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Relationship between natural occurrence of banana streak badnavirus and symptom expression, relative concentration of viral antigen, and yield characteristics of some micropropagated *Musa* spp.

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Micropropagated plants of 36 *Musa* genotypes with diverse genetic backgrounds, including 14 tetraploid plantain (TMPx) and banana (TMBx) hybrids, were evaluated for their response to banana streak badnavirus (BSV) infection under three environments from 1995 to 1997 in Nigeria. The characteristics evaluated were the natural incidence of BSV based on symptoms and virus indexing, relative concentration of BSV antigens in leaf tissues determined by ELISA, and some growth and yield descriptors. Virus occurrence and symptom expression, as well as the relative concentration of BSV antigens, fluctuated greatly between seasons during the cropping cycle, being high during the rainy season and low or negligible during the hot dry season. The natural incidence of plants with symptoms and BSV-infected plants varied between genotypes. Incidence of BSV on most International Institute of Tropical Agriculture (IITA) TMPx hybrids and three Fundación Hondureòa de Investigación Agrícola (FHIA) hybrids was high in the three environments, with some variation. Most landraces and some FHIA or Empresa Brasileira de Pesquisa Agropecuaria (EMBRAPA) hybrids were not BSV-infected under either environment at Onne. However, a few expressed some foliar symptoms at Ibadan and indexed BSV positive. The relative concentration of BSV antigens in leaf samples was also high in most TMPx and some FHIA hybrids, but low in most landraces. While BSV infection had no significant effect on most growth characteristics, it had a highly variable effect on bunch weight loss among the genotypes. There was no relationship between the natural incidence of BSV, concentration of viral antigen and bunch weight loss among the 11 TMPx hybrids, three FHIA hybrids and three plantain landraces. Despite the high natural BSV incidence and the high relative antigen concentration in their leaf tissue, TMPx 548-9, TMPx 2637-49, TMPx 7002-1 and FHIA 21 suffered less than 15% bunch weight loss, and TMPx 548-4 and FHIA 22 suffered no loss. These results suggest that under the conditions specified in this study, these hybrids could be tentatively classified as 'field tolerant' to BSV.

Keywords: banana streak badnavirus, banana, BSV, *Musa*, plantain, symptom expression

Introduction

Plantains and bananas (*Musa* spp.) are one of the world's most important, yet poorly investigated, staple food crops (Frison *et al*., 1997). They provide a significant source of carbohydrate for more than 400 million people in the tropical world (Swennen *et al*., 1995). In the humid forest and mid-altitude zones of sub-Saharan Africa, 35% of the world's *Musa* is produced, providing 25% of the carbohydrate requirement for approximately 70 million people (IITA, 1992; INIBAP, 1993). In addition, banana and plantain are a major source of income for small-scale farmers (Nweke *et al*., 1988). Due to intensification of crop production during the past 20 years, an increasing number of new pests and diseases have been identified that can cause a significant reduction in yield (Wilson, 1988; Swennen & De Langhe, 1989; Swennen *et al*., 1989; Ortiz & Vuylsteke, 1994; Ortiz, 1996). This, together with rising population pressure on the land, has led to altered farming

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practices (Ortiz & Vuylsteke, 1994). To address these problems, the International Institute of Tropical Agriculture (IITA) has focused its research on developing appropriate and ecologically sustainable resource, crop and pest management strategies in *Musa* farming systems (Vuylsteke *et al*., 1993). During the past decade, IITA scientists have developed plantain and banana hybrids (tropical *Musa* plantain or banana hybrids, respectively, designated TMPx and TMBx) that are resistant to black sigatoka disease caused by *Mycosphaerella fijiensis* (Vuylsteke *et al*., 1993; Vuylsteke & Ortiz, 1995). These hybrids are being evaluated for their yield performance and response to biotic and abiotic stresses (Ortiz *et al*., 1995; Ortiz, 1996). Recently, the occurrence of a streak disease caused by banana streak virus (BSV; genus *Badnavirus*) in these improved hybrids (Ortiz, 1996; Pasberg-Gauhl *et al*., 1996) and difficulty in obtaining virus-free plantlets through tissue culture (Crouch, 1996) have impeded their safe movement. Currently, BSV is considered a major constraint to banana and plantain improvement (Ortiz, 1996) and poses a threat to *Musa* production worldwide.

The first report of BSV was from Côte d'Ivoire in 1974 (Lassoudiere, 1974). It is now reported to occur in 43 countries of Africa, Asia, Europe, Oceania and Tropical America (Diekmann & Putter, 1996; Pasberg-Gauhl *et al*., 1996; Vuylsteke *et al*., 1996). BSV has bacilliform particles of 30×130–150 nm (Lockhart, 1986) with a circular dsDNA genome of 7·4 kbp (Lockhart, 1990), and exhibits genomic and serological heterogeneity (Lockhart & Olszewski, 1993). Although many naturally occurring isolates of BSV are known to occur (B. E. L. Lockhart, unpublished data), to date isolates from Costa Rica, Honduras, Morocco, Rwanda and Trinidad have been recognized as serologically and genomically distinct (Lockhart, 1994; Ndowora & Lockhart, 1999). Characteristics of a BSV isolate from southern Nigeria have been reported elsewhere (Dahal *et al*., 1998a; Thottappilly *et al*., 1998). The most common visual symptoms of BSV-infected plants include broken chlorotic streaks or spindle-shaped patterns on the lamina (Lockhart, 1986). Symptom expression varies depending upon virus isolate, host cultivar and environmental conditions (Lockhart, 1994; Gauhl & Pasberg-Gauhl, 1995; Dahal *et al*., 1998a,b).

Natural spread of BSV is by vegetative propagation of infected plant material and by mealybug vectors (Jones & Lockhart, 1993). BSV is semipersistently transmitted by the citrus mealybug (*Planococcus citri*) from banana to banana, and by the pink sugarcane mealybug (*Saccharicoccus sacchari*) from sugarcane to banana (Jones & Lockhart, 1993; Lockhart & Olszewski, 1993). Several species of mealybug, including a new species, *Planococcus musae*, have been identified from plantain fields in Nigeria (Matile-Ferrero & Williams, 1995). However, their role in BSV transmission and disease epidemiology has not been studied. Transmission of BSV through seeds has been reported (Danielles *et al*., 1995). The selection and deployment of host-plant resistance is considered the most sustainable approach for the management of pests and diseases of *Musa* spp. (Vuylsteke *et al*., 1993). While evaluating TMPx hybrids for yield performance and sigatoka resistance in multilocation trials, some with no symptom expression or low incidence of BSV symptoms under natural conditions were classified as 'resistant' to BSV (Ortiz, 1996). In addition, some hybrids with low BSV symptom incidence (e.g. TMPx 4479-1, TMPx 7152-2, TMBx 1378 and TMBx 5295-1) were classified as 'tolerant' (Ortiz & Vuylsteke, 1998a,b; Ortiz *et al*., 1998). However, some of these 'field resistant' or 'field tolerant' genotypes have a high incidence of typical BSV symptoms during the rainy season or when grown at subambient temperatures such as 22–24°C in a controlled environment (Dahal *et al*., 1999a,b). There are no reports of the possible mechanism of the field resistance or field tolerance of these genotypes. As resistance and tolerance of a host to viruses are independent mechanisms, it is essential to determine whether the poor expression or absence of symptoms in the breeding materials is due to resistance or tolerance (Bos & Parlevliet, 1995). For plant virus diseases, the term 'resistance' has been used to refer to the ability of a host to hinder 'infection, multiplication and invasion by virus(es)', whereas the term 'tolerance' has been used to refer to a host 'that a specific virus can infect and in which it can replicate without causing severe symptoms or greatly diminishing the rate or amount of growth or marketable yield' (Cooper & Jones, 1983). In this paper the term 'symptoms' is used to refer only to foliar symptoms. Symptom incidence alone may not truly reflect the extent of virus invasion in plants because of the possibility of variation in invasion, replication or cell-to-cell spread of a virus among infected genotypes (Cooper & Jones, 1983; Pataky *et al*., 1990). This, together with an erratic distribution of BSV symptoms and antigens over individual leaves as well as between different leaves of the same plant (Lockhart, 1986; Dahal *et al*., 1998b), may explain why some genotypes suffer lower yield loss than others. In such cases, additional characteristics such as symptom severity, virus titre and yield have been used to determine and differentiate between 'tolerance' and 'resistance' (Pataky *et al*., 1990; Kerns & Pataky, 1997). Therefore, the objective of this study was to evaluate plantain and banana hybrids, based on symptom incidence, virus indexing and relative BSV antigen concentration in the infected tissues, and to determine the effects of these parameters on the growth and yield characteristics of some plantain hybrids.

Materials and methods

Micropropagated plants

In vitro plantlets of 11 *Musa* genotypes (Pisang Ceylan, Pelipita, Saba, Yangambi km-5, EMB 402, EMB 403,

EMC 602, FHIA-1, FHIA-2, FHIA-3 and FHIA-23), derived from 'virus-free' mother plants (Frison & Putter, 1989), were received from the International Network for the Improvement of Banana and Plantain (INIBAP) Virus Indexing Centre, Montpellier, France. Micropropagated plants of 25 other genotypes (Table 1) were produced at IITA High Rainfall Station at Onne, south-eastern Nigeria (Ortiz *et al*., 1997). The mother plants in plantain fields were sampled randomly and indexed for BSV by immunosorbent electron microscopy (ISEM) (Dahal *et al*., 1999a) and, whenever possible, BSV-negative plants were used for micropropagation. In some cases mother plants without symptoms, which are normally BSV-negative (Dahal *et al*., 1998b), were also used. All the micropropagated plants were established in soil as described by Vuylsteke (1989) and kept in a polyethylene tunnel for 2–3 months before being planted in the field.

Virus isolates

The BSV isolates studied occurred in naturally infected plantain hybrids at the IITA High Rainfall Station at Onne (Pasberg-Gauhl *et al*., 1996). Some biological and molecular characteristics of an isolate (BSV-Onne) collected from this station have been described elsewhere (Harper *et al*., 1996; Dahal *et al*., 1998a; Dahal *et al*., 1999b; Thottappilly *et al*., 1998). Nigerian isolates of BSV are serologically distinct from several previously described BSV isolates (Lockhart & Olszewski, 1993) but they remain to be characterized. All these isolates can be efficiently and reliably detected

Table 1 Average percentage BSV incidence or symptom incidence (in parentheses) of 36 plantain and banana genotypes during October 1996, under three environments (sole crop at Ibadan, sole and alley crop at Onne) in Nigeria

Genotypes	Genome	Ibadan (sole)	Onne (alley)	Onne (sole)	Average	Student–Newman–Keul's Test[a]
TMPx 7002-1	AAB×AA	80(80)	70(100)	70(90)	73(90)	A a
TMPx 2637-49	AAB×AA	90(90)	40(60)	70(60)	67(70)	AB b
TMPx 548-4	AAB×AA	70(60)	50(30)	60(50)	60(47)	ABC bcd
TMPx 7152-1	AAB×AA	80(70)	50(40)	40(30)	57(47)	ABCD bcd
TMPx 5511-2	AAB×AA	60(60)	60(50)	40(30)	53(47)	ABCD bcd
TMPx 548-9	AAB×AA	70(80)	50(40)	30(40)	50(53)	ABCDE bc
TMPx 1112-1	AAB×AA	40(30)	10(20)	70(20)	40(23)	BCDEF cdefg
TMPx 1658-4	AAB×AA	20(10)	30(10)	30(0)	27(7)	DEFGH fg
TMBx 5295-1	AAB×AA	10(10)	20(10)	40(30)	23(17)	DEFGH efg
TMPx 2796-5	AAB×AA	30(50)	20(30)	20(30)	23(37)	EFGH cdef
TMPx 6930-1	AAB×AA	60(40)	0(0)	0(0)	20(13)	FGH fg
TM3x 15108-6	[AAB×AA]×AA	30(10)	0(20)	0(0)	10(10)	FGH fg
TMBx 612-74	ABB×AA	10(10)	0(0)	0(0)	3(3)	H fg
TMBx 1378	ABB×BB	0(0)	0(0)	0(0)	0(0)	H g
EMBRAPA 402	AAB×AA	20(10)	0(0)	0(0)	7(3)	GH fg
EMBRAPA 403	AAB×AA	0(0)	0(0)	0(0)	0(0)	H g
EMCAPA 602	Tetraploid from AAB?	0(0)	0(0)	0(0)	0(0)	H g
FHIA 22	AAB×AA	50(40)	30(40)	30(50)	37(43)	BCDEF bcde
FHIA 21	AAB×AA	50(50)	60(60)	0(0)	37(37)	CDEFG cdef
FHIA 1	AAB×AA	10(10)	0(0)	0(0)	3(3)	H fg
FHIA 2	AAA×AA	0(0)	0(0)	0(0)	0(0)	H g
FHIA 3	{[ABB×BB]×AA}×AA	0(0)	0(0)	0(0)	0(0)	H g
FHIA 23	AAA×AA	0(0)	0(0)	0(0)	0(0)	H g
SH 3436-9	AAA×AA	0(0)	0(0)	0(0)	0(0)	H g
SH 3640	AAB×AA	0(20)	0(10)	0(10)	0(13)	H fg
Agbagba	AAB	20(10)	20(40)	0(10)	13(20)	FGH defg
UNN Dbl Bunch	AAB	30(40)	0(0)	0(0)	10(13)	FGH fg
Saba	ABB (or BBB)	20(20)	0(0)	0(0)	7(7)	FG fg
Obino l'Ewai	AAB	10(10)	0(0)	10(0)	7(3)	GH fg
Fougamou	ABB	10(20)	0(0)	0(0)	3(7)	H fg
Bluggoe	ABB	10(10)	0(0)	0(0)	3(3)	H fg
Cardaba	ABB	0(0)	0(0)	10(0)	3(0)	H g
Pisang Ceylan	AAB	0(0)	0(0)	0(0)	0(0)	H g
Pelipita	ABB	0(0)	0(0)	0(0)	0(0)	H g
Yangambi km-5	AAA	20(10)	0(0)	10(0)	10(3)	FGH fg
Valery	AAA	0(0)	10(0)	10(0)	7(0)	H g
Standard error for means		–	–	–	6(7)	5(6)

[a]Based on arc-sin-transformed data; same letters between rows are not significantly different at $P < 0{\cdot}05$.

by ISEM and by triple antibody sandwich ELISA (TAS-ELISA) using polyclonal antisera prepared from a pool of BSV isolates (Ndowora *et al*., 1999).

Field experiments

The field experiments were conducted from August 1995 to 1997 under three environments at two locations (Ibadan and Onne) in Nigeria, as a part of multilocation evaluation of improved black sigatoka-resistant *Musa* germplasm. The multilocation trial included 10 tetraploid TMPx, three tetraploid TMBx and one secondary triploid (TM3x) developed at IITA, three tetraploid banana hybrids (EMBRAPA or EMCAPA) from Brazil, eight tetraploid hybrids from Fundación Hondureòa de Investigación Agrìcola (FHIA), five triploid Asian exotic bananas (Bluggoe, Cardaba, Fougamou, Pelipita, Saba), three triploid African plantain landraces (Agbagba, Obino l'Ewai, UNN Double Bunch) and three triploid dessert bananas (Pisang Ceylan, Valery, Yangambi km-5). The experiments were carried out using five plants of each genotype in two replications at each site in a simple lattice-square design.

Assessment of symptoms

BSV symptoms on all plants were monitored at monthly intervals. To quantify symptom severity, individual leaves were scored on a 0–3 scale (Dahal *et al*., 1999b), where 0 indicates no visible symptoms; 1, very few streaks or chlorotic flecks on the leaf lamina (<10% of the lamina affected); 2, streaks or chlorotic flecks covering a moderate portion of the lamina (>10% but <50%); 3, most of the leaf lamina covered with streaks or chlorotic flecks (>50%). The total number of leaves and number of leaves with symptoms were recorded from each plant. The symptom severity index (SSI) (Dahal *et al*., 1999b) was calculated from the severity score of individual leaves as $SSI = (0a + 1b + 2c + 3d)/n$, where a–d are the number of leaves with scores of 0–3, respectively, and n = total number of leaves observed. The SSI of individual plants was averaged to give an average symptom severity index (ASSI) of each genotype according to Dahal *et al*. (1999b). During the rainy season, when BSV symptoms were more conspicuous, all the plants were indexed for BSV by ELISA.

BSV indexing

For the confirmation of BSV infection, the plants were indexed by ELISA as described by Ndowora *et al*. (1999). For all indexing, small portions of tissue (2 × 5–10 cm) from 3 to 4 fully expanded leaf laminae (excluding old, dying leaves and the unrolling 'cigar' leaf) were made into 2–3 g composite samples. The samples were cut into fine pieces and about 1 g of this tissue was ground in 3 mL PBS (100 mM phosphate pH 7·0 containing 2% NaCl, 1% Na_2SO_4 and 0·05% v/v Tween 20) using a leaf and bud press (Erich Pöllahne, Wennigsen, Germany). The extract was used directly or after a brief (2–3 min) centrifugation in microtitre plates (Dynatech Laboratories) previously coated with 1 mg mL^{-1} BSV IgG. The trapped BSV antigens were detected using a 1 : 2000–1 : 4000 dilution of IgG–alkaline phosphatase conjugate in PBS with 2% w/v polyvinyl pyrrolidone (PVP) and 0·2% w/v BSA. The reactions were visualized by addition of 0·5 mg mL^{-1} *p*-nitrophenyl phosphate substrate. Tissues from BSV-infected TMPx 4698-1 plants with typical streak symptoms (the presence of BSV was confirmed by ISEM) were used as the positive control, and symptomless plants in which no virus particles were seen by ISEM as the negative control. Absorbance values (A_{405}) greater than twice the mean value (from at least four replicate wells) of healthy controls were considered to be positive for BSV. Results of previous studies (G. Dahal, unpublished data) showed that plantain hybrid TMPx 4698-1 consistently expressed severe foliar and fruit symptoms and contained high concentrations of BSV antigens in infected tissues. Therefore BSV-infected tissue from this genotype was used as susceptible control in ELISA assays (Dahal *et al*., 1998a, 1999a). To determine the relative BSV antigen concentration (with respect to TMPx 4698-1) in infected tissues of the various genotypes, composite samples were used from at least five plants of each genotype. Because of no or less BSV infection in most landraces, the number of plants used varied (one to four) depending on the availability of BSV-infected plants. The composite samples (0·5 g) were homogenized in 1·5 mL PBS-T buffer and 200 μL was added to three wells of the microtitre plates as described above. The average absorbance (A_{405}) value was used as an indicator of the relative BSV antigen concentration in the tissues.

Effect of BSV on yield characteristics

Four growth characteristics (days to flowering, plant height, total number of leaves and height of the tallest sucker) were recorded at monthly intervals, and four bunch descriptors (bunch weight, number of hands, number of fruits and fruit weight) were recorded at harvest. Due to differences in maturity, the harvesting dates varied considerably between plants of the same genotype and between genotypes. Because there were insufficient infected plants of most of the landraces, the effect of BSV infection on growth and yield characteristics was determined primarily on TMPx and two FHIA hybrids. The percentage yield reduction (YR), as measured by bunch weight per plant (Ortiz, 1995) relative to the symptomless and BSV-negative plants of the same genotype, was calculated as YR% = 100 [(yield of BSV-positive plants – yield of BSV-negative plants)/yield of BSV-negative plants].

Data analysis

The percentage incidence data based on symptoms and BSV indexing were transformed to angles (Gomez & Gomez, 1984) and the transformed data were

analysed using the GLM procedure in Statistical Analysis System software (SAS, 1996). Growth and yield characteristics of BSV-positive and BSV-negative plants of the same genotype were analysed within location or across the locations using the GLM procedure and the means were compared by Student–Newman–Keul's test.

To enable a comparison of symptom development on different genotypes, the area under the disease progress curve (AUDPC) plotted for the ASSI, transformed values of the percentage of plants with symptoms, and number of leaves per plant with symptoms over time were determined using the mid-point rule for area estimation (Campbell & Madden, 1990). Other characteristics associated with disease development (Y_f, final incidence of plants with symptoms; X_0, days from transplanting to first appearance of symptoms; X_{50}, days to reach 50% incidence of plants with symptoms; and r, apparent rate of increase in symptom development) were calculated and analysed using principal components analysis.

Results

Incidence based on symptoms and virus indexing

The analysis of variance for a lattice design, for incidence based on plants with symptoms or BSV indexing, showed that the intrablock error was greater than the interblock error in each independent environment except for BSV indexing at Ibadan. The lattice design was not more efficient than the randomized complete block design at controlling the experimental error across the two environments at Onne. No mean adjustments were made for the BSV scores of the 36 genotypes, because the suggested lattice adjustments were negative for genotypes not infected with BSV. The results of BSV incidence based on plants with symptoms and BSV indexing by ELISA were generally similar across the environments (Table 1) and there was a significant phenotypic correlation ($r = 0{\cdot}95$, $P < 0{\cdot}001$) between the two scoring systems. Both the incidence of plants with symptoms and of BSV-infected plants as determined by ELISA were high for most TMPx hybrids in the three environments, with some apparent variation between environments. While the genotypes differed significantly ($P < 0{\cdot}05$) in their response to symptom expression and BSV infection, neither the environments nor the genotype–environment interactions were significant ($P > 0{\cdot}05$) for the incidence of symptom-bearing and BSV-infected plants.

Irrespective of location, nine hybrids (TMPx 548-4, TMPx 548-9, TMPx 5511-2, TMPx 7002-1, TMPx 2637-49, TMPx 7152-1, TMPx 111-2, FHIA21 and FHIA22) had significantly higher (>37%; $P < 0{\cdot}05$) incidence of plants with symptoms than the other hybrids (<27%) and most of the landraces (<13%). For the other seven hybrids the incidence of plants showing symptoms was generally comparable with that of most landraces. While most TMPx hybrids expressed symptoms in all three environments, TMPx 6930-1, TMP3x 15108-6, TMBx 612-74, FHIA 1, FHIA 21 and EMBRAPA 402 expressed symptoms in only one or two environments; seven hybrids (FHIA 2, FHIA 3, FHIA 23, EMBRAPA 403, EMCAPA 602, TMBx 1378 and SH 3436-9) did not express symptoms in any environment (Table 1). Likewise, nine of the 11 landraces expressed symptoms in only one or two environments, and Pisang Ceylan and Pelipita did not express symptoms in either environment. Some plants of Agbagba expressed symptoms at Ibadan and Onne (alley crop), while other landraces (Fougamou, Obino l'Ewai, Saba, Bluggoe, Yangambi km-5, Valery and UNN Double Bunch) expressed symptoms only at Ibadan and only in one to three plants (Table 1).

While most plantain-derived TMPx hybrids had a slightly higher BSV incidence (as determined by ELISA) than symptom incidence, some other hybrids (TMPx 7002-1, TMPx 2637-49, TMPx 548-9, TMPx 2796-5 and FHIA 22) had a slightly higher incidence of plants with symptoms (Table 1). Nonetheless, the difference in the average BSV incidence between the two scoring systems was not significant ($P < 0{\cdot}05$). When the anomalous samples were re-indexed by ELISA, most of the plants with symptoms that gave ELISA-negative samples were indexed as BSV positive in the second test. Despite these disparities, generally BSV antigens were not detected in samples collected from symptomless plants of either hybrids or landraces. Most plants of the landraces expressed no visible symptoms, and BSV antigen was detected from only a few of the composite samples (Table 1). However, one or two symptomless leaf samples of five landraces (Agbagba, Obino l'Ewai, Cardaba, Yangambi km-5 and Valery) were indexed BSV positive by ELISA.

Concentration of BSV antigens in infected tissues

The relative concentration of BSV antigens in samples taken from infected plants varied depending on the genotype. It was relatively high in most TMPx and some FHIA hybrids, but low in most landraces. A biplot of BSV incidence (%) vs. absorbance value (A_{405}) indicated that most IITA and FHIA hybrids that had a higher BSV incidence (>20%) than most of the landraces also had a higher A_{405} value (>1·0) (Fig. 1). The relative antigen concentration in the landraces, except in Agbagba, was very low, often being only slightly above the detection threshold. In Agbagba, the BSV antigen concentration was comparable with that of some of the TMPx and TMBx hybrids. In these experiments, the concentration of virus antigens in TM3x 15108-6 and TMPx 6930-1 was low and comparable with those of the landraces (Fig. 1). Most of the plantain hybrids that had a high A_{405} value also expressed severe symptoms under field conditions, at least during the rainy season, suggesting a relationship between symptom severity and BSV antigen concentration.

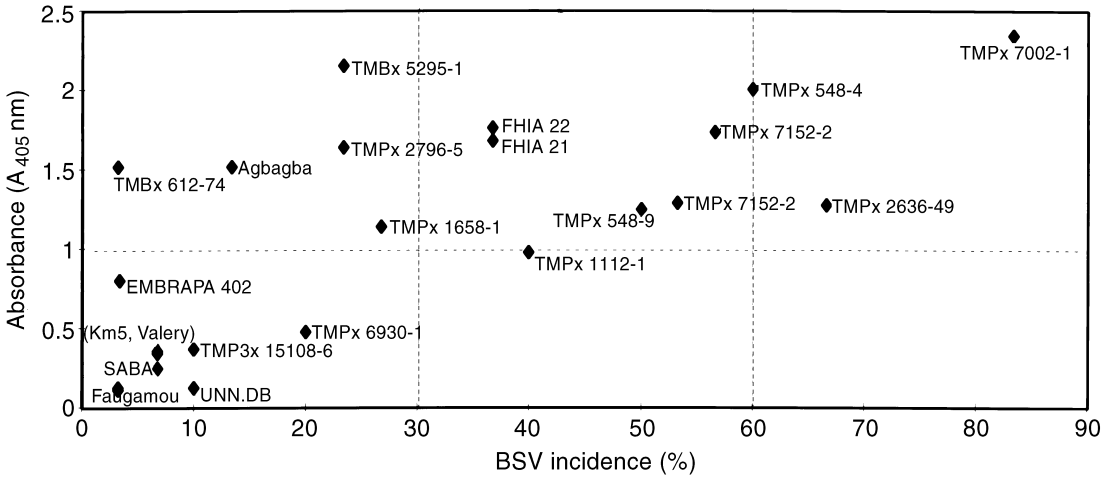

Figure 1 Relationship between average percentage of BSV-infected plants and mean BSV antigen concentration (A_{405}) of 16 hybrids (13 TMPx, two FHIA and one Brazilian) and six plantain/banana landraces grown during 1996 under three environments in Nigeria. Each point for absorbance (A_{405}) represents the mean of five composite samples, except for the following genotypes where means were obtained from the number of samples indicated: TMBx 1612-74 = 1; TM3x 15108-6 = 3; EMBRAPA 402 = 2; FHIA = 1; Obino l'Ewai = 2; Saba = 2; Agbagba = 4; Yangambi km-5 = 3; Valery = 2. The values for BSV incidence are as shown in Table 1.

Seasonal fluctuations in symptom expression and BSV antigens

Symptom incidence, percentage of leaves per plant with symptoms, and ASSI values of most TMPx hybrids fluctuated according to the season (data not shown). Symptom incidence was high between August and November during the rainy season, and low during the dry season between December and April (Table 2). In October 1996 (rainy season), the incidence of plants that tested positive by ELISA was also high and generally comparable with symptom incidence. In April 1997 (dry season), the number of plants with symptoms and the number of BSV-positive plants were low.

Effect of BSV on growth and yield characteristics

There was variable effect of BSV on some yield parameters (Fig. 2a–c). Despite some variation between the genotypes, BSV-infected plants generally had significantly lower bunch weight ($P < 0{\cdot}0105$), fewer hands ($P < 0{\cdot}0309$) and fewer fruits ($P < 0{\cdot}0006$) than apparently healthy plants. For bunch weight, the second-order interactions between BSV infection and genotype ($P = 0{\cdot}4156$) or location ($P = 0{\cdot}5974$) were not significant, but the third-order interaction between BSV infection, genotype and location was significant ($P < 0{\cdot}0309$). In these experiments, BSV infection had no significant effect on days to flowering ($P = 0{\cdot}7408$) and plant height ($P = 0{\cdot}1764$) (data not shown).

Table 2 Seasonal fluctuation in percentage symptom incidence (± SE) and BSV-positive plants (± SE) of 13 naturally infected plantain and banana hybrids during 1996–97 at Ibadan, Nigeria

	Symptom incidence (%)			BSV-positive plants (%)	
Genotype	October 1996	January 1997	April 1997	October 1996	April 1997
TMPx 7002-1	80 ± 12·6	30 ± 14·5	10 ± 9·5	80 ± 12·7	20 ± 12·7
TMPx 7152-2	70 ± 14·5	30 ± 14·5	20 ± 12·6	80 ± 12·7	10 ± 9·5
TMPx 1112-1	70 ± 14·5	40 ± 15·5	30 ± 14·5	40 ± 15·5	20 ± 12·6
TMPx 548-4	60 ± 15·5	70 ± 14·5	10 ± 9·5	70 ± 14·5	0 ± 0
TMPx 548-9	80 ± 12·6	40 ± 15·5	0 ± 0	70 ± 14·5	10 ± 9·5
TMPx 2637-49	60 ± 15·5	30 ± 14·5	0 ± 0	80 ± 12·7	10 ± 9·5
TMPx 5511-2	60 ± 15·5	10 ± 9·5	0 ± 0	60 ± 15·5	0 ± 0
TMPx 2796-5	50 ± 15·8	40 ± 15·5	0 ± 0	30 ± 14·5	0 ± 0
TMPx 1658-4	40 ± 15·5	0 ± 0	0 ± 0	20 ± 12·6	10 ± 9·5
TMPx 6930-1	20 ± 12·6	0 ± 0	0 ± 0	30 ± 14·5	0 ± 0
TM3x 15108-6	10 ± 9·5	0 ± 0	0 ± 0	30 ± 14·5	10 ± 9·5
TMBx 612-74	10 ± 9·5	10 ± 9·5	10 ± 9·5	10 ± 9·5	0 ± 0
TMBx 5295-1	10 ± 9·5	0 ± 0	0 ± 0	10 ± 9·5	10 ± 9·5

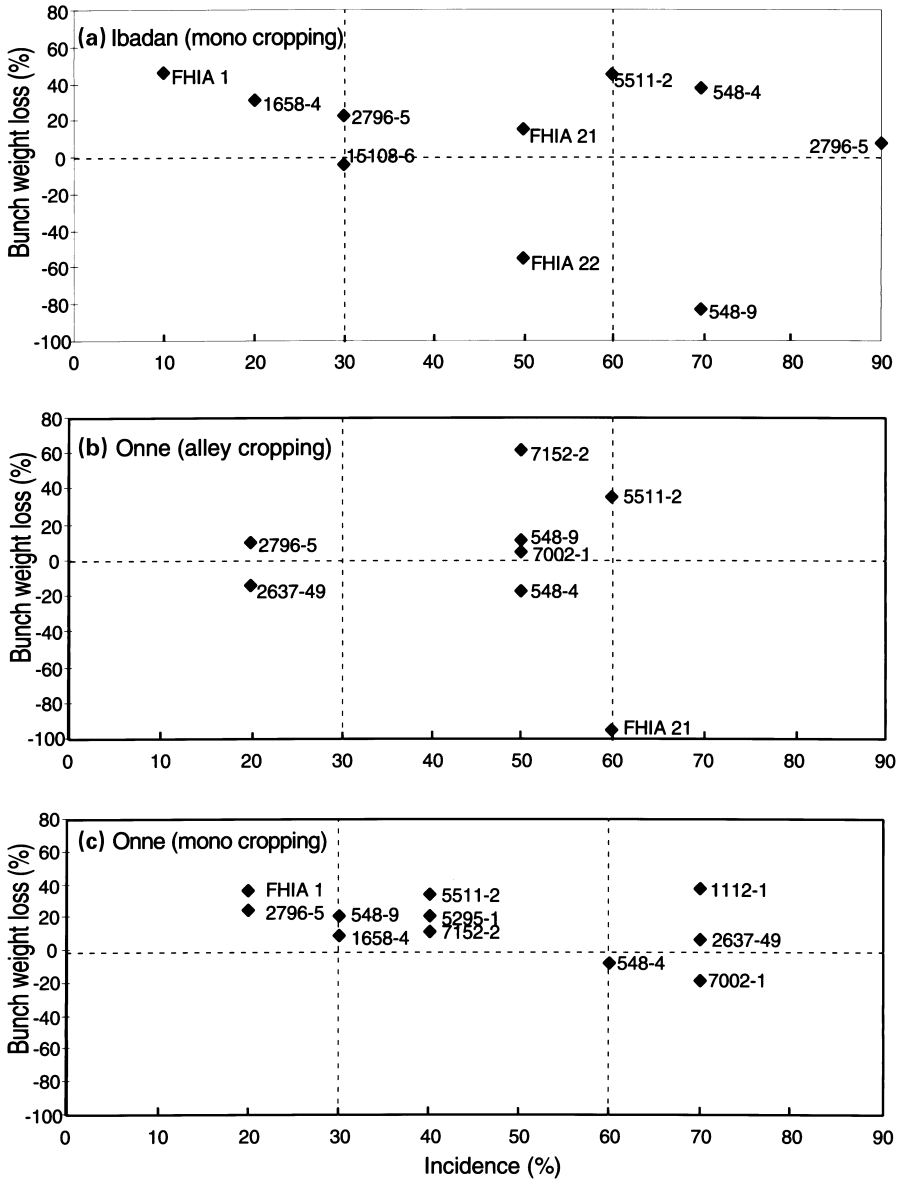

Figure 2 Relationship between the percentage natural incidence of banana streak badnavirus infection and bunch weight loss (%) of 10 TMPx and three FHIA hybrids grown during 1996 under three environments in Nigeria. (a) Ibadan (monocropping); (b) Onne (alley cropping); (c) Onne (monocropping). The values for incidence are as shown in Table 1. The values for bunch weight loss were calculated from ELISA-positive and ELISA-negative plants shown in Table 1.

The relationships between the average A_{405} and percentage bunch weight loss of 11 IITA hybrids, four FHIA hybrids and three landraces are shown in Fig. 3. Some IITA hybrids suffered up to 82% reduction in bunch weight. Average BSV incidence in TMPx 1658-4 and TMPx 2796-5 was 23 and 27%, respectively, and there was only a 9% reduction in bunch weight. In contrast, TMPx 7002-1, TMPx 548-4 and TMPx 2637-49 had much higher BSV incidence and concentration of BSV antigens, yet suffered no, or less than 7%, reduction in bunch weight. TM3x 15108-6 had few symptoms, a low antigen concentration and no yield loss. The FHIA hybrids that had moderate (37%) BSV incidence and a high antigen concentration had no, or less than 15%, yield loss (Fig. 3).

Relationships between components of disease development

Analysis of eight characteristics of banana streak disease development indicated significant correlation ($r > 0{\cdot}7$, $P < 0{\cdot}001$) of A-PROP (area under progress curve of percentage of leaves with symptoms per infected plant) with A-ASSI (area under progress curve derived from

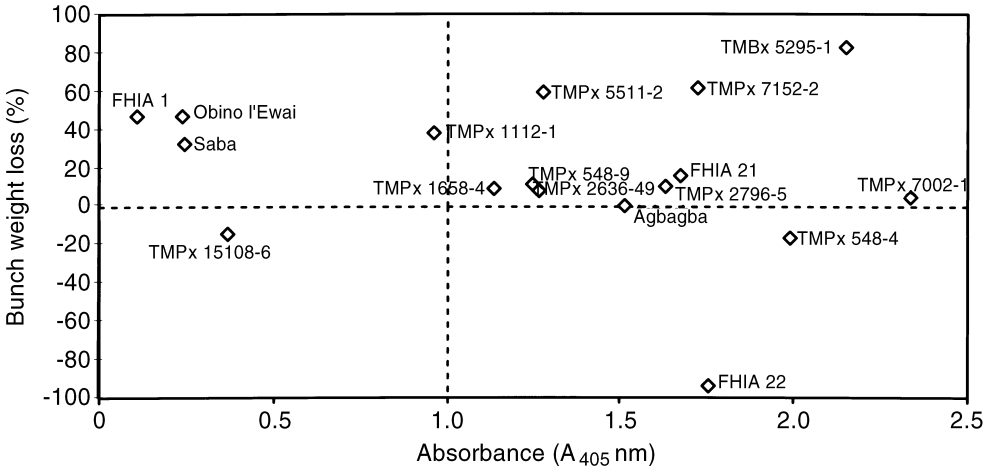

Figure 3 Relationship between the relative BSV antigen concentration (absorbance, A_{405}) and percentage bunch weight loss of 11 TMPx hybrids, three FHIA hybrids and three plantain landraces grown during 1996 under three environments in Nigeria. Each point for A_{405} represents mean of five composite samples, except for the following genotypes where means were obtained from the number of samples indicated: FHIA = 1; Obino l'Ewai = 2; Saba = 2; TM3x 15108-6 = 3; Agbagba = 4. Bunch weight values were the average of BSV-infected (ELISA-positive) plants shown in Table 1.

average symptoms severity index) and A-INC (area under progress curve derived from percentage of plants with symptoms), but not with X_0 and X_{50} (Table 3).

Discussion

Breeding for resistance to BSV has been less successful than breeding for resistance to other pathogens such as black sigatoka. Although BSV was described about a decade ago (Lockhart, 1986), until recently efficient and reliable diagnostic techniques were not available. In addition, the recent discovery that BSV genomic sequences are integrated into the *Musa* genome (LaFleur *et al*., 1996; Ndowora *et al*., 1999) have further complicated the issue. A few reports on resistance to BSV infection (Crouch, 1996; Ortiz, 1996; Ortiz &

Table 3 Pearson correlation coefficients among nine disease-development components associated with banana streak badnavirus of plantain and banana hybrids grown under three environments in Nigeria, 1995–97

Parameters[a]	Y_0	Y_f	X_0	X_{50}	A-ASSI	A-INC	A-PROP	BSV-T
Y_f	0·2484							
	0·025[b]							
X_0	−0·0192	−0·2644						
	0·8646	*0·0170*						
X_{50}	−0·3226	−0·2261	0·2551					
	0·0238	0·1182	0·0769					
A-ASSI	0·0142	0·3433	0·1629	0·4773				
	0·8997	*0·0017*	0·1462	*0·0005*				
A-INC	0. 1491	*0·7122*	−0·1142	0·0679	*0·7469*			
	0·1839	*0·0001*	0·3102	0·6431	*0·0001*			
A-PROP	0·0292	0·3631	0·1511	0·4372	*0·9678*	*0·8009*		
	0·7956	*0·0009*	0·1781	*0·0017*	*0·0001*	*0·001*		
BSV-T	−0·0981	−0·4204	0·3115	0·2257	−0·2054	−0·2729	−0·1762	
	0·6986	0·0824	0. 2082	0·5046	0·4134	0·2731	0·4844	
Bunch wt	−0·2005	−0·1979	0·3460	0·0755	−0·1594	−0·3520	−0·1892	−0·3173
	0·3262	0·2226	0·0834	0·7586	0·4366	0·0778	0·3547	0·2690

[a] Y_0 = initial incidence of plants with symptoms; Y_f = final incidence of plants with symptoms; X_0 = time (days) from transplanting to first appearance of symptoms; X_{50} = time (days) to reach 50% incidence of plants with symptoms; A-ASSI = area under progress curve derived from average symptom severity index; A-INC = area under progress curve derived from percentage of plants with symptoms; A-PROP = area under progress curve derived from percentage of leaves with symptoms per infected plant; BSV-T = concentration of BSV antigens (A_{405}); Bunch wt = average bunch weight of BSV-infected plants.

[b] Probability of correlation coefficients being equal to zero; significant values are shown in italics.

Vuylsteke, 1998a,b; Ortiz *et al*., 1998) are based on symptoms alone. In Nigeria, symptom expression by BSV-infected plants was highly affected by both genotype and environment; some genotypes, such as TMPx 4698-1, TMPx 548-9 and TMPx 548-4, expressed severe symptoms during the cooler rainy season but no symptoms during the hot dry season (Dahal *et al*., 1998a). Even under conditions that are conducive to symptom expression, most landraces remained symptomless but virus particles were detected occasionally by ISEM (Dahal *et al*., 1998a). This, and the gross similarity between the symptoms caused by BSV and cucumber mosaic virus, makes symptom-based evaluation for BSV unreliable. Techniques have recently been developed for the serological detection of BSV in leaf tissues by ELISA (Thottappilly *et al*., 1998; Ndowora & Lockhart, 1999), and this is the first report on the application of ELISA for evaluating *Musa* genotypes for resistance to BSV.

In this study, more than 36 genotypes with diverse genetic backgrounds, including 14 registered TMPx hybrids, were evaluated for natural occurrence of BSV based on symptom expression, virus indexing, relative antigen concentration, bunch weight loss and components of the disease progress curve. Preliminary tests with these virus isolates showed a good correlation between A_{405} absorbance in ELISA and the number of virions observed by ISEM using partially purified extracts (B.E.L. Lockhart, unpublished data), suggesting that A_{405} values were a reliable indication of virus titre. With a few exceptions, symptom incidence and virus indexing by ELISA were correlated; however, some symptomless plants had detectable BSV antigens (Dahal *et al*., 1998a, 1999a). In the current study, the incidence of BSV varied greatly between the genotypes and locations, and was significantly higher in most TMPx hybrids than in the landraces. Despite the variation in BSV incidence in most of the TMPx hybrids, the BSV antigen concentration in infected tissues was uniformly high. In TM3x 15108-6, the antigen concentration was low and comparable with most BSV-infected landraces. In addition, the results of this study showed that BSV infection had significant effects on bunch weight and on number of hands and fruits. In contrast to earlier reports (Lassoudiere, 1974, 1979), bunch characteristics were compared between plants of the same genotypes that tested positive and negative for BSV by ELISA. Yield loss, as measured by bunch weight per plant (Ortiz, 1995), varied greatly between most of the BSV-infected TMPx (7–61%) and FHIA hybrids. The FHIA hybrids that had moderate symptom incidence (37%) and a high antigen concentration had only 15% yield loss. Therefore the results of this study suggest that an effective evaluation of *Musa* genotypes for BSV should be based on a combination of symptom incidence, BSV indexing, antigen concentrations and other parameters for disease development, including yield characteristics.

Although BSV is transmitted by mealybugs (Lockhart, 1995) and through seed (Danielles *et al*., 1995), there is no evidence that horizontal transmission of BSV by these means occurs to any significant extent in nature (Lockhart, 1995). Based on the mean incidence of plants with symptoms among parental genotypes (Obino l'Ewai × Calcutta 4; Bobby Tannap × Pisang lilin) and their progenies, Ortiz (1996) suggested that epistatic genetic combinations arising from a specific sexual cross probably induce apparent susceptibility to BSV and symptom expression. However, recent reports suggest that BSV infection in the *Musa* genotypes used in this and other studies (Ortiz, 1996; Dahal *et al*., 1998b) probably resulted either from vegetative propagation from infected source plants, or from tissue culture-induced activation of integrated viral sequences (Ndowora *et al*., 1999). If so, the BSV isolates described in this study may differ in several respects, including pathogenecity, towards different *Musa* genotypes. The results therefore need to be interpreted with caution. They suggest that some *Musa* genotypes are able to tolerate infection by a given isolate over a single growth cycle. How these *Musa* genotypes would respond to infection by the same BSV isolate over a longer period of time, and to infection by other BSV isolates, remains to be determined. The data presented above indicate that within the *Musa*–BSV pathosystem there may be differential host–virus interactions that can be exploited by plant breeders, even in the case of *de novo* episomal virus infection arising from integrated viral sequences. To take practical advantage of these opportunities, it will be important to define these virus–host interactions more clearly at both field and molecular levels.

Because of the integration of BSV DNA sequences into the *Musa* genome (Ndowora *et al*., 1999) and lack of understanding of the mechanisms of integration and expression, it is difficult at present to develop an effective strategy for breeding improved *Musa* hybrids with resistance to BSV. Therefore, short-term alternatives are required for the management of BSV in farmers' fields. For this purpose, the genotypes in this study have been grouped based on BSV incidence as follows: (i) genotypes showing less than 30% infection; (ii) genotypes whose infection rate varied between 30 and 60%; (iii) genotypes with an infection rate exceeding 60%. Four TMPx hybrids (15108-6, 2796-5, 5295-1 and 1658-4) are in category (i); four TMPx (1112-1, 548-9, 5511-2, 7152-2) and two FHIA hybrids (FHIA 21 and FHIA 22) in category (ii); and three TMPx hybrids (548-4, 2637-49, 7002-1) in category (iii). Despite their high relative antigen concentration, six genotypes (TMPx 548-9, TMPx 548-4, TMPx 2637-49, TMPx 7002-1, FHIA 21 and FHIA 22) from categories (ii) and (iii) had less than 15% bunch weight loss. Therefore under the conditions specified in this study (same virus isolates and growing conditions), the genotypes in category (i) and the six genotypes with relatively high BSV incidence but low or no yield loss could be regarded as 'field tolerant' to BSV according

to the definition of Cooper & Jones (1983). Further evaluation of these genotypes to determine their true response under controlled conditions and to ensure their continued yield performance in subsequent crop cycles is important because, if the yield gains are removed from these 'field tolerant' genotypes, they become similar to susceptible genotypes.

BSV appears to be widely distributed throughout Africa and elsewhere (Diekmann & Putter, 1996). Nevertheless, until new genotypes with resistance are identified using appropriate techniques, yield losses could be avoided by growing these field tolerant genotypes under warmer conditions as described in this study. While doing so, appropriate precautions should be taken as there is considerable risk that field tolerance may be overcome if plants are grown in cooler conditions that are conducive to BSV development. This can occur due to distribution of the field tolerant germplasm, for example by farmers and breeding programmes. Alternatively, symptom expression could be triggered by a local change in environmental conditions. However, owing to the lack of information on the interaction between different BSV isolates and various *Musa* genotypes, extreme care should be taken in the distribution and cultivation of these field tolerant genotypes. They may also provide a virus source in the field and thus create some risk of spread, though there is no documented evidence of large-scale field spread of BSV (Lockhart, 1995). If these field tolerant genotypes perform well under the specified warmer environmental conditions, such tolerance can be combined with resistance to BSV infection when available. One possibility is that tolerant genotypes can be transformed with BSV-derived genes for additional protection. In this way, the risk of further spread of BSV could be minimized. Under very high selection pressure, resistance to the virus alone may not be stable, and it is conceivable that a combination of field resistance and field tolerance must be exploited to protect plantain and banana germplasm from BSV infection.

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