木瓜輪點病毒鞘蛋白之N端對植物系統感染之影響

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摘 要

木瓜輪點病毒(Papaya ringspot virus; PRSV)為RNA病毒，可對應一條複合大蛋白，其中P1和helper component-proteinase (HC-Pro)蛋白可裂解自身的C端，其他蛋白則由nuclear inclusion protein a (NIa)所剪切。由Nla蛋白的切位規則可發現NIb與鞘蛋白(coat protein; CP)間存在有兩個NIa可認知的切位，因此可對應出兩個同一讀框(open reading frame)的異質鞘蛋白，兩者之差別僅在N端的20個胺基酸，本實驗目的即探討此異質鞘蛋白的產生對病毒感染植物的影響。利用PCR突變方式共構築12個CP突變病毒株，其中8個突變株分別在第一個及第二個切位上(CP1及CP2)進行胺基酸的取代，獲得CP1QS、CP1MS、CP1GS、CP2ES、CP2MS、CP2GS、CP12QS/ES及CP12MS/GS突變病毒，另外，4個突變株分別在鞘蛋白N端進行5、10、15和20個胺基酸的刪除，得到CPΔ5、CPΔ10、CPΔ15及CPΔ20突變病毒。將野生型病毒及此12種突變病毒接種到木瓜上，其中野生型病毒35S-HA及35S-HAGFP分別在接種後第10天及第14天的木瓜上位葉出現病徵，而突變病毒CP1QS、CP1GS與CPΔ10則於接種後第21天才在木瓜上位葉出現病徵。摘取木瓜植株上位葉進行酵素連結免疫吸附法、RT-PCR、北方墨點法及西方墨點法分析，結果發現接種CP1QS、CP1GS、CPΔ5與CPΔ10突變株的木瓜植物皆可偵測到病毒，其他突變病毒株則未偵測到有系統感染的情形。以上結果顯示，CP1及CP2切位的改變皆會影響木瓜輪點病毒的感染能力，具較長N端的鞘蛋白對病毒系統性的感染是必需的，而且其N端第11-15個胺基酸(SNNTH)有可能是影響木瓜輪點病毒系統性感染最關鍵的區域。而CP2切位所產生的較短N端的鞘蛋白，則是病毒感染植物所必需。

關鍵詞 : 木瓜輪點病毒、NIa蛋白裂解?、鞘蛋白、突變、酵素連結免疫吸附法、反轉錄聚合?連鎖反應

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