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Transient Expression of the β-Glucuronidase Gene in Shoot Primordia of *Haplopappus gracilis* by Use of a Pneumatic Particle Gun

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Expression of the β-glucuronidase (GUS) gene in shoot primordia of *Haplopappus gracilis* was obtained by particle bombardment. The efficiency of gene delivery was tested by accelerating the pressure from 115 to 200 kg/cm^2, the number of bombardments from one to three, the amount of gold particles from 0. 1 to 0. 4 mg per projectile, and the amount of plasmid DNA from 0. 2 to 0. 8 μg/mg gold particles. Double bombardments of 200 kg/cm^2 with projectiles having 0. 2 mg of gold particles coated with 8 μg DNA/mg of gold gave 884±103 blue spots per petri dish (5. 2 cm diameter).

Introduction

Particle bombardment[1] has been proved useful for the transformation of plant cells. Shoot primordia[2] are suited for particle bombardment-mediated transformation because they can be proliferated clonally while maintaining genetic stability and are easily regenerated into plantlets[3]. No reports of gene delivery into shoot primordia by particle bombardment, however, have been published. We here report the successful expression of the β-glucuronidase (GUS) gene in shoot primordia of *Haplopappus gracilis* by particle bombardment.

Materials and Methods

1. *Plant materials and plasmid DNA*

Shoot primordia of *Haplopappus gracilis* were initatad as described earlier[2], and subcultured in a test tube (30 mm×200 mm) with Murashige and Skoog (MS) medium[4] containg 3% sucrose and 2. 0 mg/*l* 6-benzylaminopurine (BAP) every 2 weeks at 22°C under continuous illumination of 2, 000 to 10, 000 lux, on rotary culture equipment (2 cycles/min.). Chimaeric plasmid DNA, pBI 221 (Clontech, Palo Alto/CA), which has the β-glucuronidase (GUS) gene under the control of the cauliflower mosaic virus 35S (CaMV 35S) promoter and nopaline synthetase (NOS) polyadenylation signal, was used.

2. *Gene delivery to shoot primordia*

The pneumatic particle gun device and the method used for coating the plasmid DNA on the gold particles (1. 1 μm in diameter, Tokuriki Honten Co., LTD., Tokyo, Japan) were essentially as

reported previously[5]. Standard bombardment conditions were as follows : acceleration pressure of the projectile, 200 kg/cm^2; the amount of DNA-coated gold particles, 0. 2 mg per projectile ; and the amount of plasmid DNA, 4 μg/mg of gold particles. A single bombardment was given the tissue sample unless otherwise stated. The distance between the sample and the stopper was fixed at 10 cm. Five to nine days after subculture, shoot primordia were placed so as to form a circle (3. 5 cm diameter) on agar that contained the culture medium (0. 8%) in a plastic petri dish (5. 2 cm internal diameter) then bombarded.

3. *Assay for GUS expression*

After bombardmant, the shoot primordia were kept on the agar medium and cultured for 24 h, after which they were transferred to plastic well plates (Corning Co., Cell Wells) containing 1 m*l* of filter-sterilized GUS substrate mixture. The substrate mixture consisted of 0. 5 mM potassium ferricyanide, 0. 5 mM potassium ferrocyanide, 1. 9 mM 5-bomo-4-chloro-3-indolyl-β-D-glucuronide (X-gluc, the substrate of GUS) (Sigma Chemicals), and 0. 3% (v/v) Triton X-100. Samples were incubated for 24 h at 37°C, after which 3 m*l* of 70% (v/v) ethanol was added to the tissue-GUS substrate mixture to stop the reaction and to maintain the aseptic condition. GUS-expressing cells were detectd as blue-colored spots. The number of these spots was counted under a binocular microscope (×40, Nikon, SMZ-10).

Results and Discussion

Fig. 1 shows typical results of the GUS assay of shoot primordia of *Haplopappus gracilis* that had been bombarded with DNA-coated or non-coated gold particles. The many blue spots indicate that GUS - expressing cells are present in the shoot primordia bombarded with DNA - coated gold particles (**Fig. 1-B**) ; whereas, none are present in the control *H. gracilis* cells bombarded with non-coated gold particles (**Fig. 1-A**). These results show that particle bombardment provided successful delivery of plasmid DNA and expression of the gene in the shoot primordia of *H. gracilis*.

At high magnification, many blue spots can be seen on the tips of shoot primordia (**Fig. 2**). Each blue GUS-expressing cell is comprised of a densely stained blue central core and a less densely stained region that extends radially from the core. Similar results have been reported in suspension-cultured cells of tobacco[5] and maize[6], and in the leaves and roots of *Arabidopsis thaliana*[7].

The numbers of blue spots present after a single bombardment at accelerating pressures of 115, 150, and 200 kg/cm^2 respectively were 146±64, 279±55, and 349±85 (mean of 3 experiments ± SD). An accelerating pressure of 200 kg/cm^2 therefore gave the best results for *H. gracilis* shoot primordia.

As reported elsewhere, optimal accerating pressure varies depending on the type of plant cell or tissue, being 150 kg/cm^2 for cultured tobacco cells[5], and 115 to 150 kg/cm^2 for leaves and 150 to 200 kg/cm^2 for roots of *A. thaliana*[7]. These results clearly indicate the importance of optimizing the acceleration pressure in bombardment-mediated transformation.

When a sample of *H. gracilis* shoot primordia was bombarded one to three times at an accelerating pressure of 200 kg/cm^2, the respective numbers of blue spots after the single, double, and triple bombardments were 349±85, 816±106 and 570±85. Double bombardment gave the best results; whereas, triple bombardment produced fewer blue spots. This differs from the results reported by Wang *et al.*[8] for suspension-cultured cells of rice and wheat, in which the number of blue cells increased with the number of bombardments up to three.

Two bombardments of 200 kg/cm^2 each were given to a sample tissue, the amount of DNA-coated gold particles being changed from 0. 1 to 0. 4 mg per projectile. The number of blue spots

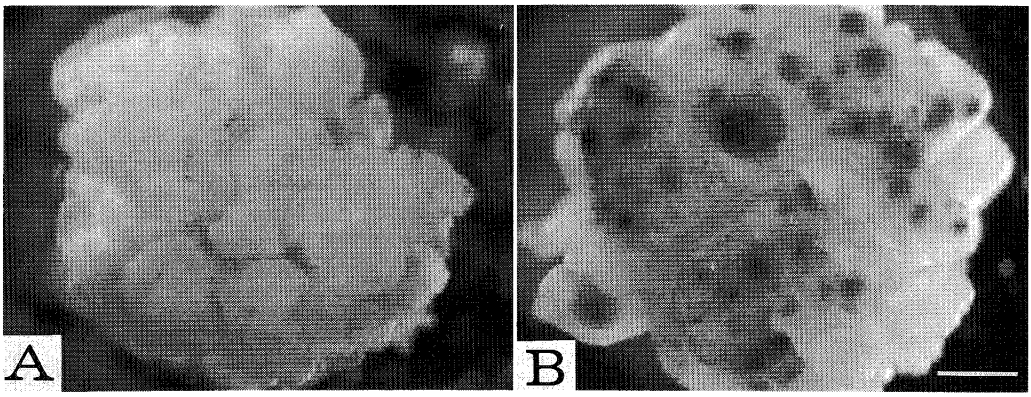

Fig. 1 Microphotographs showing GUS expression in bombarded shoot primordia of *Haplopappus gracilis*.
Shoot primordia were bombarded under optimal bombardment conditions with DNA-coated (B) and non-coated (A) gold particles then assayed for GUS expression. A number of blue spots that represent GUS-expressing cells are present in B, but none in A. Scale bar=1 mm.

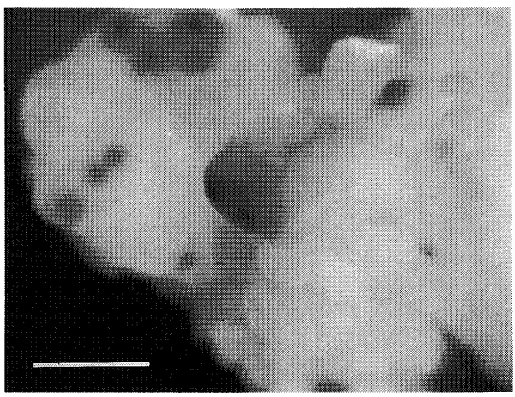

Fig. 2 Microphotograph of the blue spots of GUS-expressing cells on the tip of a shoot primordium of *Haplopappus gracilis*.
Scale bar=0.5 mm.

produced were a functicn of the amount of gold particles ; 254±52, 816±106 and 306±37, respectively, for 0.1, 0.2 and 0.4 mg gold particles per projectile. Thus, under these bombardment conditions, 0.2 mg gold particles per projectile gave the best results for *H. gracilis* shoot primordia.

The effect of the amount of DNA used also was studied. Two bombardments of 200 kg/cm^2 each with 0.2 mg gold particles per projectile were given a sample tissue, the amount of DNA used being varied from 2 to 8 μg/mg gold particles. The respective numbers of blue spots were 851±116, 816±106 and 884±103 for 2, 4 and 8 μg/mg gold particles. Although the highest amount of DNA (8 μg/mg gold particles) gave the best results, the amount of DNA in the range used did not markedly effect GUS-expression efficiency in *H. gracilis* shoot primordia. This differs from our previous results for cultured tobacco cells obtained with a nitrogen-pressure-driven particle gun device[9].

In the light of the present results, the tentative optimal bombardment conditions for shoot primordia of *H. gracilis* are two bombardments of 200 kg/cm^2 with projectiles having 0.2 mg of gold particles coated with 8 μg of DNA/mg gold. We are currently studying the production of transgenic

H. gracilis plants produced by bombardment-mediated transformation of shoot primordia. Results will be published elsewhere.

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《和文要約》

圧縮空気圧式パーティクルガンによるハプロパップス苗条原基における
GUS 遺伝子の一過的発現

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パーティクルガン法によりハプロパップス苗条原基へ GUS 遺伝子を導入し，一過的発現に成功した．加速圧力 200 kg/cm², 0.2 mg 金粒子/プロジェクタイルおよび 8 μg DNA/mg 金粒子の撃ち込み条件で 2 回撃つと，1 シャーレあたり最大 884±103 (3 回の実験の平均値±標準偏差)個の青色スポットが得られた．