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Effect of environment and cultivar on the expression of banana streak disease symptoms in Kenya

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Banana (*Musa* spp.) is grown for subsistence and income generation by 80% of small scale farmers all year round in Kenya hence it is an important food security crop. However viral diseases such as banana streak disease (BSD), caused by Banana streak virus, hamper the production of the crop. BSV has been reported to be present in all the commercial banana cultivars in Kenya. Tissue culture materials of 15 cultivars were evaluated for BSV expression so as to determine the tolerant cultivars under field and greenhouse conditions and stages of disease expression. A number of factors including plant age, cultivar, season and growth site were investigated in the greenhouse and field to assess their effect on BSD symptom expression. Statistical analysis system (SAS) for ANOVA was used for analysis of data for the experiments. Results indicated that BSD symptom expression is significantly influenced by all the factors under investigation. Significant correlation was also observed between symptom severity and plant height, girth, and number of leaves. Findings of this study will be invaluable to stakeholders and researchers in banana industry by laying a foundation for development and adoption of viable BSD management strategies.

Key words: Banana streak virus, Banana streak disease, symptoms expression, tissue culture, ELISA.

INTRODUCTION

The banana industry in Kenya is entering a new era based on the demands of the farmers and consumers. There has been interest in the use of *in vitro* produced material to re-invigorate the banana industry. Reports point to the superior performance of tissue culture plants compared to suckers (Robinson, 1993; Qaim, 1999). Food quality, environmental degradation, increased pest and disease pressure and market competition are key issues favouring use of *in vitro* propagated banana material over the suckers (Qaim, 1999.; KARI, 1998). However, the occurrence of banana streak badnavirus (BSV) in recent years has negatively affected *in vitro* production and is considered a major constraint to banana improvement worldwide (Cote et al., 2010).

Due to integration of the BSV sequences into the plant genome and lack of understanding of the mechanism of integration and expression, it is difficult to develop an effective strategy in developing *Musa* hybrids with resistance to BSV. Therefore short term alternatives are required for the management of BSV in the farmers' fields. Nine Cavendish and six East Africa Highland cultivars were evaluated for BSV symptom expression at Njoro, Kokoto and Rongai in Nakuru district in Kenya and in a greenhouse in 2005/2006. The objective was to establish the effect of the environment on the expression of BSV symptoms in tissue culture banana cultivars.

MATERIALS AND METHODS

In vitro micropropagation of banana

Two suckers were selected from each mother plant of 15 cultivars: Chinese Dwarf, Dwarf Cavendish, Giant Cavendish, Gold Dessert, Gold finger, Grand Naine, Jamanga, Lacatan, Muraru, Namukhila, Ng'ombe, Ntobe, Kibuzi, Uganda Green, and Valary. Shoot tips (30 explants) were micropropagated as described by Vuylsteke (1989)

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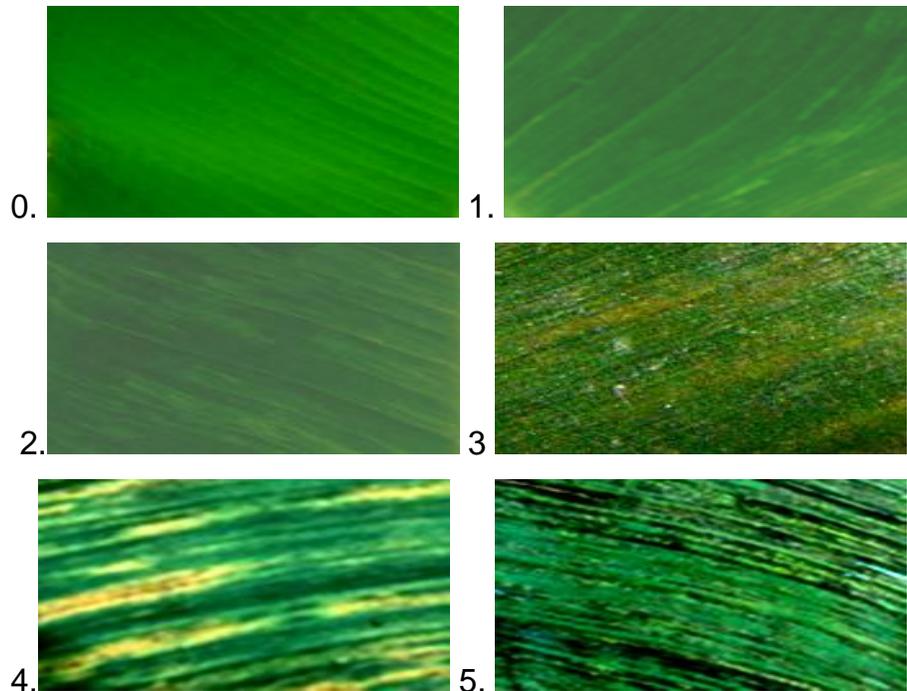
Figure 1. BSV disease rating scale, based on symptomatic expression where 0 = no symptoms, 1 = localised flecks, 2 = scattered discontinuous streaks, 3 = continuous streaks covering moderate portion of lamina, 4 = continuous and conspicuous chlorotic streaks, and 5 = necrotic streaks.

and adapted by Acuña (1996). The photoperiod was fixed at 16 h of light (15500 Lux) and 8 h of darkness. Temperature was maintained at 26°C.The mother plants of each cultivar were sampled and indexed for BSV using enzyme-linked immune sorbent assay (ELISA) protocol as described by Clark and Adams (1977). Only BSV negative materials were selected for micropropagation. All the micropropagated plants were established in polythene bags containing 2 kg of soil.

Effect of seedling age on BSV symptom expression

The objective of the experiment was to determine the stage in months of growth at which BSV expression is highest and the cultivars which are most affected. Out of the total 15 micro-propagated cultivars, five dessert cultivars that are most common were selected and tested in the greenhouse for BSV expression. Three months old micropropagated plants were used. A completely randomized design (CRD) was used. The layout was composed of two treatments, cultivars and time. The cultivar treatment included Giant Cavendish, Gold Dessert, Gold Finger, Lacatan and Grand Naine while the time treatment had six months, month 1, 2, 3, 4, 5, and 6. All the seedlings were planted in 30 by 24 cm polythene bags. Cultivar Grand Naine was used as a healthy control in this experiment. No virus inoculation was done. The soil used was mainly forest soils. The plants were treated with Furadan at 5 g per seedling in each bag to control nematodes. Watering was done daily and the plants were sprayed with fungicide and miticide fortnightly.

The study was conducted during the six months growing period before on-set of flowers. Plants were observed for six months and data was collected monthly. The variables measured were symptom severity where individual leaves were scored on a disease rating scale of 0 - 5 (Figure 1) and the total number of leaves and number

of leaves with symptoms were recorded for each plant.

The effect of cultivar and environment (location, season) on BSV symptom expression

The objectives of the experiment were to identify the effect of cultivar and environment on BSV symptom expression. Njoro, Kokoto and Rongai fall in the following agro-ecological zones: KARI - Njoro lies at an altitude of 2166 m above sea level (ASL) and is located within the agro-ecological zone low highlands (LH_3) (Jaetzold and Schmidt, 1983), longitude 0° 20' South and 35° 56' East with a mean annual rainfall of 931mm per annum. Temperature ranges from 7.9 - 21.9°C with a mean of 14.9°C (Jaetzold and Schmidt, 1983). Kokoto is in upper midland (UM_4) zone at an altitude of 1830 ASL with annual rainfall of 700 mm and mean temperatures of 20°C. Rongai is in upper midland (UM_6) at an altitude of 1620 ASL and receives annual rainfall of 600 mm with mean temperatures of 19°C. The soils are fertile vitric mollic Andosols that are well drained, deep to very deep, dark reddish brown in colour consisting of heavy textured friable silty clay to clay humid top soils (Jaetzold and Schmidt, 1983). Evaluation of fifteen *in vitro* propagated cultivars was carried out in the three sites from January, 2005 - December, 2006. The experiment was planted in a randomized complete block design (RCBD) replicated three times for each cultivar per site. The spacing and depth used was 3 m by 3 m and depth of 4 ft. Twenty tons per ha of manure and 250 kg/ha of diammonium phosphate fertiliser was used at planting time which are the current recommended quantities. Fortnight spraying was done with Bullock® containing active ingredients Beta-cyfluthrin 12.5g/l and Chlorpyrifos 250g/l for aphid control. The concentration used for spray was 20 ml/45 L of water. Mitigan® with an active ingredient Cypermetrin was used for the control of mites. The rates used for spray were 20mls/30 L of water. Rindomil® with active ingredients Mancozeb 640 g and Metalaacyl-M 40 g was used for

Table 1. Serological analysis of 15 banana cultivars before and after *in vitro* micropropagation.

Cultivar	No of initial suckers tested	No. of *in vitro* seedlings tested after potting	No of positive plants	Negative mean A_{405nm} values of ELISA for the initial suckers	Positive mean A_{405nm} Values of ELISA for *in vitro* seedlings	Positive or negative
Ng'ombe	2	5	2	0.094	0.169	+
D/ Cavendish	2	5	2	0.093	0.132	+
G/Cavendish	2	5	2	0.098	0.111	+
Grand Naine	2	5	1	0.095	0.124	+
Namukhila	2	5	1	0.070	0.128	+
Valary	2	5	1	0.093	0.110	+
C/Cavendish	2	5	1	0.098	0.131	+
Gold finger	2	5	2	0.093	0.120	+
Lacatan	2	5	1	0.100	0.112	+
Gold Dessert	2	5	0	0.100	0.100	-
Jamanga	2	5	0	0.083	0.084	-
Ntobe	2	5	0	0.080	0.083	-
Uganda Green	2	5	2	0.089	0.111	+
Kibuzi	2	5	0	0.074	0.076	-
Muraru	2	5	1	0.098	0.365	+
Negative control (PBS buffer)				0.082	0.091	-

ELISA protocol was used to screen for BSV and leaf samples with and without symptoms were used. All samples with absorbance levels of above 0.100 were considered positive while those with 0.100 or less were considered negative.

fungal infection at the rate of 20 g / 40 L of water. Weeds were removed by uprooting regularly to maintain a clean stand. Infection and symptom expression was monitored as described in Figure 1.

Data analysis

All data on symptom severity, plant height, width, number of leaves and absorbance levels were subjected to ANOVA using the General Linear Model (GLM) of SAS computer package version 6.12 (SAS, 1999) and statistical model as described by Gomez and Gomez (1984). Means were separated using the Least Significant difference (LSD). Data were recorded on a monthly basis and observations done over a period of six months. Correlations between variables were done using Pearson's correlation coefficient in SAS statistical package. All data were transformed before analysis with ANOVA procedure.

RESULTS

Serological diagnostics of micropropagated seedlings

When the potted seedlings were screened after 3 months and before transplanting into the field, results indicated that from the five seedlings of each cultivar screened, 0 - 40% were BSV positive even though the seedlings were initially symptomless. Over 73% overall of the micro-propagated cultivars showed BSV positive symptoms. However the absorbance value (A_{405nm}) of BSV antigen varied with cultivar, Muraru had the highest followed by Ng'ombe and Kibusi had the lowest A_{405nm} (Table 1). It is possible that during the initial screening the virus A_{405nm}

of some of the cultivars might have been too low for the ELISA technique to detect especially the dessert bananas. Also, there is a possibility of presence of many different isolates with varying A_{405nm}. However for the balbisiana (B genome)-containing cultivars such as Gold finger, there is a possibility of integrated viral sequences being activated after tissue culture (Ndowora et al., 1999). For all the cultivars three seedlings with absorbance levels of less than 0.1 were selected and planted in the field.

BSV expression as influenced by age

The results showed there was significant (P < 0.05) variation in symptom expression among the five cultivars during the third and fourth month of growth (Table 2). Cultivar, Gold finger, had highest symptom expression whereas Gold dessert had the lowest severity in comparison to the rest of the cultivars. There was variation in symptom expression for all the five genotypes with significantly (P < 0.05) high BSV expression at sixth month compared to first month when the expression was lowest. Symptom expression fluctuated from month to month intermittently with appearance and disappearance of symptoms. Fluctuating temperatures in the green house may possibly have played a role (Dahal et al., 1998). With maturity of plants, there is a possibility of accelerated virus replication and increased virus concentration therefore higher symptom expression could easily be detected.

Table 2. Effect of time and cultivar on expression of banana streak virus.

Time (months)	Cultivar					
	Giant cavendish	Gold dessert	Gold finger	Grand naine	Lacatan	Time means
1	1.00[c]	1.00[c]	1.00[c]	1.14[bc]	1.00[c]	1.03[c]
2	1.28[abc]	1.00[c]	1.28[abc]	1.24[abc]	1.28[abc]	1.21[abc]
3	1.24[abc]	1.00[c]	1.38[ab]	1.00[c]	1.14[bc]	1.15[bc]
4	1.24[bc]	1.00[c]	1.63[a]	1.14[bc]	1.24[abc]	1.25[ab]
5	1.00[c]	1.24[bc]	1.14[bc]	1.24[abc]	1.00[c]	1.13[bc]
6	1.38[ab]	1.28[abc]	1.52[a]	1.28[abc]	1.58[a]	1.41[a]
Cultivar means	1.19[xy]	1.09[y]	1.32[x]	1.17[xy]	1.21[xy]	

Seedlings were grown from micropropagated plants. Disease severity rating was done as described in Figure 1.Numbers within the columns followed by the same letter (a, b, c) are not significantly different at 5% and numbers within the rows followed by the same letter (x, y) are not significantly different $P < 0.05$. LSD for cultivar is significant ($P < 0.05$); LSD for time is significant ($P < 0.01$).

Table 3. Correlation between the growth characteristics and infection of banana streak virus under greenhouse conditions.

Parameter	Symptom	Height	Girth	Leaves	Time
Symptom	1.000	-0.1408	0.1178	-0.2329*	0.280**
Height		1.0000	0.05015	0.4285**	-0.525**
Girth			1.0000	0.2110*	-0.30311**
Leaves				1.0000	-0.68695**
Time					1.0000

Micropropagated plants were 6 months at determination of these parameters. *Significant at 5% level of probability.

Gold finger cultivar (AAAB_FHIA 01) had significantly ($P < 0.05$) higher expression of BSV during its growth period and Gold Dessert (AAA) had the lowest. The high incidence of BSV expression in Gold finger is probably due to the presence of a 'B' genome which has integrated viral sequences and could have been activated by micropropagation process. These results affirm those reported by Dallot et al. (2001) and Geering et al. (2005) who observed that expression of episomal BSV during *in vitro* procedure is correlated to the presence of an integrated form. However Gold Dessert (AAA genotype) might have had episomal virus with very low concentration or the meristem culture could have eliminated any BSV infection as has been reported before (Helliot et al., 2002). The higher expression shows that virus concentration increases with plant growth. Therefore cultivar and age of banana plants have a bearing in BSV expression.

Correlation between the growth characteristics and infection of banana streak virus under greenhouse conditions

A significant ($P < 0.05$) negative correlation was observed between BSV symptoms and number of leaves produced ($r = -0.23$) (Table 3). This indicated that with higher infection of BSV leaf numbers were reduced due to interruption of nutrient synthesis for the development of leaves. Highly significantly and ($r = 0.43$) positive correlation were also recorded between number of leaves and plant height; number of leaves and girth ($r = 0.21$). This indicated that taller plants and those with wider girth had more leaves. Leaf number can therefore be used to estimate the photosynthetic capacity and to predict the performance of a banana crop (Valladares et al., 2003). The significant ($r = -0.140$) negative correlation observed between symptom expression and height was an indication that BSV infection affected the growth rate in that there was slower growth rate with higher infection. These results gave an indication that with more infection and expression of symptoms there is reduced height of the plant which directly affects synthesis of nutrients for development of banana bunches.

In the analysis of the five growth characteristics, significant and positive correlation ($r > 0.28$) was observed between BSV symptom expression and time taken. There was less infection and expression during the early months of growth but this progressively increased with age of the plant. This was an indication that the age of the plant contributed directly to expression of BSV. Significant negative correlations ($r = 0.525; -0.303; -0.687$) were observed between plant height and time,

Table 4. Banana streak virus symptom incidence in Njoro, Kokoto, and Rongai over two years' period.

Cultivar	Njoro[b]		Kokoto[a]		Rongai[ab]	
	2005	2006	2005	2006	2005	2006
Chinese Cavendish	1.00[e]	1.28[cd]	1.00[e]	1.41[bc]	1.41[b]	1.41[bc]
Dwarf Cavendish	1.14[de]	1.14[d]	1.28[cd]	1.41[bc]	1.28[cd]	1.28[cd]
Giant Cavendish	1.00[e]	1.41[bc]	1.14[de]	1.52[b]	1.28[cd]	1.41[bc]
Gold Dessert	1.14[de]	1.47[b]	1.14[de]	1.61[a]	1.14[de]	1.14[d]
Gold Finger	1.00[e]	1.14[d]	1.28[cd]	1.41[bc]	1.28[cd]	1.41[bc]
Grande Naine	1.00[e]	1.14[d]	1.14[de]	1.61[a]	1.14[de]	1.28[cd]
Jamanga	1.41[b]	1.28[cd]	1.41[b]	1.41[bc]	1.41[b]	1.41[bc]
Lacatan	1.00[e]	1.28[cd]	1.14[de]	1.41[bc]	1.14[de]	1.28[cd]
Muraru	1.00[e]	1.52[b]	1.00[e]	1.41[bc]	1.00[e]	1.00[e]
Namukhila	1.28[cd]	1.41[bc]	1.75[a]	1.41[bc]	1.00[e]	1.00[e]
Ng'ombe	2.24[a]	1.41[bc]	2.24[a]	1.28[cd]	2.24[a]	2.24[a]
Ntobe	1.14[de]	1.52[b]	1.14[de]	1.00[e]	1.00[e]	1.00[e]
Kibuzi	1.00[e]	1.41[bc]	1.00[e]	1.14[d]	1.00[e]	1.00[e]
Uganda Green	1.14[de]	1.28[cd]	1.00[e]	1.41[bc]	1.14[de]	1.28[cd]
Valary	1.14[de]	1.14[d]	1.00[e]	1.28[cd]	1.14[de]	1.14[d]
Means for Locations	1.17[xyz]	1.32[w]	1.25[wx]	1.38[w]	1.24[wx]	1.28[wx]

Seedlings were grown from micropropagated plants. Disease severity rating was done as described in Figure 1. Numbers within the columns followed by the same letter (a, b, c) are not significantly different at 5% using LSD and numbers within the rows followed by the same letter (w, x, y, z) are not significantly different at 5% using LSD.

plant girth and time, and number of leaves and time (Table 3). Growth rate of all the three characteristics reduced with the time factor. With each passing month there was reduction in the rate growth in height width and number of leaves. BSV virus infection could have slowed down growth.

Determining the effect of cultivar, location and season in banana streak virus (BSV) symptom expression

Results showed significant (P < 0.05) differences for infection and symptom expression based on 15 cultivars in Njoro, Kokoto and Rongai locations (Table 4). The significant effect of cultivar by location indicates that screening should be done at each location.

The cultivar by location effect also indicates that the potential for BSV infection and expression might be influenced by location due to differences in virus isolates within locations. BSV symptom expression was significantly (P < 0.05) higher in Kokoto compared to Njoro or Rongai locations (Table 4). In the first year, symptom expression was significantly (P < 0.05) lower compared to the second year in all the 15 cultivars However, high expression levels were observed predominantly in cooking varieties (Ng'ombe, Jamanga, and Namukhila) of genotype (EAH-AAA) across the locations. During the same period, there was low moisture recorded of approximately 778.7 mm.

There is a possibility that dry conditions played a role in activation of BSV in the cooking cultivars (EAH-AAA) as had been also observed earlier by Daniells et al. (2001). In the second year when rainfall level recorded was approximately 1178.1 mm, highest expression was observed on the dessert bananas especially in Kokoto location.

Wet conditions could have contributed to the activation of BSV in dessert cultivars as had also been reported by Dahal et al. (1998). However, more studies are required to establish the relationship of cultivar and moisture levels in BSV activation and symptom expres-sion. Ng'ombe, a cooking variety (EAH-AAA genotype) had relatively high BSV infection and expression in comparison to the rest of the varieties while Valary had the lowest infection and expression across the locations and years (Table 4). Most interesting however was that Ng'ombe cultivar had vigorous vegetative growth across the locations. Further investigations arerequired to confirm tolerance to BSV aspect in Ng'ombe cultivar.

Based on the eight dessert cultivars and seven cooking cultivars evaluated in the three locations during the second year, the response of the cooking and dessert cultivars in Njoro indicated 88% dessert bananas (Cavendish and Gold finger) had lower infection in Njoro but high infection in Kokoto and Rongai locations while the cooking varieties (Muraru, Ntobe and Kibuzi) had significantly high infection in Njoro and relatively lower infection in Kokoto and Rongai location during the second

year, 2006 (Table 4). The rest of the cooking cultivars however had either lower or similar rate of infection as was observed with Ng'ombe, Uganda Green, Namukhila and Jamanga cultivars across the locations and seasons.

There were fluctuations in the BSV incidences from location to location and cultivar to cultivar. For example while Chinese Dwarf was less infected in Njoro there was high BSV infection in Kokoto and Rongai during the second season. From this study there is an indication that BSV expression might be affected by cultivar, location and year (wet or dry). It might therefore be possible to select cultivars suitable for the three locations.

DISCUSSION

Detection in the field of *de novo* BSV infections in micropropagated *Musa* spp has led to the hypothesis of a possible effect of the *in vitro* multiplication procedure on BSV expression (Dahal et al., 1999). Factors contributing to the expression of BSV in the greenhouse and the field were identified. These factors included cultivars, plant age, season of the year and location (site). It was observed that under the greenhouse conditions, infected Gold finger cultivar expressed high symptoms during the third and fourth month, while Gold dessert, Giant Cavendish, Grand Naine and Lacatan had lower expression during the same period of time (Table 2). Infection of some isolates of BSV may arise *de novo* from viral sequences that are integrated into the 'B' genome of *Musa* (Ndowora et al., 1999; Harper et al., 1999). Tissue culture has been considered as one of the trigger factors for episomal expression of these integrants (Geering et al., 2001; Dallot et al., 2001; Ndowora and Lockhart, 1997). Gold finger, which is a cultivar of the AAAB genotype which contains the 'B' genome, may have been activated by the micropropagation process leading to high infection and expression of BSV. However, the integrants are absent from Cavendish banana (Geering et al., 2001) and this could have contributed to low infection or symptom expression observed in the rest of the five cultivars in the greenhouse. Optimum infection and symptom expression for the Gold finger in the greenhouse was observed 4 months after planting of seedlings. Plants that are four months old could be used for screening greenhouse material for BSV before field planting. However, more studies are required to establish the right time for final screening before field establishment of micropropagated seedlings.

Although there were minimal differences in BSV severity amongst cultivars, Ng'ombe had a higher expression of BSV compared to Valary (AAA genotype) across the three locations (Table 4). There is need to establish why Ng'ombe which is an EAH - AAA genotype had higher expression than the Cavendish Valary. It was observed that moisture level was a critical factor in BSV expression (Table 4). Dry conditions could have

activated the high symptom expression. These results contradict those obtained from a study carried out in Nigeria where greater symptom expression were observed when plants were grown in wet season compared to those grown in dry season (Dahal et al., 1998). This information could be used in the management of the BSV by ensuring no water stress (Daniells et al., 2001).

The interaction between cultivar and location was significant (P < 0.01). It was observed that the response of the banana cultivars to BSV expression varied with the location where it was planted. This probably was because the three locations fall under different ecological zones with different altitudes, rainfall and temperatures as was described previously. The information will be valuable in future for cultivar recommendation of specific areas. Additionally, more studies are required to validate this study.

These data suggest that specific genetic factors in cultivar (such as nature of integrated BSV sequences) and environmental conditions (moisture and type of isolates) may be key factors in the expression of BSV. The findings could be used as a guide in the management of banana streak disease in Kenya.

Conclusion

During the screening of the 15 micropropagated banana cultivars, it was found that the BSV concentration varied with cultivars. When 5 banana cultivars were exposed to the greenhouse conditions they expressed varying degrees of BSV symptom expression. Symptoms were however not changing drastically within the six months because the initial screened samples could have had very low virus load which was activated by environmental factors with time. BSV infection affected the height, girth, and number of leaves negatively. This could have been due to toxicity and death of cells caused by the virus. As a result there were fewer resources available to the plant for growth.

In the current study, BSV varied greatly between cultivars and locations and was significantly higher in landraces of cooking varieties which included Ng'ombe, Jamanga and Namukhila. These expressed severe symptoms under low moisture conditions while the dessert cultivars mostly Cavendish expressed severe symptoms under high moisture levels. This is of great concern since most landraces are of the AAA genome. From these findings, it may be possible that reducing moisture for Cavendish may result into low BSV symptom expression, while increasing moisture levels for landraces may also reduce BSV symptom expression. Moisture control can only be achieved either through irrigation or planting the appropriate cultivars in the right environment.

Although Ng'ombe expressed high BSV levels, vigorous vegetative growth was observed showing the level of tolerance. As was described previously, good vegetative

growth would eventually translate into fruit production.

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