具感染力之蕃茄黃化捲葉病毒之構築與含其複製?¯鈰稭]菸草之抗病分析

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摘 要

番茄黃化捲葉病毒(Tomato yellow leaf curl virus；TYLCV）屬於雙生科(Geminiviridae)中的Begomovirus屬，基因體包含單一種環狀DNA核酸(monopartite genomes)，由銀葉粉蝨(Besisia argentifoli)傳播，對番茄收成造成嚴重之危害。本實驗利用病原誘導抗病機制(parasite-derived resistance, PDR)以台灣品系之TYLCV的部分複製蛋白?基因(C1)，依不同排列方式，分別構築正股(sense)、反意股(antisense)及兩段反意股相連(double antisenses)之轉基因質體，再以農桿菌方式轉殖到植株上，並挑戰接種含全長度TYLCV質體之農桿菌。本研究先以煙草為模式，進行抗病分析，以作為將來轉殖到番茄的參考。實驗首先在彰化縣，包括溪湖鎮、永靖鄉、大村鄉及員林鎮八個番茄園區，採集疑似受病毒感染之番茄葉片，再以聚合?連鎖反應(polymerase chain reaction；PCR)針對病毒之複製?基因(C1)，進行番茄黃化捲葉病毒之偵測，在溪湖鎮40個樣本中，共偵測到5個染病材料，其罹病率為12.5%；在永靖鄉、大村鄉及員林鎮各採集8個樣本，亦各偵測到1個染病植株，罹病率為12.5%。將針對TYLCV的PCR產物進行選殖與解序後，發現無論是C1基因或是全長度基因體，溪湖品系的TYLCV與泰國品系TYLCV最為相近，可達98%。所轉殖含C1基因的擬轉基因煙草，則先利用針對病毒C1基因及NPTII專一性引子進行PCR增幅，可獲得預期DNA片段，證明轉基因確實有送入煙草中，總共每種構築分別得10-15個擬轉基因煙草品系。由於TYLCV無法以機械接種方式感染植物，因此為了進行轉基因煙草的抗病分析，本實驗另外再構築具感染力的TYLCV質體。首先以溪湖採集之TYLCV為材料，以PCR分別放大三段DNA片段，涵蓋全長度病毒基因體，以適當酵素進行剪接後，得到一個含病毒全長度基因體1.27倍TYLCV質體(TI0380AE5)，其在5’端多出346核?酸，在3’端多出416核?酸，此質體藉由農桿菌，接種到非轉基因煙草及番茄後，取接種植物之上位葉進行PCR及解序，確定此病毒質體(TI0380AE5)確實具有感染力，比正常感染TYLCV的煙草病徵較弱。此具感染力的TYLCV質體即可用來挑戰接種於轉基因煙草。由本實驗在煙草上的研究經驗，將可應用到未來番茄的轉殖上，以達抗番茄黃化捲葉病毒之目的。

關鍵詞 : 番茄黃化捲葉病毒 ; 基因轉殖 ; 農桿菌 ; 聚合?連鎖反應

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