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A thesis submitted to the Graduate School in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Agronomy (Crop Protection option)

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DECLARATION

This Ph. D. thesis is my original work and has not been submitted or presented to any other university for an award of a degree.

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DEDICATION

To my beloved family Anthony, Annabel, Harrison and Esther who have patiently moved with me through the journey of making my dream a reality.

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ABSTRACT

Banana streak virus (BSV) is a serious constraint to production of banana and plantain (*Musa spp.*) in sub-Saharan Africa. BSV has been found to be present in virtually all the commercial banana cultivars in Kenya. Various studies have reported that banana streak virus is caused by a range of BSV isolates. The ability to quickly and reliably detect BSV is a pre-requisite to the management of the virus. A variability study of BSV in Kenya was carried out to develop suitable diagnostic techniques. Transmission of the disease is through several species of mealy-bug (*Planococcus spp*) but more significant transmission is through infected vegetative propagation materials. In some *Musa* hybrids and derived cultivars, integration of BSV into the plant genome can cause subsequent infection when triggered by stress factors such as drought, tissue culture, temperature and genomic hybridization. *In vitro* propagation has been identified as one of the main activation triggers of integrated sequences to cause systemic (episomal) banana streak disease (BSD). This was especially observed in the B genome containing AAAB hybrids (FHIA 18 and FHIA 01/Goldfinger). The heterogeneous nature of the virus in the field hinders reliable detection by serological techniques. Integrated BSV sequences cause problems in direct molecular detection of virus particles by polymerase chain reaction (PCR). Hence, deoxyribonucleic acid (DNA) -based techniques, following serological trapping of the virus, immune-capture polymerase chain reaction (IC-PCR) has been found most reliable in detection of BSV. The main objective of this study was to identify BSV isolates in Kenyan banana cultivars. Factors affecting the infection and expression of BSV were studied to optimize the diagnostic protocol in order to help with virus indexing and consequently mass production of BSV-free banana seedlings. The work was accomplished through random collection of field banana material from various banana growing regions, serological analysis using Enzyme Linked Immunosorbent Assay (ELISA) kit which confirmed 87% BSV infection. Isolation of nucleic acid from infected leaf samples, molecular characterization of BSV and sequence analysis of the clones enabled identification of about seven isolates. Three of the seven isolates, were new isolates. Genome-based methods of detection such as nucleic acid hybridization and PCR amplification were used. Through screening of various antibodies and primers, their effectiveness in detecting BSV was determined. Tissue culture materials of 15 cultivars were evaluated for BSV re-infection and expression so as to determine the tolerant cultivars under field and greenhouse conditions and stage of infection and expression. Environmental factors such as nutrition that affect the expression of BSV were also studied. Statistical analysis system (SAS) for ANOVA and various bioinformatics packages were used for analysis of data for the experiments. Results indicated that BSV is associated with increase of growth time, decrease of plant height, girth, number of leaves and high nitrogen levels. Cultivar, location and season also played a critical role in the expression of BSV. The study also confirmed that detection of new isolates was influenced by antibody type, concentration and antigen concentration. The available antibodies have limitations in comprehensive detection of BSV. However, uses of genomic techniques were more effective than serological techniques. Based on amplification, cloning, sequencing, phylogenetic analyses and pairwise comparison of the nucleotide sequence similarities of 80 % and above (proposed criteria by International committee on Taxonomy of viruses (ICTV), three new isolates of BSV were identified and confirmed for the first time in Kenya. The isolates were Khabusi2 from Mt. Elgon region (EAH - AAA genome), Lisulya from Kisii region (EAH - AAA) and Nshule from Kisii region (EAH - AAA).

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LIST OF ABBREVIATIONS

A	Absorbance levels at 405nm
A/AA/AAA/AAAA	Acuminata/ Diploid/ Triploid/ Tetraploid
AFLP	Amplified Restriction Fragment Length Polymorphism
ANOVA	Analysis of variance
AP	Aspartic protease
μl	Microlitre
ASL	Above sea level
ATPS	Africa Technology Policy Studies
B	Balbisiana
BA	Benzylamine purine
BanMMV	Banana mild mosaic virus
BBTV	Banana bunchy top virus
BBrMV	Banana bract mosaic virus
BEV	Banana endogenous virus
Bp	Base pair
BSA	Bovine Serum Albumen
BSUgAV	Banana Streak UgandaA Virus
BSUgBV	Banana Streak UgandaA Virus
BSUgCV	Banana Streak UgandaC Virus
BSUgDV	Banana Streak UgandaDVirus
BSUgEV	Banana Streak UgandaEVirus
BSUgFV	Banana Streak UgandaF Virus
BSUgGV	Banana Streak UgandaG Virus
BSUgHV	Banana Streak UgandaHVirus
BSUgIV	Banana Streak UgandaI Virus
BSUgJV	Banana Streak UgandaJ Virus
BSUgKV	Banana Streak UgandaK Virus
BSUgLV	Banana Streak UgandaL Virus
BSUgMV	Banana Streak UgandaM Virus
BSGfV	Banana streak Goldfinger virus
BSImV	Banana Streak Imove Virus
BSMysV	Banana Streak Mysore Virus

BSOEV	Banana Streak Obino L'ewai Viru s
BSV	Banana Streak Virus
BSD	Banana Streak Disease
BSV-Cav	Banana Streak Virus Cavendish
BSV-DC/Mdg	Banana Streak Virus Dwarf Cavendish, Madagasca
BSV-MA	Banana Streak Virus Grand Nain, - Morocco
BSV-Mad/DC	Banana Streak Virus Dwarf Cavendish Madeira
CaYMV	Canna yellow mottle badnavirus
ComYMV	Commelina Yellow Mottle Virus
CYMV	Citrus yellow mosaic badnavirus
CIRAD	Centre de Cooperation Internationale en Recherche Agronomique pour le Development
CRD	Completely randomised design
CSSV	Cacao Swollen Shoot Virus
DAS	Double antibody sandwich
DBV	Dioscorea Bacilliform Virus
DNA	Deoxyribonucleic acid
(ds) DNA	Double-stranded deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
EAH-AAA	East Africa Highland banana - AAA
FAO	Food Agricultural Organisation
FHIA	Fundacion Hondurena de Investigacion Agricola
FHIA - 01	<i>Musa</i> cultivar synonym Goldfinger
GLM	General linear model
IC-PCR	Immuno-capture polymerase chain reaction
IgG	Immunoglobulin
INIBAP	International Network for Banana and Plantain
ISEM	Immunosorbent electron microscopy
KARI	Kenya Agricultural Research Institute
kbp	Kilo base pair
kDA	kilo dalton
KTSV	<i>Kalanchoe top-spotting badnavirus</i>
LH	Low highland
LSD	least significant difference

NaCl	Sodium chloride
ORF I/ II/ III	Open reading frame/ one/ two/ three
PBST	Phosphate Buffered Saline + 0.05% (v/v) Tween 20
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PMEDAP	2, 6-diaminopurine analogue of adefovir
PNP	Para-Nitrophenyl phosphate
PYMV	Piper yellow mottle badnavirus
PVP	Polyvinylpyrrolidone
RCBD	Randomised complete block design
RNA	Ribonucleic acid
RSV	Schefflera ringspot
RT	Reverse transcriptase
RTBV	Rice Tungro Bacilliform Virus
SAS	Statistical Analysis System
SCBV	Sugarcane Bacilliform Virus
SSI	Symptom Severity Index
SSRNA	Single stranded ribonucleid acid
TBE	Tris-borate-EDTA
TBS-T	Tris Buffered Sulphate + Tween 20
UV	Ultra violet

CHAPTER ONE

INTRODUCTION

1.1 Background

The cultivated bananas are predominantly triploids that evolved from intra – and interspecific crosses involving two diploid wild progenitor species, *Musa acuminata* (genome AA) and *Musa balbisiana* (genome BB) originating in Malaysia and India, respectively (Simmond 1976). A haploid genome of these diploid wild species is represented by the letters A and B respectively. Most domesticated cultivars have diploid genotypes such as AA and AB, triploid genotypes such as AAA, AAB, and ABB, and tetraploid genotypes such as AAAA, AAAB, and ABBB, with $2n=22$, $3n=33$, and $4n=44$ chromosomes, respectively. The basic haploid number is 11 chromosomes (Appendix 1).

Banana (*Musa* spp.) are grown in over 120 countries (FAO, 2001) providing a major source of carbohydrates and calories for over 400 million people in tropical countries (Swennen *et al.*, 1995; Sharock and Lusty, 2000). Over 102 million metric tonnes are produced yearly (FAO, 2001).. Bananas can be consumed in various ways, fresh, cooked or dried (Dadzie, 1998). They are a rich source of vitamin A, C, calcium and iron (FAO, 1983). All banana varieties derived from *M. acuminata* are eaten as dessert while *M. balbisiana* are either cooked, roasted for food, or are brewed for alcoholic beverage. Other uses include puree for baby food, banana flour, juice, jam, sauce and savoury chips (Robinson, 1996).

Apart from food *Musa spp* is also a major source of income for small scale farmers in various African countries (Nweke *et al.*, 1988). Many small-holder farmers particularly in East, Central and West Africa grow them for subsistence and as a cash crop. In Kenya, banana plantation covers 1.7% of the total arable land and is grown in various agro-ecological zones ranging from sea level to 2000 meters above sea level (Qaim, 1999). Currently there are a total of 80,000 ha under bananas with approximate production of 210,000 metric tonnes annually (FAO 2004). Nyanza and Western Provinces produce 64.6%, of the total banana production while Central and Eastern Provinces produce 26%, Rift Valley Provinces produce 3.9%, Coast Province 5.0%, North Eastern and Nairobi Provinces produce the remaining 0.5%. Ninety percent of this production is by small scale farmers (Anon., 2006). The total production is only 30% of the potential (KARI t, 1998; Qaim, 1999). Due to intensification of

crop production in the last thirty years, an increasing number of new pests and diseases have been identified that cause significant reductions in yield (Swennen and De Langhe, 1989; Ortiz, 1996). Wide use and distribution of infected materials also contribute to low crop yields. Environmental degradation, low levels of farm inputs and poor farming practices aggravate the problem (Karembu, 2001).

Bananas are susceptible to numerous pests and pathogens including banana bunchy top virus (BBTV, a nanovirus), banana bract mosaic virus (BBBrMV, potyvirus), banana mild mosaic virus (BanMMV, an assigned member of the family *Flexiviridae*), cucumber mosaic virus (CMV, a cucumovirus) and, most recently recognized, banana streak virus (BSV). BSV is a plant pararetrovirus in the family *Caulimoviridae* and the genus *Badnavirus*. BSV has an open-circular double-stranded (ds) DNA encapsidated in bacilliform-shaped particles (Harper and Hall, 1998; van Regenmortel *et al.*, 2000). Like several other plant pararetroviruses, BSV is integrated into the host genome of some *Musa* genotypes. This integrated form of BSV is associated with the B-genome of banana and plantain (Geering *et al.*, 1999). Episomal infection observed in the propagation of virus-free source plants may originate from this integrated BSV. The occurrence has been associated with tissue culture practice, a process that is considered to activate the viral sequences that are integrated into the host genome (Ndowora *et al.*, 1999). Various studies have confirmed integrated BSV genome sequences in various genotypes of bananas that give rise to a range of episomal isolates (Harper *et al.*, 1999b; Dallot *et al.*, 2001; Geering *et al.*, 2005).

Banana streak disease symptoms were first reported from Ivory Coast in 1968 and the causal virus was first isolated and characterised from banana in Southern Morocco (Lockhart, 1986). BSV symptoms have since been observed worldwide in every producing area of banana and plantain (Dallot *et al.*, 2001). BSV is transmitted semi-persistently by various species of mealy bugs (*Planoccocus* spp) and through vegetatively propagated plant material but not mechanically or through soil (Jones and Lockhart, 1993). BSV infects banana and causes chlorotic and necrotic streak symptoms on leaves and pseudo-stem (Lockhart, 1986). Affected plants have lethal stem necrosis, pseudostem splitting, and choking in which fruit bunches may fail to emerge from the pseudo-stem, stunted plants with small bunches, and a yield reduction of up to 90% (Lassoudiere, 1974; Daniells *et al.*, 2001).

1.2 Distribution, Diagnosis and control of BSV in Musa-Producing Countries

BSV is a major concern world-wide in all banana growing areas (Remans *et al.*, 2007) and is also becoming a threat to banana industry in Kenya. BSV was first identified in Kenya in 1996 with no confirmatory tests (Diekmann and Putter, 1996) but was later confirmed in a few parts of Kenya (Personal comm. with Prof. Hull). Preliminary studies carried out in most banana growing regions of Kenya confirmed the presence of the virus and the disease through symptom observation and serological tests (Wangai *et al.*, 2002). Various management approaches have been suggested and used including chemical control of the mealy bug vector of the disease and roguing of infected plants. These are very helpful approaches but insufficient, particularly because even when plants are infected the presence or absence of symptoms is highly variable and because the disease can be induced in apparently asymptomatic plants from integrated virus sequences within the plant genome. BSV elimination from infected banana plants is also minimal. A few of the reported methods used to eliminate the virus include cryopreservation which gave the percentage of virus free plants as 59-76% in control or untreated plants while it increased in the treated plants from 90-95% (Helliot *et al.*, 2002). It has also been found that acyclic nucleoside phosphate analogues and selective inhibitors of *Hepatitis B virus* replication, adefovir (Adefovir dipivoxil HepseraTM), tenofovir (tenofovir disoproxil fumarate, VireadTM) and PMEDAP (2, 6-diaminopurine analogue of adefovir), efficiently eliminated BSV from banana (Helliot *et al.*, 2002). However, these methods are not applicable for routine use on large scale production of tissue culture material. One of the more practical approaches which have been reported is in development and use of reliable diagnostic techniques for routine virus indexing of banana material (Harper *et al.*, 1998; 2002). The method reduces distribution of diseased plants to the growers. Reducing virus infection will contribute to yield increase from the current 10 tons per hectare to the potential 60 tons per hectare (Qaim, 1999; Karembu, 2001).

1.3 Statement of the Problem

Banana streak virus (BSV), a causal agent of banana streak disease (BSD), has been identified as a major problem in all the banana growing regions and affects all commercial cultivars grown by farmers (Wangai *et al.*, 2002; 2003). BSV isolates are highly variable and their viral DNA is integrated into the banana plant genome making detection difficult (Harper *et al.*, 2004). Tissue culture technique, which is currently used for mass propagation of

banana seedlings in Kenya, has also been identified as one of the major stress factors which activate episomal BSV infection. Therefore BSV is a major threat to the banana industry in Kenya. Management of the BSV depends on quick and reliable detection of the virus. To date, the variability of the BSV isolates in Kenya has not been documented. Identification of molecular and serological diversity of BSV isolates will enable development or optimization of a diagnostic protocol for better virus indexing procedures for both conventional and tissue culture bananas. Understanding of the factors affecting expression of symptoms will contribute to better management of the virus.

1.4 Objectives

1.4.1 Broad objective

To enhance productivity of bananas in Kenya by putting in place appropriate technologies for effective detection, screening and indexing of *Banana streak virus*

1.4.2 Specific objectives

- (i) To evaluate the effect of cultivar, environment and nitrogen levels on the infection and expression of BSV symptoms in tissue culture bananas under field and greenhouse conditions
- (ii) To determine the serological variation of BSV isolates from different types of banana cultivars grown in various regions of Kenya.
- (iii) To evaluate the molecular variation of BSV isolates from banana cultivars grown in various regions of Kenya
- (iv) To determine the relationship between morphological expression, serological and molecular detection of BSV in bananas

1.5 Hypotheses (H₀)

- (i) BSV symptom expression is not influenced by different cultivars, environment and nitrogen levels on tissue culture bananas.
- (ii) There are no differences in BSV isolates detected serologically, among banana cultivars from various regions in Kenya.

- (iii) There are no differences in BSV isolates, detected through molecular tools, among banana cultivars from various regions in Kenya.
- (iv) There is no relationship between morphological expression, serological and molecular detection methods of BSV in various cultivars of bananas

1.6 Justification

Results of preliminary surveys carried out in various parts of the country have indicated the presence of BSV through symptom expression and serological detection in all the banana cultivars (Wangai *et al.*, 2002). Currently, the tissue culture propagated bananas are widely grown in Kenya. Approximately 50% of the small scale farmers grow tissue culture bananas of Cavendish, FHIA, and East Africa Highland cooking cultivars. Use of tissue culture to propagate bananas has been reported to trigger episomal BSV expression in *Musa* hybrids with B genome (Dallot *et al.* 2001). As a result, there has been a major setback on the distribution of high quality *Musa* cultivars. There is, therefore, great concern on the purity of tissue-cultured propagation material that is being produced and availed to the farmers. Due to heterogeneous nature of the isolates, the serological technique most widely used for quick detection is insensitive and may not be able to identify some of the isolates, therefore, it also needs optimisation for effective detection.. DNA- based techniques such as IC-PCR have been found suitable for amplification of episomal BSV (Harper *et al.*, 1998) and could be developed to aid selection of low or no risk banana plants.

At present, there is limited documentation on the virus isolates found locally, the mechanisms underlying their potential activation and the epidemiology of the virus in Kenya. A study on the variability of isolates will facilitate better management of BSV. Knowledge of the type of isolates will help in the development of an effective diagnostic protocol for routine virus indexing. This will facilitate mass propagation of virus-free tissue culture seedlings that will ensure establishment of virus-free, high yielding banana orchards by farmers. Hence Kenya will be self-sufficient in banana production with surplus for export and an improved farm.

1.7 Expected Outputs

1. An appropriate technology for routine indexing of BSV developed.
2. The effects of cultivar, environment and nitrogen levels on BSV expression will be documented
3. Information on serological and molecular variation of BSV isolates in Kenya generated
4. Documentation of morphological, molecular and serological relationship in BSV detection methods.

CHAPTER TWO

LITERATURE REVIEW

2.1 The banana history

The banana, Family *Musaceae* and genus *Musae* originated from Indo-Malasia region in the 3rd Century BC where a rich source of genetic diversity is found. (Morton, J. 1987) A high number of seeded varieties, mainly the wild species of *Musa acuminata* and *Musa balbisiana* still exist in Asia. It is thought that the edible diploid (AA) and triploid (AAA), crossed with wild species resulting in genomes with AB, AAB, ABB (Simmonds, 1976).

Banana is thought to have been introduced to East Africa in AD 500 (Simmond, 1976). Other studies show that banana had been cultivated on the Atlantic side of the African continent 2500 years ago (Vrydaghs *et al.*, 2002). By the end of the eleventh century, banana occurred widely throughout the tropics. It is believed that bananas were brought to Kenya through Mt. Elgon area between AD 500 -1000 and they originated from regions around the sources of the rivers Zaire and Zambezi (Wainwright, 1952; MacMaster, 1962b; Karamura, 1999). The original cultivars were mainly for cooking and are now commonly known as East Africa Highlands or 'Mutika' (Karamura, 1999). East Africa Highland bananas (EAH-AAA) are triploid (genomic AAA) containing the A genome of *Musa acuminata* (Stover and Simmond, 1987). Five clone sets have been identified which include Mbidde, Nakitembe, Nakabululu, Musakhala and Nfuka with a total of 84 clones bearing local names (Karamura, 1999). The East Africa Highland bananas (EAH-AAA) are found only in East Africa and are cultivated under banana subsistence systems mainly for food security (Karamura, 1999). The more recent introductions grown for commercial purposes in Kenya are the Cavendish group of cultivars Valary, Williams, Dwarf and Giant Cavendish and Grand naine mainly used for dessert. (Kwach *et al.*, 1996)

2.2 The botany of banana plant

Banana belongs to the family *Musaceae* and genus *Musae*. The plant is herbaceous and is composed of an underground rhizome with several shallow roots, leaf bases and leaves. The leaves are arranged in a concentric manner above the pseudo-stem. Leaves are initiated from the apical growing point of the meristem where flowers emerge from. Fruits referred to as

fingers develop from each flower. A collection of fingers is known as a hand and collectively the hands are referred to as a 'bunch' (Jones, 1999; Robinson, 1993).

2.3 Propagation

Commercially cultivated *Musa* species do not produce seed. More than one sucker develops from a rhizome but only one or two sword suckers are selected and left for continuity. Propagation is usually done through suckers, bits, corms or micropropagated tissue culture seedlings (Robinson, 1993). *In vitro* propagation via tissue culture is widely practiced in Kenya and has the advantage of a uniform plantation with uniformity in growth, flowering and harvest maturity (Robinson, 1993). When multiplying *Musa* by tissue, culture, large numbers of plants can be generated from a single meristem. It is therefore important to ensure that the meristem tissues are free of any disease especially viruses. The use of tissue culture banana seedlings has been widely recommended in Kenya to address the banana production decline (KARI, 1998). Since the principal method of spread is by vegetative propagation, disease control must be based on the use of virus-free stock plants for propagation by suckers or *in vitro* propagation (Lockhart, 1995). Production of BSV-free tissue cultured material can be achieved by identifying the prevalent BSV isolates and developing isolate specific diagnostic protocols, which would then be used for routine indexing before dissemination to growers. Isolate variation has been identified in different banana genotypes worldwide and their primers developed (Geering *et al.*, 2000; Daniells *et al.*, 2001) but this has not yet been done in Kenya (Wangai, *et al.*, 2002).

2.4 Distribution and economic impact of BSDV

Banana Streak Virus disease (BSD) occurs worldwide wherever bananas are grown, primarily in the tropics and subtropics (Lockhart, 1995). BSD caused by BSV was first reported from Ivory Coast in 1968. The causal virus was first isolated and characterized from banana in Southern Morocco (Lockhart, 1986). Subsequently the disease has been recorded in most *Musa*-producing regions and in many of the numerous clones of *Musa* and its sub-groups (Harper *et al.*, 1998). The disease has been identified in over 40 countries of South-East Asia, Australia, Europe (Canary Islands and Madeira), Central and South America and Pacific Islands (Diekmann and Putter, 1996; Lockhart and Jones, 2000), Mauritius (Jaufeerally-Fakim, *et al.*, 2006) and Africa, (Agindotan *et al.*, 2006; Harper *et al.*, 2002; 2004; 2005).

BSV infection in banana may be important for one of three reasons; its effect on plant growth, fruit yield and quality; it is a hindrance to germplasm exchange and the need for certification of *in vitro* plantlets for international trade. The occurrence of BSV in banana is likely to have an adverse effect on the international exchange of *Musa* germplasm. This is particularly true of the tetraploid hybrids recently produced by several *Musa* breeding programmes (Lheureux, *et al.* 2003). These recently developed hybrids have been bred for resistance to fungal pathogens (black leaf streak) and nematodes, but the high risk of occurrence of BSV in a number of them has led to restrictions on their distribution to and acceptance by some countries. Finally, the awareness of potential infection by BSV, BBTV and other viruses has led to a need for methods to verify that *in vitro* plantlets are virus free. The production and international movement of these plantlets has greatly increased in recent years.

Yield losses of up to 90% in banana due to BSD were reported in Cote de'Ivoire on variety Poyo (Lassoudiere, 1974). In Australia, infection caused a delay in harvest by 18 days and a yield loss of up to 11% (Lockhart, 1994; Daniells *et al.*, 2001). In Nigeria and southern Cameroon, yield losses have been reported of up to 15% through internal necrosis and death of plants (Gauhl *et al.*, 1999a). In Costa Rica and Ecuador, severe symptoms of the disease on fruits make them unmarketable (Lockhart and Jones, 1999). The range of yield losses may vary due to different cultivars and cultural conditions. However the extent of damage by BSV in Kenya has not yet been assessed.

2.5 Banana streak virus disease

The pathogenicity of the BSVD is variable ranging from latency to plant mortality. Foliar symptoms are periodic in expression and follow a distinct pattern but the mechanism is not clear. The most characteristic foliar symptoms include chlorotic and necrotic streaks on the leaves (Lockhart and Jones, 1999). Additional symptoms include narrowing and thickening of the leaf, internal pseudo-stem necrosis, splitting of the base of the leaf sheaths and detachment of leaves, overall stunting of the plant; chocking of the bunch on emergency and plant death (Lockhart and Jones, 1999). Symptoms are most severe in the primary stage following inoculation but become less pronounced and may disappear for a long period (Lockhart and Jones, 1999). The disease affects vegetative, flowering and fruiting stages.

Severely diseased plants may have reduced bunch size (Daniells *et al.*, 2001). Other symptoms include altered phyllotaxis and abnormally shaped fruits with a thinner fruit peel that is prone to splitting (Lockhart and Jones, 1999; Daniells *et al.*, 2001). In some cases, bunches burst out midway up the pseudo-stem.

Symptoms of BSV were first observed in Kenya by Diekmann and Putter (1996) without confirmatory test. Hull (Personal comm.) also confirmed its presence in a few farms in Kisumu (Nyanza Province), Murang'a (Central Province) and Kilifi (Coast Province). BSV has been reported within the East African Region (Uganda, Tanzania, Rwanda and Zanzibar) since 1994 (Kubiriba *et al.*, 1997; 2001a; Harper *et al.*, 2002). A status evaluation of the disease carried out in most of the banana growing regions of Kenya (Western, Central, Eastern and Coastal) confirmed wide spread infection of the disease (Wangai *et al.*, 2002). In the disease diagnostic survey, field symptoms such as chlorotic streak, leaf deformation, stunting, cigar leaf death, distortion of peduncle, bunch or fruits and internal pseudo-stem necrosis were observed in both tissue culture and sucker-produced bananas. Virus characteristics, occurrence and symptom indexing were determined using a serological test, the Enzyme Linked Immunosorbent Assay (ELISA) techniques (Lockhart, 1986).

Symptomatic identification has been confirmed in Kenya (Wangai *et al.*, 2002). Similar investigations carried out in Uganda by John Innes scientists (Harper *et al.*, 2002) provided a valuable resource for comparison. BSV was isolated from infected plants across Uganda's *Musa* growing areas and the isolates analysed using molecular and serological techniques. These analyses showed that BSV is highly variable in Uganda (Harper *et al.*, 2005).

2.6 The Banana Streak Virus (BSV)

2.6.1 Badnavirus species

BSV is an important and most frequently occurring pathogen of *Musa* spp. worldwide and has been associated with substantial yield losses (Lassoudière, 1979; Lockhart *et al.* 1998; Davis *et al.*, 2000; Geering *et al.*, 2000; Daniells *et al.*, 2001). BSV belongs to the genus *Badnavirus*, one of the six genera in the *Caulimovirus* family. Other species found in the genus *Badnavirus* include *Cacao swollen shoot badnavirus* (CSSV) (Patiot *et al.*, 1978; Brunt, 1970), *Canna yellow mottle badnavirus* (CaYMV) (Lockhart, 1988); *Dioscorea*

bacilliform badnavirus (DBV) (Harrison and Roberts, 1973; Phillips *et al.*, 1999), *Kalanchoe top-spotting badnavirus* (KTSV) (Hearon and Locke, 1984), *Piper yellow mottle badnavirus* (PYMV) (Lockhart, 1997), *Schefflera ringspot badnavirus* (SRV) (Lockhart and Olszewski, 1996), *Sugarcane bacilliform badnavirus* (SCBV) (Lockhart and Autrey, 1988) and *Citrus yellow mosaic badnavirus* (CYMV) (Huang and Hartung, 2000). In addition, there are a number of tentative members of the genus (Appendices 2 A and B).

2.6.2 Genome and particle properties

Badnaviruses are restricted to the cytoplasm and are not associated with any inclusions. BSV has an open-circular double-stranded (ds) DNA genome of 7.4 kilo base pair (kbp) in size (Harper and Hull, 1998). The DNA is encapsidated in bacilliform-shaped particles, approximately 150×30 nm (Harper and Hull, 1998; van Regenmortel *et al.*, 2000). The particles have an electron dense core and the tubular portion of the particle has a structure based on an icosahedron cut across its three fold axis with a structural repeat of 10 nm and nine rings of hexamer subunits per 130 nm length. Complete genomes of some of the badnaviruses have been sequenced. These include CoYMV (Medberry *et al.*, 1990), CSSV (Hagen *et al.*, 1993), different isolates of BSV (Harper and Hull, 1998), SCBV (Bouhida *et al.*, 1993) and a member of a very closely related genus RTBV (Qu *et al.*, 1991).

BSV replicates via a single stranded ribonucleic acid (ssRNA) intermediate, which is converted back to dsDNA through the action of a virus-encoded reverse transcriptase (RT). The use of RT in their replication can lead to a high degree of variation between isolates and different group members (Harper and Hull, 1998; Lockhart and Jones, 1999).

The genome organization of most *Badnaviruses* consists of three major open reading frames (ORFs) that are positioned exclusively on one strand (Fig.1). *Badnaviruses* have only one transcriptional initiation site which is present in the intergenic region between the end of ORF III and beginning of ORF I (Pooggin *et al.*, 1999). The functions of ORF I (encoding 20.8 kDa protein) and ORF II (encoding 14.5 kDa protein) are not well understood, although evidence suggests that ORF II protein participates in assembly of the virus particle (Herzog *et al.*, 2000) and could function as an insect transmission factor (Stavalone *et al.* 2001). ORF III encodes a polyprotein of 208 kDa with movement protein, coat protein, aspartic protease, reverse transcriptase, and ribonuclease H (RNase H) functions (Hohn and Fütterer, 1997).

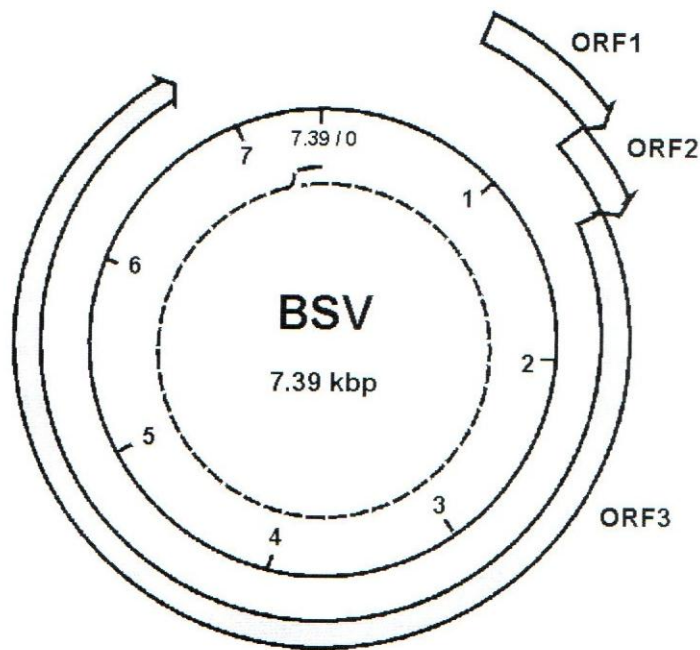


Fig. 1. Organization of the BSV genome. The complete circle represents the double-stranded DNA genome. The inner circle shows the mapping of the RNA transcript with the position of the 3' end being estimated. The outer arcs indicate the positions of the three open reading frames (Harper and Hull, 1998)

2.6.3 Integration of BSV into the host genome

BSV infection in the absence of external sources of infection is thought to originate from activation of viral sequences that are integrated in the host's nuclear genome (Ndowora *et al.*, 1999; Harper *et al.*, 1999b; Lheureux, *et al.*, 2003). Integration of BSV sequences has been identified only in the *M. balbisiana* (B) genome (Ndowora *et al.*, 1999; Harper *et al.*, 1999b; Geering 2001; 2005; Lheureux *et al.*, 2003) though the (A) genome contains an incomplete integrant that is closely related to CSSV (Ndowora *et al.*, 1999).

Four very distinct and different BSV episomal viruses have been discovered, that also exist as integrated sequences within different *M. balbisiana* (B) subspecies *Banana streak Mysore virus* (BSMysV), *Banana endogenous virus* (BEV) (Geering *et al.*, 2001, 2005a); *Banana streak obino L'ewai* (BSOEV) (Harper *et al.*, 1999b; Ndowora *et al.*, 1999); and *Banana streak Imove virus* (BSImV) (Lheureux *et al.*, 2003). These different viruses are distinct and

different enough to warrant species level differentiation. They have also been associated with the production of virus disease in the absence of external sources of infection. As yet, there is only limited information about *Banana streak goldfinger virus* (BSGfV), (Geering *et al.*, 2001), *Banana streak Imove virus* (BSImV) (Lheureux *et al.*, 2003) and *Banana streak Mysore virus* (BSMysV) (Geering *et al.*, 2001a, b).

One type (BSOEV) carries the full virus genome interspersed with non-contiguous, inverted sequences and is capable of undergoing recombination and excision to produce an episomal form of the virus (Harper *et al.*, 1999a; Ndowora *et al.*, 1999). One theory suggests that integration occurs by illegitimate recombination of these plant genomic BSV sequences, induced by processes known to induce genomic rearrangements, for example tissue culture (Ndowora *et al.*, 1999). Such an excision could be triggered during *in vitro* plant regeneration processes (Lheureux *et al.*, 2003; Dallot *et al.*, 2001). This hypothesis has been supported by Dallot *et al.* (2001) who found that the proliferation stage of a common micropropagation procedure triggered episomal BSV expression in a tetraploid AAAB.

A second type of integrant is also very common in the *Musa* genome. However, it does not appear to be related to an infectious disease either because the integrated sequence is incomplete, e.g. the cocoa swollen shoot-like Calcutta 4 integrant within the A genome of *Musa* (Ndowora *et al.*, 1999), or because it contains mutations rendering it defective and hence referred to as “dead” sequences. Geering *et al.* (2005a) reported that banana contains a diverse array of endogenous badnaviruses. This does indicate that there has been a very close relationship between *Musa* and badnaviruses throughout evolution.

In plant pararetroviruses, unlike true retroviruses, integration into the host genome is not required for replication. However, as for BSV, the genome sequences of several other badnaviruses are suspected to be integrated in their host plant, and the sequences for a number of related virus genera within the *Caulimovirus* family including tobacco vein-clearing virus (Lockhart *et al.*, 2000), petunia vein clearing virus (Richert-Poggeler *et al.*, 1996) and *Rice tungro bacilliform virus* in the rice genome (Motoyuki *et al.*, 2004), are found integrated in their plant host genome and are thought to be capable of activation to cause disease.

BSV is therefore a limiting factor in the development of new *Musa* hybrids by breeding programmes across the world. Recently introduced inter-specific *Musa* hybrids bred for improved yield and resistance to disease have been found to be widely infected with BSV isolates such as BSV-Obino l'Ewai (BSOEV); BSV-Imove (BSImV) and BSV-Gold Finger (BSVGfV) (Harper and Hull, 1998; Geering *et al.*, 2000; Lheureux *et al.*, 2003). It has not yet been established in Kenya which of the integrants are present in the banana cultivars. Expansion of banana plantation may be affected if the BSV is not detected early.

2.7 Factors of banana streak virus activation

The disease severity is variable and depends on the environmental conditions, level of management, the host, and virus genotypes (Lockhart, 1986; Dahal *et al.*, 1998a; Lockhart and Jones, 2000). There is little known about the effect of host genotype on symptom expression and this needs further investigation. However, the environmental conditions such as low temperature, wet environment and *in vitro* conditions have been observed to enhance the symptom expression (Dahal *et al.*, 1998a). Symptom expression has also been correlated with virus titre in the leaves, where there are severe symptoms the titre levels are higher (Dahal *et al.*, 1998b). In the field experiments, however, there has not been a clear correlation between temperature and symptom development while wet environment and high soil moisture increased the symptom expression (Daniells *et al.*, 2001).

2.7.1 Effect of Environment on BSV symptoms: temperature and moisture

Temperature and soil moisture are some of the stress factors, which increase BSV expression in the field (Dahal *et al.*, 1998a; Daniells *et al.*, 2001). Symptom expression has been noted to follow a distinct pattern but the underlying reasons are still not known. Generally symptoms have been observed under low temperatures and short-day conditions (Lockhart and Jones, 1999). In Nigeria, greater, symptom expression was observed with temperatures of 22° C while temperature of 28-35° C in a screen-house inhibited virus activation (Daniells *et al.*, 2001) and mostly during wet rather than dry season (Dahal *et al.*, 2000). Other environmental conditions associated with wet season such as low light intensity may trigger symptom expression. Rapid plant growth due to favourable conditions may also accelerate virus replication (Daniells *et al.*, 2001).

The relationship between wet season and cool temperature on the expression of BSV has not been established. However, in Australia and Morocco, BSV expression was observed in leaves that were initiated or produced during hotter times of the year (Daniells *et al.*, 2001). Symptom expression seems to be associated with the change of season. Fluctuating temperatures may presumably play a role (Dahal *et al.*, 1998a). Some infected land races show no symptoms even under fluctuating environmental conditions (Dahal *et al.*, 1998a). Precise correlation between symptom appearance and / or severity; and temperature changes has not been experimentally determined although the intermittent symptom expression has been correlated with virus titre in the leaves with symptomatic leaves containing higher levels of virus (Dahal *et al.*, 1998b). Identification and understanding of the factors responsible for symptom expression and their interaction is important as detection is more reliable from symptomatic than from asymptomatic samples (Dahal *et al.*, 1998b). It is also important to determine the appropriate time of sampling for more reliable detection of the virus (Dahal *et al.*, 1997).

2.7.2 Effect of nutritional levels on expression of BSV

BSV symptoms tend to be more severe in poorly managed plantations (ASPAC Newsletter, 1998). The ideal soils for production of bananas are alluvial soils (inceptisols) or volcanic soils (andisols) with an average nutritional requirement of 200 g per plant for Nitrogen (N), 50-70 g per plant Phosphorous (P) and 300 g per plant Potassium (K). Soils deficient in major nutrients especially nitrogen have been reported to activate BSV expression in banana plantations. Greater BSV expressions have also been observed with advancement of the crop possibly due to depletion of nutrients resulting into reduced yields (Daniells *et al.*, 2001). Suppressive levels of N however have not yet been determined. More work is required to determine the relative contribution of the soil nutrients to virus replication, infection and symptom expression. Symptom observation and identification is not a reliable method because plants may become asymptomatic with certain changes in temperature (Dahal *et al.*, 1998a; 2000).

2.7.3 The host: *Musa* and *Ensete ventricosum*

Musa spp and *Ensete ventricosum* are the only known hosts of banana streak virus. *Ensete ventricosum* is a natural host of the virus. Attempts to transmit BSV to other varieties in the family such as *Musa textiles*, *Heliconia* and *Strelitzia* have not been successful. However many genotypes of *Musa acuminata* (*Musa* AA, and AAA groups), *M. balbisiana* (*Musa* BB groups) and hybrids of the two species (*Musa* AAB, ABB and ABBB groups) are susceptible to BSV infection (Harper *et al.*, 1999a; Lockhart and Jones, 1999; Geering *et al.*, 2000). Studies have indicated that some banana cultivars, for example Cavendish groups, are more susceptible to the BSV than others, a fact that could pose great threat to the banana industry (Hwang *et al.*, 1998). Information on banana genotypes could be used to identify and recommend appropriate cultivars in Kenya. The information is important because it may be possible to “cure” A genotype *Musa* of all BSV types by tissue culture (Heliot *et al.*, 2002) which obviously cannot be done for B containing *Musa*.

2.7.4 Virus genotype

The virus is serologically and genomically highly variable. Several distinct species and isolates of BSV have been identified through PCR amplification with degenerate and specific primers followed by DNA hybridization analysis. Lockhart and Olszewski (1993) reported four distinct isolates of BSV which showed significant sequence diversity. The 7.4 kb genome of one isolate of BSOEV from Nigeria (BSV Onne) has been completely sequenced (Harper and Hull, 1998). The isolate originated from a tetraploid hybrid of ObinoL'Ewai x Calcutta 4. In Uganda, studies on BSV identified wide diversity of the pathogen, about 15 different species (Harper *et al.*, 2005). Four Australia isolates from Williams banana cultivar (Cavendish) ‘Mysore’, ‘Goldfinger’, and ‘Red Decca’ were designated BSV-Cav, BSV-Mys, BSV-GF and BSV-RD, respectively (Geering *et al.*, 2000). The isolates were divergent in nucleotide sequence with 68 to 78% sequence similarity in the conserved RNase H domain (Geering *et al.*, 2000). Other isolates include BSV-IM (Imove) from IRFA 910. In most occurrences the isolates are plant cultivars specific, an indication that there could be as many isolates as there are cultivars. This diversity causes a problem in virus indexing and recommendation of general diagnostic protocols.

2.8 Transmission of BSV

BSV is transmitted in a semi-persistent manner (Lockhart and Jones, 1999; Kuriba *et al.*, 2001 b) through a vector (mealy-bug), seed and pollen, planting material and viral sequences (Lockhart and Olszewski, 1993; Kubiriba *et al.*, 2001 b). Attempts to transmit BSV by mechanical inoculation using abrasion have been unsuccessful possibly due to high levels of phenolic compounds and latex present in *Musa* plants (Lockhart, 1986). It is therefore unlikely to be transmitted on cutting tools or during cultural operations. It is also not soil-borne (Lockhart, 1995). BSV is also not transmitted through mechanical inoculation of sap extracts. Use of infected plant material poses a greater risk of dissemination.

2.8.1 Vectors

The epidemiology of banana streak disease (BSD) is unclear. BSV is transmitted by several species of mealy-bugs (*Homoptera pseudococcidae*). In the greenhouse, BSV has been transmitted from banana to banana by *Planococcus citri* (Lockhart, 1995) and this species has also been observed in field-grown *Musa* in Queensland, Australia (J. E. Thomas, unpublished results). Although the family *Musaceae* is the only known hosts of BSV, sugarcane bacilliform virus (SCBV) has been experimentally transmitted from sugarcane to banana by *Saccharicoccus sacchari* (Diekmann and Putter, 1996), where it elicits typical symptoms of BSD (Bouhida *et al.*, 1993). It can therefore be speculated that at least some cases of BSD could have arisen from transmission of *Badnaviruses* from sugarcane to banana or vice versa. BSV was detected in field-captured *Dysmicoccus brevipes* and *S. sacchari* and a strong correlation observed in banana plantations between the abundance of *D. brevipes* and the incidence of BSV, implicating this species as a vector of BSV (Kubiriba *et al.*, 2001a). Transmission of the virus is achieved after a 5 minute acquisition feeding. Vectors retain the ability to transmit virus for up to 72 hours (Lockhart and Jones, 2000; Kubiriba *et al.*, 2001b). All life stages of the vector can acquire and transmit the virus. However, the early instar-nymphs, crawlers that are actively moving between plants and sometimes blown by wind or carried by ants cause most damage (Lockhart, 1995). Field observation suggests that the rate of spread by mealy-bugs is however slow (Daniells *et al.*, 2001).

2.8.2 Planting material

Significant transmission of BSV is likely to be through infected vegetative propagation material such as corms, suckers and tissue culture-derived plants (Lockhart and Jones, 2000). BSV causes systemic infection and all tissue cultures derived from meristems of diseased 'B' containing *Musa* plants have been found to carry BSV, so integrated sequences would be present in the meristems, but in general all caulimoviruses are thought to be actually excluded from meristematic regions (Lockhart and Jones, 1999). In countries where the BSV is widespread, the epidemiology of BSV is still poorly understood. Therefore a detailed study is required using other detection measures to understand epidemiology of BSV in Kenya.

2.8.3 Transmission by seed

Since BSV is also transmitted through seed, breeders should consider this fact when selecting parental stocks for breeding programs. Studies in Australia using Mysore plants with BSV in the breeding crosses have shown that the virus is almost certainly seedborne (Daniells *et al.*, 1995). Mysore contains the 'B' genome, and the Mysore virus is known to be integrated (Geering *et al.*, 2005b) therefore the virus is in all tissues including seeds and fluorescence. Further, analysis of BSV incidence in F₁ triploid (AAB) population produced by hybridisation between virus- and disease-free *M. balbisiana* and *M. acuminata* indicated that a genetic mechanism is involved in BSV appearance. Amplified Restriction Fragment Length Polymorphism (AFLP) markers segregating with the presence or absence of episomal viral particles were identified in the *M. balbisiana* genome only (Lheureux *et al.*, 2003). Knowledge of the presence or absence of BSV in banana genotypes with 'B' genome may guide breeders in developing suitable varieties to avoid spread of the disease in Kenya. Selection and deployment of host-plant resistance is considered as the most sustainable approach for the management of pests and diseases of *Musa* spp. (Vuylsteke *et al.*, 1993).

2.8.4 Viral sequence transmission

It has recently been shown that BSV genomic sequences are integrated into the genomic DNA of *Musa* (Harper *et al.*, 1999a; Ndowora *et al.*, 1999; Geering *et al.*, 2001b; 2005b). BSV-related sequences have been found in more than 400 *Musa* genotypes by PCR amplification (LaFleur *et al.*, 1996). However, the nature of the sequences is variable.

Infection of some isolates of BSV may arise *de novo* from viral sequences that are integrated into the *Musa* genome (Ndowora *et al.*, 1999; Harper *et al.*, 1999b). BSV integrants are linked to the 'B' genome of cultivated *Musa* and are absent from Cavendish banana (Geering *et al.*, 2001b). Tissue culture has been considered as one of the trigger factors for episomal expression of these integrants (Ndowora and Lockhart, 1997). Further work, however, is needed to characterise activatable integrants. This will enable development of diagnostics, which will show the potential risk of cultivars becoming infected following stress. Understanding of the integrants will help to determine approaches to controlling the problem and this suggests that successful management of BSV requires accurate detection of the virus to avoid the use of infected material for planting, tissue culture propagation, and breeding (Harper and Hull, 1998). Therefore, it is important to use a reliable method for detecting the virus. There is no appropriate method for mass detection of BSV in Kenya and this needs to be put in place.

2.9 Characterisation of BSV

Several BSV isolates have been identified around the world (Geering *et al.*, 2000). The entire genome of BSOEV from cultivar Obino-L'Ewai has been sequenced and is in the Genbank database (Harper and Hull 1998). Other incomplete sequences of isolates include BSMysV from cvs Mysore; BSGfV from cvs Gold finger; BSI_mV from CIRAD hybrids and BSCavV from cvs Cavendish (Geering *et al.*, 2000; Geering *et al.*, 2001a). Earlier studies by Lockhart and Olszewski (1993) examined five other isolates that included BSV-Mad/DC from cv. Dwarf Cavendish Madeira; BSV-MA from cv. Grand Nain, Morocco; BSV-DC/Mdg from cv. Dwarf Cavendish, Madagascar; BSV-Mys/T from cv Mysore, Trinidad and BSV-Rw from Rwanda. BSOEV, BSI_mV and BSGfV are most common in new hybrids in Africa, Central and South America (Geering *et al.*, 2001a; Lheureux *et al.*, 2003). On the basis of conserved RT sequences, 15 BSV species have been recently identified in Uganda these include BSUgIV, BSUgLV, BSUgKV, BSUgJV, BSUgHV, BSUgGV, BSUgFV, BSUgDV, BSUgEV, BSUgCV, BSUgBV, BSUgAV, BSOEV, BSI_mV, BSGfV (Harper *et al.*, 2005), in addition to some of the species already described. This indicates that a vast number of highly variable isolates may be present in the banana plants. Most recently a genome of BSV-Mys from cultivar Mysore AAB has been completely sequenced and is distinctly different from BSOEV (Geering *et al.*, 2005b).

Genomic characterisation is a prerequisite to the management of virus diseases. Since a number of banana cultivars is grown in Kenya, there is a possibility of several isolates, which might need specific detection and differentiation. Based on the current information, only BSOEV, BSIImV, BSGfV and BSMYV isolates are considered to arise from integrated sequences (Geering *et al.*, 2001a; Lheureux, *et al.*, 2003, Geering *et al.*, 2005b). Geering *et al.* (2005a) has published other *Badnavirus* integrated sequences referred to as banana endogenous viruses (BEV). This however, needs further investigation. Use of phylogenetic analyses to compare relationship of isolates has been recorded to reveal great genetic distances sufficient to be identified as distinct species (Geering *et al.*, 2000; 2001a; Geering *et al.*, 2005b; Harper *et al.*, 2005). This procedure was used in the identification of the Kenya isolates.

2.10 Diagnostic Methods

Detection of BSV on symptoms alone is unreliable as symptomless infection can occur (Dahal *et al.*, 1998b). The most widely used techniques are enzyme linked immuno sorbent assay (ELISA), immunosorbent electron microscopy (ISEM) and polymerase chain reaction (PCR) (Ndowora and Lochart, 1997; Thottappily *et al.*, 1998; Ndowora and Lochart, 1999; Geering *et al.*, 2000).

2.10.1 Serological diagnostics tests

Efficient management of BSV virus requires quick and reliable detection of the virus. Commonly used methods for other viruses might not be reliable for BSV. Visual detection through symptoms is unreliable because of the occurrence of the disease in asymptomatic plants; long incubation periods before symptom expression; similarity with the cucumber mosaic disease symptoms; and variation of BSV expression with environmental changes (Lockhart, 1994; Dahal *et al.*, 1998a).

The very high level of variation between virus isolates has implications for serological analysis. Individual antibodies often fail to detect some BSV isolates (Lockhart and Mierzewski, 1993). Ndowora (1998) reported a preparation of an antibody using BSV-Mys and a mixture of 32 isolates of sugarcane bacilliform virus for broad-spectrum detection of

BSV isolates. In spite of this improvement of reliability in BSV detection by ELISA, some virus isolates still remain either weakly or not detected by this method.

An alternative method, which has been reported, and is effective in detection of most of the isolates, is immunosorbent electron microscopy (ISEM). However, the technique is expensive, laborious and requires specialized equipment and skills (Lockhart and Jones, 1999). Hence use of a serological method, enzyme-linked immunosorbent assay (ELISA), would be a better option for BSV diagnosis.

Serological methods are widely used in plant virus diagnosis because they are simple, practical and specific. Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) was first described for use in plant virus detection by Clark and Adams (1977). Direct-virus specific antibody bound to a specific solid phase traps the virus and the bound virus is detected with virus specific enzyme-conjugated IgG. Lockhart and Olszewski (1993) however reported that individual antibodies often fail to detect some BSV isolates. The BSV isolates have high genomic and serologic viability and this makes detection difficult. For better efficiency of serological methods, Ndowora *et al.* (1998) investigated on a cocktail of antibodies (PMX2RC) using BSV-Mys and a mixture of 32 isolates of sugarcane mosaic virus, representing the widest serological diversity of the virus, for broad-spectrum detection of BSV isolates.

2.10.2 Immunosorbent Electron Microscopy

The technique involves trapping of virus specific antibodies on carbon coated grids after which sample is added. Virus specific antibodies recognize and bind to specific virus. Bound antibodies are observed as dense area around the particle under the microscope. Lockhart (1986) successfully detected BSV in ISEM tests where antiserum prepared in rabbit was used. ISEM is a sensitive detection method; though it is expensive, laborious and requires specialized equipment and skills (Lockhart and Jones, 1999).

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2.10.3 Enzyme-linked immunosorbent assay (ELISA)

The enzyme-linked immunosorbent assay (ELISA) has been very popular for detection of viruses in plant material, insect vectors, seeds, and vegetative propagules since it was introduced to plant virology by Clark and Adams (1977). Due to its adaptability, sensitivity, and economy in use of reagents, ELISA is used in a wide range of situations, especially to test a large number of samples in a relatively short period of time. Many variations of ELISA have been developed (Clark and Bar-Joseph 1984; Cooper and Edwards, 1986; Van Regenmortel and Dubs, 1993). For this work we used mainly double antibody sandwich (DAS) ELISA which is highly strain-specific.

In the process of ELISA, antibody specific to virus in question is bound to solid phase surface. After an incubation period of 2 h at 37 ° C, the infected sample is added to trapping or binding with the antibody. After the second incubation at 37 ° C a second virus specific antibody which is conjugated with an enzyme is added. Reaction is measured by conversion of specific substrate by enzyme.

Isolates of SCBV which are serologically closely related to BSV were used by Ndowora and Lockhart (2000) to prepare a broad spectrum polyclonal antiserum against BSV. The seroagents were developed against 32 SCBV isolates from sugarcane cultivars representing a wide diversity of viruses BSV-Mys isolates were added for production of antibodies. The antiserum detected 3 isolates of BSV which were serologically unrelated.

Recent studies of Agindotan *et al.* (2003) showed that monoclonal and polyclonal antibodies raised against a Nigerian BSV isolate did not detect the virus by Triple antibody sandwich (TAS) - ELISA in several samples despite the fact that they had typical BSV symptoms. There was need to investigate and optimise factors which could possibly affect the performance of BSV detection such as antigen and antibody dilutions and antibody types would also need to be investigated and optimised.

2.11 DNA-based diagnostic tests

2.11.1 Polymerase chain reaction (PCR)

Genome based methods of BSV detection such as nucleic acid hybridization and PCR amplification are highly sensitive. However, a major problem in using PCR-based diagnostics is that BSV sequences are integrated into the host genome of certain banana genotypes (LaFleur *et al.*, 1996; Harper *et al.*, 1999b; Ndowora *et al.*, 1999). Hence it is essential to ensure that the sample is not contaminated with chromosomal DNA. Detection of BSV has been successfully done through the use of serological trapping of the virus by IC-PCR technique which detects specifically episomal BSV in a reliable and sensitive manner (Harper *et al.*, 1999a). The technique avoids false positives due to integrated viral sequences and, hence it is a potential tool in diagnosing BSV infection (Harper *et al.*, 1999a).

2.11.2 Immunocapture -polymerase chain reaction (IC-PCR)

As most *Musa* cultivars contain integrated BSV sequences, a standard PCR assay will amplify both integrated and episomal copies of the viral DNA. Hence the technique can give false positives for episomally-replicating virus if plant genomic DNA containing integrated BSV sequences is present in the sample. IC-PCR is preferred as it should amplify only episomal virus because immunocapture is used to specifically bound virions, allowing contaminating plant genomic DNA to be washed away (Harper *et al.*, 1999a). Studies done by Harper *et al.* (1999a) showed that antisera used in the IC-PCR are capable of binding a large number of BSV serotypes and under the conditions selected for IC-PCR, nuclear, mitochondrial or chloroplast genomic sequences are not amplified and thus only episomal BSV is captured. They also reported that the technique is suitable for large-scale screening of *Musa* for BSV. Therefore, it could be useful in identification of the banana isolates in Kenya. After characterisation of the isolates, specific IC-PCRs will be developed for efficient routine BSV-indexing of tissue culture banana material before distribution to farmers.

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Study site description

Laboratory; greenhouse and fields experiments were conducted at KARI- Njoro, Kokoto and Rongai near Nakuru. Molecular and electron microscopy work was done at the John Innes Centre Laboratory in Norwich, United Kingdom.

KARI – Njoro lies at an altitude of 2166m above sea level (ASL) and is located within the agro-ecological zone low highlands (LH3) (Jaetzold and Schmidt 1983), longitude 0° 20' South and 35° 56' East with a mean annual rainfall of 931 mm per annum. Temperature ranges from 7.9 °C to 21.9 °C with a mean of 14.9 °C (Jaetzold and Schmidt 1983). Kokoto is in upper midland (UM4) zone at an altitude of 1830 ASL with annual rainfall of 700 mm and mean temperatures of 20°C. Rongai is in 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).

3.2 Sample collection and population

Two hundred and fifteen plant samples were collected from ten locations within five provinces in the major banana-growing regions of Kenya using simple random sampling method. Approximately 10-90 samples were collected per site for the experiments on serological and molecular diversity. The areas included Kisii (Nyanza), Mt Elgon, Kakamega and Bungoma (Western), Thika, Murang'a, Kirinyaga and Nyeri (Central), Nakuru (Rift Valley) and Embu (Eastern) Provinces (Fig. 2). The leaf tissue samples were from about 50 different cultivars (Appendix 7). They varied in expression within and between cultivars from mild to severe expression. Site selection and choice of the plant material collected was based on major banana growing regions and preliminary survey which had shown infection in all selected regions (Wangai *et al.*, 2002). Samples were collected randomly from genotypes showing varying levels of BSV (Fig. 3). For each sample, the locality, age of plant, the symptoms and the cultivar were recorded. Samples were maintained in freeze-dried state in

the ultra- freezer -72°C. During the collection in the field the second last leaf was harvested because it is the stage when the virus is the most actively multiplying and the cell wall can easily break to release virus. The collected material was preserved in the cool-box under ice. Once in the laboratory, leaves for immediate use were kept at 4 ° C but for long storage they were freeze-dried or preserved at -72°C.

3.3 Growth and maintenance of mother –plants

Suckers with disease symptoms collected from various geographical regions were planted in polythene bags and maintained in the green house where they were watered regularly and sprayed with Bullock® containing active ingredient Beta-cyfluthrin 12.5g/l and Chlorpyrifos 250 g/l for aphid control. The concentration used for spray was 20 ml/45 litres of water. Mitigan® with an active ingredient Cypermethrin was used for the control of mites. The rates used for spray were 20mls/30 litres of water. Rindomil® with active ingredient Mancozeb 640g and Metalaacyl-M 40g was used for fungal infection at the rate of 20g / 40 litres of water nb check on rates/ha check spelling for metalaacy

3.4 *In vitro* micropropagation of bananas

Two suckers were selected from each mother plant for different types of varieties and shoot tips were micropropagated according to a routine procedure of banana *in vitro* culture as described by Vuylsteke (1989) and adapted by Acuña (1996) with slight modification to suit local conditions. This procedure followed three stages: first the shoot tips were trimmed and transferred to the culture medium for the initiation stage; this was followed by proliferation stage on a medium with cytokinin. This stage had 6 cycles of multiplication; finally shoot development and rooting of individual plants (plantlets) were obtained during the rooting stage. The plantlets were transferred to nursery for acclimatisation. One and 2.5 mg/l of 6-benzylaminopurine (BA), were used in the MS medium (Murashige and Skoog, 1962) (Appendix 3), for the initiation stage (one month) and proliferation stage (three to six cycles of three weeks each), respectively. During the rooting stage, the plantlets were grown in MS medium without BA. During the stages of initiation and rooting, the photoperiod was fixed at 16 h of light (15500 Lux) and 8 h of darkness. In both cases, the temperature was maintained at 28° C.

3.5 Potting of greenhouse plants

The medium used for potting was at a ratio of 2:1:1 (2 parts of forest soil, 1 part of manure and 1 part of sand). It was put in polythene bags of 30 cm by 24 cm size. Plantlets were transferred and hardened gradually in the greenhouse for a period of three months.

3.6 Monitoring of symptom expression

BSV symptoms on all plants were monitored at monthly intervals. To quantify symptom severity, symptoms on individual plants were scored on a scale of 0-5 which was a modification of 0-3 used by Dahal *et al.* (1999), where 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 chlorotic streaks, and 5 is necrotic streaks (Fig 3). The total number of leaves and number of leaves with symptoms were recorded for each plant. The symptom severity Index (SSI) (Dahal *et al.*, 1999) was calculated from the severity score of individual leaves as $SSI = (0a + 1b + 2c + 3d + 4e + 5f) / n$, where a-f are the number of leaves with scores of 0-5, respectively and n is the total number of leaves observed. The SSI of individual plants was averaged to give a mean symptom severity index (ASSI) of each variety according to Dahal *et al.* (1999). For height and girth, the total measurements for the plant was taken, subtracted from the previous month measurements to obtain the rate of increase for the month.

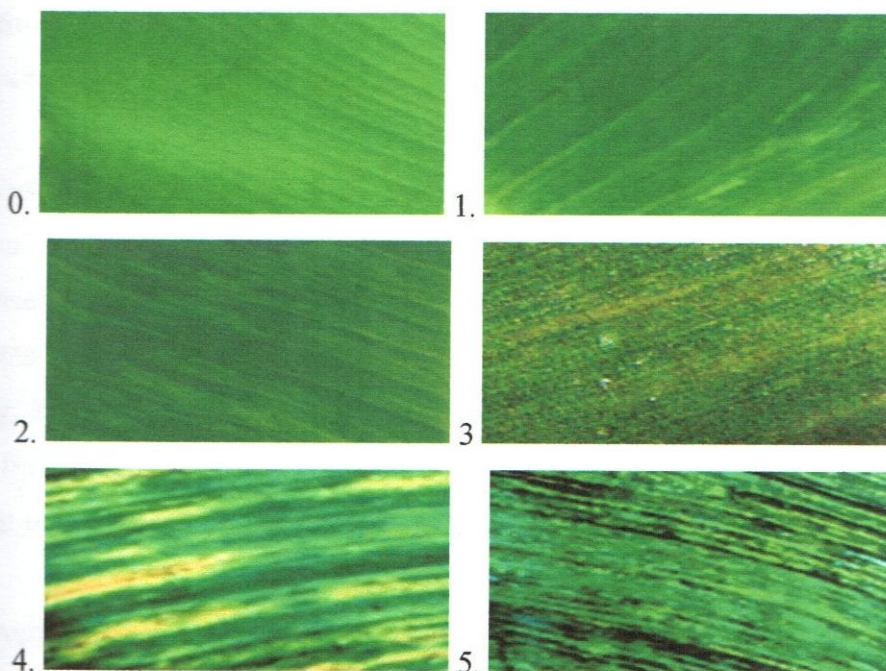


Fig. 3 BSV disease rating scale, based on symptomatic expression where 0 = no symptoms, 1 = localised flecks, 2 = scattered discontinuous streaks, 3 = continuous streaks covering moderate portion of lamina, 4 = continuous and conspicuous chlorotic streaks, and 5 = necrotic streaks.

3.7 Serological diagnostics (ELISA).

While carrying out serological tests a double antibody sandwich - enzyme linked immunosorbent assay (DAS-ELISA) was used (Clark and Adams, 1977).with polyclonal antibodies against BSV (PMX2RC) (Ndowora and Lockhart, 2000)

Sap extraction was done from fresh- frozen leaves of the collected samples with or without symptoms. Small portions of leaves (5-10 cm) from 2-3 fully expanded 2nd leaf lamina were used per sample (excluding unrolling 'cigar' leaf, old and dying leaves). Samples were cut into fine pieces and about 1 g of the tissue was ground into general extraction buffer of 4 ml of x 1 Phosphate Buffered Saline (PBS) (100 mM PBS pH 7.4, containing 2% NaCl, 1% Na₂SO₄) with the addition of 0.05% v/v 2-Mercaptoethanol using pestle and mortar (Appendix 4i). The extract was left to stand for about 30 min - 2 hrs in 4°C before it was used. To detect presence of BSV, polystyrene micro-titer plates (Titertek Multiscan Plus, Huntsville, Alabama, USA) were coated with 1 µg/ ml - 2 µg/ ml purified BSV IgG of a polyclonal BSV antibody (PMX2RC) (Ndowora and Lockhart, 2000) in coating buffer (Appendix 4ii). Wells were filled with 100µl of antiserum diluted at 1:1000. The plates were sealed in plastic bags, put in a humid lunch box and incubated overnight at 4°C.

were filled with 100 μ l of antiserum diluted at 1:1000. The plates were sealed in plastic bags, put in a humid lunch box and incubated overnight at 4°C.

Plates were washed four times with PBS + 0.05% Tween 20 (PBS-T) (Appendix 4iii). After each washing of plates they were left to stand for two minutes then tapped on paper towel and completely dried after the final wash. Micro-plates were coated with 100 μ l of antigen in duplicate wells and incubated at 4°C overnight. Plates were again washed as for the antibody coating. Five % of dried milk diluted in distilled water was added to the washed plates to block the background of the plates. To each well 100 μ l 5% of the milk was added, left to stand at room temperature for 30 min then washed as for antigen coating.

The trapped BSV antigens were detected using a 1:1000 dilution of IgG – alkaline phosphatase conjugate in PBS with 2% w/v polyvinyl pyrrolidone (PVP) and 0.2% w/v bovine serum albumin (BSA) and incubated at 37° C for 2 hrs (Appendix 4iv). Plates were washed with PBS -T. The reactions were visualized by addition of 0.5 mg mL⁻¹ *p* – nitro – phenyl phosphate substrate (PNP) (Sigma Chemical Co).

Absorbance values were determined at wavelength of 405 nm where readings were taken after every 30 min up to 2 hours. Where necessary, plates were left overnight incubated at 4° C and read the following day. Mean absorbance values which were one and a half times or more than the mean of the healthy samples and were greater than 0.100 were considered to be BSV infected (Thottappilly *et al.*, 1998). Colour development in the plates was also observed and noted for confirmation of the readings. Plates were read on ELISA Reader (E-Max Molecular Devices, Sunnyvale, CA, USA). Tissues from BSV infected samples were used as the positive control and PBS buffer as negative control in all the serological analysis experiments.

3.8 DNA-based diagnostics

3.8.1 Immunocapture-polymerase chain reaction (IC-PCR)

During IC-PCR process, sterile thin walled 0.5 ml polypropylene microfuge tubes were coated with polyclonal antibodies at a dilution of 1:100 in carbonate buffer for 2 h at 37°C. The tubes

were then washed three times with PBS-T and 100 µl samples in PBS/0.1% 2-mercaptoethanol prepared as had been described earlier was placed in each tube. This was incubated at 37°C for 2h. The tubes were then washed twice with PBS-T, once with sterile distilled water and then dried briefly. PCR was carried out directly in the tubes as described below. The antibodies used in this study were a mixture of different BSV and SCBV isolates (Ndowora and Lockhart, 2000).

3.8.2 Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) was carried out using primer pairs in Table 1. A 100 µl PCR mix used contained 10x reaction buffer, 10 mM each dNTP, 10 pM each primer 1U Taq polymerase (Invitrogen, Billingham, UK) and 50 mM Magnesium Chloride. Viral DNA trapped onto the PCR tubes was used as the DNA source. The negative control used was PBS buffer and the positive was BSOEV DNA. PCR cycle conditions were an initial denaturation at 94°C for 5 min, five cycles of 94°C for 30 s, 37°C for 30 s and 72°C for 1 min, followed by 30 cycles (94°C for 30 s, 50°C for 30 s, 72°C for 1 min) and a final extension of 72°C for 10 min. The purpose for the two annealing temperatures were to ensure low temperatures capture all related sequences and higher temperatures minimizes amplification of non-specific sequences.

3.8.3 Detection of PCR -amplified DNA fragments

Agarose gel electrophoresis was used to separate the DNA fragments as described by Harper and Hull (1998). Ten microliters of PCR product was loaded onto a 1 % agarose gel in 1 x Tris-borate-EDTA (TBE, 90 mM Tris-borate, 2 mM EDTA, pH 8.0), run at 80 V for 30 min, and the gel stained with 0.5 µl ethidium bromide in 30ml of gel and visualized under UV transilluminator for detection of the DNA bands (Sambrook *et al.*, 1989).

Table 1 Primers used in the amplification of various regions of HSV isolates. Both forward and reverse sequences are indicated.

Primer	Primer Sequence ^a	Virus isolate ^b	Source	Product	Product length
D - F1	5'- ATCTGAAGGTGTGTTGATCAATGC-3'	BSV-RD	GenBank- (AF215816) (Geering <i>et al.</i> , 2000)	ORFIII polypeptide	522
D - R1	5'-GCTCACTCCGCATCTTATCAGTC-3'				
av-F1	5'-AGGATTGGATGTGAAGTTTGAGC-3'	BSV-Cav	GenBank- (AF215815) (Geering <i>et al.</i> , 2000)	ORFIII polypeptide	782
av-R1	5'-ACCAATAATGCAAGGGACGC-3'				
F-F1	5'-ACGAACTATCACGACTTGTTC AAGC-3'	BSGFV	GenBank- (AF215814) (Geering <i>et al.</i> , 2000)	ORFIII polypeptide	476
F-R1	5'-TCGGTGAATAGTCTGAGTCTTC-3'				
SV4673-F1	5'-GGAATGAAAAGAGCAGGCC-3'	BSOEV	GenBank- (AJ002234) (Harper <i>et al.</i> , 1999a)	Aspartic protease	644
SV5317-R1	5'-AGTCATTGGGTCAACCTCTGTC-3'				
A-F1	5'CTNTAYGARTGGYTNATGCCNTTYGG3'	Badna	(Harper <i>et al.</i> , 2002)		597
-R1	5'TCC AYT TRC ANA YNS CNC CCC ANC C3'				
KOL1-F1	5'- GGACAGAGGTTGACCCCAATGA-3'	BSV-OL	(Unpublished) (Designed by Kimani, E. of KARI)		363
KOL2-R1	5'-CGATTGTGATTCACTTCTCGGC-3'				

NB: F1 - forward, R1 - reverse

^aPosition of degenerate nucleotides is indicated by the following codes: N = A, C, G, or T; Y = C or T

^bVirus from which sequence was obtained for primer design

3.9 Data analysis

All data on symptom severity, plant height, width, number of leaves and absorbance levels were subjected to ANOVA using the the General Linear Model (GLM) of SAS computer package version 6.12. The statistical model used was: $Y_{ijk} = \mu + \alpha_i + \gamma_{j(i)} + t_k + \beta_{ik} + \epsilon_{ijk}$ (SAS, 1999; Gomez and Gomez, 1984).

Where Y_{ijk} = the observation of the i^{th} Nitrogen level/ antibody type and k^{th} cultivar of j^{th} sample; μ = General mean, α_i = Effect of i^{th} N levels/ antibody type, $\gamma_{j(i)}$ = Error due to Replicate j in the i^{th} N level/ antibody type (Error a), t_k = Effect of the k^{th} variety, β_{ik} = Interaction effect of N levels/ antibody type and cultivar, ϵ_{ijk} = Error (b), $i = 1 \dots s$, $j = 1 \dots r$, $k = 1 \dots t$. Means were separated using the Least Significant difference (LSD). Test with correlation coefficients between expression or detection of banana streak virus and factors such as cultivar/ nitrogen levels/ antibody type.

CHAPTER FOUR

EFFECT OF ENVIRONMENT AND NITROGEN APPLICATION ON THE EXPRESSION OF BSV SYMPTOMS IN BANANA CULTIVARS

4.1 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-vitalize 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 affected negatively *in vitro* production and is considered a major constraint to banana improvement worldwide (Ortiz, 1996).

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 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 and in a greenhouse in 2005/2006. The objective was to identify the effect of the environment and nitrogen application on the infection and expression of BSV symptoms in tissue culture banana cultivars.

4.2 Materials and Methods

4.2.1 *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, Jamaga, Lacatan, Muraru, Namukhila, Ng'ombe, Ntobe, Sibusi, Uganda Green, and Valary. Shoot tips (30 explants) were micropropagated as described in section 3.4.

The mother plants of each cultivar were sampled and indexed for BSV ELISA protocols in section 3.7. Only BSV negative materials were selected for micropropagation. All the micropropagated plants were established in polythene bags containing 2 kg of soil.

4.2.2 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 micropropagated 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 cm by 24 cm of the polythene bags. Cultivar Grand Naine was used as a healthy control in this experiment. No virus inoculation was done. The soil used was sterilized and 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 as previously described.

The study was conducted during the six months growing period before on set of flowers. Plants were observed for six months whereby data was collected monthly. The variables measured were symptom severity where individual leaves were scored on a disease rating scale of 0-5 (Fig. 3) and the total number of leaves and number of leaves with symptoms were recorded for each plant.

4.2.3 The effect of cultivar and environment (location, season) on BSV symptom expression and re-infection

The objectives of the experiment were to identify the effect of cultivar, and environment on BSV symptom expression. Evaluation of fifteen *in vitro* propagated cultivars was carried out in three sites; KARI Njoro, Kokoto and Rongai all in Nakuru District between January 2005 to 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 per ha of diammonium phosphate fertiliser was used at planting time which are the current recommended quantities. Fortnight spraying with fungicides and miticide was as reported in section 3.3. Weeds were removed by uprooting regularly to maintain a clean stand. Infection and symptom expression was monitored as described in section 3.6.

4.3 The effect of nitrogen levels on BSV expression

The objective of the experiment was to evaluate the effect of nitrogen on the BSV symptom expression of micropropagated seedlings. Two cultivars Ng'ombe and Uganda green were selected because they were found to be quite susceptible to BSV infection during the preliminary field surveys and are popular commercial cooking cultivars, of genotype EAH-AAA. A CRD experimental design was used with 3 replications. The layout was composed of three levels of nitrogen fertiliser. The nitrogen fertiliser used was Urea (46:0:0 N: P: K). Three levels of application were 0, 100, and 200 g/plant. The recommended quantity is 100 g per plant during the first six months. The soil was analysed for micronutrients at the start of the experiment (Appendix 5). The application of nitrogen commenced when seedlings were two months old after transplanting from tissue culture stage. Data recording and analysis was done monthly after plants had attained 3 fully expanded leaves.

4.4 Data analysis

All data analysis was as reported in section 3.9. In the above experiments on effect of age, environment and nitrogen, 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 was transformed before analysis with ANOVA procedure

4.5 Results and Discussion

4.5.1 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 symptomless (Table 2). Over 73% of the micropropagated 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 2). 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 positive tetraploids such as Gold finger, there is a possibility of integrated viral sequences being activated after tissue culture (Ndowora *et al.*, 1999; Karanja *et al.*, 2008b). For all the cultivars three seedlings with absorbance levels of less than 0.1 were selected and planted in the field.

4.5.2 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 3). Cultivar, Gold finger, had highest symptom expression where as 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.*, 1998a). 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. Serological analysis of 15 banana cultivars before and after *in vitro* micropropagation

Cultivar	No of initial suckers tested	No. of <i>in vitro</i> seedlings tested after potting	No of positive plants	Negative mean A405 _{nm} values of ELISA for the initial suckers	Positive mean A405 _{nm} values of ELISA for <i>in vitro</i> seedlings	Positive or negative
g'ombe	2	5	2	0.094	0.169	+
/Cavendish	2	5	2	0.093	0.132	+
/Cavendish	2	5	2	0.098	0.111	+
rand naine	2	5	1	0.095	0.124	+
amkhila	2	5	1	0.070	0.128	+
alary	2	5	1	0.093	0.110	+
/Cavendish	2	5	1	0.098	0.131	+
ld finger	2	5	2	0.093	0.120	+
ecatan	2	5	1	0.100	0.112	+
ld Dessert	2	5	0	0.100	0.100	-
manga	2	5	0	0.083	0.084	-
obe	2	5	0	0.080	0.083	-
ganda Green	2	5	2	0.089	0.111	+
buzi	2	5	0	0.074	0.076	-
uraru	2	5	1	0.098	0.365	+
gative control (BS buffer)				0.082	0.091	-

Note: 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.

Table 3. Effect of time and cultivar on expression of banana streak virus

Time (months)	Cultivar					
	Giant	Grand Naine	Lacatan	Time means		
	Cavendish	Gold Dessert	Gold Finger	Grand Naine	Lacatan	Time means
	1.00 ^c	1.00 ^c	1.00 ^c	1.14 ^{bc}	1.00 ^c	1.03 ^c
	1.28 ^{abc}	1.00 ^c	1.28 ^{abc}	1.24 ^{abc}	1.28 ^{abc}	1.21 ^{abc}
	1.24 ^{abc}	1.00 ^c	1.38 ^{ab}	1.00 ^c	1.14 ^{bc}	1.15 ^{bc}
	1.24 ^{bc}	1.00 ^c	1.63 ^a	1.14 ^{bc}	1.24 ^{abc}	1.25 ^{ab}
	1.00 ^c	1.24 ^{bc}	1.14 ^{bc}	1.24 ^{abc}	1.00 ^c	1.13 ^{bc}
	1.38 ^{ab}	1.28 ^{abc}	1.52 ^a	1.28 ^{abc}	1.58 ^a	1.41 ^a
	1.19 ^{xy}	1.09 ^y	1.32 ^x	1.17 ^{xy}	1.21 ^{xy}	
cultivar means						

Note: Seedlings were grown from micropropagated plants. Disease severity rating was done as described in section 3.6. 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 ($P<0.01$).

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.* (2005b) 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 interaction of cultivar by time was also not significantly different for all the cultivars (Table 3). The higher expression shows that virus concentration increases with plant growth. Therefore cultivar and age of banana plants have a bearing in BSV expression.

4.5.3 Effect of BSV infection on growth characteristics (girth, height and number of leaves) of banana plants

4.5.3.1 Effect of BSV infection on girth

The data reported in this section was on the rate of increase of the girth of the plants. There was significant ($P < 0.05$) variation in the rate of increase of the girth of banana seedling. The greatest increase was observed in the first three months but there was a decline in growth in the fifth and sixth month. The girth increase was significant at ($P < 0.05$) between 1 and 5; and 6 months (Table 4). Infection and expression was lower in the first month compared to the sixth month. There was a relationship between BSV infection and expression and growth (pseudostem girth) whereby reduced growth was associated with more defined symptoms. In both cultivar Gold finger and Lacatan highest infection and expression was observed during the sixth month and when reduced growth in girth was observed while during the first month for both the cultivars there was least infection and most growth in girth. Since the banana pseudostem is the food storage for the developing fruits, there is an indication that BSV infection would reduce food for the developing crop and hence poor production. The girth reduction with infection for the five cultivars was not significantly ($P < 0.05$) different. However, each cultivar had progressively reduced rate of growth in girth with time.

Table 4. Effect of BSV infection on growth of (pseudostem girth (increase per month - cm).

Months	Giant Cavendish	Gold Dessert	Gold Finger	Lacatan	Grand Naine
(Apri-2006)	1.14 ^{ab}	1.24 ^{ab}	1.33 ^a	1.38 ^a	1.28 ^a
(May-2006)	1.14 ^{ab}	1.00 ^b	1.28 ^a	1.14 ^{ab}	1.14 ^{ab}
(June-2006)	1.52 ^a	1.33 ^a	1.24 ^{ab}	1.24 ^{ab}	1.00 ^b
(July-2006)	1.14 ^{ab}	1.14 ^{ab}	1.14 ^{ab}	1.00 ^b	1.14 ^{ab}
(Aug-2006)	1.00 ^b	1.24 ^{ab}	1.00 ^b	1.00 ^b	1.00 ^b
(Sept-2006)	1.00 ^b	1.14 ^{ab}	1.00 ^b	1.00 ^b	1.00 ^b
Cultivar means	1.16 ^x	1.18 ^x	1.17 ^x	1.13 ^x	1.09 ^x

Note: Seedlings were grown from micropropagated plants. The figures indicate average girth increase in cm per month. 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 at 5%. LSD for cultivar is not significant; LSD for time is significant ($P<0.01$).

4.5.3.2 The effect of BSV infection on plant height of five banana cultivars over a period of six months growth

The results reported in this section were on the rate of increase in height of the plants monthly. There was significant variation in plant height during the six months of growth in all the five cultivars. Highest growth was experienced in the first two months and least growth in the sixth month. BSV was least expressed in the first month compared to the sixth month. Significant ($P < 0.01$) differences in growth and BSV expression were observed amongst the cultivars (Table 5). All the five cultivars had fast growth in the first two months but the growth rate reduced significantly ($P < 0.05$) in the remaining four months. This is an indication that BSV reduced growth (plant height) and could be used as an indicator for BSV. Overall, there was no significant growth reduction between cultivars. The observations in this study showed a direct relationship between symptom expression and reduced growth as manifested in height. In all the cultivars and specifically Giant Cavendish, Gold dessert and Lacatan, there was significant ($P < 0.05$) plant height reduction with higher symptom expression.

4.5.3.3 The effect of BSV infection on the development of leaves in five commercially grown banana cultivars

In this section, data shows the rate of increase in number of leaves of the plants every month. The results indicate that there was no significant difference between the five cultivars, Giant Cavendish, Gold Dessert, Gold finger, Grand Naine and Lacatan in the number of leaves produced in each month (Table 6). However, there was a significant ($P < 0.01$) reduction in the number of leaves from first to the sixth month.. From the third month, the BSV infected plants were not able to produce more leaves. This probably could have been due to death of leaves as a result of the BSV infection. Fewer leaves would eventually affect overall plant growth. The observations made in this study indicated that there was no cultivar difference in the way leaf production was affected.

Table 5: Effect of BSV infection on increase in plant height of five cvs of banana cultivars over a six months period. Figures show growth height increase in cm.

No of Months	Giant Cavendish	Gold Dessert	Gold Finger	Lacatan	Grand Nain (Healthy control)
	2.92 ^a	2.35 ^a	3.41 ^a	1.89 ^b	2.77 ^a
	2.99 ^a	2.52 ^a	2.65 ^a	2.34 ^a	2.71 ^a
	1.00 ^c	1.96 ^b	1.33 ^c	1.55 ^{bc}	1.69 ^{bc}
	1.28 ^c	1.67 ^{bc}	1.24 ^c	2.02 ^b	2.09 ^b
	1.67 ^{bc}	1.41 ^c	1.33 ^c	2.72 ^a	2.11 ^b
	1.00 ^c	1.00 ^c	1.61 ^{bc}	1.00 ^c	1.41 ^c
cultivar means	1.81 ^x	1.82 ^x	1.93 ^x	1.92 ^x	2.13 ^x

Note: Seedlings were grown from micropropagated plants. Numbers within the columns followed by the same letter (a, b, c) is not significantly different at 5% and numbers within the rows followed by the same letter (x) are not significantly different at 5% using LSD and numbers within rows followed by the same letter (x, y) are not significantly different at 5%

Table 6. Effect of HSV on the number of leaves produced per month for five banana cultivars over a six month period. Figures show average growth number of leaves per month.

Months	Cultivar Number of leaves				
	Giant Cavendish	Gold Dessert	Gold Finger	Lacatan	Grand Nain (Healthy control)
	3.0 ^a	3.0 ^a	3.0 ^a	3.0 ^a	3.0 ^a
	2.0 ^b	2.0 ^b	2.0 ^b	2.0 ^b	2.0 ^b
	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^c
	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^c
	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^c
	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^c	1.0 ^c
	2.0 ^x	2.0 ^x	2.0 ^x	2.0 ^x	2.0 ^x
variety means					

Note: Seedlings were grown from micropropagated plants.

Numbers within the columns followed by the same letter (a, b, c) were not significantly different at 5% using LSD and numbers within the rows followed by the same letter (x) were not significantly different at 5% using LSD.

4.5.4 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 7). 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 (Stover and Simmonds, 1987). 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, plant girth and time, and number of leaves and time (Table 7). 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.

Table 7. Correlation between the growth characteristics and infection of banana streak virus under greenhouse conditions

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

Micropropagated plants were 6 months at determination of these parameters

- Significant at 5% level of probability.
- ** Significant at 1% level of probability.

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

4.6.1 Relationship between occurrence of BSV infection in 15 cultivars and season of the year under field conditions

Incidence of the number of banana plants with symptoms varied with time of the year (season) and cultivar. Symptom expression from BSV infection was slightly higher between October and December, 2006 with 73% of the total cultivars expressing BSV (1.28-1.79 score) (Table 8). During this period there was low rainfall (2.6 mm – 44.6 mm) (Appendix 6). The BSV expression reduced drastically to 27% cultivars with symptoms between March and August 2006 (Table 8) when the moisture level was high (68.0 mm and 127.6 mm) of rain. The results differ from those reported by Dahal *et al.* (2000) which reported high symptom expression during rainy season and low expression during the dry season. Significant ($P<0.01$) cultivar and season variation at were observed on BSV symptom expression during the 10 months period. However, the interaction between cultivar and season on symptom expression was not significant. The peaks for expression for each cultivar were independent of the season (Table 8).

4.6.2 Relationship between occurrence of BSV infection on 15 cultivars, location and year

Results showed significant ($P<0.05$) differences for infection and symptom expression based on 15 cultivars in Njoro, Kokoto and Rongai locations. (Table 9). Njoro, Kokoto and Rongai fall in agro-ecological zone described in section 3.1. The significant effect of cultivar by location indicates that screening should be done at each location.

Table 8. Response of banana cultivars to BSV infection in the field

Cultivar	Oct-05	Nov-05	Dec-05	Jan-06	Feb-06	Mar-06	May-06	Aug-06	Sep-06
Alamy	1.14 ^c	1.14 ^c	1.14 ^c	1.24 ^{bc}	1.58 ^a	1.00 ^c	1.24 ^{bc}	1.00 ^c	1.14 ^c
Ururu	1.52 ^a	1.28 ^{bc}	1.24 ^{bc}	1.14 ^c	1.14 ^c	1.24 ^{bc}	1.00 ^c	1.00 ^c	1.00 ^c
Grande Naine	1.14 ^c	1.14 ^c	1.14 ^c	1.14 ^c	1.24 ^{bc}	1.24 ^{bc}	1.38 ^{abc}	1.14 ^c	1.14 ^c
Old Finger	1.14 ^c	1.28 ^{bc}	1.14 ^c	1.00 ^c	1.14 ^c	1.38 ^{abc}	1.24 ^{bc}	1.28 ^{bc}	1.14 ^c
Gombe	1.41 ^{ab}	1.14 ^c	1.38 ^{abc}	1.52 ^a	1.00 ^c	1.41 ^{ab}	1.52 ^a	1.28 ^{bc}	1.14 ^c
Warf Cavendish	1.14 ^c	1.52 ^a	1.49 ^{ab}	1.24 ^{bc}	1.28 ^{bc}	1.24 ^{bc}	1.14 ^c	1.38 ^{abc}	1.00 ^c
Old Dessert	1.47 ^{ab}	1.41 ^{ab}	1.47 ^{ab}	1.24 ^{bc}	1.28 ^{bc}	1.52 ^a	1.14 ^c	1.00 ^c	1.14 ^c
Acatan	1.28 ^{bc}	1.00 ^c	1.00 ^c	1.00 ^c	1.24 ^{bc}	1.14 ^c	1.55 ^a	1.00 ^c	1.00 ^c
Ganda Green	1.28 ^{bc}	1.47 ^{ab}	1.28 ^{bc}	1.14 ^c	1.14 ^c	1.33 ^{abc}	1.41 ^{ab}	1.14 ^c	1.24 ^{bc}
Plant Cavendish	1.41 ^{ab}	1.00 ^c	1.00 ^c	1.00 ^c	1.14 ^c	1.00 ^c	1.28 ^{bc}	1.28 ^{bc}	1.14 ^c
Ibuzi	1.41 ^{ab}	1.41 ^{ab}	1.66 ^a	1.14 ^c	1.24 ^{bc}	1.00 ^c	1.00 ^c	1.14 ^c	1.24 ^{bc}
Tobe	1.52 ^a	1.41 ^{ab}	1.79 ^a	1.14 ^c	1.33 ^{abc}	1.00 ^c	1.00 ^c	1.38 ^{abc}	1.24 ^c
Amukhila	1.41 ^{ab}	1.28 ^{bc}	1.52 ^a	1.52 ^a	1.14 ^c	1.00 ^c	1.00 ^c	1.52 ^a	1.00 ^c
Manga	1.28 ^{bc}	1.28 ^{bc}	1.00 ^c	1.00 ^c	1.47 ^{ab}	1.00 ^c	1.00 ^c	1.28 ^{bc}	1.00 ^c
Chinese Cavendish	1.28 ^{bc}	1.14 ^c	1.00 ^c	1.00 ^c	1.58 ^a	1.00 ^c	1.00 ^c	1.14 ^c	1.14 ^c
Mean of Months	1.32 ^w	1.26 ^{wx}	1.28 ^{wx}	1.16 ^{xyz}	1.26 ^{wx}	1.17 ^{xyz}	1.19 ^{xy}	1.19 ^{xy}	1.11 ^{xy}

Note: Seedlings were grown from micropropagated plants. Disease severity rating was done as described in section 3.6. 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.

Table 9 Banana streak virus symptom incidence in Njoro, Kokoto, and Rongai over two years' period.

Cultivar	Njoro ^b			Kokoto ^a			Rongai ^{ab}	
	2005	2006	2005	2005	2006	2005	2006	2006
Chinese Cavendish	1.00 ^e	1.28 ^{cd}	1.00 ^e	1.41 ^{bc}	1.41 ^{bc}	1.41 ^b	1.41 ^{bc}	1.41 ^{bc}
Dwarf Cavendish	1.14 ^{de}	1.14 ^d	1.28 ^{cd}	1.41 ^{bc}	1.41 ^{bc}	1.28 ^{cd}	1.28 ^{cd}	1.28 ^{cd}
Plantain Cavendish	1.00 ^e	1.41 ^{bc}	1.14 ^{de}	1.52 ^b	1.52 ^b	1.28 ^{cd}	1.41 ^{bc}	1.41 ^{bc}
Gold Dessert	1.14 ^{de}	1.47 ^b	1.14 ^{de}	1.61 ^a	1.61 ^a	1.14 ^{de}	1.14 ^d	1.14 ^d
Gold Finger	1.00 ^e	1.14 ^d	1.28 ^{cd}	1.41 ^{bc}	1.41 ^{bc}	1.28 ^{cd}	1.41 ^{bc}	1.41 ^{bc}
Grande Nain	1.00 ^e	1.14 ^d	1.14 ^{de}	1.61 ^a	1.61 ^a	1.14 ^{de}	1.28 ^{cd}	1.28 ^{cd}
Amanga	1.41 ^b	1.28 ^{cd}	1.41 ^b	1.41 ^{bc}	1.41 ^{bc}	1.41 ^b	1.41 ^{bc}	1.41 ^{bc}
Acatan	1.00 ^e	1.28 ^{cd}	1.14 ^{de}	1.41 ^{bc}	1.41 ^{bc}	1.14 ^{de}	1.28 ^{cd}	1.28 ^{cd}
Muraru	1.00 ^e	1.52 ^b	1.00 ^e	1.41 ^{bc}	1.41 ^{bc}	1.00 ^e	1.00 ^e	1.00 ^e
Mamukhila	1.28 ^{cd}	1.41 ^{bc}	1.75 ^a	1.41 ^{bc}	1.41 ^{bc}	1.00 ^e	1.00 ^e	1.00 ^e
Jig'ombe	2.24 ^a	1.41 ^{bc}	2.24 ^a	1.28 ^{cd}	1.28 ^{cd}	2.24 ^a	2.24 ^a	2.24 ^a
Itobe	1.14 ^{de}	1.52 ^b	1.14 ^{de}	1.00 ^e	1.00 ^e	1.00 ^e	1.00 ^e	1.00 ^e
Libuzi	1.00 ^e	1.41 ^{bc}	1.00 ^e	1.14 ^d	1.14 ^d	1.00 ^e	1.00 ^e	1.00 ^e
Uganda Green	1.14 ^{de}	1.28 ^{cd}	1.00 ^e	1.41 ^{bc}	1.41 ^{bc}	1.14 ^{de}	1.28 ^{cd}	1.28 ^{cd}
Alery	1.14 ^{de}	1.14 ^d	1.00 ^e	1.28 ^{cd}	1.28 ^{cd}	1.14 ^{de}	1.14 ^d	1.14 ^d
Means for Locations	1.17 ^{xyz}	1.32 ^w	1.25 ^{wx}	1.38 ^w	1.38 ^w	1.24 ^{wx}	1.28 ^{wx}	1.28 ^{wx}

Note: Seedlings were grown from micropropagated plants. Disease severity rating was done as described in section 3.6. 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.

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 with locations.. BSV infection and symptom expression was significantly ($P<0.05$) higher in Kokoto compared to Njoro or Rongai locations (Table 9). In the first year, infection was significantly ($P<0.05$) low compared to the second year in all the 15 cultivars. However, high infection was 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 (Appendix 6). 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 1178.1mm, highest infection 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). These results also agree with the earlier observations made in section 4.6.1 on a study which was conducted in Njoro location over a period of 10 months in two seasons - wet and dry. However, more studies are required to establish the relationship of cultivar and moisture levels in BSV activation and symptom expression. 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 9). Most interesting however was that Ng'ombe cultivar had vigorous vegetative growth across the locations. Further investigations are required to confirm tolerance to BSV aspect in Ng'ombe cultivar.

Significant ($P<0.05$) interaction between cultivar, site and year was observed in all the 15 cultivars, 3 locations and 2 years of experimentation (Table 10). 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. The rest of the cooking cultivars however had either lower or similar rate of infection as was observed with Ng'ombe, Uganda Green, and 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 infection and 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.

4.7 The effect of amount of nitrogen on BSV symptom expression for two cultivars of banana seedlings over a period of six months

Results on the effect of nitrogen (N_2) on Ng'ombe and Uganda Green are presented in Table 10. Both cultivars belong to East Africa Highlands – triple A (EAH-AAA) genotype and are used for cooking and do not contain a 'B' genome. Significant differences ($P < 0.01$) of severe BSV expression were observed in cultivars Ng'ombe and Uganda Green during the first two months of N application. While Uganda Green had high symptom expression when 100 g and 200 g N was applied in the first two months, symptom expression in Ng'ombe cultivar was high only in the first month but reduced significantly at ($P < 0.05$) in the subsequent months up to the sixth month (Table 10).

Soil analysis results indicate that there was inadequate level of N in the soil (Appendix 5). The recommendation is 200g per plant per year applied in 2 splits of 100 g per plant after six months. The high symptom expression observed in the first and second month could have been due to a temporary stress caused by concentrated N nutrients before they were fully utilized by the plant. The concentrated N could have triggered expression of the BSV to express symptoms. However, with time the nutrient concentration was diluted and hence less stress and as a result reduced infection and BSV expression. The plant age after application had significant ($P < 0.01$) effect on all the three levels of N application (Table 10). During the first and second month of the plant establishment, the symptom expression was quite significant ($P < 0.01$) but decreased after a period of 3 months.

The BSV streaks on the leaves remained on Uganda Green cultivar throughout the observation period but for Ng'ombe, it kept on fluctuating with months. The interactions of cultivar and N by time were not significant while that of cultivar by time was highly significant at $P < 0.01$.

Table 10 Effect of nitrogen on the expression of banana streak virus in two banana cultivars over a six months period

N	Uganda Green						Ng'ombe					
	M 1	M 2	M 3	M 4	M 5	M 6	M 1	M 2	M 3	M 4	M 5	M 6
0	1.76 ^a	1.38 ^{bc}	1.19 ^d	1.34 ^c	1.48 ^b	1.28 ^{cd}	1.62 ^a	1.22 ^d	1.29 ^{cd}	1.13 ^a	1.27 ^{cd}	1.11 ^d
100	1.79 ^a	1.75 ^a	1.53 ^b	1.18 ^d	1.43 ^b	1.29 ^{cd}	1.88 ^a	1.25 ^{cd}	1.25 ^{cd}	1.47 ^b	1.36 ^{bc}	1.08 ^d
200	1.88 ^a	1.69 ^a	1.22 ^d	1.04 ^d	1.41 ^b	1.34 ^{bc}	1.63 ^a	1.18 ^d	1.23 ^d	1.09 ^d	1.37 ^{bc}	1.22 ^d

Note: Seedlings were grown from micropropagated plants. Disease severity rating of 0-5 means scores of Ng'ombe and Uganda Green banana cultivars for six months in the greenhouse. The rating was done as in section 3.6.

Numbers within the columns followed by the same letter (a, b, c) are not significantly different at 5% using LSD. LSD for cultivar is significant ($P < 0.01$); LSD for nitrogen is significant ($P < 0.05$), Cultivar x nitrogen interaction is not significant ($P > 0.05$). N=Nitrogen; M1-M6 = Indicates months 1-6.

4.8 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 varieties, plant age, season of the year and location (site). It was observed that in 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 infection during the same period of time (Table 3). 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.*, 1999b). Tissue culture has been considered as one of the trigger factors for episomal expression of these integrants (Geering *et al.*, 2001a; Dallot *et al.*, 2001; Ndowora and Lockhart, 1997). Gold finger, which is a cultivar of the AAAB genotype and therefore 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.*, 2001a) and this could have contributed to low infection or symptom expression observed in the rest of the five varieties in the greenhouse (Table 3). 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 infection and expression of BSV compared to Valery (AAA genotype) across the three locations (Table 9). There is need to establish why Ng'ombe which is an EAH - AAA genotype had higher expression than the Cavendish Valery (AAA). It was observed that moisture level was a critical factor in BSV infection and expression (Table 8). Infection and symptom expression of BSV was slightly higher between October and December with 73% of the total varieties expressing BSV (1.28-1.79 score) (Table 8). During this period there was low rainfall (2.6 mm – 44.6 mm) (Appendix 6). Dry conditions could have activated the high infection and expression. These results contradict those obtained from a study carried out in Nigeria where infection and greater symptom expression were observed when plants were grown in wet season compared to those grown in dry season (Dahal *et al.*, 1998a; 2000).

This information could be used in the management of the BSV by ensuring no water stress (Danielle *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 infection and 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. It was observed that high nitrogen application possibly activate expression of BSV. Before a conclusion is derived, there is need to carry out more studies with other cultivars especially the Cavendish.

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

4.9 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 infection and 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 was less nutrients availability to the banana plant.

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 infection and

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. Symptom expression caused by N concentration was a good indicator of the importance of sufficient moisture to avoid BSV activation.

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. Further, studies are required on various levels of nutrients to assess the yield in BSV infected Ng'ombe cultivar.

There was intermittent infection and symptoms expression observed throughout the growing season (Table 8). Other possible cultivars which had relatively low BSV scores across the locations included Valary, Uganda Green, Lacatan and Kibuzi. Results from this study show that micropropagated plants should be screened for BSV in a greenhouse test, two to four months after transplanting (Karanja *et al.*, 2008b).

SEROLOGICAL DETECTION OF BANANA STREAK VIRUS ON SAMPLES OF BANANAS FROM THE FIELD

5.1 Introduction

Plant viruses cause major losses to several agricultural and horticultural crops around the world. Unlike other plant pathogens, there are no direct methods available yet to control viruses and consequently, the current measures rely on indirect tactics to manage the viral diseases. Hence, methods for detection and identification of viruses, both in plants and vectors, play a critical role in virus disease management.

Since an efficient sensitive and reliable diagnostic assay is imperative for evaluation of germplasm against BSV, in the following study antibodies from Dr. Lockhart of USA (Refereed to as Lockhart), Dr. Winter of Germany (Refereed to as Winter), Agdia Company from Britain (refereed to as Agdia Co.) and Dr. Pieterse of South Africa (refereed to as Meyer G1F-Bleed 2 and Meyer R210-Bleed 1) were evaluated through DAS-ELISA. Different antigen and antibody concentrations and four different types of antibodies were evaluated for optimum BSV detection and the optimised protocol was used to detect BSV on infected banana cultivars in Kenya. The main objective of the experiment was to detect BSV from different cultivars of bananas grown in various regions of Kenya using serological techniques.

Some of the current detection methods based on coat protein include enzyme-linked immunosorbent assays (ELISA), and (ISEM). Availability of these diagnostic methods provides greater flexibility, increased sensitivity, and specificity for rapid diagnosis of BSV. In this chapter, application of the ELISA technique for detection of BSV in field bananas will be discussed.

Virus detection based on biological properties and serological assays are by far the most widely used methods in many programs (Bock, 1982). As a prerequisite for application of the ELISA technique in indexing for BSV, antibody and antigen concentrations and the type of antibodies were optimised. This made it possible to design a protocol suitable for the BSV isolates in Kenya.

5.2 Materials and Methods

5.2.1 Study site

This study was carried out at the plant protection laboratory of KARI – Njoro Kenya. The collected samples were maintained in greenhouses and experimental fields. Materials were collected from various parts of the country as was previously described.

5.2.2 Enzyme-linked immune sorbent assay (ELISA)

Serological tests were performed using polyclonal antibodies against BSV (PMX2RC) (Ndowora and Lockhart, 2000). Double antibody sandwich - enzyme linked immunosorbent assay (DAS-ELISA) tests were carried out (Clark and Adams, 1977). The sensitivity of antibody-antigen concentrations and different types of antibodies were evaluated as described below.

5.2.3 Standardisation of antibody and antigen concentrations for detection of BSV

DAS-ELISA was performed according to the procedure described by Clark and Adams (1977). Concentrations of antibody coating and antigen for use in the DAS-ELISA were determined by coating a plate with antibody concentrations of 1:1000, 1:2000 and 1:3000. Serially diluted antigen at the concentrations of 1:2, 1:4, and 1:8 was used. Apple banana genotype (AB) with unambiguous BSV symptoms was used as the source of antigen for standardisation.

5.2.4 Comparison of antibodies from different sources in detection of BSV

Four sets of antibodies were used – Winter (DSMZ DAS-ELISA kit (DSMZ, Braunschweig, Germany), Agdia Co. (AgDia ELISA kit), Meyer G1F-Bleed 2 (developed from the serum of the female goat) and Meyer R210-Bleed 1 (Developed from the serum of a newzealand rabbit) (Meyer *et al.*, 2005). All were used at suppliers' recommended concentration rates. Six banana cultivars with typical BSV symptoms were also used to compare the sensitivity of

the antibodies in the detection of BSV. These included Chirume, Lisulya, Likhaho, Nshule, Ng'ombe and Bururu. DAS – ELISA procedure was followed in all the tests as outlined previously in general materials and methods.

5.2.5 Detection of Banana streak virus (BSV) in field samples of Musa spp. using DAS - ELISA

Thirty three banana cultivars showing typical BSV symptoms were collected and analysed for BSV using DAS- ELISA protocol as described previously. Plates were coated with antibody Lockhart PMX2RC at 1:1000 concentration and antigen concentration used was 1:4. PBS buffer was used as negative controls. Two wells per sample were used as replicates. The absorbance (A_{405}) value was used as an indicator of the relative concentration in the tissue. The negative control used for all the experiments was PBS buffer and not healthy plants because of the integration nature of the virus which might show no symptoms in banana leaves even when present.

5.3 Data analysis

Data collected was analyzed using the General Linear Model (GLM) with SAS computer package version 6.12 to determine ANOVA for symptom absorbance levels.

5.4 Results and Discussion

5.4.1 Sample collection for serological studies of banana streak virus

A total of two hundred and fifteen banana leaf samples were collected from various locations across Kenya. These were from twenty farms in nine different sites (Figure 1A and B), covering the major banana growing regions of the country. All samples had BSV-like symptoms described in Fig. 2 and details were recorded on the locality, the type of symptoms and the cultivar by the local names (Appendix 7). Most of them had chlorotic streaks, but some were necrotic. The intensity varied with cultivars and collection region.

5.4.2 Standardisation of antibody and antigen concentrations on BSV detection

Results of the two sources of antibodies, from Lockhart and Winter showed significant differences at ($P < 0.01$) (Table 11). The absorbance values of Lockhart antibody were higher than those of the Winter antibody and therefore the Lockhart antibody could be considered more sensitive in its detection of the isolates in the samples. The differences in the antibody detection could have been due to the type and number of isolates used in the development of the specific batch of antibodies. Studies have shown that due to serological diversity of BSV, a broad spectrum polyclonal antibody developed from a wide range of isolates is more sensitive in detection than an antibody developed from a few isolates which would fail to detect some isolates (Lockhart and Olszewski, 1993; Ndowora, 1998). This possibly was the reason for better detection by the Lockhart antibody which was developed using more than 30 isolates (Ndowora, 1999). In both batches of antibodies, there was no significant difference in the absorbance values with the concentrations of 1:1000, 1:2000, and 1:3000 (Table 11). Any one of the three concentrations could be used effectively in detection of BSV. However, concentration of 1:1000 was adopted because it was more accurate to use.

Antigen concentrations were significantly ($P < 0.05$) different between the antibody source and the 3 different levels of concentrations; that is 1:2, 1:4, and 1:8. The best concentration for detection of BSV was 1:2 (Table 12) with the Lockhart antibody. The high concentration could possibly have enabled more accumulation of the virus in the sap hence higher trapping of the virus on the surface of the micro-titre wells.

Results show that there was high significance ($P < 0.05$) between the antibody source and their concentrations; antibody type and the 3 antigen concentrations; antibody concentrations and antigen concentrations. When antibody from Lockhart was used to detect BSV on infected cultivar apple banana, the highest mean absorbance value of 0.134 at $A_{405\text{nm}}$ was achieved with the antibody concentration of 1:1000 and antigen concentration of 1:2. The antibody from Winter however achieved the highest mean absorbance value of 0.132 with antibody concentration of 1:2000 but with similar antigen concentration of 1:2 (Table 13). Significantly high interaction was observed between antibody type and antibody concentration at $P < 0.05$. When antibody type was changed from Lockhart to Winter there was reduced effect in the detection by the second antibody type in all concentrations except 1:2000 (Table 13).

Table 11. Evaluation of the effect of antibody concentrations on the absorbance (A_{405}) value of BSV infected banana.

Antibody source	Antibody concentration	Absorbance level (A_{405nm})	Reaction
Lockhart	1:1000	0.134 ^a	+
	1:2000	0.130 ^a	+
	1:3000	0.132 ^a	+
Winter	1:1000	0.126 ^b	+
	1:2000	0.132 ^a	+
	1:3000	0.127 ^b	+

Note: Means followed by the same letter in the column are not significantly different at $P \leq 0.05$. Means are separated by LSD at 5%.

Table 12. Evaluation of the effect of antigen concentrations on the absorbance (A_{405}) value of BSV infected banana.

Antibody source	Antigen concentration	Absorbance level (A_{405nm})
Lockhart	1:2	0.147 ^a
	1:4	0.131 ^a
	1:8	0.126 ^b
Winter	-ve control (PBS buffer)	0.123 ^b
	1:2	0.128 ^b
	1:4	0.124 ^b
	1:8	0.129 ^b
	-ve control (PBS buffer)	0.125 ^b

Note: Means followed by the same letter in the column are not significantly different at $P < 0.05$. Means are separated by LSD at 5%.

Table 13. The effect of antibody and antigen concentrations on the detection of BSV.

Antibody Type	Antibody Concentrations	Antigen concentrations				Antibody means
		-ve	1:2	1:4	1:8	
Lockhart	1:1000	0.122 ^b	0.140 ^a	0.140 ^a	0.129 ^b	0.134 ^a
	1:2000	0.127 ^b	0.140 ^a	0.121 ^b	0.127 ^b	0.130 ^a
	1:3000	0.119 ^b	0.160 ^a	0.132 ^b	0.122 ^b	0.132 ^a
Winter	1:1000	0.124 ^b	0.128 ^b	0.121 ^b	0.128 ^b	0.126 ^b
	1:2000	0.128 ^b	0.132 ^a	0.127 ^b	0.138 ^a	0.132 ^a
	1:3000	0.124 ^b	0.125 ^b	0.122 ^b	0.122 ^b	0.127 ^b
Antigen means		0.124 ^b	0.137 ^a	0.127 ^b	0.128 ^b	

Note: Means followed by the same letter in the column are not significantly different at P<0.05. Means are separated by LSD at 5%.

5.4.3 Comparison of antibodies from different sources in detection of BSV isolates in Kenya

Absorbance (A_{405nm}) readings differed significantly ($P<0.01$) among the 4 antibodies namely Winter, Agdia Co. Meyer G1F-Bleed 2 and Meyer R210-Bleed 1 (Table 14). Detection using Winter and Agdia Co. antibodies was significantly high across all cultivars compared to Meyer G1F-Bleed 2 and Meyer R210-Bleed 1. Comparison between Meyer G1F-Bleed 2 and Meyer R210-Bleed 1 showed significantly ($P<0.05$) differences with Meyer G1F-Bleed 2 detecting higher absorbance values. These differences were possibly due to specificity of the antibodies to the many isolates of BSV.

5.4.4 Detection of BSV in field samples of *Musa* spp. using DAS – ELISA

A total of 215 samples were tested. BSV analysis performed on the *Musa* samples from the nine sites in Kenya using Lockhart antibody revealed that 87% of these samples were positive for BSV by ELISA testing (Appendix 7). While in Western and Nyanza province, the majority of the affected bananas were cooking cultivars, those from Rift Valley, Central, and Eastern were mainly dessert cultivars (Appendix 8). The largest number (61%) of infected samples was collected from Western and Nyanza provinces. Out of the total samples testing BSV positive in ELISA Kisii, had 43%; Bungoma 12%; Mt Elgon 4%; Kakamega 2%; Nakuru 4%; Thika 12%; Embu 2%; Nyeri 5% and Murang'a had 1% (Appendix 8).

The cultivars showed significant ($P<0.05$) differences and the virus absorbance level varied with cultivar (Table 15). Out of the total cultivars tested, 66% had absorbance level (A_{405nm}) that was one and half times that of the negative control (buffer) and therefore were considered positive. The 34% cultivars that were negative for BSV could have been infected with CMV, which also produces chlorotic streaks that may be similar to those caused by BSV (Lockhart, 1994). Another possibility is that these leaves were infected with serotypes of BSV not detected by the polyclonal antibodies used. This could confirm serotype diversity, a situation similar to that reported by Harper *et al.* (2005) in a neighboring country, Uganda. This also confirms findings reported by Geering *et al.* (2000) indicating that BSV susceptibility varies with cultivar.

Table 14. The sensitivity of antibody types on detection of BSV on various EAH-AAA cooking Banana genotypes.

	Genotypes					
	Chirume	L ibururu	Likhaho	Lisulya	Ngombe	Nshule
Antibodies						
Winter	1.558 ^a (+)	1.730 ^a (+)	1.566 ^a (+)	1.533 ^a (+)	1.686 ^a (+)	1.678 ^a (+)
Agdia Co.	1.539 ^a (+)	1.558 ^a (+)	1.633 ^a (+)	1.560 ^a (+)	1.753 ^a (+)	1.719 ^a (+)
Meyer G1F-Bleed 2	0.105 ^{ab} (+)	0.292 ^{ab} (+)	0.095 ^c (-)	0.115 ^{ab} (+)	0.087 ^c (-)	0.077 ^c (-)
Meyer R210-Bleed 1	0.061 ^c (-)	0.243 ^{ab} (+)	0.082 ^c (-)	0.063 ^c (-)	0.179 ^{ab} (+)	0.083 ^c (-)
Negative control (Buffer)	0.071 ^c (-)	0.071 ^c (-)	0.071 ^c (-)	0.071 ^c (-)	0.071 ^c (-)	0.071 ^c (-)

Note: The six cultivars used were all field samples from Mt. Elgon region Lockhart antibody was not used it was not available from the source. Absorbance values of 0.100 and above are positive (+) while those values below 0.100 are negative (-). Means followed by the same letter in the column are not significantly different at P≤0.05. Means are separated by LSD at 5%.

Table 15. Detection of BSV in field samples of *Musa* spp. using DAS – ELISA with Lockhart antibody

Sample No	Variety	Absorbance value (n=2) at 405 nm at 2 hour substrate incubation time.		
		Mean Absorbance	Genotype	Type
1	Grand naine	0.34750 ^a	AAA	Dessert
2	Giant Cavendish	0.29600 ^{ab}	AAA	Dessert
3	Lacatan	0.24875 ^{bcd}	AAA	Dessert
4	Muraru	0.24675 ^{bcde}	AA	Cooking/ Dessert
5	Nshule	0.24050 ^{bcdef}	EAH-AAA	Cooking
6	PAZ	0.24000 ^{bcdef}	AAA	Dessert
7	Gachuru	0.21725 ^{bcdefg}	AA	Dessert/cooking
8	Uganda Green	0.19067 ^{cdefgh}	EAH-AAA	Cooking
9	Mysore	0.18500 ^{cdefghi}	AAB	Cooking
10	Bogoya	0.17075 ^{cdefghij}	AAA	Dessert
11	Golden Beauty	0.16850 ^{cdefghij}	AAA	Dessert
12	Apple	0.16483 ^{defghij}	AB	Dessert
13	Gold Finger	0.16433 ^{defghij}	ABB	Dessert
14	Valary	0.14825 ^{fghij}	AAA	Dessert
15	Namkhila	0.14433 ^{ghij}	EAH-AAA	Cooking
16	Ng'ombe	0.14425 ^{ghij}	EAH-AAA	Cooking
17	Dwarf Cavendish	0.14300 ^{ghij}	AAA	Dessert
18	Likhaho	0.14000 ^{ghij}	EAH-AAA	Cooking
19	Chinese Cavendish	0.13633 ^{ghij}	AAA	Dessert
20	Williams	0.13500 ^{ghij}	AAA	Dessert
21	Uganda Kisii	0.13250 ^{ghij}	EAH-AAA	Cooking
22	Sukari ndizi	0.13100 ^{ghij}	AB	Dessert
23	Lisulya	0.12825 ^{ghij}	EAH-AAA	Cooking
24	Sialamule	0.12400 ^{ghij}	EAH-AAA	Cooking
25	Nasirembe	0.12200 ^{ghij}	EAH-AAA	Cooking
26	Lisumba	0.11750 ^{hij}	EAH-AAA	Cooking
27	Turbo	0.11600 ^{hij}	EAH-AAA	Cooking
28	Apple Giant	0.11200 ^{hij}	AB	Dessert
29	Kasukari Kisii	0.10700 ^{hij}	AB	Dessert
30	Libururu	0.10062 ^{hij}	EAH-AAA	Cooking
31	Murure	0.09900 ^{hij}	EAH-AAA	Cooking
32	Suriat	0.08900 ^{ij}	EAH-AAA	Cooking
33	Maffukha	0.08800 ^{ij}	EAH-AAA	Cooking
34	Chilume	0.08625 ^{ij}	EAH-AAA	Cooking
35	Control	0.08550 ^j	EAH-AAA	Buffer

Samples were collected from nine banana growing regions in Kenya. Antisera used were Lockhart antibody. Absorbance values of 0.100 and above were considered positive with banana streak virus. Means with the same letter are not significantly different at ($p < 0.05$). Means are separated by LSD at 5%.

3.5 Discussion

BSV is composed of isolates that are both serologically and genomically variable (Lockhart and Olszewski, 1993). An antibody produced and optimized for a specific isolate might not react with different isolates (Lockhart and Olszewski, 1993). One approach for detecting new isolates from diverse regions is to optimize the concentrations and the detection sensitivity of the already existing antibody against a range of diseased plant samples and then run PCR with a range of primers both degenerate and specific.

In this study, antigen concentrations of 1:2; 1:4 and antibody concentration of 1:1000 gave the optimum absorbance value in comparison with the other concentrations (Table 12). The reason is that at these concentrations there are no non-specific reactions which could prevent the virus from binding onto the ELISA plate. Lower concentrations of the sap reduced the sensitivity of the technique as was also observed by both Agindotan *et al.* (2003) and Jordan and Hammond (1988). For the purpose of accuracy, the concentrations of 1:1000 and 1:4 antibody: antigen respectively was adopted for all serological analysis experiments

Of the 4 tested antibodies, the absorbance values of Winter and Agdia Co. were higher than Meyer G1F-Bleed 2 and R210-Bleed 1 antibodies. The different response by the four polyclonal antibodies is an indication of detectability of some serotypes by a specific antibody and not others. Although the most effective antisera were Winter and Agdia Co. for Kenyan BSV isolates, the various polyclonal antibodies could be pooled together for broad-spectrum detection as was suggested by both Agindotan *et al.*, (2003) and Ndowora *et al.* (1998). These results above confirmed that there is need to develop antibodies with the infected local banana cultivars so as to incorporate as many isolates for detection as possible.

Infected materials collected from the 9 sites in Kenya had distinct BSV symptoms but ELISA testing only confirmed 87% positive. The detecting antibody only detects homologous virus but fails to detect heterologous viruses (van Regenmortel, 1982). Other reasons could be absence of the specific isolate in the antibody used or very low virus titre which could not be detected by the antibodies used. Therefore ELISA technique still has limitations in comprehensive detection of BSV in the field. Hence there is still need for confirmatory tests with molecular techniques.

3.6 Conclusions

Standardisation experiments concluded that detection for antibodies differed depending on the source and concentration of both antibody and antigen. A concentration of 1:1000 for antibody and 1:2 for antigen gave an optimum detection. Comparison of four different antibodies from 4 different sources (Table 14) confirmed their specificity in detection of BSV isolates in that some had higher absorbance levels than others for the same plants tested. These results confirmed a need to develop antibodies with the local banana cultivars for effective detection of BSV. Development of the antibody using Kenya BSV infected cultivars would possibly identify more isolates and hence reveal the true diversity of BSV in Kenya.

ELISA testing of 171 samples collected from various parts of the country revealed that there was variation in BSV absorbance values depending on cultivars and source of collection (Sites) (Appendix 6). It also showed that BSV infection was more prevalent in the Western and Nyanza provinces.

CHAPTER SIX

MOLECULAR IDENTIFICATION OF BANANA STREAK VIRUS

ISOLATES IN KENYA

6.1 Introduction

Recent studies in Kenya have shown that BSV is prevalent in all the banana growing regions and affects all the popular cultivars grown by farmers (Wangai *et al.*, 2002). However, the presence of the BSV has not been confirmed through molecular techniques and the variability of the isolates is not known. Integration of BSV into the genome of the *Musa* plants and isolate diversity was identified in a neighbouring country, Uganda, (Harper *et al.*, 2005) and hence needed to be taken into consideration during identification of BSV isolates and optimisation of a diagnostic protocol in this study. The major objective of the work were therefore to identify the variability of banana streak virus (BSV) isolates in Kenya and to optimise a diagnostic protocol which can be used routinely and efficiently on large numbers of banana plants.

6.2 Specific Materials and Methods

6.2.1 Sample collection

Plant materials for the experiments on molecular diversity showing varying levels of BSV symptoms were collected randomly from five provinces in the major banana-growing regions of Kenya. The areas included Kisii (Nyanza), Mt Elgon, Kakamega and Bungoma (Western), Thika, Murang'a, Kirinyaga and Nyeri (Central), Nakuru (Rift Valley) and Embu (Eastern) (Fig. 1). The samples were kept at 4 °C but for long term storage they were freeze-dried or kept at -72°C.

6.2.2 Virus purification

Mini-preps of virus were prepared from both fresh-frozen and freeze-dried material using a modification of the procedure by Harper *et al.* (2002). Leaf tissues, 20 g of fresh and 4 g of

freeze dried materials were ground in liquid nitrogen and thawed in extraction buffer (1:4 wt/vol ratio) 50 mM sodium phosphate, pH 6.1, 1 M urea, 4% polyvinyl pyrrolidone (PVP, MW 40 000) and 0.5% 2-mercaptoethanol. Celluclast (Novo Nordisk, Princeton, NJ, USA) was added to give a final concentration of 2% dilution after blending for 1 min. The mixture was incubated at 30°C for 3 h, passed through two layers of muslin and then centrifuged at 6000 g for 20 min using GSA rotor with rubber cushions. Triton X-100 (20%) was added to the supernatant to give a final concentration of 2%, and the mixture stirred at 4°C overnight. After further centrifugation at 6000 g for 10 min the supernatant was layered over a 5 ml cushion of 20% sucrose in extraction buffer with 2-mercaptoethanol and centrifuged in an ultra centrifuge at 45,000 g for 90 min. Pellets were re-suspended in a total of 250 µl of 0.01 M sodium phosphate, (pH 7.0) to give the partially purified preparation.

6.2.3 Sap extraction

One gram of fresh - frozen or freeze - dried leaf was ground in 1x phosphate buffered saline (PBS) + 0.1% 2-mercaptoethanol extraction buffer (1:4 wt/vol ratio). The sap extract was concentrated by precipitation with 4% polyethylene glycol (PEG, MW 6000) in 0.1 M sodium chloride (NaCl). The sample mixture was stirred for 2 h, at room temperature (RT) then the sap was sedimented in a microfuge for 20 min at 16,000 g. Final re-suspension was in 1/10th of the original extraction buffer volume.

6.2.4 Immunosorbent electron microscopy (ISEM)

A total of 10 purified virus samples were examined by electron microscopy using the procedure of Ahlawat *et al.* (1996). Polyclonal antibodies were trapped on carbon coated grids. This was followed by addition of sample then another layer of antibodies. Grids were viewed using a JEOL JEM 1200EX electron microscope at 15,000x magnification. Particle counts were conducted on 10 fields for each analysed sample.

6.2.5 Immunocapture - polymerase chain reaction (IC-PCR)

Antibody and antigen optimisation was carried out in concentrations of 5:1000, 10:1000 and 15:1000 in carbonate coating buffer. Sap extraction was prepared from Lisulya and Khabusi

banana cultivars and antigen concentrations of 10, 50 and 100 µl in 100 ml reaction were used. The sap was extracted and concentrated as previously described (Harper *et al.*, 2002). The optimisation was done before the IC-PCR procedure was carried out on a total of 80 infected samples. During IC-PCR, thin walled polypropylene microfuge tubes were coated with Lockhart polyclonal antibodies. The antibodies used were generated against 32 Sugarcane Bacilliform Virus (SCBV) and BSV Mysore isolates PMX2RC as described in Ndowora and Lockhart (1997). The concentration 1:100 in carbonate buffer was used and 100 µl was added in each tube and incubated for 2 h at 37°C. The tubes were then washed three times with PBS-T. A 100 µl of sap extract from the samples were added to each tube and incubated at 37°C for 2 h. The tubes were again washed twice with PBS-T, once with sterile distilled water and then dried briefly. Polymerase chain reaction (PCR) was carried out directly in the tubes (Harper *et al.*, 2002).

PCR was carried out using degenerate primer pair: 1A (5'-CTNTAYGARTGGYTNGTNGTATGCCNTTYGG-3') and 4' (5'TCCAYTTRCANAYNSCNCCCCANCC-3') where N=A,C,G or T; Y=C or T (Harper *et al.*, 2002) designed to amplify the "conserved" sequences in the reverse transcriptase/ RNase H region of the *Badnavirus* open reading frame (ORF) III. A 100 µl PCR mix contained 10x reaction buffer, 10 mM each dNTP, 10 pM each primer 1U Taq polymerase (Invitrogen, Billingham, UK) and 50 mM magnesium chloride (MgCl₂) with the final concentration of 3 µl in 100 µl reaction. The negative control used was PBS buffer and the positive control was BSOEV DNA. PCR cycle conditions were an initial denaturation at 94°C for 5 min, five cycles of 94°C for 30 s, 37°C for 30 s and 72°C for 1 min, followed by 30 cycles (94°C for 30 s, 50°C for 30 s, 72°C for 1 min) and a final extension of 72°C for 10 min. The low annealing temperature (37°C) was used to capture all related sequences including non-specific and artifacts while higher temperatures (50°C).

6.2.6 Direct (Standard) PCR versus IC-PCR

To compare the efficiency of the two methods, direct PCR and IC-PCR, 9 samples were prepared from cultivar Khabusi (EAH-AAA). Virus purification was done by sucrose gradient fractionation as described by Walkey (1985). The purpose of using sucrose gradient technique was to increase yield of purified virus. Sucrose gradient for ultra-centrifugation of a 10 to 40% (W/V) gradient was prepared by dissolving sucrose in distilled water. The solution was added in a 9 cm x 2.5 cm cellulose nitrate centrifuge tubes. The concentration were added to the in

ascending order starting with 10, 20, 30 then 40% at the bottom of the tube. The 7 mls of the first 3 concentrations and 15mls of the 4th concentration were added. The 10-40% gradient was allowed to stand overnight then 0.5 ml partially purified virus was layered onto the surface of the gradient. The rest of the procedure was as previously described in 6.2.2. IC-PCR was performed as previously described by Harper *et al.* (2002). However, for the direct PCR method 10 µl of purified virus prep was added directly into the uncoated tubes. PBS buffer was used for negative control. The rest of the procedure was the same for both as previously described (Harper *et al.*, 2002).

6.2.7 Cloning, Sequencing and phylogenetic analysis

PCR products obtained from eight samples, (Likhaho1, Lisulya1 and Lisulya2, Khabusi1 and Khabusi2, Nshule, Gold finger and Bogoya) were purified using QIAquick PCR purification kit (QIAGEN, Crawley, UK) and cloned into the vector pCRII-TOPO (Invitrogen) which was used to transform competent *Escherichia coli* (*E. coli*) Top10 (Invitrogen). During cloning 1µl TOPO salt solution, 1µl TOPO vector and 4µl PCR sample were added into thin walled PCR tubes and incubated at RT for 5min then placed onto ice. This was followed by addition of 2µl reaction/sample mix to vial of TOP10 E.coli. The reaction mixture was stirred gently and incubated on ice for 30min, heat shocked at 42°C for 30secs and placed back on ice. Finally 250µl of room temperature SOC medium was added and incubated in orbital incubator at 37°C for 1hr. For selection of transformants 'L' agar ampicillin plates (1:1000 ampicillin 100mg/ml) with 50µl x-gal (20mg/ml) was used. One plate was spread with 25µl of contents of vial and second plate with rest of vial then Incubated at 37°C overnight

Transformants were identified and selected, and plasmids were prepared, using a proprietary kit (QIAGEN, Crawley, UK), from the selected colonies. Clones containing inserts of the expected size were identified by restriction analysis, digested with *EcoR* 1. The digest products were electrophoresed in 1% agarose gel in a 0.5 x TBE (Sambrook *et al.*, 1989). Both strands of selected clone inserts were sequenced separately with bacteriophage M13 forward and reverse primers (5'– GTAAAACGACGGCCAG-3') and (5'– CAGGAAACAGCTATGAC-3'), using Big Dye 3.1 polymerase and fluorescent dye terminators (Applied Biosystems). The products were analysed on an Applied Biosystems automatic sequencer (Lark Technologies, Essex, UK). Sequences were compared with each

other and with published and other available *Badnavirus* and BSV sequences (EMBL). Caulimoviridae sequences were also used to compare with the sequences of the Kenyan BSV isolates (Appendix 9). Nucleotide sequence identities were calculated using either GCG-Gap or MEGA version 2.1 (Gibson *et al.*, 1999; Kumar *et al.*, 2001) or Wemboss software – water program for local alignment (Smith and Waterman, 1981). Sequence alignments and construction of bootstrapped, neighbour-joining trees were carried out using Clustal X (Thompson *et al.*, 1997).

6.2.8 Southern Blotting and Hybridisation

Due to BSV integration into the banana genome, it is possible to get false positives for virus even during IC-PCR amplification. To determine whether there is integrated BSV in the genome; DNA was extracted from BSV infected banana leaves of cultivar Lisulya using the Nucleon Phytopure (RPN 8511) extraction kit (Amersham Biosciences). Southern blotting was performed as described in Harper *et al.* (1999b). Approximately 2 µg of genomic DNA of Likhako banana cultivar was digested with 5 units of EcoRI (Promega) for 2 h at 37 °C. Following concentration by ethanol precipitation, digested DNA was then separated on a 0.8% agarose gel in 1 X TBE. The DNA was partially depurinated then denatured by first soaking in the gel in 0.25 N HCl for 30 min, then 0.5 M NaOH + 1.5 M NaCl for 30 min. Finally the gel was neutralized by soaking in 1.0 M Tris-HCl, pH 8.0 + 1.5 M NaCl for 20 min. The DNA was then transferred to a nylon membrane (MSI Magnacharge) by upward capillary transfer (Sambrook *et al.*, 1989). This immobilised target DNA was hybridised to a radiolabelled probe, in this case prepared from a clone of a BSV sequence isolated from *Musa* Lisulya. Following gel-purification, the excised Lisulya fragment DNA was then labelled with [a-³²P] dCTP using a Prime-a-Gene Labelling Kit (Promega) following the manufacturer's instructions. Pre-hybridization and hybridization were both done using QuickHyb Hybridization Solution (Stratagene) following manufacturer's instructions. Following hybridization, membranes were subjected to two 15 min washes with 5 x SSC, 0.5% SDS at room temperature, followed by two 15 min washes with 1 x SSC, 0.5% SDS at 42 °C, and three 15 min washes at 65 °C with 0.1 x SSC, 1.0% SDS at 65 °C. Bound probe was detected using a PhosphorImager system (Molecular Dynamics, Sunnyvale, CA, USA). Membranes were stripped of probe by washing for 2 h at 75 °C in 1 mM Tris-HCl, pH 8.0,

containing 1 mM EDTA and 0.1 x Denhardt's reagent, then rinsing in 0.1 x SSC (Sambrook *et al.*, 1989).

6.2.9 Comparison of symptom expression, ISEM, ELISA and IC-PCR techniques in BSV indexing

Banana leaves from six banana cultivars exhibiting chlorotic to necrotic streak symptoms were collected from field plants in Mount Elgon, Kisii, and Thika (Jomo Kenyatta University of Agriculture and Technology) and from the green house in Njoro- Nakuru. Symptoms were identified using the score of 0-5 as previously described. Leaf samples were ground 1 to 4 (w/v) in BSV extraction buffer and tested by DAS-ELISA as previously described using Lockhart antibodies. Screening of *Musa* samples for BSV by ISEM was also done as previously described. In performing IC-PCR, specific primers and antibodies were used which included primers for BSV Badna 1A-4', BSV -Gold finger, BSV – Dacca, BSV-UgA and BSV - Ol Primers and 2 antibodies Lockhart and Meyer G1F-Bleed 2.

6.3 Results and Discussion

6.3.1 Virus purification, sap extraction and use of PCR and IC-PCR virus detection

Initial attempts to purify the virus using the virus mini-preps gave low virus yield because the technique was complex, time consuming and required very high centrifugation speeds. It also required highly skilled manpower and could not be used for the rapid processing of multiple samples. Only four samples could be processed per week. The sap extraction method however, was relatively simple and ten times the number of processed samples was manageable. It also did not require expensive laboratory equipment. Subsequently the sample preparation technique used was the sap-extraction technique which allowed virus extraction from 80 banana leaf samples.

Results of antibody and antigen concentration showed that among the three concentrations evaluated for antibody 5:1000, 10:1000 and 15:1000 (Fig. 4), the optimum concentration was

10:1000. For the antigen, three concentrations were evaluated 10 μ l, 50 μ l and 100 μ l (Fig. 5) and the optimum concentration was 50 μ l. IC-PCR amplified 600 bp products from leaf sap extracts of a total of 80 BSV infected samples from Kenya confirming them positive for BSV infection.

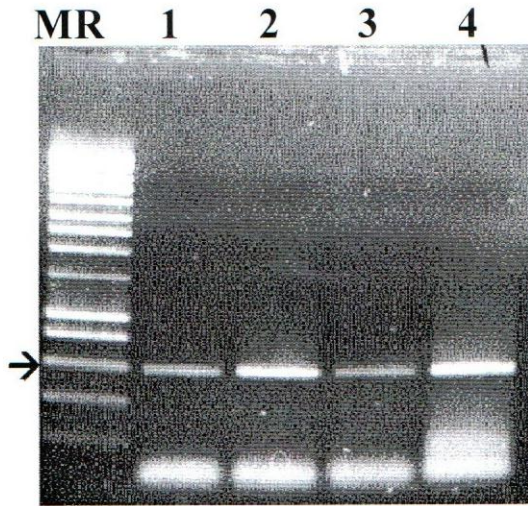


Fig. 4. Defining optimum antibody concentration for use in IC-PCR. IC-PCR amplification of BSV from virus sap extracted from cultivar Khabusi. Lane 1-3 varied antibody concentrations. Lane 1 = 5:1000; Lane 2= 10:1000; lane 3=15:1000; Lane 4= Positive control BSOEV=10:1000. The arrow points at 600bp virus band. Lane MR is BiolineDNA Marker (Bioline 1 kb DNA ladder).

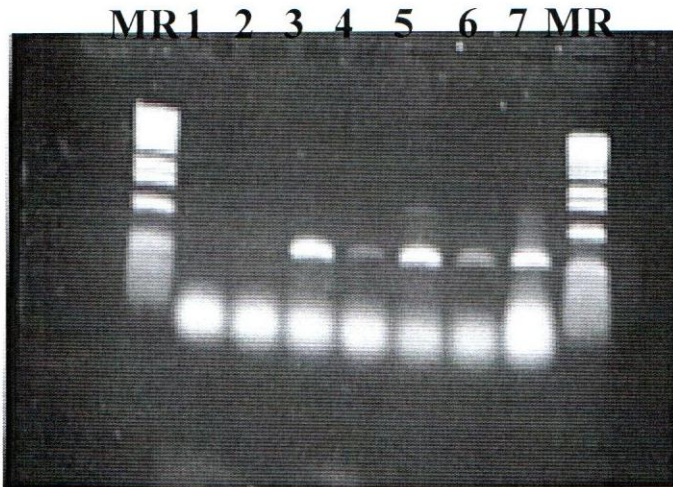


Fig. 5. Defining optimum antigen concentration for use in IC-PCR. IC-PCR amplification of BSV from virus sap extracted from cultivar Khabusi. The bands show optimisation of antigen concentrations. MrR is DNA marker (Bioline 1kb DNA ladder); Lane 1-6 varied antigen concentrations. Lane 1 is negative control; lane 2 is 10 μ l virus sap; Lane 3 is 50 μ l; Lane 4 is 100 μ l; lane 5 is 50 μ l; lane 6 is 100 μ l; lane 7 positive control BSOEV DNA

Results of BSV detection using both direct PCR and IC-PCR technique are shown in Table 16. Direct PCR detected BSV in 22 samples out of the BSV infected 80 (28%) while IC-PCR technique detected BSV in 33 samples out of a total of 80 (41%). IC-PCR technique used in this study combined technical advantages of PCR with the practical advantages of serology. The technique had an advantage over ISEM of rapid and sensitive detection of BSV.

Results of both molecular and serological detection showed that BSV varied from site to site. In Mount Elgon 57% of the samples were BSV positive while 43% were BSV negative. In Bungoma 80% of the samples were BSV positive while 20% were negative. In Kisii 27% were BSV positive but 73% were negative. In Nakuru 41% were BSV positive but 59 were BSV negative. Samples collected from Thika were all negative with IC-PCR (Table 16).

The intensity of symptoms also varied with site and genotypes. Samples collected from Mount Elgon and Kisii where most of the genotypes were EAH - AAA had symptoms ranging from moderate to severe (Disease score of 3-5). Samples collected from Nakuru and Thika where predominant genotypes were AAA and AAAB, the symptoms ranged from mild to moderate (Disease score of 1-3). These results show that location and genotype have an effect on the BSV infection and symptom expression. Cultivars also responded differently to BSV infection depending on location. Lisulya cultivar from Mount Elgon and Kisii tested positively while those in Bungoma and Nakuru tested negative. Another example was Uganda Green which tested positive in Bungoma and Nakuru but negative in Kisii. Both freeze dried and fresh material was used in the extraction of the virus (Table 16). There was no significant difference in the virus concentration produced. However, the freeze dried material had the advantage of keeping longer before use outside the freezer.

In comparing the two protocols, the results showed that IC-PCR had better amplification than direct PCR. Direct PCR amplifies both integrated and episomal virus. Hence the technique might give false positives while IC-PCR generally amplifies only the episomal virus (Fig. 6a and b).

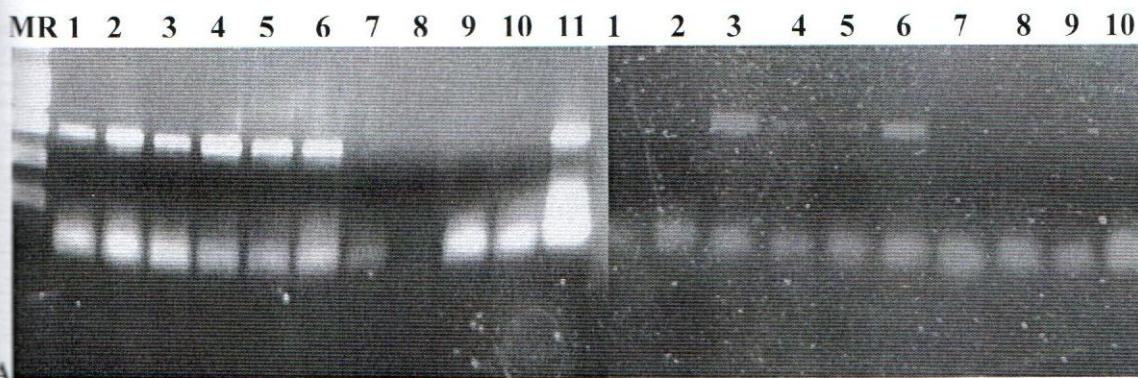


Fig. 6a IC-PCR gel picture

Fig 6b Direct PCR gel picture.

Defining amplification of BSV from virus sap extracted from cultivar Khabusi (EAH-AAA). Fig 6(a) shows the use of IC-PCR protocol while Fig.6 (b) shows the use of direct PCR protocol to amplify the same samples. In both gels lanes 1 to 9 are for the samples and 10 for negative control (PBS buffer). In gel (a) 11 is positive control (BSOEV DNA) and MR is DNA marker (Bioline 1kb DNA ladder)

Both the protocols had identical samples from different fractions of sucrose density gradient. The IC-PCR protocol had better results in comparison to direct PCR. Only four lanes have bands while with IC-PCR there are six lanes distinctly amplified. Samples were from different fractions of sucrose density gradient. Lanes 1-9 were viral DNA samples from Khabusi cultivar. Lane 10 was negative control of PBS buffer and lane 11 was positive control from BSOEV DNA. Mr is DNA marker (Bioline 1kb DNA ladder)

Table 16. Analysis of banana cultivars for the presence of BSV from major banana growing regions in Kenya. The antibody used for ELISA and ISEM was Lockhart.

Sample number	Locality	Cultivar	Genotype	FD/F	Symptom Expression "Score of 0-5 as previously described"	Serology		PCR	
						ELISA	ISEM	Direct PCR	IC-PCR
1	Mt. Elgon	Likhaho	EAH-AAA	FD	5	+	+	+	+
2	Mt. Elgon	Likhaho	EAH-AAA	F	5	nd	+	+	+
3	Mt. Elgon	Likhaho	EAH-AAA	F	5	+	nd	+	+
4	Mt. Elgon	Likhaho	EAH-AAA	F	5	+	nd	-	-
5	Mt. Elgon	Likhaho	EAH-AAA	F	5	+	nd	-	-
6	Mt. Elgon	Lisulya	EAH-AAA	FD	5	+	+	-	+
7	Mt. Elgon	Lisulya	EAH-AAA	FD	5	+	+	-	-
8	Mt. Elgon	Lisulya	EAH-AAA	FD	3	+	nd	-	-
9	Mt. Elgon	Lisulya	EAH-AAA	FD	3	+	nd	nd	+
10	Mt. Elgon	Lisulya	EAH-AAA	F	3	+	nd	+	+
11	Mt. Elgon	Khabusi	EAH-AAA	F	5	+	+	+	+
12	Mt. Elgon	Khabusi	EAH-AAA	F	5	+	nd	+	+
13	Mt. Elgon	Khabusi	EAH-AAA	F	5	+	nd	-	-
14	Mt. Elgon	Bururu	EAH-AAA	F	5	+	+	nd	+
15	Mt. Elgon	Bururu	EAH-AAA	F	5	+	+	-	+

Sample number	Locality	Cultivar	Genotype	FD/F	Symptom Expression "Score of 0-5 as previously described"	Serology		PCR	
						ELISA	ISEM	Direct PCR	IC-PCR
16	Mt. Elgon	Bururu	EAH-AAA	F	5	+	+	-	-
17	Mt. Elgon	Bururu	EAH-AAA	F	5	+	+	+	+
18	Mt. Elgon	Crown	EAH-AAA	F	5	nd	nd	-	-
19	Mt. Elgon	Murure	EAH-AAA	FD	5	+	-	-	-
20	Mt. Elgon	Chilume	EAH-AAA	FD	3	+	nd	nd	+
21	Mt. Elgon	Chilume	EAH-AAA	FD	1	+	nd	-	-
22	Bungoma	Valary	AAA	FD	3	+	nd	nd	+
23	Bungoma	Nasirembe	EAH-AAA	FD	3	+	nd	nd	+
24	Bungoma	D/Cav	AAA	FD	3	+	nd	nd	+
25	Bungoma	Suriat	EAH-AAA	FD	3	+	nd	nd	+
26	Bungoma	G/Nain	AAA	FD	3	+	nd	nd	-
27	Kakamega	Mysore	EAH-AAA	FD	5	+	nd	nd	-
28	Kisii	Sialamule	EAH-AAA	FD	3	+	+	+	+
29	Kisii	Nshule	EAH-AAA	FD	5	+	+	+	+
30	Kisii	Nshule	EAH-AAA	FD	5	+	-	-	-
31	Kisii	Nshule	EAH-AAA	FD	5	+	-	+	+
32	Kisii	Ngombe	EAH-AAA	FD	5	+	-	-	-

Sample number	Locality	Cultivar	Genotype	FD/F	Symptom Expression “Score of 0-5 as previously described”	Serology		PCR	
						ELISA	ISEM	Direct PCR	IC-PCR
33	Kisii	Ngombe	EAH-AAA	FD	5	+	-	-	-
34	Kisii	Ngombe	EAH-AAA	FD	3	+	-	nd	-
35	Kisii	Kisukari	AB	FD	3	+	nd	nd	-
36	Kisii	A/ banana	AB	FD	3	+	nd	nd	-
37	Kisii	Bogoya	AAA	FD	3	+	nd	nd	-
38	Kisii	Kampala	AAA	FD	3	+	nd	nd	+
39	Kisii	U/Green	EAH-AAA	FD	3	+	nd	nd	-
40	Kisii	Spambia	EAH-AAA	F	3	nd	nd	+	-
41	Kisii	Mbuki	EAH-AAA	F	3	nd	nd	+	-
42	Kisii	Lisumba	EAH-AAA	FD	3	+	nd	nd	-
43	Nakuru	G/Finger	AAAB	FD	2	+	-	nd	+
44	Nakuru	Lisulya	EAH-AAA	FD	2	+	nd	nd	-
45	Nakuru	Lacatan	AAA	F	1	nd	nd	nd	+
46	Nakuru	Lacatan	AAA	FD	2	+	nd	nd	-
47	Nakuru	Namukila	EAH-AAA	F	5	nd	nd	+	+
48	Nakuru	Namukila	EAH-AAA	F	5	nd	nd	-	-
49	Nakuru	Namukila	EAH-AAA	F	5	nd	nd	-	-

Sample number	Locality	Cultivar	Genotype	FD/F	Symptom Expression “Score of 0-5 as previously described”	Serology		PCR	
						ELISA	ISEM	Direct PCR	IC-PCR
0	Nakuru	G/ finger	AAAB	F	2	nd	nd	nd	-
1	Nakuru	G/ finger	AAAB	F	2	nd	nd	nd	
2	Nakuru	G/ finger	AAAB	F	2	nd	nd	nd	-
3	Nakuru	Williams	AAA	F	5	nd	nd	-	-
4	Nakuru	Williams	AAA	F	5	nd	nd	+	-
5	Nakuru	Williams	AAA	F	5	nd	nd	-	+
6	Nakuru	Williams	AAA	F	2	nd	nd	-	-
7	Nakuru	Valary	AAA	F	2	nd	nd	-	-
8	Nakuru	Valary	AAA	F	1	nd	nd	+	+
9	Nakuru	Valary	AAA	F	1	nd	nd	+	+
60	Nakuru	Valary	AAA	F	1	nd	nd	-	+
61	Nakuru	U/Green	EAH-AAA	F	2	nd	nd	+	+
62	Nakuru	U/Green	EAH-AAA	F	1	nd	nd	+	+
63	Nakuru	U/Green	EAH-AAA	F	1	nd	nd	-	-
64	Nakuru	U/Green	EAH-AAA	F	1	nd	nd	-	-
65	Nakuru	A/ banana	AA	F	2	nd	nd	-	-
66	Nakuru	A/banana	AA	F	2	nd	nd	-	-

Sample number	Locality	Cultivar	Genotype	FD/F	Symptom Expression "Score of 0-5 as previously described"	Serology		PCR	
						ELISA	ISEM	Direct PCR	IC-PCR
67	Nakuru	Lacatan	AAA	F	1	nd	nd	+	+
68	Nakuru	Lacatan	AAA	F	1	nd	nd	+	+
69	Nakuru	Lacatan	AAA	F	1	nd	nd	-	-
70	Nakuru	G/Nain	AAA	F	1	nd	nd	+	+
71	Nakuru	G/Nain	AAA	F	1	nd	nd	-	+
72	Nakuru	G/Nain	AAA	F	1	nd	nd	-	+
73	Nakuru	G/Dessert	AAA	F	2	nd	nd	-	-
74	Nakuru	G/Dessert	AAA	F	2	nd	nd	-	-
75	Nakuru	D/Cav	AAA	F	2	nd	nd	-	-
76	Nakuru	C/Cav	AAA	F	2	nd	nd	+	-
77	Thika	G/Beauty	AAA	FD	5	+	-	nd	-
78	Thika	C/Cav	AAA	FD	2	+	nd	-	-
79	Thika	Valary	AAA	FD	2	+	nd	nd	-
80	Thika	Williams	AAA	FD	2	+	nd	nd	-

Note: Abbreviations FD stands for freeze dried tissue; F- fresh-tissue. For ELISA/ ISEM detection, - = Negative/ No particles; + = presence of virus particles/field. Direct virus PCR =direct PCR without antibody coating; IC-virus prep = immune capture of purified virus on antibody coated tubes; IC-sap = immune capture of virus in the sap through antibody coated tubes; nd= not done. EAH-AAA= East Africa Highland AAA, A/banana= Apple banana, C/Cavendish= Chinese Cavendish, D/Cav = Dwarf Cavendish, G/Beauty = Golden Beauty, G/ Dessert= Gold dessert, G/Finger = Gold Finger, G/Nain =Grand Nain, U/Green = Uganda Green. 1=mild, 2/3=moderate, 5= severe symptom expression. Lockhart antisera was used in all experiments.

6.3.2 Immunosorbent Electron Microscopy (ISEM)

Characteristic bacilliform particles of approximately 120-130 nm x 30 nm trapped on BSV - antibody coated carbon grids and detected in seven out of ten samples by ISEM (Fig. 7). Particles could be observed in samples from Likhaho1, Likhako2, Lisulya1, Lisulya2, Khabusi, Bururu, and Nshule. The number of particles observed was between 1 to 10 particles per field. The ISEM technique provided useful information on the morphology of the virus particles. However, the technique can only be used for virus detection when facilities are readily available (Baker *et al.* 1985; Milne 1993). It also requires highly skilled manpower and it cannot be used for the rapid processing of multiple samples. Nonetheless, it is ideal for confirmatory tests using small numbers of samples.

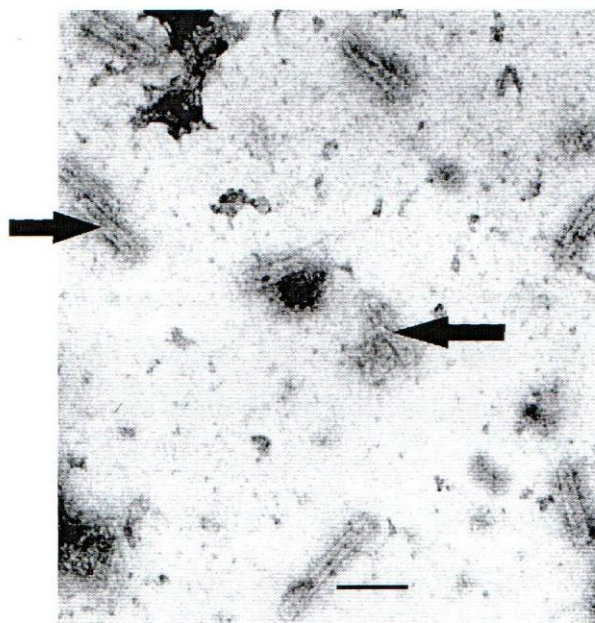


Fig. 7. ISEM of a virus preparation from Kenyan banana leaf cv. Likhaho collected from Mt. Elgon. The arrow indicates a bacilliform particle of approximate size 120 nm x 30nm. Scale bar =100 nm.

6.3.3 Cloning, Sequencing and phylogenetic analysis

Thirty three partial BSV sequences were obtained from eight samples (Likhaho1, Lisulya1 and Lisulya2, Khabusi1 and Khabusi2, Nshule, Sukarindizi and Bogoya), and could be classified into seven categories. The phylogenetic analyses in Fig. 8 show the seven categories as 1-7. Category 2 showed a high similarity of 100% with a previously known

species from Uganda, *Banana Streak Uganda A Virus* (BSUgAV). Category 5, 6 and 7 had similarities with isolates *Banana Streak Imove Virus* (BSImV), *Banana Streak Obino L'Ewai Virus* (BSOEV) and *Banana Streak Gold finger Virus* (BSGfV) respectively. Category 1, 3, and 4 were all new isolates and pairwise comparison with BSUgAV and between themselves showed similarities with sequence identities ranging from 80 – 97% (Table 17) while pairwise comparison with Ugandan species *Banana Streak Uganda B Virus* (BSUgB) to *Banana Streak Uganda H Virus* (BSVUgH) and other *Badnaviruses* had sequence identities ranging from 58 – 69% (Table 17).

Sequence comparison by GAP showed similarities between the isolates in the Polymerase (RT + RNaseH) nucleotide sequence region greater than 80%. The proposed criterion by International Committee on Taxonomy of viruses (ICTV) (Büchen-Osmond, C., 2003) for new virus species within the *Badnavirus* is based on a sequence similarity of 80% or less. Since the three categories from Kenya had sequence similarities of 80% and above, they were considered as new isolates. The proposed names were as follows: Category 1 sequence was entered into the EMBL database as accession number AM905903, (2008) and was given the name Khabusi2, ; similarly, category 3 was named isolate Lisulya (AM905901, 2008); and category 4 was named isolate Nshule (AM905904, 2008). The first five isolates were all from East Africa Highlands banana genotype (EAH - AAA) a cooking banana while category 6 was an AB genotype and category 7 was an AAA genotype mainly dessert bananas. (See appendix 10 for full sequences of the seven isolates found in Kenyan banana samples).

Although categories 1-5 were from EAH - AAA genotype they, were all affected by a diverse range of isolates. However the three new isolates were specifically found on EAH - AAA genotypes. Representatives from different isolates were found in the individual plants indicating that there can be very extensive variation of BSV sequences within the plant. An example is a Khabusi cultivar with sequences from isolates of Khabusi2, BSImV and BSGfV. Similar observation had also been made by Harper *et al.* (2005). Category 6 affected only genotype AB while category 7 affected both EAH - AAA genotype and AAA genotype.

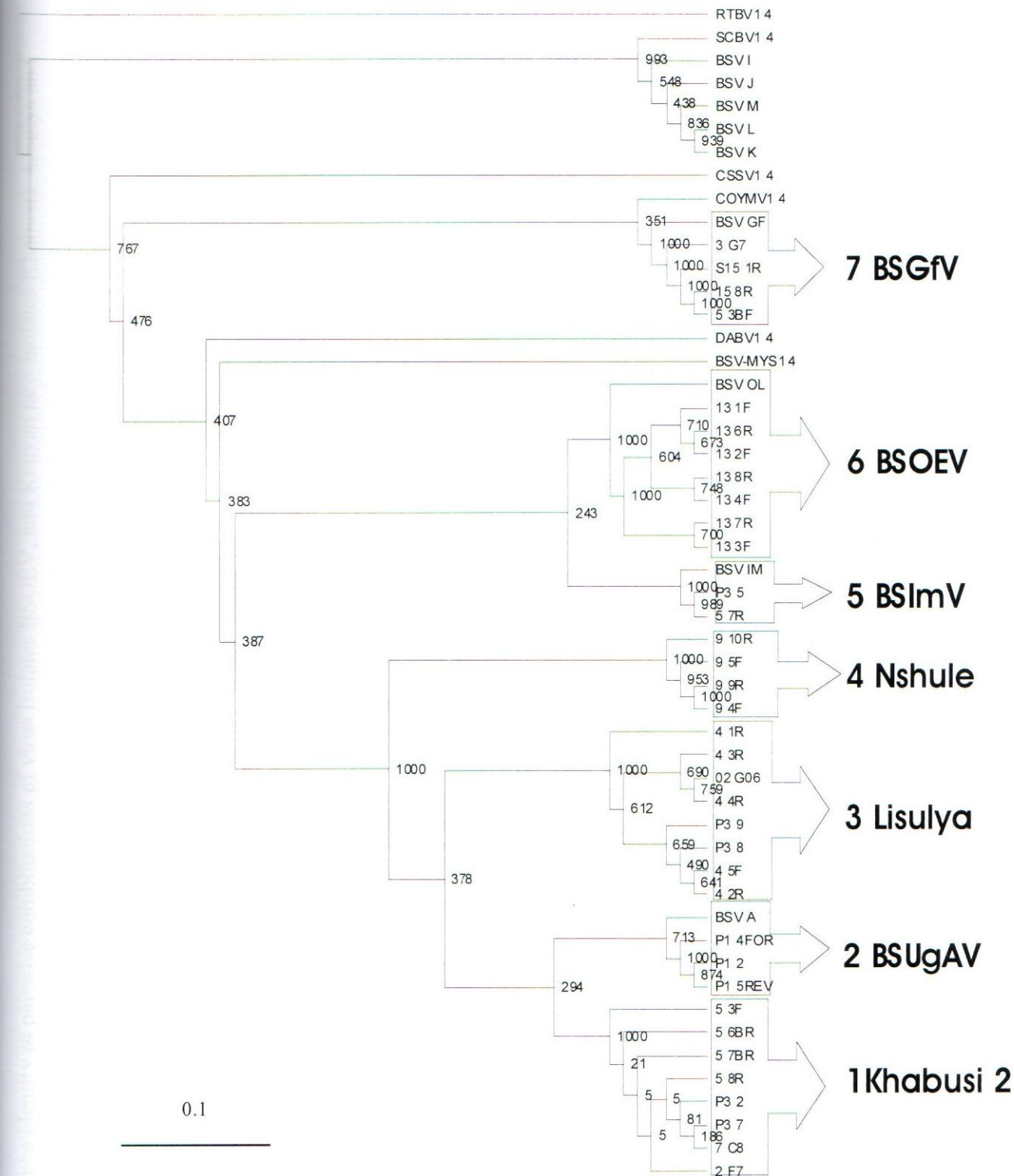


Fig 8: Phylogenetic analysis of the partial ORF III region sequences from the Kenya isolates compared with other known *Badnaviruses*. Amplified sequences were aligned with other database *Badnavirus* sequences, listed in appendix 9. Neighbour-joining trees were calculated and bootstrapped using Clustal X. The phylogenetic tree was rooted with RTBV, the *Caulimovirus* most closely related to the *Badnaviruses*, as an outgroup. The 33 Kenyan sequences are grouped into 7 isolates Khabusi 2, BSUGAV, Lisulya, Nshule, BSIImV, BSOEV and BSGfV. Bootstrap values are on the nodes. Branches which had values below 25% bootstrap support were collapsed. Scale bar = 100% amino acid sequence identity

Table 17. Amino acid sequence identity of pairwise combinations of virus isolates BSOEV, and Kenyan new isolates Khabusi2, Lisulya and Nshule for a portion of the open reading frame III.

BSV Isolates	% Amino acid sequence identity ^a										
	BSUgA	Khabusi2	Lisulya	Nshule	BSUgB	BSUgC	BSUgD	BSUgE	BSUgF	BSUgG	BSUgH
BSUgA	100	80	97	96	64	64	69	68	65	67	67
Khabusi2		100	80	80	60	64	68	67	63	65	66
Lisulya			100	95	63	63	68	66	64	66	66
Nshule				100	60	58	66	66	61	63	64
BSUgB					100	81	80	81	82	77	78
BSUgC						100	78	81	83	75	76
BSUgD							100	86	87	82	80
BSUgE								100	89	79	81
BSUgF									100	83	83
BSUgG										100	82
BSUgH											100

^aBSV isolates are BSUgA to H, Khabusi2, Lisulya and Nshule.

^bSequence identity is based on the alignment of the ribonuclease H domain of the ORF III polypotein.

There was a difference in isolate distribution between cultivars. While Khabusi2, BSI_{Im}V, Lisulya and BSG_fV isolates were found in more than 2 different cultivars, Nshule and BSOEV were consistently found in only one cultivar (Fig. 8).

An important observation made in this study was that the EAH-AAA had a diverse range of isolates which could only be detected by use of degenerate primers 1A -4'. This study also established that Khabusi2, BSU_gAV, Lisulya, Nshule and BSI_{Im}V isolates were found specifically in Kisii and Mount Elgon while BSOEV and BSG_fV isolates were found in all the banana growing regions with those specific cultivars. The phylogenetic analysis indicates that the isolates in category 1-4 were closer to the Ugandan BSU_gAV than to category 5 and 6. This could have been due to the geographical location.

Appendix 11 shows alignment of protein sequences obtained from the three new Kenyan isolates and BSU_gA to H Ugandan isolates. All sequences in the analysis were translated to give a protein sequence residue of between 165 to 187 amino acid residues.

6.3.4 Southern Blotting and Hybridisation

Results in Fig. 9 show faint bands at 7.5 kbp, and a partial band at 5.5 kbp. It is possible that this could have been as a result of less than complete digestion of the virus DNA. The rest of the virus may be one or more bands with a maximum size of 2 kbp. Small bands always give a fainter image as they have less radioactivity and so may be missed or not seen. The sample used was from Likhaho cultivar of EAH-AAA genotype and, from what has been reported previously (Harper *et al.*, 2005), it seems very unlikely that it has integrated sequences. BSU_gAV which has 100% similarity with Likhaho cultivar was not shown to be integrated (Harper *et al.*, 2005). What was observed could have been incompletely digested viral DNA hence the larger band is undigested virus and the lower band digested virus. The "missing" bits of the digested virus may be very small depending on the number of *ECOR* 1 restriction sites and could not be easily identified. More work however, is needed to resolve this issue.

Likhaho cultivar was specifically found at the border of Kenya and Uganda. It had high similarities with the previously identified virus species BSU_gA, which is unique in comparison to all other BSV species in having a (non-consequential) 2 amino acids deletion

within the RT-RnaseH sequence and also has a very high virion titre. The distribution of this virus is geographically limited within Kenya and Uganda, suggesting a very recent introduction of the virus or a recent mutation. The high virus titre suggests its potential threat to Kenyan Musa stocks and its potential for movement between plants by vectors. More survey are needed to establish the mode of spread of the virus and the economic impact on the yields of bananas in the affected areas. As a short term measure there should be restriction on Musa movement of material from the infected stocks.

The occurrence of isolates BSI_mV, BSOEV and BSGfV as observed in the Kenyan isolate 5, 6, and 7 previously (Fig 8) in primarily A genome banana cultivars, suggests that the B genome source could have originated from Musa introduced into Kenya/Uganda which were containing integrated sequences (Harper *et al.*, 2005). For example if Gold finger, which contains B genome, had activated BSV sequences, these could have been transmitted to other A genome cultivars through mealybugs, hence the spread of the virus

BSV sequence integration causes a serious obstacle to movement of Musa germplasm as well as its utilization for breeding programmes (Diekmann and Putter, 1996). Evidence has shown that when integrated sequences are activated by factors such as tissue culture process (Dallot *et al.*, 2001) or environmental changes (Dahal *et al.*, 2000) they produce infectious particles (episomal). This presents a challenge to production of tissue culture banana seedlings. Availability of an efficient diagnostic protocol will enable production of BSV free tissue culture seedlings and improve BSV status of plantations.

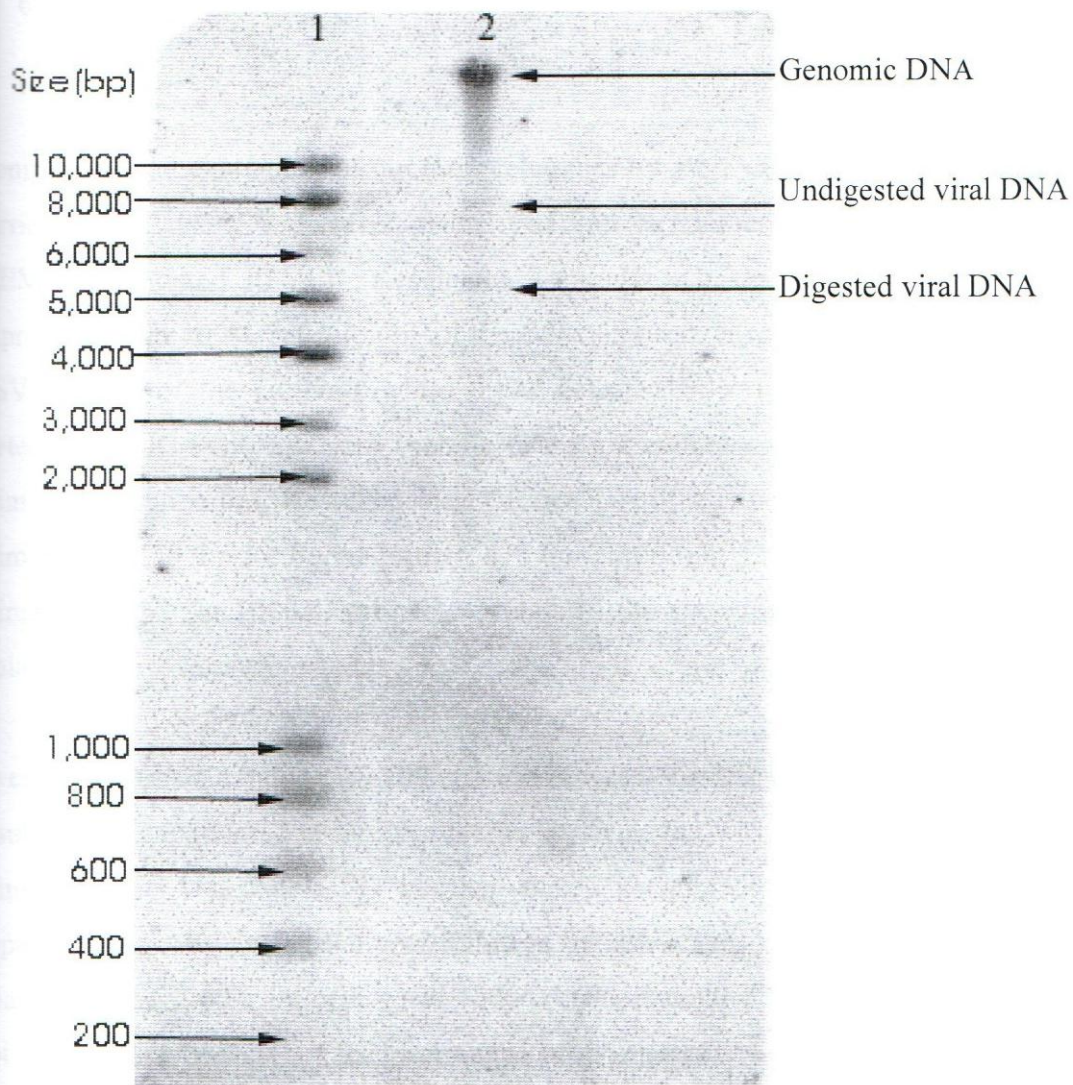


Fig. 9. Southern blot analysis of sample 2 (Likhaho). Hybridisation with a radiolabelled probe from sample 4 (Lisulya) revealed episomal viral DNA band with several fragments of both undigested and digested viral DNA. Track 1 is the DNA marker showing various sizes starting from 200 kbp to 10,000 kbp. Track 2 is the banana sample (Likhaho) showing undigested episomal viral band at 7.5kbp and digested viral band at 5.5 kbp.

6.3.5 Comparison of the sensitivity and efficiency of the four BSV screening techniques

Comparison and evaluation of the four techniques for BSV detection and identification were carried out on six BSV diseased plants. The four techniques were BSV symptom analysis, ISEM, ELISA, and IC-PCR. Results on Table 18 indicate moderate to severe symptom expression with 100% detection in all the cultivars used. However, ELISA protocol detected BSV in 67% of the cultivars while ISEM detected BSV in 67% of the cultivars tested. Detection by IC-PCR was very specific in the six cultivars and was dependent on specific primers and antibodies. However, the PCR generated the expected product depending on the primers used (597bp for Badna primer, 476 for GF primer, 522 for RD primer, 782 for DCav primer and 363 for EK-OL primer). Overall all the six cultivars had from one up to four isolates of the viruses detected.

Average absorbance value of 0.100 and above was considered positive for BSV. From these results Nshule expressed severe symptoms but detection was possible with only one type of primer Badna 1A-4' (Table 18). In other studies, symptomatic leaves have been observed to appear sporadically during the year followed by new leaves expressing few or no symptoms (Daniells *et al.*, 2001; Fargette *et al.*, 2006). Thus indexing by visual inspection alone may not be efficient because of the occurrence of asymptomatic as well as the long incubation periods before symptom expression. Symptoms may also be confused with those caused by *Cucumber Mosaic Virus* (CMV) (Lockhart and Olszewski, 1993) hence giving false results.

In the two cultivars Chirume and Ng'ombe, the leaf samples with BSV symptoms tested negative for BSV in DAS-ELISA and the four techniques tested positive for BSV when IC-PCR technique was used. When there is low number of virus particles and low virus titre the high specificity of IC-PCR is able to capture and amplify very few virus particles. Use of specific primers indicated that IC-PCR was considerably more sensitive than ISEM for detecting typical BSV, while ISEM proved to be of similar sensitivity as DAS-ELISA. Out of the 6 banana cultivars that were BSV positive in all tests taken together, 4 cultivars were positive by ISEM, 6 by IC-PCR and only 4 by DAS-ELISA. Typical bacilliform BSV-like particles were clearly recognized (Fig. 7).

Table 18. Comparison of the sensitivity and efficiency of four banana streak virus detection techniques.

Cultivar	Symptom expression (Score of 0-5 as previously described)	ELISA A_{405nm}	ISEM	IC-PCR											
				BSV-BADNA			BSV-GF			BSV-Dacca			BSV-Dcav		
				Meyer	Lockhart	Meyer	Meyer	Lockhart	Lockhart	Meyer	Lockhart	Meyer	Meyer	Lockhart	Meyer
				GIF- Bleed 2		GIF- Bleed 2	GIF- Bleed 2			GIF- Bleed 2		GIF- Bleed 2	GIF- Bleed 2		GIF- Bleed 2
Chirume	4	0.086	-	+	+	+	-	-	-	-	+	+	-	-	-
Lisulya	5	0.128	+	+	+	+	+	+	+	+	+	+	+	+	-
Likhaho	5	0.140	+	+	+	-	-	-	-	-	+	+	-	-	-
Nshule	5	0.240	+	+	+	-	-	-	-	-	-	-	-	-	-
Bururu	5	0.101	+	-	+	-	-	-	-	+	+	-	-	-	-
Ng'ombe	5	0.047	-	+	-	-	-	-	-	-	+	+	-	-	-
Control	Buffer	0.048	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: The six banana cultivars used in the experiment were collected from Mt. Elgon and Kisii regions. Three different detection methods including symptom expression, ELISA, and IC-PCR were used. ELISA mean absorbance value ($n=2$) at 405 nm was taken after 2 h substrate incubation time. IC-PCR antibody incubation time was 2h at 37° C. Presence or absence of BSV is indicated with + or -. Symptom expression disease severity score of 0-5.

6.4 Discussion

This study confirms the presence of BSV in 9 banana growing regions in Kenya, in different cultivars. The presence of BSV was confirmed using ISEM, IC-PCR and sequencing techniques. The 80 analysed samples had symptoms ranging from mild, moderate to severe within the range described by Lockhart and Jones (2000).

ISEM confirmed that samples with typical BSV symptoms contained bacilliform particles using BSV antibodies (Fig. 7). Modified antibody concentration of 1:100 rather than the recommended 1:1000 (Lockhart 1986) was used to trap the virion particles for IC-PCR. A possible reason for the initial poor results using this technique is that the antibody concentration used initially was too low for detection of Kenya BSV isolates, for example the antibodies specific for the isolates present in the Kenyan samples could be at low abundance in this particular antibody cocktail produced by Dr Lockhart. The optimal antibody concentration was applied for the analysis of all 80 samples.

The results confirm that episomal virus can be trapped by the antibodies and the viral DNA detected by IC-PCR with high sensitivity and specificity. BSV DNA fragments were not amplified from some leaf samples using degenerate primers 1A and 4' despite apparently very clear BSV symptoms. This may be because the symptoms were not due to BSV and other viruses might have been involved (Tushemereirwe *et al.*, 1996) or that the virus associated with these samples is a different BSV isolate that did not anneal to the primers used. Degenerate primers are universal but may not be able to pick all BSV isolates and species.

Comparison of the four techniques, BSV symptom analysis, ISEM, ELISA, and IC-PCR techniques in their sensitivity and specificity to identify and detect BSV in Kenyan samples confirmed symptom analysis and IC-PCR as the optimum technique with identification and detection of 100% infection while ISEM and ELISA had 67% detection.

Symptoms on plants are commonly used to characterize a disease having viral aetiology; and for roguing of diseased plants in order to control the disease. Visual inspection is relatively easy when symptoms clearly are characteristic of BSV. However, many factors such as virus strain, host plant cultivar, time of infection, and the environment can influence the symptoms

exhibited (Matthews, 1980). Plants can also exhibit virus-like symptoms as a response to unfavorable weather conditions, soil mineral/nutrient imbalances, infection by nonviral pathogens, damage caused by insect/mite/nematode pests, air pollution, and pesticides (particularly herbicides). Sometimes BSV may induce no apparent symptoms or cause symptomless infection depending on whether the virus is integrated or episomal. In addition, different viruses, for example CMV, can produce similar symptoms as BSV which might be confusing. While symptoms provide vital information on BSV identification, adequate field experience is required when making a decision on symptomatology alone.

The ISEM technique, a serological technique, used in this study combines the specificity of serological assays with the visualization capabilities of the electron microscop (EM). Although ISEM is a sensitive technique in detecting BSV, as was shown in the results of the study, it is labor intensive, expensive, time consuming and it cannot be easily used for the rapid processing of multiple samples.

ELISA, another serological technique, was shown in this study to be as efficient as the ISEM technique in BSV detection. Due to its adaptability, sensitivity, and economy in use of reagents, ELISA has been widely used in BSV detection of a large number of samples in a relatively short period of time. However, although ELISA is versatile and individual steps are simple, the assay is complex in that several steps with different reagents are involved. Many factors can therefore influence the sensitivity and reliability of the assay. Another disadvantage of serological techniques is that they are based on the antigenic properties of the virus coat protein which represents only about 10% of the total virus genome (Gould and Symons, 1983) and thus does not take into account the rest of the virus genome. Therefore it is possible to get false negatives with the technique, possibility why there was only 67% detection instead of 100%.

IC-PCR is a PCR based technique that combines the technical advantages of PCR with the practical advantages of ELISA. As was shown in the results in section 3.6.5, there was 100% BSV detection. The technique therefore has greater sensitivity compared to the other techniques. Since the technique is nucleic acid-based, it has an advantage that any region of a viral genome can be targeted for detection of BSV, hence making the technique more sensitive and specific.

Considering the advantages and disadvantages of the four techniques discussed above, IC-PCR was selected as the procedure for analysis of all BSV identification and detection work in all Kenyan banana plant material. Since there is minimal information on BSV work in Kenya, a technique is needed with high level of specificity and sensitivity that can detect a large number of BSV infected samples. Currently there is availability of basic resources; laboratory facilities, reagents, expertise and skills to execute the IC-PCR technique competently.

The study revealed wide variation in BSV found in Kenya. Some BSV isolates probably originated from integrated sequences and either the virus or the germplasm were introduced. The virus then spread as episomal virus through mealybugs transmission or vegetative propagation.. Others are not associated with integrated sequences. Through sequence alignment, interesting sites were identified with the Ugandan BSV species (BSUgAV) being more similar to Kenya BSV than the other Uganda species from other geographical regions (Fig.8). BSUgAV has previously been detected as an episomal virus in Uganda which was geographically restricted to the Mt. Elgon region (Harper *et al.*, 2005). This one very unusual virus was distinctive because of its high titre within infected plants and an unusual two amino acid sequence deletion (Harper *et al.* 2005). Mutation within the virus population, that serves to increase replication competence, cannot be ruled out. Movement of the plant material across the Kenya/ Uganda borders, however, is most likely the most significant means of dispersal of the virus (es). This virus requires surveillance and further work to identify its origin, its disease potential and propensity for further dissemination. Transmission tests are needed to establish the ability of the virus to be transmitted and causes of its high titre in plants. Using the alignment based on the collection of Kenya sequences, it should be possible to exploit the deletion to design primers to monitor the distribution of this particular isolate.

Plants can harbour more than one isolate or species. In this study, some of the banana samples of cultivars Lisulya and Khabusi, two or three BSV isolates were identified in a single plant. It was noted that symptom expression was more severe in those cultivars. It is possible that the more the number of isolates the higher the disease symptom expression. Reports indicate that the effect of symptom expression on yield loss varies with cultivar and location (Dahal *et al.*, 2000).

6.4.1 Conclusion

From the information on the variable Kenyan isolates and the nature of the virus, it is essential to develop a BSV diagnostic kit which can quickly and reliably detect BSV in the field, greenhouses and commercial banana tissue culture laboratories in the near future. This will contribute to improvement of banana productivity in Kenya.

CHAPTER SEVEN

GENERAL CONCLUSION AND RECOMMENDATION

7.1 Conclusion

The effect of cultivar on expression of banana streak badnavirus was observed in all the vegetative stages of plant 6 months in the greenhouse and 2 years in the field experiments. In the greenhouse, disease severity varied with age and leaf stage. The third leaf stages to fully expanded fourth leaf stage was noted as an effective period for screening greenhouse material for the virus before they are moved to the field. Indexing may not have detected BSV in very young seedlings at growth room since they still expressed BSV after transplanting in the greenhouse. Ng'ombe cultivar had higher infection and expression while Valary had lower infection and symptom expression of BSV across Njoro, Kokoto and Rongai locations. Other cultivars which had relatively low BSV scores across the locations included Valary, Uganda Green, Lacatan and Kibuzi.

The ELISA standardization experiment showed the antigen and antibody concentration of 1:2 and 1:1000 respectively, to be the optimum for use in serological analysis for banana streak virus in banana plants. Variation in response to BSV was also noted with different antibodies, with the ones sourced from Dr. Winter and AgDia Co. showing better response compared to those from Meyer-G1F-Bleed 2 and Meyer-R210-Bleed1. However, due to unavailability of the first two antibodies, Meyer-G1F-Bleed 2 is recommended for use until a permanent solution is found.

The presence of BSV in Kenyan samples was confirmed using ISEM, IC-PCR and sequencing techniques. ISEM confirmed bacilliform particles of 30nm x 120 - 130nm. PCR was effective at amplifying BSV sequences from partially purified virus and concentrated sap extraction and degenerate primers were used to determine uncharacterized strains of BSV. Modified antibody concentration of 1:100 rather than the recommended 1:1000 was found the most effective in trapping the BSV virion particles. IC-PCR technique was observed in all experiments to have high sensitivity and specificity.

Through sequence alignment, three new isolates were identified for the first time in Kenya (Karanja *et al.*, 2008a). The three new sequences have already been submitted to the EMBL

database and have been assigned the accession numbers AM 905901 (Lisulya); AM 905903 (Khabusi2); AM 905904 (Nshule). Separate four sequences were identified to be similar to Ugandan BSV species BSUgAV, BSIImV, BSOEV and BSGfV. Movement of the plant material across the Kenya/ Uganda borders was considered to be the most likely and significant means of dispersal of the virus (es). Some BSV isolates probably originated from integrated sequences and either the virus or the germplasm were introduced. The virus then spread conventionally. In some of the cultivars with B genome, activation of endogenous sequences of BSV through tissue culture may have contributed to the episomal BSV identified. The unusual virus isolate BSUgAV, warrants further investigation and surveillance to identify its origin, its disease potential and propensity for further dissemination. Lisulya and Khabusi cultivars had multiple infections of BSV isolates and two to three isolates were identified in a single cultivar. It was noted that symptom expression was more severe in those cultivars. It is possible that the more the number of isolates the higher the disease symptom expression in the infected plant. This study was able to identify and to confirm six new isolates present in Kenya and initiate evaluation of management options which could be used to achieve high productivity in banana orchards.

Results from this study will enable to contribute to:

1. Molecular variability studies of BSV in that new antibodies and primers can be developed and designed from the 3 new isolates.
2. Improvement of efficiency of screening techniques in that optimized IC-PCR technique can be used in screening banana tissue culture seedlings before their distribution to growers.
3. Application of the knowledge on the effect of cultivar, nitrogen levels and environment to manage the BSV disease.

7.2 Recommendations

1. Indexing of BSV should be done after two to four months of seedling growth in the greenhouse to avoid false results with seedlings that are too young
2. Moisture level is a critical factor in the infection and expression of the symptoms. Therefore application of sufficient water in the banana orchards could be used to avoid higher infection and expression where the disease is already present
3. High doses of nitrogen fertilizer beyond recommendation of 200g per plant per year could activate expression of BSV resulting into death of the plantation. Therefore, application of recommended optimal rates of fertilizer is critical in the management of the BSV. Use of organic fertilizers should be encouraged for restructuring of the soils and moisture conservation.
4. In the isolate identification, Khabusi and Lisulya cultivars were found to harbour more than one isolate therefore a more robust protocol should be developed in future which can capture a diverse range of isolates.
5. Most of the banana orchards in Kenya are established with A-genome, B- genome and EAH - AAA genome from both tissue culture and sucker propagated material which have not been indexed for viruses. From the preliminary survey results there is a possibility of BSV infection in most of the orchards. Farmers and private laboratory managers need to be educated on banana streak virus and the risk that 'B' genome cultivars present in banana production
6. Currently a practical control measure recommendation would be to avoid propagating identified infected mother plants and use of BSV screened tissue culture material. Rouging of diseased plants should also be done as a management practice Farmers can work closely with extension workers on the identification of diseased plants and cultural practices for maintenance of virus free banana plants.
7. It will be ideal in the near future to develop a diagnostic kit which can quickly, reliably and cheaply detect BSV in a diverse range of isolates
8. Further research is necessary to provide information on the effect of other plant nutrients on BSV infection
9. BSV isolates should be evaluated to determine the corresponding damage they cause in order to advise farmers appropriately
10. BSV-free mother blocks should be established by Research Institutions to supply clean banana suckers to both commercial and private plant tissue culture laboratories

in the country. The mother blocks should be screened routinely to rogue out any BSV infected plants

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APPENDICES

Appendix 1. Commonly grown banana genotypes showing their genome composition, and chromosome number.

Cultivar name	Genome Type	Chromosome No	Type	Reference
FHIA 01	AAAB	4	Sweet-acid	Meyer <i>et al.</i> 2006
FHIA02	AAAB	4	Sweet	Meyer <i>et al.</i> 2006
FHIA03	AABB	4	Cooking	Meyer <i>et al.</i> 2006
FHIA 17	AAAA	4	Sweet	Meyer <i>et al.</i> 2006
FHIA 18	AAAB	4	Sweet-acid	Meyer <i>et al.</i> 2006
Bluggoe	ABB	3	Beer	Banana Germplasm Report, 1997
Mysore	AAB	3	Sweet	Banana Germplasm Report, 1997
Kampala	AAA	3	Sweet	Banana Germplasm Report, 1997
Valary	AAA	3	Sweet	Banana Germplasm Report, 1997
Bogoya	AAA	3	Sweet	Banana Germplasm Report, 1997
Gross Michel	AAA	3	Sweet	Banana Germplasm Report, 1997
Sukari Ndizi	AAA	3	Sweet	Banana Germplasm Report, 1997
Lacatan	AAA	3	Sweet	Banana Germplasm Report, 1997
Grande Naine	AAA	3	Sweet	Banana Germplasm Report, 1997
Pelipita	AAA	3	Sweet	Banana Germplasm Report, 1997
Dwarf Cavendish	AAA	3	Sweet	Banana Germplasm Report, 1997
PAZ	AAA	3	Sweet	Banana Germplasm Report, 1997
Poyo	AAA	3	Sweet	Banana Germplasm Report, 1997
William	AAA	3	Sweet	Banana Germplasm Report, 1997
Chinese Dwarf	AAA	3	Sweet	Banana Germplasm Report, 1997
Muraru	AA	2	Sweet	Banana Germplasm Report, 1997
Mboki	AB	2	Sweet	Banana Germplasm Report, 1997
Wangae	AB	2	Sweet	Banana Germplasm Report, 1997
Sukarindizi	AB	2	Sweet	Banana Germplasm Report, 1997
Spambia	AAB	3	Roasting	Banana Germplasm Report, 1997
Matumbo	ABB	3	Cooking	Banana Germplasm Report, 1997

Nasirembe	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Bululu	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Murure	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Kiganda	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Mbwazirume	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Lisulya	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Likhaho	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Ngombe	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Kibuzi	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Namukhila	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Jamanga	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Murure	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Turbo	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Maffukha	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Ntobe	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Nshule	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Khabusi	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Mtahato	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Solio	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997
Mukia wa chui	EAH-AAA	3	Cooking	Banana Germplasm Report, 1997

Appendix 2. (A). Full list of ITCV Species in the Genus (Brunt *et al.*, 1996)

	Virus name	Acronym
1	banana streak virus	(BSV)
2	Cacao swollen shoot virus	(CSSV)
3	Canna yellow mottle virus	(CaYMV)
4	Commelina yellow mottle virus	(ComYMV)
5	Dioscorea bacilliform virus	(DBV)
6	kalanchoe top-spotting virus	(KTSV)
7	Piper yellow mottle virus	(PYMoV)
8	rice tungro bacilliform virus	(RTBV)
9	Schefflera ringspot virus	(SRV)
10	sugarcane bacilliform virus	(SCBV)

Appendix 2. (B). Tentative Species in the Genus

	Virus name	Acronym
1	Aucuba bacilliform virus { <i>Aucuba japonica</i> }	(AuBV)
2	mimosa bacilliform virus { <i>Albizzia julibrissin</i> }	(MBV)
3	taro bacilliform virus { <i>Colocasia esculenta</i> }	(TaBV)
4	Yucca bacilliform virus { <i>Yucca elephantipes</i> }	(YBV)

Appendix 3. Composition of modified Murashige and Skoog medium for *in vitro* propagation of *Musa* plants

Nutrient components		(mg/L)
Macronutrients	Ammonium nitrate	1650.0
	Potassium nitrate	1900.0
	Calcium chloride.2H ₂ O	440.0
	Magnesium sulfate.7H ₂ O	370.0
	Potassium phosphate	400.0
Micronutrients	Boric acid	6.2
	Manganese sulfate. H ₂ O	16.9
	Zinc sulfate.7H ₂ O	8.6
	Potassium iodide	0.83
	Molybdcic acid (sodium salt). 2H ₂ O	0.24
	Cobolt chloride. 6H ₂ O	0.024
	Cupric sulfate. 5H ₂ O	0.025
Iron	Na ₂ -EDTA	37.26
	Ferrous sulfate. 7H ₂ O	27.26
Vitamins	Glycine (free base)	2.0
	Nicotinic acid (free base)	0.1
	Pyridoxine.HCl	0.5
	Thiamine .HCl	0.5
Growth regulators	N ⁶ -benzylaminopurine	2.5
Carbon	Sucrose	30 000
Gelling agent	Gelrite	2 000
Antioxidant	Ascorbic acid	10.0

Appendix 4. Buffer formulations for ELISA and SSC

i) General extraction buffer: (pH 7.4)

1X PBS buffer	1 liter
Polyvinyl pyrrolidone (PVP), (MW 40,000)	10 g (1%)
2 – Mercaptoethanol 0.5%	5 ml
Tween - 20 0.05%	0.5 g

ii) Coating buffer: (pH 9.6)

Sodium carbonate (anhydrous) (Na_2CO_3)	1.59 g
Sodium bicarbonate (NaHCO_3)	2.93 g
Make up to 1 litre with distilled water	

iii) PBST Buffer (wash buffer) (7.4)

Sodium chloride	8 g
Sodium phosphate dibasic (anhydrous)	1.15 g
Potassium Phosphate, monobasic anhydrous	0.2 g
Potassium chloride	0.2 g
Tween – 20	0.5
Make up to 1 litre with distilled water	

(iv) x10 TBS buffer (7.5)

Tris HCL 400mM	500ml
Sodium Chloride	80g
Potassium Chloride	2g
Dissolve to make 1 litre with distilled water	

(vi) ECL Buffer:

Bovine serum albumin (BSA)	2 g
Polyvinyl pyrrolidone (PVP) 1% MW 40,000	10 g

(v) BSV Extraction buffer

PBS	1 litre
Polyvinyl pyrrolidone (PVP), (MW 40,000)	20.0 (2%)
Na_2SO_3 , add just before use	10.0 (1%)

(vi) SSC Buffers

Buffer Type	Concentration	Formulation
High Salt Transfer Buffer (20X SSC)	3 M NaCl, 0.3 M CH_3COONa , pH 7.0	175.5 g NaCl + 88.2 g CH_3COONa per liter of ultrapure water, pH 7.0 with NaOH or glacial CH_3COOH
High Salt Transfer Buffer (10xSSC)	1.5 M NaCl, 0.15 M CH_3COONa , pH 7.0	87.75 g NaCl + 44.1 g CH_3COONa per liter of ultrapure water, pH 7.0 with NaOH or glacial CH_3COOH
High Salt Alkaline Transfer Solution for DNA	0.4 N NaOH, 1.5 M NaCl	16 g NaOH + 87.7 g NaCl per liter of ultrapure water
High Salt Alkaline Transfer Buffer for RNA	0.01N NaOH, 5X SSC	400 mg NaOH + 43.88 g NaCl + 22 g CH_3COONa per liter of ultrapure water

Appendix 5. Soil analysis data used in the greenhouse potted plants from the forest

Fertility results		Value	g/100g of soil	Class
Soil pH		6.71		near neutral
Nitrogen %		0.32	0.32	Low
Org. Carbon %		3.20	3.20	Adequate
Phosphorus ppm		84		Adequate
Potassium me%		2.82	0.109	High
Sodium me%		0.34	0.008	Adequate
Calcium me%		7.6	0.152	Adequate
Magnesium me%		4.46	0.535	High
Manganese me%		2.37		Adequate
Copper ppm		3.47		Adequate
Iron ppm		67.1		Adequate
Zinc ppm		388		Adequate

Appendix 6. Rainfall data for Njoro Location from January 2003 to December 2006

YEAR	JAN	FEB	MAR	APRIL	MAY	JUNE	JUL.	AUG.	SEPT.	OCT.	NOV.	DEC	TOTAL
2003	24.6	12.2	129.6	186.0	199.3	93.2	85.1	213.7	102.3	78.2	79.2	27.4	1230.8
2004	102.2	17.3	85.1	169.1	158.8	57.2	58.3	116.9	50.0	48.5	43.3	49.9	956.6
2005	46.6	22.0	51.6	84.1	166.4	61.8	57.1	84.8	115.3	44.6	41.8	2.6	778.7
2006	15.3	13.0	68.0	122.1	98.3	64.7	50.3	127.6	27.4	29.5	269.6	292.3	1178.1

Appendix 7. BSV field observation of 215 samples from 9 Districts in 5 provinces of Kenya also shown in Fig. 1

Accno	Origin	Variety	Symptoms
1	Kakamega	Mysore	Short chlorotic, necrotic streaks
2	Bungoma	Nasala	Short chlorotic streaks
3	Bungoma	Nasala	Short and long chlorotic and necrotic streaks
4	Bungoma	Gross mitchel	White streaks, short leaves
5	Bungoma	Bokoboko	Short chlorotic and necrotic streaks
6	Bungoma	Nasala	Clean
7	Bungoma	Valary	Moderate streaks
8	Bungoma	Nasirembe	Moderate streaks
9	Bungoma	Dwarf Cavendish	Moderate streaks
10	Bungoma	Suriat	Moderate streaks
11	Bungoma	Grand Naine	Necrosis and chlorosis
12	Bungoma	Sweet banana	Long chlorotic and necrotic streaks
13	Bungoma	Nasirembe	Long chlorotic and necrotic streaks
14	Bungoma	Nasirembe	Long chlorotic and necrotic streaks
15	Bungoma	Nasirembe	Long chlorotic and necrotic streaks
16	Bungoma	Nasirembe	Long chlorotic and necrotic streaks
17	Bungoma	Nasirembe	Long chlorotic and necrotic streaks
18	Nyeri	Kiganda short	Choking
19	Nyeri	Williams	Necrosis and choking
20	Nyeri	Kiganda short	Necrosis
21	Nyeri	Kiganda tall	Short chlorotic streaks
22	Nyeri	Muraru tall	Short chlorotic streaks, choling, necrosis
23	Nyeri	Gachuru	Necrotic and chlorotic streaks
24	Nyeri	Kiganda short	Choking
25	Nyeri	Muraru	Short necrotic and chlorotic streaks
26	Nyeri	Kiganda short	Choking, necrosis
27	Nyeri	Muraru tall	Choking, necrosis
28	Nyeri	Mutondo	Short necrotic streaks
29	Nyeri	Njuru	Necrosis
30	Nyeri	Kiganda tall	Necrosis

Appendix 7 continued

Accno	Origin	Variety	Symptoms
31	Nyeri	Kiganda tall	Necrotic short streaks
32	Nyeri	Uganda Green	Short chlorotic, long necrotic streaks
33	Nyeri	Uganda Green	Long and short necrosis
34	Nyeri	Uganda Green	Wide chlorotic streaks
35	Nyeri	Kampala	Clean
36	Nyeri	Mutahato	Long chlorotic and necrotic streaks
37	Thika	Lacatan	Brown sigatoka
38	Thika	Lacatan	Short chlorotic
39	Thika	Lacatan	Short necrotic and chlorosis
40	Thika	Dwarf Cavendish	Necrosis
41	Thika	Valery	Short chlorotic and necrotic streaks
42	Thika	Dwarf Cavendish	Long chlorotic streaks
43	Thika	Lacatan	Necrosis
44	Thika	Dwarf Cavendish	Short chlorotic and necrotic streaks
45	Thika	Lacatan	Short necrotic streaks
46	Thika	Golden Beauty	Severe
47	Thika	Chinese cavendish	Moderate streaks
48	Thika	Valary	Moderate streaks
49	Thika	Williams	Moderate streaks
51	Kisii	Uganda Green	Short chlorotic streaks
52	Kisii	Uganda Green	Choking
53	Kisii	Uganda Green	Long chlorotic streaks
54	Kisii	Namkhila	Short chlorotic streaks
55	Kisii	Uganda Green	Clean
56	Kisii	Kibuzi	Short chlorotic streaks
57	Kisii	Uganda Green	Short chlorotic and necrotic streaks,
58	Kisii	Uganda Green	Short chlorotic streaks

Appendix 7 continued

Accno	Origin	Variety	Symptoms
59	Kisii	Uganda Green	Long chlorotic streaks
60	Kisii	Jamaga	Long chlorotic streaks
61	Kisii	Uganda Green	Short chlorotic streaks
62	Kisii	Uganda Green	Short necrotic streaks
63	Kisii	Uganda Green	Long chlorotic streaks
64	Kisii	Uganda Green	Long chlorotic streaks
65	Kisii	Uganda Green	Long chlorotic streaks
66	Kisii	Uganda Green	Long chlorotic and necrotic streaks
67	Kisii	Uganda Green	Necrosis
68	Kisii	Apple banana	Long chlorotic streaks
69	Kisii	Uganda Green	Long chlorotic streaks
70	Kisii	Uganda Green	Sun scorching
71	Kisii	Uganda Green	Necrosis and choking
72	Kisii	Nshule	Long continuous chlorotic streaks
73	Kisii	Apple banana	Long chlorotic,necrotic streaks
74	Kisii	Giant Cavendish	Short chlorotic streaks
75	Kisii	Namkhila	Long chlorotic and necrotic streaks
76	Kisii	Vallery	Short chlorotic streaks
77	Kisii	Muraru	Short chlorotic streaks
78	Kisii	Grand Naine	Necrosis and choking
79	Kisii	Mysore	Short and long necrotic, chlorotic streaks
80	Kisii	Ngombe	Short chlorotic streaks
81	Kisii	Dwarf Cavendish	Short chlorotic streaks
82	Kisii	Gold dessert	Short chlorotic streaks and choking
83	Kisii	Sibusi	Short chlorotic streaks and choking.
84	Kisii	Ntombe	Short chlorotic streaks and choking
85	Kisii	Namkhila	Short chlorotic streaks
86	Kisii	Jamaga	Short chlorotic streaks
87	Kisii	Lacatan	Short chlorotic streaks

Appendix 7 continued

Accno	Origin	Variety	Symptoms
88	Kisii	Uganda green	Short chlorotic streaks
89	Kisii	Giant cavendish	Short chlorotic streaks
90	Kisii	Chinese Cavendish	Short chlorotic streaks
91	Kisii	Grand nain	Short chlorotic streaks
92	Kisii	Jamaga	Short chlorotic streaks
93	Kisii	Namkhila	Short chlorotic streaks
94	Kisii	Ntombe	Short chlorotic streaks
95	Kisii	Sibusi	Short chlorotic streaks
96	Kisii	Gold dessert	Short chlorotic/necrotic streaks
97	Kisii	Vallery	Short chlorotic streaks
98	Kisii	Dwarf Cavendish	Leaf chlorosis
99	Kisii	Ngombe	Short necrotic streaks and choking,
100	Kisii	Grand nain	Short chlorotic streaks
101	Kisii	Uganda green	Wide necrotic streaks
102	Kisii	Vallery	Short necrotic streaks and choking
103	Kisii	Muraru	Leaf folding
104	Kisii	Lacatan	Short chlorotic streaks
105	Kisii	Giant Cavendish	Short chlorotic streaks
106	Kisii	Chinese Cavendish	Short chlorotic streaks
107	Kisii	Uganda green	Short chlorotic streaks
108	Kisii	Dwarf Cavendish	Short chlorotic streaks
109	Kisii	Sibusi	Short chlorotic streaks
110	Kisii	Ng'ombe	Short chlorotic streaks
111	Kisii	Dwarf Cavendish	Short chlorotic streaks
112	Kisii	Gold dessert	Short chlorotic streaks
113	Kisii	Vallery	Short chlorotic streaks
114	Kisii	Grand Naine	Short chlorotic streaks
115	Kisii	Ntobe	Long necrotic, short chlorotic streaks
116	Kisii	Namkhila	Short chlorotic streaks

Appendix 7 continued

Accno	Origin	Variety	Symptoms
117	Kisii	Lacatan	Short chlorotic/necrotic streaks
118	Kisii	Muraru	Long necrotic streaks
119	Kisii	Sibusi	Long chlorotic/necrotic streaks
120	Kisii	Jamaga	Short chlorotic/necrotic streaks
121	Kisii	Uganda green	Choking
122	Kisii	Giant Cavendish	Short chlorotic streaks
123	Kisii	Chinese Cavendish	Short chlorotic streaks
124	Kisii	Uganda Green	Short chlorotic streaks
125	Kisii	Grand Naine	Short chlorotic streaks
126	Kisii	Sialamule	Moderate streaks
127	Kisii	Nshule	Severe streaks
128	Kisii	Nshule	Severe streaks
129	Kisii	Nshule	Severe streaks
130	Kisii	Ngombe	Severe streaks
131	Kisii	Ngombe	Severe streaks
132	Kisii	Ngombe	Moderate streaks
133	Kisii	Kisukari	Moderate streaks
134	Kisii	Apple banana	Moderate streaks
135	Kisii	Bogoya	Moderate streaks
136	Kisii	Kampala	Moderate streaks
137	Kisii	Uganda Green	Moderate streaks
138	Kisii	Spambia	Moderate streaks
139	Kisii	Mbuki	Moderate streaks
140	Kisii	Lisumba	Moderate streaks
141	Nakuru	Lacatan	Short chlorotic streaks
142	Nakuru	Sweet banana	Short and long chlorotic streaks
143	Nakuru	Sweet banana	Long chlorotic and necrotic streaks
144	Nakuru	Sweet banana	Short broad chlorotic streaks
145	Nakuru	Gold finger	Moderate streaks

Appendix 7 continued

Accno	Origin	Variety	Symptoms
146	Nakuru	Lisulya	Moderate streaks
147	Nakuru	Lacatan	Mild streaks
148	Nakuru	Lacatan	Moderate streaks
149	Nakuru	Namukila	Severe streaks
150	Nakuru	Namukila	Severe streaks
151	Nakuru	Namukila	Severe streaks
152	Nakuru	Gold finger	Moderate streaks
153	Nakuru	Gold finger	Moderate streaks
154	Nakuru	Gold finger	Moderate streaks
155	Nakuru	Williams	Severe streaks
156	Nakuru	Williams	Severe streaks
157	Nakuru	Williams	Moderate streaks
158	Nakuru	Valary	Moderate streaks
159	Nakuru	Valary	Mild streaks
160	Nakuru	Valary	Mild streaks
161	Nakuru	Valary	Mild streaks
162	Nakuru	Uganda green	Moderate streaks
163	Nakuru	Uganda green	Mild streaks
164	Nakuru	Uganda green	Mild streaks
165	Nakuru	Uganda green	Mild streaks
166	Nakuru	Apple banana	Moderate streaks
167	Nakuru	Apple banana	Moderate streaks
168	Nakuru	Lacatan	Mild streaks
169	Nakuru	Lacatan	Mild streaks
170	Nakuru	Grand Naine	Mild streaks
171	Nakuru	Grand Naine	Mild streaks
172	Nakuru	Grand Naine	Mild streaks
173	Nakuru	Gold Dessert	Mild streaks
174	Nakuru	Gold Dessert	Moderate streaks

Appendix 7 continued

Accno	Origin	Variety	Symptoms
176	Nakuru	Chinese Cavendish	Moderate streaks
177	Embu	Sweet banana	Long chlorotic and necrotic streaks
178	Embu	Chinese Cavendish	Necrosis
179	Embu	Grand Naine	Necrosis
180	Embu	Uganda Green	Long chlorotic streaks
181	Embu	Chinese dwarf	Alternaria
182	Embu	Chinese dwarf	Clean
183	Embu	Grand naine	Short chlorotic streaks, choking
184	Embu	Grand naine	Alternaria and choking
185	Embu	Paz	Broad long continous chlorotic and necrotic streaks
186	Embu	Grand naine	Short chlorotic streaks
187	Mt. Elgon	Likhaho	Severe streaks
188	Mt. Elgon	Likhaho	Severe streaks
189	Mt. Elgon	Likhaho	Severe streaks
190	Mt. Elgon	Likhaho	Severe streaks
191	Mt. Elgon	Likhaho	Severe streaks
192	Mt. Elgon	Lisulya	Severe streaks
193	Mt. Elgon	Lisulya	Severe streaks
194	Mt. Elgon	Lisulya	Moderate streaks
195	Mt. Elgon	Lisulya	Moderate streaks
196	Mt. Elgon	Lisulya	Moderate streaks
197	Mt. Elgon	Khabusi	Severe streaks
198	Mt. Elgon	Khabusi	Severe streaks
199	Mt. Elgon	Khabusi	Severe streaks
200	Mt. Elgon	Bururu	Severe streaks
201	Mt. Elgon	Bururu	Severe streaks
202	Mt. Elgon	Bururu	Severe streaks
203	Mt. Elgon	Bururu	Severe streaks
204	Mt. Elgon	Crown	Severe streaks

Appendix 7 continued

Acc. No	Origin	Variety	Symptoms
205	Mt. Elgon	Murure	Severe streaks
206	Mt. Elgon	Chilume	Moderate streaks
207	Mt. Elgon	Chilume	Mild streaks
208	Murang'a	Muraru	Necrotic streaks
209	Murang'a	Apple banana	Short chlorotic streaks
210	Murang'a	Ug	Long chlorotic streak
211	Murang'a	Lacatan	Choking
212	Murang'a	Lacatan	Necrosis
213	Murang'a	Lacatan	Necrosis
214	Murang'a	Gold dessert 1	Short chlorotic and necrotic streaks
215	Murang'a	Gold dessert 2	Choking

Appendix 8. BSV infected banana samples from the nine banana growing regions of Kenya of the country. The table show the cultivar and number tested positive

Sample No	Site	Cultivar	Total no. tested	No. tested positive
1	Kisii	Ng'ombe	4	4
2		Apple	4	4
3		Bogoya	4	4
4		Goldfinger	7	7
5		Sukarindizi	3	3
6		Nshule	9	9
7		Mysore	3	3
8		Lacatan	4	4
9		Dcavendish	3	3
10		Namkhila	3	2
11		Uganda Green	8	8
12		Lisulya	4	4
13		Lisumba	2	2
14		Sialamule	3	3
15		Gcavendish	2	2
16		Kasukari	3	2
17		Maffukha	2	2
18		Libururu	3	2
19		CCavendish	2	2
20		Turbo	2	2
21	Bungoma	Apple Giant	3	2
22		Valary	4	4
23		Nasirembe	4	4
24		DCavendish	3	2
25		Uganda Green	4	4
26		Gbeauty	2	2
27		Murure	2	0
28		Lisulya	2	0
29		Libururu	2	0

Appendix 8 continued

Sample No	Site	Cultivar	Total no. tested	No. tested positive
30		Suriat	4	2
31		Gnaine	4	3
32		Zirume	2	1
33	Mt. Elgon	Chilume	2	0
34		Lisulya	2	2
35		Murure	2	2
36		Libururu	3	1
37		Likhaho	2	2
38	Kakamega	Sialamule	2	2
39		Apple	2	2
40	Nakuru	Goldfinger	2	2
41		Gcavendish	2	2
42		GNaine	2	2
43	Thika	GCavendish	2	2
44		Gfinger	3	3
45		CCavendish	4	4
46		Valary	4	4
47		Gbeauty	4	4
48		Williams	2	2
49		Gnaine	2	2
50	Embu	PAZ	2	2
51		GNaine	2	2
52	Nyeri	Muraru	4	4
53		Gachuru	4	4
54	Murang'a	Muraru	2	1
55		Apple banana	2	1
56		Gold Dessert	1	0
57		Lacatan	1	0
Total			171	148

Appendix 9. Caulimoviridae sequences used in the study to compare with the sequences of the Kenyan BSV isolates

Virus	Acronym	Genbank accession number	Reference
Rice Tungro Bacilliform Virus	(RTBV)	D10774	Hay <i>et al</i> (1991)
Banana Streak Obino L'ewai Virus	(BSOEV)	AJ002234	Harper G, Hull R (1998)
Banana Streak Mysore Virus	(BSMysV)	AF214005	
Banana Streak Imove Virus	(BSImV)		A.D.W. Geering, unpublished data
Banana streak Goldfinger virus	(BSGfV)	AF215814	Geering <i>et al</i> (2000)
Cacao Swollen Shoot Virus	(CSSV)	L14546	Hagen <i>et al</i> (1993)
Commelina Yellow Mottle Virus	(ComYMV)	X52938	Medberry <i>et al</i> (1990)
Dioscorea Bacilliform Virus	(DBV)	X94576	Briddon <i>et al</i> (1999)
Sugarcane Bacilliform Morocco Virus	(SCBMV)	M89923	Harper <i>et al</i> (2005)
Banana Streak UgandaA Virus	(BSUgAV)		Harper <i>et al</i> (2005)
Banana Streak UgandaC Virus	BSUgCV		Harper <i>et al</i> (2005)
Banana Streak UgandaDVirus	BSUgDV		Harper <i>et al</i> (2005)
Banana Streak UgandaEVirus	BSUgEV		Harper <i>et al</i> (2005)
Banana Streak UgandaF Virus	BSUgFV		Harper <i>et al</i> (2005)
Banana Streak UgandaG Virus	BSUgGV		Harper <i>et al</i> (2005)
Banana Streak UgandaI Virus	(BSUgIV)		Harper <i>et al</i> (2005)
Banana Streak UgandaJ Virus	(BSUgJV)		Harper <i>et al</i> (2005)
Banana Streak UgandaK Virus	BSUgKV)		Harper <i>et al</i> (2005)
Banana Streak UgandaL Virus	(BSUgL V)		Harper <i>et al</i> (2005)
Banana Streak UgandaM Virus	(BSUgMV		Harper <i>et al</i> (2005)

Appendix 10. BSV isolates from Kenya sequences

1. Khabusi2 isolate - 905903

gaagcatctt aagaagttcc tggaaatttg caaagcaa	atg gggcttggtc tcagcccaac	60
aaaaatgaag attggcacac cggaaattga cttccttgga	gcaaccattg gtaactccaa	120
aatcaaatta cagccccaca tcatcaagaa ggtcattgaa	acaagggatg aggagctgaa	180
agaagttaaa ggattaagga aatgggttggg agtcctta	at tatgccaggg catacatccc	240
taacctcggg aaaacattgg gaccgctcta ttcaaaaact	tctggcaacg gagagagaag	300
aatgaatagt caagattgga agattgtaca aaacatcaaa	actcag	346

2. BSUGAV isolate

ttgaagaacg ccccagcaat ctttcagagg aaaatggatc	attgtttcca	1
tgaagacttc atagcagtat acattgatga tatcttggtc	ttctcagaaa	51
ctgttcagca gcacaggcaa catctgaagg tgttctttga	tatctgtgaa	101
aaaaatgggt tggattaag cccaacaaag atgaagatag	ggacaagaca	151
agtggatttt cttggagcca caattggaaa ttccaaaatc	aagcttcaac	201
ctcatataat caagaagata gctgatatta aggatgagga	actaaaggaa	251
gttagaggat taaggaaatg gcttggaaatc ctcaattatg	ccagaaatta	301
catccetaat cttggaaaaa ccttaggacc cctatatcca	aagacaagtc	351
ctaattggga aagaaggatg aatggccagg actgggctat	agtcaggaag	401
attaaggaac aagtccttaa cttaccagac atggaactcc	ctccaaagga	451
agcagtcatg ataatagaaa cggatgggtg catggaa		501

3. Lisulya isolate - 905901

atgcctttcg gtttgaagaa tgccccagca atctttcaga	ggaaaatgga ccaactgttc	60
catgaagact tcatagcagt atatattgat gatattcttg	tcttctcaga aaccgtccag	120
cagcacagac aacatctgaa ggtattcttt gatatttctg	aaaaaaaaatg gttggtatta	180
agtccaacaa agatgaagat aggaacaaga caaatagatt	ttcttgagc tacaattgga	240
gattccagaa tcaagcttca acctcatata atcaaaaaaa	tagctgatat taaggatgag	300
gaactaaagg aagtcagagg attgaggaaa tggcttgga	tccttaatta tgccagaaat	360
tatatcccta atcttgga aaccttagga cccctgtatt	caaagacgag tctaatggg	420
gaaagaagga tgaatagcca ggaactgggt atagtcagga	agattaagga gcaagtcctt	480
aacttaccag acatggaact cctccaaaag gaagcagtc	tgataattga aacagatggt	540
tgcattggaag gatggggtgg c		561

4. Nshule isolate - 905904

atggatcact gttttcatga agacttcata gcagtatata	ttgatgatat cttggtcttc	60
tcagaaactg tccagcagca cagacaacat ctgaagggtg	tctttgatat ttgtgagaaa	120
aatgggttgg tattaagccc acaaaaaatg aagataggga	caagacaagt ggattttctc	180
ggagccacaa ttggaaattc caaaatcaag cttcagctc	atataatcaa gaagatagct	240
gatattaagg atgaggaact aaaggaagtc agaggattga	ggaaatgggt tggaatcctc	300
aattatgcca gaaattatat ccctaactctg ggaaaaac	ct taggacctt atattcaaaa	360

acaagtccta atgggggagag aaggatgaat agccaggatt ggactatagt caagaagatc 420
aaggaacaag tccttaacct accagacatg gaactccctc caaaggaagc aatcatgata 480
attgaaacag atg 493

5. BSimV isolate - 905902

gaagcatctt aagaagttcc tggaaatttg caaagcaaatt gggcttggtc tcagcccaac 60
aaaaatgaag attggcacac cggaaattga ctctcttgga gcaaccattg gtaactccaa 120
aatcaaatta cagccccaca tcatcaagaa ggtcattgaa acaagggatg aggagctgaa 180
agaagttaaa ggattaagga aatgggttgg agtccttaat tatgccaggg catacatccc 240
taacctcggg aaaacattgg gaccgtctta ttcaaaaact tctggcaacg gagagagaag 300
aatgaatagt caagattgga agattgtaca aaacatcaaa actcag 346

6. BSOEV - 905905

atggacaatt gcttttagagg gacagaggat tttattgcgg tatacattga tgacatactg 60
gtattctctg aaacaatata ccaacataaa gagcatttga agaaatttat gataatctgc 120
gagaaaagtg gtttagtctt aagtccgaca aaaatgaaaa ttggaacaag acagattgac 180
ttcttaggtg caactattgg aaactcaaaa attaagttgc agcctcatat tattaataaag 240
atcatcgaag tgaaagatga agaactgaag gaagtaaaaag gattaagaaa atggctggga 300
atccttaatt atgctaggag ctacattccg aaactgggaa aaatccttgg accactgtat 360
gccccaacga gtcctaattg agaaagaaga atgaatactc aggactggaa aattgtcaaa 420
gaggtaagg aagttgtagc taatctgcca gaacttgagt tccccccga aaatgctatc 480
atgataattg aaacggatgg ct 502

7. BSGfV isolate - 9050900

atggacgact gcttccgagg tacagaaaaa tttatagctg tttacataga tgatattcta 60
atcttttcag atagcaggga agcccatcga acccatctca gacaattcat caccatattg 120
gaagaaaatg ggctgttact aagcccaacg aagatgaaga taggagtcca acaagtggat 180
ttcttgggtg caaccattgg cgattctaaa gtaaggcttc agcctcacat agtcaaaaag 240
gtgctagaaa caaaggaaga aagcctgtct gaaacaaaagg cottaagaag atggttgggc 300
atactcaatt atgccagagc atatattcct gatcttggaa agatcctagg tcctttatac 360
tcaaaaactt caggaaaagg ggagcgaaaa ctcaatcacc aagacatgaa gataattcac 420
cagatcaagg agaag 435

Appendix 11. Multiple alignment of protein sequences of three new Kenyan virus isolates and BSUGA to H isolates

BSVA_1	1	----LKNAPA	IFQRKMDHCF	H--EDFIAVY	IDDILVFSET	VQQHRQHLKV
Khabusi2	1	-----	-----	-----	-----	-----KHLKK
Lisulya	1	****LKNAPA	IFQRKMDHCF	H--EDFIAVY	IDDILVFSET	VQQHRQHLKV
Nshule	1	-----	-----MDHCF	H--EDFIAVY	IDDILVFSET	VQQHRQHLKV
BSVB_1	1	----LKNAPA	VFQRKMDECF	KGTGEFIAIY	IDDILVFSKN	EKDHAHRLAR
BSVC_1	1	----LKNAPA	IFQRKMDNCF	KDTEEYIAVY	IDDILVFFEN	ESDHTRHQAQ
BSVD_1	1	----LKNAPA	IFQRKMDNCF	RGTEQFIAVY	IDDIHVFSEN	EKEHGQHLTR
BSVE_1	1	----LKNAPA	VFQRKMDNCF	KGTEQLIAFY	IDDILVFSEN	EREHEQHLTK
BSVF_1	1	----LKNAPA	IFQRKMDNCF	KGTEQFIAVY	IDDILISLEN	ERDHKQHLAK
BSVG_1	1	----LKNAPA	IFQRKMDDCF	RGMEQFIAVY	IDDILVFSEN	MRDHAKHLKR
BSVH_1	1	----LKNAPA	IFQRKMDACF	KGTEQFIAVY	IDDILVFSEN	TKDHAKHLHK
BSVA_1	45	FFDICEKNGL	VLSP TKMKIG	TRQVD FLGAT	IGNSKIKLQP	HIIKKIADIK
Khabusi2_2	6	FLEICKANGL	VLSP TKMKIG	TPEID FLGAT	IGNSKIKLQP	HIIKKVIETR
Lisulya_1	49	FFDICEKNGL	VLSP TKMKIG	TRQID FLGAT	IGDSRIKLQP	HIIKKIADIK
Nshule_1	34	FFDICEKNGL	VLSP TKMKIG	TRQVD FLGAT	IGNSKIKLQP	HIIKKIADIK
BSVB_1	47	MLEICQKHGL	VLSP SKMKVA	IKEIEFLGAI	LGNS*-ELQP	HIIKRITEYQ
BSVC_1	47	MLKICQENSL	VLSP SKMKIA	VKEIKFLGAI	LGNSKIKLQP	HIIKKITEYQ
BSVD_1	47	MLEICQENGL	ILSP TKMKIA	VREIEFLGAV	LGNSKIKLQP	HIIKKIAEYQ
BSVE_1	47	MLKICQENGL	VLSP TKMKIA	VKEIEFLGAV	LGNSKIKLQP	HIIKKITDYR
BSVF_1	47	MLEICQENGL	VLSP TKMKIA	VKEIEFLGAV	LGNSKIKL*P	HIIKRITEYQ
BSVG_1	47	MIEICQENGL	VLSP TKMKIA	VQEIEFLGAV	LGNSRIKLQP	HIIKRIMEYN
BSVH_1	47	MLEICQKNGL	VLSP TKMKIA	VKGIEFLGAV	LGNSRIKLQP	HIVKKFTEFK
BSVA_1	95	DEELKEVRGL	RKWL GILNYA	RNYIPNLGKT	LGPLYSKTSP	NGERRMNGQD
Khabusi2_2	56	DEELKEVKGL	RKWL GVLNYA	RAYIPNLGKT	LGPLYSKTSG	NGERRMNSQD
Lisulya_1	99	DEELKEVRGL	RKWL GILNYA	RNYIPNLGKT	LGPLYSKTSP	NGERRMNSQD
Nshule_1	84	DEELKEVRGL	RKWL GILNYA	RNYIPNLGKT	LGPLYSKTSP	NGERRMNSQD
BSVB_1	96	EENLTTKKGL	RSWL GILNYA	RNYIPNLGKL	LGPLYAKTSP	TGEKRFNEQD
BSVC_1	97	EEDLTTKKGL	RS*LGILNYA	RNYIPNLGRL	LGPLYAKTSP	IRERRLNEQD
BSVD_1	97	EKDLMTKKGL	RSWL GILNYA	RSYIPNLGKL	LGPLYSKTSP	TGEKRMNEQD
BSVE_1	97	EEDLTTKKGL	RSWL GILNYA	RSYIPNLGRL	LGPLYSKTSP	IGEKRMNNQD
BSVF_1	97	EEDLTTKKGL	RSWL GILNYA	RSYIPNLGRL	LGPLYSKTSP	TSEKRLNEQD
BSVG_1	97	EEDLTTKRSM	RSWL GILNYA	RSYIPNLGKL	LGPLYSKTSP	TREKRMNEQD
BSVH_1	97	EEDLTTKRGL	RSWL GILNYA	RNYIPNLGKL	LGPLYSKTSP	TGEKRLNEQD
BSVA_1	145	WAIVRKIKEQ	VLNLPDMELP	PKEAVMIET	DGCME----	
Khabusi2_2	106	WKIVQNIKTQ	-----	-----	-----	
Lisulya_1	149	WAIVRKIKEQ	VLNLPDMELP	PKEAVMIET	DGCME****	
Nshule_1	134	WTIVKKIKEQ	VLNLPDMELP	PKEAIMIET	D*-----	
BSVB_1	146	WELIRKIKSL	VKNLPDLEVP	PEECFIILET	DGCME----	
BSVC_1	147	WKLIRNIKNL	VKNLLDLEVP	PEGSFIILET	DGCME----	
BSVD_1	147	WKLVRRIKSL	VQNLPDLEVP	PEECFIILET	DGCIE----	
BSVE_1	147	WELIKKIKSL	VQNLPDLEVP	PEDCFIILET	DGCME----	
BSVF_1	147	*KLIRKIKSL	VQNLPDLEVP	PEDCFIILET	DGCME----	
BSVG_1	147	WKLIREIKGI	VNKLPDLEVP	PQECFIILEV	DGCME----	
BSVH_1	147	WKLIKEVKSK	VQQLPDLEVP	PPNCYIILEV	DGCME----	

Appendix 12. Publications

1. **Laura Karanja**, Anne Wangai, Glyn Harper, Ram Sumer Pathak. (2008a). Molecular identification of Banana Streak Virus isolates in Kenya” *Journal of Phytopathology* 156, 678–686. Blackwell Verlag, Berlin
2. **L. Karanja**, A. Wangai, G. Harper, R. S. Pathak. (2008b). Evaluation of expression banana streak virus in field and greenhouse grown tissue culture bananas in Kenya. *Proceedings of Mini-KARI Conference* 2007
3. **L Karanja**, A. Wangai, G Harper, R. S. Pathak. (2008). Sequences for new BSV isolates Kenya. *www.ncbi.nlm.nih.gov/entrez*
4. **L. Karanja**, A. Wangai, G. Harper, J. Stanley, R.S. Pathak. (2007). Epidemiological studies on the variability of banana streak virus in Kenya. Book of Abstract in Biotechnology, Breeding & Seed Systems for African Crops. The Rockefeller Foundation & Instituto de Investigação Agrária de Moçambique 26-29 March, 2007; Joaquim Chissano International Conference Centre, Maputo
5. **Laura Karanja**, Anne Wangai, Glyn Harper, John Stanley & Ram Pathak. (2006). Epidemiological Studies of Banana Streak Virus Isolates: Studies on the Variability of Banana Streak Virus in Kenya. ACORBAT XVII Reuniao Internacional da Associacao Para a Cooperacao nas Perquisas sobre Banana no Caribe e na America Tropical 15 a 20 de outubro de 2006 – Joinville – Santa Catarina- Brasil, pp 764-769
6. **Karanja, L.** Wangai, A.; Kilonzo, A.W.; Nguthi, F.N.; Ndungu, J. (2003). Distribution and characterisation of banana streak virus isolates in Kenya. Plant and Animal Genomes Conference, San Diego (USA). 2003/01/11-15
7. Wangai, A. **Karanja, L.**; Ndung'u, J. Kimani, E.; Kilonzo, S. 2003 In : Advances in plant virology : A three day International conference at CIRAD, Montpellier, France on 29 September - 1 October 2003 - Cirad-Inra, 1 p. Association of Applied Biologists, Wellesbourne, A three day International conference at CIRAD, Montpellier, France, Montpellier, 2003/09/29-10/01
8. **L Karanja**, A Wangai, G Harper. (2007) Evaluation of expression of Banana streak viruses in field and greenhouse-grown banana in Kenya. ISHS/ProMusa symposium. Recent advances in banana crop protection for sustainable production and improved livelihoods Greenway Woods Resort, White River, South Africa September 10-14, 2007. Abstract