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Full Length Research Paper

Management of viral disease in banana using certified and virus tested plant material

El-Dougdoug, Kh. A.[1] and M. M. El-Shamy[2]

[1]Department of Microbiology, Faculty of Agriculture, Ain Shams University, P. O. Box 68 Hadayek Shobra 11241 Cairo, Egypt.
[2]Department of Botany, Faculty of Science, Menoufia University, Egypt.

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Viruses are major limitations to cultivation. These viruses were detected by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using specific polyclonal antibodies for Banana bunchy top virus (BBTV) and Cauliflower mosaic virus (CMV). Polymerase chain reaction, (PCR) based detection of a 500 bp amplicon from BBTV infected tissues and or a 600 bp amplicon from infection Brome mosaic virus (BMV) infected tissues confirmed the presence of the viruses in these plants. As well as the major deoxyribonucleic acid (DNA) fragments of expected size, 500 bp was amplified from BBTV infected tissues and the size of the major amplified product in BMV infected tissues was 600 bp. The application of banana meristem tip (0.3 mm) is more effective for BBTV and or BMV eradication *in vitro*. Chitosan (0.12%), treatment for infected plants was more effective for BBTV and BMV eradication *in vivo*. The results proved that there is five important precautions for success of the rouging program of banana viral control included: (1) To ensure that the nursery stock is clean and free from latent virus infection via starting tissue culture seedlings virus tested or suckers treated with 0.12% chitosan, (2) Detecting infected plants periodically every month by fortnightly inspection via external symptoms and every season by a DAS-ELISA test for the presence viral diseases (3) Rouging the infected plants after two inspections. The rouged plants were destroyed by burning at the end of growing season, (4) Spraying the plants and weeds with malathion and cilecron every two weeks alternatively to kill the aphid vectors from the first April to end of growing season is December, (5) Eradication of woods and grasses from plantations (secondary virus hosts) by digging up and inherbicide. Dealing with this problem as a community.

Key words: Banana, nursery, orchard, banana bunchy top virus (BBTV), Brome mosaic virus (BMV) *in vitro, in vivo*, eradication, PCR, ELISA.

INTRODUCTION

Banana is one of the most important fruit in Egypt and cultivated in wide areas. Banana production increased to 512.5 thousand metric tons and the average crop was 11.27 to 13.71 feddan (Ministry of Agriculture, ARE, 1996) as a result of cultivation of new varieties high in production. Two viruses, Banana bunchy top virus (BBTV) and banana Cauliflower mosaic virus Bean common mosaic virus (BCMV) are considered able as one of the limiting factors in the production of banana crop. The virus causes serious losses in many countries (about 20 to 30% and occasionally reaching 50 to 80%). They are usually spread from plant to plant in nature by insect vectors, but often are also transmitted over long distances and from one crop cycle to another in vegetative planting material. The use of healthy planting materials and destruction of infected or diseased plants are essential for the control of viruses. The control strategy using pathogen-free stocks is to dilute the effects of disease through the supply of large quantities of healthy planting material. An important feature of this approach is the maintenance of pathogen-free foundation materials, which are protected from re-infection (Ang and

*Corresponding author. E-mail: magdyelshamy@yahoo.com.

Ong, 1998).

Four factors influenced the success of a rouging program for the control of BBTV. These are incubation period of the virus, relative infection rate, detection efficiency and eradication efficiency (Allen, 1978). The control measures of BBTV consist of: (a) Early disease recognition and prompt eradication of infected plants, (b) Control of its insect vector, Pentalonia nigronervasa, (c) use of virus-free planting materials and (d) Quarantine for areas that are free from disease (Nakahara, 2000).

This study aims to eradicate the banana viruses via detection of the virus from naturally infected banana plants and produced virus-free banana plants by applying two programs: Establishment of adeptic culture *in vitro* and continuation of banana plants growth and control the vectors and weeds in open field orchards and nursery.

MATERIALS AND METHODS

Source of plant materials

The banana seedlings and suckers (200 samples with 20 to 30 cm) were collected from mother plants *Musa* spp. cv. Williams, Cavendish subgroup cultivated in Meet El-Attar, Benha, Qualubia Governorate. The mother plants exhibited BBTV and Brome mosaic virus (BMV) distinct external symptoms. The selected plants were investigated depending on serological and molecular detection.

Virus detection

The viruses were detected in naturally infected and treated banana plants, in this study, by their external symptoms. This was also by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and polymerase chain reaction (PCR). The virus isolates (BBTV and BMV) were detected in banana plants by DAS-ELISA according to Clark and Adam (1977) using specific poly clonal IgG BBTV and BMV. Enzyme-Linked Immunosorbent Assays (ELISA) Kits were provided by Sanofi Sante Animal Paris, France. PCR and reverse transcriptase PCR (RT-PCR) techniques were used to detect BBTV and BMV nucleic acids in banana plants. Total DNA of infected banana leaves was extracted using a version of CTAB (Cetyl trimethyl ammonium bromide) according to Dellaporta et al. (1983). Total Ribonucleic acids (RNA) of infected banana leaves were extracted according to Gibbs and Mackenzie (1997).

PCR amplification of BBTV

Oligonucleotide primers (Table 1) for PCR were derived from the published sequences of BBTV- DNA N (Harding et al., 1993). The PCR reactions were performed according to condition and parameters described by Harding et al. (1993). The complementary DNA (cDNA) of BMV-RNA was done using the CMV/CP complementary primer (Table 1) of the conserved ultimate of CMV-RNA-3 and Avion Myeloblastosis virus reverse transcriptase (AMV-RS). The PCR reaction was performed according to conditions and cycling parameters described by Quenmada et al. (1991).

Preparation of chitosan

Chitosan with a degree of deacetylation 72% was obtained from Aldrish Chemical Company. Chitosan was dissolved in 0.05% (W/V) acetic acid and pH was adjusted to 5.5 with NaOH (Mahmoud et al., 2003).

Two programs of virus controlling in banana plants were carried out.

Establishment of an aseptic culture of banana

Micropropagtion of banana in vitro

The infected banana plants cv. Williams confirmed by PCR technique was used as source of meristems for tissue culture.

Meristems tip

The meristems were excised from shoot apices with rhizomatous base (about 2.5 cm^2 × 5 cm length). Individual meristem (the dome with 2 to 4 leaf primordial with rhizomatous base) were then excised with 0.3 mm under the binocular using fragments of a razor blade attached to a scalpel handles. The meristem tip was soaked in ethanol 76% for sec. before transferred to the culture medium. Individual meristem tip was cultured on MS starting medium. The cultured jars were incubated in growth room under incubation conditions at 3 weeks. The meristems were transferred on MS multiplication medium. Monthly subcultured of the plantlets to a fresh multiplication medium was carried on at subculture fourth. After that, the plantlets were transferred on MS rooting medium (Table 2).

Virus indexing

To be sure of virus free banana plantlets resulted by meristem tip culture confirmed using DAS-ELISA.

$$\text{Percentage of virus-free plantlets} = \frac{\text{No. of virus free plantlets}}{\text{Total No. of survived plantlets}} \times 100$$

Acclimatization

Healthy plantlets that showed negative results by DAS-ELISA were removed from the culture jars. The roots were rinsed with tap water and shortened to 3 cm. The roots of plantlets were immersed in penlate solution (1 gL^{-1}) and transferred into steam sterilized soil (peat: sand: vermiculite mixture by 2:1:1 ratio) in pots (12 cm Q) and covered with wet polyethylene for 10 days under greenhouse conditions. The air humidity exceeds 90% during the first days and decrease gradually.

Production of banana seedlings under nursery

Naturally infected banana plants cv. Williams exhibited typical bunchy top and stunting or mosaic symptoms (+ve results with DAS-ELISA) as well as healthy one was used to produce virus free banana seedlings. The corm of these plants was subjected to treatment with 0.5 to 1.0 ml of 0.12% chitosan. The treated seedlings were planted in clay soil at farm (30 × 20 m^{23}) in Meet El-Attar contains 200 lots and designed to produce virus-free banana seedlings. The distance between lots was 1 m^2. The seedlings were fixed to a 25 cm depth in the lots at the first march.

The seedling (healthy and infected) were treated with chitosan by two ways. a- Injected by syringe in the corms and with paraffin wax

Table 1. Oligonucleotide primers for BBTV and CMV.

Virus	Nucleotide sequence
BBTV Reverse	5`GCTAGGTATCCGAAGAATC-3`
Forward	5`-TCAAACATGATATGTAATTC-3`
CMV Reverse	5`-CCCCGGATCCTGGTCTCCTT-3
Forward	5`-CCCCGGATCCACATCAYAGTTTTRAGRTTCAATTC-3

Table 2. Chemical constituents of MS media for different growth stages of banana production *in vitro*.

Constituents	Medium of growth stages		
	Starting	Multiplication	Rooting
* Stock salts gL^{-1}	4.5	4.5	4.5
Sucrose gL^{-1}	30	30	30
6-benzyl amino purine mgL^{-1}	3	5	-
Nphthalene acetic acid mgL^{-1}	-	-	2
pH	5.8	5.8	5.8
Phytagel gL^{-1}	2.2	2.2	-
Agar gL^{-1}	-	-	7
Muo-inositol gL^{-1}	0.1	0.1	0.1
Culture's containers	250 ml	500 ml	500 ml
Size of media/container	30 ml^3	40 ml^3	40 ml^3

* Stock salts

days for 2 months). After 6 months data were recorded on survival before planting. B- Sprayed with chitosen periodically (each 15 percentage, average of shoot length or pant; number and leaf area, diameter of pseudostem, number and thickness of roots. Leaves tissues were obtained from the plants and tested by DAS-ELISA for the presence of BBTV and BMV.

Eradication of banana virus in orchards

The second content program was done in banana groves (about-five feddan including 200 lots) in banana groves, Meet Attar Benha, Qualubia Governorate. Mother banana plants were grown under natural conditions. The control program was based on:

Detecting infected plants periodically every month by fortnightly inspection via external symptoms and DAS-ELISA test for the presence viral diseases. BBTV and BMV rouging the infected plants after two inspections. The rogued plants were destroyed by burning at the end of growing season. The plants and weeds were sprayed with malathion cilecron with 1.5% alternatively to the end of growing season in December. Eradication of woods and grasses from plantations (secondary virus hosts) by digging up and insecticide. The percentage of virus infection was determined four times by DAS-ELISA through this a program.

RESULTS

Virus detection

It is easy to detect the viral infection on banana plants in the nursery and orchards because the external symptoms are clear and distinctive.

Symptoms of BBTV

The symptoms of infected banana with BBTV were dark green streaks on the midrib, reduced size, brittle of the leaves and gather at the top of plant making a resetting shape. Some leaves, veins are dark green colored and form a "hook" shape, as the midrib is approached (Figures 1a and b).

Symptoms of CMV

The symptoms of infected banana with Cauliflower mosaic virus (CMV) are characterized by a conspicuous molting and mosaic of the leaves, green streaks on midrib of leaves and are wavy (Figure 1c). Common observation of infected pants is stunted growth. In severe cases this is accompanied by rotting of the heart and central cylinder.

Using DAS-ELISA, indicate the presence and the percentage of BBTV, BMV and mixing of them naturally infected banana cv. Williams was 73.6; 46.6 and 20%, respectively. It also noticed that banana plants infected with BBTV or and BMV gave less number of suckers compared with corresponding healthy ones. As well as,

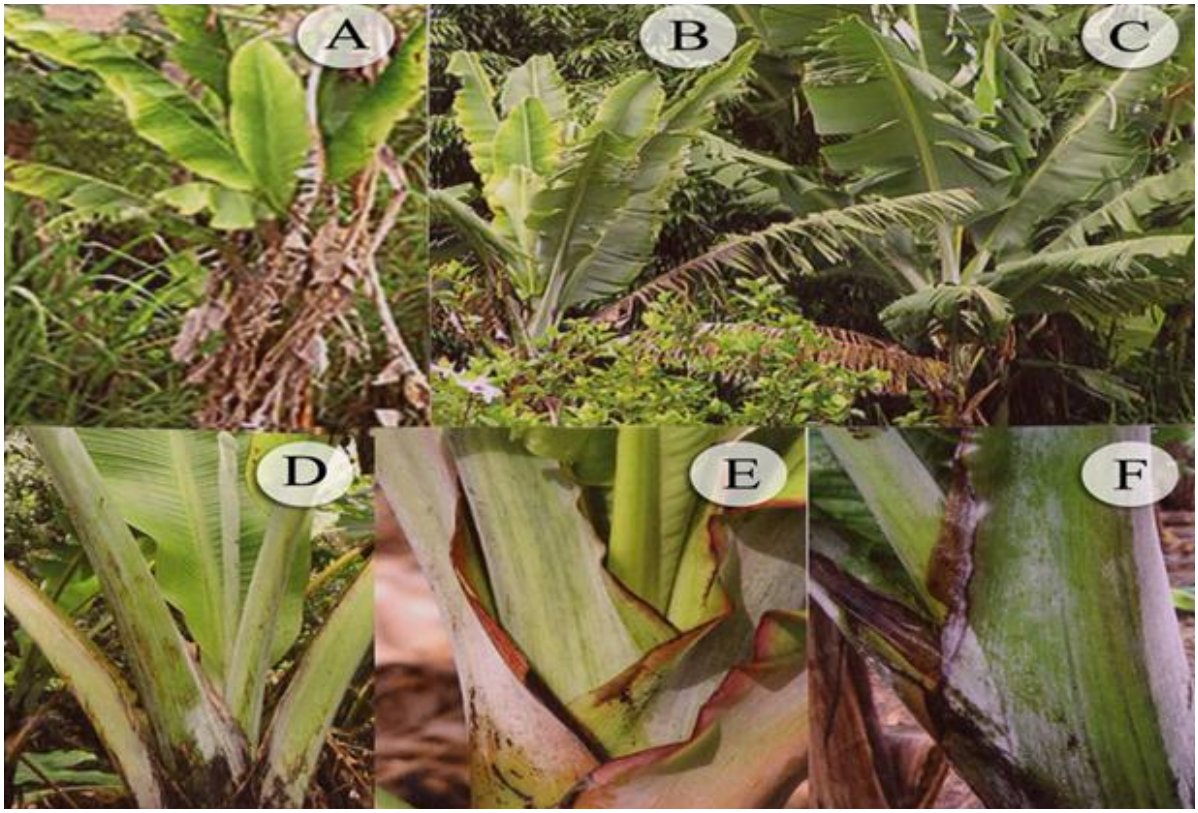

Figure 1. Naturally infected banana plants exhibited different viral symptoms. Leaves are bunched up, narrow, stiff, upright and with yellow and irregular or wavy leaf margin (A, B and E). Petioles & leaf sheaths are mottled, streaked (A, D, and F). Healthy plant (C).

the percentage of healthy suckers 26.5% resulted from infected banana plants.

Molecular detection

The total DNA of BBTV infected banana leaves was determined spectrophotometrically as 240 µg/0.02 g of tissues. The total RNA of infected banana leaves was measured spectrophotometrically as 150 ng/0.02 g of tissues as well as the purity of total DNA and Ribonucleic acid (RNA) as indicated by A_{260}/A_{280} ratio was 1.72 and 1.52, respectively.

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 naturally infected leaves (Figure 2A) as amplicons of expected size, 500 bp were seen in only the infected tissues (Lane 1 and 2). No amplification was obtained with uninfected banana leaves samples (Lane 3).

The CMV-RNA was reverse transcriptased by Moloney Murine Leukemia Virus (MMLV) using the oligo-dt (5`-CCCCGGATCCTGGTCTCCTT-3`) as minus sense primers.

The resulting complementary DNA (cDNA) was amplified by PCR using primers (CM1 and CM2) for coat protein gene. The PCR product was investigated using agarose gel electrophoresis analysis (Figure 2b). The size of the major amplified product in all samples was600 bp (Lane 3 and 4). This product was not detected in uninfected leaves (Lane 3)

Establishment of virus-free banana plants

Production of virus-free banana seedlings in vitro

This experiment aimed to study the meristem tip size related to virus elimination from BBTV and BMV infected banana plants.

Meristem tip sizes of 0.3, 0.5 and 1.0 mm was excised from diseased banana plants BBTV or BMV under steromicroscope. They were cultured on starting MS medium and incubated under convenient conditions. After 6 weeks post-cultivation the meristems were developed to the shoot (Figure 3) and tested against BBTV and CMV virus-using with DAS-ELISA. The

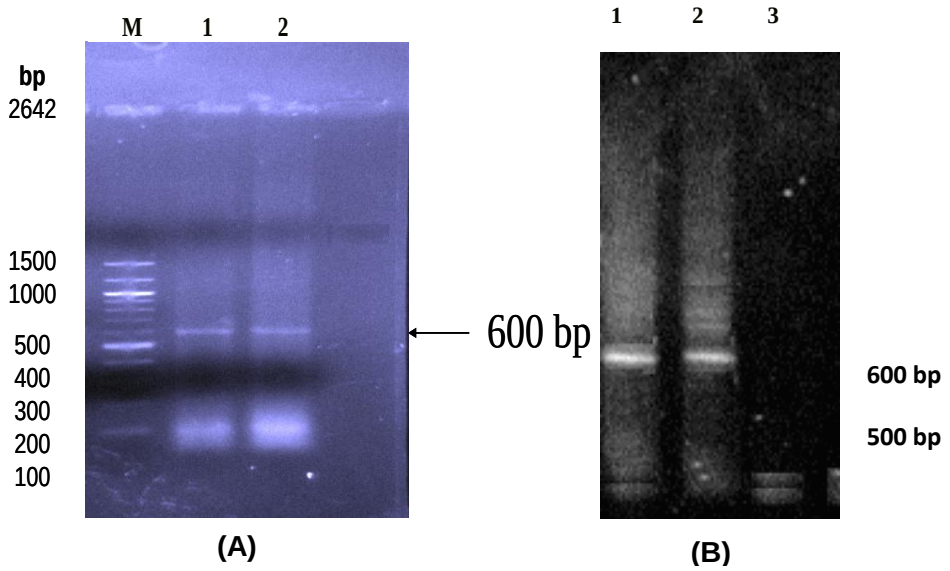

Figure 2. Agrose gel (0.7%) showing PCR products (CP gene amplified) of BBTV and BMV. (A)BBTV-PCR products Lanes 1,2 infected banana plants and Lane 3 healthy ones, (B) BMV-PCR products, Lanes 1,2 infected banana plants and Lane 3 healthy ones. M: DNA Molecular weight marker (XVI, Roche). The arrow indicates the correct size of amplified PCR products.

Figure 3. Different stages of healthy banana production *in vitro* from infected plants using (0.3 min) meristem tip culture.

Table 3. Production of virus-free banana seedlings using meristem tip culture *in vitro*.

Size of Meristem (mm)	Parameter						
	BBTV				BMV		
	Survival (%)	Virus free No.	Virus infected No.	Virus free (%)	Virus free No.	Virus infected No.	Virus free (%)
0.3 mm	75	65	10	86.66	70	5	93.33
0.5 mm	85	45	40	52.94	50	35	58.82
1.0 mm	100	10	90	10	12	88	12.00

smallest size (0.3 mm) gave 75% survival with 86.66 and 93.33% virus free (BBTV and BMV, respectively) plants. But using the size of 0.5 mm, gave 85% survival with 52.94 and 58.82 virus free plants (BBTV and BMV respectively). While using the size 1.0 mm gave 100% survival with 10 and 12% of virus free plants (BBTV and BMV, respectively; Table 3).

The explants (subculture 1) virus tested were transplanted on multiplication medium and incubated under convenient conditions. The explants (sub culture 2) were generated at subculture 6 on multiplication medium. The explants (subculture 6) were transplanted on rooting medium and incubated under convenient conditions at about 3 to 4 weeks until formation of roots.

Acclimatization

The plantlets were adapted into steam-sterilized soil in pots and grown under greenhouse conditions (Figure 3).

Production of virus-free banana seedling in nursery

All banana suckers (200 samples) were tested against BBTV and BMV in nursery through two seasons via external symptoms and confirmed with DAS-ELISA test. The results revealed that, BBTV and BMV were detected in about 75 and 45% (about 200 plants), respectively. The suckers treated by injection and spraying with 0.12% chitosan solution did not have any external viral symptoms. While, the percentage of BBTV infected plants in 1st and 2nd seasons were 9 and 5% as well as BMV infected plants were 5 and 2% respectively by using DAS-ELISA test. So, chitosan showed actively against viral infection and induction of the plants growth: Whereas, the chitosan treatment due to increasing in survival of suckers with 14.5 and 10% compared with BBTV and BMV infected plants respectively. As investigation results of suckers excised from infected banana mother plants, it was found that the BBTV or BMV infection due to reduction in suckers growth whereas reduction in morphological characters (Table 4) compared with suckers excised from healthy mother plants. Data in Table 4 show the effect of chitosan an morphological characters of infected banana plants after 6 months post-chitosan treatment *in vivo*, data revealed that, the increasing of shoot length/plant; no. of leaves/plant, leaf area; diameter of pseudostem; corm diameter; No. of roots and root diameter of infected banana suckers with BBTV or BMV compared with pre-chitosan treatment (Table 4). As well as, increasing in chlorophyll a and b and carotenoids contents of infected banana plants treated with 0.12% chitosan, than un-treated banana ones.

Continuation of growth of banana plants and virus control in orchards

The application of the procedures as described in materials and methods very effective in controlling banana viruses and producing virus free suckers. The viruses were detected via external symptoms and confirmed by DAS-ELISA test. The data in Table 5 showed that, the previous procedures due to reduction of BBTV and BMV infected banana plants were 73.66, 29.2 and 15.5% in the first year to 4.25 and 1.75% in the second year, respectively.

The present investigation also clearly indicated that, BBTV or BMV naturally infected banana mother plants gave lowest number of suckers compared with corresponding healthy plants. It also be noticed the lowest number of healthy suckers (1 and 3 suckers, respectively) compared with 6 sucker per healthy plant based on DAS-ELISA test. The percentage of virus in infection in cv. Williams was decreased season after season. It was 73.3 and 46.6% in first season(start experiment), 10.75 and 2.5% second season and 3.50 and zero% in third season for BBTV and BMV, respectively. In addition, the number of healthy suckers per plant was increased 1, 4, 5 (BBTV); 2, 5, 6 (BMV) and 3.5.6 (BBTV + BMV) at

Table 4. Effect of chitosan solution in growth of banana plants infected with BBTV and BMV*.

Morphological parameters	Treatments				
		BBTV		BMV	
	Healthy	Without chitosan	Post chitosan treatment	Without chitosan	Post chitosan treatment
Survival (%)	100	85.5	100	90	100
Shoot length/plant (cm)	75	34	65	52	70
No. of leaves/plant	5	7	8	5	6
Leaf area (cm)	450	200	350	300	400
Pseudostem diameter (cm)	20	9	15	12	17
No. of roots/plant	10	8	9	8	10
Thickness of roots	0.7	0.3	0.5	0.4	0.6
Corm diameter (cm)	22	15	18	17	19
Chlorophyll a	3.75	1.50	3.15	1.25	3.25
Chlorophyll b	2.25	0.91	2.10	0.75	1.95
Carotenoids	3.25	1.85	2.95	1.65	2.50

* First season.

Table 5. Percentage of BBTV and BMV infection in banana plants and their suckers growing under environmental conditions in orchards.

Growing seasons	Parameters				
	Percentage of infection				
	Mother plants		Suckers per plant		
	BBTV	BMV	BBTV	BMV	BBTV + BMV
1st season	73.66*	46.6	5/6**	4/6** 1/6 0/6	3/6**
2nd season	10.75*	2.5	2/6		1/6
3rd season	3.50*	0.0	1/6		0/6

* Average of sucker plant^{-1} calculated from 100 mother plants. ** No. of infected suckers/No. of total sucker plat, calculated from 100 plants based on DAS-ELISA test.

1st, 2nd season respectively, (Table 5).

On the other hand, the suckers associated of mother plants not appeared viral like symptoms as those of healthy ones in the field. The rate of infection was higher in the winter season than in the summer season. Young plants that were virus infected early after planting showed severe symptoms and never grow more than one meter at the end of growth season. Old plants that were virus infected showed no significant change in the growth. It was also observed that the suckers in the same lot may exhibit infection. But if one of the suckers is in one lot, the lot became infected after 3 month of planting. The mother plant rarely exhibited any symptoms by the end of growth

season.

The eradication of viruses in banana groves in this experiment was based on: 1) periodically detection of BBTV and BMV via external symptoms and yearly by random method using ELISA test. 2) Rouging and destruction of the infected plants outside in the groves and +ve ELISA tested. The rouged plants destroyed by burning at the end of growing season. 3) At the same time control of aphid vectors by spraying with (0.2%) an effective insecticide. 4) Eradication of weeds and grasses by using glyphosate.

DISCUSSION

BBTV and BMV are of the most widespread banana viruses in different countries of the world (Smith et al., 1990) which are concerned with banana cultivation in Egypt (Allam et al., 1988). In Egypt, the most threatening viral diseases are those caused by BBTV and BMV, these viruses are considered as limiting factors in banana production (Allam et al., 1988; El-Dougdoug et al., 2002). Banana plantations are propagated asexually by suckers since almost all of their cultivars are seedless or seed sterile. Banana diseases subjected to many natural caliseases constitute a major problem, virus diseases are serious as insect vectors are abundant and there are many alternate hosts.

We noticed that, the first symptoms of banana infected with BBTV were dark green streaks on the lower portions of the midrib of the leaf; the fresh infected leaves were brittle, reduced in the size and gather at the top of plant making a resetting shape. These symptoms reported also by Allam et al. (1988), El-Sayed (1994), Othman et al. 1996) and El-Dougdoug et al. (2006). The symptoms of BMV are characterized by a conspicuous interveinal chlorosis of the leaves. Common observation of infected (plants stunted growth. In severe cases this is accompanied by rotting of the heart leaf and central cylinder as stated by Nurhadi and Setyobudi (1998), Allam et al. (2000) and El-Dougdoug et al. (2006).

All banana plants used for starting this work were found to be infected with either BBTV or BMV and were detected in leaf samples by different methods as the biological, serological and molecular. DAS-ELISA was used to detect BBTV and BMV because of their sensitivity, specificity and speed (Clark and Adam, 1977; El-Dougdoug et al., 2002, 2006). We obtained the same conclusion.

A polymerase chain reaction (PCR) assay was developed for detection of BBTV and BMV of banana plants as well as single aphid (Xie and Hu, 1995). They added that, dot blot hybridization assay were as sensitive as ELISA, while PCR was 1.000 times more sensitive than dot blot immunoassay and ELISA. Furthermore El-Sayed (1994) found that PCR and dot blot hybridization were more sensitive than other traditional methods for the detection of BBTV and BMV.

The smallest size of meristem tip 0.3 mm more effective for elimination of banana viruses than 0.5 and 1.0 cm meristem size, whereas gave largest number of virus-free plants.

The active growing points of the plant shoot are meristem and nodel cuttings. They contain the truly meristemic cells, which surrounded by leaf primordial and primary leaves. Since more differentiated vascular tissues are found in meristem from a distance. Vascular elements of the leaf primordial are still incipient, and have not yet made contact with main strand system in the stem. Therefore, virus particles can reach the meristematic region of the apex only through cell to cell movement slowly. For this reason, virus concentration decreases in both apical axillary buds of infected plants (Perez et al., 1999).

Recent investigations of tissue culture methods proved that the number of virus free plants produced is inversely proportional to the size of the explants cultured. Thus, in some instances it is possible to excise a meristem tip free of the virus present in the infected parent and regenerate them into a healthy plant (Zilkah et al., 1992). Moreover, they indicated the importance of explants size in the successful elimination of some viruses and the role of certain host virus combination in determining the success of virus elimination (George, 1993). Virus eradication procedure depends partly on the nature of viruses; some of these viruses are more readily eliminated than others. The mechanism of such *in vitro* virus inactivation remains unknown, but whatever the explantation it seems probable that this type of virus eradication is more likely to occur if low, rather than high concentrations of virus particles are present in the tip (Walkey, 1991).

Four factors influence the success of a rouging program for the control of BBTV. These are incubation period of the virus, relative infection rates, detection efficiency and eradication efficiency (Allen, 1978). The virus activity might be prevented by alternation of the charge on the virus particles caused by polybasic substances. Such effects could be produced by chitosan (Mahmoud et al., 2003). Usually chitosan is obtained from decaylation of chitin crusts in crustacea. The chitosan possess a wide biological activity including induction of many plant defense responses such as accumulation of chitinases, production of phytoalexin (Walter-Simmons et al., 1983); synthesis of proteinase inhibitors (Walter-Simmons et al., 1989), lignification (Barber et al., 1984) and callose synthesis (Lienart et al., 1993). Chitosan was found to inhibit plant viral infections such as *alfalfa mosaic virus, tobacco mosaic virus; potato virus X, peaut stunt virus, tobacco necrosis virus; cucumber mosaic virus* and BBTV and BMV (Posppieszny et al., 1991 and Mahmoud et al., 2003). In addition, it was exhibited antiviroid action (Posppieszny, 1997). Mahmoud et al. (2003) postulated that, chitosan may be substitute for the virus particles when attached to

cell receptors. Compared to suckers, the use of plantlets grown by tissue culture has many advantages. Tissue culture plantlets are cheaper and easier to propagate and transport. They have a higher survival rate in the field. They reduce the cost of controlling foliar diseases by 50%. Their uniformity of growth makes it possible to control the time of flowering and harvesting and give a significant increase in yield and fruit quality (Hwang et al., 1984).

Tissue culture is now standard practice in banana propagation to ensure that the nursery stock is clean and free from latent infection of BBTV and BMV (Magnaye and Valnayor, 1995). For banana which is propagated vegetative, production of virus-free starting material (seedling and rhizomes) is very important to reduce yield loss due to over viral infection in field. Espino et al. (1998) reported that a control measure of BBTV consists of a) early disease recognition and prompt eradication of infected plants, b) Control of insect vector *Pentalonia nigonervosa*, c) use of virus-free planting materials and d) quarantine for areas that are free from the disease.

Quarantine laws should be revised and enforced on the import of new suckers and on the movement of infected suckers within the country. With the use of tissue culture techniques disease free planting material (suckers) can be produced and used for new plantation (Leghari, 2002). To control banana viruses removing the infected plants, control woods and aphid vectors during the growing period, use virus-free planting materials and continuous monitoring and inspection of banana plants (Calo, 2005).

It was noticed that for established plantings, effective control of the diseases requires early detection and immediate eradication of infected plants followed by replanting with disease free planting materials. As most banana virus produces character symptoms on the eaves, eradication was commonly done under symptomatology basis as stated by Nurhadi and Steyobudi (1998).

The control program can be summarized as follows: Detecting infected mother plants periodically every month by fortnightly inspection via external symptoms and ELISA test for the presence of viral diseases. Rouging the infected plants after two inspections the rouge plants were destroyed by burning at the end of growing season. Spraying the plants and weeds with malathion to kill the aphid vectors every two weeks from first of April to the end of growing season in December), eradication of weeds and grasses from plantations (secondary virus hosts) by insecticide. Quarantie regulation must be implemented. This control program is similar to that done by Allam et al. (1988).

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