Original Papers Plant Tissue Culture Letters, **11**(3), 206-210 (1994)

Optimization of Gene Delivery Conditions in Roots of *Arabidopsis thaliana* by Bombardment-mediated Transformation

Motoaki SEKI and Hiromichi MORIKAWA

Department of Gene Science, Faculty of Science, Hiroshima University,
Higashi-Hiroshima 724, Japan

(Received April 22, 1994)
(Accepted July 15, 1994)

Expression of the β-glucuronidase (GUS) gene in roots of *Arabidopsis thaliana* collection number C24 was obtained by particle bombardment. Effects of accelerating pressure of projectiles (115 to 200 kg/cm^2), amount of gold particles (0. 2 to 0. 6 mg per projectile), type of gold particles and number of bombardments (1 to 3 times) on the efficiency of gene expression were studied. Three bombardments of 200 kg/cm^2 with projectiles having 0. 4 mg of gold particles ("Tokuriki-3" particles) gave 4856±204 blue spots per *ca.* 150 mg root sections.

Introduction

Recently, we[1,2,3] and Bruce *et al.*[4] have reported bombardment-mediated transformation of *Arabidopsis thaliana*. This method may broaden the spectrum of experimental possibilities in this important plant model system[5], allowing the performance of large scale transformation towards gene targeting and organellar transformation.

We have previously studied gene delivery conditions in roots of *A. thaliana* bombarded with the β-glucuronidase (GUS) gene using the pneumatic particle gun[1]. We have shown that a preculture period (2 to 3 days) is a vital factor for biolistic transformation of root sections. However, only *ca.* 200 blue spots were observed at the partially optimized bombardment conditions studied. To improve gene expression efficiency, in this study, we optimized some of the bombardment conditions (*i.e.*, accelerating pressure of projectile, amount of gold particles, type of gold particles, number of bombardments) in roots of *A. thaliana*.

Materials and Methods

1. *Plant materials*

A. thaliana collection number C24 seeds were kindly provided by Dr. D. Valvekens (Laboratorium voor Genetica, Rijksuniversiteit Gent, Belgium). Surface-sterilized seeds of *A. thaliana* were sown on Gamborg B5[6] agarose (0. 6%) medium containing 3% sucrose and plants were aseptically grown for 4 to 6 weeks. Roots were harvested from the plants and excised into sections (0. 5-1. 0 cm long), and the sections were placed in a circle (45 mm diameter) on filter paper (ADVANTEC TOYO No. 2, 5. 5 cm diameter) on the 0. 5/0. 05 agar (0. 8%) medium, consisting of B5 inorganic salts, B5 vitamins, 3% sucrose, 0. 5 mg/*l* 2, 4-dichlorophenoxyacetic acid and 0. 05 mg/*l* kinetin as described previously[1].

2. *Plasmid DNA and gold particles*

Chimeric plasmid DNA, pBI221(Clontech, Palo Alto, CA, USA), which has the β-glucuronidase (GUS) gene under the control of the cauliflower mosaic virus (CaMV) 35S promoter and nopaline synthase (NOS) polyadenylation signal, was used. Three types of gold particles (Tokuriki-1; Tokuriki-2; Tokuriki-3) purchased from Tokuriki Honten Co. (Tokyo, Japan) were used. The shape of particles was irregular in the case of "Tokuriki-1" particles and spherical for "Tokuriki-2" and "Tokuriki-3" particles. The average diameter of "Tokuriki-1", "Tokuriki-2", and "Tokuriki-3" particles was 1.2, 1.1, and 0.8 μm, respectively.

3. *Gene delivery to the root sections*

The particle acceleration device and the methods for gene delivery to the cells using this device were essentially as reported previously[1,7]. Standard bombardment conditions were as follows: accelerating pressure of the projectile, 150 kg/cm^2; the amount of plasmid DNA, 4 μg/mg of gold particles; the amount of DNA-coated gold particles, 0.2 mg per projectile; the number of bombardments, 1. After being cultured for 3 days[1] on the 0.5/0.05 agar medium, the root sections were subjected to the particle bombardment.

After being bombarded, the filter papers with *A. thaliana* roots were kept on the agar medium and cultured for 24 h in 16 h light/8 h dark cycle at 26°C, after which they were assayed for transient GUS expression.

4. *Assay for GUS expression*

The GUS activity assay was carried out essentially as reported previously[1]; the filter papers with *A. thalina* root sections were transferred to glass dishes (6 cm internal diameter) and *ca.* 800 μl of filter-sterilized GUS substrate mixture was added onto the root sections. The tissues were incubated for 24 h at 37°C, and then 3 ml of 70% (v/v) ethanol was added to the cell-GUS substrate mixture in order to stop the reaction and to keep aseptic conditions. GUS-expressing cells were detected as blue-colored spots. Each spot, regardless of its size, was considered as one GUS-expression unit, and the number of spots was counted under a binocular microscope ($\times$40, Nikon, SMZ-10).

Results and Discussion

Fig. 1 shows typical results of the GUS enzyme assay of the *A. thaliana* collection number C24 roots that were bombarded with the plasmid pBI221-coated gold particles. Many blue spots or clumps of GUS-expressing cells were observed. No blue spots were detected after bombardment with noncoated gold particles (data not shown). These results show that successful delivery of the GUS gene and expression of the gene in *A. thaliana* collection number C24 roots were obtained by our device.

Table 1 shows the effects of accelerating pressure of projectiles and the amount of gold particles on the number of blue spots in *A. thaliana* collection number C24 roots. The root sections were bombarded once with "Tokuriki-2" particles. The accelerating pressure was varied between 115 and 200 kg/cm^2, and the amount of gold particles was changed from 0.2 to 0.6 mg per projectile. The optimum accelerating pressure was 150 or 200 kg/cm^2 regardless of the amount of gold particles (**Table 1**). This is consistent with our previous results that the optimal accelerating pressure was 150 or 200 kg/cm^2 with *A. thaliana* race "Landsberg *erecta*" roots[1].

As reported elsewhere, optimal accelerating pressure varies depending on the type of plant cells or tissues, being 150 kg/cm^2 for cultured tobacco cells[7], 200 kg/cm^2 for shoot primordia of *Haplopappus gracilis*[8], and 115 to 150 kg/cm^2 for leaves and 150 to 200 kg/cm^2 for roots of *A. thaliana*[1]. These results clearly indicate the importance of optimizing the acceleration pressure in bombard-

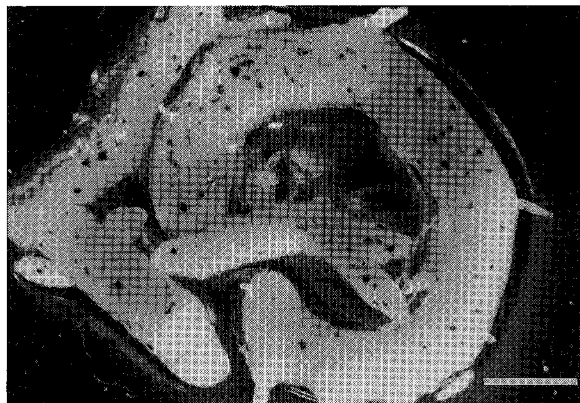

Fig. 1 Microphotograph of *Arabidopsis thaliana* collection number C24 roots that were bombarded with DNA-coated gold particles and assayed for the GUS expression. Bombardment conditions were as follows: accelerating pressure of the projectile, 150 kg/cm^2; the amount of plasmid DNA, 4 μg/mg of gold particles; the amount of DNA-coated gold particles, 0. 2 mg per projectile; the number of bombardments, 2. The bombarded roots showed many blue spots indicative of GUS expression. Scale bar=1 mm.

Table 1. Effects of accelerating pressure and amount of gold particles on the number of blue spots in *Arabidopsis thaliana* collection number C24 roots*1.

Amount of gold particles (mg/projectile)	Accelerating pressure (kg/cm^2)		
	115	150	200
0. 2	270±158*2	695± 47	800±188
0. 4	1004± 81	1556±272	1477±193
0. 6	1070±122	1345±157	1463± 77

*1 Roots were precultured for 3 days.
*2 Average of two experiments±deviation.

ment-mediated transformation.

Also, the optimal amount of gold particles per projectile was 0. 4 or 0. 6 mg (**Table 1**). We have previously reported that 0. 2 mg gold particles per projectile gave the best results for *Haplopappus gracilis* shoot primordia[8]. These results show that adjustment of the amount of gold particles depending on the type of the tissues is a vital factor for biolistic transformation.

Furthermore, effects of the type of gold particles and number of bombardments were studied. The root sections were bombarded one to three times with three types of gold particles (Tokuriki-1; Tokuriki-2; Tokuriki-3) at the accelerating pressure of 200 kg/cm^2, for 0. 4 mg gold particles per projectile. In order to prevent more aggregates of gold particles in the center of target tissues, with double and triple bombardments, the region that was *ca.* 1 cm away from the center of the target tissues was placed right above the barrel, bombarded one time with DNA-coated gold particles. With double bombardment, the bombarded roots were then rotated in a 180-degree arc and bombarded. With triple bombardment, the bombarded roots were rotated in a 120-degree arc, bombarded, and repeated again. "Tokuriki-2" and "Tokuriki-3" particles were more efficient for number of blue spots (GUS-expression efficiency) than "Tokuriki-1" particles regardless of the number of bombardments (**Table 2**). Spherical gold particles ("Tokuriki-2" and "Tokuriki-3" particles) used here seemed to have advantages in GUS-expression efficiency compared with irregu-

Table 2. Effects of multiple shots and type of gold particles on the number of blue spots in *Arabidopsis thaliana* collection number C24 roots*[1].

Type of gold particles	1 shot	2 shots	3 shots
Tokuriki-1	176±112*[2]	435± 18	621± 67
Tokuriki-2	1019±145	2378±132	3674± 60
Tokuriki-3	1397±272	2907±250	4856±204

*[1] Roots were precultured for 3 days.
*[2] Average of two experiments±deviation.

lar shaped gold particles ("Tokuriki-1" particles).

The number of blue spots increased with the number of bombardments up to three times in *A. thaliana* roots (**Table 2**). Wang *et al.*[9] reported that the number of blue spots increased with the number of bombardments up to three times for suspension-cultured cells of rice and wheat. Jin *et al.*[8] reported that double bombardments gave the best results and that triple bombardments produced fewer blue spots. These results show that adjustment of the number of bombardments depending on the type of the tissues is also a vital factor for biolistic transformation.

The highest number of blue spots (4856±204 per *ca.* 150 mg root sections) was observed at an accelerating pressure of 200 kg/cm^2, an amount of gold particles of 0. 4 mg/projectile, type of gold particles of Tokuriki-3, and 3 bombardments (**Table 2**). We are now studying production of transgenic *A. thaliana* plants by bombarding with neomycin phosphotransferase II (*nptII*) gene, hygromycin phosphotransferase (*hpt*) gene, or *bar* gene encoding phosphinothricin acetyltransferase (PAT) under these optimized gene delivery conditions. Results will be published elsewhere.

Acknowledgement

This research was supported by Grants-in Aid from the Ministry of Education, Science and Culture of Japan.

References

1) Seki, M., Y. Komeda, A. Iida, Y. Yamada, H. Morikawa, 1991. Plant Mol. Biol., **17**: 259-263.
2) Seki, M., N. Shigemoto, Y. Komeda, J. Imamura, Y. Yamada, H. Morikawa, 1991. Appl. Microbiol. Biotechnol., **36**: 228-230.
3) Sawasaki, T., M. Seki, H. Anzai, K. Irifune, H. Morikawa, Transgenic Research, (in press).
4) Bruce, W. B., A. H. Christensen, T. Klein, M. Fromm, P. H. Quail, 1989. Proc. Natl. Acad. Sci. USA, **86**: 9692-9696.
5) Meyerowitz, E. M., 1989. Cell, **58**: 263-269.
6) Gamborg, O. L., R. A. Miller, K. Ojima, 1968. Exp. Cell Res., **50**: 151-158.
7) Iida, A., M. Seki, M. Kamada, Y. Yamada, H. Morikawa, 1990. Theor. Appl. Genet., **80**: 813-816.
8) Jin, Y., T. Tanaka, M. Seki, K. Kondo, R. Tanaka, H. Morikawa, 1993. Plant Tissue Culture Letters, **10**: 271-274.
9) Wang, Y. C., T. M. Klein, M. Fromm, J. Cao, J. C. Sanford, R. Wu, 1988. Plant Mol. Biol., **11**: 433-439.

《和文要約》

パーティクルガン法によるシロイヌナズナ根組織への至適な遺伝子導入条件の検討

関　原明・森川弘道

広島大学理学部遺伝子科学

パーティクルガン法によりシロイヌナズナ C24 株の根組織へ GUS 遺伝子を導入し，一過的発現に成功した．加速圧力 200 kg/cm², 0.4 mg 金粒子(Tokuriki-3 粒子)/プロジェクタイルの撃ち込み条件で 3 回撃ち込むと，1 シャーレあたり最大 4856±204(2 回の実験の平均値±偏差) 個の青色スポットが得られた．