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Improving transformation efficiency through the control of ethylene on *Agrobacterium tumefaciens*-mediated gene transfer to 'Fuji' apple

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Objectives

We have tried to find the effect of ethylene controlling on transformation efficiency of gene transfer mediated by *Agrobacterium tumefaciens* in apple (*Malus domestica* cv. 'Fuji').

Materials and Methods

1. Material

Plant - *Malus domestica* cv. 'Fuji'

Agrobacterium strain - AGL1/pCAMBIA3301

2. Methods

After treatments of either AVG (0.001, 0.01, 0.1, or 0.5 mg/L) or $AgNO_3$ (0.1, 0.5, 1.0 or 3.0 mg/L) during on *Agrobacterium* inoculation or selection media; ethylene production, infection and regeneration efficiency were measured.

Results and Discussion

The effect of ethylene controlling on transformation efficiency of gene transfer mediated by *Agrobacterium tumefaciens* (AGL1/pCAMBIA3301) was investigated in apple (*Malus domestica* cv. 'Fuji'). During three days of *Agrobacterium* inoculation, leaf explants excised from apple shoots produced ethylene, the production of which was increased, and inhibited by the addition of 0.001, 0.01, 0.1, or 0.5 mg/L aminoethoxyvinylglycin (AVG). However, the treatment of Silver nitrate ($AgNO_3$) (0.1, 0.5, 1.0 or 3.0 mg/L) increased the production of ethylene. After three days of co-cultivation with *Agrobacterium*, gene transfer into the explants was assessed by transient GUS-expression assay. Either treatments of AVG or $AgNO_3$ increased *Agrobacterium* infection efficiency by about 60%. However, there is no significant difference in the *Agrobacterium* infection efficiency at the different concentration of AVG or $AgNO_3$. Reapplication of AVG and $AgNO_3$ on selection media resulted in ethylene production and regeneration efficiency differently. The production of ethylene was increased with the addition of $AgNO_3$ and inhibited with AVG during four weeks in general. AVG treatments on selection media resulted in regeneration efficiency increasing (AVG 0.001 mg/L) or similar (AVG 0.01, 0.1, or 0.05 mg/L) to the control. However, $AgNO_3$ inhibited the regeneration efficiency about 30%. These results suggest that the efficiency of *Agrobacterium* infection to be significantly increased by both AVG and $AgNO_3$ treatments. However, the shoot regeneration rate was increased with 0.001 AVG, but inhibited with $AgNO_3$. Among three steps (1. gene transfer to explant cells, 2. selection of transformed cells, and 3. plant regeneration from transformed cells) of 'Fuji' apple transformation mediated by *Agrobacterium*, ethylene synthesis inhibitor, AVG was positively working on step 1 and step 3.

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