Note

Plant Biotechnology, 15(2), 109-111(1998)

Expression of the β-Glucuronidase Gene Introduced into Intact Leaves Attached to *Arabidopsis thaliana* Plants by Particle Gun

Gen-ichiro ARIMURA*, Hiromi BANJO*, Kohei IRIFUNE*, Keng-Hock PWEE**, Misa TAKAHASHI* and Hiromichi MORIKAWA*

Received 10 November 1997; accepted 27 February 1998

Particle bombardment has proved to be useful for the transformation of plants. We have previously reported successful transient expression of *uidA* under cauliflower mosaic virus 35S (CaMV 35S) promoter in cultured plant cells [1,2] and the stable transformation of various plants [3-6] through the use of a pneumatic particle gun.

We here report an *in planta* transient expression assay method using a particle gun device, in which transgenes are introduced into the cells of leaves that are attached to intact plants. This method is simple and the leaves into which genes have been introduced are not cut out from the plants. Therefore, the *in planta* transient expression assay is advantageous over the assay using detached leaves, in that subsequent biochemical analyses can immediately assess the whole plant in regard to such things as the response to gaseous environments or signal transport between organs. This assay will be useful as a simple method for researching gene function prior to producing the transformant.

Young seedlings of *Arabidopsis thaliana* ecotype C24 were used as the plant material. Plasmids pBI221 and pPC8 were used, in which the *uidA* gene, encoding β-glucuronidase (GUS), is driven by the cauliflower mosaic virus 35S (CaMV 35S) promoter and pea plastocyanin promoter (*petE* - 784), respectively. Plasmid pDO432 [8], in which the *luc* gene, encoding the firefly luciferase (LUC) is driven by the CaMV 35S promoter, was also used.

Arabidopsis thaliana ecotype C24 plants were planted in vermiculite: perlite (1: 1, v/v) in plastic pots placed in a growth chamber (Model ER-20A, Nippon Medical & Chemical Instruments Co., Osaka, Japan). The plants were irrigated with a half strength of inorganic salts of Murashige and Skoog medium [9] every 4 days, and grown under continuous light (70 $\mu Em^{-2}s^{-1}$) at 22±1°C for 5 to 7 weeks. Prior to bombardment, the leaves of the *Arabidopsis thaliana* seedlings in the pots were held onto the surface of an agarose gel plate. The 0.6% agarose gel, which was in an aluminum foil tray in a petri dish (*ca.* 5 mm in thickness, 60 mm diameter), was taken from the petri dish, cut through the center, and placed on the surface of the soil in the pot growing a plant as shown in **Fig. 1**. The seedling in the pot was then placed into the bombardment box of the particle gun device (Rehbock model 260, Rehbock Co., Japan) [10] and bombarded with gold particles coated with plasmids, as reported previously [11, 12] with slight modifications. Four micrograms of pBI221 alone or an equimolar mixture of pBI221 and pDO432, and that of pPC8 and pDO432 were precipitated separately with 1 mg of gold particles (1.1 μm, diameter, Tokuriki Honten Co., Tokyo, Japan). Then, 0.1 or 0.2 mg of DNA-coated gold particles were placed on a projectile. The sample-to-stopper distance was kept at 10 cm, and the initial velocity of the projectile was 375 m s^{-1}. Two to six shots were given to each target plant.

After bombardment, the plants in the pots were removed from the agarose gel plate and then placed again in the growth chamber and incubated for 24 h at

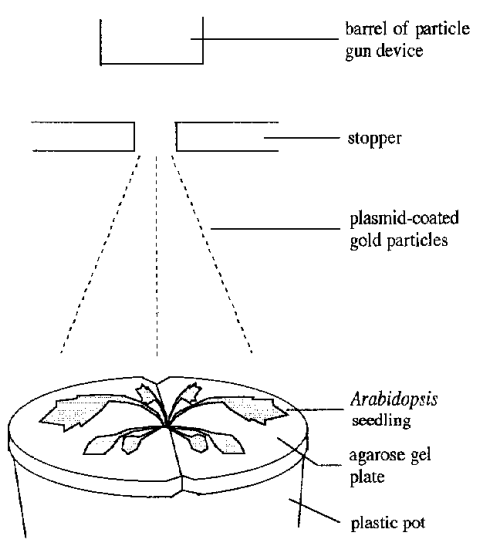

Fig. 1 Schematic diagram for microprojectile bombardment to the leaves of a seedling of *Arabidopsis thaliana*. The agarose gel plate (0.6%, *ca.* 5 mm in thickness, 60 mm in diameter) in a aluminum foil tray is put on the surface of the soil in a pot during the bombardment manipulation.

* *Graduate Department of Gene Science, Faculty of Science, Hiroshima University, Higashi-Hiroshima, 739-8526 Japan*
** *Dept. Bot., Natl. Univ. of Singapore, Lower Kent Ridge Rd. Singapore, S-0511 Singapore*

Table 1. Transient expression of the GUS gene in leaves attached to *Arabidopsis thaliana* plants.

Experiment No.	Holding of leaves*1	mg Au/projectile	No. of shots	No. of GUS-expression units/plant*2
1	−	0.1	2	4
2	−	0.1	4	2
3	−	0.1	4	2
4	−	0.1	4	6
5	−	0.2	2	3
6	−	0.2	2	4
7	−	0.2	2	5
8	−	0.2	2	9
9	+	0.1	4	63
10	+	0.1	4	148
11	+	0.1	4	222
12	+	0.1	4	292
13	+	0.1	6	225
14	+	0.2	2	125
15	+	0.2	2	502
16	+	0.2	2	515
17	+	0.2	3	108
18	+	0.2	3	157
19	+	0.2	3	380

*1 The leaves, which were attached to the seedlings, were held (+) or not held (−) on a disk of agarose gel, and bombarded with pBI221-coated gold particles of 0.1 or 0.2 mg.

*2 The number of GUS-expression units (blue spots) per plant.

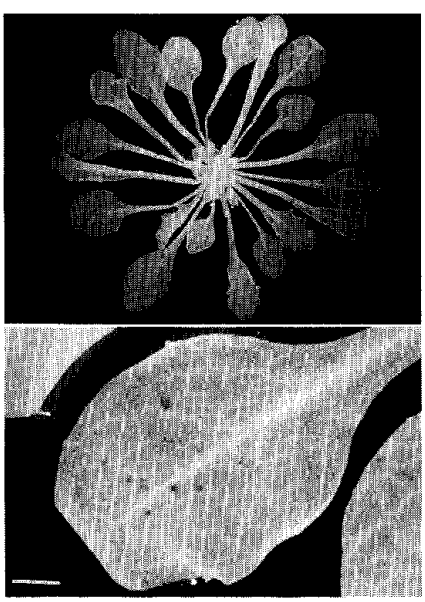

Fig. 2 Typical photomicrograph of *Arabidopsis thaliana* leaves that show blue spots of GUS-expressing cells. Leaves of 42-day-old seedlings of *A. thaliana* were bombarded with pBI221-coated gold particles, as shown in **Fig. 1**, and assayed for GUS expression. Bar=2 mm.

22±1°C under continuous light. The plants were then subjected to fluorometric [13] or histochemical [14] GUS assay and LUC assay [15]. The protein content of the leaf extracts was determined according to the method of Bradford [16], with BSA as a standard.

The effect of holding the leaves attached to intact seedlings onto the agarose gel plate as shown in **Fig. 1** was studied. The leaves that were being held or not held onto the gel plate were bombarded with pBI221 and then subjected to histochemical assay for GUS expression. The results are summarized in **Table 1**. Clearly, in the bombarded leaves holding onto the agarose gel plate, there were more blue spots of GUS-expressing cells than in the leaves not held to the plate. At most, a total of 515 blue spots per plant were observed for two shots of 0.2 mg gold particles per projectile each (see **Table 1**). More than 50 of the blue spots on GUS expression cells per leaf were detected (**Fig. 2**). In the following experiments, bombardments were made to the attached leaves that were being held to the agarose plate.

Table 2 shows the GUS activity detected in leaves from the *Arabidopsis thaliana* plant that were bombarded with an equimolar mixture of *uidA*-harboring pBI221 and *luc*-harboring pDO432, with that of *uidA*-harboring pPC8 and pDO432. The GUS activity was normalized against the LUC activity so that possible differences in the bombardment efficiency (or gene delivery efficiency) could be cancelled out. We found that the relative GUS activity (GUS activity/LUC activity) was *ca.* 1.7 times higher with pPC8 than with pBI221. This indicates that the activity of the

Table 2. Activity of GUS and LUC enzymes detected in bombarded *Arabidopsis thaliana* leaves.

Enzyme activity*2	Leaves*1 bombarded with		
	Noncoated	pBI221 and pDO432	pPC8 and pDO432
GUS activity*3	21±2	407± 114	893± 215
LUC activity*4	51±4	1385± 348	1167± 310
GUS activity/LUC activity		0.47±0.08	0.80±0.21

*1 Leaves were bombarded three times with 0.2 mg of gold particles each. Gold particles were not coated or coated with an equimolar mixture (4 μg DNA per projectile) of pBI221 and pDO432, or that of pPC8 and pDO432.

*2 Values are the average of three to five experiments±SD.

*3 pmol 4-methyl umbelliferone (MU)/h/mg protein.

*4 Light unit/mg protein.

petE-784 promoter is somewhat higher than that of the CaMV 35S promoter in *Arabidopsis thaliana* leaves. Pwee and Gray (1993) have shown that the *petE*-784 promoter, which contains light-responsive elements, positive regulatory elements include putative binding sites of AT-1 and the G-box, and the previously defined *cis*-elements such as the octopine synthase enhancer consensus and a putative hexameric sequence, is the strongest promoter in its deletions in transgenic tobacco plants. The *petE*-784 promoter-GUS constructs directed expression in transgenic tobacco, and GUS activity was *ca.* 11 times higher than that of the 35S promoter-GUS constructs in the leaves of transgenic tobacco in greenhouse-grown plants [7].

The present results demonstrate that the rapid analysis of gene expression in plants is possible after *in planta* transient expression assay. The introduction of transgenes into intact tissues attached to intact plants by a particle gun (*in planta* transient expression assay) is made possible by holding the leaves onto an agarose gel plate. This assay paves the way to a rapid and useful method for the study of gene expression in plant tissues.

Acknowledgments

This work was supported by the program of Research for the Future from the Japanese Society for the Promotion of Science (JSPS-RFTF96L00604).

References

[1] Seki, M., Komeda, Y., Iida, A., Yamada,Y., Morikawa, H., 1991. Plant Mol. Biol., **17**: 259-263.

[2] Jin, Y., Tanaka, T., Seki, M., Kondo, K., Tanaka, R., Morikawa, H., 1993. Plant Tissue Culture Letters, **10**: 271-274.

[3] Morikawa, H., Nishihara, M., Seki, M., Irifune, K., 1994. J. Plant Res., **107**: 117-123.

[4] Nishihara, M., Seki, M., Kyo, M., Irifune, K., Morikawa, H., 1995. Transgenic Research, **4**: 341-348.

[5] Sawasaki, T., Seki, M. , Anzai, H., Irifune, K., Morikawa, H., 1994. Transgenic Research, **3**: 279-286.

[6] Seki, M., Shigemoto, N., Komeda, Y., Imamura, J., Yamada, Y., Morikawa, H., 1991. Appl. Microbiol. Biotechnol., **36**: 228-230.

[7] Pwee, K. -H., Gray, J. C., 1993. Plant J., **3**: 437-449.

[8] Ow, D. W., Wood, K. V., DeLuca, M., de Wet, J. R., Helinski, D. R., Howell, S. H., 1986. Science, **234**: 856-869.

[9] Murashige, T., Skoog, F., 1962. Physiol. Plant., **15**: 473-497.

[10] Morikawa, H., Chiba, K., Irifune, K., Iida, A., Seki, M., Nishihara, M., Tanaka, T., Yamashita, T., Asakura, N., 1994. In "Particle Bombardment Technology for Gene Transfer" (eds. by Yang, N. S., Christou, P.), p. 52-59, Oxford University Press, New York.

[11] Morikawa, H., Iida, A., Yamada, Y., 1989. Appl. Microbiol. Biotechnol., **31**: 320-322.

[12] Iida, A., Morikawa, H., Yamada, Y., 1990. Appl. Microbiol. Biotechnol., **33**: 560-563.

[13] Jefferson, R. A., Kavanagh, T. A., Bevan, M. W., 1987. EMBO J., **6**: 3901-3907.

[14] Klein, T. M., Gradziel, T., Fromm, M. E., Sanford, J. C., 1988. Biotechnol., **6**: 559-563.

[15] Yamamoto, S., Nishihara, M., Morikawa, H., Yamauchi, D., Minamikawa, T., 1995. Plant Molecular Biology, **27**: 729-741.

[16] Bradford, H. M., 1976. Anal. Biochem., **72**: 248-254.