03-1-48

Development of *Agrobacterium* Mediated Stable Transformation Method for Pepper (*Capsicum annuum* L.)

Moon Kyung Ko, Hye Ja Kang, Kyung Hoan Im[1], Young Soon Kim*

Kumho Life & Environmental Science Laboratory, Korea Kumho Petrochemical Co., Ltd. Kwangju
[1]Department of Biology, University of Incheon, Incheon

Objectives

Establish a stable transformation method for pepper
Confirm the genetic stability of the transferred genes in the progeny

Materials and Methods

1. Material
   pepper (*Capsicum annuum* L.)
   Agrobacterium tumefaciens GV 3101 carrying pCAMBIA 1301 or 1304
2. Methods
   Precultured pepper explants were inoculated with *Agrobacterium*. Then, calli and shoots were induced under the selection condition. Finally, the regenerated shoots were acclimated in soil and the progeny of the transgenic plants was analyzed.

Results and Discussion

Being one of the most important vegetable crops, the pepper has received much attention for the possible introduction of agronomically important traits into the genome. However, the pepper is generally known as an extremely recalcitrant species for genetic transformation because no stable transformation method has been established so far.

To establish stable transformation method for pepper, we used highly morphogenetic tissues derived from hypocotyls and cotyledons of the aseptic plant. Hormone concentration was also adjusted to guarantee maximal occurrence of the cell division. Then, the pepper cells that were transformed were able to proliferate under the selection regime and a number of hygromycin resistant pepper calli were isolated which expressed a co-transferred *gusA* gene.

Since the explants were derived from seedlings, they retain great morphogenetic potentials for shoot development. Particularly, *de novo* regeneration of shoots, which may not be transformed, occurred in the upper part of the explants. Therefore, it is very important to select the transformed callus and shoots on the strict condition to inhibit the growth of 'false positive shoots'. After all, phenotypically normal shoots were regenerated from the resistant calli. Various tissues from these primary transgenic (T_0) plants showed GUS activity indicating the proper integration of transformed gene into pepper genome. The integration and the expression of *gusA* gene were also confirmed by Southern and Northern hybridization, respectively. All the progeny (T_1) of transgenic plants showed the expected segregation pattern of hygromycin resistance indicating that the T-DNA was stably maintained in the progeny through the generation.

* Corresponding author: Young Soon Kim, TEL: 062-970-2667, E-mail: youngskim@kkpc.com