BIOLOGY, CYTOPATHOLOGY AND MOLECULAR IDENTIFICATION 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. Zucchini yellow mosaic potyvirus (ZYMV) is considered as one of the most important viruses infects squash. We used the biological, serological and cytopathological studies to identify an Egyptian isolate of ZYMV. Results showed that the Egyptian isolate of ZYMV showed 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-cells infected was also observed. In addition, the nucleotide of *CI* gene of the viral isolate under investigation was determined and its similarities to some overseas isolates were addressed.

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). Presently, three *Potyvirus* species are most commonly reported in surveys of virus infecting cucurbits in different parts of the world like *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) and at the same time it was also observed in France, where it was named as *Muskmelon yellow stunt potyvirus* (MYSV) (Lecoq *et al*., 1981 and 1983). It was firstly described in Egypt in 1983 (Provvidenti *et al*., 1984b). It has been observed in about 50 countries in both traditional and intensive growing conditions since its first report (Desbiez and 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 and dan Sumpena, 1994).

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

MATERIALS AND METHODS

Virus isolate confirmation and maintenance: Leaves exhibited ZYMV-like symptoms suspected to be virus naturally infected were collected in January 2006 from *Cucurbita pepo* (Zucchini squash) cv. Eskandarani from commercial fields of Kafr Saad City, Damietta Governorate, Egypt. These samples were found by direct antigen coating (DAC)-ELISA (Converse and 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 2ml of 0.02M sodium phosphate buffer (SPB), pH 7.2 and kept overnight at 4˚C. Density-gradient centrifugation was carried out as described by Delgado and Grogan (1966) and Abdel-Halim *et al.,* (2000). The final pellet was then resuspended in 500µl of 2mM SPB, pH 7.4.

Electron microscopy: Negative staining as described by Milne and 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 d.H_2O to remove the excess stain. The grids were left for 5 min to dry and then examined by electron microscope, JEOL JEM-100S in the Electron Microscope Unit, Egyptian Organization for Biological Products and Vaccines (VACSERA), Agouza, Giza, Egypt.

To examine the cytopathological modifications,we mechanically inoculated *C. pepo* cv. Escandarani predusted with carborundum with the purified ZYMV. Three plants were kept uninoculated as control, and the plants were maintained in a greenhouse at 22° to 30°C. Four weeks post inoculation, sample leaves from both control and infected plants were harvested separately.

Small pieces of sampled leaves were fixed in a cold 3%-glutaraldehyde, post-fixed in 1% osmium tetra-oxide (OsO_4), dehydrated in increasing concentrations of ethanol, then kept in pure propylene oxide solution for 30 min. The propylene oxide was then replaced with a mixture of propylene oxide and resin medium 1:1 (v/v) for 2 h, 1:2 (v/v) for 4 h at 4°C, 1:3 (v/v) overnight and finally pure resin for 4 h at 4°C. The ultrathin sections were prepared using the ultramicrotome with a diamond knife. The selected ultrathin sections were stained with a mixture of 2% uranyl acetate and acetone (1:1, v/v) for 20 min at RT, followed by staining with Reynold's lead citrate for 20 min before observations.

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 virus was partially purified. 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).

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 (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.

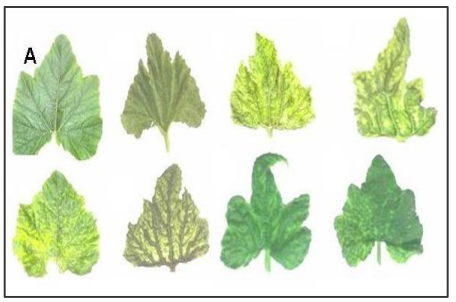

Figure-1: External symptoms caused by ZYMV-Eg in *C. pepo* cv. Escandarani.

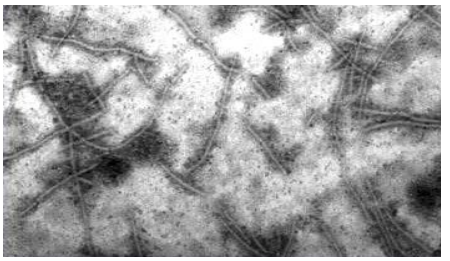

Figure-2: Electron micrograph of partially purified ZYMV-Eg negatively stained with 2% uranyl acetate. (X-40,000).

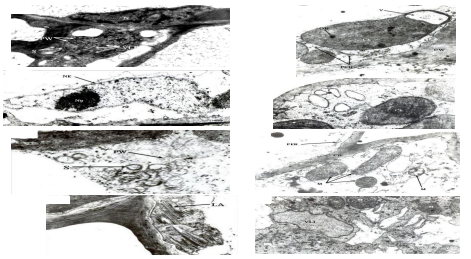

Figure-3: Electron micrographs of ultrathin sections from ZYMV-Eg infected leaves of *C. pepo* cv. Eskandarani showing cytopathological effects of our isolate.

DISCUSSION

ZYMV disease is a major constraint in the production of cucurbits world-wide. The virus can cause massive damage (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 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-Ghaffar *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 negatively stained with 2% uranyl acetate proved 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 and Lecoq (1997) and Svoboda and Polák (2002). The particle width was found to be about 13 nm. Similar results were obtained by Abdel-Ghaffar *et al*., (1998) and Mahmoud *et al*. (2004). Also, these results are similar to that reported by 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 CCI in the cytoplasm of virus-infected cells during the infection cycle (Lesemann, 1988 and Edwardson and 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 and 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 ultra-structure changes. It was obvious that chloroplast, mitochondria and nucleus were severely affected by ZYMV-infection. The changes included disorganized chloro-plasts with significantly decreased amount of thylakoids, degeneration of mito-chondria where they found to be partially or completely disintegrated and abnor-malities in the shape and size of the nucleus as it appeared elongated with dense chromatin organized into discrete areas located at periphery. Other cyto-pathological 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 modi-fications in the number and ultrastructure of chloroplast, whereas mitochondria, nuclei and peroxisomes remained unaffected.

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