西瓜銀斑病毒核鞘蛋白與矮南瓜黃化嵌紋病毒及木瓜輪點病毒西瓜系統鞘蛋白轉機因西瓜之構築

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摘 要

台灣瓜類栽培面積廣大且種類繁多，其中以甜瓜及西瓜為大宗。本研究鑑於甜瓜作物受胡瓜嵌紋病毒 (Cucumber mosaic virus , CMV) 之危害，常造成嚴重損害，由於目前缺乏此種抗病材料，而傳統方法對於此病毒的防治迄無良方，本研究利用遺傳工程方法構築具CMV鞘蛋白轉基因甜瓜，預期應可作為防治病毒之一極具價值的方法。本研究以構築含 NPTII與胡瓜嵌紋病毒鞘蛋白基因的農桿菌為轉殖媒介，以銀輝甜瓜商用栽培種子之子葉進行轉殖，試圖建立一套適合銀輝甜瓜栽培品種之組織培養再生與基因轉殖系統。 先以成熟種子子葉為材料，經去殼消毒後，每片子葉切割成四等份，感染農桿菌後，經四天的共同培養，在含有 kanamycin, carbenicillin, cefozdin 抗生素的培養基中進行初步的篩選，直到形成擬轉基因芽體。研究數據顯示，此階段的擬轉基因芽體再生率為48.3 %，再將擬轉基因芽體移入芽體篩選培養基進行二次的篩選培養，在培養的過程中也一併的記錄了芽體黃化 (35.4 %) 及水浸狀 (33.3 %) 的發生率，等形成單一芽體後，經 PCR分析，由外觀正常的二十個擬轉基因芽體內可偵測到 NPTII基因確實在約 1.0 kb的位置有明顯之位帶。而十八個 CMV CP 基因確實在約 0.65 kb的位置有明顯之位帶。南方點漬法發現，共七個轉基因株系 (1、2、3、9、27、33及 42)，其中除了1、2為單一 copy，其餘均帶有多個重複序列數目(copy number)；且由西方點漬法發現，確實在約 23 KDa 的位置有明顯之位帶。待芽體形成後再進行發根 (發根率84 %) 及馴化處理，於溫室中進行抗病評估的分析。結果顯示，轉基因植株直到接種病毒後第四周都沒有病徵的現象。

關鍵詞 : 銀輝甜瓜 ; 農桿菌 ; 基因轉殖 ; 組織培養 ; 再生

目錄

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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