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Biological and Serological Studies on an Isolate of Dwarf Mosaic Potyvirus Infecting Maize Plant

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ABSTRACT

In this study, an isolate of maize dwarf mosaic potyvirus (MDMV) infecting maize (*Zea mays* L.) was subjected to indirect-enzyme-linked immunosorbent assay (ELISA) detection using polyclonal antibodies (PAbs); biological and serological studies. The virus was successfully transmitted from maize to Sorghum (*Sorghum bicolor* var. Rio) and its characteristic symptoms were confirmed 15 days post virus inoculation. Ultra-thin sections of the virus-infected leaves of maize plant were prepared and subjected to electron microscopy. Results showed the presence of cytoplasmic cylindrical inclusion bodies, which appeared as pinwheels, scrolls and laminated aggregates. In addition, flexuous filamentous virus-like particles were found in the infectious sap. The virus was purified and a yield of 2.1 mg of the purified virus was obtained from 100 gram virus-infected tissue. In the following specific PAbs were raised in rabbits. The antiserum titer was determined using indirect-ELISA. MDMV-antiserum titer was 1/500, 1/1500 and 1/1000 of first, second and third bleedings, respectively.

Key Words: Biological study; Serological study; MDMV; Electron microscopy; Polyclonal antibodies; ELISA.

INTRODUCTION

Maize dwarf mosaic potyvirus (MDMV) was described by Williams and Alexander (1965). It infects numerous species in the Gramineae and induces the formation of cytoplasmic, cylindrical (pinwheel and scroll) inclusions in host cells. Field and sweet corn (*Zea mays*), grain and fodder sorghum (*Sorghum bicolor*), and Johnsongrass (S. *halepense*) are important natural hosts, developing mosaic and occasional necrotic foliage and occasionally dwarfing symptoms. The virus possibly occurs wherever maize and sorghum are grown throughout the world with the exception of Australia. Sap transmission tests show that most species of *Gramineae* are susceptible, some being infected symptomlessly (Ford & Tosic, 1972; Tosic & Ford, 1974). Cultivated wheat, barley, oat, rye and rice are non-hosts.

The MDMV is transmissible mechanically and by several aphid species in a non-persistent manner. The virus is characterized with flexuous filamentous particles *c.* 750 nm long and 13 nm in diameter containing single-stranded RNA (Shukla *et al.*, 1989). The virus was also previously included as a strain of sugarcane mosaic potyvirus (SCMV) (Pirone & Anzalone, 1966), but Shukla *et al.* (1989) reported it as an independent member of the potyvirus group.

El-Morsi *et al*. (2003) reported that the cultivated area of maize and sorghum was greater than 1636014 and 359930 feddens, which give 36764655 and 5473336 ardebs as total production per year, respectively. Diseases caused by the viruses were found to be the most serious ones as they reduced the yield as well as the quality of such crops (Broadbent, 1964; Tosic *et al.,* 1990).

Potyviruses are considered the most economically important virus groups (Van Regenmortel, 1981; Hollings & Brunt, 1981). MDMV was first discovered in Southern Ohio in 1963 (William & Alexander, 1965). In this study, an unidentified isolate of MDMV was biologically propagated on maize or sorghum plants as the most important differential hosts as reported by Rangel *et al.* (1995) for raising a specific antiserum for virus detection.

MATERIALS AND METHODS

Source of virus isolate. An isolate of MDMV under investigation was kindly provided by Dr. Ahmed I. Abdel-Fattah, Sugar Crops Institute, ARC, Giza, Egypt. This isolate was maintained on *Z. mays* L.

Serological confirmation. The presence of MDMV in virus-infected maize leaf samples was detected by PAbs specific to SCMV, which was kindly provided by Dr. Ahmed I. Abdel-Fattah, Sugar Crops Institute, ARC, Giza, Egypt *via* indirect-enzyme-linked immunosorbent assay (I-ELISA) as described by Koenig and Paul (1982).

Infectious sap extraction. The infectious sap was extracted using the method of El-Morsi *et al.* (2003) from a weight of 100 grams of virus–infected leaves of *Z. mays* L. with the characteristic symptoms of MDMV and showed positive ELISA result. The extracted sap was then stored at –20°C until use.

Virus propagation. A number of 20 maize (*S. bicolor* cv. Rio) were inoculated with the prepared sap in the presence of carborandum (600mesh) as a abrasive according to the method given by Allam *et al*. (1987). As a control, leaves of

the same plant at the two or three leaf stage were left without any inoculation. Leaves with mosaic symptoms were harvested 15 days post inoculation and divided into two groups for further studies.

Electron microscopy. The first group of the virus-infected leaves was used for preparation of ultrathin sections according to the method of El-Morsi *et al.* (2003). The gold sections picked into the cupper nylon coated grids, stained with a mixture of 2% uranyl acetate and Reynold's lead citrate as showed by Abdel Ghaffar (1994) and finally dried grids were subjected to electron microscopy.

MDMV purification. The virus also purified from the second group of virus-infected leaves as described by von Baumgarten and Ford (1981) that modified by El-Morsy *et al.* (2003). The yielded purified virus was also determined according to the equation given by Noordam (1973) using extinction coefficient of 2.4 for tobacco etch potyvirus (TEV) (Purcifull, 1966) and 20 X as a dilution factor.

Raising of antiserum. In this experiment, PAbs specific to MDMV were raised using 12 weeks old New Zealand white rabbits and 1.5 mg purified virus as recommended by van Regenmortel (1982) and Dresser (1986). The antigen was then injected subcutaneously, followed by intramuscular booster injections in the presence of incomplete adjuvant. The mode of injecting was similar to that reported by McDaniel and Gordon (1989).

Evaluation of raised antiserum. I-ELISA procedure that described by Koening and Paul (1982) was sued for evaluation of the specificity of the raised PAbs collected from three bleedings (1st, 2nd & 3rd) as mentioned by Abdel-Ghaffar *et al.* (1998).

RESULTS AND DISCUSSION

ELISA confirmation. The experimental results of I-ELISA detection of MDMV (Table I), using PAbs specific to SCMV, in the virus-infected maize leaves plant confirmed the presence of MDMV as a strain of SCMV infecting maize. The positive samples gave ELISA values ranged from 0.98 to 0.65 at A_{405} nm compared to the healthy ones, which gave value of 0.230. This finding is in full agreement with that reported by El-Morsi *et al.* (2003).

Symptomatology of MDMV. Results in Fig. 1 showed that *Z. mays* L. mechanically inoculated with the confirmed isolate of MDMV showed severe mosaic symptoms, which appeared as longitudinal streak running parallel to veins, or rectangular dark green areas 21 days post virus inoculation. These results were close to that reported by Allam *et al.* (1987), Tosic *et al.* (1990) and El-Morsy *et al.* (2003). This result confirmed that the MDMV isolate transmitted mechanically (Ragel *et al.*, 1995; Kegler *et al.* 1997). MDMV infects maize and sorghum, causes mosaic and dwarfing symptoms (Panayotou, 1980; Allam *et al.*, 1987; Rangel *et al.*, 1995) with significant reduction in the fresh weight and yield reduction of about of 12.5–20% (Allam *et al.*, 1987).

Table I. I-ELISA detection of MDMV in virus-infected maize leaf samples using PAbs specific to SCMV

Leaf samples	ELISA detection	
	Value at A_{405} nm	Result
1	0.654	+
2	0.479	+
3	0.765	+
4	0.487	+
5	0.978	+
6	0.895	+
7	0.542	+
8	0.654	+
9	0.784	+
10	0.667	+
Negative control	0.230	-

+: Positive. -: Negative.

Table II. I-ELISA evaluation of the produced PAbs specific to MDMV

Dilutions	Evaluation of MDMV-antiserum via I-ELISA					
	1st Bleeding		2nd Bleeding		3rd Bleeding	
	ELISA value	Result	ELISA value	Result	ELISA value	Result
1/50	1.265	+	1.870	+	1.652	+
1/100	0.865	+	1.345	+	0.965	+
1/500	0.621	+	0932	+	0601	+
1/1000	0.219	-	0.654	+	0.300	+
1/1500	0.200	-	0.421	+	0.280	-
1/2000	0.121	-	0.258	-	0.213	-
1/2500	0.100	-	0.200	-	0.178	-
1/3000	0.090	-	0.187	-	0.110	-
1/3500	0.001	-	0.115	-	0.005	-
Healthy (10^{-1})	0.118	-	0.150	-	0.145	-

Note: ELISA value at 405 nm is an average of 2 replicates, and measured 20 min post incubation at 37°C. Healthy: Sap obtained form leaves showing no symptoms of *Z. maize* L.

Fig. 1. Severe mosaic symptoms as longitudinal streak running parallel to veins, or rectangular dark green areas 21 days post MDMV inoculation.

Electron microscopy of ultra-thin sections of virus-infected leaf. The MDMV isolate under investigation induced cylindrical inclusions (CI) which appeared as

pinwheel, scroll, and bundles (Fig. 2 & 3) and similar to those typical of potyvirus group sub-division III (Achon *et al*., 1996). This result is in full agreement with those reported by several investigators (An *et al*., 1992; Garrido *et al*., 1993; Abdel Ghaffar, 1994; El-Morsi *et al*., 2003). Edwardson and Christie (1991) reported that these types of inclusions are considered as the most important phenotypic criteria for assigning viruses to the Potyviruses. The pinwheels inclusion were scattered or in loose aggregates in the cytoplasm, but occasionally occur in monolayers adjacent to the tonoplast in the cytoplasmic bridges transferring vacuoles, or within plamsodesmata (Abdel Ghaffar, 1994; El-Morsi *et al*., 2003).

Purification of MDMV. Morphological investigations on MDMV revealed that its particles are flexuous filaments or rods with length about 690–800nm and 15nm width (McDaniel, 1982; Garrido *et al*., 1996). The findings of this study are in close conformity to that reported by Gordon and Gingery (1973), von Baumgarten and Ford (1981), Chen *et al.* (1992) and Garrido *et al.* (1993). The ultraviolet absorption profile of purified virus was similar to the potyvirus nucleoproteins (Langenberg, 1973). The $A_{260/280}$ ratio was 1.2, whereas, the ratio of $A_{max/min}$ was 1.1.

Fig. 2. Electron micrograph showing pinwheel, scroll and laminated aggregates inclusions in the cytoplasm of virus-infected cells of maize (*Z. mays* L.) (X-20,000).

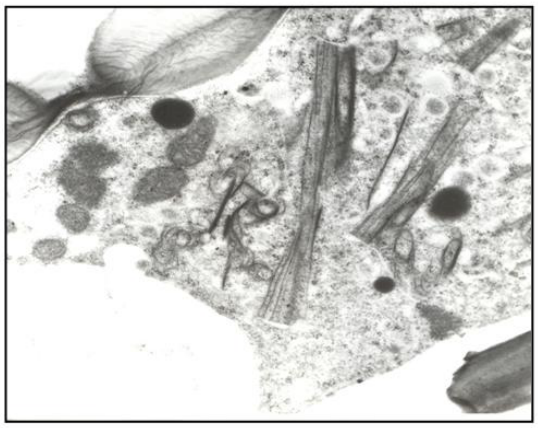


Fig. 3. Electron micrographs showing pinwheel, laminated aggregates and scroll inclusions in the cytoplasm of virus-infected cells of maize (*Z. mays* L.) (X-20,000)

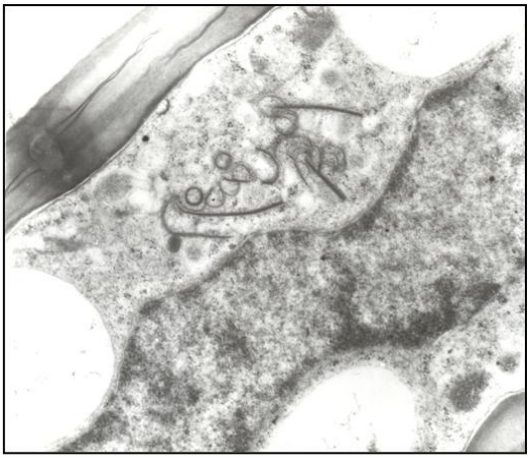
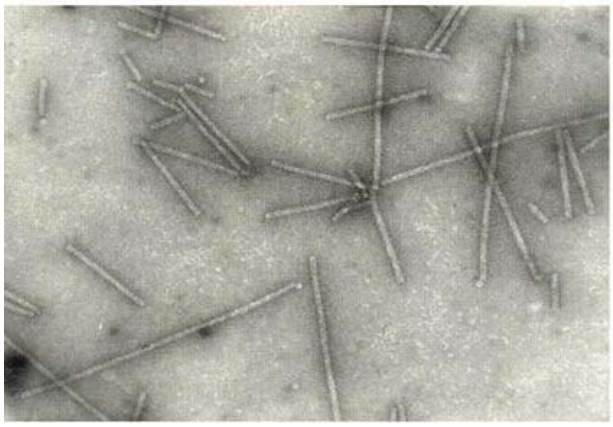


Fig. 4. Negative staining of partially purified–MDMV particles (X–46,000)



The virus preparation was negatively stained with 2% uranyl acetate. They were flexuous filaments particles ranging in length of 720–750 nm and 13 nm in width (Fig. 4), whereas no such particles were observed in the preparation of the healthy maize plants. Similar data has been reported by McDaniel and Gordon (1989), Chen *et al*. (1992), Garrido *et al*. (1996) for length while width was (13 nm) in concurrence to those of Abdel Ghaffar (1994) and El-Morsi *et al.* (2003).

Raising of antiserum. Results for PAbs specific to MDMV that evaluated at 1st, 2nd, and 3rd bleeds by I-ELISA (Table II) revealed that the efficiency of tested bleeds for virus detection was up to 1/500, 1/1500 and 1/1000, respectively. Abdel Ghaffar (1994) reported that MDMV-antiserum titer was 1/1500, 1/7000 and 1/3000 at first, second and third bleeds using R-ELISA, respectively. Several reports by Clark and Adams (1977); Triolo *et al.* (1996); Basalp *et al*. (1997) and El-Morsi *et al.* (2003) showed that ELISA was a powerful serological tool for virus detection. Therefore, the production of such specific PAbs will encourage the idea for producing ELISA kits for detection of MDMV.

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