網紋洋香瓜基因轉殖

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摘 要

中文摘要　　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。此二作物受矮南瓜黃化病毒 (Zucchini yellow mosaic virus, ZYMV) 及木瓜輪點病毒西瓜系統 (Papaya ringspot virus W type, PRSV-W) 之危害，造成嚴重損害，由於缺乏抗病材料，傳統方法對於此二病毒的防治迄無良方，本研究乃利用遺傳工程方法構築具 ZYMV及PRSV-W病毒之鞘蛋白轉基因瓜類，預期能得到抗病毒的種原。本實驗以含NPTII和同時帶矮南瓜黃化嵌紋病毒 (Zucchini yellow mosaic virus, ZYMV) 及木瓜輪點病毒西瓜型 (Papaya ringspot virus W type, PRSV-W) 相連鞘蛋白基因的農桿菌為轉殖媒介，並以網紋洋香瓜商用栽培種的種子進行轉殖，試圖建立一套適合本土洋香瓜栽培品種之基因轉殖與組織培養再生系統。　　經由建立組織培養叢生苗過程發現，預備試驗中，分別探討添加不同的維他命至基本培養基中，植株產生不正常的癒合組織及嚴重水浸狀現象，將培養基中thiamine HCl成分濃度提高後，確實有效降低水浸狀的現象。使植株生長正常，其最適合的濃度為50 mgl-1。基因轉殖及再生的實驗，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，經四天的共同培養後，在含有抗生素的培養基中進行篩選，直到形成擬轉基因芽體後，再移入芽體篩選培養基繼續進行篩選培養。結果顯示，單獨添加BA 0.5 mg l-1的再生培養基再生率為51.7 %，並且以添加抗生素carbenicillin作為篩選，效果最佳，並得到十個以上正常的擬轉基因株系。由PCR放大偵測到病毒鞘蛋白基因及NPTII確實在約1.0 kb的位置有明顯之位帶。並且由南方點漬法發現，共三個轉基因株系(ZW-2、ZW-3及ZW-4)為2個重複序列數目(copy number)。形成單一芽體後，再進行後續的發根及馴化處理，至溫室中進行溫室抗病評估的分析，期望能成功構築具有抗病性的轉基因網紋洋香瓜。 關鍵字：網紋洋香瓜、組織培養、基因轉殖、再生、農桿菌

關鍵詞 : 網紋洋香瓜 ; 組織培養 ; 基因轉殖 ; 再生 ; 農桿菌

目錄

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