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Simplified construction and performance of a device for particle bombardment

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Abstract

A modified particle inflow gun (PIG), that utilized a plastic vacuum chamber, was compared with a conventional PIG by bombarding cantaloupe (*Cucumis melo* L.) cotyledon basal quarters with plasmid pBI221 (Clontech Inc.) containing a β-glucuronidase (GUS) gene adsorbed onto tungsten particles. Both guns produced an equivalent number of transient GUS foci when tested at 410 kPa (60 psi), 620 kPa (90 psi) or 830 kPa (120 psi) helium and at a 10, 15 or 20 cm gap between the specimen and DNA/particle holder screen. For both guns, treatments utilizing the lower pressure and/or the greater distance generally produced significantly fewer GUS-positive foci. The plastic PIG was convenient to operate and could be built with simple hand tools in less than 40 minutes, using readily available parts.

Introduction

DNA transfer via particle bombardment (or ballistics), wherein microscopic metal particles coated with genetically engineered DNA are explosively accelerated into plant cells, has become the second most widely-used vehicle for plant genetic transformation, after *Agrobacterium*-mediated transformation (Gray & Finer 1993). Several distinct 'particle guns' have been described (McCabe & Christou 1993; Oard 1993; Sautter 1993; Vain et al. 1993a), including the Biolistic PDS 1000/He (Kikkert 1993), which is the only commercially available device. The most attractive of the non-commercial devices is the particle inflow gun (PIG), which was based on a flowing helium device described by Takeuchi et al. (1992), since it can be fabricated with readily available parts and offers performance on par with the Biolistic PDS 1000/He (Brown et al. 1994).

However, fabrication of the conventional PIG still requires specialized welding and engineering skills that are beyond reach of many scientists interested in exploiting particle-mediated transformation technology. This prompted us to develop another version of the PIG that is composed of parts readily available from technical equipment supply companies and hardware stores. The new 'plastic PIG' can be assembled in less that 40 minutes using only simple hand tools. The purpose of this article is to detail the construction and performance of the plastic PIG.

Materials and methods

Construction of the plastic pig

For convenience, specific brands and suppliers for the following required components are specified (Table 1); however, this does not constitute an endorsement of brands or suppliers, since others may be as suitable. Although 115 VAC versions of electrical parts are specified here, 220 VAC valves and timers are avail-

Table 1. Parts list for the plastic PIG and specimen holders.

Part	Catalog No.	Supplier
Vacuum chamber	5305-0910	Nalge Co., Rochester, NY
Solenoid valve	S24C-4V, NC, 115/60V, 1/4″	Atkomatic Valve Co., Inc., Indianapolis, IN
Interval timer	G-08683-90	Cole-Parmer Instrument Co., Niles, IL
Apparatus positioner	G-08056-10	ditto
Vacuum pump	G-07055-04	ditto
Gas pressure regulator	G-98200-70	ditto
Barbed fitting	G-06362-40	ditto
Tee connector	G-06455-15	ditto
Tubing clamp	G-06833-00	
13 mm Plastic filter holder	4312	Gelman Sciences, Inc., Ann Arbor, MI
0.2μm air filter	4464	ditto
Plastic tubing	01T252PE-RDT	Ark-Plas Products, Inc., Flippin, AR
Helium tank, lab grade		Local supplier
Miscellaneous hardware		Local hardware supply
Metal hose clamps		ditto
2-prong electrical appliance cord		ditto
Brass close nipple w. flange, 1/4″-18 SAE		ditto
Brass cap, 1/4″-18 SAE		ditto
Brass bushing, 1/4″ × 1/8″ NPT		ditto
Steel jam nut, 9/16″ SAE		ditto
Teflon and electrical tape, rosin- core solder, silicone sealing glue		ditto
Specimen holders		
PC petri dishes	5502-0010	Nalge Co.
Spectra/mesh PP screen	08-670-185	Fisher Scientific, Pittsburgh, PA
Magnets 3 mm diameter (6)		Radio Shack, Inc.
Marine-tex epoxy putty		Travaco Laboratories, Inc., Chelsea, MA

Table 2. Tools required to assemble plastic PIG and specimen holders.

Electric hand-held drill
7/64″ & 1/4″ drill bits for metal
7/16″ drill bit for masonry
5/16″-24 NF & 1/4″-18 NPT thread taps
5/16″-24 NF thread die
Adjustable hole saw
Hobby vise
Adjustable wrench
Pliers
Soldering iron

able through the same suppliers. Similarly the closest metric-equivalent fittings may be substituted for the English measurements given below. Electrical connection to supply current should be through a ground fault protected circuit. Tools required to accomplish fabrication are listed in Table 2. Figures 1, 2 detail construction and use of the plastic PIG. Assembly steps are as follow:

1. Drill pilot hole in center top of vacuum jar. Enlarge hole with 7/16″ masonry bit and thread the hole with 1/4″-18 NPT tap.
2. Drill pilot hole in center of brass cap, then enlarge to 1/4″. Use 5/16″-24 NF tap to cut threads into hole.
3. Screw jam nut tightly onto close nipple, add teflon tape to threads on short end of nipple and screw tightly into the # 2 port of the solenoid valve. Screw the brass bushing, then the barbed fitting into the # 1 port of the valve, using teflon tape to seal.

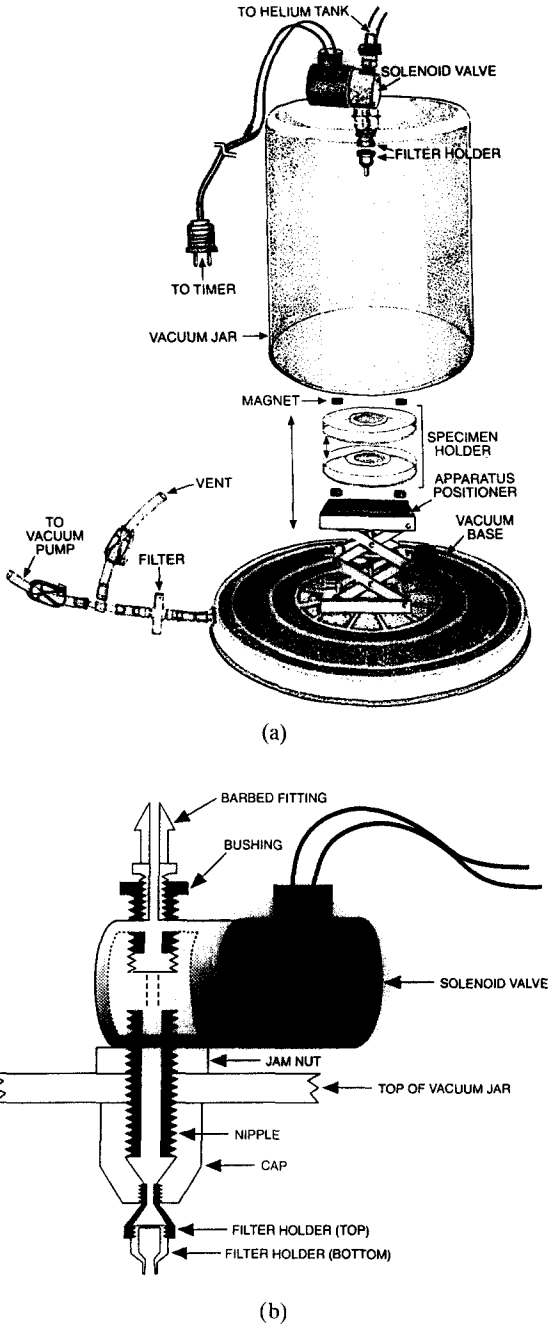

Fig. 1. Construction and setup of the plastic PIG: *(a)* Exploded view of plastic PIG and specimen holders; *(b)* Detail of solenoid valve assembly.

4. Add a small amount of silicone glue to the exposed threads of the close nipple and screw the valve assembly snugly into the top of the vacuum jar. Add more silicone glue to the exposed threads protruding into the jar before screwing the brass cap tightly onto the nipple (see Fig. 1b).
5. Use the 5/16″-24 NF die to cut carefully threads over the luerlock end of the plastic filter holder. Add teflon tape to the threaded end and screw the filter holder into the brass cap. Allow silicone glue on the assembled valve-vacuum jar unit to cure overnight before subjecting to vacuum.
6. Solder the two-prong electrical appliance cord onto exposed wires of valve and wrap securely with electrical tape. Plug cord into interval meter.
7. Clamp one end of plastic tubing to barbed fitting and the other end to the helium regulator.
8. Attach one end of vacuum-vent tubing to vacuum plate as shown in Fig. 1a and 2, and the other end to the vacuum pump.
9. Place the apparatus positioner in the center of vacuum plate.

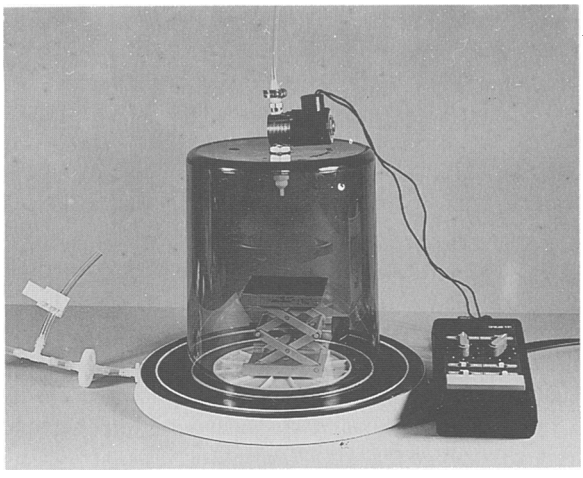

Fig. 2. Setup of plastic PIG and timer.

Construction of specimen holders

Specimen holders consist of the top and bottom of autoclavable plastic petri dishes, in which holes have been drilled to accept screens (see Fig. 1a and Table 1 for parts list).

1. Use hole saw on drill to cut 4 cm diameter hole in center of petri dish tops and bottoms.
2. Autoclave screen material to pre-shrink. Cut into 5 cm diameter discs. Use Marine-tex to attach screen to the *inside surface* of the bottom and the *outside surface* of the top. The petri dish surface under the screen should be roughened with sandpaper prior to attachment.

3. Cover glued surface with plastic wrap and add a weight to hold screen tightly in place. Allow to cure overnight.

For bombardment, specimens are held between screens by nesting the top piece of the dish over the inverted bottom, both of which are then clamped together with two-to-three pairs of magnets (Fig. 1a).

Preparation of plasmid-particle mixture

The following procedure was modified from Finer et al. (1992). Autoclave M17 tungsten particles (Bio-Rad Laboratories, Inc., Hercules, CA) and place 50 mg in 0.5 ml 95% ethanol, incubate 20 minutes, then vortex well. Centrifuge (ca. 10,000 rpm) for 5 min and wash with 0.5 ml sterile water – repeat 5 times. Vortex, then pipette 25 µl of tungsten stock into Eppendorf tube. Add 5 µl DNA (1 $\mu g \cdot \mu l^{-1}$) and mix well. Add 25 µl 2.5 M $CaCl_2$, and vortex. Quickly add 10 µl 100 mM spermidine (free base) and vortex. Incubate on ice for 5 minutes. Without centrifugation, remove about 50 µl of solution, leaving the particles. Use immediately.

Operation cycle of the plastic pig

Clamp tissue in a specimen holder using magnets, center on the positioner adjusted to the desired height. It is imperative that tissue not be 'wet', i.e., not covered with a film of liquid, in order for the procedure to be successful. Place a 2 µl drop of DNA/particle mixture on the middle of a plastic filter holder screen and screw tightly into the vacuum jar. Place vacuum jar on base and evacuate the chamber to 90 kPa (27 in. Hg) vacuum. Set timer to 0.1 sec and fire valve, then vent the chamber and replace specimens on culture medium.

Comparison of plastic pig with conventional pig

An experiment was conducted to compare the performance of the plastic PIG with a conventional PIG, using transient expression of β-glucuronidase (GUS) as an indicator. The conventional PIG was constructed following the specifications of Finer et al. (1992), except that a different solenoid valve-timer apparatus was used and the luerlock fitting was replaced with a threaded brass cap as described above. This latter modification eliminated the constricted gas passage imposed by the luerlock fitting. The basal quarters of cantaloupe (*Cucumis melo* L. 'Eden Gem') cotyledons (3 × 4 mm) were prepared as previously described (Gray et al. 1993) and used after 4 days of culture.

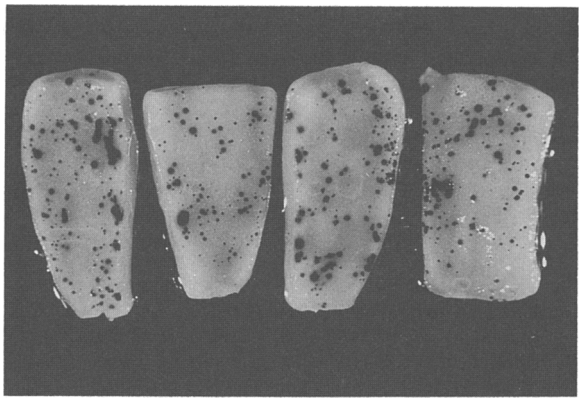

Fig. 3. Transient expression of GUS in four basal cotyledon quarters bombarded at 830 kPa and a 15 cm particle-to-specimen gap. Discrete GUS foci, shown in this photograph as black spots of various sizes, were dark blue in coloration; bar <equals> 2 mm.

The plasmid was pBI221 (Clontech Laboratories, Inc., CA), which consisted of a 3kb HindII-EcoRI fragment of pBI121 containing the CaMV 35S promoter, GUS gene and NOS-terminator (Jefferson et al., 1987) cloned into pUC19. The plasmid was maintained in DH5alpha bacterial host and purified according to the QIAGEN plasmid purification kit. Treatments compared for each device consisted of three distances between the filter screen and specimens (10, 15 or 20 cm), each at 410 kPa (60 psi), 620 kPa (90 psi) or 830 kPa (120 psi) helium pressure. Four replicates, consisting of eight cotyledon quarters per exposure, were tested for each treatment. At 24 h after treatment, cotyledon quarters were placed in the X-gluc solution as previously described (Jefferson et al. 1987). The number of dark blue spots per 8 cotyledon quarters were determined for each replicate. Data were subjected to analysis of variance.

Results and discussion

Transient GUS activity was visualized as discrete dark blue foci across the surface of basal cotyledon quarters (Fig. 3). Microscopic examination suggested that each focus was due to the bleeding of blue stain from one or few cells into adjacent cells. Comparison of the plastic PIG with the conventional PIG tested at three different pressure and distance settings demonstrated that there was no statistical difference in transient GUS expression when subjected to analysis of variance ($p < 0.01$). However, both pressure and distance had a significant effect on GUS expression ($p < 0.01$). There were no

Table 3. Transient expression of GUS in cantaloupe cotyledon quarters treated at three different helium pressures and distances in a plastic PIG versus a conventional PIG.[1]

Pressure (kPa)/distance(cm)	Average no. GUS-positive foci	
	Plastic PIG	Conventional PIG
410/10	326 bcd[2]	369 abcd
410/15	199 cd	341 abcd
410/20	112 d	143 cd
620/10	521 ab	494 ab
620/15	600 a	505 ab
620/20	149 cd	128 cd
830/10	535 ab	471 ab
830/15	293 bcd	387 abc
830/20	160 cd	205 cd

[1]Data reflect average number of GUS-positive foci per 8 cotyledon quarters for each exposure. Each treatment was replicated four times.
[2]Means within or between columns followed by the same letter were not statistically different according to Student–Newman–Keul's test, p <equals> 0.01.

PIG × pressure × distance interactions. Average number of GUS-positive foci per treatment are given in Table 3. Generally, the lower pressure (410 kPa) and greater distance (20 cm) produced the fewest GUS-positive foci. The highest number of GUS-positive foci were obtained at 620 kPa and 15 cm (600 per exposure). However, this treatment was statistically similar to several others, depending on the gun used.

The plastic PIG is simple to operate. The solenoid valve serves as a convenient handle for lifting the vacuum jar (Fig. 2). The specimen height is infinitely adjustable using the positioner. The specimen holders effectively position tissue for exposure to DNA-coated particles. Friable callus and similar material can be treated by utilizing finer screens or pieces of filter paper in the specimen holder bottoms. We found that the time needed to complete one cycle was equivalent to that of a conventional PIG (e.g., Vain et al. 1993a). The plastic PIG is actually simpler and less expensive to operate than the commercially available Biolistic PDS 1000/He device (see Kikkert 1983), which requires additional manipulations and consumes several dollars of disposable items for each operation cycle. While only transient expression of GUS is reported here, stable transformation was obtained with the conventional PIG (Vain et al. 1993a,b), suggesting that the plastic PIG will be equally effective. The plastic PIG was particularly easy to build. It required only simple tools and mechanical skills to construct and was assembled to completion on our laboratory bench in less than 40 minutes. Cost of components was approximately $400 U.S. The low cost and simplicity of this design should make particle bombardment technology available to more scientists.

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