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Factors Affecting Transient Expression of *gus* Gene in Wheat Immature Embryos

Chang Hyun Choi^{1,2*}, Sang-Soo O², Myung-Ho Lim², Cheol Ho Hwang¹, Choong-Hyo Yun²

¹School of Bio-Resource Sciences, Dankook University

²National Institute of Agricultural Bio-technology, Suwon 441-707, Korea

Objectives

The development of particle bombardment for the delivery of a gene cassette into regenerable wheat explants revolutionized wheat transformation. It is well known that the particle bombardment parameters are important for the transformation efficiency. In this study, to evaluate the effect of parameters on the wheat transformation efficiency, immature embryos were subjected to particle bombardment in combinations of several bombardment parameters and GUS activity was observed.

Materials and Methods

1. Explant: Wheat (*Triticum aestivum* cv: Bobwhite)

Immature embryos(0.8 mm-1.2 mm) harvested 14-20 days post

anthesis.

2. Plasmid: pAHC25 (GUS)

3. Medium: Callus induction medium (MS with 2.4-D 2.5 mg/l, sucrose 60 g/l, L-asparagine 0.15 g/l), Regeneration medium and Rooting medium (MS with 2% sucrose)

Results and Discussion

- Using 400 µg of gold particle (0.6 µm) showed GUs spots in all DNA amounts used
- Combination of 1, 4 µg of DNA and 400 or 700 µg of gold particle (0.6 µm) revealed highest transformation efficiency.
- In case of 1.0 µm of gold particle, 1100 psi is better than 900 psi for transformation efficiency.

Table 1. A number of blue spot in several amount of GUS gene and gold particle (µg)

Gold \ DNA	0.5	1	4	7	10
100	0	50	25	5	35
400	70	110	110	90	120
700	35	90	95	80	90
1000	55	50	90	55	80

Table 2. A number of blue spot in several condition (Gold particle size, helium gas psi, distance)

Particle size	Psi	Distance	Gus transient assay number of Blue spots (+: About 25)
0.6 µm	1100	6	++++
		9	+
		12	+
	900	6	+++++
		9	+
		12	+
1.0 µm	1100	6	+++++
		9	+++++
		12	+++++
	900	6	+++++
		9	+++
		12	+

*Corresponding author. Tel 031-299-1668 E-mail chyun5311@rda.go.kr