

IDENTIFICATION OF A VIRUS INFECTING SQUASH

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SUMMARY

A mechanically transmissible virus was isolated from a squash showing a leaf mosaic symptoms. Virus particles observed with transmission electron microscopy were filamentous particles characteristic of potyviruses. Of 29 particles measured in leaf dips from squash, 65 % ranged from 782 - 813 nm long. This isolate induced systemic mottle or mosaic symptoms on squash and pumpkin. Cylindrical inclusions were seen in cytoplasm of epidermal cells of squash. The SDS-Immunodiffusion tests indicated that an antiserum prepared to an isolate of Watermelon Mosaic Virus - 2 reacted identically with the unknown. These properties and serological tests showed the mosaic disease was caused by Watermelon Mosaic Virus - 2.

INTRODUCTION

Mosaic viruses are important on cucurbits, some of them are Cucumber Mosaic Virus (CMV), Watermelon Mosaic Virus - 2 (WMV - 2), Watermelon Mosaic Virus - 1 (WMV - 1) and Squash Mosaic Virus (SqMV).

WMV, a member of the potyvirus group is a pathogen of worldwide importance in cucurbit crops (Webb and Scott, 1965; Van Regenmortel, 1971; Fischer and Lockhart, 1974). Webb and Scott (1965) divided WMV isolates from the USA into two distinct groups which were distinguished by host range and serological properties. An antiserum prepared to an isolate of WMV - 1 reacted strongly with WMV - 1 type isolates but not reacted with WMV - 2 isolates. Purcifull and Hiebert (1979) detected that the WMV complex consists of three serologically different groups of viruses, based on studies with antisera prepared to Florida isolates of WMV - 1 and WMV - 2.

A mechanically transmissible virus was isolated from a squash (*Cucurbita pepo melopepo* L.) showing a leaf mosaic symptoms. The objective of this study was to identify and characterize the unknown virus.

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MATERIALS AND METHODS

Light Microscopy

Epidermal strips were removed from infected leaves of the host plant and were stained with "Luxol" brilliant - green and calco-mine orange for the presence of inclusion bodies, then were prepared for microscopic examination (Christie, 1967).

Host Range and Symptomatology

Pieces of leaf tissue from squash that had mosaic symptom were grounded in a mortar containing phosphate buffer (0.05 M, pH 7.4) using 1 ml buffer per gram of leaf. The crude sap was rubbed on leaves with a pinch of 600-mesh carborandum on virus - free plant of Cucurbitaceae, Leguminosae, Amaranthaceae and Solanaceae that were obtained from seed. Plants were kept in a greenhouse for observation.

Electron Microscopy

A symptomatic area of squash leaf was prepared for examination in an electron microscope. The tissue was sliced up on slide in 1 % potassium phosphotungstate, pH 6.8, containing 0.1 % bovine serum albumin which was used as a negative stain (Purcifull and Hiebert, 1979). Philips 200 electron microscope were used to photograph the particles that were measured by comparison with a diffraction grating.

Serological Tests

The SDS - Immunodiffusion tests (Purcifull and Batchelor, 1977) were conducted in a medium consisting of 0.8 % Noble agar (Difco), 1.0 % sodium azide (Sigma), and 0.5 % SDS (Sigma). Test antigens were prepared by grinding tissues in water (1 mg/g tissue), followed by additional of 1 ml of 3 % SDS per gram of tissue. The samples were pressed out through cheesecloth and held at room temperature until use, but not longer than 15 min. In some cases, freeze - dried antigens were used (Purcifull et al., 1975). Some antigens and antisera were obtained from F. W. Zettler and D. E. Purcifull (Univ. of Florida, Gainesville, USA). For each test normal serum and healthy sap were used as controls. The plates were incubated for 24 hrs at 25°C, than were examined for precipitate lines.

RESULTS

Light Microscopy

Cylindrical inclusions were seen in the cytoplasm of epidermal cells of squash (Fig. 1.). This observation together with electron microscopy evaluation, determined that the unknown virus was belong to potyvirus group.

Host Range and Symptomatology

The responses of the unknown virus to the test host plants were shown on Table 1. This mechanically transmissible virus induced systemic mottle or mosaic symptoms on squash and pumpkin. No symptoms were induced by the unknown virus on the other plants in the test (Table 1.).

Electron Microscopy

Virus particles observed with transmission electron microscopy (TEM), were filamentous particles characteristic of potyviruses. Of 29 particles measured in leaf dips from squash 65 % ranged from 728 - 813 nm long.



Fig. 1. CI = Cylindrical inclusions in the cytoplasm of epidermal cell.

Table 1. Host plants and their reactions to the unknow virus.

Hosts	Reaction to Virus
Cucurbitaceae	
<i>Cucumis sativus</i>	-
<i>Cucurbita pepo</i>	+
<i>Cucurbita pepo melopepo</i>	+
<i>Luffa acutangula</i>	-
Leguminosae	
<i>Cassia occidentalis</i>	-
<i>Cassia tora</i>	-
<i>Pisum sativum</i> "Alaska"	-
<i>Pisum sativum</i> "Little Marvell"	-
Amaranthaceae	
<i>Gomphrena globosa</i>	-
Solanaceae	
<i>Nicotiana megalosiphon</i>	-
<i>Datura stramonium</i>	-
<i>Nicotiana tabacum</i> CV. NN Samsun	-
<i>Nicotiana tabacum</i> CV. Turkish	-

Serological Tests

The SDS - Immuno diffusion tests indicated that an antiserum prepared to an isolate of WMV - 2 reacted identically with the unknow virus, and an antiserum of WMV - 1 did not react identically (Fig. 2.). Other reactions were obtained and presented at Table 2.

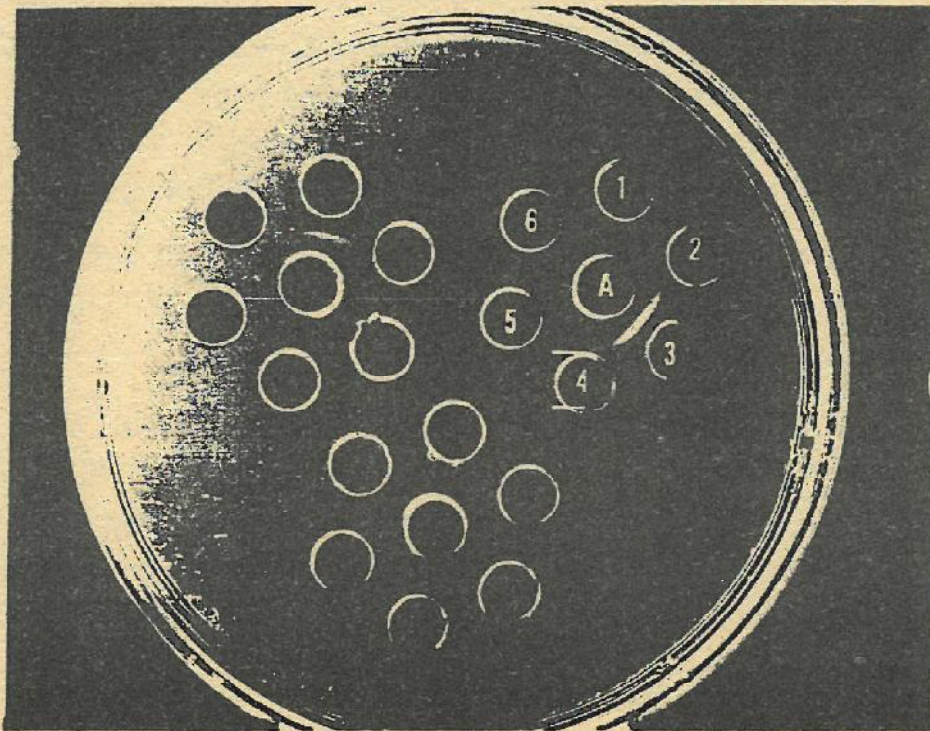


Fig. 2. A = Antiserum WMV - 2, 1 = WMV - Moroccan Ag(Antigen), 2 = Unknow virus Ag, 3 = WMV - 2 Ag, 4 = Papaya Ringspot Virus Ag, 5 = Healthy squash sap Ag, 6= WMV - 1 Ag. Serologic reactions of WMV - 2 antisera to unknow virus and other viruses.

Table 2. + = Identical reaction, - = Unidentical reaction, WMV - M = WMV - Moroccan, PRSV = Papaya Ringspot Virus, WMV = Watermelon Mosaic Virus. Reactions of Watermelon Mosaic Virus isolates.

Antisera	Antigens				
	Unknown	WMV - 2	WMV - 1	WMV - M	PRSV
WMV - 2	+	+	-	-	-
WMV - 1	-	-	+	-	+
WMV - M	-	-	-	+	-

The antigen of the unknow virus reacted only with antisera of WMV - 2. The Papaya Ringspot Viruses gave identical reaction when compared with WMV - 1, but they failed to react with the WMV - 2 and the unknow. The normal serum and healthy sap from squash did not from any precipitates and there were no non - specific precipitations.

DISCUSSION

A search of literature shows that mechanically transmissible WMV - 2 has filamentous particles, with a modal class of 780 nm, also induce systemic mottle or mosaic symptoms in squash and pumpkin. Sometimes systemic necrosis and premature death of the plants in pea has been recorded with WMV - 2 (Purcifull and Hiebert, 1979).

According to Christie and Edwardson (1977) WMV - 2 induces cylindrical inclusions in the cytoplasm of epidermal cells of squash. Our results support these observations.

Purcifull and Hiebert (1979) had showed that there were at least 2 and possibly 3 serologically distinct types of WMV. Our serology findings gave the same results. Light and electron microscopy, host range and serological tests showed that the mosaic disease was caused by WMV - 2.

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ÖZET

KABAK BİTKİSİNİ İNFEKTE EDEN BİR VIRUSUN TANIMLANMASI

Yaprakta mozaik belirtileri gösteren, mekanik olarak bulaşabilen bir virus kabak bitkisinden izole edilmiştir. Elektron mikroskopta gözlenen virus partikülleri, potyvirus grubunun özelliği olan uzun ince yapıda olan partiküllerdir. Ölçülen 29 partikülden % 65'i 781-813 nm uzunluğunda bulunmuştur. SDS - İmmunodifüzyon testleri sonucu, izole edilen virus WMV - 2 virusu ile benzer reaksiyon vermiştir. Bulunan özellikler ve seroloji testlerinin sonucu, izole edilen virusun WMV - 2 olduğunu göstermiştir.

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