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BIOLOGICAL AND SEROLOLGICAL STUDIES ON AN ISOLATE OF MAZIE DWARF MOSAIC POTYVIRUS INFFECTING MAIZE PLANT BY

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ABSTRACT

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 was confirmed 15 days post virus inoculation. Ultrathin 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 by electron microscopy. 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, cytoplasmic inclusions, purification, polyclonal antibodies, ELISA.

INTRODUCTION

In 1965 maize dwarf mosaic potyvirus (MDMV) was described by Williams and Alexander. 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 (maize; *Zea mays*), grain and fodder sorghum (*Sorghum bicolor*), and Johnsongrass (S. *halepense*) are important natural hosts, developing mosaic and occasional necrotic foliage symptoms. The virus possibly occurs wherever maize and sorghum are grown throughout the world except that it is not reported in Australia. The natural host range is restricted to maize, sorghum and Johnsongrass which develop mosaic and occasionally dwarfing symptoms. Sap transmission tests showed that most species of Gramineae are susceptible, some being infected symptomlessly (Ford and Tosic, 1972 & Tosic and Ford. 1974). Cultivated wheat, barley, oat, rye and rice are non-hosts.

The virus is transmissible mechanically, and by several aphid species in a non-persistent manner. MDMV is a virus 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 and Anzalone, 1966), but Shukla *et al.* (1989) reported it as an independent member of the potyvirus group.

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

In this investigation, an isolate of MDMV infecting *Z. mays* L. was subjected to biological studies followed by purification for producing polyclonal antibodies (PAbs) specific for such virus.

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 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 sorghum plants (*S. bicolor* cv. Rio) were inoculated with the prepared sap in the presence of carborandum (600 mesh) as a abrasive according to the method given by Allam *et al.* (1987). Leaves of the same plant at the two–three leaf stage were left without any inoculation as a control. 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-Morsi *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 and 3th) as mentioned by Abdel-Ghaffar *et al.* (1998).

RESULTS AND DISCUSSIONS

Potyviruses considered the most economically important virus groups (Van Regenmortel, 1981 and Hollings and Brunt, 1981). MDMV was first discovered in Southern Ohio in 1963 (William and Alexander, 1965). In this study, an unidentified isolate of MDMV was subjected to biological or serological studies using maize and sorghum plants as the most important differential hosts as reported by Rangel *et al.* (1995).

ELISA confirmation

The experimental results of I-ELISA detection of MDMV (Table 1), 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 0.978 to 0.654 at A_{405} nm compared to the healthy ones, which gave value of 0.230. This result in full agreement with that reported by El-Morsi *et al.* (2003).

Table (1): 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	-

- = Negative. +: Positive.

Symptomatology of MDMV

Results in Figure (1) show 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 confirm that the MDMV isolate transmitted mechanically as showed by Rangel *et al.* (1995) and Kegler *et al.* 1997). MDMV infects maize, sorghum, cause mosaic, and dwarfing symptoms (Panayotou, 1980; Allam *et al.* 1987 and Rangel *et al.* 1995) with significant reduction in the fresh weight with yield reduction of about of 12.5–20 % (Allam *et al.* 1987).

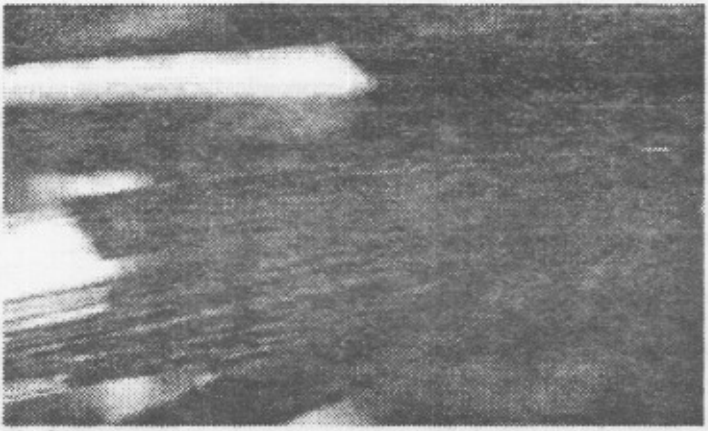

Figure (1): Severe mosaic symptoms as longitudinal streak running parallel to veins, or rectangular dark green areas 21 days post MDMV inoculation.

Electron microscopy of ultrathin sections of virus-infected leaf

Results in Figures (2) and (3) show that the MDMV isolate under investigation induced cylindrical inclusions (CI) which appeared as pinwheel, scroll, and bundles and similar to those typical of potyvirus group subdivision III (Achon *et al.*, 1996). The obtained results are in full agreement with those reported by several investigators (An *et al.* 1992; Garrido *et al.* 1993; Abdel Ghaffar, 1994 and El-Morsi *et al.* 2003). Edwardson and Christie (1991) reported that this type of inclusions, i.e., CI induced by virus-encoded protein and considered as the most important phenotypic criterion for assigning viruses to the Potyvirus group. The pinwheel inclusions 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) and El-Morsi *et al.* (2003) obtained similar result.

Figure (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).

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

Purification of MDMV

Several investigators studied the morphology of MDMV. They showed that its particles are flexuous filaments or rods with length about 690–800 nm and 15 nm width (McDaniel, 1982 and Garrido *et al.* 1996).

In this study, the MDMV was purified as described in the material and methods. As mentioned by El-Morsi *et al.* (2003), the applied procedure avoided the problems of MDMV purification, i.e., the low concentration of virus in host, adsorption of viral particles on normal cell constituents, instability, aggregation of the virus particles, remaining of insolubly aggregates after sedimentation by high speed and contamination with host proteins in purified preparations. From a

weight of 100 g virus-infected tissues of Z. *mays* L., a yield of 2.1 mg of virus based on 2.4 as the extinction coefficient at 260 nm was obtained. This result too close to that reported by Gordon and Gingery (1973) (15-30 µg/g); Von Baumgarten and Ford (1981) (10-12 µg/g); Chen *et al.* (1992) (7-12 µg/g) and Garrido *et al.* (1993) (10-15 µg/ml). 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.

In the purified virus preparation negatively stained with 2% uranyl acetate, flexuous filaments particles ranging in length of 720–750 nm and 13 nm in width were found (Figure 4). No virus particles observed in the preparation of the healthy maize plants. Similar results obtained by McDaniel and Gordon (1989); Chen *et al.* (1992); Garrido *et al.* (1996), while width was 13 nm closed to that obtained reported similar results by Abdel Ghaffar (1994) and El-Morsi *et al.* (2003).

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

Raising of antiserum

In this work, PAbs specific to MDMV were raised as mentioned in the materials and methods. Similar to that reported by McDaniel ad Gordon (1989) the virus was emulsified with Freund's complete adjuvant, which was recommended to potentiate the immune response and as a depot for slow release of antigen (Dresser, 1986) and then injected subcutaneously, followed by intramuscular booster injections in the presence of incomplete adjuvant.

Results in Table (2) revealed that the produced antiserum was evaluated at 1st, 2nd, and 3rd bleedings by I-ELISA. The efficiency of tested bleedings 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 bleedings 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 viruses

detection. Therefore, the production of such specific PAbs will encourage the idea for producing ELISA kits for detection of MDMV. El-Morsi *et al.* (2003) reported similar observation.

Table (2): I-ELISA evaluation of the produced PAbs specific to MDMV.

Dilution	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 from leaves showing no symptoms of *Z. maize* L.

As a conclusion, sorghum and maize plants reported to be susceptible to many viral diseases, i.e., dwarfing and mosaic diseases. Among important viruses that caused devastating losses by reducing either the yield or the quality of the crops is MDMV. Therefore, the present investigation aimed to determine some biological properties of an isolate of MDMV followed by raising specific antiserum that could be used for production of ELISA kits for virus detecting.

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دراسات بيولوجية وسيرولوجية علي عزلة من فيروس تبرقش وتقزم الذرة تصيب نبات الذرة

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تم الكشف عن عزلة من فيروس تبرقش وتقزم الذرة تصيب نبات الذرة بواسطة الأليزا الغيرمباشره باستخدام أجسام مضادة عديدة النسل متخصصه لفيروس موزيك قصب السكر. وقد تم وبنجاح نقل الفيروس ميكانيكيا بالعصير من الذرة الي نبات السورجم صنف ريو وقد ظهرت الأعراض المميزة للفيروس بعد ١٥ يوم من الحقن. وباستخدام الميكروسكوب الأليكتروني تم فحص القطاعات الفائقة الدقة والتي تم تجهيزها من أوراق الذرة المصابة وقد ثبت تواجد أجسام محتواه من النوع الأسطواني والتي ظهرت في صورة شكل العجلة وشكل دائري وصفائح متجمعه في سيتوبلازم الخلايا المصابة. كما تم تنقية الفيروس حيث ظهرت جزيئات خيطية الشكل مرنة في العصير المعدي. وقد تم الحصول علي كمية تقدر بـــ ٢,١ مليجرام فيروس من ١٠٠ جرام أوراق نباتات مصابه. وقد تبع ذلك إنتاج أجسام مضادة في الأرانب والتي تم تقويمها في ثلاثة ادماءات هي ١، ٢، ٣ بواسطة الأليزا الغير مباشرة وقد ثبت أن الأدماءات الثلاثة كانت فعالة حتى تخفيفات ١/٥٠٠، ١/١٥٠٠، ١/١٠٠٠ علي التوالي.