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Special section on particle bombardment

Development of the Particle Inflow Gun

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Abstract

A simple and inexpensive particle acceleration apparatus was designed for direct delivery of DNA to plant cells. The Particle Inflow Gun (PIG) is based on acceleration of DNA-coated tungsten particles directly in a helium stream. High levels of transient expression of the β-glucuronidase gene were obtained following bombardment of embryogenic suspension cultures of maize and soybean, and leaf tissue of cowpea. Stable transformation of soybean and maize has also been obtained using this bombardment apparatus.

Abbreviations: 2,4-D – 2,4-dichlorophenoxyacetic acid, GUS – β-glucuronidase, NOS – nopaline synthase, PCV – packed cell volume, PPT – phosphinothricin

Introduction

Particle bombardment technology has become very widespread due to its value in transient gene expression (Ludwig et al. 1990) and stable transformation studies (Christou et al. 1988). The physical nature of DNA introduction permits reliable and effective gene transfer to major agronomic monocotyledonous species and to intact plant and animal tissues. Many versions of particle gun have been built (Christou et al. 1988; Sautter et al. 1991; Takeuchi et al. 1992) and several of these versions have been commercialized (Williams et al. 1991). Nevertheless, expansion of particle gun technology is still limited by accessibility of devices due to high cost and complexity. The development of an inexpensive and efficient device that is simple to build and operate would aid in the distribution and utilization of this technology.

An inexpensive and easy to assemble particle gun was first described by Takeuchi et al. (1992). The 'flowing helium gun' accelerated particles directly in a stream of low pressure helium. Macrocarriers did not support or carry the particles and the force necessary to accelerate the particles was therefore reduced. The absence of macrocarriers reduced consumables, cleanup time and cycle time. In addition, this device gave transient transformation of a variety of different plant tissues. The flowing helium gun was used as the basis for development of the Particle Inflow Gun (PIG). Several new features were added to the basic design of the flowing helium gun to make it more efficient and compatible with biological targets:

- A vacuum chamber was used to reduce the drag on the particles and also lessen tissue damage.
- A timer relay-driven solenoid (Morikawa et al. 1989) replaced the manual syringe stopcock. The solenoid provided more consistent accelerations by permitting better control of the amount of helium released through the use of a

timer relay. The amount of helium released could be further controlled using a prechamber, upstream of the solenoid. By reducing the amount of helium used to accelerate the particles, tissue damage/displacement was also reduced. Unlike a membrane rupture system (Williams et al. 1991) no preparation was required with the solenoid and it functioned at low helium pressures, which was less damaging to the target tissue.
- The PIG was constructed using equipment that were readily available from supply companies. The components of the PIG permit ease of construction and operation and contribute to consistent bombardment results. The overall cost of the PIG was less than 500 US dollars.

In addition to the physical process of particle bombardment, considerable efforts were also placed on evaluating the biological parameters relating to the status of target tissue. Improvement of the starting material quality as well as a reduction of stresses to the target tissue occuring during bombardment resulted in major improvements of plant transformation efficiency.

In this paper, we describe in detail particle bombardment-mediated transformation of the following 3 different biological systems using the PIG:
- small (100–500 μm) embryogenic cell groups obtained from maize (*Zea mays* L.) suspension cultures,
- large (1–8 mm) embryogenic clumps obtained from soybean (*Glycine max* Merrill.) suspension cultures,
- leaf tissue of cowpea.

Transient transformation efficiencies of some other plant tissues are also briefly noted.

Description of the Particle Inflow Gun

The vacuum chamber (Fig. 1a) consisted of a 5-sided steel box and measured 16.5 × 16.5 × 30.5 cm. The box could be welded from either 6.4 mm steel or stainless steel and the front of the vacuum chamber was ground smooth to provide a good seal with the door. The door was constructed from 2.5 cm thick plexiglass and a 6.4 mm thick soft neoprene rubber gasket was glued to and recessed in the door. Two small steel plates were welded to the left side of the chamber to aid in the attachment of hinges for the plexiglass door. Two collars were welded into holes drilled in the top and left side of the box. Care was taken to place the top collar perpendicular to the top of the box. Deviations from this will skew the particle projection. All of the fittings (pipes, nipples, compression fittings) used in construction of the PIG were 1/4 inch (6.4 mm) I.D. National Iron Pipe. The vacuum/gauge/vent assembly, which consisted of two high pressure needle valves (Trerice; Detroit, MI, #735–2) and a vacuum gauge (Marshall town, Hastings, NE, #G14489), was connected to the collar in the left side of the box (Fig. 1a) using a cross fitting. The vacuum gauge displayed the vacuum settings down to 29 in. Hg. A 2-way solenoid (ASCO, Florham Park, NJ, Red Hat II, #JKF8262G22, with type I splice box) was connected to the vacuum chamber by the collar on the top of the chamber (Fig. 1b). The solenoid was controlled by a timer relay which was always set for the minimum timer duration of 50 ms. The helium prechamber which consisted of a 10 cm portion of pipe, was attached to the top of the solenoid on one side and to a needle valve on the other side. Different prechambers could be evaluated by using various size of pipes. A copper line from a helium tank set at 60 PSI was connected to the other end of the prechamber needle valve using a compression fitting.

On the inside top of the vacuum chamber, a stainless steel, male Leur-lok needle adaptor (Clay Adams, Parsippany, NJ, #7553) was connected to the collar using a compression fitting (Fig. 1b). Because this model of needle adaptor is no longer in production, it may be necessary to substitute needle adaptors from other suppliers (Sigma, St Louis, MO #C3399 or Aldrich, St Louis, MO #Z11,797–8). A 13 mm stainless steel (Fisher Scientific, Pittsburgh, PA, #09–753–10A) or plastic (Gelman, Ann Arbor, MI, #4317) syringe filter unit could be readily attached to and removed from the device using the Leur-lok needle adaptor. A 3/8 inch (9.5 mm) polypropylene insert was designed to fit just inside of the vacuum chamber and grooves for a plexiglass shelf were cut into the left and right walls of the insert at every 1.5 cm. Target tissue was bombarded either unprotected or covered

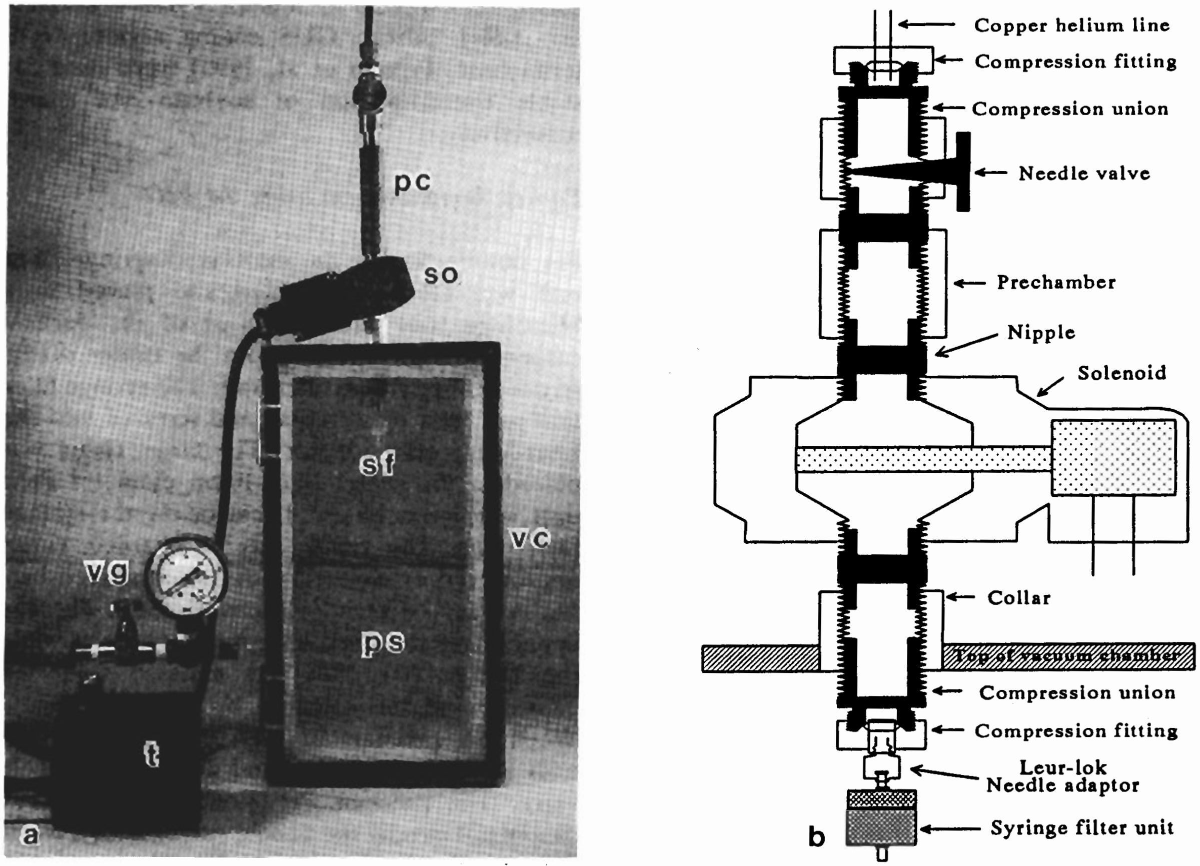

Fig. 1. (*a*) Particle Inflow Gun: prechamber (pc), plexiglass shelf (ps), syringe filter unit (sf), solenoid (so), timer relay (t), vacuum chamber (vc) and vacuum/gauge/vent assembly (vg) (*b*) Schematic of the connections from the helium line through the syringe filter unit.

with a baffle. The baffle was made by cutting off the bottom of a 400 ml disposable polypropylene beaker and melting a 500 μm nylon screen (Tetko, Inc., Elmsford, NY) to the bottom of the beaker using a hot plate. The beaker was then inverted and placed over the tissue prior to bombardment, putting the screen 9 cm above the target tissue.

Operation of the Particle Inflow Gun

Plant tissue preparation

Embryogenic suspension cultures of soybean and maize were obtained as described previously (Finer & McMullen 1991, Finer et al. 1992). Suspension cultures that contained the highest amount of embryogenic cell clumps compared to nonembryogenic tissues were selected for transformation studies.

Various cell preparation/conditioning methods were evaluated for enhancement of transformation efficiency. Embryogenic tissue of soybean was partially dried by placing it in a Petri dish, uncovered, in a laminar-flow hood for 15 min (Finer & McMullen 1991). Following bombardment, soybean tissue was maintained in a covered Petri dish for an additional 30 min before resuspension in liquid culture medium. Embryogenic maize cells were filtered through a 500 μm filter, collected on a 100 μm filter and evenly dispersed on a 7 cm filter paper disc (100 μl PCV on a Whatman #4 filter) (Vain et al. 1993). Prior to bombardment, discs were stored on a solidified medium for short periods of time.

For osmotic conditioning of maize cells, filter discs were stored on a medium containing 0.2 M sorbitol and 0.2 M mannitol 4 h prior to and 16 h after bombardment.

Leaf tissue of cowpea (*Vigna unguiculata* (L.) Walpers) was obtained from greenhouse-grown plants. Leaves were not sterilized prior to bombardment.

Particle preparation and precipitation

Tungsten particles (M10; provided by Sylvania Chemicals/Metals, Towanda, PA) were sterilized for 15 min by placing 50 mg particles in 500 μl 95% ethanol in a 1.5 ml microfuge tube. Particles were rinsed 3 times in sterile distilled water by repeated vortexing, centrifugation and resuspension in 500 μl of water. Particle suspensions were made fresh for each experiment. For soybean and cowpea, the DNA was precipited onto the particles by mixing 25 μl of tungsten particle suspension (2.5 mg), 5 μl of DNA (5 μg), 25 μl of 2.5 M $CaCl_2$, and 10 μl of 100 mM spermidine (free base). After allowing the particles to settle for 5 min at 4°C, 50 μl of the supernatant was removed and discarded. For maize, 10 μl of tungsten (1 mg), 20 μl of DNA (20 μg), 25 μl of 2.5 M $CaCl_2$, and 10 μl of 100 mM spermidine (free base) were mixed and placed at 4°C. After 5 min, 45 μl of supernatant were removed and discarded. For each precipitation, the microfuge tube was vortexed after the addition of each component and the total procedure was performed as rapidly as possible.

Plasmids

The plasmid pUCGUS (CaMV35S promoter :GUS coding region:NOS terminator; Finer & McMullen 1991) and pGB5 (CaMV35S promoter:*Sh-1* intron: GUS coding region:NOS terminator; Finer et al. 1992) were used for transient expression in soybean/cowpea and maize respectively. Bombarded tissues were assayed for GUS activity 2 days after bombardment (Jefferson 1987) and the number of blue foci were determined. The plasmid pHygr (CaMV35S promoter:AphIV coding region:NOS terminator; Finer & McMullen 1990) and pBARGUS (CaMV35S promoter:*Adh-1* intron: BAR coding region:NOS terminator + *Adh-1* promoter: *Adh-1* intron: GUS coding region: NOS terminator; Fromm et al. 1990) were used for stable transformation of soybean and maize respectively.

Particle bombardment using the PIG

For bombardment, an autoclaved syringe filter unit was first disassembled and placed in a microfuge tube rack. Two μl of the particle suspension was pipetted onto the center of the screen of the syringe filter unit. The syringe filter unit was then reassembled and screwed into the Leur-lok needle adaptor. The target tissue was placed in the center of a 10 cm diameter Petri dish which was placed 17 cm below the syringe filter unit. The tissue was bombarded either unprotected or covered with an autoclaved baffle. A vacuum of approximately 29 in Hg was applied and the particles were discharged when the helium (60 PSI) was released following activation of the solenoid by the timer relay. When the prechamber was used, it was filled with helium by opening and closing the needle valve. The prechamber could be bypassed by simply not closing the needle valve. With a 50 ms opening of the solenoid (prechamber bypassed), approxiately 500 ml of expanded helium was released at 1 atm (vacuum chamber volume is approximately 8 l). With the various sized prechambers, this amount was reduced to 50 to 170 ml of expanded helium. From 12 to 16 bombardments could be performed per hour including the time required for precipitation of DNA onto the particles.

Selection for stable transformation

Post-bombardment treatments varied depending on the target tissues and selective agents.

For soybean cultures, which consisted of large clumps of tissue, selection for stable transformants was performed in liquid culture in order to allow better contact of the tissue with the selective agent (Finer & McMullen 1991). Thirty min after bombardment, clumps of soybean tissue were placed in liquid proliferation media without antibiotics. After 1–2 weeks of culture, clumps of tissue were transfered to a liquid culture medium containing $50\ \mu g\ ml^{-1}$ hygromycin and subcultured weekly. After 6–8 weeks of maintenance in hygromycin-containing media, embryogenic tissues that were yellow-green were removed and separately subcultured. Non-transformed tissues that did not survive selection were either white or brown.

For maize, because of the small size of the cell clumps, selection could be performed directly on solidified culture media (Vain et al. 1993). For small cell groups, this technique of selection was preferred, because of the ease of selection and isolation of resistant clones. Selection for PPT-resistant maize lines was initiated 2 days after bombardment by placing the filter carrying the bombarded cells on a solidified MS medium containing $3–5\ mg\ l^{-1}$ of bialaphos or glufosinate. Filters were transferred to fresh herbicide-containing media every 14 days and resistant clones were isolated after 6–8 weeks.

Transformation efficiency

The bombardment parameters that we described in the text (60 PSI pressure, 17 cm distance target-syringe filter, 500 μm baffle placed 9 cm above the target) provided good transient GUS expression for numerous plant tissue using the PIG (Table 1). These parameters need to be optimized for each system.

1. Transient expression

Vacuum

Evaluation of the importance of vacuum was performed using soybean hypocotyl tissue and particle preparation as described by Takeuchi et al. (1992). With no vacuum, optimum transient expression was obtained in soybean hypocotyls if the tissue was 3 cm from the tip of the syringe filter unit (160 ± 40 blue foci per bombardment). Distances less than 3 cm resulted in severe tissue damage while distances greater than 5 cm gave no transient expression. The use of a vacuum led to a dramatic increase in transient expression in bombarded soybean hypocotyls at distances greater than 3 cm. With the vacuum, tissue could be bombarded at greater distances, thereby reducing tissue damage and increasing transient transformation.

Particle distribution

In order to measure particle distribution and levels of transient expression, leaf tissue of cowpea and embryogenic cells of maize were subjected to particle bombardment. Leaf tissue and embryogenic cells were used because they provided uniform and consistent target tissue for bombardment. After bombardment, the majority of the cells expressing GUS were found within a 5 cm diameter circle. A zone of death was clearly observed on bombarded cowpea leaves (Fig. 2) and a slight reduction of blue foci

Table 1. Transient and stable transformation of several plant tissues using the Particle Inflow Gun.

Species	Target tissue (quantity)	Bombardment protocol[w]	Transient expression: # blue foci per shot	Stable transformation # transformed clones per shot
maize	susp[x](100 mg FW)	(1)	8,702[y]	3.4 PPT^{r}[z]
soybean	susp (1 g FW)	(2)	3,297	11.5 Hygr^{r}
soybean	hypocotyl (1)	(5)	380	–
cowpea	leaf (1)	(3)	15,000	–
wheat	susp (100 mg FW)	(1)	880	–
cotton	susp (1 g FW)	(2)	2,047	–
sunflower	susp (500 mg FW)	(2)	780	–
Chlamydomonas	cells (10^7)	(4)	–	1,000 Spec^{r}

[w] Tissue preparation and bombardment parameters described in the text for (1) maize, (2) soybean, (3) cowpea, (4) *Chlamydomonas* (R. Sayre, Columbus OH, USA; personal communication) and (5) soybean (Takeuchi et al. 1992) using the PIG.

[x] Susp: suspension culture.

[y] Each value is the mean of 5 to 34 replications.

[z] PPT^{r}: phosphinothricin resistant clones; Hygr^{r}: Hygromycin resistant clones; Spec^{r}: Spectinomycin resistant clones.

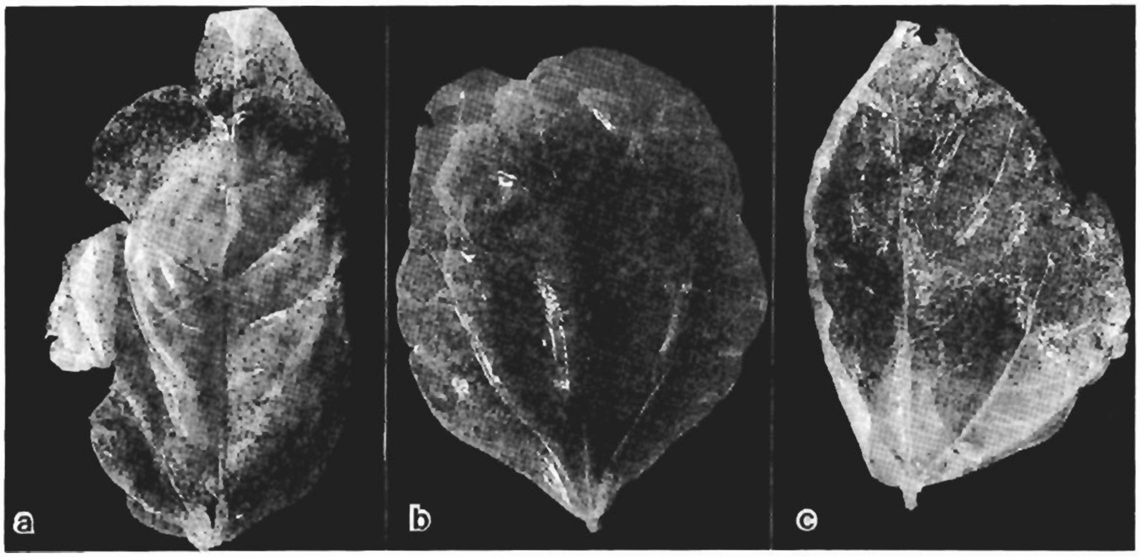

Fig. 2. Transient expression of the GUS gene in bombarded cowpea leaves using (*a*) no helium prechamber and no baffle (*b*) a 50 ml helium prechamber and no baffle and (*c*) no helium prechamber and a 500 μm baffle placed 9 cm above the target. Other bombardment parameters: 60 PSI helium pressure and 17 cm distance target-syringe filter.

density could also been observed in the center of the bombarded maize cells (Fig. 3). The occurrence of a central necrotic area was also reported by Klein et al. (1988) for tobacco leaves and suspension culture cells using an older gunpowder version of the Biolistic device. Damage in the center of the blast area could be totally eliminated by using a helium prechamber or baffle with the PIG (Fig. 2).

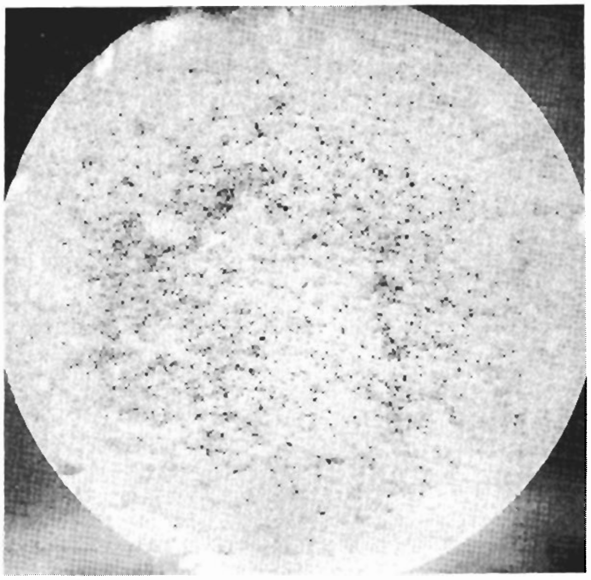

Fig. 3. Transient expression of the GUS gene in bombarded embryogenic maize cells (100 μl PCV) using no helium prechamber and a 500 μm baffle. Other bombardment parameters: 60 PSI helium pressure and 17 cm distance target-syringe filter.

Pressure, distance, prechamber and baffle

Pressure, distance, prechamber and baffle were interactive relative to tissue displacement, particle penetration and cell survival. Optimization of these parameters was influenced by the nature of the target tissue, the nature and quantity of particles used and the intensity of the vacuum. In general, targets such as plant leaves or large clumps of tissue required more gentle bombardment conditions to lessen cell damage or tissue displacement. Either lower pressures, longer distances or use of a baffle or helium prechamber was necessary with these tissues.

The effects of different helium pressures and bombardment distances were evaluated in detail for the maize system where the target was made up of a fine layer of cells (cell clumps <500 μm). Transient expression of the GUS gene significantly increased (2.4-fold) with increasing pressure from 40 to 80 PSI but no significant differences in bombardment efficiency was observed if the tissue was between 14 and 23 cm from the syringe filter (Finer et al. 1992). However, the highest pressure (80 PSI) could not be used with the closest distance (14 cm) for bombardment of maize tissue consisting of clumps greater than 500 μm because of massive tissue displacement. Large clumps of tissue protrude farther from the filter paper surface and may therefore be more vulnerable to displacement by the helium burst.

The use of a baffle was indispensable for bombarding large clumps of embryogenic tissues like soybean suspension culture tissue. The baffle slightly reduced dislodgement but, most importantly, kept the dislodged tissue in a restricted area where it was possible to recover it aseptically. In addition to the baffle playing a protective role, it may also have been helpful in aiding particle dispersion (Gordon-Kamm et al. 1990). For the more sensitive tissues like cowpea leaves, use of a baffle or prechamber dramatically reduced cell damage in the blast area center (Fig. 2) resulting in a more even distribution of transient expression (approximately 15,000 blue foci were obtained with and without prechamber/baffle). Although we did not observed a significant effect of the helium prechamber on the level of transient GUS expression, the prechamber may be valuable for stable transformation work due to a reduction of damage to the target tissue. For maize, which is less susceptible than soybean to tissue displacement and less fragile than cowpea leaves, the baffle and the helium prechambers did not significantly improve transient transformation (no baffle: 7718 blue foci ; baffle: 8,702 blue foci ; helium prechamber: 5416 blue foci).

Particles

Particles from several different sources were evaluated for transient GUS expression using embryogenic maize cells as the target tissue. Use of Sylvania M10 particles gave the highest transient GUS expression (Table 2). Direct observation of the different particles using scanning electron microscopy revealed a large variation in the size of the tungsten particles compared to the gold. This variation was confirmed by size distribution analysis of tungsten particles (Table 3). The overlap in the sizes of M10 and M17 particles and the differences observed in transient expression assays suggest that a purification of particles of narrower size distribution may be beneficial.

Evaluation of the syringe filter unit

A comparison between the stainless steel and plastic syringe filter units (Takeuchi et al. 1992)

Table 3. Size distribution analysis of Sylvania M10 and M17 particles[x].

Size range (μm)	M10 Frequency (%)	M17 Frequency (%)
0.1–0.2	0	4.5
0.2–0.3	5.0	3.0
0.3–0.4	8.0	3.5
0.4–0.5	11.0	6.0
0.5–0.6	10.5	8.0
0.6–0.7	11.0	10.0
0.7–0.8	10.0	10.0
0.8–0.9	9.0	8.0
0.9–1.0	6.0	7.5
1.0–2.0	23.0	36.5
2.0–3.0	3.0	2.5
3.0–4.0	1.5	0.3
4.0–5.0	0.5	0.2
5.0–6.0	0.5	0
6.0–7.0	0.5	0
7.0–8.0	0.5	0

[x] Physical testing laboratory sedigraph obtained from Sylvania Chemicals/Metals.

Table 2. Effect of particle type on transient GUS expression in maize embryogenic suspension culture after bombardment[x].

Particle type	#Blue foci for 100 μl PCV (100 mg) of cells[y]
Tungsten M10 Sylvania (Towanda, PA)	8,196[a]
Tungsten M17 Sylvania (Towanda, PA)	5,447[b]
Gold Alfa Chemicals (Danvers, MA), #00766, 1.5–3.0 μm	3,255[c]
Gold Heraus (Germany), # 009150, 0.4–1.2 μm	1,367[c]
Gold 1 μm[z]	853[d]

[x] Bombardment was performed using 60 PSI helium pressure, 17 cm distance target-syringe filter, 500 μm baffle placed 9 cm above the target. [y] Each value is the mean of 6 replications. [z] A. Nagasawa, Sumitomo Chemical Co. Ltd., Hyogo, Japan. [abcd] Entries followed by different letters are significantly different at $p = 0.05$ by one way analysis of variance.

gave insignificant differences in the number of blue foci obtained per bombardment (Finer et al. 1992). Plastic syringe filters were preferred because of lower cost and lower reactivity of the plastic with solutions.

Other factors such as the nature of the plasmids and the method and volume of precipitation used for bombardment may also influence particle gun mediated transformation but these factors have not yet been evaluated quantitatively using the PIG.

Cell preparation or conditioning

One of the largest enhancements in transient expression resulted from altering the preparation methods or the state of the target tissue. When the target consisted of a fine layer of cells, as with embryogenic maize cultures, transformation efficiency depended in part on the physical characteristics of the layer. The use of small clumps of tissue provided a larger surface area for a lower PCV and the clumps were less likely to be dislodged from the filter paper by the impact of the helium burst. Use of maize suspension culture cells filtered through a 500 μm instead of a 1 mm filter led to a 2.6-fold increase in transient expression of the GUS gene (Finer et al. 1992). The amount of target tissue also influenced the level of transient expression. For maize, transient transformation was fairly proportional to the PCV up to 400–500 μl PCV, at which point the bombardment efficiency was reduced due to layering of cells (Finer et al. 1992). The amount of bombarded cells may also influence subsequent selection for stably-transformed tissues. The quantity of cells must be maintained as low as possible to prevent cross-feeding during the selection process.

Osmotic treatment or drying of tissue tremendously influenced transient transformation of maize and soybean respectively. A 2- to 3-fold increase in transient GUS expression was obtained following placement of embryogenic maize cells on a medium containing both 0.2 M sorbitol and 0.2 M mannitol prior to and after bombardment (Vain et al. 1993). With an osmotic treatment, an average of 8,702 blue foci could be obtained from 100 μl PVC (100 mg FW) of bombarded maize embryogenic tissue. With embryogenic soybean tissue, a similar 2- to 3-fold increase in transient expression was observed following a drying treatment (Table 4). With drying treatment, an average of 3,280 blue foci could be obtained from 1 g FW of bombarded soybean embryogenic tissue. We believe the basis of osmotic and drying enhancement of transient expression resulted from plasmolysis of cells, which may have reduced cell damage by preventing extrusion of the protoplasm from bombarded cells (Vain et al. 1993).

Benefits of other pretreatments such as culture venting (Russell et al. 1992) and the use of cells in a particular phase of growth (Armaleo et al. 1990) have also been reported for different species using the particle gun.

Shot-to-shot variation

Both qualitative and quantitative differences in transient expression were observed in all plant tissues bombarded using the PIG. The shot-to-shot coefficient of variation (standard deviation/ mean) was 21% for maize, 16.4% for soybean

Table 4. Effect of drying treatments on transient GUS expression in soybean embryogenic tissue after particle bombardment[x].

Drying treatment (min)[y]		#Blue foci for 1 g of cells[z]
Before bombardment	After bombardment	
0	0	1,156[a]
0	30	1,952[a]
0	90	1,333[a]
15	0	1,354[a]
15	30	3,315[b]
15	90	3,280[b]

[x] Bombardment was performed using 60 PSI helium pressure, 17 cm distance target-syringe filter and a 500 μm baffle placed 9 cm above the target. [y] Drying treatment was performed by placing tissue in a Petri dish in a laminar-flow hood uncovered before bombardment and covered after bombardment. [z] Each value is the mean of 3 replications.

[ab] Entries followed by different letters are significantly different at $p = 0.05$ by one way analysis of variance.

and 15.9% for cotton. A major portion of the variability was due to inconsistencies in precipitation of DNA onto the particles. Variability was also influenced by the quality and heterogeneity of the target tissue.

2. *Stable transformation*

Stable transformation of soybean and maize was obtained using the PIG (Finer et al. 1992; Vain et al. 1993). An average of 11.5 stably-transformed, hygromycin-resistant soybean clones and 3.4 herbicide-resistant maize clones were obtained for each bombardment of embryogenic tissue (Table 1). The number of transgenic soybean and maize clones obtained using the PIG were greater than 3 and 10 times more than previously reported for other bombardment devices using equivalent quantities of starting material (Finer et al. 1991; Gordon-Kamm et al. 1990). Transformed soybean and maize plants have been regenerated from the transformed clones.

A limited number of bombardment parameters was quantitatively evaluated for their influence on stable transformation. In general, protocols that gave the highest levels of transient expression were used for stable transformation experiments. The effect of osmoticum treatment was evaluated for both transient expression and stable transformation.

Osmotic treatment (0.2 M sorbitol and 0.2 M mannitol) of maize cells for 4 h before and 16 h after bombardment resulted in a 6- to 7-fold increase in the number of stable transformants obtained from 100 μl PCV of tissue (Vain et al. 1993). From each group of 8,702 GUS-positive foci, 3.4 stably transformed embryogenic maize clones were recovered, resulting in a transient-to-stable conversion frequency of 0.04% compared to 0.015% with no osmoticum treatment. The increase in transient-to-stable conversion frequency suggests that the osmotic treatment may also be benefical for selection by reducing the cell growth and therefore improving selection efficiency.

Recommendations

For recovery of transgenic plants using particle bombardment, both physical (particle introduction) and physiological parameters (survival and growth of bombarded cells) need to be considered. One critical aspect of stable transformation is the development of a selection process adapted to the material used as the target. The method of selection will often influence the choice of the type and quantity of plant tissue used for bombardment. After the target tissue and selection conditions are properly defined, the bombardment parameters can be initially optimized using transient expression studies. Bombardment conditions providing the highest levels of transient expression should be considered as a good starting point for development of stable transformation procedures. This strategy has been used with the PIG to obtain transgenic soybean and corn.

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