J. Plant Biotechnology(2002) *Vol. 4(4). pp. 135~141*

Review

Journal of Plant Biotechnology

Overcoming of Barriers to Transformation in Monocot Plants

Toyama Koichi[1], Chang-Hyu Bae[1], Mi-Suk Seo[2], In-Ja Song[1], Yong-Pyo Lim[3], Pill-Soon Song[4], Hyo-Yeon Lee[1]*

[1]College of Agriculture and Life Science, Sunchon National University, Sunchon, Chonnam, 540-742, Korea; [2]College of Agriculture, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan; [3]Kumho Life and Environmental Science Laboratory, 1, Oryong-dong, Buk-gu, Kwangju, 500-712, Korea; [4]College of Agriculture, Chungnam National University, 305-764, Korea

Key words: *Agrobacteriam*, riu Zoysiagrass, transformation

Abstract

***Agrobacterium*-mediated transformation has been unsuccessful for monocot plants except for a few important crops such as barley, rice, maize and wheat. We discussed here that a successful transformation of monocots demands certain critical conditions. The requirements for an efficient transformation are a selection of target tissues competent for plant regeneration and *Agrobacterium*-infection, and various factors promoting *Agrobacterium*-infection. The factors were divided into two to activate *Agrobacterium* and to increase plant cell's susceptibility against *Agrobacterium*. Optimization of these factors significantly increased transformation efficiency of zoysiagrass and rice plants. A technical improvement in transformation system for monocots will promote improvement of the breed as well as a study of gene functions in monocots.**

Introduction

Monocot is very important for mankind life. All over the world, people live on poaceae, such as rice, maize, barley and wheat. Grass species is used as main forage for livestock. Also turfgrass is essential for improvement of living condition, used in traffic and athletic field.

Although the breed for monocot plants have been improved by classical breeding methods for a long time, the genetic engineering as breeding tool attract a breeder's attention. Recently many interest genes that would provide the strong traits for improvement of agriculture were isolated from various plants. However that will not be significant without system for gene transfer to economical important plants. Furthermore monocot plants-derived genes often do not express in dicot plants. Therefore, it is necessary to establish the transformation system of monocot plants for the purpose of studying the monocots-derived gene expression in various monocots.

Gene transfer method to plants is mainly classified to direct and indirect transformation system. Until now, nearly all genetic transformations in monocot plants had been performed by using direct DNA delivery system (Lee, 1996; Chai and Stiklen, 1998; Cho et al., 2000). Because monocots have been generally considered outside the host range of *Agrobacterium tumefaciens* (De Cleene and Deley, 1976), so far indirect transformation, *A. tumefaciens*-mediated transformation has been unsuccessful for monocot plants (Chai and Sticklen, 1998) except for barley (Tingay et al., 1997), rice (Hiei et al., 1994; Rashid et al., 1996), maize (Ishida et al., 1996) and wheat (Cheng et al., 1997).

Because *Agrobacterium*-mediated transformation has the strong advantages, no necessary of protoplasts, stable integration to genomic DNA and simple segregation pattern by low copy number, it is preferred by breeders more than direct transformation method (Hiei et al., 1994).

Recently, for tissue culture-recalcitrant grass species, zoysia-grass (*Zoysia japonica* Steud.) we established seed-derived four callus types and developed an efficient plant regeneration system (Bae et al., 2001), and developed a *Agrobacterium*-mediated transgenic plant by modification

* Corresponding author, E-mail; hyoyeon@sunchon.ac.kr
Received Oct. 28, 2002; accepted Nov. 15, 2002.

of several factors (Toyama et al., unpublished). We discuss here an application of the effective factors and a prospective on *Agrobacterium*-mediated transformation of monocot plants, zoysiagrass and rice.

Brief review of monocot transformation

For Poaceae transformation various methods were used (Table 1). Most popular gene-delivery method is Particle gun technology, and embryogenic calli or suspension cells were main target. Recently Cho et al. (1998; 1999; 2000; 2001) used highly regenerable green tissues as target for particle bombardment and obtained many transgenic plants in various Poaceae plants. Although transformation via protoplasts needs high techniques for tissue culture, the first transgenic rice was obtained by the method (Toriyama et al., 1988). Another gene-delivery method, *Agrobacterium*-mediated transformation has become popular in rice after establishment of efficient system by Hiei et al. (1994). While there are a few reports in other monocot species because of an outside the host range of *Agrobacterium tumefaciens* (De Cleene and Deley, 1976).

Donor materials for an efficient transformation

Successful transformation of plants demands that target tissues is competent for propagation and regeneration of green plants. Because various mature organs of dicot plants such as tobacco leaf and cauliflower hypocotyls have a high regenerability to induce shoots directly, they could be used for transformation experiments without callus induction pathway (Horsch et al., 1985; Ding et al., 1998). However, a direct organogenesis from the mature organ is difficult in monocot plants, and for successful transformation an efficient induction system of regenera-

Table 1. Examples of transformation for Poaseae

Plants	Method	Donor tissues	Reference
Bahiagrass	Particle bombardment	Embryogenic calli	Smith et al. (2002)
Baley	Particle bombardment	Green tissue	Cho et al. (1998)
Barley	*A. tumefaciens*	Immature embryos	Tingay et al. (1997)
Barley	Particle bombardment	Shoot meristematic culture	Zhang et al. (1999)
Bentgrass	Electroporation	Protoplasts	Asano et al. (1998)
Bentgrass	Particle bombardment	Embryogenic suspension cells	Xiao and Ha (1997)
Blue grama grass	Particle bombardment	Highly chlorophylous embryogenic cells	Aguado-Santacruz et al. (2002)
Indica rice	*A. tumefaciens*	Embryogenic calli	Rashid et al. (1996)
Italian ryegrass	Particle bombardment	Embryogenic suspension cells	Dalton et al. (1999)
Japonica rice	electroporation	Protoplasts	Toriyama et al. (1988)
Japonica rice	*A. tumefaciens*	Embryogenic calli	Hiei et al. (1994)
Japonica rice	Synthetic polycationic amino polymer	Protoplast	Tsugawa et al. (1998)
Japonica rice	*A. tumefaciens*	Embryogenic calli	Seo et al. (2002)
Javanica rice	*A. tumefaciens*	Embryogenic calli	Dong et al. (1996)
Maize	*A. tumefaciens*	Immature embryos	Ishida et al. (1996)
Maize	Whisker	Type 2 calli	Petolino et al. (2000)
Maize	Particle bombardment	Type 1 calli	Wright et al. (2001)
Oat	Particle bombardment	Shoot meristematic culture	Zhang et al. (1999)
Oat	Particle bombardment	Green tissue	Cho et al. (1999)
Orchardgrass	Particle bombardment	Green tissue	Cho et al. (2001)
perennial ryegrass	Particle bombardment	Embryogenic suspension cells	Spangenberg et al. (1995b)
perennial ryegrass	Particle bombardment	Embryogenic suspension cells	Dalton et al. (1999)
Red fescue	Particle bombardment	Embryogenic saspension cells	Spangenberg et al. (1995a)
Red fescue	Particle bombardment	Green tissue	Cho et al. (2000)
Tall fescue	Particle bombardment	Embryogenic saspension cells	Spangenberg et al. (1995a)
Tall fescue	Particle bombardment	Green tissue	Cho et al. (2000)
Wheat	Particle bombardment	Embryogenic calli	Vasil et al. (1992)
Wheat	*A. tumefaciens*	Immature embryos, embryogenic calli	Cheng et al. (1997)
Wheat	Particle bombardment	Green tissue	Kim et al. (1999)
Zoysiagrass	PEG	Protoplsts	Inokuma et al. (1998)
Zoysiagrass	*A. tumefaciens*	Organogenic Type 3 calli	Toyama et al. (Unpubl.)

ble callus is necessary. Particle gun technology does not choose target tissue and have been most used for genetic transformation in monocot. However, it would be not useful method for production of transgenic monocot plants, provided that regenerable callus or tissue was not induced as target cell.

For barley transformation by using particle gun technology, Cho et al. (1998) induced highly regenerable green tissues derived from immature scutellar tissues by modifying hormonal combination and cupric sulfate level and by culturing under dim light condition in callus induction media. Furthermore, the green tissues were produced from immature scutellar tissues of wheat (Kim et al., 1999) and from mature seed-derived embryogenic callus tissues of oat (Cho et al., 1999), tall and red fescues (Cho et al., 2000) and orchardgrass (Cho et al., 2001). These tissues regenerate multiple shoots, can be maintained for more than a year with minimal loss in regenerability and can be used for the successful transformation of previously recalcitrant cultivars of, for example, barley and wheat (Cho et al., 1998; Kim et al., 1999).

Maize can form two different types of callus (Tomes and Smith, 1985) The callus type affects to transformation frequency in maize. Depending on the transformation method elected, it is possible to favor the initiation of one type of callus over another. Type I callus is convenient for biolistic transformation (Koziel et al., 1993), whereas a certain type II callus is preferentially chosen to produce protoplasts (Sillito et al., 1994).

Hiei et al. (1994) reported, for the first time in monocot plants, an efficient *Agrobacterium*-mediated transformation system for rice. They examined various tissues; namely, shoot apices and segments of roots from young seedlings, scutella, immature embryos, calli induced from young roots and scutella, and cells in suspension cultures induced from scutella, as donor for *A. tumefaciens*. The most production of transgenic cell was obtained from scutellum callus. In addition, scutellum callus have high ability of propagation and regeneration, which showed scutellum callus suitable for donor material of *A. tumefaciens*-mediated transformation.

In the background of importance to establish regeneration system in monocot transformation, we established highly regenerable culture system for *A. tumefaciens*-mediated trans-formation of zoysiagrass (Toyama et al., Unpublished). When zoysiagrass seed-derived calli were cultured under light for 3 days before every subculture, green colored calli emerged, from which little albino plants regenerated on shoot induction medium (Figure 2A). When the green colored calli were subcultured on fresh callus growth media, highly regenerable callus that regenerate green shoots propagated. Furthermore, zoysiagrass seed-derived calli changed to four callus types by cultured on various hormonal combinations. Four morphological types of callus were: Type 1; most of which were whitish-yellow, compact and non-friable callus (Figure 1-1): Type 2; all of which were white, compact and friable callus (Figure 1-2): Type 3; all of which were yellow, compact and very friable callus (Figure 1-3): Type 4; all of which were translucent, soft, and watery callus (Figure 1-4).

Table 2. Effect of callus type and co-cultivation period on *A. tumefaciens*-mediated transformation of *Z. japonica*.

Callus types	Co-cultivation perio (days)	GUS spots per 1 g callus
1	3	23±0.2
	9	60±13.4
2	9	0
3	3	41±16.5
	6	834±162.0
	9	1310±157.0

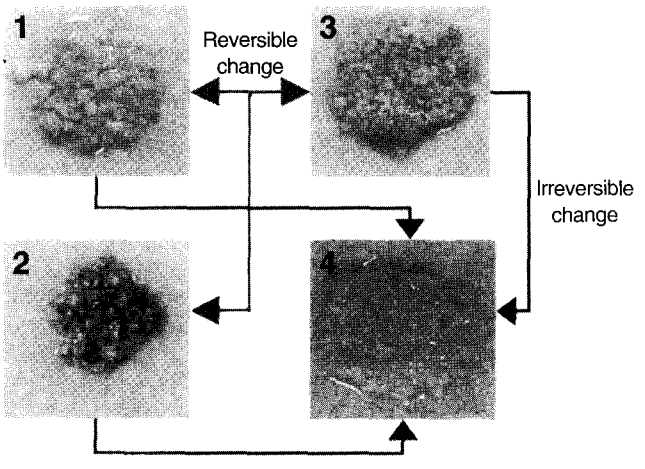

Figure 1. Four morphological types of callus were: Type 1; most of which were whitish-yellow or pale green, compact and non-friable callus, and multiple shoot primordia were seen on the surface of some calli (1): Type 2; all of which were white, compact and friable callus (2): Type 3; all of which were yellow, compact and very friable callus (3): Type 4; all of which were translucent, soft, and watery callus. The best proliferation yields for Type 1 and Type 2 calli and Type 3 callus were observed on MS medium containing 0.01 mg/L BA in combination with 1 and 4 mg/L 2,4-D, respectively. Types 1, 2 and 3 calli were regenerable, while Type 4 callus was not. The proliferation yield of non-regenerable Type 4 callus was reduced by the addition of 0.01 mg/L BA in combination with 2,4-D. Each of Types 1 through 3 calli resulted in mixed patterns of callus proliferation of all Types 1-4. However, Type 4 callus alone resulted in propagation of only Type 4 itself.

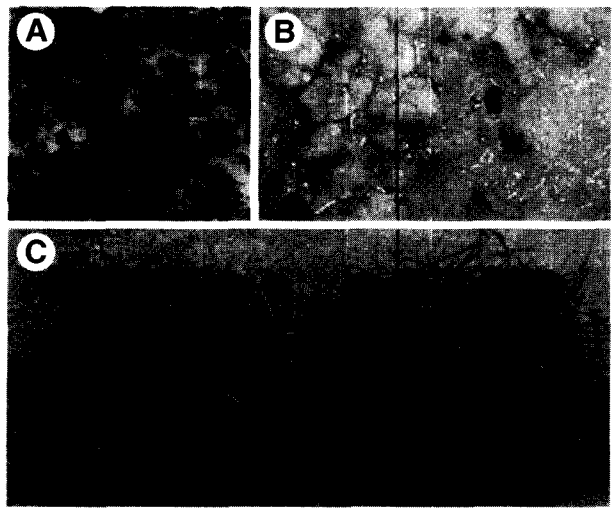

Figure 2. Transgenic GUS expression in *A. tumefaciens*-infected calli and steps in the regeneration of transgenic plants. (A) Efficient shoot induction on antibiotics-free shoot induction media-impregnated filter paper. (B) Transient GUS expression observed after 9 days of co-cultivaition on Type 3 calli. from *A. tumefaciens*-infected callus. (C) Herbicide applications in the transformed plant (right) and non-transformed plants (left). After one week of 10 g/L Herbiace-application, the transgenic plants survived herbicide spraying, while the control plants stopped growing and died.

Types 1, 2 and 3 calli were regenerable, while Type 4 callus was not. The proliferation yield of non-regenerable Type 4 callus was reduced by the addition of 0.01 mg/L BA in combination with 2,4-D. Furthermore, As the result of suppressing Type 4 callus occurrence and selecting green colored calli on every subculture, the callus lines maintained regenerability of green plants over 3 years. These callus types affected to *Agrobacterium*-mediated transformation efficiency significantly. We investigated relationship between callus types (regenerable callus type: 1, 2 and 3) and transformation efficiency. The number of GUS spots on Type 3 callus in 6-9 days-co-cultivation period was 20-30 times more abundant than Type 1 callus (Figure 1B). No GUS expression was detected in all co-cultivation period on Type 2 callus. Although effect of callus type on *Agrobacterium*-mediated transformation has not been discussed so far, our result shows that classification of callus type is the most basic and important factors besides *Agrobacterium* activation.

Various factors promoting *A. tumefaciens*-infection

Factors to enhance *Agrobacterium*-mediated transformation efficiency are largely divided to two types, namely, factors to activate *Agrobacterium* and to increase the susceptibility of plant cells against *Agrobacterium*. The former contains acetosyringone (Stachel et al., 1985), monosaccharides such as D-glucose (Shimoda et al., 1990) and betaine (Vernade et al., 1988), and the latter contains hormonal combination (Toyama et al., Unpublished; Seo et al., 2002), $CaCl_2$ (Montoro et al., 2000) and osmotic treatment (Uzé et al., 1997). By now various possible factors were evaluated or applied for *Agrobacterium*-mediated transformation of both dicot and monocot plants. In this chapter we introduce some evidences for application of gene delivery-enhancing factors.

In genetic transformation, small phenolic compound such as acetosyringone is required for the transcriptional activation of the *Agrobacterium* virulence machinery (Stachel et al., 1985). Acetosyringone as a phenolic signal are transduced through a receptor *Vir*A protein in the inner membrane of the bacterial cell. The expression of these genes triggers the transfer of a specific DNA segment, called transferred DNA (T-DNA), from the Ti plasmid to plant cell, and its integration into their nuclear DNA. However, the major crop species, the monocotyledonous cereal plants, have generally proven refractile to *Agrobacterium* infection (De Cleene and Deley, 1976). In this background, the use of acetosyringone during infection and co-cultivation has been shown to be critical for *Agrobacterium*-mediated transformation in monocot plants, such as japonica and indica rice (Hiei et al., 1994; Rashid et al., 1996; Seo et al., 2002), pharaenopsis orchid (Belarmino and Mii, 2000) and agapanthus (Suzuki et al., 2001). In zoysiagrass, *Agrobacterium*-infection and gene expression in plant cell required adequate endogenous acetosyringone level (Toyama et al., Unpublished).

Shimoda et al. (1990) reported that a group of aldoses (L-arabinose, D-xylose, D-lyxose, D-glucose, D-mannose, D-idose, D-galactose and D-talose) could markedly enhance acetosy-ringone-dependent expression of *vir* genes when the concentration of acetosyringone is limited (10 μM). In rice 10 g/L glucose was routinely used for efficient transformation (Hiei et al., 1994; Seo et al., 2002).

Glycine betaine allows enhanced induction of the *Agrobacterium tumefaciens vir* genes by acetosyringone at low pH (Vernade et al., 1988). We added betaine to co-cultivation media, on which *Agrobacterium*-infected rice calli were cultured. After co-cultivation, the GUS expression was in the presence of 120 mg/L betaine higher than absence (Seo et al., 2002).

In dicot *Hevea brasuliensis*, the effect of exogenous calcium on *A. tumefaciens*-mediated gene transfer has been discussed, and removal of calcium from infection medium enhanced the transformation efficiency (Montoro et al., 2000). Therefore the influence of $CaCl_2$ on *A. tumefaciens*-

mediated gene transfer was investigated in monocot zoysiagrass and rice calli. The GUS activity peaked on $CaCl_2$-free co-cultivation medium, and decreased as the $CaCl_2$ concentration increased in *Z. japonica* (Toyama et al., Unpublished). In rice the GUS activity peaked when the $CaCl_2$ was reduced to 50 mg/L from 440 mg/L contained in MS (Murashige and Skoog, 1962) media (Seo et al., 2002). In zoysiagrass also, calcium in co-cultivation medium inhibited GUS transient expression. Although the effect of calcium on *Agrobacterium*-infection to plant cell is poorly understood (Montoro et al., 2000), calcium plays a crucial role in resistance response to pathogenic microorganisms (Dierk, 1998). The calcium-induced plant defense machinery may operate in monocot callus against *Agrobacterium*-infection. Therefore, the removal of calcium from co-cultivation medium may weaken the plant's resistance to microbial-infection, resulting in acceleration of *Agrobacterium*-mediated transformation.

Two to three days of co-cultivation period have been used for general Gramineae transformation (Hiei et al., 1994; Rashid et al., 1996; Dong et al., 1996; Ishida et al., 1996; Cheng et al., 1997), while a prolonged co-cultivation period of 5-7 days has been shown to increase *Agrobacterium*-mediated transformation in *Lilium usitatissimum* and agapanthus (Dong and McHughen, 1991; Suzuki et al., 2001).

In zoysiagrass type 1 and 3 callus little GUS expression was observed after 3 days co-cultivation. However, prolongation of co-cultivation period to 6 and 9 days increased GUS expression only on Type 3 callus (Toyama et al., Unpublished). Also in rice, GUS expression increased 2.3 times, when co-cultivation period was prolonged from 3 to 10 days (Seo et al., 2002).

Osmotic treatment is essential for microprojectile mediated gene transfer to monocot plant cell (Hamilton et al., 1992; Iglesias et al., 1994). And Uzé et al. (1997) reported, in *Agrobacterium*-mediated transformation of rice, that plasmolysis as pretreatment was effective for cells expressing the marker gene.

In transformation of bar gene for zoysiagrass we applied various factors that contained using Type 3 calli as donor, removal of $CaCl_2$, and addition of 100 mg/L betaine and 50-100 mg/L acetosyringone in co-cultivation media and co-cultivation period of 10 days. And we got stable transgenic plants resistant to 5 g/L bialaphos (Figure 1C). A DNA gel-blot analysis for the bialaphos resistant plant confirmed the integration of transgene into the zoysiagrass genome.

In rice transformation we applied various factors that contained reduced $CaCl_2$ level (50 mg/L) and addition of 120 mg/L betaine in co-cultivation media and co-cultivation period of 10 days. After 4 weeks of co-cultivation with *A. tumefaciens*, calli were selected on 50 mg/L hygromycin-containing regeneration media. The number of regenerated plants was 35.1-53.6% of used calli and increased remarkably. Regenarated plants were identified as positive by DNA blot analysis.

Prospects for poaceae transformation

Plant transformation technology has become a versatile tool for an improvement of breed as well as for a study of gene functions in plants. A majority of monocot transformants obtained to date has been generated by the particle bombardment technique. For a while, *Agrobacterium*-mediated transformation is rapidly becoming the method of choice. However, most of important monocot plants besides rice are recalcitrant to *Agrobacterium*-mediated transformation. As described here, an obvious improvement of transformation frequency was obtained by using optimum donor plant materials and by optimizing factors to promote *Agrobacterium*-infection. In addition to conditions we discussed here, *Agrobacterium* strains, marker genes and promoters are also important factors to improve transformation efficiency in monocots. A perfect understanding of all factors related to *Agrobacgerium*-infection makes ultimately possible to develop *in planta* transformation system over all species. The developments will save labor, time and cost to make transgenic plants, leading to speed up an improvement of breed, to fall in price of agricultural products, and ultimately to increase a level of human life by avoidance of a food crisis.

References

Aguado-Santacruz GA, Rascón-Cruz Q, Cabrera-Ponce JL, Martínez-Hernández A, Olalde-Portugal V, Herrera-Estrella L (2002) Transgenic plants of blue grama grass, Bouteloua gracilis (H.B.K.) Lag. ex Steud., from microprojectile bombardment of highly chlorophyllous embryogenic cells. Plant Cell Rep 104: 763-771.

Belarmino MM, Mii M (2000) *Agrobacterium*-mediated genetic transformation of a phalaenopsis orchid. Plant Cell Rep 19: 435-442.

Bae CH, Toyama K, Lee SC, Lim YP, Kim HI, Song PS, Lee HY (2001) Efficient plant regeneration using mature seed-derived callus in Zoysiagrass (*Zoysia japonica* Steud.). Kor J Plant Tissue Cult 28: 61-67.

Chai B, Sticklen MB (1998) Applications of biotechnology in turfgrass genetic improvement. Crop Sci 38: 1320-1338.

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.

Cho M-J, Jiang W, Lemaux PG (1998) Transformation of recalcitrant barley cultivars through improvement of regene-rability and decreased albinism. Plant Sci 138: 229244.

Cho M-J, Jiang W, Lemaux PG (1999) High-frequency transformation of oat via microprojectile bombardment of seedderived highly regenerative cultures. Plant Sci 148: 917.

Cho M-J, Ha CD, Lemaux PG (2000) Production of transgenic tall fescue and red fescue plants by particle bombardment of mature seed-derived highly regenerative tissues. Plant Cell Rep 19: 1084-1089.

Cho M-J, Choi H-W, Lemaux PG (2001) Transformed T0 orchardgrass (*Dactylis glomerata* L.) plants produced from highly regenerative tissues derived from mature seeds. Plant Cell Rep 20: 318-324.

Dalton SJ, Bettany AJE, Timms E, Morris P (1999) Co-transformed, diploid *Lolium perenne* (perennial ryegrass), *Lolium multiflorum* (Italian ryegrass) and *Lolium temulentum* (darnel) plants produced by microprojectile bombardment. Plant Cell Rep 18: 721-726.

De Cleene M, Deley J (1976) The host range of crown gall. Bot Rev 42: 389-466.

Dierk S (1998) Resistance response physiology and signal transduction. Curr Biol 1: 305-310.

Ding L-C, Hu C-Y, Yeh K-W, Wang P-J (1998) Development of insect-resistant transgenic cauliflower plants expressing the trypsin inhibitor gene isolated from local sweet potato. Plant Cell Rep 17: 854-860.

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

Dong J-Z, McHughen A (1991) Patterns of transformation intensity on flax hypocotyls inoculated with *Agrobacterium tumefaciens*. Plant Cell Rep. 10: 555-560.

Hamilton DA, Roy M, Rueda J, Sindhu RK, Sanford J, Mascarenhas JP (1992) Dissection of a pollen-specific promoter from maize by transient transformation assays. Plant Mol Biol 18: 211-8.

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

Horsch RB, Fry JE, Hoffmann NL, Eichholtz D, Rogers SG, Fraley RT (1985) A simple and general method of transferring genes into plants. Science 227: 1229-1231.

Inokuma C, Sugiura K, Imaizumi N, Cho C (1998) Transgenic Japanese lawngrass (*Zoysia japonica* Steud.) plants regenerated from protoplasts. Plant Cell Rep 17: 334-338.

Ishida Y, Saito H, Ohta S, Hiei Y, Komari T, Kumashiro T (1996) High efficiency transformation of maize (*Zea mays* L.) mediated by *Agrobacterium tumefaciens*. Nature Biotechnol 14: 745-750.

Kim H-K, Lemaux PG, Buchanan BB, Cho M-J (1999) Reduction of genotype limitation in wheat (*Triticum aestivum* L.) transforma-tion. Congress on *In Vitro* Biology, New Orleans, p 1021.

Koziel MG, Beland GL, Bowman C, Carozzi NB, Crenshaw R, Crossland L, Dawson J, Desai N, Hill M, Kadwell S (1993) Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. Biotechnology 11: 194-200.

Lee L (1996) Turfgrass biotechnology. Plant Sci 115: 1-8.

Montoro P, Teinseree N, Rattana W, Kongsawadworakul P, Michaux-Ferriere N (2000) Effect of exogenous calcium on *Agrobacterium tumefaciens*-mediated gene transfer in *Hevea brasiliensis* (rubber tree) friable calli. Plant Cell Rep 19: 851-855

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

Petolino JF, Hopkins NL, Kosegi BD, Skokut M (2000) genetic *transformation and hybridization*: Whisker-mediated trans-formation of embryogenic callus of maize. Plant Cell Rep 19: 781-786.

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

Stachel SE, Messens E, Van Montagu M, Zambryski P (1985) Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. Nature 318: 624-629.

Seo M-S, Bae C-H, Choi D-O, Rhim S-L, Seo S-C, Song P-S, Lee H-Y (2002) Investigation of transformation efficiency of rice using *Agrobacterium tumefaciens* and high transformation of GPAT (glycerol-3-phosphate acyltransferase) gene relative to chilling tolerance. Korean J Plant Biotechnlogy 29: 85-92.

Shimoda N, Toyoda-Yamamoto A, Nagamine J, Usami S, Katayama M, Sakagami Y, Machida Y (1990) Control of expression of *Agrobacterium vir* genes by synergistic actions of phenolic signals molecules and monosaccharides. Proc Natl Acad Sci 87: 6684-6688.

Sillito R, Carswell G, Kramer CM (1994) Maize protoplast culture, in the maize handbook (Freeling M and Wabot V, eds), pp 695-700, Springer.

Smith RL, Grando MF, Li YY, Seib JC, Shatters RG (2002) Transformation of bahiagrass (*Paspalum notatum* Flugge). Plant Cell Rep 20: 1017-1021.

Spangenberg G, Wang ZY, Wu XL, Nagel J, Iglesias VA, Potrykus I (1995a) Transgenic tall fescue (*Festuca arundinacea*) and red fescue (*F. rubra*) plants from microprojectile bombardment of embryogenic suspension cells. J Plant Physiol 145: 693-701.

Spangenberg G, Wang ZY, Wu XL, Nagel J, Potrykus I (1995b) Transgenic perennial ryegrass (*Lolium perenne*) plants from microprojectile bombardment of embryogenic suspension cells. Plant Sci 108: 209-217.

Suzuki S, Supaibulwatana K, Mii M, Nakano M (2001) Production of transgenic plants of the Liliaceous ornamental plant *Agapanthus praecox* ssp. *orientalis* (Leighton) Leighton via *Agrobacterium*-mediated transformation of embryogenic calli. Plant Sci 161: 89-97.

Stachel SE, Messens E, Van Montagu M, Zambryski P (1985) Identification of the signal molecules produced by wounded plant cells that activate T-DNA transfer in *Agrobacterium tumefaciens*. Nature 318: 624-629.

Tingay S, McElroy D, Kalla R, Fieg S, Wang M, Thomton S, Brettel R (1997) *Agrobacterium tumefaciens*-mediated barley trans-formation. Plant J 11: 1369-1376.

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

Tomes DT, Smith OS (1985) The effect of parental genotype on initiation of embryogenic callus from elite maize (*Zea maize* L.) germplasm. Theol Appl Genet 70: 505-509.

Toriyama K, Arimoto Y, Uchimiya H, Hinata K (1988) Transgenic rice plants after direct gene transfer into protoplasts. Bio/Technol 6: 1072-1074.

Toyama K, Bae C-H, Kang J-G, Lim Y-P, Adachi T, Song P-S, Lee H-Y An efficient transformation system for herbicide-tolerant zoysiagrass (*Zoysia japonica* steud.) mediated by *Agrobacterium tumefaciens*. Unpublished.

Tsugawa H, Otsuki Y, Suzuki M (1998) Efficient transformation of rice protoplasts mediated by a synthetic polycationic amino polymer. Theol Appl Genet 97: 1019-1026.

Uzé M, Wunn J, Puonti-Kaerlas J, Ptrykus I, Sautter C (1997) Plasmolysis of precultured immature embryos improves *Agrobacterium* mediated gene transfer to rice (*Oryza Sativa* L.). Plant Sci 130: 87-95.

Vasil V, Castillo AM, Fromm ME, Vasil IK (1992) Herbicide resistant fertile transgenic wheat plants obtained bymicroproj-ectile bombardment of regenerable embryogenic callus. BioTechnology 10: 667-674.

Vernade D, Herrera-Estrella A, Wang K, Van Montagu M (1988) Glycine betaine allows enhanced induction of the *Agrobacterium tumefaciens vir* genes by acetosyringone at low pH. J Bacteriol 170: 5822-9.

Wright M, Dawson J, Dunder E, Suttie J, Reed J, Kramer C, Chang Y, Novitzky R, Wang H, Artim-Moore L (2001) Efficient biolistic transformation of maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) using the phosphomannose isomerase gene, *pmi*, as the selectable marker. Plant Cell Rep 20: 429-436.

Xiao L, Ha S-B (1997) Efficient selection and regeneration of creeping bentgrass transformants following particle bombard-ment. Plant Cell Rep 16: 874-878.

Zhang S, Cho M-J, Koprek T, Yun R, Bregitzer P, Lemaux PG (1999) Genetic transformation of commercial cultivars of oat (*Avena sativa* L.) and barley (*Hordeum vulgare* L.) using *in vitro* shoot meristematic cultures derived from germinated seedlings. Plant Cell Rep 18: 959-966.