African Journal of Biotechnology Vol. 12(1), pp. 27-31, 2 January, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2536
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

The influence of host and pathogen genotypes on symptom severity in banana streak disease

M. C. Wambulwa[1]*, F. N. Wachira[2], L. S. Karanja[3], S. M. Kiarie[1], and S. M. Muturi[1]

[1]Department of Biochemistry and Molecular Biology, Egerton University, P. O. Box 536, Egerton, Kenya.
[2]Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA), P. O. Box 765 Entebbe, Uganda.
[3]Kenya Agricultural Research Institute, P. O. Private Bag, Njoro, Kenya.

Accepted 10 October, 2012

Banana is the fourth most important food crop worldwide. However, its production has been threatened by banana streak disease, caused by banana streak virus (BSV). Despite this situation, little is known about the inter-relationships between symptom severity and cultivar/virus genotypes. Symptom severity assessment for sixty five symptomatic samples was carefully done. The rolling circle amplification technique was used to identify the virus species (isolate) infecting each sample. The Mysore virus isolates (BSMysV) and the banana cultivars containing the balbisiana (B) component were associated with the most severe banana streak disease symptoms.

Key words: Banana streak disease, symptom severity, banana streak virus (BSV) isolate, rolling circle amplification.

INTRODUCTION

Banana streak disease, caused by Banana streak virus (BSV), has been reported to occur in most banana-growing regions worldwide (Fargette et al., 2006). The virus elicits a wide range of symptoms which may include foliar chlorotic and necrotic streak, altered phylotaxis, pseudostem splitting, abnormal bunch development, and death of the meristematic tissue (Dahal et al., 2000a). Banana streak disease accounts for yield losses in the range of 6 to 15% (Dahal et al., 2000b; Daniells et al., 2001) depending on cultivar type, virus species, and environmental conditions.

BSV is a member of the genus Badnavirus and family Caulimoviridae. The genome comprises of non-covalently closed, double-stranded DNA of about 7.4 kb. Badnaviruses are bacilliform and do not contain the translational trans-activator protein found in other caulimoviruses (Lockhart and Olszewski, 1999). All members of Caulimoviridae have an open-circular, double-stranded DNA genome, whose replication occurs through a reverse transcription (RT) step (Hohn, 1999). In plant pararetroviruses, unlike true retroviruses, integration into the host genome is not required for replication (Hohn, 1999).

Three BSV species are currently recognised, namely; *Banana streak Mysore virus* (BSMyV), *Banana streak OL virus* (BSOLV) and *Banana streak GF virus* (BSGFV) (Hull et al., 2005). A fourth species, *Banana streak acuminata Vietnam virus* (BSAcVNV), has also recently been proposed based on full length sequence analyses (Lheureux et al., 2007). Although partial sequences have been reported for several other putative virus species including the *Banana streak Cavendish virus* (BSCavV) (Geering et al., 2000), *Banana streak Imové virus* (BSImV) and *Banana streak Uganda A-M viruses* (Harper et al., 2005), the taxonomic status of these viruses remains unresolved.

So far, there is little, contradictory information on the virulence of the various BSV isolates in different banana genotypes and the degree to which symptoms would be expressed. This has significantly hindered the capacity to successfully manage banana streak disease through such strategies as quarantine measures. The focus of the

*Corresponding author. E-mail: mcwambulwa@gmail.com.

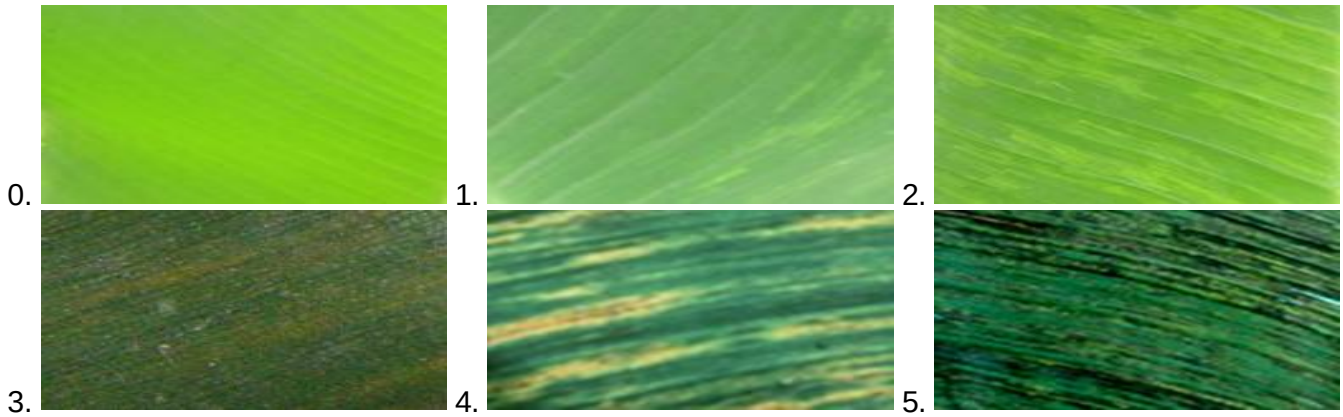

Figure 1. Banana leaves depicting symptoms severity scoring scale. 0 is no symptoms, 1 is localised flecks, 2 is scattered discontinuous streaks, 3 is continuous streaks covering moderate portion of lamina, 4 is continuous and conspicuous chlorotic streaks, 5 is necrotic streaks according to Karanja (2009).

study was to determine the inter-relationship among BSV isolate, symptom severity and host genotype by means of the novel and sensitive rolling circle amplification approach (James et al., 2011).

MATERIALS AND METHODS

Sample collection

Banana leaf samples of different cultivars in banana fields expressing banana streak disease symptoms were collected. This sampling was carried out in farmers' fields in five major banana growing regions in Kenya: Nyanza, Western, Central, Eastern and Rift Valley Provinces. Samples of different banana cultivars of varying genotypes were collected from each region. Samples with varying degree of banana streak disease symptom expression were collected. The sampling was based on the assumption that the variance in the degree of symptom severity is due to the differences in the type (or number) of isolate(s). BSV symptom descriptions on all the samples were recorded. For quantification of symptom severity, each sample was scored on a scale of 0 to 5 (Karanja, 2009), where 0 is no symptoms, 1 is localized flecks, 2 is scattered discontinuous streaks, 3 is continuous streaks covering moderate portion of lamina, 4 is continuous chlorotic streaks, and 5 is necrotic streaks as shown in Figure 1. Details of the locality and the cultivar genotype were recorded for all collected samples. The samples were then packed in silica gel bottles and transported to Kenya Agricultural Research Institute (K.A.R.I.), Njoro. Indexing was done for BSV using the rolling circle amplification method.

Nucleic acids extraction

Total banana nucleic acid was isolated using the Cetyltrimethylammonium bromide (CTAB) protocol as modified by James et al. (2011). Dry leaf tissue (0.05 g) was ground in 300 ml of extraction buffer and liquid nitrogen. During buffer mix preparation, all ingredients except CTAB were mixed and made up to 450 ml using deionised water. 10 g of CTAB was then dissolved in 50 ml of water in a falcon tube and mixed gently to avoid foaming. The CTAB was then combined with the rest of the buffer solution and mixed well. The slurry of ground leaf tissue in extraction buffer was incubated at 65°C for 15 min, spinned at 13,000 rpm and 750 µl transferred to a fresh tube. This was then mixed with an equal volume of chloroform/isoamyl alcohol (24:1) and centrifuged for 5 min at 13,000 rpm. Nucleic acids in the aqueous phase was then pelleted by isopropanol and centrifuged for another 5 min at 13,000 rpm. The DNA pellet was washed with 500 µl of 70% ethanol and re-suspended in 100 µl of nuclease-free water. The DNA was left at 4°C overnight to fully dissolve. The nucleic acids were then stored at -20°C.

Rolling circle amplification

The full Banana streak virus genome was amplified and isolated using the TempliPhi Kit (GE Healthcare, Buckinghamshire, United Kingdom) according to James et al. (2011). Two mixes were prepared. For master mix 1 (MM1), 5 µl of TempliPhi sample buffer was mixed with 1 µl of the isolated sample and 1 µl of a 50 µM stock solution (4.16 pmol/ul of each primer) of TempliPhi degenerate primers (Table 1). The mix was then heated at 95°C for 3 min to denature the DNA followed by cooling to room temperature or 4°C. Denaturation was necessary because BSV genome is double stranded. Master Mix 2 (MM2) was prepared by mixing 5 µl of TempliPhi reaction buffer and 0.2 µl of TempliPhi Enzyme Mix. 5 µl of the TempliPhi premix (mix 2) was transferred to the cooled, denatured sample (MM1) then incubated at 30°C for 18 h. After the incubation period, the enzyme (*Phi29* DNA polymerase) was heat-inactivated at 65°C for 10 min. The samples were then cooled and stored at 4°C.

Restriction enzyme digestion and gel electrophoresis

Ten (10) microlitres of the TempliPhi reaction product from each of the symptomatic samples were incubated separately with the restriction enzyme *Stu* I (Gibco BRL, Eggenstein) for 2 h. A 20 µl aliquot of the digested TempliPhi product was mixed with 2 µl of 5× gel loading dye (Biolabs) and electrophoresed for about 20 min at 100 V on a 1% SYBR Safe-stained agarose gel using 1× TAE as the running buffers. The gel was visualised under ultra violet (UV) illumination with Gel Doc (BIO-RAD) Software (USA). Internal standards (positive controls) for BSV isolates generated using *Stu*I (New England BioLabs) were used to identify the isolate(s) present in each sample based on the published isolates (James et al.,

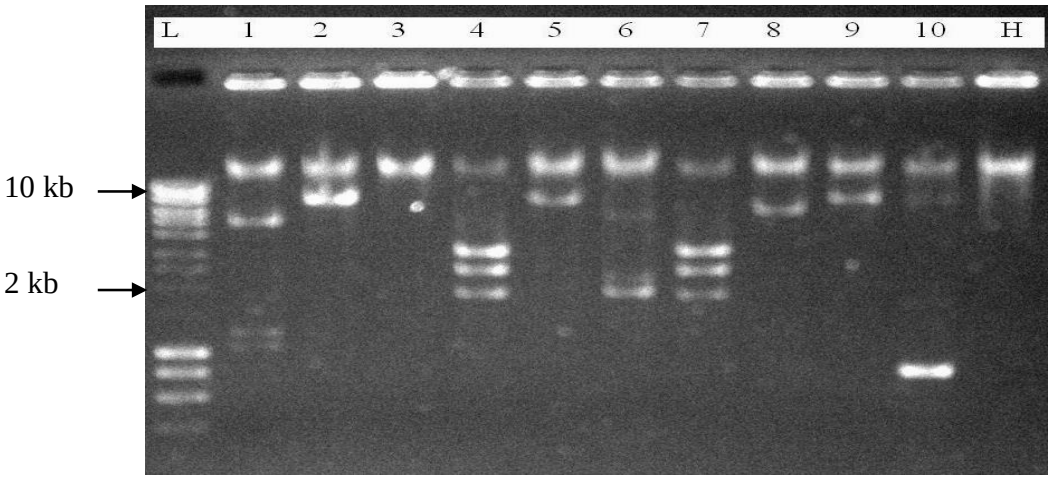

Figure 2. TempliPhi-RFLP for samples 1 to 10 using *Stu*1. Lane H represents a healthy (negative) control (DNA extract of a BSD-free *Musa* tissue). Lane L is a molecular marker (Bioline Hyperladder).

Table 1. Rolling circle amplification degenerate primer mix composition.

Primer name	Primer sequence (5'-3')
1A	CTNTAYGARTGGYTNGTNATGCCNTTYGG
4'	TCCAYTTRCANAYNSCNCCCCANCC
BadnaFP	ATGCCITTYGGIITIAARAAYGCICC
BadnaRP	CCAYTTRCAIACISCICCCCAICC
BSV2292	ATGARYTAHATWAGRTGYTMSCC
BSV2826	TYYWGAAARCATGGTGGGRGARGA
BSV3298	YTCCCAYCTTTCRAAKACYTT
BSV3517	KRATMTTYTWTYTDGAARATCC
BSV3700	KTGGBAGTTTKGTRAAGARYTC
BSV4030	TGCARRTGYTWYGCYTGYGGAGA
BSV6652	GAAAARRTMTGYGCNTAYGCVAG

2011). The relationships between isolate type/banana cultivar genotype and symptom expression were determined using the restriction fragment data. Analysis of variance (ANOVA) was done using the Statistical Package for Social Sciences (SPSS) to determine the relationships among symptom expression, BSV isolate and host genotype.

RESULTS

Identification of BSV isolates

Various restriction fragment profiles were obtained when Rolling Circle Amplification (TempliPhi) products were digested with *Stu*1 as shown in Figure 2. Most of the indexed samples (83.1%) gave observable profiles on agarose gel after *Stu*1 restriction enzyme digest. About 17% of the samples were consistently negative. The obtained profiles were compared to the available *Stu*1 standards (James et al., 2011) and only 2 of the positive samples gave profiles that could not be identified with any of the available standards.

Relationship between genotype and symptom severity

Symptom severity of individual leaf samples were scored on a scale of 0 to 5; a modification of Karanja (2009). The analysis was done based on the assumption that the samples were infected at almost the same time.

Results shown in Table 2 indicate that symptoms were more severe in *Musa* cultivars with AAB (with highest symptom expression mean of 3.97 ± 0.251). Of the 28 samples with the AAA genotype, 28.6% merely expressed localized flecks and mild chlorosis (category 1). For the samples with AAB genotypes, only two out

Table 2. Mean symptom expression degree (based on scale 0 to 5) for samples of three banana genotypes (AAA, AAB and AAAB).

Genotype	N**	Mean*	95% Confidence interval for mean	
			Lower	Upper
AAA	28	2.61±0.283	2.03	3.19
AAB	29	3.97±0.251	3.45	4.48
AAAB	7	2.57±0.612	1.07	4.07
Total	64	3.22±0.197	2.82	3.61

*Mean symptom expression was determined based on a scale of 0 to 5 (Karanja, 2009). **N as number of sample.

Table 3. Mean symptom expression degree (based on scale 0 to 5) for banana samples infected with three common BSV isolates (BSMysV, BSGfV and BSOEV).

BSV Isolate	N	Mean	95% Confidence interval for mean	
			Lower	Upper
BSMysV	25	4.04±0.280	3.46	4.62
BSGfV	16	2.38±0.301	1.73	3.02
BSOEV	11	3.09±0.415	2.17	4.01
Total	52	3.33±0.209	2.91	3.75

of twenty nine samples expressed category 1 symptoms. The relative sample size for the AAAB cultivars was not sufficient to make a conclusion as to how these tetraploids respond to BSV infection with regard to symptom expression. Analysis of variance (ANOVA) with regard to symptom expression showed that the three cultivar groups differed significantly ($P < 0.05$).

Symptom severity and isolate type

The results presented in Table 3 suggest that the Mysore isolate generally causes more severe symptoms than the other BSV isolates (with highest mean of 4.04 ± 0.280). The proportion of the samples infected with the Mysore isolate which expressed category 5 symptoms (necrotic streaks) was 56% compared to 18.75 and 18.2% for Gold finger and Obino l' Ewai, respectively. The mean symptom expression degree for BSOEV was 3.09 ± 0.415 while that for BSGfV was the lowest at 2.38 ± 0.301. Analysis of variance (ANOVA) with regard to symptom expression showed that the three BSV isolates differed significantly ($P < 0.05$).

DISCUSSION

Results of this study show that some correlation exists between genotype of the sample/isolate and the degree of symptoms observed. However, each site was only visited once, and since many factors appear to influence BSV symptom expression including environmental conditions and plant growth stage (Mobambo et al., 1996; Dahal et al., 1998; Daniells et al., 2001), it is unlikely that a complete picture was obtained. Additionally, the ideal way was to start with clean plants in the field, followed by inoculation with the various BSV isolates. However, there have been consistent failure reports regarding this approach.

The results obtained in a study in Nigeria (Dahal et al., 1998) showed that symptom expression of BSV-infected plants at two temperature regimes (in a cool room and screen house) varied by genotype. Plantain hybrids (genome AAB × AA) generally expressed more severe symptoms, whereas most cooking bananas did not express symptoms under either temperature regime.

The most likely reason for greater expression of banana streak disease symptoms in cultivars with a balbisiana genome component is that the endogenous pararetroviruses (integrated sequences) in these cultivars contain all of the genetic information needed for "reconstruction" of functional BSV genome very similar to that of the infectious BSV virus. This phenomenon was particularly true for the Gold finger isolate (Gayral et al., 2008).

Gayral et al. (2008) demonstrated that BSGfV sequences are integrated only in the genome of *M. balbisiana* cultivar cv. PKW and are absent from two other common cultivars of *M. acuminata* tested. Other studies have also shown that species of the BSV clade

sensu stricto, to which BSGfV belongs, are integrated mainly in the B genome (Geering et al., 2001, 2005), whereas a minority of BSV species such as *Banana streak Cavendish virus* (BSCavV) (Iskra-Caruana et al., 2009) and *Banana streak acuminate Vietnam virus* (BSAcVNV) (Lheureux et al., 2007) are thus far reported as being specific to the A genome.

Generally, the Mysore isolate appeared to elicit more severe BSV symptoms in this study. In an earlier study to determine the infection dynamics of the Mysore isolate (Daniells et al., 2001), it was observed that symptoms of BSMysV infection in cv. 'Williams' were very severe, and infection would probably substantially reduce yield of the plant. In comparison to BSMysV, BSV-Cav was comparatively benign in the same cultivar. A BSV-infected plant of the Mysore cultivar was used as a source of inoculum on healthy plants. Only one out of eight plants became infected as determined by Immunocapture polymerase chain reaction (IC-PCR).

In a subsequent test, using infected 'Williams' cultivar plant as a source of inoculum, all six cv. 'Williams' test plants became infected. Symptoms of infection were very severe with chlorotic streaks covering a large proportion of the leaf. This observation can be attributed to difference in antigenic properties of the various BSV isolates. It is possible that the Mysore isolate carries epitopes that are more immunogenic than the other isolates hence elicits more severe symptoms. Other than genotype, the variance in symptom severity can also be attributed to other factors. Mixed infections, which have been reported in Kenya (Karanja et al., 2008), could be one of the causes of the variance in degree of symptoms observed.

Karanja et al. (2008) observed that symptoms were more severe in samples which were infected with more than one isolate; the more the number of isolates, the more severe the symptoms. Previous studies have also shown that environmental and nutritional factors can influence banana streak disease symptom expression. The best studied of such factors is temperature (Dahal et al., 1998). This Nigeria-based study found out that symptoms are more severe at lower (22 to 28°C) than at higher temperatures (35°C).

Since increased symptom expression was observed in *Musa* cultivars containing a balbisiana component in their genomes, it would be important to make use of such cultivars in experiments aimed at studying BSV symptom expression in *Musa*. The fact that Mysore isolate elicits most severe symptoms calls for further research into its precise mechanism.

ACKNOWLEDGEMENTS

We are grateful to KARI-Njoro Biotechnology staff for technical assistance. We also thank Queensland University of Technology (QUT), Australia, for funding this research through the Banana 21 Project.

REFERENCES

Dahal G, Hughes J, Gauhl F, Pasberg-Gauhl C, Nokoe KS (2000a). Symptomatology and development of banana streak, a disease caused by banana streak badnavirus, under natural conditions in Ibadan, Nigeria. Acta Hortic. 540:361-375.

Dahal G, Hughes JD'A, Thottappilly G (1998). Effect of temperature on symptom expression and reliability of Banana streak badnavirus detection in naturally infected plantain and banana (*Musa* spp.). Plant Dis. 82:16-21.

Dahal G, Ortiz R, Tenkouano A, Hughes JD'A, Thottappilly G, Vuylsteke D, Lockhart BEL (2000b). Relationship between natural occurrence of banana streak badnavirus and symptom expression, relative concentration of viral antigen, and yield characteristics of some micro-propagated *Musa* spp. Plant Pathol. 49:68-79.

Daniells JW, Geering ADW, Bryde NJ, Thomas E (2001). The effect of Banana streak virus on the growth and yield of dessert bananas in tropical Australia. Ann. Appl. Biol. 139:51-60.

Fargette D, Konaté G, Fauquet C, Muller E, Peterschmitt M, Thresh JM (2006). Molecular ecology and emergence of tropical plant viruses. Annu. Rev. Phytopathol. 44:235-260.

Gayral P, Noa-Carrazana J, Lescot M, Lheureux F, Lockhart BEL, Matsumoto T, Piffanelli P, Iskra-Caruana M (2008). A single *Banana Streak Virus* integration event in the banana genome as the origin of infectious endogenous pararetrovirus. J. Virol. 82:6697-6710.

Geering ADW, Mcmichael LA, Dietzgen RG, Thomas JE (2000). Genetic diversity among Banana streak virus isolates from Australia. Phytopathology 90:921-927.

Geering ADW, Mcmichael LA, Dietzgen RG. Lockhart BEL (2001). Analysis of the distribution and structure of *Banana streak virus* DNA in a range of *Musa* cultivars. Mol. Plant Pathol. 2:207-213.

Geering ADW, Olszewski NE, Harper G, Lockhart BEL, Hull R. Thomas JE (2005). Banana contains a diverse array of endogenous badnaviruses. J. Gen. Virol. 86:511-520.

Harper G, Hart D, Moult S, Hull R, Geering A, Thomas J (2005). The Diversity of *Banana streak virus* isolates in Uganda. Arch. Virol. 150:2407-2420.

Hohn T (1999). Plant pararetroviruses-Caulimoviruses: Molecular biology. In: Encyclopedia of Virology. Granoff A. and Webster R.G. eds. Academic Press, San Diego. pp. 1281-1285.

Hull R, Geering A, Harper G, Lockhart BE, Schoelz JE (2005). Family Caulimoviridae. In Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses. Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U. and Ball L. A. eds. London: Elsevier pp. 385-396.

Iskra-Caruana ML, Gayral P, Galzi S, Laboureau N (2009). How to control and prevent the spread of banana streak disease when the origin could be viral sequences integrated in the banana genome? In Proceedings on Banana Crop Protection, Sustainable Production and Improvement of Livelihoods. Jones D. and Van den Bergh I. eds. Int. Soc. Hortic. Sci. pp. 77-84.

James AP, Geijskes RJ, Dale JL, Harding RM (2011). Development of a novel rolling-circle amplification technique to detect banana streak virus which also discriminates between integrated and episomal virus sequences. Plant Dis. 95:57-62.

Karanja L (2009). Diversity of *Banana streak virus* in Kenya. Ph. D. Thesis. Egerton University.

Karanja L, Wangai A, Harper G, Pathak RS (2008). Molecular identification of *Banana streak virus* isolates in Kenya. J. Phytopathol. 156:678-686.

Lheureux F, Laboureau N, Muller E, Lockhart BE, Iskra-Caruana ML **(**2007). Molecular characterization of banana streak acuminata Vietnam virus isolated from *Musa acuminata siamea* (banana cultivar). Arch. Virol. 152:1409-1416.

Lockhart EBL, Olszewski NE (1999). Plant pararetroviruses-Badnaviruses. In: Granoff A. and Webster R. G. eds. Encyclopedia of Virology. Academic Press, San Diego, pp. 1296-1300.

Mobambo N, Gauhl F, Swennen R, Pasberg-Gauhl C (1996). Assessment of the cropping cycle effects on black leaf streak severity and yield decline of plantain and plantain hybrids. Int. J. Pest Manag. 42:1-7.