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Effects of Sonication and Vacuum Infiltration on *Agrobacterium*-Mediated Transformation in Immature Embryos of Korean Wheat Genotypes

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ABSTRACT: The effects of sonication and vacuum infiltration on transformation efficiency was investigated by using immature embryos of Korean wheat as explants. Two *Agrobacterium tumefaciens* strains, KYRT1 and EHA105, carrying pCAMBIA 1305.1 were used. Transformation efficiency was demonstrated by the detection of ß-glucuronidase (GUS) activity. GUS expression showed clear difference among Korean wheat cultivars. Geurumil showed higher GUS expression efficiency 79.1 % compared with other cultivars. The effects of the duration of vacuum infiltration and sonication treatment showed a tendency high GUS expression efficiency by their combination. In comparison with other *Agrobacterium* strains, KYRT1 showed high efficiency in most Korean cultivars.

Keywords: Korean wheat cultivar, *Agrobacterium tumefaciens*, β-glucuronidase, transformation, vacuum infiltration, sonication.

Agrobacterium-mediated transformation is the most commonly used method for plant genetic engineering. The transfer of T-DNA is influenced by several factors including plant genotype, type of explant, tissue culture medium, *Agrobacterium* strain and plasmid vector, cell density in inoculation, and the conditions of inoculation and co-cultivation. The optimal level of each of these elements needs to be determined for every transformation system.

The improvements of the transformation method such as sonication and vacuum infiltration treatments were reported in plants. The first publication using sonication for transformation was that of Joersobo & Brunstedt (1990, 1992). They demonstrated that mild sonication of sugar beet (*Beta vulgaris* L.) and tobacco (*Nicotiana tabaccum* L.) protoplasts facilitated the uptake of plasmid DNA with some loss in viability of the protoplasts. Transient expression of the foreign DNA was increased by 7 - 15 fold over expression following electroporation. No long-term deleterious effects of sonication were observed. Trick & Finer (1997, 1998, 1999) sonicated cotyledons of *Glycine max* Jack, embryonic cultures of *Aesculus glabra* and *Glycine max* Merrill, leaf tissue of *Vigna unguiculata*, seedlings of *Picea glauca* and *Triticum aestivum* and immature embryos of *Zea mays* and cocultivated with *Agrobacterium*. Lee (2001) reported that sonication treatment resulted in significant increase in the transformation efficiency.

One of the simplest available plant transformation systems involves the infiltration of *Agrobacterium* cells into *Arabidopsis* plants before flowering and direct selection for rare transformant in the resulting seedling population (Chang *et al.*, 1994). Recently, the components of the transformation method were assessed and improvements to the method were reported (Clough & Bent, 1998) Ye *et al.* (1999) reported the *Arabidopsis* ovule was the target for infiltration transformation. The effects of vacuum infiltration and sonication in wheat inflorescence tissue were reported (Amoah *et al.*, 2001).

The purpose of this paper was to determine the effects of sonication and vacuum infiltration on transformation and to develop an efficient method of *Agrobacterium*-mediated transformation by using immature embryos of Korean wheat cultivars.

MATERIALS AND METHODS

Plant materials

Immature embryos of six genotypes (cvs. Alchanmil, Geurumil, Gobunmil, Keumkangmil, Tapdongmil, Urimil) were used as the target materials for transformation efficiencies. The cultivars were planted in the field during the normal wheat growing season (2002 - 2003) at the National Institute of Crop Science, Suwon, Korea. Immature embryos measuring 1.0 ~ 2.0 mm in diameter were aseptically removed from surface-sterilized immature seeds.

Inoculation, co-cultivation and histochemical β-glucuronidase analysis

Agrobacterium tumefaciens strains, KYRT1 (Torisky *et al.*,

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1997) and EHA105 (Hood *et al.*, 1993), carrying the vector pCAMBIA1305.1 contained β-glucuronidase (GUS) as a reporter gene were cultured at 28°C for 3 days in liquid YEB medium containing 50 mg/l kanamycin and 100 mg/l rifampicin in the dark with shaking at 250 rpm. The *A. tumefaciens* cell density was adjusted to give an OD_{600} of 1.0 to 1.5 for inoculation. Acetosyringone was added just two hours prior to inoculation to a final concentration of 200 μM.

Immature embryos were placed in petri dishes containing the *A. tumefaciens* suspension and incubated at room temperature for 1 hour on a shaker at 70 - 80 rpm as a control. The immature embryos were transferred to co-cultivation medium (MS salts including vitamins, 500 mg/l glutamine, 100 mg/l casein hydrolysate, 40 g/l maltose, 2 mg/l 2,4-D, 2.2 mg/l picloram, 200 μM acetosyringone, pH 5.8) without blotting, and then co-cultivated at 25°C in the dark for three days. After co-cultivation, immature embryos were washed in ddH_2O containing 250 mg/l cefotaxime.

Immature embryos were assayed for transient GUS expression. Histochemical staining of immature embryos was performed as described by Jefferson (1987).

Sonication and vacuum infiltration

For sonication treatments, 20 immature embryos transferred into glass round bottom tubes containing 5 ml of *Agrobacterium* suspension. The test tubes were capped and placed in the center of a bath sonicator (BRANSONIC 5210R-DTH).

Immature embryos immersed in *Agrobacterium* suspansion were placed in the desiccator and the vacuum applied at 760 mm Hg, for periods 0.5 h and 1 h. Immature embryos were placed on a sterile filter paper to blot off excess bacteria, and then co-cultivated on co-cultivation medium at 25°C in the dark for 3 days.

RESULTS AND DISCUSSION

The transformation efficiencies in Korean wheat cultivars were investigated by sonication and vacuum infiltration treatments and their combinations. Efficiency of *Agrobacterium*-mediated transformation was determined by GUS gene expression in immature embryos of wheat. Transient GUS expression in immature embryos showed four different patterns, individual blue spots, blue spots grouped slightly, blue staining grouped broadly and GUS expressed as a whole (Fig. 1).

Table 1 showed differences of GUS expression among the cultivars and treatments and between *Agrobacterium* strains. In comparison of *Agrobacterium* strains, KYRT1 showed higher in most cultivars (Fig. 2). The ability of different

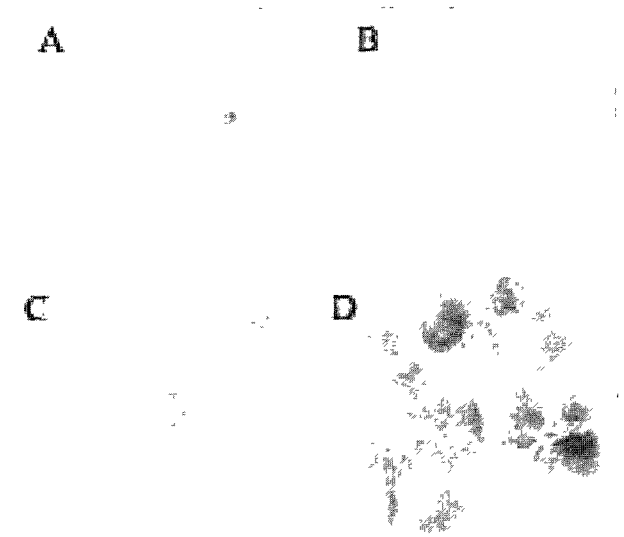

Fig. 1. Grades of transient GUS expression in wheat immature embryos (A) + Individual blue spots, (B) ++ Blue spots grouped slightly, (C) +++ · Blue staining grouped broadly, (D) ++++ . GUS expressed as a whole

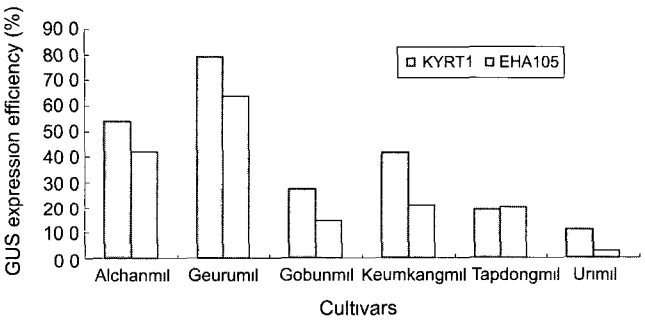

Fig. 2. The effect of Agrobacterium strains on GUS expression in immature embryos.

Agrobacterium strains, in the same vector, to transfer T-DNA into plant tissues in soybean have been reported in Torisky *et al.* (1997). This experiment also demonstrates the difference between *Agrobacterium* strains to T-DNA transfer in wheat.

In barley cvs. Golden Promis (Tingary *et al.*, 1997), Dissa (Wu *et al.*, 1998) and Schooner (Wang *et al.*, 2001), *Agrobacterium*-mediated transformation has been achieved mainly in genotypes selected for their good response in tissue culture. Although there has also been progress in wheat (Cheng *et al.*, 1997; Weir *et al.*, 2001; Wu *et al.*, 2003), it has been confined mainly to a few responsive varieties, and published methods have proved difficult to follow. In experiments described here, the frequency of GUS expression showed clearly difference among the wheat cultivars with least significant differences at 7.41%. Geurumil showed the

Table 1. Effects of *Agrobacterium* strains and treatments on GUS expression in immature embryos of Korean wheat genotypes

Strain	Treatment[b]	Proportion of explants showing GUS expression (%)[a]					
		Alchanmil	Gobunmil	Geurumil	Keumkangmil	Tapdongmil	Urimil
KYRT1	NT	44 44+[c]	25.00+	100.00+++	87.50+++	25.00+	0.00−
	30 sec	0.00−	15 00+	0.00−	35.29+	0.00−	0.00−
	60 sec	5.00+	5.00+	10.00+	30 00+	10 00+	0 00−
	90 sec	5 88++	50 00+	25.00+	70.00++	5.00+	0.00−
	0.5 h	15 00++	60.00+	100 00++++	35 00++	25 00+	0 00−
	1 h	100 00++	10.00+	100.00++++	50 00+	10 00+	60 00+
	1.5 h	100 00+	50.00+	90.00++	20.00+	10.00+	35.00+
	30 sec+0 5 h	47.37++	10 00+	100.00++++	100.00+++	0.00−	20 00+++
	60 sec+0 5 h	45.00+	25.00+	100.00++	85.00+++	85.00++	15.00+
	90 sec+0.5 h	20 00+	10.00+	100.00+++	0.00−	35.00+	0 00−
	30 sec+1 h	100 00++	10.00+	50.00+	75.00++	15 00+	10 00+
	60 sec+1 h	90 48+	25.00+	100.00+++	25 00+	20 00+	0 00−
	90 sec+1 h	100.00++	50 00+	100.00++++	10 00+	50.00+	0.00−
	30 sed+1 5 h	83 33+	35.00+	100.00+++	0.00−	0.00−	10.00+
	60 sec+1.5 h	63 16+	10 00++	100 00++++	20 00++	10.00+	15 00+
	90 sec+1 5 h	42.86+	50.00+	850 00+	20 00+	5.00+	10.00+
EHA105	NT	68 42+	10.00+	75 00+	50 00++	60 00++	0.00−
	30 sec	10 00+	10.00+	85.00++	25.00+	0.00−	0 00−
	60 sec	11.76+	5.00+	10 00+	0.00−	5.00+	0 00−
	90 sec	0.00−	10 00+	15 00+	5 00+	0 00−	0.00−
	0 5 h	100.00+++	0.00−	100.00+++	5.00+	15.00+	0.00−
	1 h	100 00++	25 00+	75.00+	15.00+	85.00++	5 00+
	1.5 h	71.43+	75.00++	90.00+	5 00+	0 00−	0 00−
	30 sec+0 5 h	0 00−	10.00+	100.00++	95.00+++	5 00+	10.00+
	60 sec+0.5 h	100.00++	10.00+	100 00++	90.00+++	5.00+	5.00+
	90 sec+0.5 h	100.00++	5 00+	100 00++++	30.00+++	0.00−	5.00+
	30 sec+1 h	30.00+	5.00+	10 00++	0 00−	85.00++	5 00+
	60 sec+1 h	5 00+	35.00+	85 00++	5 00+	20.00+	0.00−
	90 sec+1 h	26 32+	15.00+	75.00+	10.00+	35.00+	5 00+
	30 sed+1.5 h	20.00+	0.00−	90.00+++	0 00−	0 00−	5.00+
	60 sec+1.5 h	16.67+	15.00+	5 00+	0 00−	0 00−	5.00+
	90 sec+1.5 h	10.00++	0.00−	0.00−	0.00−	0.00−	0.00−

[a] (Number of GUS expression immature embryos / Number of immature embryos) × 100
[b] NT : No treatment; 30, 60, 90 sec : Sonreation treatment, 0 5, 1, 1.5 h Vacuum infiltration treatment.
[c] GUS expression in immature embryos of wheat (Same as Fig. 1).

higher GUS expression compared with other cultivars (Table 1, Fig 2).

This experiment was thus designed to investigate the effects of the duration of vacuum and sonication treatments on GUS expression in Korean wheat cultivars. There treatments significantly increased the number of explants producing GUS expression (Table 1). Sonication is thought to enhance T-DNA delivery through the microwounding of the tissues which permit the *Agrobacterium* to travel deeper and more completely throughout the tissue normal co-cultivation will permit (Trick & Finer, 1997), thus enhancing the *Agrobacterium* colonization and infection of the tissues. The microwounding may also result in the active division of the cells and the accompanying DNA synthesis may enhance

the incorporation of the T-DNA into the plant genome (Amoah *et al.*, 2001). Vacuum infiltration has been used in transformation protocols of several plant species including *Arabidopsis* (Clough & Bent, 1998). Also called germ-line transformation, it has been used to transform *Arabidopsis* ecotypes and mutants that were recalcitrant to *Agrobacterium* transformation (Mysore *et al.*, 2000). The inoculation intensity is increased either by the vacuum infiltration, by the combinations of vacuum infiltration and sonication, and determines GUS expression activity.

In conclusion, optimal T-DNA delivery as measured by GUS activity was obtained in KYRT1 treatment and combination of sonication and vacuum infiltration treatments. Especially the frequency of GUS expression showed clearly difference among the wheat cultivars.

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REFERENCES

Amoah, B. K., H Wu, C. Sparks, and H. D. Jones 2001 Factors influencing *Agrobacterium*-mediated transient expression of *uidA* in wheat inflorescence tissue. Journal of Experimental Botany 52 . 1135-1142.

Chang, S. S., S. K Park, B. C. Kim, B J. Kang, and D. U. Kim. 1994 Stable genetic transformation of *Arabidopsis thaliana* by *Agrobacterium* inoculation in planta The Plant J 5 : 551-558

Cheng, M , J. E. Fry, S. Pang, H. Zhou, C, M. Hironaka, D R. Duncan, T W. Conner, Y. Wan 1997 Genetic transformation of wheat mediated by *Agrobacterium tumefaciens* Plant Physiol. 115 : 971-980.

Clough, S. J. and A. F. Bent. 1998 Floral dip· a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. The Plant J. 16 : 735-743.

Hood, E. E., S. B. Gelvin, L. S. Melchers, and A. Hoekema. 1993. New *Agrobacterium* helper plasmids for gene transfer to plants Transgenic Res. 2 208-218

Jefferson, R A 1987. Assaying chimeric genes in plants: the *GUS* gene fusion system. Plant Mol. Biol Rep. 5 : 387-405.

Joersbo, M. and J. Brunstedt 1990. Direct gene transfer to plant protoplasts by mild sonication Plant Cell Rep. 9 : 207-210

Joersbo, M. and J. Brunstedt. 1992 Sonication· A new method for gene transfer to plants. Physiology Plantarum 85:230-234.

Lee, B. M. 2001. Effect of sonication and vir genes on transient gene expression in Agrobacterium-mediated transformation. Korean J of Life Sci 11 : 316-320

Mysore, K. S., C T R. Kumar, and S. B. Gelvin. 2000. *Arabidopsis* ecotypes and mutants that are recalcitrant to *Agrobacterium* root transformation are susceptible to germ-line transformation The Plant J. 21 : 9-16.

Tingay, S., D. McElroy, R Kalla, S. Fieg, M. B. Wang, S. Thornton, and R. Brettell. 1997. *Agrobacterium tumefaciens*-mediated barley transformation The Plant J 11 . 1369-1376

Trick, H N and J. J Finer. 1997. SAAT: sonication-assisted *Agrobacterium*-mediated transformation Transgenic Res. 6 . 329-336.

Trick, H N and J. J. Finer. 1998 Sonication-assited *Agrobacterium*-mediated transformation of soybean [*Glycine max* (L.) Merrill] embryonic suspension culture tissue Plant Cell Rep. 17 : 482-488.

Trick, H N. and J J Finer. 1999 Induction of Somatic embryogenesis and genetic transformation of Ohio buckeye (*Aesculus glabra* Willd.). In Vitro Cell Dev. Biol. 35 : 57-60.

Torisky, R. S., L. Kovacs, S. Avdiushko, J. D. Newman, A. G. Hunt, and G. B. Collins 1997. Development of a binary vector system for plant transformation based on the supervirulent *Agrobacterium tumefaciens* strain Chry5 Plant Cell Rep. 17 · 102-108.

Wang, M , D. C. Abbott, N. M Upadhyaya, J. V. Jacobsen, and P M. Waterhouse 2001. *Agrobacterium tumefaciens*-mediated transformation of an elite Australian barley cultivar with virus resistance and reporter genes Aust. J. Plant Physiol. 28 . 149-156.

Weir, B., X. Gu, M. B. Wang, N Upadhyaya, A. R. Elliott, and R. I S. Brettell. 2001 *Agrobacterium tumefaciens*-mediated transformation of wheat using suspension cells as a model system and green fluorescent protein as a visual marker Aust J. Plant Physiol. 28 807-818.

Wu, H., A C. McCormac, M. C. Elliott, and D. F. Chen 1998 *Agrobacterium*-mediated stable transformation of suspension cultures of barley (Hordeum vulgare L.). Plant Cell Tissue Organ Cult. 54 : 161-167

Wu, H., C. Sparks, B. Amoah, H. D Jones. 2003 Factors influencing successful *Agrobacterium*-mediated genetic transformation of wheat. Plant Cell Rep 21 : 695-668.

Ye, G N , D Stone, S. Z Pang, W. Creely, K. Gonzalez, and M. Hinchee. 1999. *Arabidopsis* ovule is the target for *Agrobacterium* in planta vacuum infiltration transformation The Plant J 19 . 249-257