, SacI; Xa, XbaI; Xh, XhoI.

expressed in rice plants, especially in actively proliferating tissues (unpublished data). The promoter activity of *OsTubA4* is similar to that of the 35S promoter. We constructed the binary vector pGA1894 that contains the promoter region from the *Os-TubA4* gene (Fig. 2). The *Os-Tub4* promoter region (nucleotides 556 to 1152 in accession number AF 182523) was amplified by PCR, and the fragment was inserted into the BamHI and HindIII sites of pGA1611, thus replacing the *ubi* promoter.

We also constructed the plant expression vector pGA1723 that contains the 35S promoter (Fig. 2). This vector was constructed by replacing the BamHI-SacI of pGA1605 with a 1.7-kb fragment of pGA1178 carrying the 35S promoter and the transcription termination region of gene 7 of pTiA6 (T7). The constructed vector contained the 35S promoter, cloning sites (HindIII, MluI, XhoI, ClaI, and BglII), and the transcription termination region of gene 7 of pTiA6 (T7).

We also constructed an additional plasmid, pGA1891, which contained the *nos* promoter (Fig. 2). The *nos* promoter was isolated from pGA629 (unpublished data) by digestion of HincII and HindIII, and inserted into the klenow-treated BamHI and HindIII sites of pGA1605. The *nos* promoter is weakly active in rice. Therefore, this vector can be used for expressing a foreign gene at a low level to avoid side effects.

Rice Transformation

Plant transformation efficiency is determined by several factors, including choice of starting plant materials, vectors, *Agrobacterium* strains, selective agents, and culture media. One of the most important elements for efficient rice transformation is the plant material. Using scutellum-derived calli provides high transformation efficiency (Hiei et al. 1994). A chemical generated from rice scutellum has induced expression of *Agrobacterium vir* genes and T-strand generation (Vijayachandra et al., 1995).

In the current study, we also used scutellum-derived calli, which were induced from mature seeds by culturing them for four weeks on a medium containing 2,4-D (Fig. 3B). The calli were subcultured on new 2N6 media before co-cultivation by *Agrobacterium*. This step caused the calli to divide rapidly and, consequently, made them more competent for transformation. We also examined anther-derived calli. However, more than half of the regenerated plants were albino, which indicates that anther-derived calli probably are not good starting materials (data not shown).

During co-cultivation, the addition of acetosyringone and glucose, and the low pH of the medium were important factors in encouraging efficient transformation. Vernade et al. (1988) reported that glycine betaine enhanced the induction of *Agrobacterium vir* genes by acetosyringone. This suggests that the naturally occurring osmoprotectant may accelerate adaptation of the bacteria to these conditions. Therefore, we induded 1mM betaine in the co-cultivation medium (Fig. 3C). Transient expression analysis showed that adding the glycine betaine increased *gus*

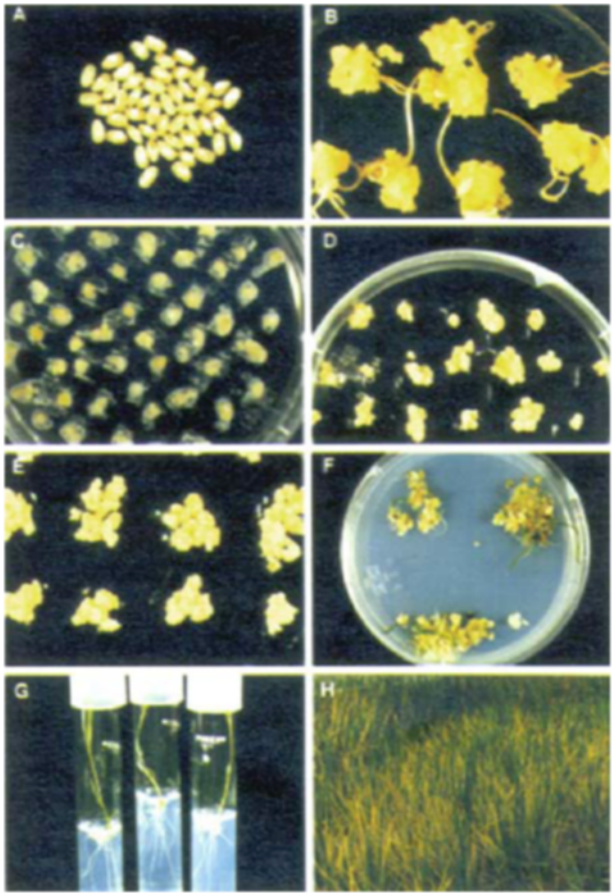

Figure 3. Production of transgenic rice plants by *Agrobacterium*-mediated transformation. **A.** Mature rice seeds after dehusking. **B.** Induction of calli from scutellums of rice seeds on the 2N6 medium **C.** Co-cultivation of *Agrobacterium* with calli. **D.** Selection of transgenic calli on the 2N6-CH30 medium after co-cultivation. Actively growing calli (arrows) appeared within three to four weeks after culturing. **E.** Proliferation of transgenic calli on the 2N6-BA medium. **F.** Transgenic rice plants regenerating from the transformed calli on the MSR16 medium. **G.** Further growth and root development of transgenic plants before transfer to soil. **H.** Transgenic rice plants growing in the greenhouse.

expression (data not shown).

The supervirulent *Agrobacterium* strains and super binary vectors have been used most frequently for rice transformation. However, the vectors are not convenient for handling and delivery of a foreign gene because of their larger size. In addition, the supervirulent strains are significantly less sensitive to several antibiotics compared with LBA4404 (Shackelford and Chlan, 1996). In the present study, therefore, we used *Agrobacterium* strain LBA4404 and ordinary binary vectors to optimize the transformation conditions.

Hygromycin is a useful antibiotic because most monocot tissues show a higher sensitivity to it (Meijer et al., 1991). Using hygromycin allows us to clearly distinguish between transformed and untransformed rice tissues, and reduces the problems related with albinos or the fertility of regenerated plants (reviewed in Hiei et al., 1997). In our experiments, approximately 20 to 40% of the co-cultivated calli produced hygromycin-resistant cells on the first selection medium (Fig. 3D). Most of the cells that recovered from the first selection actively grew on the second selection medium (Fig. 3E). These two rounds of selection significantly reduced escapes.

Regeneration of fertile plants is one of the critical steps during plant transformation. In general, calli induced on a 2,4-D-containing medium regenerated into plants after subsequent culturing on media containing a reduced amount of auxin and enhanced levels of cytokinin (Morris and Altmann, 1994).

In addition, regeneration frequency is also affected by genotype, starting materials, carbohydrate sources, hormonal composition of the regeneration medium, use of feeder cells, and water stress (Jain et al., 1996). In our procedures, the choice of composition in the second selection medium was an important contributor to successful regeneration because it included a low level of auxin, a high concentration of cytokinin, and sorbitol. In this medium, the high ratio of kinetin to NAA, and the high concentrations of sucrose, sorbitol, and agar were important factors. Increasing the phytagel concentration by two-fold slightly increased regeneration efficiency. Using 1.6% agar as a gelling agent in the medium was the most effective in plant regeneration. We routinely obtained 50 to 85% regeneration within 2 to 10 weeks (Fig. 3F). Regenerants were grown in test tubes (Fig. 3G) and then planted in soil. Approximately 80 to 90% of the regenerants produced fertile seeds (Fig. 3H).

In this study, we transformed 10 japonica varieties, Daisan, Dongjin, Hwayoung, Milyang 99, Milyang 109, Milyang 151, Nakdong, Palgong, Shingeumobyeo, and YR 17104 Acp5. Although there were differences in transformation efficiency, all the varieties were efficiently transformable with our vector system. Dongjin and Milyang 151 showed relatively higher frequencies of transformation compared with the other varieties.

ACKNOWLEDGEMENTS

We thank Gi-Hwan Yi for providing the rice seed. We also thank Kyungsook An and Mee-Young Lee for technical assistance, and Hirofumi Uchimiya for donating the pUBA. This work was supported in part by a grant from the Korea Institute of Science and Technology Evaluation and Planning (KISTEP).

Received December 14, 1999; accepted December 15, 1999.

LITERATURE CITED

Aldemita RR, Hodges TK (1996) *Agrobacterium tumefaciens*-mediated transformation of japonica and indica rice varieties. Planta 199: 612-617

An G (1995) Binary Ti plasmid vectors, *In* KMA Gartland, MR Davey, eds, *Agrobacterium* protocols, Humana Press, New Jersey, pp 47-58

An G, Ebert PR, Mitra A, Ha SB (1988) Binary vectors, *In* SB Gelvin, RA Schilperoort, eds, Plant Molecular Biology Manual, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp A3/1-19

An G, Watson BD, Stachel S, Gorden MP, Nester EW (1985) New cloning vehicles for transformation of higher plants. EMBO J 4: 277-284

Bennetzen JL (1999) Plant genomics takes root, branches out. Trends Genet 15: 85-87

Chan MT, Chang HH, Ho SL, Tong WF, Yu SM (1993) *Agrobacterium*-mediated production of transgenic rice plants expressing a chimaeric α-amylase promoter/β-glucuronidase gene. Plant Mol Biol 22: 491-506

Chan MT, Lee TM, Chang HH (1992) Transformation of indica rice (*Oryza sativa* L.) mediated by *Agrobacterium tumefaciens*. Plant Cell Physiol 33: 557-583

Cho SK, Chung YS, Park SJ, Shin JS, Kwon HJ, Kang KH (1998) Efficient transformation of korean rice cultivars (*Oryza sative* L.) mediated by *Agrobacterium tumefaciens*. J Plant Biol 41: 262-268

Christensen AH, Sharrock RA, Quail PH (1992) Maize polyubiquitin gene: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. Plant Mol Biol 18: 675-689

Chu CC, Wang CC, Sun CS, Hsu C, Yin KC, Chu CY, Bi FY (1975) Establishment of an efficient medium for anther

culture of rice through comparative experiments on the nitrogen sources. Sci Sin 18: 659-668

Cornejo MJ, Luth D, Blankenship KM, Anderson OD, Blechl AE (1993) Activity of a maize ubiquitin promoter in transgenic rice. Plant Mol Biol 23: 567-581

Dong J, Teng W, Buchholz WG, Hall TC (1996) *Agrobacterium*-mediated transformation of javanica rice. Mol Breed 2: 267-276

Gould J, Devey M, Hasegawa O, Ulian EC, Peterson G, Smith RH (1991) Transformation of *Zea mays* L. using *Agrobacterium tumefaciens* and the shoot apex. Plant Physiol 95: 426-434

Graves AE, Goldman SL (1986) Transformation of *Zea mays* seedlings with *Agrobacterium tumefaciens*; detection of T-DNA specified enzyme activities. Plant Mol Biol 7: 43-50

Guerineau F, Mullineaux P (1993) Plant transformation and expression vectors, *In* RRD Croy, ed, Plant molecular biology labfax, BIOS Scientific, Oxford, pp 121-147

Hiei Y, Komari T, Kubo Y (1997) Transformation of rice mediated by *Agrobacterium tumefaciens*. Plant Mol Biol 35: 205-218

Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J 6: 271-282

Hoekema A, Hirsch PR, Hooykaas PJJ, Schilperoort RA (1983) A binary plant binary vector strategy based on separation of *vir*- and T-region of the *Agrobacterium tumefaciens* Ti-plasmid. Nature 303: 179-180

Jain RK, Jain S, Wu R (1996) Stimulatory effect of water stress on plant regeneration in aromatic indica rice varieties. Plant Cell Rep 15: 449-454

Jeon J-S, Chung Y-Y, Lee S, Yi G-H, Oh B-G, An G (1999) Isolation and characterization of an anther-specific gene, RA8, from rice (*Oryza sativa* L.). Plant Mol Biol 39: 35-44

Kang H-G, Jeon J-S, Lee S, An G (1998) Identification of class B and class C floral organ identity genes from rice plants. Plant Mol Biol 38: 1021-1029

Klee HJ, White FF, Iyer VN, Gordon MP, Nester EW (1983) Mutational analysis of the virulence region of an *Agrobacterium tumefaciens* Ti plasmid. J Bacteriol 153: 878-883

Lichtenstein CP, Fuller SL (1987) Vectors for the genetic engineering of plants. Genet Eng 6: 103-183

McElory D, Brettell RIS (1994) Foreign gene expression in transgenic cereals. Trend Biotech 12: 62-68

Meijer EGM, Schilperoort RA, Rueb S, van Os-Ruygrok PE, Hensgens LAM (1991) Transgenic rice cell lines and plants: expression of transferred chimaeric genes. Plant Mol Biol 16: 807-820

Morris PC, Altmann T (1994) Tissue culture and transformation, *In* EM Meyerowitz, CR Somerville, eds, *Arabidopsis*, Cold Spring Harbor Laboratory Press, New York, pp 173-222

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-479

Rachid H, Yokoi S, Toriyama K, Hinata K (1996) Transgenic plant production mediated by *Agrobacterium* in *indica* rice. Plant Cell Rep 15: 727-730

Raineri D, Bottino P, Gordon MP, Nester EW (1990) *Agrobacterium* mediated transformation of rice (*Oryza sativa* L). Bio/technology 8: 33-38

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, New York

Sasaki T (1999) Rice genomics for agro-innovation. AgBiotechNet 1 (http://www.agbiotechnet.com/)

Shackelford NJ, Chlan CA (1996) Identification of antibiotics that are effective in eliminating *Agrobacterium tumefaciens*. Plant Mol Biol Rep 14: 50-57

Toki S (1997) Rapid and efficient *Agrobacterium*-mediated transformation in rice. Plant Mol Biol Rep 15: 16-21

Toki S, Takamatsu S, Nojiri C, Ooba S, Anzai H, Iwata M, Christensen AH, Quail PH, Uchimiya H (1992) Expression of a maize ubiquitin gene promoter-*bar* chimaeric gene in transgenic rice plants. Plant Physiol 100: 1503-1507

Toriyama K, Hinata K (1985) Cell suspension and protoplast culture in rice. Plant Sci 41: 179-183

Vernade D, Herrera-Estrella A, Wang K, Montagu MV (1988) Glycine betaine allows enhanced induction of the *Agrobacterium tumefaciens vir* genes by acetosyringone at low pH. J Bacteriol 170: 5822-5829

Vijayachandra K, Palanichelvam K, Veluthambi K (1995) Rice scutellum induces *Agrobacterium tumefaciens vir* genes and T-strand generation. Plant Mol Biol 29:125-133