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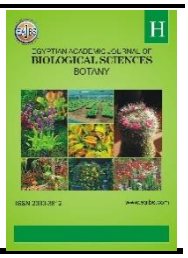

Purification and Cytopathological Effects of *cucumber mosaic virus* on *Cucurbita pepo* Plants

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ABSTRACT

Cucumber mosaic virus (CMV) is one of the most devastating viral pathogens that cause severe problems for several plants in Egypt and worldwide. In the current study, the electron microscopic observation of spherical with a diameter between 27.49 to 30.53 nm and the yield of the purified virus was 1.4 mg/100 g infected leaves. Results of the mechanical inoculation revealed severe mosaic and chlorotic symptoms and were observed on *Cucurbita pepo* after ten days, while no signs appeared on the mock-inoculated plant. The ultra-thin sections of CMV-infected *C. pepo* leave judged by I-ELISA revealed the presentence of CMV-like particles using transmission electron microscopy. In addition, accumulation of starch grain was observed as cytopathological effects of *C. pepo* leaf affected by CMV-infection. Moreover, the formation of double-membrane bodies in the parenchyma and chloroplast malformation was also reported.

INTRODUCTION

Plant diseases are to blame for significant crop output losses worldwide, posing substantial food security issues (Abdelkhalek and Hafez, 2020; Heflish *et al.,* 2021). Plant viruses are the most critical pathogens in causing massive agricultural production difficulties once they appear in the field (Abdelkhalek *et al.,* 2020a; Abdelkhalek and Sanan-Mishra, 2018; Hafez *et al.,* 2014). *Cucumber mosaic virus* (CMV) belongs to the genus *Cucumovirus* and the family *Bromoviridae* (Scholthof e*t al.,* 2011). It is considered one of the most destructive and economically important viruses wreaking havoc on crop quality and yield globally (Abdelkhalek *et al.,* 2020b). It was reported that CMV could infect more than 1200 species in over 100 plant families worldwide. Moreover, it has many plant hosts, including monocots and dicots (Lou *et al.,* 2020).

Electron Microscopy is a highly effective method for studying the shape of virus particles and is often used for virus identification because symptoms alone cannot be used as valid criteria to diagnose virus infection in planting material (Awasthi *et al.,* 2014; Milne, 2019). Many plant viruses cause characteristic intracellular inclusions or massive crystalline accumulations of virus particles, and electron microscopy can be used to confirm viral infection in a simple, quick, and very inexpensive manner (Edwardson *et al.,* 2019). Plant cells infected with tospoviruses have a large and varied variety of viral inclusions. During

cytopathological tests, numerous viral particles were discovered on ultrathin slices of infected plant cells (Ie, 1971; Otulak *et al.*, 2014).

According to the CMV infection's detrimental influence and economic importance on the plant yield worldwide, it becomes necessary to purify and characterize the viral particles. Additionally, identify the virus in infected *C. pepo* to control its spread and produce polyclonal antibodies against CMV to develop a rapid, sensitive, and specific immune-enzyme system for diagnostics and the routine detection of CMV.

MATERIALS AND METHODS

Source of the Squash Plant Materials and CMV isolate:

Virus-free seeds of the squash (*Cucurbita pepo* L.) plant were kindly provided by the Agriculture Research Center at the Ministry of Agriculture, Egypt. The cucumber mosaic virus (accession No., MN594112), isolated previously from infected cucumber plants (Abdelkhalek *et al.*, 2020b), was used as a viral source isolate. Under greenhouse conditions, CMV isolate was continuously maintained on tobacco plants.

Virus Particles Purification:

The purified preparation of the CMV isolate was obtained as described previously (Younes, 1995). Briefly, one hundred grams of fresh systemically infected *C. pepo* leaves were collected 12 days after inoculation virus-infected leaves and pulverized in liquid nitrogen. The pulverized tissues were ground with 300 ml of 0.5 M sodium citrate buffer (pH 6.5), containing 0.2 ml thioglycolic acid and 200 ml of chloroform. After stirring the mixture for 30 min, the aqueous phase was separated by centrifugation at 10000 rpm at 4°C for 10 min. A 6% polyethylene glycol 6000 and 1% sodium chloride were added to the supernatant and stirred slowly overnight at 4 °C. The pellets were collected by 30 min centrifugation at 10000 rpm, resuspended in 80 ml of 0.01 M borate buffer, pH 7.5, and left overnight at 4°C with gently stirring. To eliminate any non-soluble materials, centrifugation for 15 min at 10000 rpm at 4°C was carried out. The pellets were discarded, and the supernatant was centrifuged for 120 min at 30000 rpm at 4°C. The supernatant was discarded, and the pellets were resuspended in 2 ml of 0.01M borate buffer, pH 7.5. The purified virus was scanned, quantified spectrophotometrically in UV spectrophotometer using the extinction coefficient of 5 $(mg/ml)^{-1}$ cm^{-2} at 260 nm and examined with TEM.

Mechanical Transmission and Sample Collection:

The *C. pepo* plants at 20 days of germination were mechanically inoculated with the 20 µg/mL of purified CMV that was diluted with 0.1 M phosphate buffer, pH 7.2, before use. The upper leaves were dusted with carborundum 600 mesh and inoculated as described previously (Abdelkhalek, 2019; Abdelkhalek *et al.*, 2019). At 10 dpi, the plant samples were collected and subjected to electron microscopy examination.

Cytopathological Effect:

Pieces of infected *C. pepo* leaves were prepared for TEM using the previously reported technique (Martelli and Russo, 1984). Samples were taken from infected leaves, treated in a solution of 3 % glutaraldehyde in 50 mM phosphate buffer (pH 7.2), and stored at 4 ^{o}C overnight. Samples were rinsed in the same buffer and fixed for 2 h at room temperature in 1 % osmium tetroxide (OsO4) in the same buffer. Samples were dehydrated in a sequence of escalating acetone concentrations after OsO4 fixation. After that, the samples were dehydrated and embedded in an Epon Araldite mixture (Medina et al., 2003; Soylu et al., 2005). Diamond knives were used to cut ultra-thin pieces (70-90 nm) using an Ultracut E Microtome. After that, sections were routinely mounted on formvar-coated, 200 mesh copper grids for staining. Drops of 4.5 percent uranyl acetate were used to stain grid-mounted parts with silver-gold interference color. Grids were rinsed in dH_2O after treatments

and stained with drops of Reynold's lead citrate (Roland and Vian, 1991). TEM, JEOL-CX100, operating at 80 KV, was used to investigate the ultrathin sections (The electron microscope unit, Faculty of Science, Alexandria University, Egypt).

RESULTS AND DISCUSSION

Purification of CMV Particles and Symptoms Appearance:

The ultraviolet absorption spectrum of the purified virus showed a pattern typical for coat protein with a maximum at 259-261 nm and a minimum at 244-245 nm. The values of $A_{260/280}$ and $A_{280/A260}$ were 1.123 and 0.712, respectively. The yield of the purified virus was 1.4 mg/100 g infected leaves. The purified CMV-preparation obtained by the PEG purification method revealed that CMV particles were spherical with a diameter between 27.49 and 30.53 nm (Fig. 1). The obtained results agreed with previously reported (Chalam et al., 1986; Eni et al., 2010; Hafez et al., 2011). Under greenhouse conditions, the symptomatology identification of CMV on *C. pepo* leaves was recorded as mosaics, extensive chlorosis, and leaf necrosis, as shown in Figure 1. Our observations concerning the main symptoms associated with samples positive to CMV infection as reported previously (Megahed et al., 2012; Younes, 1995).

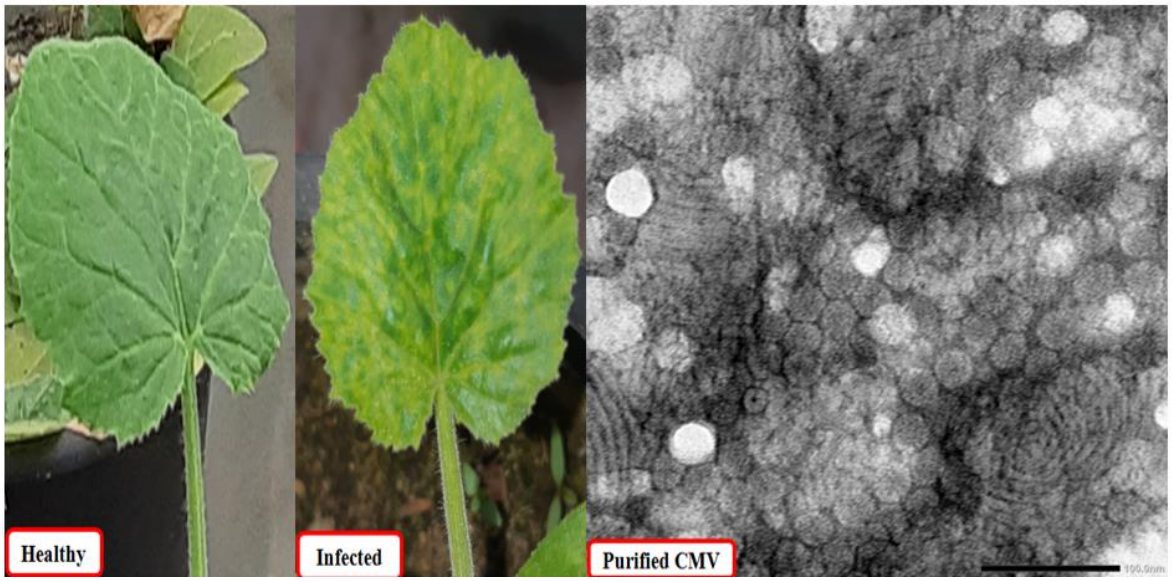

Fig. 1. Squash (*Cucurbita pepo* L.) plant showing severe mosaic symptoms after 10 days of mechanical inoculation of CMV. Transmission electron microscope image showing the purified CMV particles in a spherical shape with range 28 to 30 nm. Bar= 100 nm in diameter.

Cytopathological defects of CMV infection

Ultrathin sections produced from healthy *C. pepo* leaves demonstrated typical cell structure and chloroplast, but those prepared from CMV-infected *C. pepo* leaves revealed many cytopathological effects (Fig.2). The existence of many spherical particles and aggregates of viral particles lying side by side within the envelope was confirmed by electron micrographs of *C. pepo* plants that acquired the virus by mechanical transmission (Fig. 2A). Interestingly, electron micrographs revealed partly damaged and distorted chloroplast (Figure 2B), indicating a cytopathic consequence linked with CMV infection. These cells also showed mitochondrial degradation (Fig. 2). Figure 2 shows the existence of additional CMV-cytopathological abnormalities in CMV-infected *C. pepo* leaves, such as the development of cytoplasmic bridges and vacuolated cytoplasm. Similar malformation and chloroplast degradation were generally reported upon plant viral infections

(Abdelkhalek *et al.*, 2019; Hafez *et al.*, 2011, 2014). Upon CMV infections, the chloroplasts were destroyed, and grana were disordered and dispersed into the cytoplasm as the infection proceeded, resulting in a decrease in chlorophyll concentration in plant tissues (Montasser and El-Sharkawey, 2017).

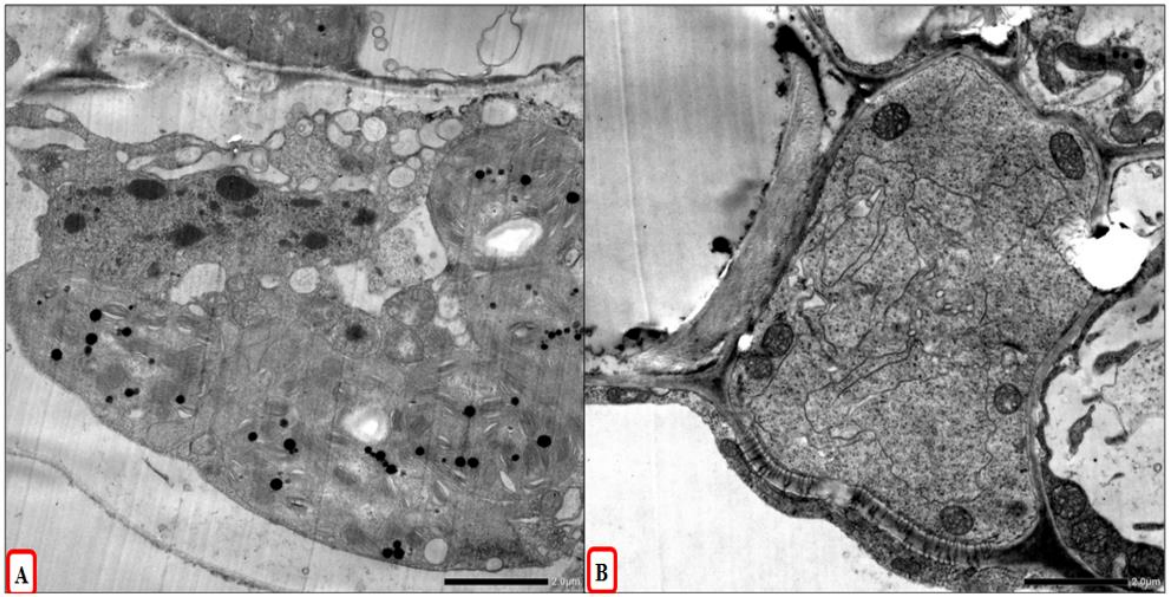

Fig. 2. Electron micrographs of ultrathin sections from CMV-infected squash (*Cucurbita pepo* L.) plant leaf starch grain accumulation (A), chloroplast malformation (B), and presence of double-membrane bodies in the parenchyma.

REFERENCES

Abdelkhalek, A., 2019. Expression of tomato pathogenesis related genes in response to Tobacco mosaic virus. *Journal of Animal and Plant Sciences*, 29, 1596–1602.

Abdelkhalek, A., Al-Askar, A.A., Behiry, S.I., 2020a. Bacillus licheniformis strain POT1 mediated polyphenol biosynthetic pathways genes activation and systemic resistance in potato plants against Alfalfa mosaic virus. *Scientific Reports*, 10, 1–16.

Abdelkhalek, A., Behiry, S.I., Al-Askar, A.A., 2020b. Bacillus velezensis PEA1 Inhibits Fusarium oxysporum Growth and Induces Systemic Resistance to Cucumber Mosaic Virus. *Agronomy*, 10, 1312.

Abdelkhalek, A., Hafez, E., 2020. Plant Viral Diseases in Egypt and Their Control, in: Cottage Industry of Biocontrol Agents and Their Applications. Springer, pp. 403–421.

Abdelkhalek, A., Qari, S.H.S.H., Hafez, E., 2019. Iris yellow spot virus–induced chloroplast malformation results in male sterility. *Journal of Biosciences*, 44, 142. https://doi.org/10.1007/s12038-019-9960-9

Abdelkhalek, A., Sanan-Mishra, N., 2018. A comparative analysis of the suppressor activity of Tobacco mosaic virus proteins in the tomato plant. *Jordan Journal of Biological Sciences*, 11(4):469- 473.

Awasthi, L.P., Singh, S.P., Chaubey, A.N., Abhishek, K.R., Rajesh, K., 2014. Molecular characterization of potato viruses through RT-PCR and electron-microscopy. *Caribbean Journal of Science and Technology*, 2, 405–410.

Chalam, T. V, Reddy, M. V, Nene, Y.L., Subbayya, J., 1986. Some properties of a strain of cucumber mosaic virus isolated from chickpea in India. *Plant Diseases*, 70, 128–

130.

Edwardson, J.R., Christie, R.G., Purcifull, D.E., Petersen, M.A., 2019. Inclusions in diagnosing plant virus diseases, in: Diagnosis of Plant Virus Diseases. CRC Press, pp. 101–128.

Eni, A.O., Hughes, J., Rey, M.E.C., 2010. Production of polyclonal antibodies against a yam isolate of Cucumber mosaic virus (Cmv). *Research Journal of Agriculture and Biological Scienceshttp,* 6, 607–612.

Hafez, E., El Morsi, A.A., Abdelkhalek, A.A., 2011. Biological and molecular characterization of the fig mosaic disease. *Molecular Pathogens,* 2(2):1-8.

Hafez, E.E., El-Morsi, A.A., El-Shahaby, O.A., Abdelkhalek, A.A., 2014. Occurrence of iris yellow spot virus from onion crops in Egypt. *Virus Disease,* 25. https:// doi.org/ 10.1007/s13337-014-0235-7

Heflish, A.A., Abdelkhalek, A., Al-Askar, A.A., Behiry, S.I., 2021. Protective and Curative Effects of Trichoderma asperelloides Ta41 on Tomato Root Rot Caused by Rhizoctonia solani Rs33. *Agronomy,* 11, 1162.

Ie, T.S., 1971. Electron microscopy of developmental stages of tomato spotted wilt virus in plant cells. *Virology,* 43, 468–479.

Lou, L., Su, X., Liu, X., Liu, Z., 2020. Transcriptome analysis of Luffa cylindrica (L.) Roem response to infection with Cucumber mosaic virus (CMV). *Gene,* 737, 144451.

Martelli, G.P., Russo, M., 1984. Use of thin sectioning for visualization and identification of plant viruses, in: Methods in Virology. Elsevier, pp. 143–224.

Medina, V., Rodrigo, G., TIAN, T., JUAREZ, M., DOLJA, V. V, Angeles Achon, M., FALK, B.W., 2003. Comparative cytopathology of Crinivirus infections in different plant hosts. *Annals of Applied Biology,* 143, 99–110.

Megahed, A.A., El-Dougdoug, K.A., Othman, B.A., Lashin, S.M., Ibrahim, M.A., Sofy, A.R., 2012. A new Egyptian satellite strain of cucumber mosaic cucumovirus. *International Journal of Virology,* 8, 240–257.

Milne, R.G., 2019. Electron microscopy of in vitro preparations, in: Diagnosis of Plant Virus Diseases. CRC Press, pp. 215–252.

Montasser, M.S., El-Sharkawey, A.E., 2017. Histopathology of chloroplast ultrastructure and inclusion bodies in CMV-infected plant tissues using electron microscopic examination and image analysis. *Vegetos* ,30, 23–35.

Otulak, K., Kozieł, E., Garbaczewska, G., 2014. Seeing is believing. The use of light, fluorescent and transmission electron microscopy in the observation of pathological changes during different plant—Virus interactions. *Microscopy Research and Technique,* 6th ed.; Mendez-Vilas, A., Ed 367–376.

Roland, J.C., Vian, B., 1991. General preparation and staining of thin sections. *Electron Microscopy of Plant Cells,* 1, 67.

Scholthof, K.G., Adkins, S., Czosnek, H., Palukaitis, P., Jacquot, E., Hohn, T., Hohn, B., Saunders, K., Candresse, T., Ahlquist, P., 2011. Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology,* 12, 938–954.

Soylu, S., Brown, I., Mansfield, J.W., 2005. Cellular reactions in Arabidopsis following challenge by strains of Pseudomonas syringae: from basal resistance to compatibility. *Physiological and Molecular Plant Pathology,* 66:232–243.

Younes, H.A.A., 1995. Studies on certain virus diseases affecting some vegetable crops under greenhouse conditions. Ph. Thesis. Fac. Agric.(Saba-Basha) Alexandria Univ. 220pp.