Plant Biotechnol Rep (2008) 2:267–270
DOI 10.1007/s11816-008-0067-2

SHORT COMMUNICATION

A low-pressure gene gun for genetic transformation of maize (*Zea mays* L.)

Chien-Yuan Kao · Shin-Hui Huang · Chiu-Mei Lin

Received: 24 June 2008 / Accepted: 1 August 2008 / Published online: 5 September 2008

Abstract We have successfully used the low-pressure BioWare gene gun, developed for gene transfer in animal cells, for plant tissues. The BioWare device is easy to manipulate. Just 50 psi helium pressure was sufficient to transfer foreign genes into the aleurone layer and embryo of maize without causing tissue damage in the impact area. As shown by expression signals from invasive histochemical β-glucuronidase (GUS) activity, the foreign reporter gene expressed well in bombarded tissues. This successful GUS-transient expression extends the application of this low-pressure gene gun from animal cells to plant tissues.

Keywords Maize · Gene gun · GUS · Transient expression · BioWare

Introduction

The stable integration of foreign genes into plant genome represents one of the most significant developments in plant biology and crop improvement. The first transgenic tobacco plant was produced by using *Agrobacterium tumefaciens* (Horsch et al. 1985). Several years later, the high-velocity microprojectile, or gene-gun technology, was invented by John Sanford and his colleagues (Klein et al. 1987; Sanford 2000). Both methods have been used extensively for genetic transformation of plants, leading to the commercialization of biotech crops. The biolistics system can be used with intact tissues and is not restricted to any particular plant group. It has revolutionized plant genetics by producing most of the world's biotech crops and has also been used extensively in transient expression assays for studying gene regulation and function (Christou 1992; Kao et al. 1996; Sambrook and Russel 2001; Vasil 2003). A number of modifications of the original biolisitcs device have been developed to improve its performance (Gray and Finer 1993; Kikkert 1993; McCabe and Christou 1993; Oard 1993; Sautter 1993; Vain et al. 1993). One of the major problems with all biolistic devices developed so far is the use of high pressure, which causes severe damage to target cells. They also suffer from several other disadvantages: (1) low transformation efficiency, (2) need to use large amounts of deoxyribonucleic acid (DNA), (3) laborious to use, (4) high noise level, (5) large size and design makes it less portable, (6) and the high cost of the basic instrument and disposable supplies needed for operation.

The low-pressure BioWare gene gun has been used for genetic transformation of animal cells and human tumor cell lines only (Chang et al. 2008; Cheng et al. 2005a, b; Tu et al. 2007; Lin et al. 2008). By gene delivery and expression in the aleurone layer and embryos of maize (*Zea mays* L.) we show for the first time that it can be equally effective in plant transformation. We found the BioWare device to be safe and flexible to use, as well as light and handy. We used it obtain 18 stable transgenic plants of the orchids *Mormodes lawrence* and *Zygopetalum mackayi,* transformed with three different plasmid constructs (manuscript in preparation).

C.-Y. Kao (✉) · S.-H. Huang
Department of Horticulture, National I-Lan University, Yilan 26047, Taiwan
e-mail: cykao@niu.edu.tw

C.-M. Lin
School of Medicine, College of Medicine, Taipei Medical University, Taipei 110, Taiwan

Materials and methods

Tissue preparation

Sweet corn (*Zea mays* L.) ears of 25 days after pollination were surface-sterilized by immersion for 1 min in 70% ethanol and 5 min in 5% commercial Clorox, followed by two rinses with sterile water. The kernels were cut horizontally, and the pericarp was peeled off to expose the aleurone layer. The developing embryos were then removed from the kernel. The kernels with exposed aleurones and isolated embryos were placed on a gelrite solidified Murashige Skoog (MS) medium (Sigma, M5519) containing 20 g/l sucrose prior to bombardment.

Particle bombardment

The preparation of the pCambia 1301 plasmid DNA/gold mixture was modified from the methods described previously (Taylor and Vasil 1991). Briefly, 37 µl of a 40 mg/ml gold stock solution (1.6 µm diameter, Biorad Inc.) was mixed in order with 25 µl water and 5 µg plasmid DNA in a 250-µl Eppendorf tube. The tubes were vortexed briefly before and after each addition. Twenty microliters of 100 mM free-base spermidine and 50 µl of 2.5 M calcium chloride ($CaCl_2$) were placed in separate drops on the side of the tube to avoid premature mixing of either solution with the DNA/gold solution. The tubes were then immediately mixed by vortexing for 10 s. At that time, the plasmid DNA was precipitated and attached to the gold particle. The tubes were centrifuged for 5 s and the supernatant fluid removed. Two hundred microliters of 100% ethanol were added and sonicated briefly. After brief centrifugation to pellet the gold/DNA, the supernatant fluid was removed. One hundred microliters of 100% ethanol were added and the tubes placed on ice. A 10-µl volume of sonicated gold/DNA mixture was used for each bombardment. The gun was pointed longitudinally to the sample under a funnel-shaped steel net (3 cm high) with a helium pressure of 50 psi. The BioWare low-pressure gene gun with its accessory parts (Fig. 1a; BioWare Technologies Co. Ltd., Taipei, Taiwan) was used for these experiments.

Histochemical localization of GUS activity

Bombarded aleurone kernels and embryos were incubated overnight at 37°C in the presence of 1 mM 5-bromo-4-chloro-3-indolyl-b-D-glucuronide (X-Gluc) and 0.5 mM potassium ferricyanide. Photographs were taken under low magnification with a light microscope (Jefferson et al. 1987).

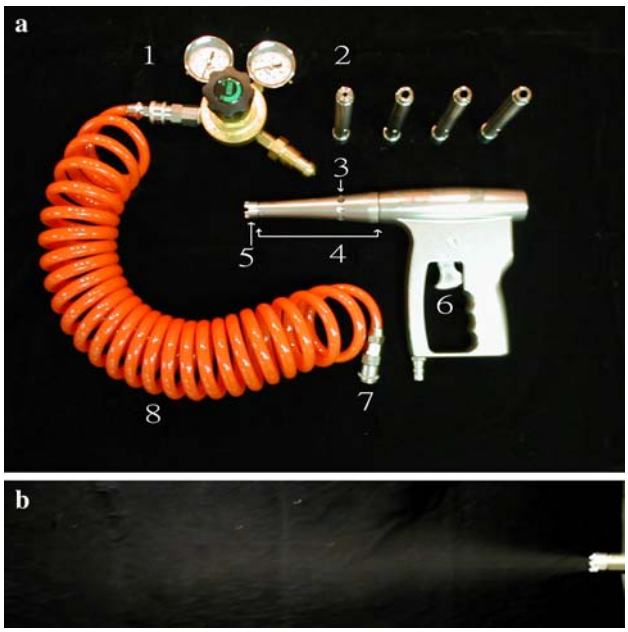

Fig. 1 BioWare low-pressure gene gun and image of sample explosion from the gun. **a** Low-pressure gene gun and its accessory parts, *1* regulator; *2* barrels with different inside diameter; *3* loading aperture; *4* sleeve; *5* spacer; *6* trigger; *7* quick coupling; *8* PU coil tube. **b** Pattern of bursting mist from the gun at 50 psi helium pressure in 20 µl distilled water simulation

Results and discussion

The direction and extent of exploding mist at the time of the explosion is shown in Fig. 1b. One of the major concerns for the application of the BioWare device for plant tissues has been the low pressure (maximum 75 psi) that is used to propel microprojectiles coated with DNA, and whether it would generate enough force to penetrate the rigid wall that surrounds plant cells. Two features incorporated in the design of the instrument overcame this limitation: (1) helium gas directly propels the DNA-coated particles, and (2) the distance between the gun hole and target tissues can be adjusted to nearly zero (Fig. 1). Without previous experience in plant tissues with this gun, we observed that edible brown pigment as loading buffer gave the best result with the filter set at 50 psi pressure and 3 cm from target tissue (Fig. 2). We then used this condition for aleurone and embryos tissue transformation.

Maize aleurone and embryo have been extensively used as ideal plant transient expression systems (Kao et al. 1996; Hattori et al. 1992; Hoecher et al. 1995; McCarty et al. 1991). In our study, 30 exposed aleurone kernels used in ten separate bombardments and 18 embryos in six separate bombardments with the pCambia 1301 construct showed strong positive β-glucuronidase (GUS) blue stain (Fig. 3). Indeed, the staining was intense. Therefore, we reduced the length of time used for incubation and GUS

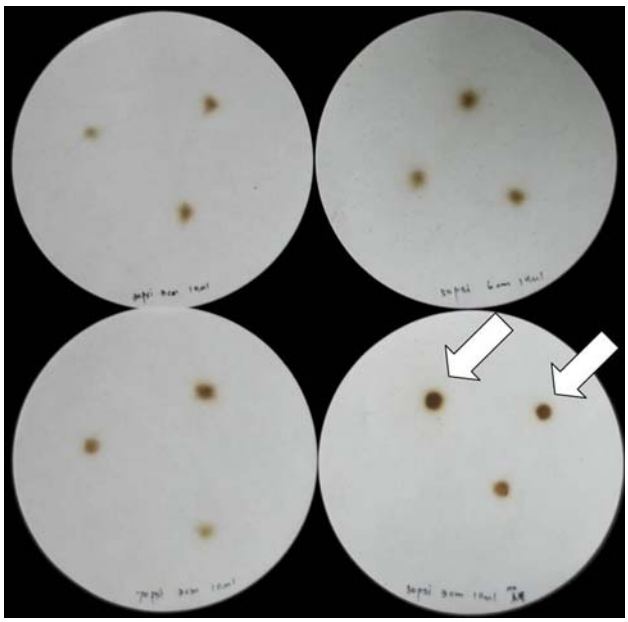

Fig. 2 Ten microliters of brown pigment as loading buffer generates the best impact area with 50 psi, with the target at 3 cm (*bottom right*; *arrow*). Other conditions tested were 30 psi, 3 cm (*upper left*); 70 psi, 3 cm (*bottom left*), and 50 psi, 6 cm (*upper right*)

Fig. 3 Strong β-glucuronidase (GUS) expression on the surface of maize aleurone layer and embryos after low-pressure gene-gun bombardment. Appearance of GUS stain on the **a** aleurone layer and **b** embryos bombarded with pCambia 1301 in contrast to no blue stain in DNA-free control on the *left*

staining to create well-defined blue spots. From the longitudinal free-hand sections of the embryo and kernels, the GUS blue stain could be seen beyond the outer surface layers of the embryo in contrast to aleurone (Fig. 4). This indicates that even at the low pressure of 50 psi, DNA-coated gold particles can penetrate several cells layers and be expressed in the inner layers of the embryo. Although the possible diffusion of β-glucuronidase and/or its blue cleavage products cannot be ruled out. It should be pointed

Fig. 4 β-glucuronidase (GUS) staining in longitudinal free-hand sections of maize embryo and kernel with exposed aleurone layer. *Deep blue* GUS stain can be seen beyond the outer surface of embryo (*bottom left*) in contrast to *deep blue* in the outer layer of endosperm (*bottom right*) and their no-DNA control, respectively (*upper left* and *right*)

out that we obtained similar results with a privately owned green fluorescent protein (GFP) plasmid under fluorescent microscope. Positive GUS histochemical staining in the aleurone layer and embryos (Figs. 3, 4) indicated the successful delivery and expression of foreign DNA by the low-pressure BioWare gene gun. We show that helium gas pressure as low as 50 psi is sufficient to drive DNA/gold particles through several layers of cells in plant tissues and successfully express the introduced gene. Compared with the 1,300 psi used for transient assays in the same material (Kao et al. 1996), and 1,100–1,350 psi used in orchid transformation (Yu et al. 1999; Davina et al. 2007) with the Biolistics device, 50 psi helium pressure is exceptionally low. Bombardment of noncarrier naked DNA (without gold particles) at 50 or 70 psi, and shorter distance to the embryo and aleurone samples, gave only negative results. However, noncarrier naked DNA bombardment at low pressure has been successfully used for transformation in animal systems (Lin et al. 2008). This suggests that the rigid plant cell wall is indeed a penetration barrier and the gold particles play an indispensable role in plant tissue transformation.

High-velocity bombardment of DNA-coated microprojectiles allows direct delivery of genes of interest into regenerable plant tissues, bypassing the complexities and limitations of *Agrobacterium* transformation. It appears to be the best method for plant transformation (Christou 1992). The biolistics device has been used for the production of most of the world's major transgenic crops and also in extensive transient expression investigations. The low-pressure gene gun used by us was invented to circumvent the many problems associated with the use of existing devices in transforming live mice. We have shown that it is also efficient in delivering DNA constructs into plant cells and has several advantages over the traditional biolistics devices used previously.

Acknowledgments The authors thank Dr. Indra K. Vasil (Associate Director, Genetics Institute, University of Florida) for his critical reading of and helpful suggestions for the manuscript.

References

Chang ML, Chen JC, Yeh CT, Chang MY, Liang CK, Chiu CT, Lin DY, Liaw YF (2008) Gene gun bombardment with DNA-coated gold particles is a potential alternative to hydrodynamics-based transfection for delivering genes into superficial hepatocytes. Hum Gene Ther 19:391–395

Cheng WF, Hung CF, Chen CA, Lee CN, Su YN, Chai CY, Boyd DA, Hsieh CY, Wu TC (2005a) Characterization of DNA vaccines encoding the domains of calreticulin for their ability to elicit tumor-specific immunity and antiangiogenesis. Vaccine 23(29):3864–3874

Cheng WF, Lee CN, Chang MC, Su YN, Chen CA, Hsieh CY (2005b) Antigen-specific CD8+T lymphocytes generated from a DNA vaccine control tumors through the Fas-FasL pathway. Mol Ther 12:960–968

Christou P (1992) Genetic transformation of crop plants using microprojectile bombardment. Plant J 2:275–281

Davina C, Lee SM, Ng JH, Yu H (2007) L-methionine sulfoximine as a novel selection agent for genetic transformation of orchids. J Biotechnol 131:466–472

Gray DJ, Finer JJ (1993) Development and operation of five particle guns for introduction of DNA into plant cells. Plant Cell Tissue Organ Cult 33:219

Hattori T, Vasil V, Rosenkrans L, Hannah LC, McCarty DR (1992) The *viviparous-1* gene and abscisic acid activate the *C1* regulatory gene for anthocyanin biosynthesis during seed maturation in maize. Genes Dev 6:609–618

Hoecker U, Vasil IK, McCarty DR (1995) Integration control of seed maturation and germination programs by activator and repressor functions of *viviparous-1* of maize. Genes Dev 9:2459–2469

Jefferson RA (1987) Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol Biol Rep 5:387–405

Horsch RB, Fry JE, Hoffmann N, Wallroth M, Eichholtz D, Rogers SG, Fraley RT (1985) Transferring genes into plants. Science 227:1229–1331

Kao CY, Cocciolone SM, Vasil IK, McCarty DR (1996) Localization and interaction of the *cis*-acting elements for abscisic acid, VIVIPAROUS1, and light activation of the *C1* gene of maize. Plant Cell 8:1171–1179

Kikkert JR (1993) The biolistic PDS-1000/He device. Plant Cell Tissue Organ Cult 33:221–226

Klein TM, Wolf ED, Wu R, Sanford JC (1987) High-velocity microprojectiles for delivering nucleic acids into living cells. Nature 327:70–73

Lin CC, Yen MC, Lin CM, Huang SS, Yang HJ, Chow NH, Lai MD (2008) Delivery of noncarrier naked DNA vaccine into the skin by supersonic flow induces a polarized T helper type 1 immune response to cancer. J Gene Med 10:679–689

McCabe D, Christou P (1993) Direct DNA transfer using electric discharge particle acceleration (ACCELL^{TM} technology). Plant Cell Tissue Organ Cult 33:227–236

McCarty DR, Hattori T, Carson CB, Vasil V, Lazar M, Vasil IK (1991) The *viviparous-1* developmental gene of maize encodes a novel transcriptional activator. Cell 66:895–905

Oard J (1993) Development of an airgun device for particle bombardment. Plant Cell Tissue Organ Cult 33:247–250

Sambrook J, Russel DW (2001) DNA transfection by biolistics. In: Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor, New York

Sanford JC (2000) The development of the biolistic process. In Vitro Cell Dev Biol Plant 36:303–308

Sautter C (1993) Development of a microtargeting device for particle bombardment of plant meristems. Plant Cell Tissue Organ Cult 33:251–257

Taylor LP, Vasil IK (1991) Histology of and physical factors affecting transient GUS expression in plant millet (*Pennisetum glaucum* (Li) R. Br.) embryos following microprojectile bombardment. Plant Cell Rep 10:120–125

Tu CF, Lin CC, Chen MC, Ko TM, Lin CM, Wang YC, Lai MD (2007) Autologous neu DNA vaccine can be as effective as xenogenic neu DNA vaccine by altering administration route. Vaccine 25(4):719–728

Vain P, Keen N, Murillo J, Rathus C, Nemes C, Finer JJ (1993) Development of the particle inflow gun. Plant Cell Tissue Organ Cult 33:237–246

Vasil IK (2003) The science and politics of plant biotechnology—a personal perspective. Nat Biotechnol 21:849–851

Yu H, Chen M, Nie L, Lu X, Ming X, Zheng H, Qu LJ, Chen Z (1999) Recovery of transgenic orchid plants with hygromycin selection by particle bombardment to protocorms. Plant Cell Tissue Organ Cult 58:87–92