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Short communication

HandyGun: An improved custom-designed, non-vacuum gene gun suitable for virus inoculation

Sidona Sikorskaite[a,1], Anssi L. Vuorinen[a,1], Minna-Liisa Rajamäki[a], Arto Nieminen[b], Victor Gaba[c], Jari P.T. Valkonen[a,*]

[a] Department of Agricultural Sciences, PO Box 27, FIN-00014, University of Helsinki, Finland
[b] The Instrument Center, PO Box 27, FIN-00014, University of Helsinki, Finland
[c] Department of Plant Pathology and Weed Science, Agricultural Research Organization, The Volcani Center, PO Box 6, Bet Dagan 50250, Israel

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ABSTRACT

Particle bombardment with a non-vacuum gene gun is an efficient method for transfection of plant cells with cloned viruses and initiation of virus infection. The HandyGun developed in this study is an improved version of a non-vacuum gene gun. Bombardment parameters were studied by inoculating an infectious, 35S promoter-driven cDNA of *Potato virus A* (PVA; *Potyvirus*) to the potato clone 'A6', *Nicotiana benthamiana* and *N. tabacum* as plasmid DNA coated on microprojectiles (gold particles). The large number of initial infection sites (necrotic local lesions) observed on inoculated 'A6' leaves and the high percentage of *Nicotiana* plants which were infected systemically with PVA following inoculation with HandyGun were not particularly sensitive to variation in the parameters tested (helium pressure and the amounts of plasmid DNA and gold particles). Data showed that HandyGun is a robust and reliable tool for obtaining high infection rates in plants reproducibly. It is easy and inexpensive to use and can be constructed from parts commonly available.

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Particle bombardment is a physical means for acceleration of nucleic acid-coated particles into plant tissue and was developed originally to overcome recalcitrance of various plant species and genotypes to *Agrobacterium*-mediated genetic transformation (Sanford et al., 1987; Klein et al., 1987). The first devices were gunpowder-powered and relied on acceleration of DNA-coated tungsten microprojectiles to velocities sufficient for penetration into epidermal cells of plant tissue placed in a vacuum chamber (Sanford et al., 1987; Klein et al., 1987). Later, modifications were introduced to the device, so that acceleration of microprojectiles could be achieved by other means such as an electric discharge (McCabe et al., 1988), compressed air (Oard et al., 1990) or flowing helium (Sanford et al., 1991; Takeuchi et al., 1992).

Sanford et al. (1991) developed also the first non-vacuum hand-held gene gun, which is an efficient, easy and flexible device for gene delivery *in vitro* and *in vivo* and whose use is not limited by a large target size. Hence, hand-held devices are preferred e.g. for inoculation of plants with cloned viruses. Gal-On et al. (1997) described a hand-held non-vacuum gene gun which was modified from previous models (Takeuchi et al., 1992; Gray et al., 1994) and could be assembled from parts available commercially. This device and its improved version (Gaba and Gal-On, 2005) were used for inoculating plants with an infectious clone of *Zucchini yellow mosaic virus* (ZYMV; genus *Potyvirus*) driven by the *Cauliflower mosaic virus* 35S promoter, or a promoter derived from the related *Strawberry vein banding virus* (Wang et al., 2000), and infection rates up to 100% were achieved.

The gene guns introduced initially to markets were based on the devices of Sanford et al. (1987, 1991) and modified subsequently to propel microcarriers by a burst of helium gas (Kikkert, 1993). We have gained positive experience from the use of the latest commercial models of hand-held gene guns for inoculation of various Solanaceous species with 35S promoter-driven infectious clones or infectious *in vitro* transcripts of *Potato virus A* (PVA; genus *Potyvirus*) (Rajamäki and Valkonen, 1999, 2002, 2009; Hämäläinen et al., 2000; Kekarainen and Valkonen, 2001). However, dependence on external maintenance services and purchase of commercial supplies and spare parts available only from the manufacturer add significantly to the costs and can be considered disadvantages. Therefore, the aim of this study was to design a non-vacuum gene gun, referred further to as HandyGun, that can be constructed easily from supplies available commonly, and to determine the parameters for its efficient use in virus inoculation of plants.

The parts used for construction of HandyGun, their sources and related technical data are in Table 1. Parts such as the timer,

* Corresponding author at: Department of Agricultural Sciences, Latokartanonkaari 7, FIN-00014 Helsinki, Finland. Tel.: +358 9 19158387; fax: +358 9 19158727.
E-mail address: jari.valkonen@helsinki.fi (J.P.T. Valkonen).
[1] These authors contributed equally.

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Table 1
Parts used for construction of HandyGun (British piping standards, BSP or BSPP; can be substituted by the American standard, NPT). Numbering of the parts corresponds to Fig. 1.

Part	Technical data	Manufacturer
1. DIN plug	To fit to the solenoid valve. Standard DIN, transparent, 24 V	Hirschmann Automation and Control GmbH, Neckartenzlingen, Germany (EN 175 301-803-A)
2. Solenoid valve	Magnetic, Dash 3, 24 VDC, 0.6 W	Dynamco, Commerce, GA, USA (cat. no. D3533KL0)[a]
3. Base plate	For the solenoid valve	Dynamco (cat. no. B03B2B)[a]
4. Aluminum tube	Machined with lathe out of a cylinder shape to fit the thread of base plate from one end and the thread of Luer adapter from the other. Length 50 mm excluding the threads.	Self-manufactured
5. Luer lock adapter	Male	Cole-Parmer, Vernon Hills, IL, USA (cat. no. 31507-73)[a]
6. Swinney filter holder	13 mm plastic or 13 mm steel	Pall, East Hills, NY, USA (cat. no. 4317 or 4042)[a]
7. Spacer	Made out of the top of a 13 mm plastic Swinney filter holder and a plastic tube glued on it.	Self-manufactured
8. Push button switch	Attached to the timer, Type LUMOTAST 75.	Rafi, Berg/Ravensburg, Germany
9 Suitable box to fit the timer	Insides of a used computer power unit box were removed.	FSP Group Inc., Taoyuan City, Taiwan, ROC (cat. no. FSP250-60GTA), self-manufactured partly.
10. Clamp stand	To attach the gun	Commonly available
11. Timer	0–100 ms, 230 V, set to 100 ms	Megatron Electronics and Controls, Haifa, Israel (cat. no. MSST-700-CPT)[a]
12. Two glass fuses	For the timer, 5 mm × 20 mm IEC127 II, 100 mA	Camden Electronics Ltd, St. Albans, UK
13. A bottle of instrument helium	50 l, compressed at 200 bar	AGA, Espoo, Finland
14. A suitable valve for the gas bottle	Unicontrol 500 HT (helium/nitrogen)	AGA (cat. no. 213 007 280/309254)
15. A pipe tubing elbow	To connect the base plate to the gas tube, CL Compact 7522-8-1/4	Camozzi, Brescia, Italy
16. Suitable plastic tubing	For connecting the gas bottle to the base plate.	Commonly available
17. Electric wires	For connecting the timer to the push button and to the solenoid valve.	Commonly available

[a] According to Gaba and Gal-On (2005).

solenoid valve, base plate, Luer lock adapter and filter holders were those used by Gaba and Gal-On (2005). Other parts were either made of electronic supplies available commonly (push button, DIN plug, fuse) or were custom-made (timer box, lathed aluminum tube and spacer). HandyGun was assembled essentially as described (Gaba and Gal-On, 2005), however, many technical improvements were introduced. In the device of Gaba and Gal-On (2005), the timer was connected to the solenoid valve by a miniature DIN plug with a two-conductor electrical wire, and a second wire was used to connect the timer with an electrical switch (push button) fixed to the base plate. However, in HandyGun the timer and the push button are placed in a box (made of a computer power supply unit) (Fig. 1), which reduces loose wiring and makes the device handy to operate. The device is not hand-held but attached to a clamp stand which facilitates its use by leaving the operator's hands free for other tasks.

The adapters between the base plate and Luer adapter were replaced with a single aluminum tube. While the exact length and diameter of the tube are not crucial, thick tube walls and a minimum length of 50 mm facilitate attachment of the gun to a clamp stand. There is no secondary gas regulator needed in HandyGun. Fuses were introduced to the timer for safer use of the device. A Swinney filter holder made of steel was tested but a Swinney filter holder made of plastic was used finally, as described (Gaba and Gal-On, 2005) because it is less expensive (and no difference in infection rates was observed using the plastic and metal filter holders; data not shown).

An important addition to the original device (Gaba and Gal-On, 2005) was a spacer which defines and maintains a constant distance between the leaf and the filter holder between bombardments. Optimization of parameters for bombardment with a commercially available hand-held gene gun has shown that the distance between the nozzle of the gene gun and the target is one of the important determinants in efficiency of the bombardment (Kekarainen and Valkonen, 2001). Therefore, a plastic spacer for maintaining a distance of 2.5 cm between the nozzle of the filter holder and the leaf was introduced (Fig. 1). Furthermore, the spacer supports the leaf from the top, while a piece of cardboard can be held underneath to support the leaf from underside. The impact of bombardment pressure hitting the leaf could be reduced by using a spacer with holes drilled through sides of it (Fig. 1).

The infectious cDNA of PVA (pBUIII) placed under the 35S promoter was used for experiments (Paalme et al., 2004). pBUIII was transformed to *Escherichia coli* DH5α, grown overnight in 2 × 500 ml volume at 37 °C and purified by a Megaprep kit (Qiagen, Hilden, Germany) and used at a final concentration of 1.2 μg/μl sterile deionized water (SDW). Initially, the non-linearized plasmid DNA was coated on microprojectiles (gold particles, diameter 1 μm; BioRad, Hercules, CA, USA) by calcium nitrate [$Ca(NO_3)_2$] precipitation as recommended (Gaba and Gal-On, 2005). Gold particles (50 mg) were washed with 1 ml fresh 70% ethanol, sonicated in an ultrasonic bath (Finnsonic, Lahti, Finland) at 40 kHz for 5 min, vortexed for 5 s and centrifuged for 5 s with a microfuge. Subsequently, microprojectiles were washed three times with 1 ml of sterile glycerol (50%) and finally resuspended in 0.5 ml of 50% glycerol to a final concentration 0.1 mg/μl. Equal volumes of plasmid DNA (200 ng/μl), 1.25 M $Ca(NO_3)_2$ and microprojectile suspension were mixed together and used immediately. An aliquot of 2.5 μl of the mixture (167 ng DNA on 0.08 mg gold) was placed at the center of the filter holder grid, through which the particles were accelerated into leaf tissue using pressurized helium gas (3 bar). Alternatively, microprojectiles were coated with plasmid DNA using sodium acetate (NaAc) and polyvinylpyrrolidone (PVP40; Sigma, St. Louis, MO, USA). In this method, gold particles (5 mg) and 50 μg of plasmid DNA dissolved at 1.2 μg/μl SDW were mixed with 105 μl (2.5 vol) of 99.5% ethanol and 4.2 μl (1/10 vol) of 3 M NaAc (pH 7.0), or DNA was diluted and used at 0.25 μg/μl SDW to reduce aggregation of microprojectiles and 200 μl of the DNA solution was added to 500 μl gold/ethanol stock (10 mg/ml) and mixed with 20 μl of 3 M NaAc (pH 7.0). Subsequently, the DNA/gold mixture was incubated at room temperature for 10 min. Supernatant was discarded and gold particles were washed three times with 1 ml of 99.5% ethanol and resuspended in 250 μl of solution containing 0.05 mg PVP/ml 99.5% ethanol. The suspension of DNA-coated particles (5 μl, 1.0 μg DNA on 0.10 mg gold) was used for bombardment of leaves as above.

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