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Genetics Transformation of *Carica papaya* by Infecting Mature Zygotic Embryos with *Agrobacterium tumefaciens* Strains LBA-4404

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Abstract: Transient expression of the *GUS* gene has been demonstrated in somatic callus of the two varieties of *Carica papaya* cv. Shahi and Ranchi after co-cultivation with *Agrobacterium tumefaciens* strain LBA-4404 carrying a binary plastid vector system containing neomycin phosphotransferase gene as the selectable marker and β-glucuronidase (*GUS*) as reporter gene. The mature embryonal axes were used as explants. The co-cultivated explants were transferred into final selection medium containing 500 mg L^{-1} carbenicillin + 200 mg L^{-1} cefotaxime + 50 mg L^{-1} kanamycin. The callus of *C. papaya* cv. Shahi showed highest *GUS* activity compared to *C. papaya* cv. Ranchi. The anatomical section of callus showed the positive *GUS* activity. All transformed callus grew vigorously in this medium and formed embryos followed by plantlets.

Key words: Genetics transformation, papaya, *A. tumefaciens*, kanamycin

INTRODUCTION

Papaya is an important fruit crop of Bangladesh and available throughout the year. The main problem of papaya production in this country is the attack of Papaya Mosaic Virus (PMV). The attack of PMV deteriorates the fruit quality, and make those unfit for human consumption. The papaya mosaic virus resistant variety can be developed through genetic transformation system for papaya using viral Coat Protein (CP) gene[1]. Transformation of papaya developed by co-cultivating papaya discs, stems and petioles with *Agrobacterium tumefaciens* strains GV3111[2]. The transgenic papaya expressing the coat protein gene showed high levels of resistance against the severe papaya ringspot virus isolates collected from Hawii[3]. The present study has, therefore, been undertaken to develop the protocol for genetic transformation of papaya using *Agrobacterium tumefaciens* strain LBA-4404.

MATERIALS AND METHODS

The seeds of two varieties of *Carica papaya* cv. Shahi and Ranchi were washed with detergent and then with the help of tap water for several times. The seeds were disinfected with 0.2% $HgCl_2$ for 5-10 min followed by five times rinse with sterilized distilled water. The embryonal axes were separated from seeds for inoculation. The medium was half strength MS salt supplemented with 5 mg L^{-1} nicotinic acid, 1.0 mg L^{-1} pyridoxin, 0.5 mg L^{-1} thiamine HCl, 100 mg L^{-1} myo-inositol, 1.0 mg NAA, 0.5 mg kinetin, 160.0 mg adenine sulphate, 1.0 mg GA_3, 1.0 g L^{-1} casein hydrolysate and 30 g sucrose per litre. *Agrobacterium tumefaciens* strain LBA-4404 was grown on Luria-Bertani (LB) medium containing 15 g NaCl, 10 g peptone, 5 g yeast extract and 50 mg kanamycine per litre at 28^0C. A single colony of *Agrobacterium tumefaciens* was inoculated in 10-15 mL liquid LB medium containing 50 mg L^{-1} kanamycin and grown at 28°C on a gyratory shaker (180 rpm) to an OD_{550} of 0.8. Mature zygotic embryos axes were immersed in *Agrobacterium tumefaciens* LBA-4404 for one minute with gentle agitation under laminar air flow cabinet. The explants were blotted on sterile filter paper and co-cultivated with *Agrobacterium tumefaciens* on regeneration medium for 6 h. Co-cultivated explants were transferred into regeneration medium containing 500 mg L^{-1} Carbenicillin and 200 mg L^{-1} cefotaxime for one week. After one week, the explants were transferred to regeneration medium containing 200 mg L^{-1} cefotaxime + 50 mg L^{-1} kanamycin for callus induction.

GUS staining: β-glucuronidase activity was assayed histochemically using a small amount of callus. Callus was stained by placing the tissue in 1 mL X-gluc staining phosphate buffer in a small vial. The sample was incubated at 37°C over night and examined under stereomicroscope for the evidence of blue colour in transformed sample.

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RESULTS AND DISCUSSION

The embryonal axis was suitable explant for genetic transformation which was reported by Rabbani[4]. The explants were placed into regeneration medium after infection with *A. tumefaciens* strains LBA-4404 for co-cultivation. The co-cultivated explants were transferred into regeneration medium containing 500 mg L^{-1} carbenicillin + 200 mg L^{-1} cefotaxime after 2 days. During cocultivation the explants became swollen and the bacteria engulfed the some of the explants. Then the explants were transferred into final selection medium containing 500 mg L^{-1} carbenicillin + 200 mg L^{-1} cefotaxime + 50 mg L^{-1} kanamycin. The maximum number explant responded in *C. papaya* cv. Shahi than *C. papaya* cv. Ranchi during the acclimatization (Table 1) Upon subculturing on same medium, the infected explants were gradually increased to form callus. The callus formation (%) was higher in *C. papaya* cv. Shahi than *C. papaya* cv. Ranchi. Some of the transformed callus and non-transformed callus were stained histochemically for the detection of GUS expression. Blue colour indicated that the transformation of genes were positive results under sterio-microscope (Fig. 1). The anatomical section of callus also showed the positive GUS activity (Fig. 1). The callus of *C. papaya* cv. Shahi showed highest GUS activity compared to *C. papaya* cv. Ranchi. The control callus did not show blue colour during the study. Ficth *et al.*[2] reported that hypocotyls of papaya were co-cultivated with *A. tumefaciens* containing binary cosmid vectors pGA482GG showed the similar results. Pang and Sanford[2] also reported the similar results from petiole explant of papaya after co-cultivation with *A. tumefaciens* containing LBA-4404. The highest somatic embryos and plantlets were obtained from explant of *C. papaya* cv. Shahi infected by *A. tumefaciens* strain LBA-4404.

Table 1: Transformation efficiency and *GUS* activity of two papaya varieties after infection by *A. tumefaciens* stains LBA-4404

Genotypes	Survivability (%)	Callus formation (%)	No. of GUS positive explants (%)	No. of somatic embryo produced/ explant	No. of regenerated plants/ explant
C. papaya cv. Shahi	5	43	26	8	5
C. papaya cv. Ranchi	4	38	22	7	4

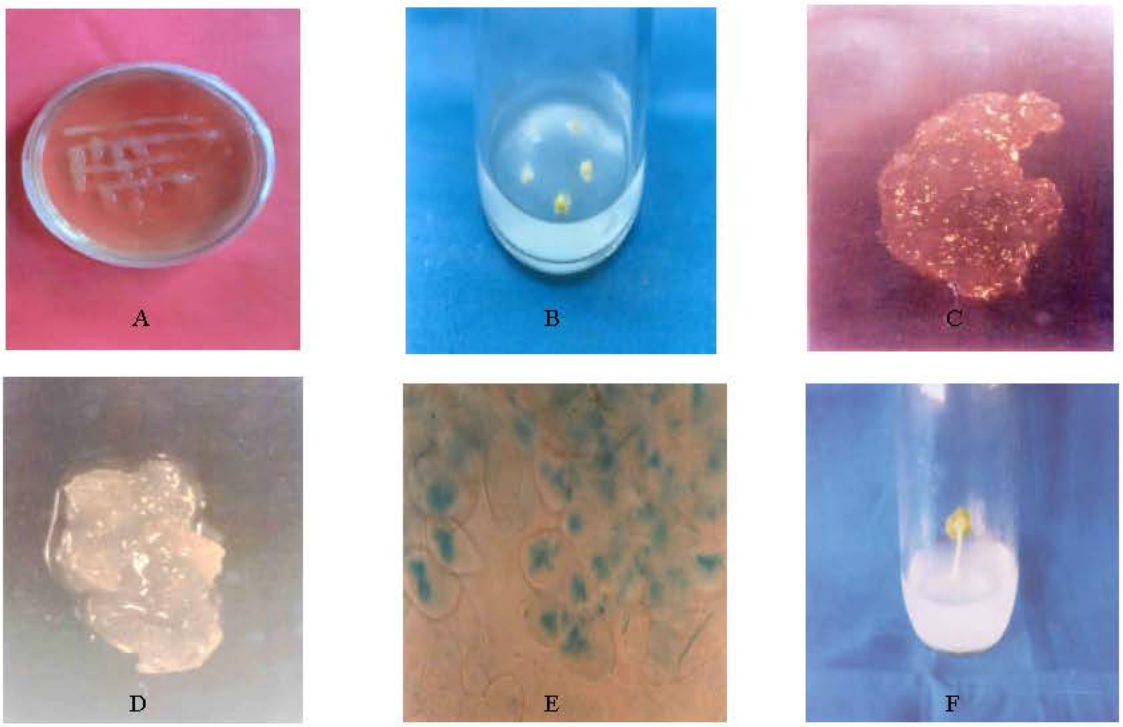

Fig. 1: A) Colony of *Agrobacterium tumefaciens*, B) Embryogenic callus obtained from embryo axis infected by *Agrobacterium tumefaciens*, C) Genetic transformed callus, D) Non genetic transformed callus, E) Cross section of genetic transformed callus and F) Transgenic plantlet

Selection of kanamycin resistant transformants: Some of the transformed callus and non-transformed callus were cultured into medium containing 50 mg L^{-1} kanamycin. All transformed callus grew vigorously in this medium and formed embryos followed by plantlets. But non-transformed callus did not grow and turned into black in this medium. This results indicated that kanamycin resistant genes were inserted in the explants and gave the positive result. Pang and Sanford[2] reported that 86% explants of papaya formed callus in the medium containing kanamycin and control explants did not form callus. Our results indicated that *A. tumefaciens* strain LBA-4404 infection of mature embryo of *C. papaya* cv. Shahi was more efficient genetic transformation compared to *C. papaya* cv. Ranchi.

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