Construction of transgenic *citrus* plants by introduction of fatty acid desaturase genes

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Abstract

Three kinds of *citrus*, 'Yoshida' navel orange (YN), 'Cara Cara Red' navel orange (CCRN), and kumquat were transformed with fatty acid desaturase genes, fad3 and fad7. To introduce DNA the microprojectile bombardment system was used for the stem microsections of kumquat. The transformants were screened on the selective medium containing kanamycin, and then DNA's of the transformants were analyzed by polymerase chain reaction. Some embryogenic clones were obtained from the transformed callus cells of YN and CCRN. Some planted were regenerated from the kumquat microsections transformed with fad7.

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Introduction

The fatty acid composition of cell membrane is well known to be closely related with the tolerance of plants to low temperature. In general plants with higher content of polyunsaturated fatty acids are more tolerant to cold stress.

The enzymes, fatty acid desaturases, catalyze the process which introduce double bonds into fatty acids. Many reports showed that modifications of the genes encoding these enzymes altered the composition of unsaturated fatty acid in membranes and changed low-temperature resistance of plants (Ishizaki-Nishizawa et al., 1996).

Recently the composition of unsaturated fatty acids in membrane lipids was reported to be important in growth, respiration and photosynthesis as well as low-temperature resistance of plants (Tasaka et al., 1996). Therefore introduction of desaturase genes might improve fruit qualities via enhancing these physiological activities of plants especially at low temperature.

In this study we tried to transform *citrus* species with fatty acid desaturase genes for improving fruit qualities.

Materials and methods

The embryogenic callus cells of 'Yoshida' (YN) and 'Cara Cara Red' (CCRN) navel orange *(Citrus sinensis)*, and the stem microsections of kumquat *(Fortunella japonica)* were used for transformation.

Two kinds of fatty acid desaturase genes, fad3 and fad7, were used. Each gene was constructed in a plant expression vector, pBI121, harboring nptII.

The PDS-1000/He Biolistic Particle Delivery System (Bio-Rad) was used for introducing DNA into callus cells of YN and CCRN orange. *Agrobacterium* system was used for kumquat stem microsections.

Transformants were screened on a selective MT medium containing 100mg/l of kanamycin. The introduced DNA of each gene in transformants was analyzed by PCR.

Results and discussion

The embryogenic callus cells of YN and CCRN were transformed by microprojectile bombardment system. For the transformation the DNA of fad3 or fad7 reconstructed in pBI121 was coated on M17 tungsten particles. The cells were bombarded with the particles at 1100 psi of helium gas pressure, 1/4" of gap distance and 7.0cm of target distance. The bombarded cells were transfered to the sective medium containing 100mg of kanamycin after two days stabilization. The cells growing continuously on the kanamycin medium were obtained. Fig. 1 showed the selective growth of YN cells transformed with fad7. We could obtained some cells of YN and CCRN in the early stage of embryogenesis. Fig. 2 showed the embryogenic

cells of YN transformed with fad7.

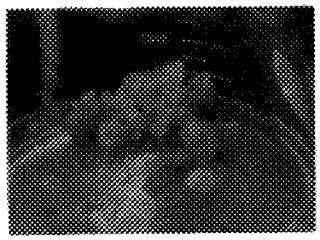

Fig. 1. Selective growths of YN callus cells on MT medium containing kanamycin after bombardment with pBI121 containing both of nptII and fad7 gene.

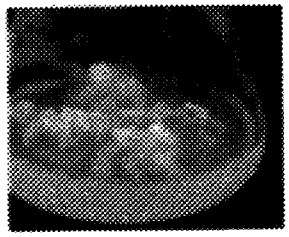

Fig. 2. The transformed YN cells under somatic embryogenesis.

The genomic DNA extracted from the selected callus clones was amplified by PCR with the specific primers for nptII or target genes, fad3 and fad7, to confirm that the clones contain the introduced DNA. Fig. 3 showed that the DNA bands of 1.0kbp for fad7 were observed in two clones (lane 8 and 9) among five from the YN cells bombarded with pBI121 harboring nptII and fad7. These two clones also showed the 795bp DNA bands for nptII (lane 13 and 14). Therefore these two clones were appeared to contain both genes of nptII and fad7. Five clones from the cells bombarded with pBI121 harboring nptII and fad3 were also tested for fad3 DNA. The two clones showed both DNA of nptII and fad3.

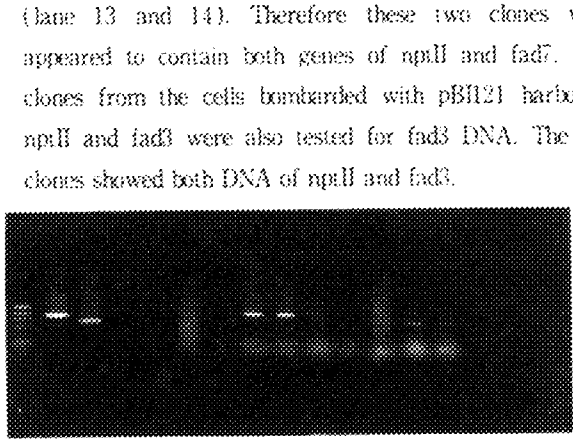

Fig. 3. PCR-amplified DNA's of nptII and fad7 gene from the clones bombarded with pBI121 containing both of nptII and fad7.
lane 1: marker, 2: positive control (fad7), 3: positive control (nptII), 4: negative control (-fad7), 5: negative control (-nptII), 6 and 7: non-bombarded clones, 8-12: fad7 amplification in bombarded clones, 13-17: nptII amplification in bombarded clones.

For the transformation of kumquat, stem microsections were used. The microsections were prepared as 400μm thickness by vibrating microtome. The DNA of pBI121 harboring nptII and fad7 were introduced by *agrobacterium* transformation system. The transformed microsections were cultured on the MT medium containing 2mg/l of BA, 0.2mg/l of NAA and 50mg/l of kanamycin. We could obtained some plantlets from the kumquat microsections (Fig. 4).

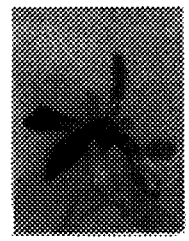

Fig. 4. A plantlet derived from the microsection of kumquat transformed with fad7.

References

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