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Foundation of optimum transformation condition using *Agrobacterium tumefaciens* at lettuce (*Lactuca Sativa L.*)

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Objectives

We have tried to foundation of optimum transformation condition using *Agrobacterium tumefaciens* at lettuce

Materials and Methods

1. Material

Plant - *Lactuca Sativa L.*

Agrobacterium strain - LBA4404/pCAMBIA1301

2. Methods:

We used five hybrid lines of lettuce for plant regeneration from cotyledon and primary leaf explants. After surface sterilization of seeds, seeds were germinated on MSO medium under an 16 h photoperiod at 26±1°C

Results and Discussion

For screening of highly regenerable lines of lettuce, we used five hybrid lines of lettuce for plant regeneration from cotyledon and primary leaf explants. After surface sterilization of seeds, seeds were germinated on MSO medium under an 16 h photoperiod at 26±1°C. Both cotyledon explants from 7-days-old (depending on the lines) seedlings and primary leaf explants from 10-12 days-old (depending on the lines) seedlings were cultured on MS medium containing 2mg/l kinetin, 0.1mg/l NAA¹, vitamins, 3% sucrose and 0.25% phytigel for shoot induction respectively. The frequency of plant regeneration was recorded after 30 days of culture on shoot induction medium. After screening of the highly regenerable line, we established the optimum transformation condition using transient expression of *gus* gene by *Agrobacterium*-mediated transformation. Strains of *A. tumefaciens*, LBA4404 (pCAMBIA1301) carrying p35s-hpt-35s and pNOS-nptII-NOS were used for this study. Based on the histochemical staining of GUS activity, transient expression was found to be dependent on acetosyringone concentration, *Agrobacterium* infection and co-cultivation time. Maximum GUS activity was obtained when acetosyringone concentration, *Agrobacterium* infection and co-cultivation time were applied to 150 µM, 15 min and 3 day, respectively.

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