In Vivo Plant Experiment Via GDS-80

INTRODUCTION

Principle of the GDS-80 gene delivery system

Low pressure gene delivery system (GDS-80), designed according to the rocket nozzle principle and aerodynamic theory, is a novel system used to deliver the bio-particles into target cells. As in *fig.* 1, when there is an input gas pressure on the left, (i.e. Helium gas) an enormous pressure difference between the two chambers will form. Once the gas passes through the throat where the sample is loaded, the gas output of the GDS-80 gives bio-particles enough kinetic energy to accelerate them near supersonic speed (about 300 m/s). During the acceleration, the sample solution will be nebulized and sprayed evenly onto the surface of the target.

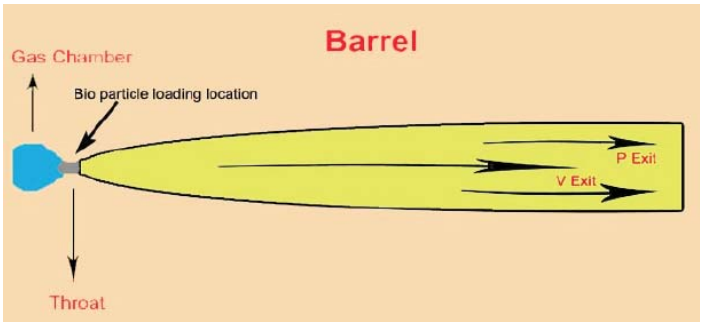


Figure 1. *Design theory of the GDS-80 delivery system.*

Factors that affect the transfection efficiency

While performing the bombardment with GDS-80, there are four major factors that affect the transfection efficiency. According to the design principle of a rocket nozzle, there are two main factors that affect the transfer result; delivery pressure setting and diameter of the barrel. By increasing the delivery pressure setting, the pressure difference within the two chambers becomes larger so that the speed of the bio-particles increases. Enlargement of the diameter of the barrel will lower the output speed of the particles. Delivery distance also plays an important part in the experiment. By

increasing the delivery distance from the barrel to target samples, bio-particles will be slowed down due to air resistance. It also reduces the possibility of damaging the targets. The last one is the total amount of delivered samples. The more amounts of samples that are delivered increase the transfer efficiency. However, adjusting the delivery distance and pressure are most important. The barrel size and the delivery sample amount should be kept at a constant making it easier to modify the experiment.

In vivo plant experiment

Genomic research toward the plant cells are now widely studied using many strategies. No matter what the main purpose of the study, it will apply to the living plant research in the end. There are many barriers while transferring genes into living plants, such as maintaining a micro-organism free environment, fragile tissue, delivery pressure setting and even the status of the plant growth. Through use of the GDS-80 system, Wealtec provides the easiest way to transfer the gene into living plants. By adjusting the delivery pressure and distance, GDS-80 can transfer the bio-particles into plant cells *in vivo* successfully.

MATERIALS

- *Nicotiana tabacum* and *Chenopodium Album* plants.
- Low-pressure gene delivery system, GDS-80. (Wealtec)
- Universal target spacer, UTS-10. (Wealtec)
- Plasmid DNA: Viral DNA construct with GFP reporter gene.
- Maestro™ *in vivo* imaging system. (CRi Inc.)

PROCEDURES

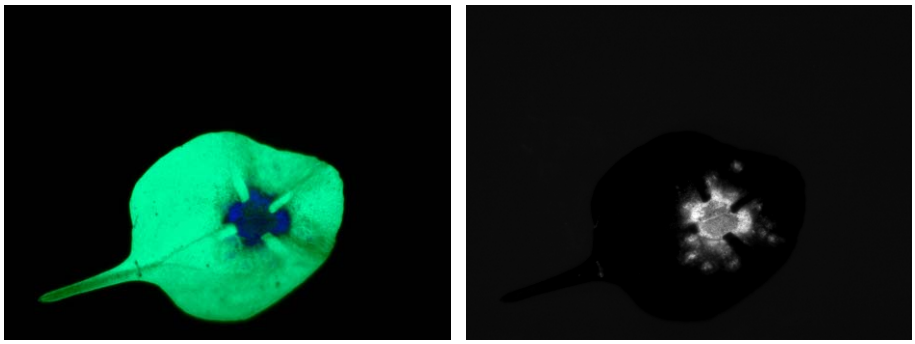
1. Plant the samples at least 30 days before experiment making sure the leaf is bigger than 5 x 6 cm^2.
2. Prepare the DNA coated gold solution with the following procedures:
   a. Add adequate sterilized distilled water with gold particle powder.

b. Wash gold particles with 100% EtOH for three times and discard the supernatant.
c. Add distilled water to resuspend the gold particles.
d. Apply DNA stock (1 μg/μl) into gold particle solution to form a concentration of 1 μg DNA/ 0.6mg gold particles.
e. Vortex the micro-centrifuge tube vigorously to mix the sample well.
f. Drop a proper volume of 0.1 M spermidine and 2.5 M $CaCl_2$ into the tube while continuously and vigorously vortexing.
g. Keep vortexing for one minute more.
h. Wash with 100% EtOH for three times and discard the supernatant.
i. Resuspend the DNA-coated gold particles in 100% EtOH.

3. Set up the GDS-80 system with UTS-10 attached as described in the instruction manual to have 40 ~ 50 psi of the deliver pressure.
4. Adjust the variable distance arm to have the delivery distance of 6 to 8 cm.
5. Clip the tetra-claw leaf clamp onto the target leaf and fix the leaf clamp with manual screw.
6. Aliquot 10 μl of DNA-coated solution into the sample loading hole.
7. Perform the bombardment onto the leaf samples.
8. Incubate the plant for more than three days to observe the fluorescence result by using the CRi Maestro™ *in vivo* imaging system.

RESULT

(A) *Nicotiana tabacum* (B) *Nicotiana tabacum*



(C) *Chenopodium Album* (D) *Chenopodium Album*

Figure 2. *Bombarded leaves after 3 days incubation. Pictures on the left are captured with true-color image and with fluorophores-of-interest image on the right through CRi Maestro™ in vivo imaging system.*

(A) *Nicotiana tabacum* (B) *Nicotiana tabacum*

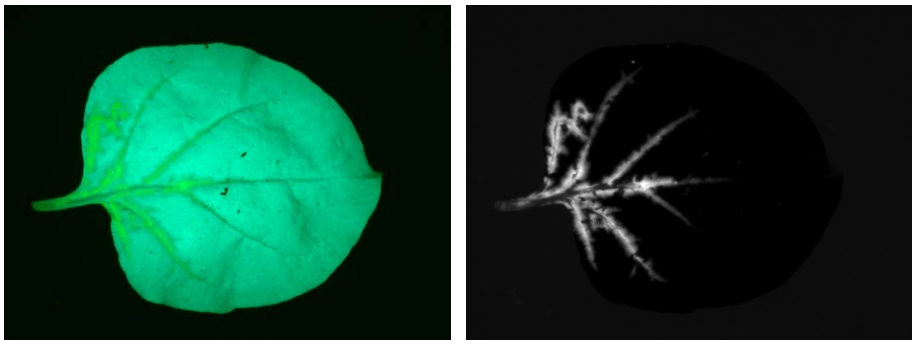


Figure 3. *Leaf which on the same plant with bombarded leaf after 7 days incubation. Pictures on the left are captured with true-color image and with fluorophores-of-interest image on the right through CRi Maestro™ in vivo imaging system.*

DISCUSSION

Characteristic and the composition of living plants tissues vary from plant to plant. While bombarding the living plants, there are many conditions that need to be consider in regard to the plant such as the phase of the plant growth, plant size, damage toward the tissue as well as which portion to deliver. Operators need to modify the transfer condition based on the characteristic of the tissue while delivering the bio-particles onto plant cells.

In this article, the structure of *Chenopodium*'s leaf is tougher than *Nicotiana*'s. It is better to shorten 1 to 2 cm of the delivery distance or to increase the deliver pressure to get a better result while transferring onto *Chenopodium*'s leaf. In *fig.* 2, the transfer condition of *Chenopodium*'s leaf is 50 psi with 6 cm distance and 40 psi with 8 cm for the *Nicotiana's* leaf with the help of UTS-10 accessory. However, the transfer efficiency is still better on the *Nicotiana*'s leaf after shortening the distance. No matter what delivery condition is performed, the rule that should be followed is tissues should not be damaged to wither after bombardment. The expression after 7 days incubation, since there has viral DNA constructed with GFP reporter gene in the plasmid DNA, the GFP gene can be expressed and spread all over the plant. In the *fig.* 3, different leaves on the same plant that were bombarded were infected by GFP gene through the capillary bundles. If incubated for longer period of time, the gene can be spread all over the entire plant.

It is very easy to adjust the delivery parameters by mainly adjusting the deliver pressure and the distance. GDS-80 is a very powerful tool to deliver gene onto living plants. With the assistant of UTS-10, whether the experiment is performed *in vivo* or *in vitro*, users can finish their plant targeting experiments more easily.

Yi-Ta, Chen
Product Manager
Email: info@wealtec.com

Wealtec Bioscience Co. Ltd.
27Fl. No. 29-1 Sec.2, Jungjeng E. Rd., Danshuei Jen, Taipei, Taiwan 25170
TEL: +886-2-8809-8587 FAX: +886-2-8809-8589; http://www.wealtec.com