木瓜畸葉嵌紋病毒具感染力轉錄體之構築

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摘 要

台灣木瓜畸葉嵌紋病毒 (Papaya leaf distortion mosaic virus, PLDMV) 大里 (DL) 品系的全長度基因體序列已解序完成，共含10153個核?酸，此分離自台灣木瓜植株的DL品系之寄主範圍與日本P品系或C品系並不相同。利用適當的酵素剪接可進一步將病毒全長度基因體序列構築於質體上，更可將不同品系的PLDMV鞘蛋白基因進行置換，以探討其是否影響病毒之寄主範圍。在構築含PLDMV全長度序列的質體時，首先利用含木瓜輪點病毒 (Papaya ringspot virus, PRSV) 全長度基因體的質體為骨架，逐一的將其序列置換為PLDMV。之前實驗室已構築好涵蓋 PLDMV 序列的四個質體，包括p35SPL2711-HA9779Not 、PL2680NarI-4361、PL4247-6604及PL5761-9810，此四個質体所含的PLDMV序列彼此有互相重疊，因此經由酵素剪接將可獲得含全長度基因體，然而在構築PL2680NarI-9810時發現，有多餘88個重複的病毒核?酸出現於第4315核?酸位置，重新利用RT-PCR擴增出對應此區域的DNA片段 (為1690 bp) 進行置換，最後經酵素剪接得到全長度PLDMV-DL質體。質體純化後接種於木瓜植株，14天後以ELISA及RT-PCR分析，皆無法測得病毒之感染。將此含全長度PLDMV的質體進行解序，發現病毒的第3900-3915核?酸位置發生16個核?酸的缺失，之後雖欲以含正確序列之PL2680NarI-4361質體進行修正，卻仍無法獲得正確之構築。之後經RT-PCR證實PLDMV-DL基因體序列可能會在大腸桿菌中表現，因而不利於質體之構築。隨後利用BPROM程式找出可能被原核細胞所認得的啟動子位置後，將TATA BOX進行點突變得到△TM8-PLDMV質體，此質體在3900-3915依然含有16個核?酸的缺失，因此需再以PL2680NarI-4361質體進行置換。另外，為了測試PLDMV的鞘蛋白是否為影響寄主範圍的關鍵因子，利用兩階段重疊PCR方式進行序列取代，最後將DL品系鞘蛋白基因置換成日本之C品系序列，目前已得到pSK+DL4296- polyA (C-CP) 之構築，待全長度PLDMV-DL質體構築完成，即可進行置換並測試是否影響寄主範圍。

關鍵詞 : 木瓜畸葉嵌紋病毒、具感染力轉錄體、隱性原核生物啟動子、鞘蛋白

目錄

封面內頁 簽名頁 授權書 中文摘要 iii 英文摘要 v 誌謝 vii 目錄 viii 圖目錄 x 表目錄 xii 1. 前言 1 1.1 木瓜畸葉嵌紋病毒之發現及其特性 1 1.2 病毒具感染力轉錄體之構築 3 1.3 實驗目的 9 2. 材料與方法 11 2.1 菌株品系、寄主植物與含PLDMV序列之質體材料 11 2.2 E. coli 勝任細胞之製備與質體的轉型作用 12 2.3 質體DNA之快速篩選 12 2.4 質體 DNA 之純化 13 2.5 膠體回收DNA 14 2.6 PLDMV 2680-9810序列之構築 15 2.7 PLDMV全長度DNA之連接 16 2.8 含PLDMV全長度基因體之質體的感染力分析 16 2.9 酵素連結免疫分析(Enzyme-Linked Immunosorbent Assay, ELISA) 17 2.10 核糖核酸萃取 18 2.11 反轉錄聚合?酵素連鎖反應 (Reverse transcription polymerase chain reaction, RT-PCR) 19 2.12 PLDMV木瓜品系與日本瓜類品系之鞘蛋白基因置換 20 2.13 大腸桿菌內偵測PLDMV-DL質體的表現 21 2.14 PLDMV序列中可能的原核生物啟動子之點突變 23 3. 結果 26 3.1 PLDMV 2680-9810序列之構築 26 3.2 PLDMV全長度 DNA之連接 27 3.3 PLDMV全長度序列質體感染力分析與核?酸解序 28 3.4 大腸桿菌內PLDMV-DL之RNA分子偵測 29 3.5 PLDMV序列中可能的原核啟動子點突變 30 3.6 PLDMV木瓜品系與日本瓜類品系之鞘蛋白基因置換 31 4. 結論 33 參考文獻 55 附錄 60

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