Construction of transgenic melon (Cucumis melo L. cv. Silver Light) carrying the coat protein gene of Cucumber mosaic

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ABSTRACT

Cucumber mosaic virus (CMV) is transmitted by aphids and cause serious economical loss to cucurbit cultivation in Taiwan. The goal of this study is to establish a variable microprogated system and produce CMV CP-transgenic plants to against CMV. Uncoated mature seeds were sterilized and used for transformation. Cotyledons were cut into 4 pieces and soaked on MS solution. All the explants were cocultured with Agrobacterium which contained CMV CP gene for four days, then transferred to the generation medium containing 100 ppm kanamycin and 200 ppm carbenicillin. Putative buds growed from the explants were recorded in the selective period. Results showed, the frequency of shoot regeneration is 48.3 % and the rate of rooting is 84 %. The chlorosis rate of buds is 35.4 % and the hyperhydricity is 35.3 % after 5 subculture for multiple shoots. After several times of subcultures, the buds gradually developed to normal-appearance multiple shoots. More then 20 putative transgenic lines were obtained. All the putative lines were tested for transgenic by PCR using specific primers to NPTII or CMV CP gene Eighteen lines had positive reaction. Seven lines were further confirmed by southern and western analysis. Transgenic lines were evaluated the resistance to CMV by mechanical inoculation under greenhouse conditions. Transgenic lines showed no symptoms 28 days after inoculation, however, the control plants displayed severe symptoms 7 days after inoculation.

Keywords : Muskmelon ; Agrobacterium ; transformation ; tissue culture ; regeneration

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縮寫 全名 B5 Gamborg ’ s medium (Gamborg et al., 1968) BA 6-benzylaminopurine CMV Cucumber mosaic virus CaMV Cauliflower mosaic virus GUS β-Glucuronidase MS Murashige & Skoog medium (Murashige & Skoog, 1962) NAA α-Naphthaleneacetic acid NOS Nopaline synthase NPTII Neomycin phosphotransferase II PCR Polymerase chain reaction PRSV-W Type W strain of Papaya ringspot virus ZYMV Zucchini yellow mosaic virus

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