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Characterization of an isolate of *Cucumber mosaic virus* associated with allamanda mosaic

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ABSTRACT

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This study reveals the occurrence of a mosaic disease in allamanda cultivated in Taiwan. The present study was undertaken to establish the etiology of the disease. The results were based on biological, serological and molecular analyses and indicated that a subgroup IB isolate of *Cucumber mosaic virus* (CMV) is the causal agent of the allamanda mosaic.

Key words: *Cucumber mosaic virus*, allamanda mosaic

Allamanda (*Allamanda carthatica* L.; family *Apocynaceae*), also called "golden trumpet vine", is native to Brazil and is a popular perennial shrub or vine ornamental worldwide. Plants showing severe mosaic, rugose and distortion in leaves (Fig. 1-A) are common in Taiwan. Examination of crude sap prepared from symptomatic leaves by transmission electron microscope (TEM) revealed the presence of spherical virus-like particles with a diameter of about 28 nm. To our knowledge, *Cucumber mosaic virus* (CMV) is the only virus that has been detected in allmanda [12], however, it has not been well characterized yet.

CMV is the type species of the genus *Cucumovirus* in the family Bromoviridae [10] and was firstly reported in 1916 as a causal agent of plant diseases [9]. It has a broad host range, a worldwide distribution and a severe impact on cultivated crops. These characteristics make it one of the economically most important plant viruses [14]. CMV contains three genomic RNAs, designated as RNA 1, RNA 2, and RNA 3, and generates at least two subgenomic RNAs, RNA 4 and RNA 4A, which are transcribed from the RNA 3 and RNA 2, respectively [7, 16]. RNA 1 and RNA 2 encode the replication associated proteins, while RNA 3 encodes the movement protein (MP) [20] and the coat protein (CP) [19]. Subgenomic RNA 4 allows the translation of the CP, while the RNA 2-derived RNA 4A encodes the 2b protein that is involved in the suppression of gene silencing, long-distance movement, and expression of systemic symptoms [2, 8]. Based on phylogenetic analysis of the CP ORF and rearrangements in the 5' non-translated region (NTR) of RNA 3, CMV strains can be currently divided into three subgroups: IA, IB, and II [1, 14, 17, 18]. We present evidence that CMV is associated with allamanda mosaic in this study. Further molecular analysis of 3'-half of RNA 3 showed that the allamanda CMV belongs to subgroup IB.

A virus culture was isolated from diseased allmanda plants showing severe mosaic, rugose and distortion in leaves (Fig. 1-A). Numerous spherical viral particles with a diameter of about 28 nm were observed in negatively stained purified preparations. The host range and symptomatology of the virus are detailed in Table 1. Local infections were found in *Chenopodium amaranticolor* (Fig. 1-B), *C. quinoa* (Fig. 1-C) and *Gomphrena globosa* 3 to 5 days post-inoculation (dpi) when plants were mechanically inoculated with leaf extracts prepared from isolated virus. Localized etched ringspots have also been observed in the inoculated leaves of *Nicotiana tabacum* cv. Van Hicks, however, the infections became systemic and caused severe mosaic later on upper new leaves (Fig. 1-D,

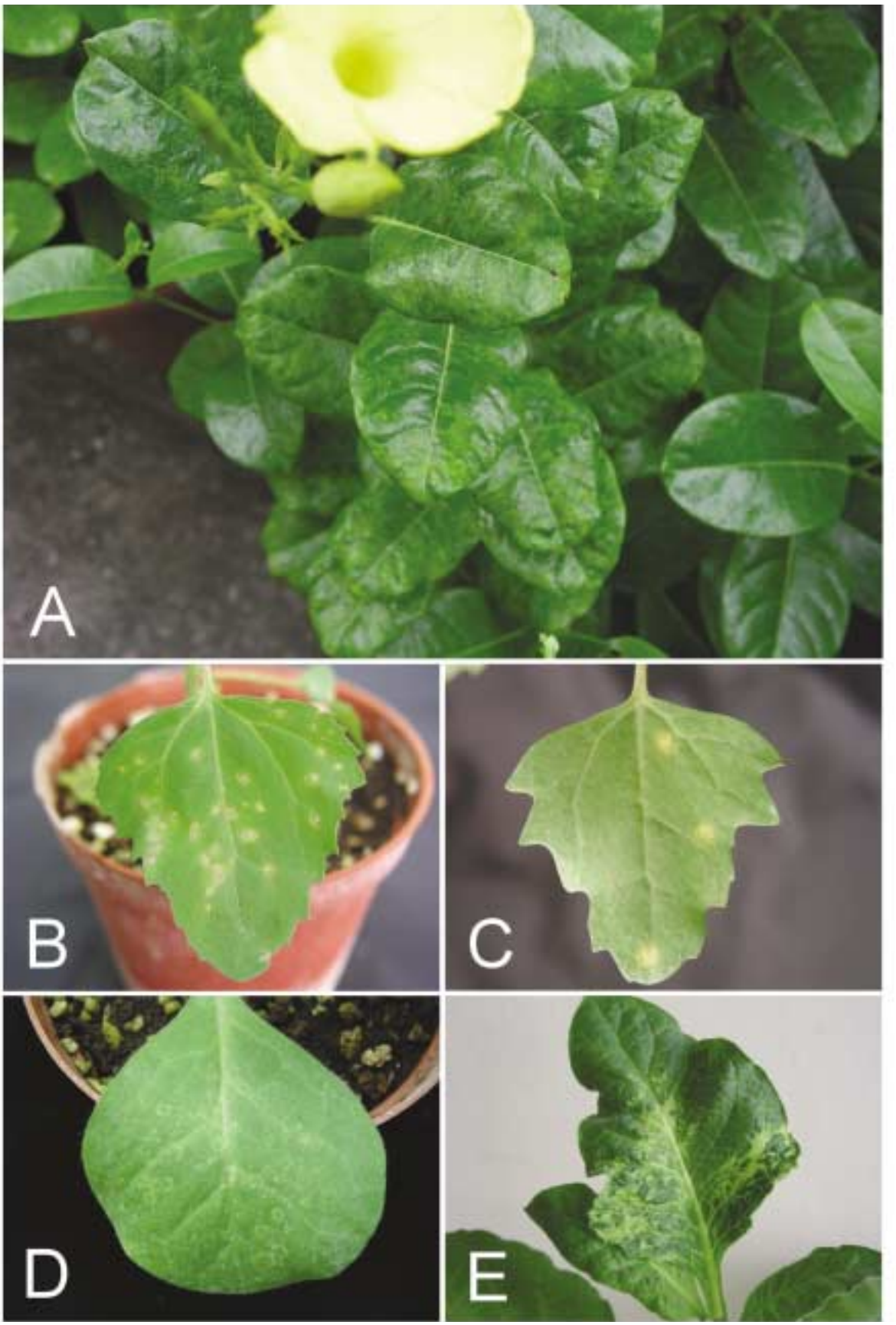

Fig. 1. Symptoms of host plants infected by *Cucumber mosaic virus* allamanda isolate (CMV-Alla). A. *Allamanda carthatica*; B. *Chenopodium amaranticolor*; C. *C. quinoa*; D. Local lesions on inoculated leaf of *Nicotiana tabacum*; E. Systemic mosaic in *N. tabacum*.

Table 1. Host range and symptomatology of *Cucumber mosaic virus* isolated from allamanda

Test plants	Reactions
Amaranthaceae	
Gomphrena globosa	Local lesion
Chenopodiaceae	
Chenopodium amaranticolor	Local lesion
C. quinoa	Local lesion
Cruciferae	
Brasica campestris	No infection
B. oleracea var. botrytis	No infection
Cucurbitae	
Cucumis sativus	Systemic mosaic
Cucurbita moschata	Systemic mosaic
Luffa aegyptiaca	Systemic mosaic
Leguminosae	
Phaseolus vulgaris	No infection
Pisum sativum	No infection
Solanaceae	
Capsicum annuum var. annuum	Systemic mosaic
Lycopersicon esculentum	Systemic mosaic
Nicotiana benthamiana	Systemic mosaic
N. glutinosa	Systemic mosaic
N. rustica	Systemic mosaic
N. tabacum cv. Van Hicks	Etched ring spots in inoculated leaves and systemic mosaic

1-E).

Polyacrylamide gel electrophoresis (PAGE) of sodium dodecyl sulfate (SDS) dissociated protein [13] of purified virus revealed a major polypeptide band of about 23 kDa which similar to that of CMV. Immunoblotting identified the protein band is immunologically reacted to the homologous antiserum and to the virus-specific monoclonal antibody [11] against *Cucumber mosaic virus*. Immunoblots further probed with CMV subgroup-specific monoclonal antibodies [11] indicated that the tested allamanda virus is an isolate of subgroup I CMV (Fig. 2). Reverse-transcription polymerase chain reaction (RT-PCR) using degenerate primers for the 3'-half of CMV RNA 3 [5] produced a cDNA fragment of about 1.1 kb. Sequence analysis revealed that the amplified cDNA fragment was 1115 nucleotides in length and consisted of the 3' part of the intergenic region of RNA 3 (158 nt), CP ORF (657 nt) and 3' non-translatable region (3' NTR, 300 nt). The nucleotide sequence of the cDNA clone of the 3'-half of RNA 3 encoding the RNA 4 subgenomic messenger from allamanda CMV is now available in the EMBL database with accession number of AJ871492. The nucleotide sequence of the amplified fragment showed 83.5-98.2% and 57.8-64.7% similar to those of CMV in subgroups I and II, respectively, confirming that the tested virus belongs to subgroup I of CMV (Table 2). The length of CP

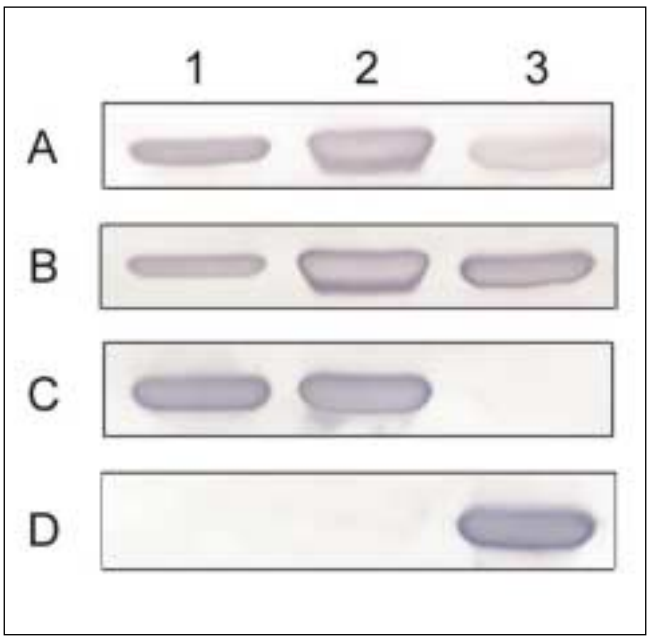

Fig. 2. Immuno-detection of allamanda virus with homologous antiserum and CMV-specific monoclonal antibodies (MAb) in transblots of SDS-PAGE. Purified allamanda virus (lane 1), subgroup I CMV (strain EUS) (lane 2), and subgroup II CMV (Strain S) (lane 3) were probed with homologous polyclonal antiserum (A), CMV-specific MAb (B), subgroup I CMV-specific MAb (C), and subgroup II CMV-specific MAb (D).

Table 2. Comparison of the nucleotide (nt) and/or amino acid (aa) sequences of the full length, coat protein gene, 5' and 3' nontranslated regions (NTR) of RNA 4 of CMV- allamanda (AJ871492) with those of some CMV isolates

Subgroup	Isolate	Accession Number	Identity (%)				
			RNA 4	5'NTR	CP ORF		3'NTR
			nt	nt	nt	aa	nt
IA	K	AF127977	89.2	66.5	95.1	99.1	88.0
	As	AF013291	85.6	67.7	95.3	99.1	78.0
	Lily	AJ131615	83.5	65.8	91.9	98.2	76.3
	Ly2	AJ296154	83.8	67.3	92.2	98.6	75.7
	O	D00385	85.4	62.4	91.8	97.2	84.3
	Fny	NC_001440	84.9	63.2	93.0	97.7	80.3
	Mf	AJ276481	86.2	63.9	93.0	97.2	83.3
	D8	AB004781	85.7	61.8	93.0	97.2	83.3
IB	NT9	D28780	90.9	73.3	94.7	98.2	91.0
	Tfn	Y16926	90.3	74.8	95.0	98.6	89.0
	DEL	AJ131626	98.2	96.2	98.8	98.6	98.0
	EUS	AJ131627	96.5	97.5	98.5	97.7	91.7
	AMA	AJ131625	97.8	93.0	98.9	98.6	98.0
II	TN	AB176847	63.6	58.9	72.5	79.8	54.0
	Trk7	L15336	63.7	66.2	71.4	78.0	54.3
	ALS-IPO	AJ276587	61.8	63.6	71.1	78.0	54.3
	WL	D00463	57.8	65.7	72.1	79.4	46.3
	Q	M21464	64.7	64.9	72.8	80.3	53.7

ORF for tested isolate is 657 nt and thus encodes a coat protein of 218 amino acids. The nucleotide sequence homology of the ORF is more than 91.8% (up to 98.9%) and 71.1% (up to 72.8%) for isolates within and between subgroups, respectively. This translates to more than 97.2% (up to 99.1%) and 78.0% (up to 80.3%) within and between subgroups for the deduced CP amino acid sequence. The nucleotide sequence identity between 5' NTR of the tested isolate and published CMVs showed high divergence between isolates of different subgroups and even between isolates of different geographical origins within the same subgroup. Sequence of 3'-NTR in the tested isolate is less divergent than that of the 5' NTR and more than 75.7% (up to 98.0%) identity can be observed between isolates within the same subgroup. Based on phylogenetic analysis, CMV strains can be divided into three subgroups: IA, IB, and II [15, 18]. Among these, most strains of subgroup IB are of Asiatic-Pacific origin [18]. The >96.5% nucleotide sequence identities between amplified cDNA fragments of allamanda CMV and domestic subgroup IB CMV strains [5], i.e. strains DEL [4], AMA [6], and EUS [3], indicated it is an isolate belonging to CMV subgroup IB which is predominant on the island.

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摘 要

陳煜焜[1,2]、楊佳津[1]. 2006. 軟枝黃蟬嵌紋病之病因鑑定. 植病會刊15:77-82. (1台中市 中興大學植物病理學系；[2] 聯絡作者，電子郵件：ykchen@nchu.edu.tw；傳真: +886-4-2287-7585)

軟枝黃蟬疑受病毒感染產生嵌紋病徵。本文提出生物學、血清學及分子層次的證據，證實該病害是由胡瓜嵌紋病毒 (Cucumber mosauc virus, CMV) 所引起，且該 CMV 是一個 IB 亞群的分離株。

關鍵詞：胡瓜嵌紋病毒、軟枝黃蟬嵌紋病