Available online at www.sciencedirect.com
ScienceDirect
Agricultural Sciences in China
2009, 8(6): 658-663
June 2009

A Simplified Seed Transformation Method for Obtaining Transgenic *Brassica napus* Plants

SONG Li[1,2], ZHAO De-gang[1,2,3], WU Yong-jun[3] and TIAN Xiao-e[3]

[1] *Guizhou Key Laboratory of Agricultural Bioengineering, Guizhou University, Guiyang 550025, P.R.China*
[2] *Key Laboratory of Green Pesticide and Agricultural Biological Engineering, Ministry of Education/Guizhou University, Guiyang 550025, P.R.China*
[3] *College of Life Sciences, Guizhou University, Guiyang 550025, P.R.China*

Abstract

We report here a seed transformation of sonication-assisted, no-tissue culture to rapidly produce transgenic *Brassica napus* plants. This method comprises the steps of treating seeds by ultrasonic wave, inoculating *Agrobacterium tumefaciens* with a recombinant *ChIFN-α* gene and germinating directly of treatment seed on wet filter papers. The obtained transformants were verified by GUS histochemical assay and nested PCR amplification. It suggests that seed transformation has a potential use in genetic transformation of rape.

Key words: *Brassica napus*, seed transformation, sonication

INTRODUCTION

Plant transgenic technique is a powerful tool to introduce the desired gene(s) into receptor in the plant bioreactor, which focuses easily on producing large amounts of recombinant protein in a short time. Oilseed rape (*Brassica napus* L.) is a potential bioreactor of pharmacological products for large area planting and easily separating recombinant protein. Transgenic plants were so far produced primarily from tissue culture of rape cotyledon petioles (Moloney *et al*. 1989; Damgaard *et al*. 1997; Zhang *et al*. 2006) and hypocotyl segments (Cardoza and Stewart 2003; Ramzan Khan *et al*. 2003; Peng *et al*. 2006; Zhang *et al*. 2006) by single *Agrobacterium*-mediated transformation, which was in general labour-intensive, time-consuming and relatively expensive. Using single *Agrobacterium*-mediated transformation, a series of tissue culture including co-cultivation, callus induction, shoot initiation, and root inducing is required to culture tissue cell and often 2-5 months are required to obtain complete transgenic plantlets in canola (Ponstein *et al*. 2002; Das *et al*. 2006). Tissue culture procedures also have some other adverse effects, such as somatic mutations (Rakoczy-Trojanowska 2002), plant chimera, losing plants in transplanting and complicated culture medium. Therefore, it is desired for developing a gene transfer of no-tissue culture to build up oilseed rape bioreactor.

The direct seed transformation is an alternative procedure as that transgene can go without tissue culture steps and generate large numbers of transgenic plants rapidly. The microscopic injuries caused by ultrasound etching on the surface and internal layers of the targeted tissue provide a channel for the DNA transfer from bacterium to plant in the infection process (Joersbo and Brunstedt 1992; Santarém *et al*. 1998; Beranová *et al*. 2008). The successful produc-

Received 5 December, 2008 Accepted 2 February, 2009
SONG Li, Ph D candidate, Tel: +86-851-3865027, Fax: +86-851-3863615, E-mail: lpsssl@126.com; Correspondence ZHAO De-gang, Ph D, Professor, +86-851-3865027, Fax: +86-851-3863615, E-mail: dgzhao@gzu.edu.cn

doi:10.1016/S1671-2927(08)60261-8

tion of transgenic plants via sonication-assisted *Agrobacterium*-mediated transformation (SAAT) has been reported in different plant species such as flax (Beranová *et al*. 2008), soybean (Santarém *et al*. 1998) and *Chenopodium rubrum* (Flores Solís *et al*. 2003) with hypocotyl, cotyledon, and seedling explants. But the SAAT method using seed explant has not been described for rape.

Chicken alpha interferon (ChIFN-α) possess powerful, and wide-range of antiviral properties, antiproliferatives, and immunoregulatory functions in birds (Schultz *et al*. 1995; Plachý *et al*. 1999; Jarosinski *et al*. 2001; Ruttanapumma *et al*. 2005). However, use of ChIFN-α was limited due to the difficulties in mass production. In order to establish an efficient plant system to produce interferon economically, we have previously shown the bioactive recombinant ChIFN-α in lettuce plant (Song *et al*. 2008). We report herein a successful seed transformation by a SAAT method for the further study of oilseed rape bioreactor. In this work, transgenic *B. napus* were generated only in 45-60 days after germinating directly from seed explants treated with ultrasonic, which didn't need tissue culture and regeneration processes.

MATERIALS AND METHODS

Expression of plasmid and bacterial strains

We constructed the plant expression vector pSFIFN-α containing *ChIFN*-α gene, reporter gene β-glucoronidase (*GUS*), and selectable neomycin phosphotransferase (*NPT*) gene for kanamycin resistance in our previous work (Song *et al*. 2008). These genes were under the control of *Cauliflower mosaic* virus (CaMV) 35S promoter and poly A terminator in the T-DNA region. The plasmid pSFIFN-α vector was mobilized into *Agrobacterium tumefaciens* strain EHA105 for the eventual transformation.

Plant materials

Mature seed of oilseed rape (*Brassica napus* L.) line Youyan 10, a local bushy cultivar, was obtained from Oil Crops Institute, Guizhou Academy of Agricultural Sciences, Guiyang, China. Healthy seeds of uniform dimension were used in the experiment.

Transformation procedure

B. napus seeds were initially dipped in 75% ethanol for 1 min, and then disinfected with 0.1% (v/v) mercuric chloride for 15 min. After being rinsed thoroughly five times with sterile distilled water to remove the mercuric chloride, the seeds were put in a 50 mL of sterile centrifuge tube containing 20 mL of sterilized water placed in an ice bath, and were sonicated repetitively with 10 min on and 10 min off for a period of time in an MSE Soniprep150 sonifier (Sanyo, Sussex, UK). 5 and 10 μm of amplitudes and 0.5, 2.0, and 2.5 h of ultrasonic time were performed respectively in the experiments. The seeds produced were kept in the 25 mL final OD_{600} 0.6-0.8 liquid culture of *A. tumefaciens* EHA105 bearing plasmid pSFIFN-α for *ChIFN*-α gene expression in an XMTD digital homoeothermic water bath (Dongfang, Yuyao, China) at 28°C for 1 or 20 h. The period of 0 μm of amplitude and 0 h of infection with *Agrobacterium* (no sonication and sonication without *Agrobacterium* inoculation applied) were used as a control variant in the experiments.

Plant growth and selection

After inoculating with *Agrobacterium* solution for 1 or 20 h, excess *A. tumefaciens* on the treated seeds were removed by blotting on a sterile filter paper. The seeds were subsequently sown on sterilized moistened wet filter paper in Petri dishes for 2 days co-cultivation in the dark at 27°C. Then they were washed with tap water and grown at 27°C in a 16 h light/8 h dark photoperiod. When the plumules were around 3-5 cm, they were directly transferred to 8-cm pots containing a commercial soil mixture (Klasmann, Germany). Due to the presence of *NPT* gene in the plasmid pSFIFN-α, 15 mg L^{-1} of kanamycin was used to smear on the leaf for selecting transgenic *B. napus* each day. After 35 days, the plants survived were transferred to the greenhouse for full development.