以定點突變HC-Pro基因構築臺灣弱系木瓜輪點病毒 = Construction of a Taiwan mild strain of papaya ringspot virus by site-dir

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摘 要

台灣於民國64年首先於高雄縣發現有木瓜輪點病毒(Papaya ringspot virus, PRSV)的感染，之後其病害迅速蔓延全台灣，嚴重衝擊台灣木瓜產業。為了防範病毒所造成的病害，交互保護 (cross-protection)為一種不錯的防治策略。交互保護為先將弱系病毒接種於植物上，可使植物有效的抵抗親源性相近的強系病毒之感染。雖然目前弱系的木瓜輪點病毒有夏威夷HA 5-1品系，但因交互保護效果僅侷限於親緣性較高的病毒，所以要能防治台灣品系的木瓜輪點病毒病害，則須選擇來自台灣本地的弱系病毒，才能有效的保護，因此本實驗的目的即利用人為方式，在台灣木瓜輪點病毒永康品系基因體上進行點突變，以獲得弱系的台灣木瓜輪點病毒，提供交互保護的使用。實驗首先比對台灣木瓜輪點病毒永康品系(PRSV-YK)與夏威夷弱病毒品系(PRSV HA 5-1)及其他不同種的馬鈴薯Y群病毒的P1及HC-Pro序列，推測在PRSV-YK品系的P1及HC-Pro基因上分別有兩個胺基酸位置可能是影響病毒毒力的關鍵點，針對這四個點設計特定引子，利用聚合?連鎖反應(polymerase chain reaction; PCR)分別進行一個及多個核?酸的點突變，所造成胺基酸的改變在P1蛋白上的有I309→S和K481→Q以及在HC-Pro蛋白上的有F753→L和D944→N。目前已獲得三種在HC-Pro蛋白突變的構築，分別為pPYK 753L、pPYK 944N及pPYK 753L944N，將其質體接種到木瓜植株一個月後，發現除了接種pPYK753L944N有出現微弱病徵外，另外pPYK753L及pPYK944N的構築在木瓜植株上均未造成感染。將此三種HC-Pro區域突變的病毒接種到單斑寄主奎藜(Chenopodium quinoa)時，皆無單斑的產生。利用酵素連結免疫吸附反應(Enzyme-Linked Immunosorbent Assay ; ELISA)進行偵測時，可偵測到pPYK 753L944N有明顯的病毒累積而pPYK753L與pPYK944N的構築，則偵測不到病毒的存在。在交互保護實驗，已被YK 753L944N重組病毒保護一個月的木瓜植株，再以YK品系病毒進行挑戰接種，一個月後發現木瓜植株上僅有輕微的病徵產生，且病毒濃度累積介於單獨接種『保護病毒』與只接種野生型YK的木瓜植株之間。另外以『保護病毒』接種0天、5天及10天後，再進行挑戰接種，之後15天觀察其病徵表現及病毒累積的濃度，發現在0天至15天的保護時間期限並不足以提供木瓜植株達到有效的保護效果。

關鍵詞 : 木瓜輪點病毒 ; 點突變 ; 交互保護作用

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