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BIOLOGY AND CYTOPATHOLOGY OF AN EGYPTIAN ISOLATE OF ZUCCHINI YELLOW MOSAIC POTYVIRUS (ZYMV-EG)

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ABSTRACT

Squash is considered as one of the most important crops in Egypt and worldwide. It is threatened with different pathogens specially viruses, which cause considerable loss in its yield. *Zucchini yellow mosaic Potyvirus* (ZYMV) is considered as one of the most important viruses infects squash. Biological, serological and cytopathological studies were carried out to identify the Egyptian isolate of ZYMV. Results showed that the Egyptian isolate of ZYMV revealed systemic symptoms in the form of severe mosaic and vein banding on *Cucurbita pepo* cv. Eskandarani under open field conditions. Direct antigen coated (DAC)-enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies was used as a diagnostic tool for detecting ZYMV in the virus-infected *C. pepo* samples. It showed positive reactions with ZYMV antiserum. Filamentous virus-like particles measuring 750X13 nm was successfully purified from ZYMV-infected squash plants, based on the use of polyethylene glycol and ultracentrifugation. The electron microscope of ultrathin sections of virus-infected leaf tissues revealed the presence of cylindrical inclusions as pinwheels, laminated aggregates and scrolls in the cytoplasm of cells infected with ZYMV. In addition, disorganization of plastids, nucleus and mitochondria in the virus- infected cells was also observed.

Key words: Squash, ZYMV, DAC-ELISA, Ultrathin sections.

INTRODUCTION

Cucurbit species include a variety of high value crops as melons, watermelon, cucumber, summer squashes, and winter squashes that play important roles both in local diets and as export crops in many countries. Viral diseases are more serious for cucurbitaceous plants compared to diseases caused by other agents. Symptoms of viral infections on *Cucurbitaceae* are mosaic, yellowing, stunting, chlorosis, leaf and fruit deformations **[Lisa *et al.*, (1981) and Lecoq *et al.*, (1983)]**. Three *Potyvirus* species were most commonly reported in surveys of virus infecting cucurbits in different parts

of the world: *Watermelon mosaic potyvirus* (WMV), *Papaya ring spot potyvirus* (PRSV), and *Zucchini yellow mosaic potyvirus* (ZYMV) **[Ullman *et al.*, (1991); Rivera *et al.*, (1993) and Luis-Arteaga *et al.*, (1998)].**

ZYMV is a member of genus *Potyvirus* in the family *Potyviridae*, was first reported in Italy in 1973 **[Lisa *et al.*, (1981)]**. It was also observed, at the same time, in France, where it was named as *Muskmelon yellow stunt Potyvirus* (MYSV) **[Lecoq *et al.*, (1981 & 1983)]**. ZYMV was firstly described in Egypt in 1983 **[Provvidenti *et al.*, (1984b)]**. It had been observed in about 50 countries in both traditional and intensive growing conditions since its first report **[Desbiez & Lecoq (1997)]**.

The Egyptian isolates of ZYMV **[Provvidenti *et al.*, (1984b) and Abdel-Ghaffar *et al.*, (1998)]** incited symptoms closely resembling those caused by European isolates of this virus **[Lecoq *et al.*, (1981) and Lisa *et al.*, (1981)]**, and the American strain ZYMV-Connecticut (ZYMV-CT) **[Provvidenti *et al.*, (1984a)]**. ZYMV in certain circumstances may completely destroy cucumber planting **(Sutarya & dan Sumpena (1994)]**.

This study concerned to identify an Egyptian isolate of ZYMV using ELISA technique and electron microscopy. Also, its morphological and cytopathological effects on the host plant were studied.

MATERIALS AND METHODS

Virus isolates confirmation and maintenance:

Leaves exhibited ZYMV-like symptoms suspected to be virus naturally infected were collected from *Cucurbita pepo* cv. Eskandarani (Zucchini squash) from commercial fields of Kafr Saad City, Damietta Governorate, Egypt. These samples were tested for ZYMV infection by specific ZYMV antiserum (provided from AGERI) using direct antigen coating (DAC)-ELISA **[Converse & Martin (1990)]** to be devoid of other viruses infecting cucurbits except ZYMV. The ZYMV isolate was maintained and propagated under greenhouse conditions in Zucchini squash (*C. pepo* cv. Eskandarani) by mechanical inoculation.

Virus purification:

ZYMV was purified from *C. pepo* cv. Escandarani using polyethylene glycol (PEG) as described by **[Abdel-Halim *et al.*, (2000)]**. The virus pellets were then immediately resuspended in 2 ml of 0.02 M sodium phosphate buffer (SPB), pH 7.2 and kept overnight at 4°C. Density-gradient centrifugation was carried out as described by **[Delgado & Grogan (1966) and Abdel-Halim *et al.*, (2000)]**. The final pellet was then resuspended in 500 µl of 2 mM SPB, pH 7.4.

Negative staining:

Negative staining as described by **[Milne & Lesemann (1984)]** was performed to determine virus morphology in the purified virus preparation as follows: fifteen microliters of the PVP were adsorbed on gold carbon coated grids for 5 min., stained with 2% uranyle acetate (w/v), and washed with distilled H_2O to remove the excess of stain. The grids were left for 5 min. to dry and then examined by JEOL JEM-100S TEM

of the Electron Microscope Unit, Egyptian Organization for Biological Products and Vaccines (VACSERA), Agouza, Giza, Egypt.

Electron microscopy:

To examine the cytopathological modifications by electron microscopy, we mechanically inoculated *C. pepo* cv. Eskandarani predusted with carborundum with the purified ZYMV. Uninoculated plants were kept as control. All plants were maintained in a greenhouse at 22° to 30°C for four weeks post inoculation. Leaf samples from both control and infected plants were harvested separately. For preparation of ultrathin sections from ZYMV-infected leaves, the method of **[Zechmann *et al.*, (2003)]** was carried out with little modifications. The ultrathin sections were prepared using ultramicrotome with diamond knife and were stained with a mixture of 2% uranyl acetate and acetone (1:1, v/v) for 20 min. at RT, then with Reynold's lead citrate for 20 min. followed by washing several times with distilled H_2O. The dried stained sections were observed by JEOL JEM-100S TEM.

RESULTS

ZYMV characterization:

The presence of ZYMV in sampled leaves of zucchini squash was detected *via* DAC-ELISA with positive absorbance reading at 405 nm in the range of 0.181 to 0.319 while healthy leaf extract had reading of 0.090. The naturally infected leaves as well as the mechanically inoculated leaves developed severe mosaic, vein banding, chlorosis, yellows and crinkling **Fig. (1).** The electron micrograph of negatively stained partially purified virus preparations shows the presence of flexuous filamentous virus particles with dimensions 750 X 13 nm **Fig. (2).**

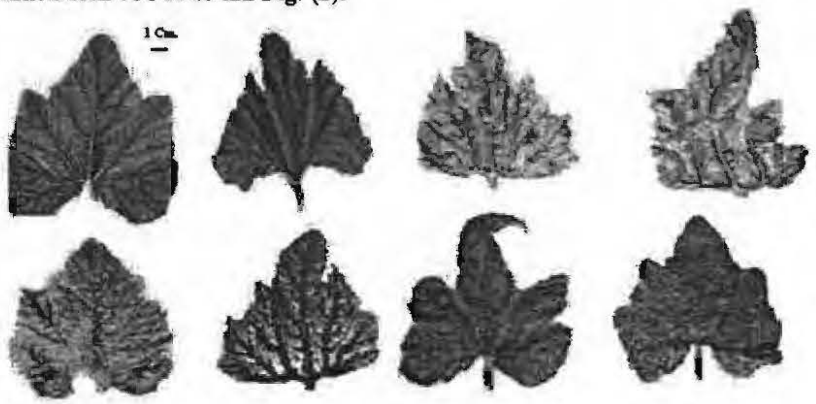

Fig. (1): External symptoms caused by ZYMV-Eg in *C. pepo* cv. Eskandarani

Fig. (2): Electron micrograph of partially purified ZYMV-Eg negatively stained with 2% uranyl acetate. (X-40,000).

Cytopathology:

The electron micrographs of ultrathin sections prepared from control sampled leaves zucchini squash appeared the absence of cytoplasmic inclusions in the cytoplasm of tested cells (Data not shown). On the other hand, cylindrical inclusions in the form of pinwheel, scrolls, and a few aggregates of virus-like particles (VLP) (Fig. 3A, 3C, 3D) were observed in the positive-ELISA leaf samples (Virus-infected leaves). Proliferated endoplasmic reticulum and other cytopathological modifications as formation of osmophilic bodies **(Fig. 3E)**, deformed chloroplast **Fig. (3F)**, degraded mitochondria Fig. **(3G, 3H)** and elongation of nucleus **Fig.(3A, 3B)** with dense chromatin located at periphery were also found.

DISCUSSION

ZYMV is a member of genus *Potyvirus*, family *Potyviridae*. ZYMV disease is a major constraint in the production of cucurbits world-wide. The virus can cause massive damage (to total loss) to cucurbit crops, and prevents the growth of some cucurbit crops in certain areas **[Gal-On (2007)]**.

Field symptoms observed in this study were similar to that of ZYMV and consisted of yellowing, yellow-green mosaic, leaf deformation, crinkling and distortion of the leaves *C. pepo* cv. Eskandarani plants and stunting of the whole plant. Similar results were obtained by **[Abdel-Gbaffar *et al.*, (1998) and Mahmoud *et al.*, (2004)]**, who reported that the characteristic observed symptoms were severe mosaic and malformation. **[Prieto *et al.*, (2001)]** also reported that infected zucchini samples showed yellow mosaic and severe leaf blistering.

The electron micrograph of the partial purified ZYMV suspension showed the presence of filamentous virus particles with length of about 750 nm. This agree with that found by **[Lisa *et al.*, (1981); Siaw *et al.*, (1985); Riechmann *et al.*, (1989); Murphy *et al.*, (1990); Desbiez & Lecoq (1997) and Svoboda & Polák (2002)]**. The

viral particle width was found to be about 13 nm similar to the one which was obtained by **[Abdel-Ghaffar *et al.*, (1998) and Mahmoud *et al.*, (2004)]**. Also, these results agree with **[Gal-On (2007)]** who reported that ZYMV virions are flexuous filaments of 11-13 nm in diameter.

Members of *Potyvirus* group are characterized by inducing cytoplasmic cylindrical inclusion (CCI) in the cytoplasm of virus-infected cells during the infection cycle **[Lesemann (1988) and Edwardson & Christie (1996)]**. The induction of such CCI formation by virus-encoded protein is the most important phenotypic criterion for assigning viruses to the potyviruses **[Edwardson *et al.*, (1984) and Milne (1988)]**. The electron microscopy of the ultrathin sections of positive-ELISA ZYMV infected leaves revealed that our Egyptian isolate induced CCI (pinwheel and scroll) inclusions of type III. A few aggregates of virus particles were also found. This result is in agreement with that found by **[Abdel-Ghaffar *et al.*, (1998)]**.

Kitajima & Lovisolo (1972) found that aggregated mitochondria have been observed in *Datura* cells infected with a *Potyvirus*. The development of abnormal membrane system within mitochondria has been described for several virus infections **[Francki (1987)]**. Data herein indicate that the infection of zucchini leaf cells with ZYMV induced several ultrastructure changes. It was obvious that chloroplast, mitochondria and nucleus were severely affected by ZYMV-infection. The changes included; disorganized chloroplasts with significantly decreased amount of thylakoids, degeneration of mitochondria where they found to be partially or completely disintegrated and abnormalities in the shape and size of the nucleus as it appeared elongated with dense chromatin organized into discrete areas located at periphery. Other cytopathological effect such as formation of osmophilic bodies was also found. The results of this investigation were in agreement with that of **[Francki *et al.*, (1985)]**, who reported that the infection with potyviruses induced characteristic changes in the nucleus. In countrary **[Zechmann *et al.*, (2003)]** reported that ZYMV-infection induced severe modifications in the number and ultrastructure of chloroplast, whereas mitochondria, nuclei and peroxisomes remained unaffected.

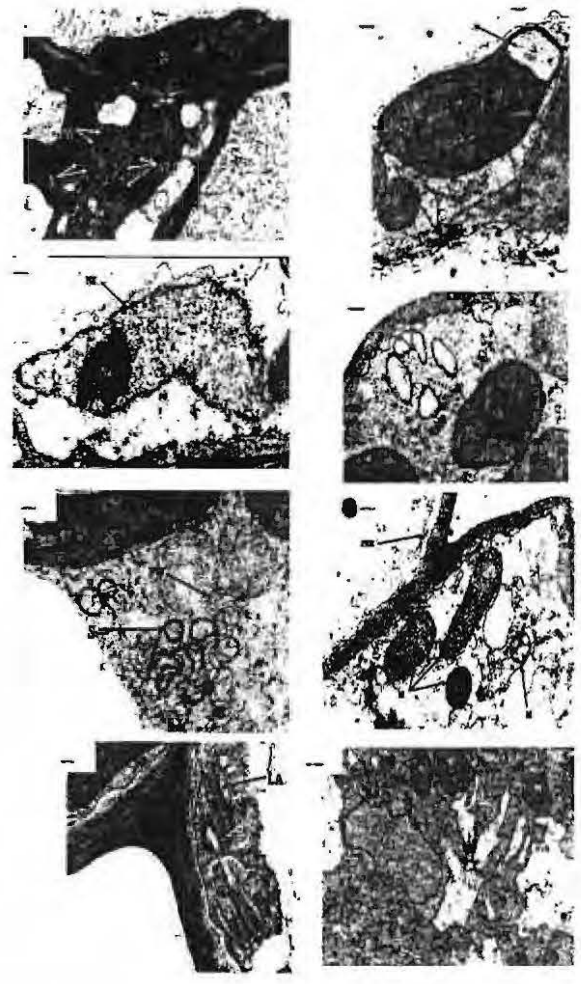

Fig. (3): Electron micrographs of ultrathin sections from ZYMV-Eg infected leaves of *C. pepo* cv. Eskandarani showing its cytopathological effects. PW: pinwheel, S: scrolls, VLP: virus-like particles, VLI: virus-like inclusion, LA: laminated aggregates, OB: osmophilic body, Ch: chloroplast, CW; cell wall, M: mitochondria, NE: nuclear envelope, Nu: nucleolus, PER: proliferated endoplasmic reticulum. (A-D: X-8,000 & E-H: X-15,000).

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الملخص العربى

دراسة بيولوجية وسيتوباثولوجية على عزلة مصرية من فيروس الموزايك الأصفر الزوكيني (ZYMV-Eg)

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تعتبر الكوسة من أهم المحاصيل الزراعية في مصر والعالم و التى تتعرض للعديد من الأمراض المختلفة وخاصة الأمراض الفيروسية التى تؤثر على إنتاجيتها. يعتبر فيروس الموزايك الأصفر الزوكيني (ZYMV) أحد أهم الفيروسات التي تصيبها. هدفت هذه الدراسة البيولوجية والسيرولوجية والسيتوباثولوجية لتعريف إحدى العزلات المصرية من فيروس الموزايك الأصفر الزوكيني (ZYMV). أكدت النتائج أن هذه العزلة تعطي إصابة جهازية فى صورة موزايك شديد وتحزم للعروق على نبات الكوسة صنف إسكندراني فى الحقل. كما أكدت نتائج الإختبارات السيرولوجية باستخدام تقنية الإليزا المباشرة أن هناك تفاعل إيجابي بين هذه العزلة وبين السيرم المضاد لفيروس الموزايك الأصفر الزوكيني (ZYMV). وجد أيضا أن جزيئات ZYMV خيطية الشكل بأبعاد ٧٥٠ x ١٣ نانومتر، وبفحص القطاعات فائقة الدقة من النباتات المصابة بواسطة الميكروسكوب الإلكترونى ثبت وجود محتويات داخلية إسطوانية الشكل تظهر فى صورة العجلة (Pinwheels) وتراكمات خيطية طولية (Laminated aggregates) وأخرى دائرية (Scrolls) فى سيتوبلازم الخلايا المصابة بفيروس الموزايك الأصفر الزوكيني (ZYMV). كذلك تم رصد بعض التأثيرات الخلوية للفيروس مثل تشوه البلاستيدات الخضراء، وإستطالة النواة، وضمور الميتوكوندريا.