

台灣木瓜畸葉嵌紋病毒全長度基因體序列之解讀

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摘要

為了進行台灣品系木瓜畸葉嵌紋病毒(PLDMV-TW DL)全長度基因體序列的解讀，本實驗根據已發表的PLDMV日本品系基因體核? 藹 C(Accession No.AB088221)及環球科技大學包慧俊博士已解讀完成的台灣品系PLDMV-TW DL 3'端1927個核? 藹 C，設計了PLDMV-TW DL上下游特定引子共17個，再以反轉錄-聚合? s鎖反應(reverse transcription-polymerase chain reaction, RT-PCR)，將台灣品系PLDMV不同部位的基因體放大並構築在TA載體上，總共得到八個互相重疊的DNA片段，可以涵蓋整個PLDMV核? 藹 C。PLDMV-TW DL的基因體不包含3'端poly(A)的部分，總共由10153個核? 藹 c成，其5'端的引導序列核? 藻?34個，而3'端非轉譯區則有208個，此基因體可對應產生單一個開放讀碼框(Open Reading Frame, ORF)，其起始密碼AUG和終止密碼UAG分別位於基因體135-137和9942-9944的位置，可轉譯出一條由3269個胺基酸所組成的大蛋白，分子量為372.895 kDa，是目前已知馬鈴薯Y群病毒(potyvirus)所對應產生的複合蛋白中第四大的。將美國國家生物技術資訊中心【National Center for Biotechnology Information (NCBI)】中已發表的40個全長度馬鈴薯Y群病毒基因體序列與台灣品系PLDMV序列進行比對時，可發現台灣品系的PLDMV與日本品系PLDMV最相近，其核? 藹 C相似度達94%，而與其他39種馬鈴薯Y群病毒的相似度則只有53-57%。當比對病毒的各個基因時，發現N1b的相似度最高(核? 藹 蘆漪荳? 蛭?1-65%，胺基酸的相似度則介於57-65%)，而P1與3'UTR的相似度是最低的。此外進一步以病毒全長度基因體、N1b、CP和P1之胺基酸序列繪製系統發生樹(phylogenetic tree)時，亦可觀察到台灣品系和日本品系的PLDMV類緣性是最相近的。而在宿主範圍的測試方面，則以機械接種之方式將其接種於木瓜、刺角瓜(Cucumis metuliferus Acc.2459)或其他葫蘆科植物Cucurbita pepo (Diner)、Cucumis sativus (Sagami-hansiro)上，結果發現PLDMV-TW DL可在木瓜上造成嵌紋及葉片扭曲變形的典型病徵，但在其他葫蘆科植物則未有明顯病徵出現。利用酵素連結血清反應(ELISA)及反轉錄-聚合? s鎖反應分析，結果證實PLDMV-TW DL確實會感染刺角瓜和南瓜Cucurbita pepo (Diner)。此外，為了獲得大量之PLDMV-TW DL病毒顆粒，也進一步以接種的木瓜植物葉片為材料，進行病毒的純化，以穿透式電子顯微鏡觀察時，可看到約780 nm長絲狀的病毒顆粒。由以上核? 藹 P胺基酸的序列比對與類緣關係的分析和寄主範圍測試，證實PLDMV-TW DL確實是PLDMV這個種中的一個新的病原型，其與日本PLDMV-J 56 P品系是屬於不相同的病毒。

關鍵詞：木瓜畸葉嵌紋病毒；反轉錄-聚合? s鎖反應；開放讀碼框；馬鈴薯Y群病毒；系統發生樹；酵素連結血清反應；穿透式電子顯微鏡；基因體；分子量；類緣性；顯微鏡；刺角瓜

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