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In Planta Agrobacterium-Mediated Transformation of Adult Arabidopsis Thaliana Plants Using pZp-Gus Plasmid

KEYWORDS

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ABSTRACT *Transformation of Arabidopsis thaliana was performed mediated by Agrobacterium tumefaciens medhod. We used adult plant of Arabidopsis because the tissue culture method and somaclonal variation could be avoided. We obtain 19 transformants and we could detect it by naked eye. This method could be used for others herbs plant.*

Introduction

Arabidopsis thaliana was transformed by directly applying Agrobacteria to the plant and recovering transformants in the progeny. This procedure offer two main advantages: tissue culture and resulting somaclonal variations are avoided and only a short time is required in order to obtain entire transformed individuals.

The following research makes use of adult plants that was infiltrated with Agrobacterium at the reproduction stage. Screening of transformants were performed *in vitro*, in growth chamber and by histochemical GUS assay.

Arabidopsis thaliana (L.) heyn. Ecotype Wassilevskija was used to perfect the infiltration protocol. Agrobacterium strain EHA 101 carrying the binary vector plasmid pZp-GUS was used. In the binary plasmid, the β-glucuronidase (*gus A*) reporter gene is nested and used as marker in transformation.

Material and Method

Material

Seed of Arabidopsis thaliana (L.)

Method

Growth of Arabidopsis plants Healthy Arabidopsis plant was grown under long days in pots (diameter: 8 cm) in soil and covered with mesh. A first blot was clipped. Plant was ready 4-6 days after clipping.

Transformation of a plasmid to Agrobacterium 5 µl of PEG-purified (or mini-preped) DNA was layered on top of 100 µl frozen Agrobacterium EHA 101 competent cell. Incubation is at 37°C for 5 minutes. Then, 1 ml of YEP broth was added. Subsequently, it was shaked at 28°C for 4 hr. Centrifugation was carried out at 12,000xg for 2 minutes. Supernatant was removed and the precipitate was resuspended in 100 µl of YEP broth. Then, 50 µl of the cell suspension was spreaded on a YEP plate containing 100 µg/ml Kanamycin (Km) and 100 µg/ml Spectinomycin (Sp). Incubation of the plate was performed at 28°C for 2 days. A single colony was picked-up and spreaded to a new YEP plate containing Km and Sp. Incubation of the plate was at 28°C for 1 day.

Result

Transformation of a plasmid to *Agrobacterium*

Plasmid of pZp-GUS was successfully transformed into *Agrobacterium tumefaciens* EHA 101 confirmed by the occurrence of several colonies in YEP plate containing 100 µg/ml Kanamycin (Km) and 100 µg/ml Spectinomycin (Sp) (Figure 1). A single colony was picked up and spreaded onto new YEP plate containing Km and Sp (Figure 2).

Infiltration

After preparation of Agrobacterium culture solution with OD 0.8-1.2, fertilized siliques from Arabidopsis plants were removed and the Agrobacterium solution was infiltrated into the Arabidopsis plant (transformation). The plants were then watered moderately until maturity and dry seeds were harvested after 4 weeks.

Screening of transformants

Firstly, the seeds were sowed on selection medium (MS half strength, B5 vitamin, supplemented with 50 µl/ml gentamycin and 100 µl/ml carbenicillin) and put in growth chamber (28 degree C) for 18 days. Nine teen transformants of Arabidopsis were growing among non transformed plants and planted on the soil for 17 days (Figure 3). Subsequently, histochemical GUS assay was performed. It was observed that 2 transformants (Plants no. 11 and 16) showed very strong activity of GUS gene. It was indicated by intense blue staining in leaves tissues. The rest showed low activity of GUS gene.

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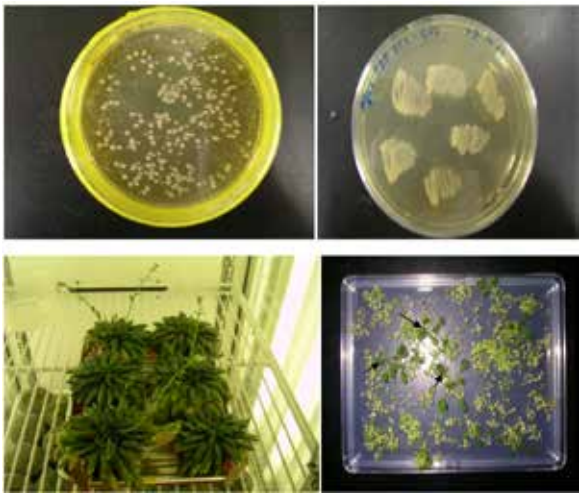

Figure 1. Agrobacterium tumefaciens grew successfully. Fig 2. Single colony of A. tumefaciens was spreaded into fresh YEP plate containing Km and Sp. Figure 3. Adult plant of Agrobacterium ready for transformation.4. Transformant of Arabidosis (arrow) are bigger and green.

We cheked the laf of ranformants and they shows GUS gene expressions.

Discussion

Since this method has been proven efficient and rapid, further research regarding In Planta Agrobacterium-Mediated Transformation of Adult *Arabidopsis thaliana* Plants using another gene will be performed.

To the best of our knowledge, we report a first experiment in transformation of adult Arabidopsis thaliana using pZp-GUS plasmid.

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