Global Journal of Biotechnology & Biochemistry 5 (1): 41-43, 2010
ISSN 2078-466X

Agrobacterium Tumefaciens-Mediated Transformation and Gus Gene Expression in Physalis Minima

[1]E. Sheeba, [2]S. Parvathy and [2]S. Palanivel

[1]E. Sheeba, Department of Microbiology,
J.J College of Arts and Science, Pudukottai, Tamilnadu, India -622404.
[2]Government Arts College, Karur, Tamilnadu, India

Abstract: Introduction of foreign genes in to plant tissues via *Agrobacterium tumefaciens* based vectors requires specific knowledge of *Agrobacterium*-host compatibility. Transformation of *Physalis minima* was achieved using *Agrobacterium tumefaciens* strain LBA 4404 pB19. The concentration of the cefotaxime 250 mg/l and kanamycin, 75 mg/l for the best growth of *Physalis minima.* Molecular analysis of the transformed plants has done by PCR techniques.

Key words: Transformation · GUS gene · *Agrobacterium tumefaciens* · *Physalis minima*

INTRODUCTION

Among the various approaches for integrative transformation, *Agrobacterium* mediated technique is most widely used. Gene transfer to plants via *Agrobacterium tumefaciens* is an efficient way to introduce desirable traits into crop plants. This transformation process depends on the combined action of bacterial and plant genotypes. The genus *Agrobacterium* belongs to the family Rhizobiaceae. *Agrobacterium tumefaciens* is a Gram negative, rod shaped, motile, aerobic and soil borne bacterial pathogen. A reporter gene encodes an enzyme with an easy assayable activity that is used to report on the transcriptional activity of a gene of interest. In order to use GUS as a reporter of promoter activity, a transgenic plant containing the promoter-gus A chimeric gene is made. Advantages of GUS gene are there is no detectable background level of β-glucoronidase activity in most higher plants, the enzyme is very stable and can be assayed at any physiological pH, with an optimum pH between 5.2 and 8.0 and β-Glucuronidase catalyzes the cleavage of a wide variety of β- glucuronides, including many chromogenic, fluorogenic substrates, thus allowing the histochemical, spectrophotometric and fluorometric measurements of GUS gene fusion expression. In the present investigation, the transformed nature of plant was studied and confirmed by PCR.

MATERIALS AND METHODS

Chemicals, Plant Material and Bacterial Strain: All of the chemicals used in this research were taken from authentic samples mainly purchased from Sigma Aldrich. The *Physalis minima* seeds were obtained from Kerala. The sterilized seeds of *Physalis minima* germinated on solid hormone free Murashige and Skoog (MS) basal medium (pH 5.8, sucrose 30g/l, agar 10g/l) at 25 ±1°C. The resulting plantlets were maintained under the laboratory conditions for further use.

Agrobacterium tumefaciens LBA 4404 pB19 was provided by the bank of Microbes at Jain Institute of Vocational and Advanced Studies (JIVAS, Bangalore, Karnataka). The growth profile of *A. tumefaciens* was determined in different media preparations at 600nm after 48 hours at 28°C. Media used for the growth profile of *Agrobacterium tumefaciens* were LB Broth, Nutrient Broth, Tryptone, Yeast Mannitol Broth and Yeast Extract Peptone. *Agrobacterium tumefaciens* was proliferated in LB medium with Rifampicin (10µg/ml) and kanamycin (50µg/ml) for 48 hours on a shaker at 131 rpm and 28°C.

Plant Infection: Internodes, leaf and petiole explants of 0.5-2.0cm long were cut from the two or three week old *Physalis minima* plantlets. No specific physical or chemical treatment was carried out the explants prior to the bacterial infection. The explants were immersed in 50ml

Correspoding Author: E. Sheeba, Department of Microbiology, J.J College of Arts and Science, Pudukottai, Tamilnadu, India -622404. Tel: 919739153372

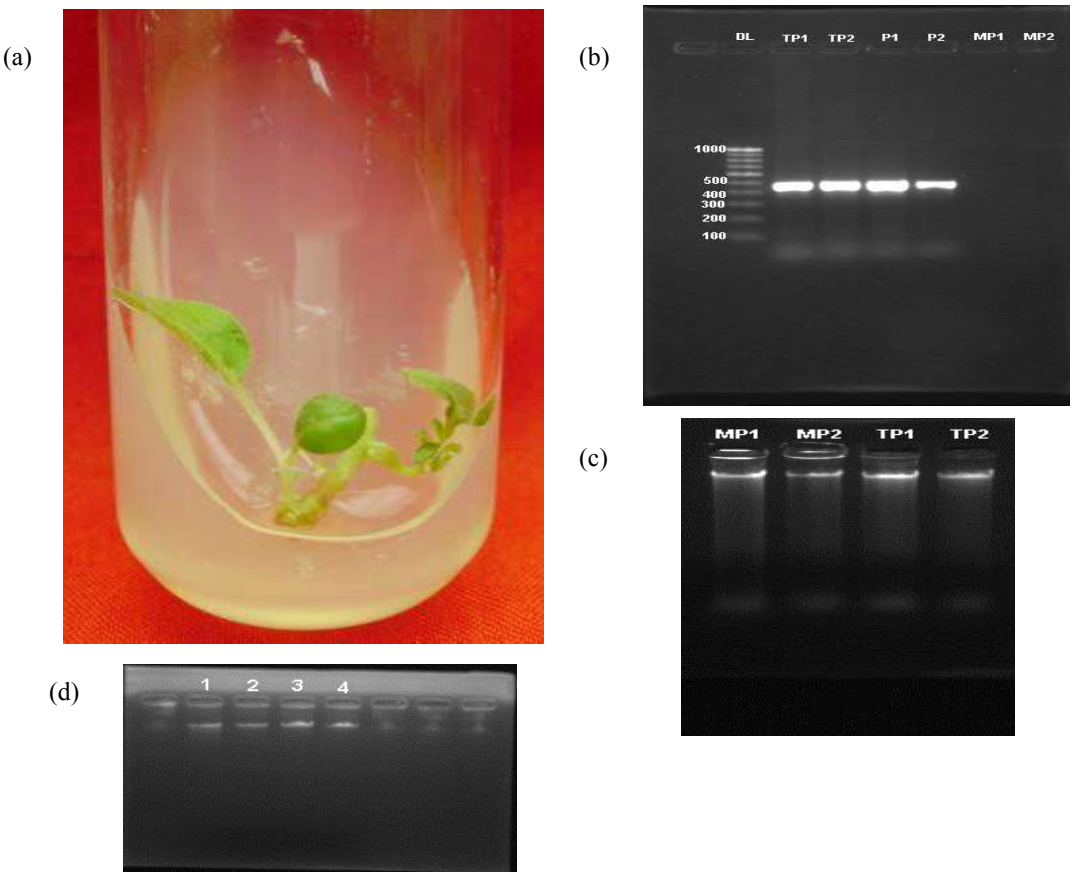

Fig. 1: *Agrobacterium tumefaciens* mediated gene transfer and GUS gene expression in *physalis minima* -
(a) *Regenerated plant after GUS gene transformation.*
(b) The PCR amplification of GUS gene of transformed plant (TP1 and TP2).
(c) Genomic DNA of untransformed plant (MP1 and MP2) and transformed plant (TP1 andTP2)
(d) Plasmid DNA of *Agrobacterium tumefaciens*

Table 1: Growth profile of *Agrobacterium tumefaciens* in different media preparation shown in

		Optical density (600nm)		
S.l No	Growth medium	Original	Duplicate	Average
1	L B Broth	1.403	1.516	1.459
2	Nutrient Broth	1.033	0.985	1.009
3	Tryptone	0.502	0.638	0.570
4	Yeast Mannitol Broth	1.187	1.163	1.175
5	Yeast Extract Peptone	0.793	0.842	0.817

O.D taken after 48 hrs.

of *Agrobacterium tumefaciens* broth culture for 2′. To remove extra bacteria, the explants were blotted on sterile filter paper and transferred on to a hormone free MS medium and kept at 25±1°C.The explants were not exposed to antibiotic at this stage. After 48 hours the explants were transferred on to fresh hormone free MS medium containing 150mg/l-250mg/l cefotaxime and 25mg/l-75mg/l kanamycin and incubated at 25±1°C for the regeneration of plants. The transformation frequency were calculated as

$$\frac{\text{Number of explants produced transgenic shoots}}{\text{Number of total explants used in the experiment}} * 100$$

GUS Gene Detection: The extraction of DNA from normal leaf and transformed leaf was carried out using the Dellaporta method (Dellaporta *et al.*, 1983). The conventional alkaline lysis method was used for extraction of the bacterial plasmid. Concentration of the extracted DNA was calculated by recording the optical density of the sample at 260nm using a Sanyo Spectrophotometer.

PCR experiments were carried out with a set of forward and reverse primers in a PTC 200 Thermal cycler.

The primers consisted of 23 and 21 nucleotides with the sequences of 5' TATCAGCGCGAAGCTTTTAT 3' and 5' CAGTTGCAACCACCTGTTGAT 3' for the forward and reverse, respectively.

The extracted bacterial plasmid DNA (140ng) and the plant genomic DNA (120ng) in 25µl was used in each PCR. The reaction mixture contained 10 picomoles each primo, dNTPs (2.5mM), Tris HCl [(pH 8.8) 10mM], KCl (500mM), MgCl2 (15mM), Gelatin (0.1%), Tween-20 (0.05%) and NP 40 (0.05%). The extension reaction was carried out in the presence of one unit of Taq polymerase. The initial denaturation of the DNA strands was peroformed at 94°C for 3' followed by 30 cycles of 30 seconds denaturation at the same temperature. Annealing and extension were done at 52 and 72 °C, respectively for one minute. Final extension was performed at 72°C for six minutes.

The electrophoresis of PCR products were performed on 1.2% agarose gel (1X TAE buffer and 5mg/ml EtBr) applying a constant voltage of 50V and observed under UV light.

RESULTS AND DISCUSSION

L B Broth was found to be the most suitable medium for growth and maintenance of the bacterial strain.

Among the various explants, only nodal explants showed positive response to the infection of *Agrobacterium tumefacien.* Necrosis of leaf segment and petiole explants observed with in 24 hours of co-cultivation. The probable reasons for this may be the delicate nature, age of the explant and virulence of the bacterium.

In vitro grown and purposely wounded nodal plants showed excellent results. During co-cultivation, a thin film of bacteria on explant surface and a thick growth along the edges of explant observed within 24 hrs. In due course of co-cultivation, no signs of explant necrosis seen, rather they remained healthy, green and swelled a bit.

After 7 days on selective agent medium, emergence of very small shoots observed, which continued to grow well. Time taken to grow the shoot system was 15-20 days. The growth of the transformed shoot was faster than the normal plant. Frequency of the emergence of shoots found to be 20%.

Some authors [6] indicated that the co-cultivation period should be not less than 4 days and some others [5] indicated that 2 days was suggested for co- cultivation period. Other authors [8] maintained that the difference in co-cultivation period was due to the different species and explants. In the present study, 2 days was taken as the co-cultivation period.

To ensure the insertion of GUS gene into the *Physalis minima* genome, PCR reaction carried out with normal plant. The result showed that GUS gene transformation was active in transformed plantlets.

REFERENCES

1. Bidney, D., C. Scelonge, J. Martich, M. Burrus, L. Sims and G. Huffman, 1992. Microprojectle bombardment of plant tissues increases transformation frequency by *Agrobacterium tumefaciens* Plant MolecularBiol., 18: 301-313.
2. Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA minipreparation: Version II. Plant Molecular Biology. Report, 1: 19-21.
3. Lacorte, C., E. Mansur, B. Timmerman and A.R. Cordeiro, 1991. Gene transfer into the peanut (*Arachis hypogaea L*) by *Agrobacterium tumefaciens.* Plant cell reports, 10: 354-357.
4. Lawrence, P.K. and K.R. Koundal, 2001. *Agrobacterium tumefaciens*-mediated transformation of pigeon pea (*Cajanus cajan L. Mills sp*) and molecular analysis of regenerated plants. Current Sci., 80(11): 1428-1432.
5. Otani, M., T. Shimada, T. Kimura and A. Saito, 1998. Transgenic plant production from embryogenic callus of sweet potato (*Ipomoea batatas (L.) Lam*) using *Agrobacterium tumefaciens*, Plant Biotechnol., 15(1): 11-16.
6. Prakash, C.S., U. Varadarajan and A.S. Kumar, 1991. Foreign gene transfer to sweet potato *Ipomoea batatas(L.)* Hortscience, 26(5): 492.
7. Soma paul and S.R. Sikdar, 1999. Expression of npt II marker and gus reporter genes and their inheritance in subsequent generations of transgenic Brassica developed through *Agrobacterium*-mediated gene transfer. Current Sci., 76(12): 1569-1573.
8. Xing, Y.J., Q. Yang, Q. Ji, Y.M. Luo, Y.F. Zhang, K. Gu, and D.Z. Wang, 2007. Optimization of Agrobacterium mediated transformation parameters for sweet potato embryonic callus β- glucuronidase (GUS) as areporter. African Journal of Biotechnol., 6(22): 2578-2584.