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Optimization of *Agrobacterium*-Mediated Transformation Protocol in Gourd

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Objectives

The purpose of this study was to establish the efficient transformation process on *Agrobacterium* mediated transformation in gourd (*Lagenaria siceraria* Standl.). Effects of inoculation temperature and time, co-culture period, acetosyringone concentration on transformation efficiency were investigated.

Materials and Methods

1. Plant materials: *in vitro* germinated 'Bulsazo' gourd
2. Selection medium: SIM(MS salts, 0.5 mg/L zeatin, 0.3 mg/L IAA), 100 mg/L Km, 200 mg/L Cf
3. Strain: *Agrobacterium tumefaciens* LBA4404 (pCAMBIA2301)
4. Treatments: inoculation time, inoculation temperature, co-culture period, acetosyringone concentration
5. Histochemical GUS assay: 10-day-old explant, incubated overnight at 37°C

Results and Discussion

The cotyledon segments of 'Bulsazo' gourd were transformed with *Agrobacterium tumefaciens* LBA4404 carrying pCAMBIA 2301 vector containing intron-*gus* and *npt* II gene. After transformation the explants were cultured on shoot inducing medium (SIM), and then transferred and cultured on selection medium. After 10 days of culture, GUS gene expression was assayed. GUS assays showed that the highest transformation efficiency was obtained when the explants inoculated onto *Agrobacterium* suspensions with OD_{600} 0.8 at 30°C for 20min, followed by co-culture for 3 days on SIM with 100 μM acetosyringone. Calli were formed around the cut edge of the cotyledon after a week in culture. Shoot regeneration was observed after 4 weeks of culture. The putatively transformed shoots were elongated and rooted successfully on selection medium with 100 mg/L kanamycin.

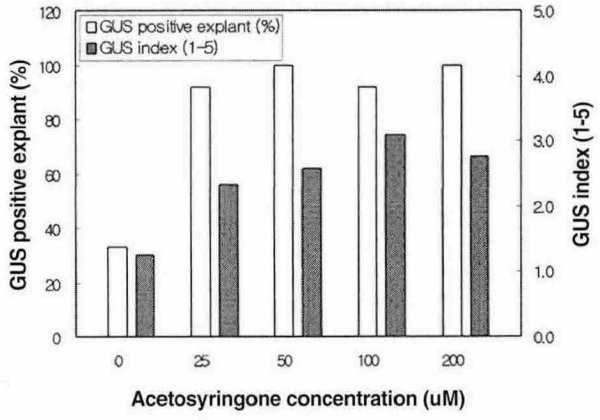

Figure 1. Effects of acetosyringone concentration during *Agrobacterium* mediated transformation on GUS expression from cotyledon in 'Bulsazo' gourd.

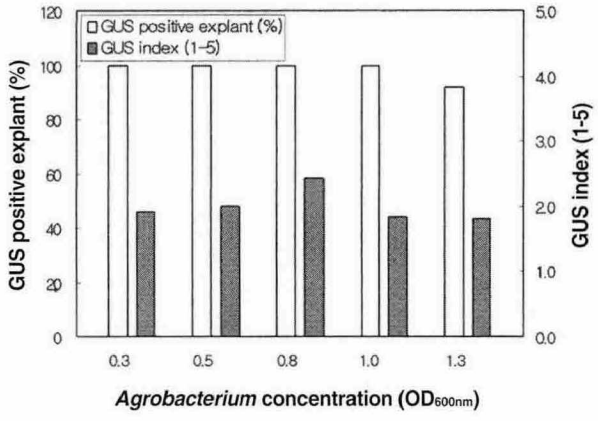

Figure 2. Effects of *Agrobacterium* concentration during *Agrobacterium* mediated transformation on GUS expression from cotyledon in 'Bulsazo' gourd.

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