

Eradication of Banana Viruses from Naturally Infected Banana Plants. 1- Biological and Molecular Detection of Cucumber Mosaic Virus and Bunchy Banana Top Virus in Naturally Infected Banana Plants

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Abstract: Bunchy top banana is a destructive viral disease in many countries including Egypt. It causes severe losses because infected plants produce no fruit. This study was designed to detect viruses affecting banana plants (bunchy top banana virus BBTV and CMV). Results revealed that the early symptoms of BBTV-infected banana were dark green streaks on midrib of the lower portions of leaves. The newly growing leaves were brittle. They gather at the top of plant making a rosetting shape. CMV-infected banana were characterized by a conspicuous interveinal chlorosis of the leaves. BBTV and / or CMV mixed infection were detected with 2% triphenyl tetrazolium chloride in discs of suckers infected. The viruses were detected via the precipitation serological reaction using specific IgG by DAS-ELISA and DIBA. BBTV was isolated by using syringe on healthy banana plants and BMV was isolated by local lesions assay on *Chenopodium amaranticolor*. BBTV has limited host rang, it reacts with mosaic on *Canna sp.* while CMV exhibited different systemic symptoms on *Cucumis sativus*, *Sorghum vulgare*, *Zea mays*, *Musa sp.*, *Nicotiana tabacum*, *Datura metel*, *Lycopersicon esculentum*, local symptoms on *Ch. amaranticolor*, *Ch. quinoa*, *Vigna sinensis*, *V. unguiculata* and local lesions followed by systemic infection on *Cucurbita pepo* and *N. glutinosa*. BBTV and CMV are transmitted by infective *Pentaloria nigronervosa* in semi- and non-persistent manner respectively. PCR as an enzymatic procedure was used successfully to detect BBTV and CMV of naturally infected banana plants. The PCR amplification of BBTV-DNA and CMV-RNA was carried out on the total DNA and RNA isolated from naturally infected plants using specific coat protein (PC gene). The results showed that BBTV and CMV respectively. Examination of ultrathin sections prepared from naturally infected banana leaves revealed disorganization and destruction of chloroplasts, degeneration of mitochondria and vacuolation in the cytoplasmic and membrane and abnormal osmophilic body in cytoplasm.

Key words: Banana plant, BBTV, BMV, DAS-ELISA, PCR, RT-PCR.

INTRODUCTION

Banana is one of the most important fruits in Egypt. In 1996, banana production was 512.5 thousand metric tons and the average crop was 11.27 & 13.71 tons /fed.

Viral infection causes a great loss in banana production with a ratio of about 20-30% loss and occasionally reaching 50-80%^[9].

Bunchy top banana is a destructive viral disease in many countries including Egypt. It causes severe losses because infected plants produce no fruit. Banana bunchy top virus is most severe on banana but also affects some ornamentals such as *Canna sp.* BBTV, are transmitted by aphids in the persistent manner^[1].

In Egypt, the most threatening viral diseases are those caused by banana bunchy top virus (BBTV) and banana mosaic virus (BMV). These viruses are considered as

limiting factors in banana production^[3,7]. These viruses cause losses in many countries. They naturally spread from plant to plant by insect vectors and from a crop cycle to another in vegetative planting material.

Banana bunchy top virus belongs to the family Circoviridae (from being small, round) genus Nanovirus (from being small, nanos=dwarf). They are isometric, small (about 18-22 nm in diameter) and contain ssDNA organized in multiple circular ssDNA components.^[1]

Cucumber mosaic virus (CMV) which was a worldwide distribution and is found in most Musa growing areas causes banana mosaic also known as infectious chlorosis or heart rot. The virus particle in shape has a wide host range which includes: cucurbits, solanaceae, corn and several broadleaf. The commercial cultivars of banana latunda, lakatan, cavendish etc. are susceptible to this disease^[24].

This study aimed to detect and identify BMV and BBTV from naturally infected banana plants.

MATERIALS AND METHODS

Source of virus isolates: CMV and BBTV viruses were isolated from infected banana plants var. Williams cultivated in Meet El-Attar, Benha (Qualubia Governorate, Egypt) during spring. One hundred plants showing signs of BBTV and CMV infection were used in the current study. Detection and identification of Egyptian BBTV and CMV isolates were based on symptomology, histochemical changes, indicator plants, serology and molecular methods.

Histochemical detection: As viruses cause some biochemical changes in the infected plants, we can detect them using color tests. We used a solution of 2% triphenyltetrazolium, took discs from corms by cork borer and put in the solution in Petri dishes for five minutes. The color of the discs was reported^[2].

The virus isolates CMV and BBTV were detected in naturally infected plants by DAS-ELISA according to Clark and Adams^[4]. Sanofi Sante Animal Paris, Farnce, provided ELISA kits for detected virus isolates.

Ch. amaranticolor was used for local lesions assay^[14]. *Ch. amaranticolor* was inoculated mechanically by CMV clarified sap from selected sample (+ve ELISA). Local lesions appeared after 15-20 days. Clarified crude sap from BBTV naturally infected banana leaves (+ve ELISA) was inoculated by syringe in 10 adapted healthy banana seedlings var. Williams produced by tissue culture. Inoculated plants were kept in an insect-proof green house until appearance of the external viral signs.

Six differential hosts *Chenopodium album*, *Ch. amaranticolor*, *Cucumis melo* L., *Cucurbita pepo* L. Eskandarani, *Vigna sinensis* and *Phaseolus vulgaris* were mechanically inoculated with CMV isolate by conventional leaf rub method with cotton swab, using carborandum (600 mesh). The crude sap of infected plants was extracted in cold 0.01M phosphate buffer (PH 7.1) containing 3% nicotine solution and 0.01M cysteine^[17]. Five plants were used as replicates. The inoculated plants were kept in an insect-proof greenhouse for 30 days and were inspected daily for development of external symptoms.

Insect transmission was done according to Leghari^[16]. Aphid species, *Pentalonia nigronervosa* Coq. was obtained from stock culture of virus free insect (Kindly plant Protection Dept. Fac. of Agric., Ain shams Univ.).

Dot immunobinding assay (DIBA): A polyclonal antibody against CMV and BBTV were obtained by D.E. Purcifil from Florida Univ. Antirabbit alkaline phosphatase conjugate (AP) was purchased from SIGMA. The DIBA assay was applied according to Cambra and colleagues^[5].

Table 1: Oligonucleotide primers for BBTV and CMV.

Virus	Nucleotide sequences
BBTV	Reverse 5'-GCTAGGTATCCAAGAAATC-3'
	Forward 5'-TCAAACATGATATFTAATTC-3'
BMV	Reverse 5'-CCCCGGATCCTGGTCTCCTT-3'
	Forward 5'-CCCCGGATCCACATCAAGTTTATGTTCAATTC-3'

Molecular detection: (RT-PCR and PCR) techniques were used to detect CMV and BBTV in naturally infected plants.

Total RNA of CMV: Infected banana leaves were extracted according to Gibbs and Mackenzie^[10].

RT-PCR amplification of CMV: Reverse transcription of CMV RNA was done using the CMV CP.5 as in table (1)^[19], complementary of the conserved ultimate 3' terminal 10 nucleotides of all CMV RNA-3 and Avian Myeloblastosis virus reverse transcriptase (AMV_RS). The PCR reaction were performed according to conditions and cycling parameters described by Quenmada and colleagues^[19].

Total DNA of BBTV: Infected banana leaves were extracted using a version of CTAB (Cetyl trimethyl ammonium bromide) method^[6].

PCR amplification of DNA-BBTV: Oligonucleotide primers for PCR were derived from the published sequences of BBTV DNA-4^[13]. The nucleotide sequences of two primers used in the PCR amplification were shown in Table (1). The PCR reactions were performed according to condition and parameters described by^[13].

Agarose gel electrophoresis analysis was carried out using a Pharmacia GN-100 submarine gel electrophoresis apparatus. DNA-PCR products were visualized on a UV trans-illuminator ($\lambda=254$ nm) and photographed with an UVB laboratory products, Epichemi II Dark room, 3UV trans-illuminator Pharmacia.

Histopathological studies: Ultrathin sections from CMV or BBTV naturally infected banana leaves were performed according to the method adapted from Spurr^[23] with further modification by Sohair^[22].

RESULTS AND DISCUSSIONS

Samples of infected banana plants exhibiting virus symptoms were collected from field. Signs of bunchy top virus infection were distinctive. The early symptoms of BBTV-infected banana were dark green streaks on the lower portions of midrib of banana leaves (Fig.1A). The new infected leaves were brittle, reduced in size and gather at the top of plant making a rosetting shape, which is diagnostic and distinct from symptoms caused by other known viruses of banana (Fig.1B and 1D). Some leaf

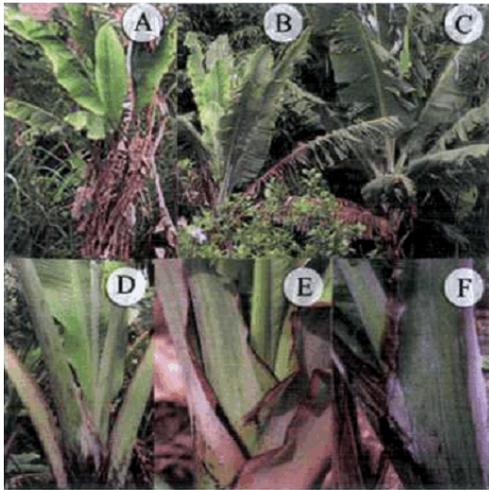


Fig. 1: Naturally infected banana plants exhibited different viral symptoms. Leaves are bunched up, narrow, stiff, upright and irregular or wavy leaf margins (A, B and E). Petioles and leaf sheaths are mottled, streaked (A, B and F). Healthy plant (C).

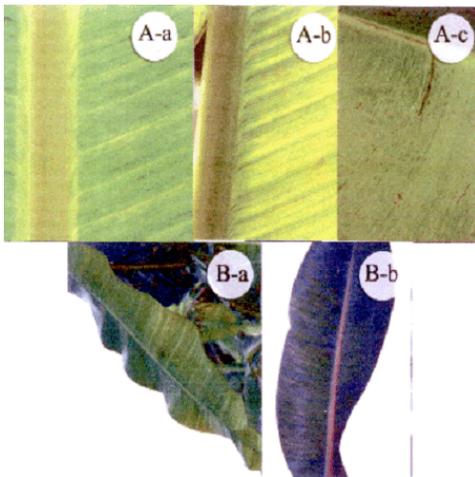


Fig. 2: Banana leaves with BBTV (A) and CMV (B): leaf veins are dark green colored and form a hook shape as the midrib is approached (a, b). Leaf showed the characteristic horse code streaking ©. Leaves with BMV appeared green streaks and wavy (a and b).

veins were dark green-colored and form a hook shape as the midrib is approached (Fig.2A [a, b]).

Samples exhibiting signs of CMV infection were characterized by a conspicuous mottling and mosaic of the leaves, leaves have green streaks and are wavy (Fig. 2B [a, b and c]). Common observation of infected plants is stunted growth that is accompanied by rotting of the heart leaf and central cylinder in severe cases.

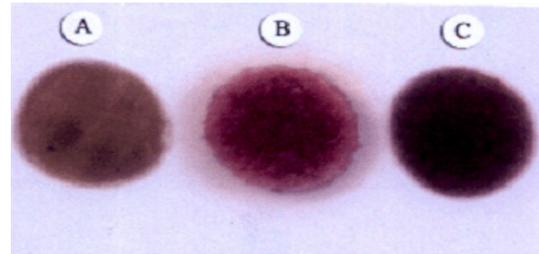


Fig. 3: Histochemical changes in discs of corms of banana plants. Var. Williams naturally viral infected Disc from healthy corm Red color from BBTV infected corm. Dark brown-red color from BMV infected corm.

Virus Detection: The discs of suckers, naturally viral infected, treated with triphenyl tetrazolium chloride appeared red with BBTV, dark brown-red with CMV and red and dark brown-red with mixed infection. Those taken from healthy plants remained colorless (Fig. 3). While these results indicated that percentage of mixed infection was 20.72%, BBTV and BMV single infection were 15.075 and 10.75% in naturally infected banana respectively.

Using DAS-ELISA, data indicated that BBTV and CMV were detected as single infection in 45 and 20 out of 100 tested plants respectively. Mixed infection was found in 25 plants. On the other hand no viruses were detected in 10 plants showing symptoms and positive reaction with BBTV or BMV using DAS-ELISA. These plants were used as a source of BBTV and BMV in the following experiments.

Virus isolation: BBTV was isolated using syringe on 10 adapted healthy banana seedlings (2 months) var. Williams produced by tissue culture. Seedlings that inoculated with BBTV infected sap were kept in an insect-proof greenhouse until developing the external symptoms. We noticed that the inoculated seedlings showed typical banana bunchy top symptoms after 25-35 days. The BBTV infection was confirmed by DAS-ELISA.

The infectious sap of CMV-infected plant was mechanically inoculated on *Ch. amaranticolor* as local lesion host. The local lesions that has similar morphological characters (minute, circular without hallow necrotic local lesion) were collected and extracted in phosphate buffer 0.2M PH 7.1. The infectious sap was re-inoculated on healthy seedlings of banana plants (about 35 day's age) as propagative and maintenance host. DAS-ELISA confirmed the acquired infection of BMV isolate.

Virus identification:

I. Host range: BBTV that was propagated on banana plants was mechanically inoculated using syringe on *Canna indica*. The inoculated plants showed mosaic

Table 2: Reaction of host range to mechanical inoculation with the isolate of CMV.

Families	Host	Symptoms	DAS-ELISA*
Chenopodiaceae	<i>Chenopodium album</i>	-----	0.075
	<i>Ch. amaranticolor</i>	Necrotic local lesions after 12- 16 days	0.285
	<i>Ch. quinoa</i>	Chlorotic local lesions after 12 days(Fig.4F)	0.325
Cucurbitaceae	<i>Cucumis melo</i> var. <i>Balady</i>	Systemic mosaic and Stunting (Fig. 4A)	0.190
	<i>Cucumis sativus</i> var. <i>Beta Alpha</i>		0.425
	<i>Cucurbita pepo</i> L. var. <i>Eskandarina</i>	Yellow spots after 6 days Followed by systemic mosaic (Fig.4B)	0.345
Graminaceae	<i>Sorghum vulgare</i> L.	Mosaic after 20 days	0.315
	<i>Zea mays</i>	Mosaic after 20 days	0.275
Leguminaceae	<i>Vigna sinensis</i> L. var. black eye	Brown lesions in inoculated leaves after 10 days (Fig.4D)	0.325
	<i>Vigna unguiculata</i> L. var. Kafr El-Sheikh	Brown lesions in inoculated leaves after 10 days (Fig.4E)	0.332
Solanaceae	<i>Phaseolus vulgaris</i> L. var. kidney	Typical mosaic after 20 days	0.175
	<i>Datura metel</i> L	Mosaic after 19 days	0.295
	<i>Nicotiana glutinosa</i> L.	Yellow spots after 16 days followed by systemic mosaic	0.421
	<i>N. tabacum</i> L. var. White barley	Mild to severe mosaic and stunting after 20 days (Fig. 4C)	0.421
Compositaeae	<i>Lycopersicon esculentum</i> L. var. Castle rock	Mosaic and stunting	0.320
	<i>Zinnia elegans</i> .		

* Optical density 405 nm

-Negative control 0.125

Temperature in greenhouse

No symptoms

symptoms after 21 days in contrast to *Colocacia esculentum* var. *esculanta* which did not show any symptoms. These results were confirmed by BBTv-IgG using DAS-ELISA.

We noticed that BBTv is transmitted from banana by using syringe and the inoculated plants showed external symptoms after 14 days.

CMV isolate that was propagated on banana plants was mechanically inoculated on 16 plant species belonging to 6 different families (table, 2). Infected and non-infected plants were checked by DAS-ELISA. According to response of tested plants, it was classified into four categories as follows:

- Plants showed different systemic symptoms, included *Cucumis sativus* (systemic mosaic and stunting), [Fig. 4A], *Sorghum vulgare* and *Zea mays* (Mosaic after 20 days), *Musa sp.* (typical mosaic after 20 days), *Datura metel* (mosaic after 19 days), *Nicotiana tabacum* var. Samsun (mild to severe mosaic and stunting 20 days) (Fig.4C) and *Lycopersicon esculentum* (Mosaic and stunting).
- Plants showed local lesions only on the inoculated leaves, included *Ch. amaranticolor* (necrotic local lesions after 12-16 days), *Ch. quinoa* (chlorotic local lesions after 12 days) (Fig. 4F), *Vigna sinensis* and *Vigna unguiculata* (brown lesions in inoculated leaves after 10 days) (Fig.4D and 4E).

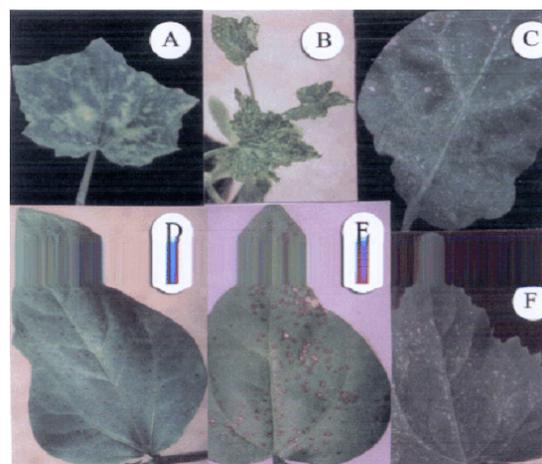


Fig. 4: Different hosts of CMV appeared variation of symptoms. (A) Systemic mosaic on *Cucumis sativus* (Cucumber). (B) Systemic mosaic and stunting on *Cucurbita pepo* (squash). (C) Yellow spots on *Nicotiana tabacum*. (D) Necrotic brown lesions in inoculated leaves of *Vigna sinensis*. (E) Large necrotic brown lesions in inoculated leaves of *Vigna unguiculata*. (F) Chlorotic local lesions on *Ch. quinoa*.

- Plants showed local lesions on the inoculated leaves followed by systemic crinkle, leaf narrow and gather at the top of plant making a rosetting shape that included *Cucurbita pepo* (yellow spots after making a followed by systemic mosaic, Fig.4A) and *Nicotiana glutinosa* (yellow spots after 16 days followed by systemic mosaic).
- Plants showed no visible reaction with BMV, included *Ch. Album*, *Cucumis melo*, *Phaseolus vulgaris* and *Zinnia elegans*.

Aphid species *Pentalonia nigronervosa* Coq. were feeded BBTV infected banana plants for 24 hours, then transferred to adapted healthy banana plants var. Williams. The results indicated that BBTV is transmitted from banana to banana by infective aphid vector (*Pentalonia nigronervosa*) in semi-persistent manner. This result was confirmed by DAS-ELISA.

CMV is transmitted from infected banana plants to banana and other plant species using infectious sap by mechanical inoculation. On the other hand, BMV is also transmitted by infective aphid vector (*Pentalonia nigronervosa*). After 15 minutes feeding on banana plants and 24 hours incubation period, CMV was transmitted in a non-persistent manner.

The precipitation serological reaction was determined using BBTV and CMV polyclonal antibodies by (DAS-ELISA) test as follows: Samples of a young fully developed leaf of each banana, cucumber, squash, tomato, *Nicotiana tabacum* var. white barely and cowpea were taken after 4 weeks of inoculation. Using polyclonal antibodies against BMV isolate gave positive reaction but the variations in optical density were due to the differences in their titer.

BBTV and CMV were detected by DIBA test using specific IgG. In dot blot immunoassay, BBTV or/ and CMV infected leaf saps were detected when the nitrocellulose membrane was probed with polyclonal antibody raised against BBTV or/and IgG diluted in TBS buffer and the anti-rabbit alkaline phosphatase conjugate diluted (1-10.000) was used as secondary antibody. The nitrocellulose membrane was color developed using NBT/BciP substrate. The colored spots show the high sensitivity of the detection of BBTV and BMV healthy control showed no colored signals as showing in (Fig. 5A and B).

Molecular detection: The total DNA was extracted from banana plants, naturally infected with BBTV isolate as described in materials and methods. The yield of total DNA was determined spectrophotometrically as 250 µg / 0.02 g of tissues. The purity of DNA samples as indicated by $A_{260/280}$ ratio was 1.75. The total RNA was extracted from banana plants naturally infected with BMV isolate by using pure RNA extraction kit (Roche Diagnostics) as described in materials and methods. The yield of total RNA was measured spectrophotometrically as 150 µg / 0.02g of tissues. The purity of total RNA samples as indicated by $A_{260/280}$.

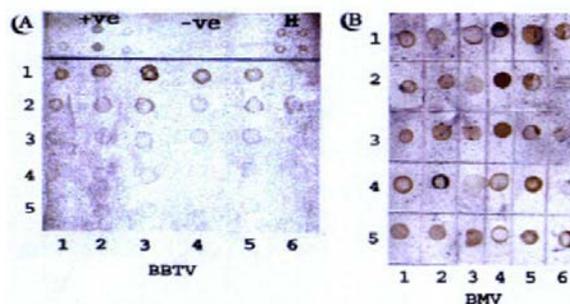


Fig. 5: DIBA test showing serological reaction of (A) BBTV-IgG and (B) CMV-IgG with naturally infected banana plants. Healthy sap (-ve control) showed no colored signals. +ve positive control -ve negative control

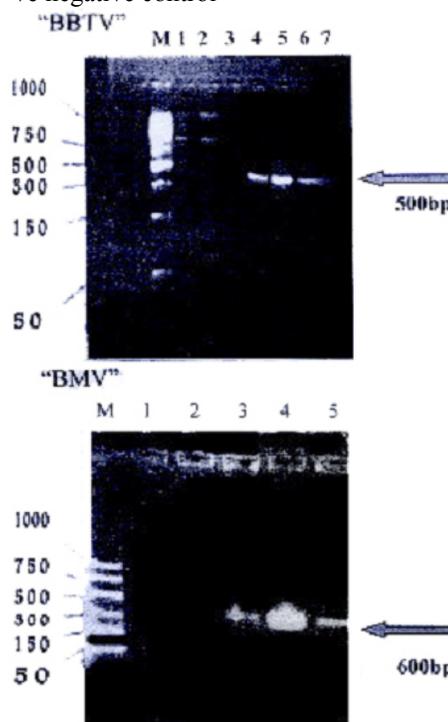


Fig. 6: Agarose gel (1%) electrophoresis analysis of PCR products (A) CR products of the CP region of BBTV using specific primer pair (B) RT-PCR products of the CP region of BMV using specific primer pair (CM1 andCM2). The arrows indicate the correct size of the amplified PCR Products 500bp "BBTV" and 600bp "CMV". Lane 1, 2, 3, 4, 5 and 6 naturally infected banana plant in field. Lane M molecular weight of marker (250 bp ladder).

PCR as an enzymatic procedure was used successfully to detect very low amounts of nucleic acid belonging to several plant viruses with high sensitivity and specificity. The results showed that BBTV was detected in leaf tissue from naturally infected banana plants in open filed. (Fig. 6A) shows agarose gel

electrophoresis analysis BBTV primers. A major DNA fragments of the expected size; 500 bp was amplified from infected tissue (lane 4, 5, 6). No amplification was obtained with uninfected banana leaves tissue samples (Lane 1, 2, 3).

The viral RNA was reversed transcribed by MMLV. The reverse transcription reaction was primed with the oligo-dt (5'-CCCCGGATCCTGGTCTCCTT-3') as minus sense primers. The resulting complementary DNA (cDNA) was amplified by PCR after adding two sets of primers (plus sense primers, CM1 and CM2) for coat protein (CP). The efficiency of DNA amplification from BMV infected leaf tissues using each of two sets of primers were investigated using agarose gel electrophoresis analysis product. Figure (6B) shows agarose gel electrophoresis analysis of BMV- isolate from naturally infected banana leaves amplified by RT-PCR. The size of the major amplified product in all samples was 600 bp (lane 3, 4, 5). This product was not detected in uninfected tissues (lane 1, 2).

Examination of mesophyll cells in ultrathin sections prepared from leaf tissues naturally mixed infection with BBTV and CMV revealed different cytoplasmic effects in comparison with healthy ones. It caused granulation of cytoplasm accompanied with multiple cytoplasmic vacuoles of different sizes formed by invagination of plasmalemma (Figs. 7 B & D) and tonoplast (Fig. 7 D). Malformation and distortion of mitochondrial matrix were observed (Fig. 7B, F & G) the most mitochondria cristae (Fig. 7E) or without cristae (Figs. G & F). In the ultrathin section prepared from naturally infected. Banana leaf with BBTV and BMV possible isometric virus particles were found in the cytoplasm (Fig. 6). Results also showed disorganization and distraction of chloroplasts (Figs. 7B, C, D, E&F). Cells filled with plastids containing protein crystals and loss round and no apical dense that may be lipid globules. Vesicles were frequently observed, appearance of abnormal large vesicles were illustrated and enlargement of chloroplast and different changes were found in ultrastructure of chloroplast (Fig. 7E&F). Disorganization of stoma lamella and grana, disappearance of plastoglobuli and inclusion bodies filled with flexuous rod-like virus particles were also seen (Fig. 7G). The chloroplasts showed conspicuous alterations in shape and in internal structure (distortion of grana and thylakoid membrane). In addition some infected cells showed vacuolation in cytoplasm (Fig. 7B&D), some abnormalities in the cell walls i.e. the cell wall protrusions, depositions of electron dense materials in or near the cell wall protrusions (Fig. 7F & G). plastoglobuli and inclusion bodies filled with flexuous rod-like virus particles were also seen (Fig. 7G). The chloroplasts showed conspicuous alterations in shape and in internal structure (distortion of grana and thylakoid membrane). In addition some infected cells showed vacuolation in

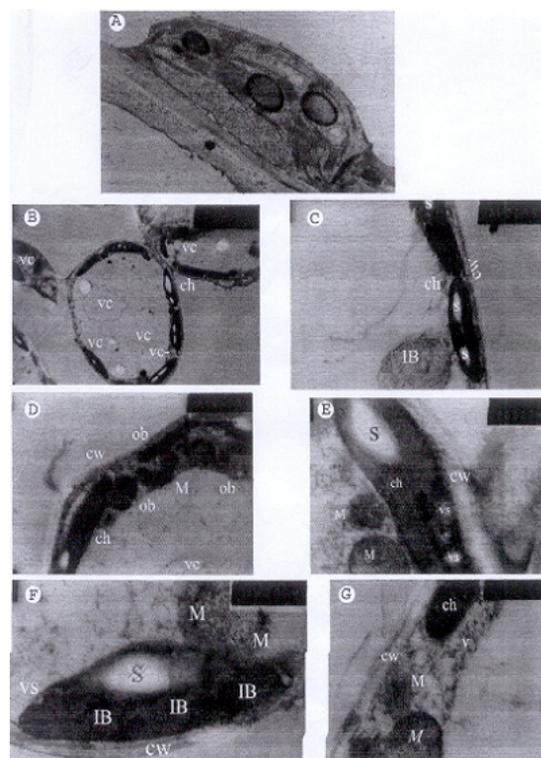


Fig. 7: Ultrathin sections of mesophyll cell in infected banana leaves Showing alternation of cell organelles (B-G) compared with Healthy cell (A) Ch = Chloroplast V = Virus particles Vs = Vesicles CW = Cell wall Vc = Vacuoles M = Mitochondria IB= Inclusion bodies Ob = Osmophilic bodies

cytoplasm (Fig. 7B&D), some abnormalities in the cell walls i.e. the cell wall protrusions, depositions of electron dense materials in or near the cell wall protrusions (Fig. 7F & G).

We noticed that the first symptoms of banana infected with BBTV were dark green streaks on the lower portions of the midrib of banana leaf, the new infected leaves were brittle, reduced in size and gather at the top of plant making a rosetting shape. These findings were reported also by Allam and colleagues^[2].

Virus causing chlorosis/mosaic disease of banana was identified as a strain of cucumber mosaic virus (CMV). Association of CMV with the disease was established to CMV-T and slot blot hybridization with nucleic acid probe of CMV-P genome^[24]. The symptoms of BMV are characterized by a conspicuous inter-veinal chlorosis of leaves. Common observation of infected plants is stunted growth. In severe cases this is accompanied by rotting of heart leaf and central cylinder^[11,18]. All banana plants used were found to be infected with either BMV or BBTV by different methods as the biological, serological and

molecular detection. Enzyme linked immunosorbent assay (ELISA) was used to detect banana viruses because of their sensitivity, specificity and speed^[4,12]. The obtained results were based on the immunobinding assay techniques (as DIBA) for virus detection. These techniques have recently proved to be very efficient for detection of many plant viruses^[15]. The virus isolate was biologically purified via single local lesion technique. Homologous single local lesion production on *Ch. amaranticolor* were cut and macerated on a glass slide and mechanically inoculated in seedling of *Ch. amaranticolor* as propagative host for BMV isolate^[7]. In contrast to other authors^[2,21], BBTV can be mechanically transmitted to some host plants as *Canna sp.* In contrast to our results, Rao^[20] mentioned that *Cucumis melo* was a new host while in our results it gave no symptoms. Similar to our results El-dougDoug and colleagues^[7] reported that BMV in banana was mechanically transmitted from banana to *N. tabacum* var. Samsun, *N. glutinosa*, *D. stramonium* with mosaic symptoms and *Ch. amaranticolor* with local necrotic lesions. Results of aphid transmission of BBTV and CMV, which normally caused systemic symptoms on banana could be attributed to the fact that the BBTV and BMV are consistently transmitted to offspring and accumulated there. Whenever these plants are propagated vegetatively, the new raised plants as well as their suckers were almost infected^[8]. Agrios^[1] found that BBTV is transmitted over long distance by propagative materials such as rhizomes, suckers, or tissue- cultured meristems and over short distance by banana aphid *Pentalonia nigronervosa* in persistent manner. Volunteer bananas growing in pervious banana plantations are often infected with BBTV and also support large aphid population that then transmit the virus to newly planted banana plantations. It was found that sections of suckers infected with BBTV turned brick red with triphenyl tetrazolium chloride stain. They also noticed that the sections obtained from infected plants with CMV appeared black while others taken from healthy plants remained colorless. The same results were obtained by Summanwar and Marathe^[25].

In the present study, examination of ultrathin sections of naturally infected banana plants with mixed infection manifested as mosaic, chlorosis on blade dark green streaks on the midrib of the infected leaves, disorganization, revealed degeneration of phloem cells as well as the appearance of necrotic cells. The ultrastructural study showed that chloroplasts of the infected leaves were partially or fully disintegrated. Chloroplasts with large vesicles having electron dense globules were also observed in infected cells. Both kinds of chloroplast abnormalities were found in banana leaves.

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