P 19 Development of Selectable Marker-free Transgenic Plants by Integration of Co-transformation and Negative Selection

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Objectives

Because of public concerns about problems caused by selectable marker genes, researchers have made several answers about how to remove them from transgenic plants. But so far, no obvious elimination methods have been developed yet which are generally applicable to plants. Plants are mostly transformed by *in vitro* regeneration methods. Here we worked on tobacco as model plant and deviced a new strategy integrating co-transformation and negative selection to develop selectable marker gene-free transgenic plants. We think that it is easy and efficient, so it can be generally-applicable to most transformable plants.

Materials and Methods

1. Materials
   - *Nicotiana tobacum* Samsun NN
   - 2 *Agrobacterium tumefaciens* LBA4404 strains harboring selectable marker genes(*nptii* and *codA*) and reporter gene (*uidA*) binary vectors, respectively
2. Methods
   - 2 *Agrobacterium* harboring each binary vectors were transformed with various ratios to get best condition to obtain high ratio of GUS(+) to Km^R and also many GUS(+). After kanamycin selection, the chosen plants were tested for their GUS activity and *uidA*-specific PCR amplification. Km^R and GUS(+) R1 plants were planted and let to set R2 seeds. R2 seeds were screened for their 5-FC resistance. Finally, I got 5-FC^R and GUS(+) transgenic plants and tested their genotype with PCR and southern blotting.

Results and Discussion

Mixture of 2 *Agrobacterium* harboring selectable marker genes (*nptii* and *codA*) and reporter gene (*uidA*) binary vectors was used to transform tobacco(*Nicotiana tobacum* Samsun NN). Varioius amount of *agrobacterium* with reporter binary vector versus fixed amount of *agrobacterium* with selectable marker binary vector was tried to test the best condition to get high ratio of GUS(+) to Km^R and also many GUS(+) if possible. It proved to be best ratio from 0.5 to 1.0 (*Agrobacterium* with selectable marker binary vector used was 1mL of Ab_{600}=1.0 among 20 mL MS infection solution.). 22 transgenic tobacco's in hands were let to set seeds. Then, several of these T2 seeds were germinated on MS 5-FC250 solid medium. This can exclude transgenic tobacco with selectable marker genes as a results of *codA* gene expression. The resistant seedlings were transplanted to pots and tested for their GUS activity and Southern blotting pattern. Form almost every lines, GUS(+) and 5-FC^R T2 progenies was obtained. Also, #13 line showed 1/3 viability on MS 5-FC250 media and 2/3 GUS(+) ratio, which perfectly matches the mendelian prediction.

We also tried *cre*/loxP mediated selectable marker gene removal system. It was proved to be efficient with *in planta* transformation method. But it didn't worked well with *in vitro* regeneration mediated transformation system unlike expectation. This is thought to be due to failure of tight control on *cre* gene expression under inducible promoters which are usually chimera of minimal promoter and several *cis*-elements. That is, long and irregular regeneration conditions *in vitro* caused incident and low, but, continuous *cre* gene expression. Possibly because of such accumulation of *cre* expression, we could get few transgenic plants. One of transgenic plants had very complex insertion patterns. According to our results, we consider that a new strategy integrating co-transformation and negative selection is easy and generally-applicable to most *in vitro* transformable plants.

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