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胡瓜嵌紋病毒(CMV)相關之沙漠玫瑰輪斑嵌紋病

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摘 要

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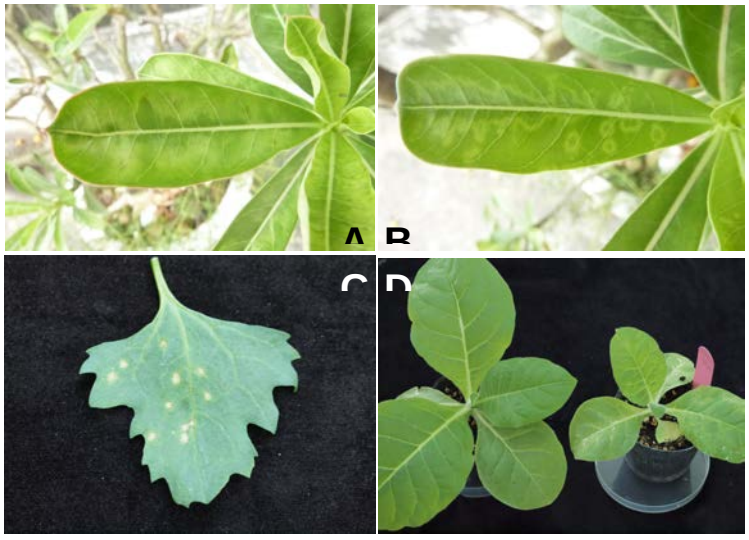
2010 年於嘉義地區發現葉片具有嵌紋、黃化輪紋與線條等病徵的沙漠玫瑰[Desert rose, *Adenium obesum* (Forssk.) Roem. & Schult, family Apocynaceae]。罹病組織粗汁液內具有直徑約 28 nm 的疑似球形病毒顆粒。 血清學反應顯示與胡瓜嵌紋病毒(*Cucumber mosaic virus*, CMV)有正相關。單離後的病毒株可機械接種於指示植物並引起與 CMV 感染後之類似病徵。以專一性引子對配合反轉錄-聚合酶連鎖反應(RT-PCR)增幅 CMV RNA 3 靠 3’ 端半段（即 RNA 4）的 cDNA。所增幅的 cDNA 經選殖與解序已取得基因資料庫登錄碼(GenBank accession number) AB667971。基因序列分析顯示外鞘蛋白基因的核苷酸與胺基酸序列與 CMV 第一亞群及第二亞群的對應序列分別有 92.2-95.0% 和 75.5-77.7% 相同度。利用番茄斑萎病毒屬的廣效簡併式引子對並未能在罹病的沙漠玫瑰檢測得番茄斑萎病毒屬的病毒感染，顯示 CMV 是沙漠玫瑰輪斑嵌紋病相關的主要因子。

關鍵詞：胡瓜嵌紋病毒、番茄斑萎病毒、沙漠玫瑰、黃化輪紋、嵌紋

沙漠玫瑰[desert rose, *Adenium obesum* (Forssk.) Roem. & Schult]為原生於非洲的夾竹桃科(family Apocynaceae)多肉植物，因其耐旱、耐熱、容易照顧且花色多等特點，成為常見的盆栽觀賞花卉。2010 年於嘉義地區發現沙漠玫瑰葉片具有嵌紋及褪綠實心或空心輪斑(圖一)等疑似病毒引起的病徵。電子顯微鏡鏡檢病組織粗汁液，可觀察到直徑約 28 nm 疑似病毒的球形顆粒。初步以酵素聯結免疫吸附分析法(ELISA)和西方轉漬法(western blotting)檢測，病組織粗汁液與胡瓜嵌紋病毒 (*Cucumber mosaic virus*, CMV)的抗血清[(8)]有正反應。胡瓜嵌紋病毒(*Cucumber mosaic virus*, CMV)最早於 1916 被發現為植物病原[(12)]，是一種寄主範圍廣泛，引起多種經濟作物嚴重損失的植物病毒[(13, 17)]，被歸類為胡瓜嵌紋病毒屬(genus *Cucumovirus*)的模式種(type species)[(16)]。 CMV 的基因體由三條單股的正意核醣核酸(single-stranded positive-sense RNA)所組成，依其分子大小分別以 RNA 1、RNA 2 和 RNA 3 稱之，另自 RNA 3 與 RNA 2 分別轉錄出兩條次基因體 RNA (subgenomic RNA) – RNA 4 和 RNA 4A [(10, 19)]。RNA 1 和 RNA 2 的基因產物組合成複製酶(replicase)，RNA 3 上有兩個基因可分別轉譯出移動蛋白(movement protein, MP)和外鞘蛋白 (coat protein, CP) [(23, 24)]。外鞘蛋白是藉由次基因體 RNA 4 的表現而產生；,而另一次基因體 RNA 4A 的表現則產生 2b 蛋白(2b protein)。 2b 蛋白與抑制寄主植物的基因靜默作用、病毒在寄主體內的長距移動、以及病徵的表現有關[(4, 10)]。CMV 的系統(strain)和病毒株(isolate)眾多，目前依其 CP 和 RNA 3 的 5’端非轉譯區 (5’ NTR) 之演化類緣分析可區

分 CMV 成 IA、IB、和 II 等三個亞群 (subgroups)[2, 18, 20, 22]。

沙漠玫瑰病組織粗汁液機械接種於奎藜（*Chenopodium quinoa*)進行單斑分離，經三次單離後得一病毒分離株，暫以 CMV-DR 稱之。CMV-DR 接種的奎藜病葉以 5 倍量(w/v) 0.1 M 磷酸緩衝液(0.1 M phosphate buffer, pH 7.2)萃取粗汁液，並機械接種於 CMV 的診斷寄主，可在奎藜(*C. quinoa*)、紅藜(*C. amaranticolor*)和千日紅(*Gomphrena globosa*)的接種葉引起壞疽性局部病斑；也可在胡瓜(*Cucumis sativus*)、南瓜(*Cucurbita moschata*)、絲瓜(*Luffa cylindrica*)、番茄(*Solanum lycopersicum*)、甜椒(*Capsicum annuum*)和數種菸草(*Nicotiana benthamiana*、*N. glutinosa*、*N. rustica*、 *N. tabacum* cv. van Hicks)引起系統性嵌紋病徵。但在十字花科 (*Brassica campestris*、*B. oleracea* var. *botrytis*、*B. juncea*)及豆科(*Phaseolus vulgaris*、*Pisum sativum*)診斷寄主則不造成感染。CMV-DR 的寄主範圍與在某些寄主植物所造成的病徵表現則與某些本土性的 CMV 病毒株所造成者極爲類似 [8]。以接種 CMV-DR 的菸草(*N. tabacum* cv. van Hicks)爲材料依 Hsu *et al.*[14]的方法進行病毒顆粒之純化，可得大量直徑約 28 nm 的球形病毒顆粒(圖二)。以 Roossinck and White[21]的方法自純化的病毒樣本中純化病毒 RNA，並以 TRIzol Reagent (Invitrogen, USA)純化罹病沙漠玫瑰組織之總量 RNA。取病毒 RNA 配合 CMV RNA 4 的簡併式引子對 (forward:5’-ACATCAYAGTTTTRAGRT TCAATTC-3’；reverse: 5’-TGGTCTCCTT-3’)進行反轉錄聚合酶連鎖反應(reverse transcription- polymerase chain reaction, RT-PCR)[7]。RT-PCR 反應可增幅出一大小約 1.1 kb 的 cDNA 片段。該片段經選殖於 pGEM-T easy (Promega, USA)載體，並送交中興大學生科中心進行序列解析。結果顯示其全長爲 1123 個核苷酸(nucleotides, nt)，包括 CMV RNA4 的 5’端 (165 nt)、CMV 外鞘蛋白基因開放轉譯架(coat protein open reading frame, CP ORF) (657 nt)和 3’ 非轉譯區(3’ nontranslated region, 3’ NTR) (301 nt)， 其在美國國家生物技術資訊中心(NCBI)基因數據庫的登錄碼(accession number)爲 AB667971 [6]。



圖一、胡瓜嵌紋病毒相關的病徵。在沙漠玫瑰葉片引起嵌紋(A)和黃化輪斑與條紋(B)，在奎藜(*Chenopodium quinoa*)接種葉上造成壞疽性局部病斑(C)，和在菸草(*Nicotiana tabacum*)接種葉引起壞疽性單斑(接種 7 天後)、隨後引起嵌紋黃斑與嚴重矮化等系統性病徵(接種 14 天後) (D、右株)。

Figure 1. *Cucumber mosaic virus*-associated symptoms on desert rose (A, B), *Chenopodium quinoa* (C), and *Nicotiana tabacum* (D). Field symptoms of mosaic (A), ringspots and line patterns (B) showed on the leaves of desert roses. Necrotic local lesions appeared on the inoculated leaves of *Chenopodium quinoa* (C) and *Nicotiana tabacum* (D, right plant) 7 days after mechanical inoculation by the CMV associated to desert rose ringspot mosaic disease. Systemic mild mosaic, faint yellowish ringspots, and severe stunting (D, right) appeared 14 days after inoculation.

選 36 個 CMV 分離株與 CMV-DR 進行序列相同度比較分析(Vector NTI Advance 10, Invitrogen, USA)，顯示 CMV-DR 與他種 CMV 的 RNA 4 全長度序列相同度介於 72.1–93.5%之間，而外鞘蛋白基因的核苷酸與胺基酸序列相同度則分別介於 75.5-95.7%與 77.6-99.5% (表一)。由外鞘蛋白基因的核苷酸序列相同度分析可知，CMV-DR 與第一亞群(subgroup I)的 CMV 較近似 (其核苷酸序列相同度高於 92.2%以上)；而與第二亞群(subgroup II)的 CMV 有較疏遠的類緣關係(核苷酸序列相同度低於 77.7%)。外鞘蛋白基因具有 657 個核苷酸可轉譯出具有 218 個胺基酸的外鞘蛋白，其演譯出的胺基酸序列和第一亞群(subgroup I)的 CMV 病毒株有高於 95.4%的相同度(表一)。類緣關係演化分析亦顯示類似的結果，且與本土性的 CMV 分離株，如 NT9 (D28780) [14]、Alla (AJ871492) [8]、AMA (AJ131625) 、DEL (AJ131627)、EUS (AJ131627) [7]同屬於第一 B 亞群 (sungroup IB)，推論 CMV-DR 應該也是本土性的病毒株。

表一、胡瓜嵌紋病毒沙漠玫瑰分離株(CMV-DR) RNA 4 基因(AB667971)各區域的核苷酸與胺基酸序列與其他胡瓜嵌紋病毒分離株之相同度比較

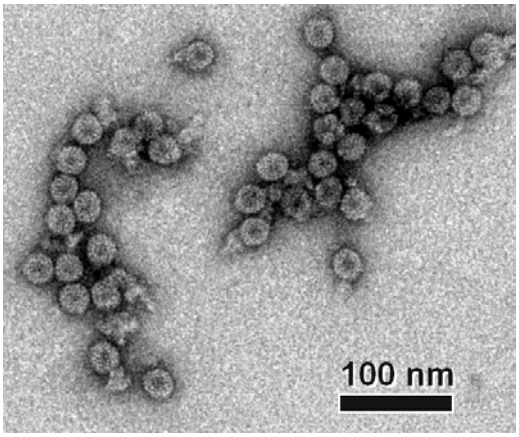
Table 1. Comparison of the nucleotide (nt) and/or amino acid (aa) sequence of the full length, 5’ nontranslated region (5’ NTR), coat protein gene open reading frame (CP ORF), and 3’ NTR of the *Cucumber mosaic virus* desert rose isolate (CMV-DR) RNA 4 (AB667971) with those of some CMV isolates

Isolate	Accession Number	Identity (%)				
		RNA 4	5’ NTR	CP ORF		3’ NTR
		nt	nt	nt	aa	nt
O	D00385	89.1	75.9	93.2	97.7	87.5
Mf	AJ276481	90.1	78.2	94.1	97.7	88.2
Rb	GU327365	90.1	77.6	94.4	97.7	87.8
Fny	D10538	89.8	78.2	94.4	98.2	86.3
Fny	NC_001440	89.8	78.2	94.4	98.2	86.3
M	D10539	89.5	78.2	93.8	95.4	86.6
M48	D49496	89.4	78.2	92.2	95.4	89.5
HL	AB049568	87.8	79.3	92.8	97.7	82.1
Lily	AJ131615	87.6	77.8	93.2	97.7	81.5
LiCB	AB506800	87.5	78.8	93.0	97.2	80.8
Li	AB506797	87.4	78.9	93.3	98.2	79.9
Ly2	AJ296154	87.8	79.9	93.5	98.2	80.5
Z	AB369269	89.8	78.2	93.6	97.7	88.2
Z1	GU327368	89.7	78.2	93.6	97.7	87.5
As	AF013291	89.1	80.4	94.5	98.2	82.6
Alla	AJ871492	93.2	83.6	95.6	98.6	93.4
AMA	AJ131625	93.5	84.8	95.7	98.2	93.4
DEL	AJ131626	93.1	84.8	95.0	98.2	93.4
EUS	AJ131627	91.9	84.8	94.7	97.2	89.8

表一、胡瓜嵌紋病毒沙漠玫瑰分離株(CMV-DR) RNA 4 基因(AB667971)各區域的核苷酸與胺基酸序列與其他胡瓜嵌紋病毒分離株之相同度比較(續)

Table 1. Comparison of the nucleotide (nt) and/or amino acid (aa) sequence of the full length, 5' nontranslated region (5' NTR), coat protein gene open reading frame (CP ORF), and 3' NTR of the *Cucumber mosaic virus* desert rose isolate (CMV-DR) RNA 4 (AB667971) with those of some CMV isolates (Cont.)

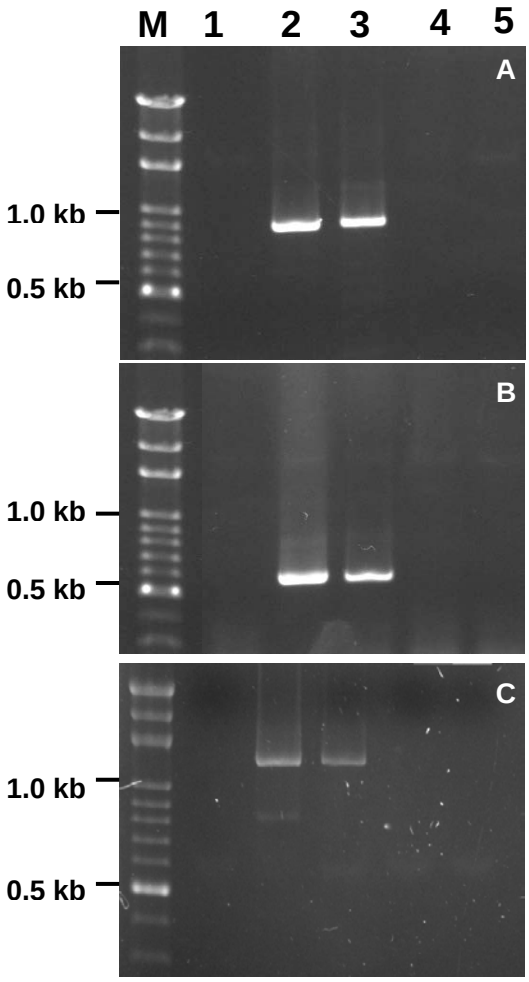
Isolate	Accession Number	Identity (%)				
		RNA 4	5' NTR	CP ORF		3' NTR
		nt	nt	nt	aa	nt
242	AJ585520	90.3	77.1	94.8	99.5	87.9
K	AF127977	91.1	79.6	95.0	99.5	89.5
Tfr-In	JF279606	91.7	84.8	94.1	98.6	90.5
NT9	D28780	92.1	83.6	94.5	98.6	91.5
Tfn	Y16926	92.4	86.7	94.8	99.1	90.2
Pl-1	AM183116	92.3	86.7	94.2	98.2	91.1
D8	AB004781	90.4	77.2	94.5	97.7	88.8
Twa	AJ585522	89.9	77.6	94.1	97.7	87.8
Alidarh	EU642567	72.4	68.5	75.5	77.6	68.3
Tss-In	JF279605	73.5	67.3	77.7	82.6	68.3
Palampur	HE583224	73.6	67.9	77.1	81.7	69.3
ALS-IPO	AJ276587	72.9	66.1	76.7	79.5	68.7
Q	M21464	73.2	66.1	77.7	81.7	67.6
WL	D00463	72.1	64.9	77.3	80.8	65.4
Trk7	L15336	73.5	71.7	76.7	79.5	68.2
M2	AB006813	73.3	66.1	77.6	81.3	68.2
TN	AB176847	73.7	69.7	77.4	81.3	68.2



圖二、胡瓜嵌紋病毒沙漠玫瑰分離株之病毒顆粒。

Figure 2. Virions of the desert rose isolate of *Cucumber mosaic virus* (CMV-DR).

感染沙漠玫瑰的病毒目前已知有 CMV [3]和番茄斑萎病毒(*Tomato spotted wilt virus*, TSWV) [1]，前者引起嵌紋病徵，而後者則引起黃斑與條紋病徵。此兩種病徵可同時在本研究的病株樣品中觀察到，顯示不能排除兩種病毒共同感染的可能性。由於進行病毒分離時僅分離得 CMV 單一種病毒，因此取沙漠玫瑰原始病株之鮮葉組織萃取總量 RNA，以多組番茄斑萎病毒屬的簡併式廣效引子對 [gL3673/gL4510c [9]、gM410 /gM870c [5]和 gL2740 / gL3920c [5]]分別進行 RT-PCR 檢測。結果顯示利用三組番茄斑萎病毒屬的簡併式引子對均可在西瓜銀斑病毒(*Watermelon silver mottle virus*, WSMoV)與 TSWV 感染的菸草植株中檢測出 WSMoV 與 TSWV，但未能自原始的沙漠玫瑰病株上檢測出任何番茄斑萎病毒屬的病毒(圖三)。



圖三、應用番茄斑萎病毒簡併式引子對以 RT-PCR 檢測番茄斑點萎凋病毒(TSWV) 感染沙漠玫瑰之可能性。A、引子對 gL3637/gL4510c [9]， B、引子對 gM410 / gM870c [5]，C、引子對 gL2740 / gL3920c (Chen *et al.*, 2012)。M 爲分子標記，行 1 -5 分別表示 RNA 之來源，1 爲沙漠玫瑰病株、2 爲西瓜銀斑病毒(WSMoV)接種之 *Nicotiana benthamiana*、3 爲 TSWV 接種之 *Nicotiana benthamiana*、4 爲 *Chenopodium quinoa* 健葉、5 爲 *N. benthamiana* 健葉。

Figure 3. RT-PCR detection of *Tomato spotted wilt virus* (TSWV) in diseased desert rose using primers gL3637/gL4510c [9] (panel A), primers gM410 / gM870c [5] (panel B), and primers gL2740 / gL3920c [5] (panel C). Lanes M indicate the molecular markers. Digits 1-5 indicate the sources of RNA used in RT-PCR were extracted from desert rose (lane 1), WSMoV-infected *Nicotiana benthamiana* (lane 2), TSWV-infected *N. benthamiana* (lane 3), healthy plant of *Chenopodium quinoa* (lane 4), and healthy plant of *N. benthamiana* (lane 5), respectively.

雖然不同的病毒在沙漠玫瑰引起不同的病徵已見諸國外的報告[1, 3]，但本研究自沙漠玫瑰的病株只分離得CMV一種病毒，也未在沙漠玫瑰病株檢測出任何可能的番茄斑萎病毒，顯示本研究的沙漠玫瑰病毒病害之病因與CMV最具相關性。長期的病徵觀察中發現黃色輪斑與線條常見於新生葉與幼葉，但新葉亦有未出現輪斑而呈現嵌紋病徵者。在生長過程中，部份的葉片的病徵會消失，待葉片漸入成熟期，病徵會再出現，但以嵌紋狀病徵爲主。

本研究探討沙漠玫瑰輪斑嵌紋病的相關病因，證據顯示胡瓜嵌紋病毒(CMV)與本病有關，但確認其病因仍須將CMV-DR 回接於沙漠玫瑰健株並產生輪斑或嵌紋病徵。

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ABSTRACT

Y.S. Chang[1], Y.W. Lin[1], T.C. Chen[2], and Y.K. Chen[1*]. 2012. *Cucumber mosaic virus*-associated ringspot mosaic disease of desert rose. Bull. Plant Pathol. Bull. 21 : 39-45. (1: Department of Plant pathology, National Chung Hsing University, Taichung, Taiwan; 2: Department of Biotechnology, Asia University, Taichung, Taiwan; * corresponding author, Email: ykchen@nchu.edu.tw, FAX: 04-22877585)

Desert rose [*Adenium obesum* (Forssk.) Roem. & Schult, family Apocynaceae] with symptoms of mosaic, chlorotic ringspots and line patterns on leaves were observed in Chiayi area in 2010. Spherical virus particles with a diameter of approximately 28 nm were observed in crude sap prepared from symptomatic leaves. Serological tests indicated that the virus reacted positively to a rabbit antiserum prepared to *Cucumber mosaic virus* (CMV). Isolated virus culture was mechanically transmissible to indicator plants and induced symptoms similar to those incited by CMV. The cDNA of the 3′-half of CMV RNA 3 has been obtained in reverse transcription-PCR with specific primers. The amplified cDNA fragment was cloned and sequenced (GenBank Accession No. AB667971). Nucleotide sequences of the coat protein open reading frame (CP ORF) had 92.2 to 95.0% and 75.5 to 77.7% sequence identity to those of CMV in subgroups I and II, respectively. Tospoviruses were not detected from infected plants of desert rose by using wide-spectrum degenerated primers for *Tospovirus* in RT-PCR indicating the CMV is possibly the main factor associated to the ringspot mosaic disease of desert rose.

Key words: *Cucumber mosaic virus*, *Tospovirus*, desert rose, chlorotic ringspot, mosaic