Agrobacterium-mediated Gene Transfer in Citrus reticulata Blanco

GAO FENG, HUA XUEJUN, FAN YUNLIU, ZHANG JINREN, AND CHEN SHANCHUN

1 Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing 630712, China
2 Biotechnology Research Center, Chinese Academy of Agricultural Sciences, Beijing 100081, China.

Introduction

Agrobacterium-mediated in vitro transformation has been applied to a growing number of plant species and used as a new technique to improve their yields and qualities (Fraley et al. 1986). However, up to now we have not seen any detailed reports about this in citrus fruit trees. In our experiment, leaf disc method developed by Horsch et al. has been simplified and extended for use with explants of leaves, stems and cotyledons of "Hongju" tangerine, to study the possibilities and stabilities of transformation and expression of T-DNA in citrus cells and to provide a new way for citrus improvement by using with genetic engineering.

Materials and methods

Leaves, stems and cotyledons were excised from grown in vitro seedlings of "Hongju" tangerine and were cut into sections (about 5 mm) as explants. The explants were briefly inoculated with wild-type A. tumefaciens strain B6 S3 grown overnight and diluted 5 times with MS medium, and then blotted dry with sterile filter papers and cultured on MT medium in the dark at 28±1°C. After 3 days of cocultivation, the explants were transferred to MT medium supplemented with carbenicillin (carb) at 500 mg/l to remove excess bacteria. The explants which were not inoculated with bacteria were used as controls. All of the explants...
were cultured with 16h photoperiod at 28±1°C. the results were investigated after 2 weeks. After 1 month, tumour tissues or calli were excised from the original explants respectively and subcultured on the same medium at about 30 days intervals. Octopine synthase activity was assayed according to the method of Xu Yao et al.

Results

After 5 - 10 days cultured on MT medium contained 500 mg/1 carb, the infected explants of leaf, stem and cotyledon initiated tumour tissues. After 2 weeks, many tumour tissues were formed from the infected explants and were visible to the eyes. The results show that the frequency of transformation is biggest in leaves (50.0%), followed by stems (43.5%) and cotyledons (9.5%), but the tumour tissues formed from cotyledons are larges. While no tumour tissue or callus is formed from control leaves and cotyledons, only a small callus is formed from a control stem section. There are some obvious differences between the callus and the tumour tissue in formative sites and appearances. The callus is formed from all of cut surface at the site of end of a stem section and is hemisphere in shape, its surface is rough and loose, while the tumour tissue is formed from a small part of cut surface and is sphere in shape just as a drop of water, its surface is smooth and compact. When subcultured on the hormone-free MT medium, callus grew slow, but tumour tissue grew quite fast.

In order to further identify whether the T-DNA was transferred and expressed in cells of "Hongju" tangerline, octopine synthase activity was assayed in the tumour tissues and callus after subcultured 3 times (about 90 days). The results show that all of the tumour tissues which taken at random exist octopine, but the callus does not. This demonstrated that the T-DNA has been transferred and expressed in the transformed cells of "Hongju" tangerine.

Discussion

There are two main kinds of functional genes in T-DNA of Ti plasmid of A. tumefaciens. One is the plant hormone synthase gene, another is the opine synthase gene (Weising, et al., 1988). Their expression in transformed cell results in that the transformed cell has characteristics of hormone autonomous growth and opine synthesis. Therefore, the two characteristics