Agrobacterium-mediated Transformation of Commercial Muskmelon (Cucumis melo L., cv.Xing Hua)

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ABSTRACT

ABSTRACT Zucchini yellow mosaic virus (ZYMV) and Type W strain of Papaya ringspot virus (PRSV-W) are transmitted by aphids and cause serious economical loss to cucurbit cultivation in Taiwan. The major objective of this study was to establish a better microprogated system and produce coat-protein-gene transgenic plants that are resistant to ZYMV and PRSV-W. Muskmelon multiple shoots exhibited hyperhydric appearance when cultured in vitro and poor survival rate under acclimatization. In this study, we tried to decrease the hyperhydric rate by adding different concentration of thiamine HCl (10 mgl-1,50 mgl-1,100 mgl-1) to the MS basal medium. The results indicated that the rate of hyperhydric shoots was reduced when the shoot culture or the medium with 50 mgl-1 thiamine HCl. In the transformation procedures, seed coats or mature seeds were removed and the seeds was sterilized as explants for transformation . Each fresh cotyledon was soaked on MS solution and cut into 4 pieces using a dull scalpel blade. A fresh overnight bacterial suspension was added 50 μl and light sharked for 10 min. All explants were transferred to the co—culture medium for four days then transplanted to the regeneration medium containing 100 ppm kanamycin and 200 ppm carbenicillin or 200 ppm cefotaxime. The putative transformed buds formed from explants cultured for 4-6 weeks on the selective medium. The best rate of bud formation was 51.7 % on the regeneration medium (MS basal medium adding 0.5 mg l-1 BA, 200 mgl-1 carbenicillin and 100 mgl-1 kanamycin). All the buds were removed and transplanted on multiple—elongation medium (0.01 mgl-1 NAA & 0.1 mgl-1 BA) containing 100 mgl-1 kanamycin and 200 mgl-1 carbenicillin. After several times subcultures the buds developed to normal-appearance multiple shoots. We produced more then 10 putative transgenic lines. All the putative transgenic lines were conformed by PCR using specific NPTII or CP gene primer. In our study, we describe the procedures for regeneration and Agrobacterium—mediated production of transgenic Muskmelon, an economically important cultivar of melon in Taiwan. Key Words : Muskmelon、tissue culture、transformation、regeneration 、Agrobacterium

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