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Eggplant blister mottled virus (EBMV): A possible new potyvirus characterized from Iraq

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A possible new potyvirus infecting eggplants (*Solanum melongena* L.) was characterized from Iraq. The virus caused characteristic mottling, crinkling, blistering and stunting accompanied by severe abnormalities on new leaves and fruits. The virus was mechanically transmitted to *Gomphrena globosa* and *Zinnia elegans* producing necrotic local lesions (NLL) within 5 days of inoculation. The virus induced systemic latent infection on *Chenopodium amaranticolor* unlike any other known eggplant virus. The other test plants reacted systemically to the virus with different symptoms. No symptoms were observed on *Chenopodium quinoa*, *Chenopodium murale*, *Vigna unguiculata*, *Capsicum annum*, and *Phaseolus vulgaris*. Results of virus-vector relationship showed that, the virus transmitted by *Myzus persicae* in non-persistent manner. Purified preparation of the virus with absorption ratio 260/280 nm of 1.26, and yield of 4.45 mg/100 g virus infected was obtained. SDS-PAGE separation of purified viral particles indicated viral coat protein of 29 Kd. Electron microscopy of negatively stained crude extracts from symptomatic eggplants revealed flexuous particles of 720 nm. An antiserum at titer of 1/1024 against the purified virus was also prepared by four intramuscular injections of the virus. Based on the differences in symptoms on herbaceous host plants and similarity to potyvirus particles and coat protein (CP) size, we propose the name eggplant blister mottled virus (EBMV) for this possible new virus, member of potyvirus genus, characterized for the first time from Iraq.

Key words: Potyvirus, blister, mottling, eggplant, *Solanum melongena*.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is one of the most important vegetable crops in Iraq. It is grown in open field during summer and in plastic and glasshouses during winter. It has been reported that eggplants are infected by several viruses causing significant damage due to plant stunting, crinkle, mottling accompanied by leaf and fruit abnormalities. Of these viruses, eggplant mottle dwarf virus (EMDV), a member of rhabdoviruses has been reported mainly from Mediterranean regions since 1969. It has also reported from northern Africa, southern

Europe and Middle East (Martelli and Russo, 1973; Martelli and Hamade, 1986; AL-Musa and Lockhart, 1990; Ghorbani, 1995; Ciffuo et al., 1999; Aramburu et al., 2006). The virus is known to be transmitted mechanically and by leafhoppers *Agalia vorobjevi* (Babaie and Izadpanah, 2003). Another virus, Eggplant mottle crinkle virus (EMCV), was first reported from Lebanon (Makkouk, 1981), then India (Raj et al., 1989), and recently from Iran (Rasoulpour and Izadpanah, 2008). The virus, having spherical particles of 37-40 nm in diameter, has been considered as a member of Tombusvirus that is transmitted mechanically (Lommel, 2000). Eggplant severe mottle virus (ESMV) was first identified in Nigeria and was reported to be transmitted by aphids *Myzus persicae* and *Aphis craccivora* in non-persistent manner (Ladepo, 1988; Cherif and Mateli, 1992; Brunt et al., 1996). Eggplant mosaic virus (EMV) in eggplants was first reported from west India by Briand et al. (1997). The virus is transmitted by sap, flea beetles *Epitrix Fuscula* (Gibbs and Harrison, 1971; Debrat et al.,

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Abbreviations: NLL, Necrotic local lesions; CP, coat protein; EBMV, eggplant blister mottled virus; EMCV, eggplant mottle crinkle virus; ESMV, eggplant severe mottle virus; EMV, eggplant mosaic virus; PVY, Potato virus Y; AMV, alfalfa mosaic virus; CMV, cucumber mosaic virus; CLL, chlorotic local lesions.



Figure 1. A, symptoms of mottle; B, associated with blister on the leaves of *S. melongena*; C, severe abnormalities on the new leaves.

1977; Dsonza et al., 1990; Rubeiro et al., 1996) and aphids *M. persicae* in non-persistent mode (Verma and Lai, 1967). Purified EMV consist of spherical particles of 28-30 nm in diameter. Potato virus Y (PVY), member of potyvirus is common in eggplants. It is transmitted by aphids *M. persicae* in non-persistent mode (Sastry et al., 1974). PVY consist of flexuous particles of 760 nm in long. Alfalfa mosaic virus (AMV) and Cucumber mosaic virus (CMV) are also reported to infect eggplants. The two viruses are transmitted mechanically to wide host plants and by several species of aphids in non-persistent manner (Kemp and Troup, 1977; Tanne and Sara, 1980; Brunt et al., 1996; Fleish et al., 2001). Purified preparation of AMV contained bacilliform particles, while CMV consisted of spherical particles measuring 30 nm in diameter.

In recent years, an outbreak of a new disease was observed on eggplants in plastic and glasshouses cultivation in Iraq. The disease suspected to be of viral origin and was characterized by leaf mottling, crinkle, plant stunting, accompanied by severe malformation of upper new leaves and fruits. The present study was conducted to identify and characterize the causal virus by biological, serological and molecular means.

MATERIALS AND METHODS

Survey and sample collection

Symptomatic eggplants leaves (Figure 1) from plastic/glasshouse cultivation in Baghdad region of Iraq were collected.

Biological characterization

Host range study

The symptomatic leaves were homogenized in 0.1 M Potassium phosphate buffer PH 7.0 containing 0.2% Na-diethyl dithiocarbamate (Na-DIECA) at 1:4 (g/ml) (Al-Ani and Rathi, 1984). The homogenate was filtered through 2 layers of cheese cloth and the filtrate was centrifuged at 3000 rpm for 10 min. The supernatant obtained was mechanically inoculated on to carborandum dusted leaves of various test plants viz.; *S. melongena* L., *C. amaranticolor*

Coste and Reyn., *Datura stramonium* L., *Datura ennoxia*, *Dolicus Lablab* L., *G. globosa* L., *Lycopersicon esculentum* Mill., *Nicotiana clevelandii* Gray., *N. glutinosa* L., *Nicotiana tabacum* cv. Turkish, *N. tabacum* cv. White burly, *Petunia hybrida* Hord., *physalis floridana* Rydb., *Vigna siensis* Endl., *Vinca rosea* = L., *Withania samnifera*, *Zinnia elegans* jacq., *Cucumis melo* L., *Cucumis sativus* L., *Cucurbita pepo* L., *Malva parviflora* L., *Solanum nigrum* L. at 3-4 leaves stage. The inoculated plants were maintained in insect-proof cages in a greenhouse for four weeks till symptom development.

Virus-vector relationship: Groups of aphids *M. persicae* and *Aphis fabae* reared on *Lactuca sativa* and *Beta vulgaris* respectively were placed on virus-infected eggplants in insect-proof cages for 0.5, 1, 5, 10, 30 and 60 min for acquisition/access periods. The insects were then transferred on healthy eggplants (5 aphids/plant) for 24 h. In other trial, viruliferous aphids were caged with healthy eggplants for 1/2, 1, 5, 10, 30 and 60 min for determination of minimum inoculation access period. The inoculated plants were maintained in growth room at $26 \pm 2^\circ\text{C}$ with 14 h photoperiods for symptoms development. Similarly, adults of whitefly *Bemisia tabaci* were collected from eggplants field by aspirator and caged with virus infected eggplants for 24 h. Then the whiteflies were transferred to healthy eggplants (5 whiteflies/plant) for 24 h for inoculation. The inoculated plants were maintained in growth room till symptoms development. The virus in the inoculated plants was confirmed by enzyme-linked immunosorbent assay (ELISA) using indigenously raised polyclonal antiserum.

Serological characterization

Virus purification: Pure isolate of virus was obtained by successive isolation of single lesion from *Z. elegans* grown in insect-proof cages in a greenhouse. The virus was then propagated in eggplants that serve as source for virus purification. The virus was purified according to the procedure described by Rowhani and stace-smith (1979) for Potato leaf roll virus.

Antiserum production and enzyme-linked immunosorbent assay (ELISA): Antiserum was obtained by 4 administrations of purified virus in to a New Zealand rabbit of 1 year age intramuscularly at 10 days intervals. For each administration 0.5 mg/ml of virus emulsified with an equal volume of Freund's incomplete adjuvant was injected. The blood was collected 15 days after the last injection through the ear marginal vein. The final bleed was allowed to clot for 1 h at room temperature and finally the antiserum was obtained by centrifugation at 3000 rpm for 10 min. The antiserum was maintained at -20°C until use. DAS-ELISA was done to detect the virus in the infected plants using polyclonal

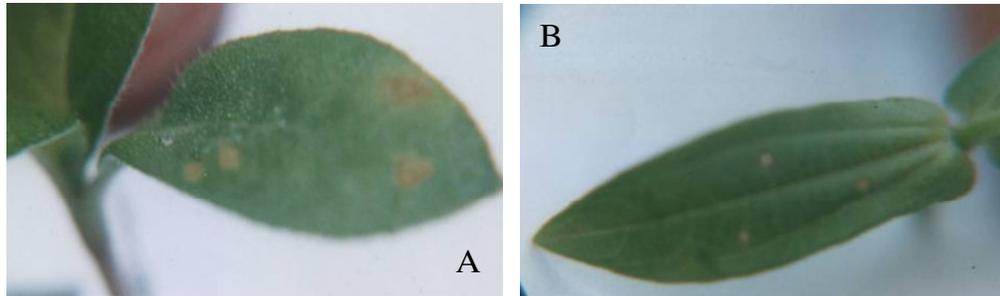


Figure 2. Necrotic local lesions on leaves of *G. globosa*. A, *Z. elegans*; B, sap inoculated by EBMV.

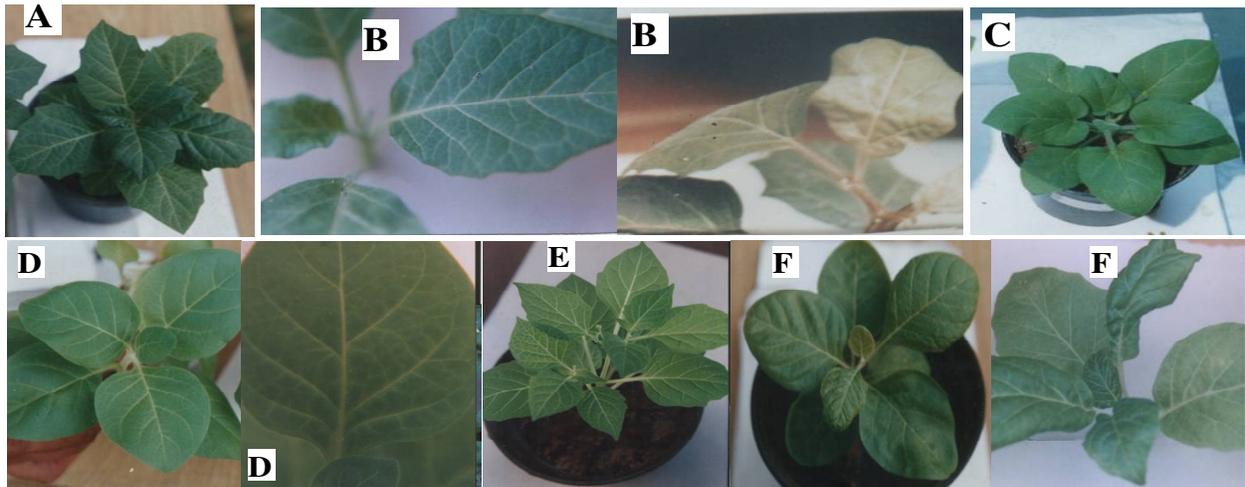


Figure 3. Symptoms of vein clearing associated with mottling and faint chlorosis on the leaves of *D. ennoxia*. A, *D. stramonium*; B, *Nicotiana glutinosa*; C, *N. tabacum* var Samsun; D, *S. nigrum*; E, *W. somnifera*; F, sap inoculated by EBMV.

antiserum prepared against the virus and linked with alkaline phosphatase according to Clark and Adams (1977).

Characterization of viral protein: The molecular weight of viral coat protein was determined by sodium dodecyl sulphate (0.1% SDS)-polyacrylamide gel electrophoresis (10% PAGE) of purified virus in according to Laemmli (1970). The gel was stained for 30 min in 0.25% comassie brilliant blue dissolved in methanol/water/acetic acid (5:5:1) and destained in 10% acetic acid with 5% methanol solution to visualize protein bands.

Electron microscopy: Copper grids (300 mesh) coated with 0.4% formvar solution in chloroform were floated on a droplet of an extract from virus infected plants. The grids were blotted dry, floated on a droplet of water, and then transferred for 1 min to a droplet of 0.1% solution of potassium phosphotungstate in 0.1 M phosphate buffer. The grids were then observed by transmission electron microscopy.

RESULTS

Among mechanically inoculated test plants, *G. globosa* and *Z. elegans* reacted to the virus by producing necrotic

local lesions on the inoculated leaves after 5 days of inoculation by extracts from leaves of virus infected eggplants (Figure 2). The virus induced systemic symptoms on *S. melongena* after 16 to 20 days of inoculation. The symptoms began as small faint chlorotic lesions on the leaves, developed to mottle or mosaic associated with blisters on the blades and finally to severe abnormalities of the new leaves associated with some degrees of necrosis on the veins and stunting of plants (Figure 1). Symptoms of vein clearing, mild mottling on the leaves developed to faint chlorosis on *D. stramonium*, *D. ennoxia*, *Lycopersicon esculentum*, *Nicotiana glutinosa*, *N. tabacum* cv. Samsun, *N. tabacum* cv. Turkish, *S. nigrum* and *Withania somnifera* after 15 days of sap inoculation from infected eggplants (Figure 3). Mottling associated with chlorosis and vein-banding on the leaves of *Malva parviflora*, *N. clevelandii*, *Physalis floridana*, *Petunia hybrida*, *Vigna sinensis* Endle., and *Vinca rosea* were observed after 10–12 days of sap inoculation. Symptom of mottling and mosaic on the leaves of *C. sativus*, *Cucumis melo*, and *Cucurbita pepo*

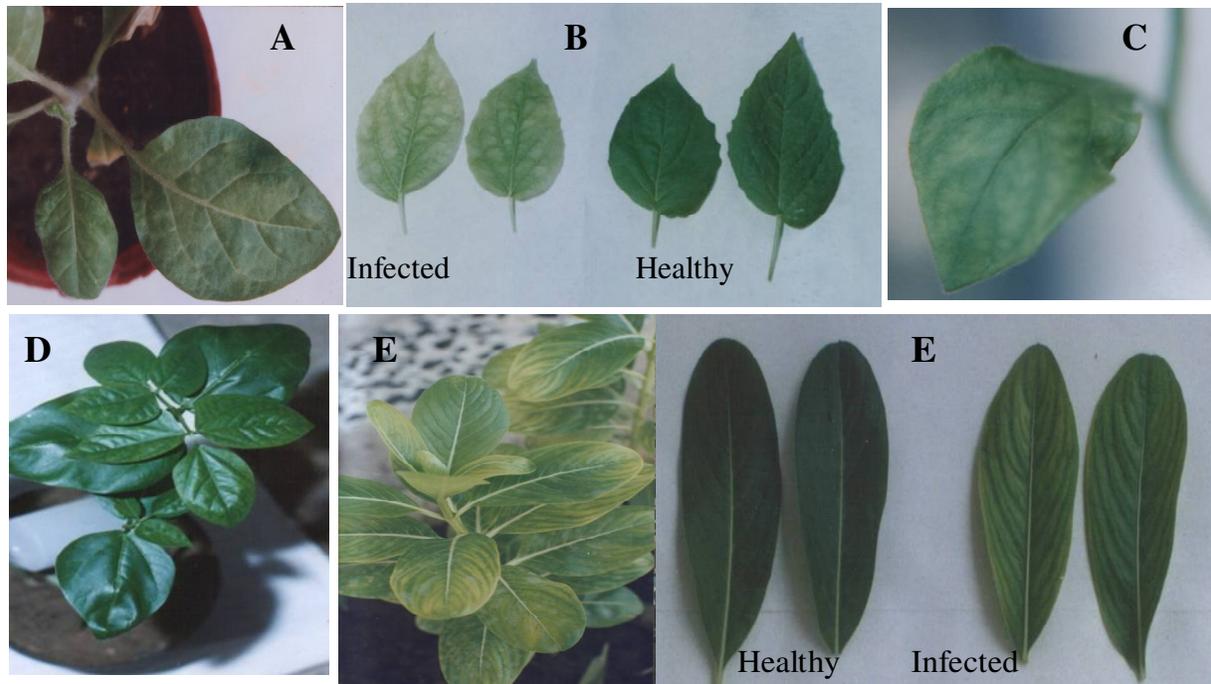


Figure 4. Symptoms of mottling, vein banding associated with chlorosis on the leaves of *Nicotiana clevelandii*. A, *P. floridana*; B, *P. hybrid*; C, *V. sinensis* Endle; D, *V. rosea*; E, inoculated by EBMV.

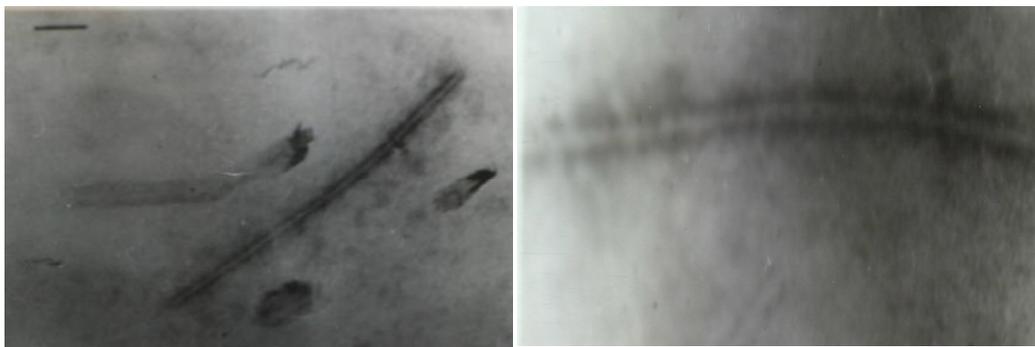


Figure 5. Electron micrographs of virus extract from infected eggplant leaves showing flexuous particle of about 720 nm long (The bar is 100 nm long).

with some degrees of stunting were observed upon mechanical inoculation by the virus (Figure 4). No symptoms were found on *Capsicum annum*, and *Solanum tuberosum*. The infection of *C. amaranticolor* by the virus as was detected by DAS-ELISA and was found to be latent mention type of symptoms observed on *C. amaranticolor*.

Virus–vector relationship: Symptoms characteristic of the virus appeared when healthy eggplants exposed to group of aphids *M. persicae* pre-caged on virus infected plants for 1/2 and 1 min. *A. fabae* and *B. tabaci* failed to transmit the virus. The same periods were required for

inoculation healthy plants. This indicated that *M. persicae* transmitted the virus in non-persistent manner.

Virus purification: Results showed that the procedure used for virus purification was suitable to obtain acceptable quantity of purified virus with absorption ratio 260/280 of 1.26. The virus yield was 4.45 mg/100 gm from infected eggplant leaves calculated using the PVY extinction coefficient = 2.8.

Electron microscopy: The observation of samples from infected eggplant leaves by transmission electron microscope revealed the presence of flexuous particles of

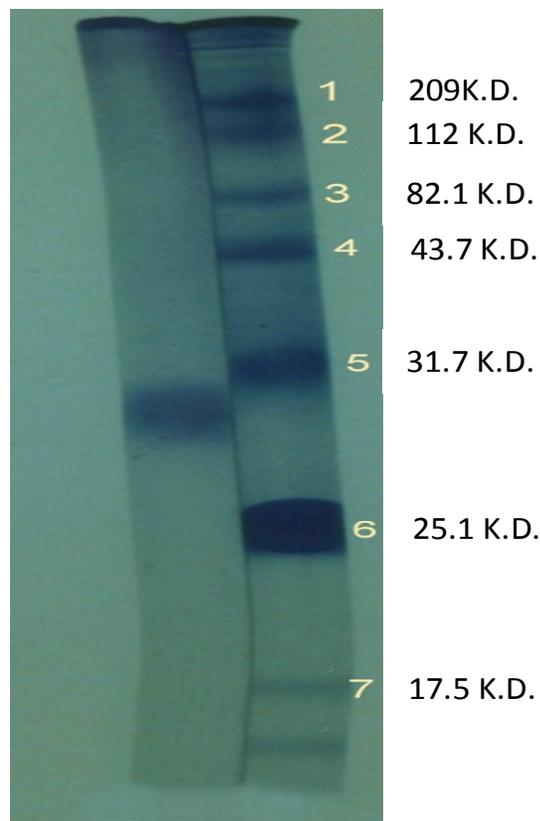


Figure 6. SDS-Polyacrylamide gel electrophoresis pattern of a dissociated sample of purified EBMV showing one band (left) represent the coat protein, of about 29 Kd estimated according to marker proteins of known molecular weight co-migrated with the viral protein (right).

about 720 nm in long (Figure 5). This indicated that the virus may be a member of potyvirus.

Serological analysis: An antiserum against the virus obtained after 4 intramuscularly administration of purified virus had a titer of 1/1024.

Viral coat protein characterization: The analysis of a sample of purified virus on SDS-PAGE revealed single protein subunit of 29 Kd determined by using proteins of known MWs co-migrating with the virus extract on the gel (Figure 6).

DISCUSSION

A possible new virus causing severe damage on eggplants was characterized by biological, serological, and molecular means. The symptoms induced on test plants upon inoculation by sap extracts from virus infected eggplants showed that, the virus seemed

different from AMV, CMV, EMCV, EMV, and PVY, these viruses induced chlorotic local lesions (CLL) on *C. amaranticolor* (Kemp and Troup, 1977; Makkouk et al., 1981, Raj et al., 1989; Brunt et al., 1996), while this plant is unsusceptible to EMDV and ESMV (Ladipo et al., 1988, Al-Musa and Lockhart, 1990, Brunt et al., 1996), but the infection of this plant by the virus in question found to be latent. Results showed that *D. stramonium* react systemically to AMV, ESMV, and EMV (Bos and Jspers, 1971; Gibbs and Harrisson, 1971) and by forming local lesions to CMV, and EMCV (Makkouk et al., 1981), immune to PVY (Sastry, 1974). To the virus in this study, *D. stramonium* reacted systemically. The virus infected *G. globosa* systemically, this host react to EMV and CMV by forming chlorotic local lesions (Gibbs and Harrisson, 1971), but not susceptible to AMV, EMV, PVY and ESMV. Unlike to all viruses infecting eggplant, discussed in this paper, the virus induced necrotic local lesions (NLL) on *Z. elegans*.

Like AMV, CMV, PVY, ESMV and EMCV, which are known to be transmitted by aphids *M. persicae* in non-persistent manner (Sastry et al., 1974; Kemp and Troup, 1977; Ladipo, 1988, Cherif and Marteli, 1992; Brunt et al., 1996; Fleysh et al., 2001), our virus was found to be transmitted by *M. persicae* in non-persistent manner.

Result of electron microscopy and coat protein analysis indicated that our virus was a member of potyvirus group like PVY and ESMV. It was reported that the MW of potyvirus genus is between 28–47 Kd (Lopez moya et al., 1994; Hull, 2002; Chang et al., 2002; Chen et al., 2006; Adhab, 2009), but this virus is different from these two viruses based on the differences in the symptoms induced on *D. stramonium*, *C. amaranticolor* and *Z. elegans*, which mean that our virus may be representing a new virus infecting eggplant in Iraq. The name proposed is EBMV due to characteristic blistering symptoms on eggplant.

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