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Viral Elimination Strategies for *Musa* spp.

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Abstract

Banana is one of the major crops in tropical parts of the world. The banana plant is susceptible to many diseases due to the less genetic diversity in its popular cultivars. Particularly viral diseases can severely destruct quantity and quality of the crop. Four major banana infecting viruses doing the damage- banana bunchy top virus, banana streak virus, banana bract mosaic virus, cucumber mosaic virus; two other viruses- banana mild mosaic virus and banana virus X causing mild infections. Treatments are not available to remove viral infections from field crop. Banana-infecting viruses readily transmit through insect vectors and via vegetative planting materials, causing diseases in germplasm storage, their exchanges and in cultivation fields. Present account gathers information on recent viral disease outbreak reports in Musa species, about their newer isolates, vector, alternate host, etc. It further reviews viral elimination approaches used to produce virus-free planting material in banana like- in vitro culture, thermotherapy, chemotherapy, and cryo-exposure, for their effectiveness, mode of action and survival rate. In vitro viral eradication approaches those found effective on the other crops also discussed; like new antiviral drugs, electrotherapy, and combinations of various therapies that may steer to formulate future strategies to protect Musa species from viral diseases.

Keywords: *Banana bunchy top virus, Banana streak virus, Banana bract mosaic virus, Cucumber mosaic virus*

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INTRODUCTION

Musa species is one of the essential crops, particularly in tropical zones based on production and utilization [1]. Banana is a major staple food crop for a huge number of people and provides income through local and international trades. Banana is the fourth most cultivated fruit in more than 130 countries belonging to Asia, America, Africa, Oceania and the Pacific [2]. Conventional *Musa* cultivation takes place by using suckers as planting material taken from the mother plant. The most vital sucker borne diseases of *Musa* planting materials are nematodes, weevils, and infections, including viruses and bacterial shrivels. Viral diseases are the main threats for the banana crop. Since plants lack the immune system, as a result, virus infection lasts generally for the complete lifespan of their hosts [3]. Popular banana cultivars are sterile and propagated vegetatively. Therefore, acquisition of viral resistance via sexual recombination is difficult. Hence, proficient techniques are required to recover selected genotypes from the infected stocks in the absence of virus resistance lines in *Musa* for safer germplasm conservation and exchange.

MUSA INFECTING VIRUSES

The banana plant is susceptible to different viral infections. Four major viruses can cause severe infections; two of them are DNA viruses- banana bunchy top virus (BBTV) and banana streak virus (BSV) while the two other contain RNA genome- banana bract mosaic virus (BBrMV) and cucumber mosaic virus (CMV). Two other RNA viruses- banana mild mosaic virus (BanMMV) and banana virus X (BVX) can cause mild infections.

BBTV is the causal agent of banana bunchy top disease (BBTD) that may destruct banana crop up to 100%, and it is the main reason for limiting cultivation areas in the Asia Pacific regions and some extent to the African continent [4]. BBTV belongs to genus *Babuvirus* of family *Nanoviridae*. BBTV is an isometric virus, 18–20 nm in width, with

genome involving at least six circular, single-stranded DNA segments, each with a size of around 1 kb [5–8]. Complete genome sequencing of two BBTV isolates from Hainan (China) has revealed that one having satellite DNA and other without it [9]. Moreover, some defective viral DNA components (mostly derived from DNA-R: replication associated protein) also identified [10]. DNA sequencing of Sri Lankan BBTV isolate recognized as a new member of Pacific Indian Ocean group [11]. Nucleotide sequence identity 98 to 100% of BBTV isolates collected from all over the Democratic Republic of Congo revealed that a single viral introduction in the 1950s resulted in its spread throughout the country [12]. Recently, the first-ever incidence of the disease was confirmed in South Africa too [13], showing its spread in new regions. Aphid-vector *Pentalonia nigronervosa* readily transmits the virus as it accumulates at higher concentrations at the anterior midgut and possibly translocates using a haemolymph-independent path [14]. Recently, another aphid *P. caladii* was also identified as a competent BBTV vector [15]. In infected plants, leaves become shorter, fragile and narrower to give a typical appearance of the bunch. Diseased plant fails to produce fruits from pseudostem. Disease management includes the use of virus-free planting material, vector control, and destruction of infected plants.

BSV is the causal agent of banana streak disease or leaf streak in banana and plantain, occurs frequently in Asia, Africa, Europe, Middle East and Oceania [16]. BSV belongs to genus *Badnavirus* of family *Caulimoviridae*, average size is 30 x 130–150 nm and contains a circular double-stranded DNA about 7.2–8.8 kb [17]. In nature, the virus transmits by the vector mealy bugs [18]. Sometimes, temperature and water stress may cause BSV infection by activating its integrated sequences present within the host *Musa* genome [19–21]. This activation is observed only in *M. balbisiana* (B) and hybrid *M. acuminata* x *M. balbisiana* (A x B) genomes but not in A genome containing bananas. The disease appears as little yellow spots on the leaf that further recline to form streaks. The streaks become necrotic and give blackish appearance on the lamina. Necrotic streaks may appear on pseudostem, midrib, and petiole. Fruit malformation is the major symptom induced by BSV, resulting in significant reduction of production up to 90% [22].

Another virus- BBrMV belongs to the genus *Potyvirus* under the family *Potyviridae* [17] and causes banana bract mosaic disease. It is a filamentous virus (660–760 x 12 nm), the genome is a positive-sense single-stranded RNA and around 10 kb long [23, 24]. BBrMV transmits by many species of aphids and through vegetative planting material too. Diseased plants exhibit spindle-shaped pinkish to reddish streaks on pseudostem, midrib, peduncle, bracts, and fingers. Apart from banana, BBrMV could also infect cardamom plants, as an alternate host [25]. The disease occurs in India, Sri Lanka and Philippines with yield losses up to 40% [26]. Due to its limited distribution and ability to quick transmission, BBrMV is a matter of concern for plant quarantine. Recently, its occurrence was reported first time from the north-east region of India too [27].

CMV is the causal agent of banana infectious chlorosis disease. Its genome contains a positive sense single-stranded RNA [28] under the genus *Cucumovirus* belonging to family *Bromoviridae* [17]. CMV also transmits by many aphid vectors and can infect more than 800 plant species, including *Musa,* is the largest host range in comparison to any other virus [29]. Infected banana plants show typical mosaic symptoms, stunted growth with deformed leaves and fruits. It severely affects quantity and quality of banana fruits. In near future, it may be a serious threat for *Musa* cultivation in India [30]; for instance, it is now first time observed in the North-East India (Sikkim state) too [31].

The two other viruses- BanMMV and BVX are belonging to family *Flexiviridae*, containing a single-stranded positive RNA genome [32, 33]. Their impact is mild on banana crop, but mixed infection along with other viruses can cause severe leaf necrosis. These two viruses are also creating serious constraints for banana germplasm exchanges. In India, Selvarajan and Balasubramanian [34] reported the first occurrence of BanMMV in the banana crop.

ELIMINATION OF VIRUSES

Virus-infected planting materials are a major problem in cultivation, exchange, and storage of banana germplasms. Most of the gene banks of banana are severely suffering due to the viral diseases. Viral resistance can confer by the transgene in banana; genetic manipulation in banana, including the introduction of viral resistance, has reviewed several times, most recently by Dale et al. [35], Ghag and Ganapathi [36]. Therefore, viral elimination therapies have discussed here like meristem-tip culture, chemotherapy and cryotherapy to produce virus-free *Musa* planting material. Some of the efficient *in vitro* approaches used for viral eradication in other crops have also discussed at the end of this section.

In vitro culture

Apical meristem or shoot tip is the choice of explant for initiation of *in vitro* clonal propagation of selected banana genotypes. The meristem-tip culture technique generally considers as a tool for virus elimination. However, meristem culture technique was not able to remove CMV from banana plants [37]. In another report, few virus-free plantlets could regenerate via *in vitro* meristem culture technique from BSV infected banana plants [38]. Further, this technique was unable to recover even a single BBTV-free banana cultivar 'Lakatan' [39]. Several other workers also reported inefficiency of the tissue culture technique, eradicating viruses in banana [40, 41]. Therefore, other therapies in the combination of *in vitro* meristem-tip culture technique also screened to regenerate virus-free planting material. Furthermore, plant tissue culture process itself may induce excision of BSV integrated sequences to cause BSV infection [40, 42]. However, this viral activation occurs only in 'B' and hybrid 'A x B' genomes containing bananas but not in 'A' genomes [43, 44]. Therefore, BSV may become a major problem for plant tissue culture industries in near future [45]. *In vitro* culture of banana using 'male-inflorescence' as explant, was also reported as a viral elimination tool [46]. More than 80% of *in vitro* cultured shoots detected free from viruses; those were regenerated via male inflorescence as explants from BSV, CMV, BBTV or BBrMV infected banana plants. However, viral indexing of six-months-old acclimatized plants is required for further confirmation, which is a set standard practice for checking of virus-free banana plantlets [16].

Thermotherapy

Application of heat treatment effectively eliminates numerous viruses from varieties of plant groups. BBTV infected banana shoot cultures of cultivar 'Lakatan' were subject to incubate at high temperature up to 40°C *in vitro* for a period of 2 months [39]. Meristems of these heat-treated shoot cultures further served as explants and could regenerate 62.5% BBTV-free plantlets (Table 1). Similarly, CMV-infected banana cultivar Williams BSJ plants were subjected to heat treatment to eliminate the viral infection [47]. The infected plants were kept in a growth cabinet under an artificial light with 16 h light/8 h dark. During light hours, the temperature rose to 40°C for a period of 4 weeks and night temperature maintained at 25°C; then, meristems of these heat-treated plants cultured *in vitro* to regenerate 38% CMV-free plants. Thus, thermotherapy in combination with *in vitro* meristem culture could eradicate BBTV and CMV, to some extent.

Chemotherapy

Incorporation of antiviral chemicals is turning out to be an important tool for virus elimination. The antiviral agents belonging to inhibitors of viral replication enzymes (inosine monophosphate dehydrogenase [IMPDH] and S-adenosylhomocysteine hydrolase) or neuraminidase (NA) inhibitors that prevent spread of viruses from infected to healthy cells [48]. Mostly used ribavirin [49] is a synthetic nucleoside analogue of guanine that inhibits IMPDH. Chemotherapy is simple and can easily combine with *in vitro* culture of meristems by incorporation of antiviral chemicals into the culture medium. Antiviral substances, for example, noncyclic adenosine analogue- (RS)-9-(2, 3-dihydroxypropyl) adenine [(RS)-DHPA] or ribavirin was added into the culture medium for the elimination of CMV in banana cultivar Williams [47]. Ribavirin treated 29% cultures recorded free from the virus, while RS-DHPA could be able to remove virus in only in 2% cultures (Table 1).

Table 1: *Examples of Successful in vitro Viral Elimination Strategies in Different Crops.*

Plant	Virus	*In vitro* therapy	Viral Elimination %
Musa spp. [37]	Cucumber mosaic virus Banana streak virus	Cryotherapy	30 90
Musa spp. [38]	Banana streak virus	Chemotherapy (Tenofovir) Chemotherapy (Adefovir) Chemotherapy (PMEDAP)	90 88 69
Musa spp. [39]	Banana bunchy top virus	Thermotherapy	62.5
Musa spp. [47]	Cucumber mosaic virus	Thermotherapy Chemotherapy (Ribavirin) Chemotherapy (RS-DHPA)	38 29 2
Allium sativum [56]	Onion Yellow Dwarf Virus Leek Yellow Strip Virus Garlic Common Latent Virus	Cryotherapy + Thermotherapy	90 100 80
Begonia spp. [53]	Prunus necrotic ringspot virus	Chemotherapy (Ribavirin) + Thermotherapy	100
Malus spp. [52]	Apple chlorotic leaf spot virus, Apple stem grooving virus & Apple stem pitting virus	Chemotherapy (Ribavirin) + Thermotherapy	95
Rosa hybrida [58]	Arabis mosaic virus & Prunus necrotic ringspot virus	Chemotherapy (Ribavirin) + Thermotherapy	85
Solanum tuberosum [59]	Potato leafroll virus & Potato spindle tuber viroid	Electrotherapy	>40
S. tuberosum [57]	Potato Y potyvirus	Electrotherapy	~90
Vitis vinifera [55]	Grapevine fleck virus	Chemotherapy (Ribavirin + Oseltamivir)	100
V. vinifera [51]	Grapevine leafroll-associated virus 1 Grapevine rupestris stem pitting-associated virus	Thermotherapy	91.2 67.6
V. vinifera [54] Cultivars: 'Agiorgitiko' 'Malagouzia'	Grapevine rupestris stem pitting-associated virus	Chemotherapy (Mycophenolic acid)	 85 53

In another experiment, anti-viral molecules adefovir, tenofovir or 9-(2-phosphonomethoxyethyl)-2,6-diaminopurine (PMEDAP) containing culture medium was used to target viral reverse transcriptase for a duration of six months [38]. Further, these treated ones were cultured to regenerate plantlets. Six-months-old plantlets in the greenhouse were exhibited 69%, 88% and 90% BSV eradication after PMEDAP, adefovir or tenofovir treatments, respectively. Thus, chemotherapy of the infected *in vitro* cultures found quite efficient for the elimination BSV. Chemotherapy may prove effective for the other viruses too in future viral disease eradication programs.

Cryotherapy

Cryopreservation mainly refers to the storage of biological samples at an ultra-low temperature (-196°C) in liquid nitrogen. Cryopreservation technique may also utilize as a pathogen eradication tool. Meristem clumps of *in vitro* cultures regenerated from the viral-infected banana plants were exposed to the ultra-low temperature in liquid nitrogen [37]. After the therapy, 30% and 90% *in vitro* raised plantlets were free from CMV and BSV, respectively (Table 1). However, just half of the cultures could survive after this therapy. In this experiment, light microscopy of cryo treated cultures revealed that during cryoexposure, only fewer vacuolated meristematic regions could survive, and rest of the more hydrated tissues died. Further, electron microscopy of cryoexposed banana meristem revealed that most of the meristematic cells injured during pretreatment, freezing and thawing steps [50]. Therefore, few cells at the periphery of the meristematic dome were able to survive to increase chances for the development of plants from virus-free cells. In addition, cryotherapy considered as a highly technical method.

Strategies in Other Crops

The blending of *in vitro* technique with other therapies successfully deployed to eliminate viruses from varieties of plants (Table 1), particularly in combination with

thermotherapy, antiviral drugs, and electrotherapy that may prove effective for the removal of banana viruses too. For example, tissue culture with a combination of *in vitro* thermotherapy (40°C during the day and 37°C at night for a period of one week) effectively eradicated 91.2% grapevine leafroll-associated virus 1 and 67.6% grapevine rupestris stem pitting-associated virus (GRSPaV) from *Vitis vinifera* cultivar [51]. Combined *in vitro* exposure of ribavirin (25 µg/ml) and thermotherapy at 36°C for 20 days to virus-infected (apple chlorotic leaf spot virus, apple stem grooving virus and apple stem pitting virus) apple shoot cultures was resulted in high viral elimination rates up to 95% [52]. Similarly, combined *in vitro* chemotherapy (ribavirin 20 mg/l) and thermotherapy (38°C for 16 h light period and 22°C for 8 h dark period) for 25 days could eradicate prunus necrotic ringspot virus with almost 100% survival in *Begonia* spp. [53]. Mycophenolic acid found most effective as well as the mildest phytotoxic antiviral IMPDH inhibitor than tiazofurin and ribavirin for eliminating GRSPaV from grapevine-infected cultures [54]. Combined *in vitro* treatment of IMPDH inhibitor (ribavirin) and NA inhibitor (oseltamivir) to *V. vinifrea* cultures resulted in 100% elimination of grapevine fleck virus [55]. Synergistic treatments of shoot-tip culture, cryotherapy and thermotherapy were given to multiple virus-infected garlic resulted in 40% survival and regeneration of more than 80 % virus-free plants [56]. Exposure to electric current may also eradicate viruses from plants probably by increasing temp inside the cells, for example, explants from potato Y potyvirus-infected potato plants were exposed to an electric current of 15 mA for 10 min followed by meristem-tip (100 µ) culture could be able to regenerate about 90% virus-free plants [57].

CONCLUSIONS

Viruses can severely damage the quality-quantity of the banana fruit, and they are continually infecting *Musa* crops in newer areas too. Simultaneously, their novel vector and alternate host have discovered. Genome sequencing reveals novel isolates of these viruses. Therefore, quest is the production of virus-free saplings for cultivation and germplasm storage/exchanges. Consequently, development of viral resistant lines of sterile banana cultivars via genetic manipulations may be the perfect solution. On the other hand, acceptance of genetically modified food is still unanswered. Meanwhile, elimination of viruses is the option to recover healthy banana plants from the infected stocks. Plant tissue culture is the widely used technique for germplasm conservation and its exchanges, as well as to produce *Musa* quality-planting material; particularly meristem-tip culture is also known for the production of disease-free plants. However, banana viruses may multiply through this technique too. Use of new antiviral drugs, thermotherapy, cryoexposur, electrotherapy and their novel combinations with meristem-tip culture may prove much effective in upcoming viral elimination programs for *Musa* spp. Thus, the information compiled here provides an insight for making future strategies to protect this important crop from the viruses.

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