# Agrobacterium-Mediated Transformation in Cucumber (*Cucumis sativus* L.)

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**Introduction.** Cucumber (*Cucumis sativus* L.) is an important horticultural crop. Its fruits are used for slicing and pickling and juice extraction worldwide. In India, both the fruits and seeds have long been used in the manufacture of traditional medicines that act as skin conditioners, diuretics and body coolants. Thus, cucumber is a potential candidate for an edible vaccine (6). In India, studies on cucumber tissue culture in general and transformation in particular are very limited (1). In the present study, a reliable transformation and regeneration protocol is reported for cv. Green Long, which is widely cultivated in India.

**Materials and Methods.** The monoecious cucumber cultivar Green Long (obtained from Ramachandra Bhageluram Maurya Co., India) was used for this study. The seeds were surface-sterilized and kept on sterile moist cotton for 24h. After 24h, the seed coats were separated and removed without disturbing the cotyledons. The cotyledons were carefully dissected eliminating the embryonic axis. The distal end of the cotyledon explants was cut and these explants were vertically inoculated in such a way that the distal end was touching the experimental medium used. MS medium containing 3% sucrose (Himedia Laboratories Co., Mumbai, India), 0.8% agar (Himedia Laboratories Co., Mumbai, India) with BA (1mg/l) was used for shoot regeneration. The pH of the medium was adjusted to 5.8 before autoclaving at 121$^{o}$C for 20min. The cultures were kept at 25±2$^{o}$C at a 16h photoperiod with the light intensity of 30 µmol $m^{-2}$ $s^{-1}$ under diffuse cool white fluorescent lamps.

The *Agrobacterium timefaciens* strain EHA 105 was used for this study (2). It is a super virulent leucinopine type, with a binary plasmid (pGA492GI, provided by Rafael Perl-Treves, Bar Ilan University, Israel) with *npt* II (kan resistant), and bar (phosphonothricin resistant) genes driven by the CaMV 35S promoter.

The proximal end of the cotyledon explants was pricked to make wounds using a sterile needle to induce *Agro* infection. The explants (80-100per treatment) were dipped in bacterial suspension containing acetosyringone (20 µl/l) for 10 min, followed by washing in sterile distilled water two to three times, and finally blotted with sterile Whatman No. 1 filter paper. The explants were co-cultivated in MS basal medium devoid of any PGR, pH 5.4, and kept at 27$^{o}$C in the dark for 48h. The explants were then transferred to shoot regeneration medium containing MS salts, 1 mg/l BA, 3% sucrose, 0.8% agar, 25 mg/l kanamycin and 300 mg/l cefotaxime, and grown for 15 days. Thereafter, the regenerated shoots were separated from explants and cultured *in vitro* on PGR free MS medium containing 100 mg/l kanamycin and 300 mg/l cefotaxime to select transgenic cucumber shoots.

*GUS assay:* The regenerated plants were assayed for the expression of gusAint gene following the histochemical procedure described by Jefferson et al. (3).

*PCR analysis:* PCR analysis of transformed cucumber shoots was carried out as per the method of Nishibayashi et al. (5) using *npt* II primer with 800 bp.

**Results and Discussion:** The factors responsible for enhancing transformation frequency in cucumber have been studied earlier (7). With the help of this study, an effort was focused on evaluating the *Agrobacterium*-mediated gene transfer in cucumber cv. Green Long, the most popular cultivar in India.

Multiple shoots were induced from the proximal end of cotyledon explants infected by *Agrobacterium* on shoot induction medium supplemented with 25mg/l kanamycin and 300mg/l cefotaxime. After 15 days of initial culture, explants with multiple shoots were

transferred onto MS medium containing 100 mg/l kanamycin and 300 mg/l cefotaxime to select transgenic cucumber shoots. The adventitious shoots from explants infected by *Agrobacterium* maintained their green colour and grew normally. The GUS assay was performed to confirm the transformation event. The GUS expression was lower in transformed shoots co-cultivated without acetosyringone than with co-cultivation with acetosyringon, whereas in the acetosyringone treatment, the transformed shoots showed strong GUS activity (Fig 1). Our results are agreement with Nishibayashi et al. (4), who reported that acetosyringone was an essential for effective cucumber transformation. The kanamycin resistant shoots were selected randomly and examined by PCR for the presence of the integrated *npt* II gene. The DNA of transformed shoots integrated with *npt* II primer and produced a band of the expected size of 800bp. The DNA of non-transformed (negative control) shoots did not exhibit the band of 800 bp. The amplified DNA from the binary vector pGA492GI (positive control) produced a band of 800 bp (Fig 2). In the present investigation, about 21% of infected explants produced shoots, out of which only 12% of the shoots were fully transformed. In conclusion, the present protocol could be applied to improve Green Long by introducing the gene of interest via *Agrobacterium*.

**Literature Cited**


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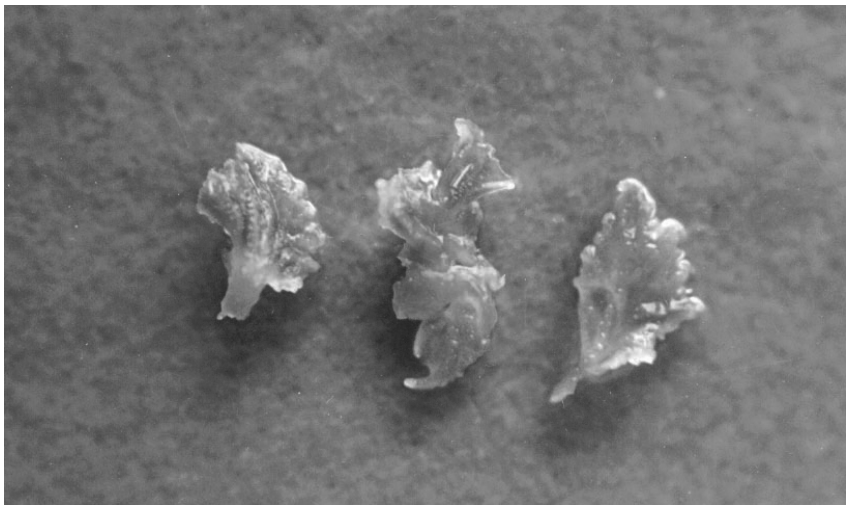

Figure 1. Transgenic shoots (2-3 weeks) showing GUS positive.

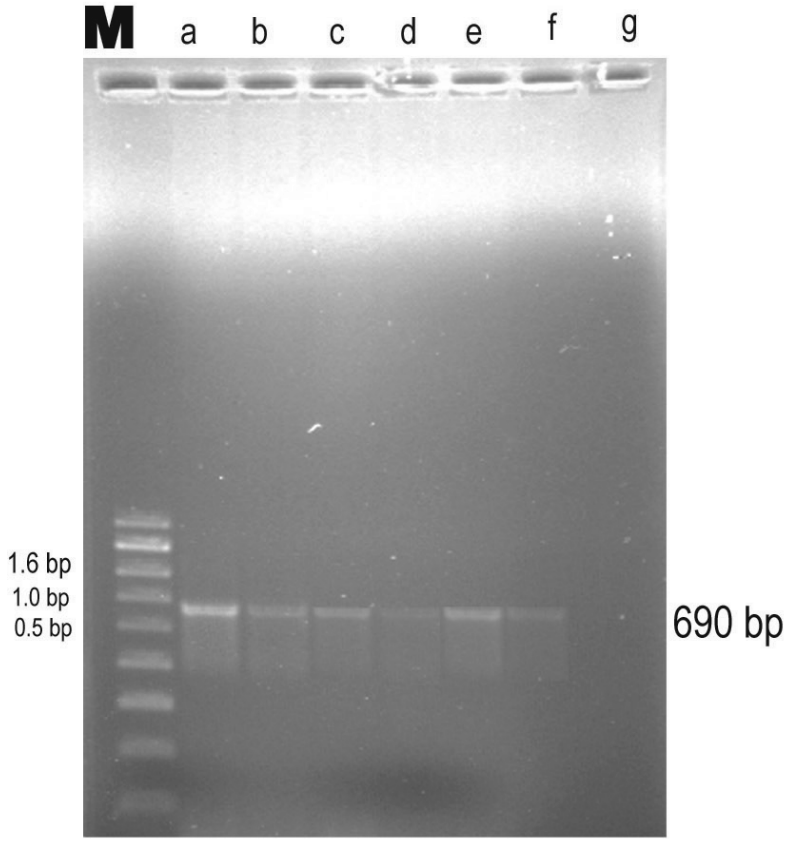


Figure 2. PCR amplification of the *npt* II gene from genomic DNA isolated from transgenic plants of EHA 105/pGA492GI (lane a, b, c, d, e,) and positive vector control (lane f). Lane g is the negative control (untransformed plant using two specific primer sequences of the *npt* II coding region).