

Disease Resistance

REVIEW ARTICLE

Banana bunchy top disease (BBTD) symptom expression in banana and strategies for transgenic resistance: A review

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Abstract

Bananas are one among the world's leading food crops after rice, wheat and maize. Banana cultivation is affected by various diseases. Among them, in globally banana bunchy top disease (BBTD) caused by the banana bunchy top virus (BBTV) and Fusarium wilt caused by the *Fusarium oxysporum* f.sp. cubense are the most serious diseases. BBTV is an isometric virus with a circular single stranded DNA (ssDNA) genome consisting of at least six components, BBTV DNA-1 to 6. The virus is transmitted by the banana aphid (*Pentalonia nigronervosa*). In this review paper, we are discussing the global status of BBTD, symptom expression in both vegetative and reproductive stage, resistant strategy and recent BBTV resistant banana clones development.

Key words: Banana bunchy top virus (BBTV), Banana bunchy top disease (BBTD), Single stranded DNA (ssDNA), Pathogen-derived resistance (PDR), Post transcriptional gene silencing (PTGS)

1. Introduction

1.1 Banana bunchy top disease

Banana is the most popular commercial fruit crop grown all over the world and also serves as a staple food in many countries (FAO, 2012). Banana production and export worldwide is affected by many viral diseases such as banana bunchy top disease (BBTD) which caused by banana bunchy top virus (BBTV) (Dale, 1987). BBTV is one of the most serious diseases of banana in Asia, Australia and the South Pacific. It is a nanovirus, single-stranded DNA virus with isometric virions 18–20 nm in diameter (Harding et al., 1991; Vetten et al., 2005). BBTV infects most banana cultivars, retards the growth of infected plants, and causes economic losses to banana production. BBTV is transmitted

by vegetative propagation and the aphid vector, *Pentalonia nigronervosa* (Hu et al., 1996). Due to the high destructive potential of the disease, the Invasive Species Specialist Group (ISSG) of the International Union for the Conservation of Nature (IUCN) listed BBTV as one of the World's 100 Worst Invasive Alien Species (Lowe et al., 2000). The objectives of this review is to study the BBTV geographic distribution, symptoms expression from juvenile stage to reproduction stage of banana, transmission, host range, genome organization and other nanoviral diseases.

1.2 History and geographic distribution

BBTD has been reported in many banana growing regions throughout the world (Table 1). BBTD was first reported in Fiji in 1889 and caused heavy destruction that threatened Fiji's banana export industry (Magee, 1927; Simmonds, 1934; Taylor, 1969). Subsequently, banana industry in Australia was affected by BBTD due to the importation of infected banana suckers from Fiji (Magee, 1927). The BBTD disease spread to southeast Queensland and resulted severe devastation to the Australian banana industry (Magee, 1927; Eastwood, 1946).

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BBTD was introduced in Sri Lanka in 1913 and later into Southern India in 1940's, where the virus spread to various banana growing areas and covered the entire country during 1970's (as been reviewed by Wardlaw et al., 1972). In Tamil Nadu India, the hill bananas [Virupakshi AAB- (G.I 124) and Sirumalai AAB-(G.I 126] were grown in multitier system (Figure 1) was highly susceptible to BBTV (Figure 2). The virus has been the main cause for drastic reduction in hill banana cultivation from 18,000 ha in 1970's to 2,000 ha at present and showed the incidence of 14-74% percentage (Kesavamoorthy, 1980; Selvarajan et al., 2010; Elayabalan et al., 2013). Vishnoi et al. (2009) reported that BBTD incidence occurred between 20-30% between 2006 to 2007 in India northern zones of Lucknow, Barabanki, Bahraich, Kanpur, and Etawah districts of Uttar Pradesh. Recently, there was a report on the newly isolate BBTV complete genome which was identified in Meghalaya, India and Pacific India Ocean group (Banerjee et al., 2014).

BBTD in Central and South Africa emerged as major constraint in banana production (New Ag, 2009). The first record of BBTD in continental Africa was reported in Egypt in year 1901 (as been reviewed by Fahmy, 1927). BBTD first discovered in sub-Saharan Africa in 1950s from Kisangani region of Democratic Republic of Congo (DRC) (Wardlaw, 1961). Between 1960 and 1980, the disease was observed in the neighbouring countries of Republic of Congo, Gabon, Burundi, Central Africa Republic, Equatorial Guinea and Rwanda (Manser, 1982; Jeger et al., 1995; Thomas and Iskra-Caruana, 2000). In the 1990s, BBTD epidemics were reported in Malawi (Kenyon et al., 1997). A decade later, BBTD was reported in Angola (Kumar et al., 2009) and most recently in Cameroon (Oben et al., 2009). However, BBTD incidence was absent in Latin America and Caribbean (Jones, 1993).

1.3 Symptoms

Typical symptoms of BBTD include the appearance of dark green broken streaks on leaf veins, midribs, petioles and pseudostem with group of clustered leaves on the top of plant looks bunchy appearance (Figures 3 and 4). Disease progression results in leaves at the apex of the plant becoming short and narrow with chlorotic, brittle leaf margins that tend to curl upwards. Banana plants infected in the late development produced small fruits,

distorted and tip of male bud are bird mouth shape like appearance (Elayabalan, 2010) (Figure 5). Although BBTD symptoms are usually very distinctive across all *Musa* spp., in some cases symptomless BBTD have been reported in Taiwan (Diekmann and Putter, 1996). In India, hill banana cultivation at higher elevation more than 7500 msl feet, symptomless BBTD in hill banana were detected (Elayabalan, unpublished data). Attenuated symptoms in banana have been reported in Fiji followed by initial severe outbreak (Diekmann and Putter, 1996). However, Magnaye and Valmayor (1995) reported that no natural resistance has been found in any banana cultivars.

1.4 Transmission

BBTD is not mechanically transmissible (Magee, 1967), but it can be transmitted locally by the banana aphid (*Pentalonia nigronervosa*) (Figure 6) or over long distances by vegetative propagation (Magee, 1927). Transmission by the aphid is in persistent, non-replicative manner, with an acquisition feeding period of at least four hours and inoculation feeding of period of at least fifteen minutes (Hu et al., 1996). The efficiency of transmission ranges between 46 and 67% (Hu et al., 1996), with nymphs than adults in transmitting the virus (Magee, 1967). Retention of infectivity in the aphid has been reported up to 20-23 days after removal from the virus source. BBTD symptoms are evident approximately in 25 days following the transmission, although this varies depending on temperature and age of plants (Allen, 1978). BBTV transmission studies through aphids under greenhouse conditions. Bioassay to screen the transgenic banana and symptom expression observation made on transgenic and non-transgenic bananas (Borth et al., 2011; Elayabalan et al., 2013).

1.5 Host range

Alternative hosts for BBTV have been investigated since the aphid vector colonies numerous plant families including Araceae, Commelinaceae, Musaceae and Zingiberaceae (Blackman and Eastop, 1984). Other host such as *Canna indica* (Canna; Cannaceae) and *Hedychium coronarium* (white ginger or garland flower; Zingiberaceae) were reported to be the host for BBTV in Taiwan (Geering and Thomas, 1997; Yasmin et al., 2001).

Table 1. BBTD in global reports.

| Continents | Countries | Reported Year | References |
|-----------------|--------------------------|--|-----------------------------|
| Africa | Burundi | 1960-80 | Sebasigari and Stover, 1988 |
| | Congo | 1958 | Wardlaw et al., 1961 |
| | Central African Republic | 1960-80 | Diekmann and Putter, 1996 |
| | Egypt | 1901 | Magee, 1927 |
| | Gabon | 1960 | Manser, 1982 |
| | Rwanda | 1960-80 | Sebasigari and Stover, 1988 |
| | Malawi | 1990 | Kenyon et al. 1997 |
| | Zaire | 1960 | Manser, 1982 |
| | Angola | 2009 | Kumar et al.,2009 |
| | Cameroon | 2009 | Oben et al.,2009 |
| Asia | Bangladesh | 1980 | Vakili, 1969 |
| | China | 1979 | Vakili, 1969 ; Dale, 1987 |
| | India | 1940 | Wardlaw 1972, Jones 1992 |
| | Indonesia | 1970 | Vakili, 1969 |
| | Japan | 1993 | Kawano and Su, 1993 |
| | Malaysia | 1969 | Jones, 1994 |
| | Myanmar | 1969 | Vakili, 1969 |
| | Pakistan | 1989 | Panhwar 1991 |
| | | | Khalid et al.,1993 |
| | Philippines | 1924 | Dale, 1987 |
| | Sri Lanka | 1913 | Bryce, 1921 |
| | Taiwan | 1961 | Dale, 1987 |
| | Thailand | 1993 | Kawano and Su, 1993 |
| | | Jones, 1993 | |
| | Vietnam | 1969 | Vakili, 1969 |
| Pacific regions | Australia | 1913 | Magee, 1927; Eastwood, 1946 |
| | Fiji | 1889 | Magee, 1927; Dale, 1987 |
| | Hawaii | 1989 | Diekmann et al. 1996 |
| | Guam | 1950 | |
| | Samoa | 1950 | |
| | American Samoa | 1960 | |
| | Tonga | 1960 | |
| | Tuvalu | 1960 | |
| | Kiribati | 1960 | |
| | The Americas | Central, North and South America and the Caribbean | No report (Free of BBTV) |



Figure 2. Banana bunchy top disease incidence in hill banana aerial field view at lower Pulaney hill range Western Ghats of Tamil Nadu in India.

Source: Elayabalan, 2010



Figure 3. Typical symptom of banana bunchy top disease in Hill banana, BBTB affected plant (A) and un-affected plant (UA) (BBTB).

Source: Elayabalan, 2010

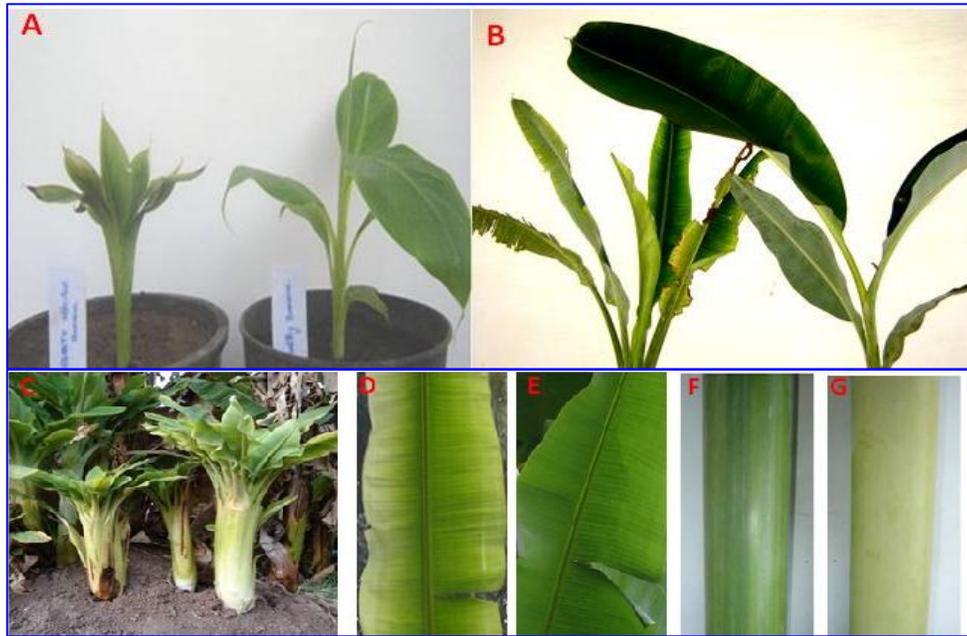


Figure 4. Vegetative stage of BBTV expression. (A) BBTV Infected banana (left), healthy banana (right) at young stage (B). BBTV Infected banana (Right) healthy banana (right) at 4-5 month old plant (C). Typical BBTVD symptoms in banana clump (D). Marginal yellowing and dark green dots and dashes (E). Healthy plant (F). Dark green streaks on the leaf petiole (G) Healthy plant.
 Source: Elayabalan, 2010.



Figure 5. Reproductive stage of BBTV expression (A, C, E healthy plant and B, D, F infected plant with BBTV expression). (A) Normal and healthy bunchy emergence (B) Abnormal bunch emergence (C) Normal fruit development and growth of bunch (D) Small size fruit and slender growth of bunch (E) Male bud having compact bract (F) Loosen and bird mouth shape of the bract.
 Source: Elayabalan, 2010

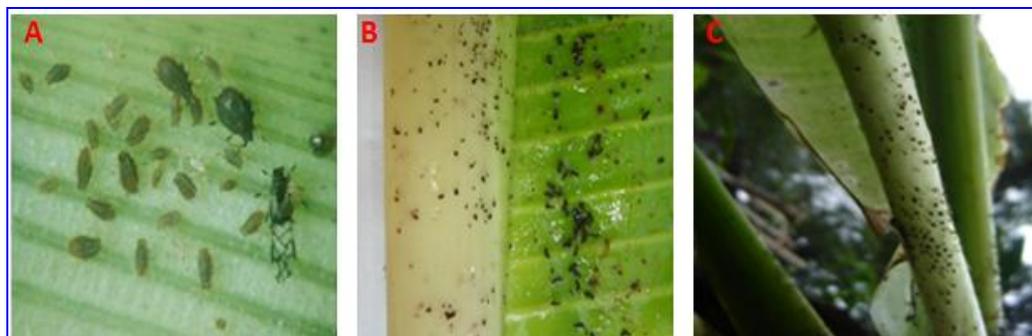


Figure 6. Banana aphid (*Pentalonia nigronervosa*). (A) Aphids colony's –Nymph, Adult and winged aphid (B) Aphids with honey dew and ants (C) Aphids on the cigar leaf and leaf sheath.

Source: Elayabalan, 2010

1.6 Genome organization of BBTV

BBTV is an isometric virus with 18-20 nm in diameter, which belongs to the genus Babuvirus in the family Nanoviridae (Harding et al., 1991; Vetten et al., 2005). Its genome consists of at least six components of circular single stranded DNA (ssDNA), designated as DNA-R, -U3, -S, -M, -C and -N, each with a similar organization and size (approximately 1 kb) (Harding et al., 1991; Burns et al., 1995; Vetten et al., 2005) (Figure 7).

The genomic components comprise an intergenic region (IR) and at least one open reading frame (ORF) that is transcribed in the virion sense (Burns et al., 1995). DNA-R encodes the master replication initiation protein (Rep) which is essential for trans-replication of the BBTV genomic components (Horser et al., 2001 a) through its nicking and joining activity (Hafner et al., 1997b). DNA-S encodes the coat protein (CP) (Wanitchakorn et al., 1997), while DNA-M and DNA-N are believed to encode the movement protein (MP) and nuclear shuttle proteins (NSP), respectively (Wanitchakorn et al., 2000). DNA-C encodes the cell cycle link protein (Clink) that has plant retinoblastoma-like binding ability to switch the host plant cells to S phase to make them more permissive for viral replication (Aronson et al., 2000; Wanitchakorn et al., 2000). The roles of the DNA-U3 gene product and that encoded by the small internal ORF of DNA-R are unknown (Burns et al., 1995; Beetham et al., 1997). Multiple satellite DNAs known as deficient DNA-R components (Bridson and Stanley, 2006) encoding non-essential Reps, which are capable of autonomous replication. However, it cannot trans-replicate any BBTV genomic component, and depends on helper virus to prepare cell conditions optimal for replication and movement within and between plants (Horser et al., 2001b).

The IR of each genomic component comprises a stem-loop common region (SL-CR), a major common region (CR-M), a TATA box and a polyadenylation signal (Burns et al., 1995). The 69 bp SL-CR shares 62% homology between all six genomic components (Burns et al., 1995), and contains a stem-loop (SL) structure which contains the nanonucleotide loop sequence (5' TATTATTAC 3') was conserved between all components (Burns et al., 1995; Hafner et al., 1997b) and iterative elements (iterons) that are potential Rep binding sites (Horser, 2000a). The CR-M varies in size from between 62 and 92 bp and shares at least 76% homology between all six genomic components (Burns et al., 1995). The CR-M comprises three relatively conserved domains (domain I, II and III) and short primer sequences that map to this region (5' of CR-M, domains I and II) have been isolated from BBTV virions (Hafner et al., 1997a), indicating its role in second strand synthesis of circular ssDNA genomic components. The promoter and terminator regions that drive the expression of encoded ORFs are located within the IR and have been shown to be active in both monocot and dicot embryogenic cells with significant activity in vascular-associated tissue (Dugdale et al., 1998; 2000).

1.7. Other plant-infecting circular ssDNA viruses

Some circular ssDNA viruses similar to BBTV have been characterised, including members of the genus Nanovirus (family Nanoviridae) and the family Geminiviridae (these are diverse group of circular ssDNA plant viruses includes four genera viz., Mastrevirus, Curtovirus, Begomovirus, and Topocovirus) (Mansoor et al., 2003). Although BBTV is almost similar to the nanoviruses, it shares similarities with the Gemini viruses in both genome organization and replication strategy (Daniela et al., 2006).

1.8 Genus Nanovirus

The nanoviruses cause diseases in many economically important dicotyledons especially in legumes throughout the world. The nanovirus type species, Subterranean clover stunt virus (SCSV) causes disease in subterranean clover, French bean, faba bean and pea was reported to cause yield losses of up to 65% in Australia (Grylls and Butler; 1956; Vetten et al., 2005). Faba bean necrotic yellows virus (FBNYV) causes disease in faba bean, lentil, chickpea and pea in west Asia, north and east Africa and Spain, while Milk vetch dwarf virus (MVDV) causes disease in Chinese milk vetch, broad bean, pea and soybean in Japan (Katul et al., 1995; Shirasawa et al., 2005; Vetten et al., 2005). All are transmissible through different aphid species in a persistent and non-propagative manner (Vetten et al., 2005).

The nanoviruses comprise at least eight genomic components of circular ssDNA (Vetten et al., 2005). Five of which contain ORFs, encoded proteins that share similarities with the BBTV components DNA-R, DNA-S, DNA-M, DNA-C and DNA-N. In addition, FBNYV DNA-U4 contains an ORF that shares similarities with BBTV DNA-U3 (Gronenborn, 2004; Vetten et al., 2005). Similar to BBTV DNA-U3, the role of FBNYV DNA-U4 is unknown. No nanovirus component has been identified that contains an ORF similar to the internal ORF encoded by BBTV DNA-R. No function has been assigned to the ORFs located on the other nanovirus-specific components (DNA-U1 and DNA-U2). Multiple satellite DNAs, encoding non-essential Reps (otherwise known as para-Reps) have also been identified associated with nanoviruses, some of which shares homology with the satellite Rep encoding components of BBTV (Katul et al., 1995; Katul et al., 1998; Sano et al., 1998).

Significant differences exist between the genomic components of the nanoviruses and BBTV. Cloning of genomic components has revealed the genomic components of the genus Nanovirus are smaller than their BBTV counterpart by approximately 100 bp (Vetten et al., 2005). Sequence analysis of the genomic components has also revealed differences between the nanoviruses and BBTV within the intergenic region, most notably the iteron sequences [Rep binding motifs

and the nanonucleotide loop sequence (5' (T/C) AGTATTAC 3'; conserved in the genus Nanovirus] (Boevink et al., 1995).

2. Transgenic strategies for developing crops resistant to DNA viruses via pathogen-derived resistance (PDR)

The PDR mechanism may involve interference of viral protein activities or viral gene expression. Full-length or truncated, wild-type or mutated viral sequences in sense, anti-sense or as inverted repeats, have all been used successfully to generate PDR (Goldbach et al., 2003). For geminiviruses, genes that encode the Rep, CP, MP, nuclear shuttle protein (NSP) and the replication enhancer protein (REnP) were used in attempts to generate PDR against gemini viruses (Hanley-Bowdoin et al., 2004a,b; Vanderschuren et al., 2007). Non-coding regions of geminiviruses have also been used to generate PDR against geminiviruses (Yang et al., 2004).

A problem of particular concern is that strain of viruses' shows variation in their virulence on different crops and even within the varieties of same crop. The virus used to protect one crop could potentially cause serious disease on other crops or varieties nearby. Several theories have been proposed to protect plants from virus infection.

Hamilton (1980) first postulated the concept of PDR in plants and was substantiated Sanford and Johnston (1985). They suggested that the transgenic expression of pathogen sequences might interfere with the replication of pathogen itself. Sanford and Johnston (1985) developed the simple concept of parasite or pathogen-derived protection using transgenic plants. The PDR proposes that the expression of certain genes of a pathogen in the host would disrupt the normal balance of viral components and thereby interfere with the virus life cycle. Hence, such type of disruption might prevent the replication and/or movement of the virus beyond the initially infected cell. Even with less effective interference in the replication cycle, PDR might modulate the disease symptoms and result in only a localized infection. The first demonstration of PDR against plant viruses was given by Powell et al. (1986), who has shown that the expression of tobacco mosaic virus (TMV) coat protein in tobacco plants has protected those plants against TMV.

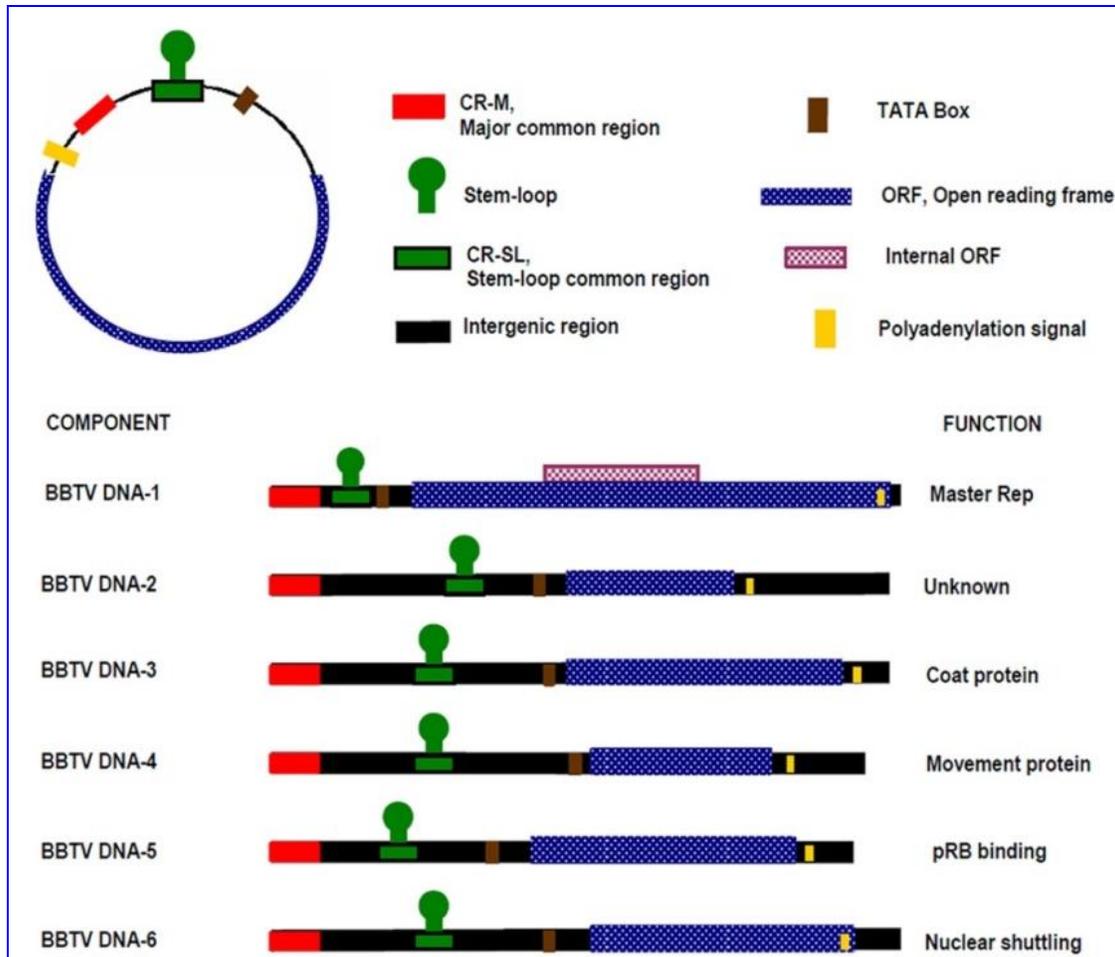


Figure 7. Genome organization of the genus Nanovirus.

Source: Elayabalan, 2010

Currently, there are two basic molecular mechanisms with PDR based on protein mediated resistance in which the expression of an unmodified or modified viral gene product interferes with the viral infection cycle and nucleic acid based protection. PDR does not involve the expression of protein product (RNA mediated resistance). Gene silencing is involved in PDR and has evolved through several stages. However, there was no correlation between level of RNA accumulation and degree of resistance (Lawson et al., 1990). Resistance was conferred by modified viral transgenes which encoded untranslatable RNAs. This post-transcriptional mechanism operates at the RNA level and would therefore have the potential to suppress the accumulation of viral RNA that shares sequence identity with the silenced transgenes.

2.1 Expression of viral proteins

2.1.1 Replicase mediated resistance

The multifunctional replication associated proteins (Reps) of viruses play an integral role in viral gene transcription regulation and the initiation and termination of virus replication. The Rep is thought to function as an oligomer and is possibly involved in regulation of host gene expression, by interacting with host proteins involved in developmental and cell cycle regulation (Gutierrez et al., 2004; Martin et al., 2008).

Gene constructs of rep genes that have been used for resistance include full-length, truncated or mutated genes. This type of resistance remains confined only to a narrow spectrum of viruses. However, the resistance generated by the use of Rep sequences is very tight; a high dosage of input virus can be resisted easily by the transgenic plant. Replicase protein mediated resistance against a virus in the transgenic plant was first shown against Tomato mosaic tobamovirus (TMV) in tobacco

plants containing a putative rep gene encoding 54 kDa replicase proteins (Golemboski et al., 1990). Similar resistance had developed for several viruses viz., Pea early browning virus (PEBV) (MacFarlane and Davis, 1992), Potato virus Y (PVY) (Audy et al., 1994) and Cucumber mosaic virus (CMV) (Hellwald and Palukaitis, 1995). In plants carrying a transgene derived from the replicase genes of Cowpea mosaic virus (CowMV) (Sijen et al., 1995) and Pepper mild mottle tobamovirus (PMMV) (Tenllado et al., 1996), it is clear with the involvement of a RNA-mediated with homology dependent resistance mechanisms.

2.1.2 CP-mediated protection

The use of viral CP as a transgene for producing virus resistant plants was one of the most spectacular successes achieved in plant biotechnology. The cp gene of TMV was used first in the demonstration of virus-derived resistance in transgenic plants (Powell et al., 1986). They suggested that the transgenic tobacco plants expressing high level of TMV-CP were more resistant to TMV virions than to TMV-RNA. It was suggested that CP-mediated protection against TMV was through the inhibition of virion disassembly in the initially infected cells. Hence, it was proposed that RNA inoculums could overcome the resistance because disassembly was not required to establish infection by naked RNA.

The most important success story related to CP-mediated resistance to a virus is against papaya ring spot virus (PRSV). Transgenic papaya (var. sunset) with cp gene was grown from 1991 to 1993 and remained virus-free for 25 months. Subsequently, it was further crossed with other popular varieties such as Rainbow, which produced 11.2 t/ha marketable fruits compared to 5.6 t/ha from the non-transgenic lines (Chen et al., 2001).

2.1.3. MP-mediated protection (MP-MP)

Movement proteins are required for their cell to cell and long distance systemic spread and have been used to engineer resistance against various plant viruses. These proteins have been shown to modify the gating function of plasmodesmata and allowed virus particles or their nucleoprotein derivatives to spread to adjacent cells (Noueir et al., 1994). CP-mediated protection is through the expression of wild type cp gene whereas MP-MP is based on the transgenic expression of dominant negative mutant forms of viral genes.

Transgenic expression of dysfunctional MP conferred resistance to TMV-MP (Lapidot et al., 1993). Resistance conferred by transgenic

expression of a dysfunctional TMV-MP is likely due to competition for plasmodesmatal binding sites between the mutant MP and the wild-type MP of the inoculated virus (Lapidot et al., 1993). MPMP exhibited broad-spectrum resistance. The protection conferred by the mutant MP of TMV mediates resistance to viruses of the potex, cucumo, and tobamovirus groups in addition to the targeted tobamoviruses (Cooper et al., 1995). This suggested that MPs of different viruses might interact with the plasmodesmatal components (Carrington et al., 1996).

Initially, transgenic plants expressing the defective movement protein were resistant to both Tobacco mosaic virus (TMV) and Cabbage leaf curl virus (CabLCV) especially the proteins share 80% amino acid sequence similarities. In a similar experiment, tomato plants transformed with a mutated Bean dwarf mosaic virus (BDMV) mp gene showed resistance to tomato mottle virus (ToMoV) virus with a movement protein sharing 93% amino acid sequence identity with that of BDMV (Hou et al., 2000). Hence, the use of MP transgenes is constrained by the fact that they are often toxic when over-expressed in plant cells are known pathogenicity determinants. Their uncontrolled expression have many undesirable effects on various aspects of plant development (Covey and Al-Kaff, 2000; Hou et al., 2000).

2.2. Pathogen derived resistance without protein expression

2.2.1 Antisense technology

Development of conceptual models of post transcriptional gene silencing (PTGS) and Pathogen derived resistance to viruses, proposed mechanisms for suppressing the accumulation of nucleus-derived RNA (gene silencing) and virus-derived RNAs with homology to the transgene. Such a mechanism would require a high degree of sequence specificity due to highly strain specific.

In principle, the interaction leading to suppression of viral RNA could involve base pairing of the sense RNA transcript of the transgene and the negative strand of the viral RNA, which is produced as an intermediate in the replication cycle of most viral RNAs. The antisense RNA could be produced by an RNA-dependent RNA polymerase (RdRP) encoded in the host genome using the transgene RNA as a template (Schiebel et al., 1993 a,b) and would have the potential to base pair with the transgenic and viral RNAs.

Formation of duplex RNA could influence accumulation of host and viral RNA to cause gene silencing and virus resistance. The base paired

region may render the duplex RNA susceptible to degradation by RNases specific for double-stranded RNA (Nicholson, 1996).

The base-paired region could also arrest the translation and consequently have an indirect effect on RNase susceptibility and translation could cause reduced accumulation of both nucleus and virus derived RNAs. The proposed involvement of antisense RNA can be considered as a part of the response of homology-dependent resistance.

Antisense RNA strategies have been successfully exploited since 1991 to target and selectively suppress the expression of virus genes. The transcripts targeted have included those of tobacco mosaic virus (TMV) rep in *N. tabacum* (Day et al., 1991), tomato yellow leaf curl virus (TYLCV) rep in *N. benthamiana* and tomato (Bendahmane and Gronenborn, 1997 and Yang et al., 2004); tomato leaf curl virus (ToLCV) rep in tomato (Bendahmane and Gronenborn, 1997); tomato golden mosaic virus (TGMV) and beet curly top virus (BCTV) rep, TrAP and REN (the last of which encodes a replication enhancer protein in begomoviruses in tobacco (Bejarano and Lichtenstein, 1994), BGMV rep, TrAP, REN, and MP in beans (Aragão et al., 1998); cotton leaf curl Kokhran virus (CLCuKV) (REP, TrAP and REN in *N. tabacum* (Asad et al., 2003), and ACMV REP, TrAP and REN in cassava (Zhang et al., 2005).

The effectiveness of this approach depends directly on base-pairing between target and antisense RNAs and will therefore only work against viruses closely related to the virus from which the transgene was derived. For broad based resistance, multiple sequences might have to be targeted-it and this could be achieved using fused antisense RNA sequences to different parts of the viral genome (Vanderschuren et al., 2007).

2.2.2. RNAi (RNA interference) mediated resistance

Sequence specific RNA degradation described as PTGS in plants, quelling in fungi and RNA interference in nematodes. Lindbo and Dougherty (1992) demonstrated that untranslatable viral RNA sequence could trigger specific, post-transcriptional RNA degradation of the mRNA and are correlated with viral protection. Operationally, PTGS is observed as high-level nuclear transcription determined from nuclear run-on experiments. There was a low and steady state levels of mRNA accumulation in the cytoplasm as detected through northern hybridization. Therefore, the gene silencing system is triggered in the cytoplasm together with any viral RNA that has same or similar sequence. Currently, there were many

examples of resistance based on expression of viral genes such as coat-protein, replicase, antisense, sense co-suppression, satellite RNA, ribosome which are functionally operated by PTGS.

RNA silencing is an evolutionary conserved mechanism protecting cell from pathogenic RNA and DNA, which is increasingly viewed as an adaptive immune system of plants against viruses (Voinnet et al., 2001). Proof of the concept that RNAi can be engineered to effectively target geminiviruses has recently been documented in transient assays, initially for mungbean yellow mosaic virus-Vigna (MYMV-Vig) (Pooggin et al., 2003) and later for African cassava mosaic virus (ACMV) (Vanitharani et al., 2003). Mansoor (2003) produced transgenic tobacco plants with constitutively expressing double-stranded (ds) RNA cognate to coding and non-coding regions of DNA b from cotton leaf curl virus (CLCV). They reported that plants were resistant to CLCuV when challenged with agro-inoculation or white fly.

PTGS used in the development of resistance against the viruses ACMV (Vanitharani et al., 2003, Chellappan et al., 2004, Vanderschuren et al., 2007), mungbean yellow mosaic virus (MYMV) (Pooggin et al., 2003), Sri Lankan cassava mosaic virus (SLCMV), East African cassava mosaic virus (EACMV) (Chellappan et al., 2004), TYLCV (Abhary et al., 2006, Fuentes et al., 2006; Zrachya et al., 2007), bean golden mosaic virus (BGMV) (Bonfim et al., 2007), and TLCV (Ramesh et al., 2007). Although only in its early stages, research utilizing this process to achieve virus resistance is very promising in that any viral coding or non-coding sequences can be targeted and no protein need be expressed. Hence, PTGS based resistance strategies could potentially have fewer deleterious effects on plant development than those in which a protein is expressed from the transgene. However, it would be impossible to predict the effects of engineered hairpin RNA sequences on endogenous gene expression unless the entire genome of the crop in question has been sequenced.

An advantage of RNA-based approaches is safer than those that rely on the expression of foreign proteins in plant material consumed by humans and animals (Waterhouse et al., 2001). However, there are some potential drawbacks to the use of PTGS-based resistance mechanisms. Broad-based resistance may be difficult to engineer using PTGS because it is homology-dependent and there is therefore likely to be only a small amount of tolerable sequence variation between PTGS inducing transgenes and their targeted viruses. In addition, some viruses express proteins that are

silencing suppressor (Vanitharani et al., 2003; Moissiard and Voinnet, 2004; Bisaro, 2006; Sharma et al., 2010) which could undermine PTGS-based resistance. Such “anti-silencing” determinants include the transcription activator proteins (TrAPs) of African cassava mosaic virus (ACMV) (Voinnet et al., 2005), tomato yellow leaf curl China virus (TYLCCNV) (Van Wezel et al., 2002), the AC4/C4 genes of Sri Lankan cassava mosaic virus (SLCMV) (Vanitharani et al., 2004), Indian cassava mosaic virus (ICMV) (Vanitharani et al., 2004), MYMV (Trinks et al., 2005), and TGMV (Wang et al., 2005); and Bendi yellow vein mosaic virus (BYVMD) (Gopal et al., 2006); the V2 of TYLCV (Zrachya et al., 2007) and the bC1 gene encoded by a satellite DNA molecule associated with many monopartite begomo viruses including tomato leaf curl Java virus (TLCJV) and bendi yellow vein mosaic disease (BYVMD) (Gopal et al., 2006; Kon et al., 2007).

2.3 Resistance due to the expression of non-pathogen derived antiviral agents

2.3.1 Virus-induced cell death

Infected plants often have an innate defensive hypersensitive reaction that limits virus movement to the site of infection by inducing the death of infected cells and their neighbours.

Such reaction can be artificially induced to provide virus resistance in transgenic plants by the combined action of the barnase and barstar proteins of *Bacillus amyloliquefaciens* (Zhang et al., 2005; Vanderschuren et al., 2007).

Barnase is a ribonuclease (RNase) and barstar is its inhibitor. In the absence of virus infection, the two transgenes expressed at similar levels, resulted the absence of RNase production. By placing barnase under the control of a viral virion-sense promoter that is activated during virus infection and barstar under the control of a viral complementary-sense promoter that is repressed during virus infection, an infected cell should over-express barnase relative to barstar and die before the infecting virus proceed for the further stage.

2.3.2 DNA binding proteins

The use of transgenically expressed DNA binding proteins to provide virus resistance relies on the identification of virus sequence-specific binding proteins that will not bind host DNA sequences. The virus Rep is a sequence-specific dsDNA binding protein (Fontes et al., 1992; Castellano et al., 1999) that recognises and binds to direct repeats in the virion strand origin of replication (v-ori) where it initiates and terminates

rolling circle replication (Fontes et al., 1992; Fontes et al., 1994; Heyraud-Nitschke et al., 1995). This sequence specific activity has been exploited by designing artificial zinc finger proteins with high affinity for the Rep-specific direct repeats in the v-ori of different geminiviruses (Sera and Uranga, 2002), with the idea that the artificial zinc finger proteins will competitively block the binding of Rep due to the higher affinity of the artificial zinc finger protein–dsDNA interaction. This resulted inhibition of viral replication. The utility of this approach was successfully demonstrated in *Arabidopsis thaliana* against beet severe curly top virus (BSCTV) (Sera, 2005).

2.4 Strategies in the pipeline

2.4.1 Peptide aptamers

A peptide aptamer is a short (20 amino acids long) recombinant protein, constrained within a scaffold protein such as thioredoxin. It strongly binds to a target protein and interferes with its intracellular function (Hoppe-Seyler et al., 2004; Baines and Colas, 2006).

Peptide aptamers were first applied to engineering virus resistance in transgenic

N. benthamiana, targeting the nucleoprotein (N) of the tospovirus which is known as tomato spotted wilt virus (TSWV) (Rudolph et al., 2003; Uhrig et al., 2003). To engineer geminivirus resistance using a similar strategy, Rep specific aptamers were selected by a yeast two-hybrid screen of a random peptide aptamer library using the N-terminal domain of TGMV Rep as bait (Lopez-Ochoa et al., 2006). Peptides were identified that bind to the Reps of diverse geminiviruses, including cabbage leaf curl virus (CaLCuV), East African cassava mosaic virus-Uganda (ACMV-Uganda) and ACMV-Cameroon (Lopez-Ochoa and Hanley-Bowdoin, 2007) demonstrating their potential in broad-spectrum resistance.

2.4.2 InPAct

Most of the expression based resistance mechanisms rely on high-level constitutive expression of recombinant proteins, a novel gene expression system called InPAct (for In Plant Activation) is likely to be part of the ‘next generation’ of inducible transgene expression technologies. The main innovation of the InPAct system is that instead of it depending on promoter transactivation, it directly exploits the extremely specific DNA nicking and joining activities of virus Reps to ensure that gene expression cassettes will only be functional in the presence of these proteins.

InPact system will also be useful for virus-induced expression of non-lethal resistance genes for several reasons: (i) constitutive expression of resistance genes is redundant when no infection occurs and is likely to add to the metabolic load of the crop, (ii) constitutively expressed genes are more likely to be the target of transgene silencing, (iii) as mentioned above, the expression of virus proteins such as Rep or movement proteins can cause developmental defects (Dugdale et al., 2014). Recent reports on the production of transgenic resistant crops were summarized in Table 2.

3. Recent BBTV resistant banana clone development through RNAi technology

Agrobacterium-mediated transformation of embryogenic banana cell suspensions with constructs that may prevent the replication of BBTV has been favoured by many research groups, as there is a much better chance of a plant developing that is not a chimera. In 2011, it was reported that some transformed clones of ‘Dwarf Brazilian’ (AAB, Pome subgroup) were resistant to BBTV under experimental conditions in Hawaii (Borth et al., 2011). Two Indian research groups have claimed success. Shekhawat et al. (2012) have published an account of tests that showed that transformed ‘Rasthali’ (AAB genome, syn. ‘Silk’) did not develop symptoms when exposed to aphids carrying BBTV. The transformation of the Indian

hill banana ‘Virupakshi’ (AAB genome, Pome subgroup) has also been reported for RNAi technology to impart BBTV resistance in banana (Elayabalan et al., 2013).

One of the most severe viral diseases of hill banana is caused by BBTV, a nanovirus transmitted by the aphid *P. nigronevosa*. Elayabalan et al. (2013) reported on the Agrobacterium-mediated transformation on a highly valued hill banana cultivar Virupakshi (AAB) for resistance to BBTV disease. The target of the RNA interference (RNAi) is the rep gene, encoded by the BBTV-DNA1. The presence of the transgenes was confirmed in the selected putative transgenic hill banana lines by PCR and reverse transcription PCR analyses. Transgenic hill banana plants expressing RNAi-BBTV-rep were obtained and shown to resist infection by BBTV. The transformed banana plants were symptomless, and the replication of challenge BBTV almost completely suppressed. Hence, the RNAi mediating resistances were shown to be effective management of BBTV in hill banana. Since environmental conditions have a strong influence on many mechanisms involved in the regulation of virus genes including those encoding suppressors of replication, hence field trials are necessary to confirm resistance level in the laboratory and glasshouse.

Table 2. Review on viral disease resistance genes for transgenic approach.

| Source of transgenic sequence | Transgene | Plant | Viruses under investigation | Reference |
|--|--------------------------|------------------------------|--|-------------------------|
| 1. Expression of viral proteins | | | | |
| Tomato yellow leaf curl virus | Truncated REP gene | <i>Solanum lycopersicum</i> | Tomato yellow leaf curl virus (TYLCV) | Antignu et al., 2004 |
| Tomato yellow leaf curl Sardinia virus | Truncated REP gene | <i>Solanum lycopersicum</i> | Tomato yellow leaf curl Sardinia virus (TYLCSV) | Brunetti et al., 1997 |
| Tomato yellow leaf curl Sardinia virus | Truncated REP gene | <i>Nicotiana benthamiana</i> | Tomato yellow leaf curl Sardinia virus (TYLCSV) and Tomato yellow leaf curl virus (TYLCV) | Lucioli et al., 2003 |
| Tomato leaf curl New Delhi virus | Truncated REP gene virus | <i>N. benthamiana</i> | Tomato leaf curl New Delhi virus (ToLCNDV); African cassava mosaic Virus (ACMV); Potato yellow and Mosaic virus (PYMV) | Chatterji et al., 2001 |
| Bean golden mosaic virus | rep gene mutants | <i>N. benthamiana</i> | Bean golden mosaic virus (BGMV) | Hanson et al., 1999 |
| African cassava mosaic virus | Full-length REP gene | <i>N. benthamiana</i> | African cassava mosaic virus (ACMV) | Hong and Stanley (1996) |
| African cassava mosaic virus | rep gene mutant | <i>N. benthamiana</i> | African cassava mosaic virus (ACMV) | Sangare et al., 1999 |
| Mungbean yellow | Full-length and | <i>N. benthamiana</i> | Mungbean yellow mosaic | Shivaprasad et al., |

| | | | | |
|--------------------------------|---|--|--|----------------------------|
| mosaic virus | truncated REP genes | | virus (MYMV) | 2006 |
| Maize streak virus | rep gene mutants and truncated rep gene mutants | <i>Digitaria sanguinalis</i> | Maize streak virus (MSV) | Shepherd et al., 2007 |
| 2. Gene silencing | | | | |
| African cassava mosaic virus | REP gene (transgene-induced PTGS) | <i>Manihot esculenta</i> | African cassava mosaic virus (ACMV), Sri Lankan cassava mosaic virus (SLCMV) and East African cassava mosaic virus (EACMV) | Chellappan et al., 2004 |
| African cassava mosaic virus | DNA-A bidirectional promoter | <i>Manihot esculenta</i> | African cassava mosaic virus (ACMV) | Vanderschuren et al., 2007 |
| African cassava mosaic virus | Chemically synthesized siRNA to REP mRNAa | <i>N. tabacum</i> protoplasts | African cassava mosaic virus (ACMV) | Vanitharani et al., 2003 |
| Mungbean yellow mosaic virus | DNA-A bidirectional promotera | <i>Vigna mungo</i> | Mungo yellow mosaic virus (MYMV) | Pooggin et al., 2003 |
| Tomato yellow leaf curl virus | Hairpin RNA derived from REP genea | <i>Solanum lycopersicum</i> and <i>N. benthamiana</i> | Tomato yellow leaf curl virus (TYLCV) | Abhary et al., 2006 |
| Tomato yellow leaf curl virus | Hairpin RNA Derived from REP gene | <i>Solanum lycopersicum</i> | Tomato yellow leaf curl virus (TYLCV) | Fuentes et al., 2006 |
| Tomato yellow leaf curl virus | Hairpin RNA derived from COAT PROTEIN gene | <i>Solanum lycopersicum</i> | Tomato yellow leaf curl virus (TYLCV) | Zrachya et al., 2007 |
| Bean golden mosaic virus | Hairpin RNA derived from REP gene | <i>Phaseolus vulgaris</i> | Bean golden mosaic virus (BGMV) | Bonfim et al., 2007 |
| Tomato yellow leaf curl virus | Hairpin RNA derived from REP and AC4 genes | <i>Solanum lycopersicum</i> | Tomato yellow leaf curl virus (TYLCV) | Ramesh et al., 2007 |
| Banana Bunchy top virus | REP | Banana (<i>Musa</i> spp) cultivar. Dwarf Brazilian | Banana Bunchy top virus (BBTV) | Borth et al., 2011 |
| Banana Bunchy top virus | REP | Banana (<i>Musa</i> spp) cultivar. Rasthali (AAB) | Banana Bunchy top virus (BBTV) | Shekhawat et al. 2012 |
| Banana Bunchy top virus | Hairpin RNA derived from REP | HillBanana (<i>Musa</i> spp) cultivar. Virupakshi (AAB) | Banana Bunchy top virus (BBTV) | Elayabalan et al., 2013 |
| 3. Antisense | | | | |
| Tomato golden mosaic virus | REP | <i>N. tabacum</i> | Tomato golden mosaic virus (TGMV) | Day et al., 1991 |
| Tomato yellow leaf curl virus | REP | <i>N. benthamiana</i> | Tomato yellow leaf curl virus (TYLCV) | Bendahmane et al., 1997 |
| Tomato yellow leaf curl virus | REP | <i>Solanum lycopersicum</i> | Tomato yellow leaf curl virus (TYLCV) | Yang et al., 2004 |
| Tomato leaf curl virus | REP | <i>Solanum lycopersicum</i> | Tomato leaf curl virus (TLCV) | Praveen et al., 2005 |
| Tomato golden mosaic virus | REP, TrAP and REN | <i>N. tabacum</i> | Tomato golden mosaic virus (TGMV) | Bejarano et al., 1994 |
| Bean golden mosaic virus | REP, TrAP, REN and MP | <i>Phaseolus vulgaris</i> | Bean golden mosaic virus (BGMV) | Araga et al., 1998 |
| Cotton leaf curl Kokhran virus | REP, TrAP and REN | <i>N. tabacum</i> | Cotton leaf curl Kokhran virus (CLCuKV) | Asad et al., 2003 |
| African cassava mosaic virus | REP, TrAP and REN | <i>Manihot esculenta</i> | African cassava mosaic virus (ACMV) | Zhang et al., 2005 |

4. Conclusion

Banana crop faces numerous environmental challenges, particularly with viral, bacteria, fungal, pests and abiotic stresses. The BBTV problem is aggravated by the limited diversity of cultivars. BBTV problems point to the necessity of developing alternate strategies for banana improvement. Biotechnological approaches such as tissue culture and genetic transformation has the potential to overcome this disease with farmers make use of disease free planting materials and creating awareness about BBTV knowledge.

Author contributions

S. E. and S. S. made a major contribution to the review paper. S. E. was involved in overall planning and supervision. R. S. was involved in selected BBTV review section.

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