DISTRIBUTION, MOLECULAR DIVERSITY AND BIOLOGICAL CHARACTERIZATION OF BEAN COMMON MOSAIC NECROSIS VIRUS IN WESTERN KENYA

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A Thesis Submitted in Fulfillment for the Requirements of the Award of the Degree of Doctor of Philosophy in Crop Protection of Masinde Muliro University of Science and Technology

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DECLARATION

This thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for a degree or any other award.

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DEDICATION

To my brother Wilfred Wafula Mangeni.

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ABSTRACT

Common bean (*Phaseolus vulgaris* L) is the main high protein legume crop in the cropping system of western Kenya. The crop is mainly grown by small-scale farmers for food and cash. Despite its importance, common bean yields are low (<1.0 t/ha) and declining. Bean production is constrained by plant viruses among other factors. Of the viruses infecting common bean, *Bean Common Mosaic Necrosis Virus* (BCMNV) is a widespread virus that causes Bean Common Mosaic Disease (BCMD) either singly or in mixed infection with *Bean Common Mosaic Virus* (BCMV). In Kenya, limited common bean varieties with resistance to BCMNV strains have been reported. In addition, there is inadequate documentation on the strains of the virus infecting common bean. Moreover, the extent of other hosts and distribution of the virus in main growing areas is still not known. This information is crucial in devising control measures. This study therefore, sought to determine the distribution and characterization of BCMNV isolates from western Kenya. The specific objectives were to determine incidence and severity of BCMD in western Kenya, to determine molecular characteristics of BCMNV and to screen local germplasm for resistance to BCMNV. In October 2016 and May 2017, two diagnostic surveys for BCMD were conducted in 7 counties of Western Kenya namely Bungoma, Busia, Homa bay, Nandi, Vihiga, Kakamega and Siaya. In total 270 bean farms were visited, 150 in the long rain and 120 in the short rain seasons, respectively. Leafy samples showing virus-like symptoms were collected and analysed by Enzyme linked immunosorbent Assay (ELISA) and next generation sequencing (NGS). Extraction of total RNA from ELISA positive samples was done using RNeasy Plant Mini Kit and NGS carried out following Illumina protocol to determine diversity of the virus. NGS data was trimmed and the sequence reads assembled into contigs, which were analyzed against virus sequence database. Phylogenetic analyses and comparisons were performed using MEGA7 program. Sixteen popularly grown bean cultivars together with cowpea, soybean and groundnut were planted in a greenhouse in a randomized complete block design with three replicates. The plants were inoculated with BCMNV isolate at 3-leaf stage. Data was taken weekly for 3 weeks on type of symptoms expressed and number of plants infected. ANOVA was used to compare disease incidence and severity means and least significant difference (L.S.D.) values were used to separate the significant different means at $P \leq 0.05$. Symptoms of mosaic, downward curling, vein necrosis, local lesions, stunting or a combination of these were observed during both surveys. Disease incidence among the counties varied significantly ($p=0.05$). Mean virus incidence was higher (41.8%) in the short rain season compared to long rain season (35.6%). Kakamega county had the highest mean virus incidence (47.6%) while Siaya had the lowest (31.6%). The mean BCMD severity was highest (2.3) in Kakamega county and lowest (0.5) in Siaya. There was a strong positive correlation between viral disease incidence and severity ($r=0.843$; $p<0.001$). Of the 240 symptomatic leaf samples collected, 59 were ELISA positive. NGS technology revealed full-length sequence of BCMNV from an isolate BG 12 from Bungoma County with a genome of 9584 nt in length. Phylogenetic analysis of full-length sequences available through the Genbank clustered the isolate with the Tanzanian isolate strain TN-1 and two USA isolates, TN1a and NL-3K. On variety resistance tests to BCMNV isolate BG 12, 10 bean cultivars were susceptible, 4 tolerant (Imbeko, KK/RIL5/Red 13, Okwoto, RIL05/CAL 194) and 2 resistant (KK RIL05 and KK 072). BCMNV is widely distributed across counties probably because of use of uncertified seeds by farmers and inoculum pressure from seed and aphid vector. However, for integrated disease control strategies, there is need to breed for multiple-virus resistance to counter the problem of mixed virus infection in beans and identify vectors. For improved yields of common bean, farmers should be advised to plant certified seed that are virus free for all legumes in the cropping system.

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LIST OF ABBREVIATIONS ACRONYMS

BCMD	Bean common mosaic disease
BCMNV	Bean common mosaic necrosis virus
BeCA	Biosciences Eastern and Central Africa
DSMZ	German Collection of microorganisms and Cell cultures
dNTPs	Deoxyribonucleoside Triphosphate
MEGA	Molecular Evolutionary Genetics Analysis
MOPS	3-morpholinopropane-1-sulfonic acid
ICTV	International Committee on Taxonomy of Viruses
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline -Tween
RAM	Rabbit Anti Mouse
RAM-AP	Rabbit Anti Mouse- Alkaline Phosphatase
ssRNA	Single stranded Ribonucleic acid

CHAPTER ONE

INTRODUCTION

1.1 Common bean plant

The common bean (*Phaseolus vulgaris* L) is an important legume crop for food and cash in Kenya. Common bean is a true autogamous diploid species, with 22 chromosomes and a haploid genome size that is estimated to be between 587 Mbp and 637 Mbp (Bernnett & Leitch, 2010). Varieties of common bean mostly mature within 65 -110 days (Buruchara, 2007). Pod formation occurs after self-pollination; however, cross-pollination is possible by insect coated with pollen grains. Seeds have two cotyledons and vary greatly in size and colour from the small black wild type to the large white, brown, red, black or mottled seeds of cultivars, which are 7-16 mm long (Buruchara, 2007).

1.1.2 Origin of Common bean

Common bean originated from Mexico but later diversified and dispersed throughout the whole American continent and the rest of the world. Although common bean was domesticated about 7000 years predominantly in two diversity centers, Mesoamerican (Mexico and Central America), and the Andean region, new genetic pools are rapidly emerging in Europe, Asia and Africa (Broughton *et. al,* 2003). In Africa, the Mesoamerican and Andean gene pools are approximately equal in frequency, even if there are striking differences between different countries; they are due to different

farmer selection preferences and the input of germplasm from national programs (Belluci *et. al.,* 2014).

1.1.3 Production of common bean

Total world bean production is 31405912 tons (FAOSTAT, 2017). Brazil is at the top of bean producers of the world together with China, India and Myanmar accounting for a world production of more than 50 % (WTO, 2018). Africa produces 6851757 tons which is about 17 % of the world total, with 4778206 tons (70 %) of production occurring in Eastern Africa. Beans form the 8th crop in terms of acreage in Sub-Saharan Africa. Top producing countries in Africa are Tanzania, Uganda and Rwanda, followed by Cameroon, Kenya and Ethiopia. Production in Kenya is 716 kg ha^{-1} (FAOSTAT, 2017). The main varieties cultivated in Kenya include, Mottled Purple and or Red (locally referred as as Rosecoco, Nyayo, Kati, Wairimu), Speckled grey and or purple (known locally as Mwezimoja), Pinto sugar beans (Mwitemani), Yellow (a greenish bean), Saitoti (medium, red mottled), Noe (Butter beans) and Kachuma (Round deep red kidney) (Mangeni *et al.,* 2014). Yield potential is 1400-2000 kg/ha or 7-9 bags/acre can be obtained when farmers use good quality seeds of the appropriate variety that are viable (Katungi *et al.*, 2010).

1.2 Constraints to bean production

Kenya's average yield of 716 kg ha^{-1} is low compared to Tanzania and Rwanda with average yield of 991 kg ha^{-1} and 830 kg ha^{-1} respectively (FAOSTAT, 2017). Yield potential is not attained due to adverse conditions such as poor agronomic practices, low input, intercropping with competitive crops, low soil fertility, periodic water stress, weed competition, lack of disease resistant bean varieties, damage caused by insect pests such as aphids (*Aphis spp*), bean stem maggot (*Ophiomyia spp*), borers (*Dectes spp*,) (Mwaniki, 2002; Wagara, 2005).

The major common bean diseases include angular leaf spot caused by *Phaeoisariopsis griseola*, Bacterial blight caused by *Xanthomonas campestris pv. Translucens*, Root rots caused by *Rhizoctonia spp, Fusarium spp and Pythium spp*, Anthracnose caused by *Colletotricum lindemuthianum*, rust caused by *Uromyces appendiculatus*, Bean common mosaic disease (BCMD) among others (Wortmann *et al*., 1998).

Diseases caused by viruses are among factors lowering common bean production in Kenya. About 20 viruses are known to infect beans in Africa. These include; *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean yellow mosaic virus* (BYMV), *Bean golden mosaic virus* (BGMV), *Cucumber mosaic virus* (CMV), *Southern bean mosaic virus* (SBMV), *Tobacco streak virus* (TSV), *Tomato aspermy virus*, *Clover yellow vein virus* (CYVV), *Tobacco ring spot virus (TSRV), Tomato ringspot viruses* (TmRSV), *Alfalfa mosaic virus* (AMV), *Soybean mosaic virus* (SMV), *Watermelon mosaic virus 2* (WMV-2), *Bean golden mosaic virus* (BGMV), *Cowpea chlorotic mottle virus (CCMV), Bean yellow stipple virus* (BYSV),

Cowpea aphid borne mosaic virus (CABMV) among others (Gad & Thottappilly, 2003). The viruses may occur singly in beans or as mixed infection with two or more. Bean common mosaic disease caused by BCMV and or its related necrotic species BCMNV is the most widespread virus disease in Kenya (Odendo *et al*., 2004).

In wild and cultivated legumes including common beans (*Phaseolus vulgaris* L.), BCMV and BCMNV are known to be the most common and most destructive viruses (Morales, 2006; Mangeni *et al.,* 2014). The viruses, BCMV and BCMNV are seed and aphid transmitted accounting for yield loss as high as 100 % in beans (Damayanti *et al.,* 2008; Saqib *et al.,* 2010; Singh & Schwartz, 2010; Verma & Gupta, 2010; Li *et al.,* 2014; Mutuku *et al.,* 2018; Mwaipopo *et al.,* 2018) coupled by unpredictable weather. Variations in weather conditions such as temperature, humidity, wind patterns, rainfall and daylight hours length due to general climate change have an effect on vector reproduction, subsequent development, feeding behaviour and distribution. These factors in combination influence virus replication and transmission (Tabachnik, 2010). Increase in temperature due to global warming is a critical determinant of increased virus transmission efficiency, symptom expression and severity (Caminade *et al.,* 2019).

1.3 Statement of the problem

Common bean production potential in Kenya is 1400 – 2000 kg Ha^{-1} (Katungi *et al.*, 2010). Attainment of common bean production potential is constrained by diseases such as Bean Common Mosaic Disease (BCMD)(Kayondo *et al.,* 2014). BCMD is caused by either or both BCMV and BCMNV from the genus Potyvirus. Plant infection as high as 100 % has been reported with yield losses of 6-100 % (Buruchara *et al.,* 2011). BCMNV

is both seed-borne and aphid-transmitted in a non-persistent manner (Hongying *et al*., 2002). Depending on the common bean cultivar and stage of development, seed transmission rate varies from less than 1 % to 50 % (Hong-Soo Choi *et al*., 2006). Therefore, this poses a major threat to common bean production as the crop becomes infected during its early growth stages. In Kenya, limited common bean varieties with resistance to BCMNV strains have been reported.

1.4 Justification

The current status of BCMD in Kenyan agro-ecological zones is not well documented; the most recent survey was done in 2013 (Mangeni *et al.,* 2014). BCMNV pose a problem to bean production, because crop losses continue to increase as the knowledge on the virus is inadequate. Therefore, there is need to study the current distribution of BCMNV in western Kenya. In addition, the knowledge of BCMNV strain spectrum in Kenya is lacking and it is an important pre-requisite in exploitation of disease management through host resistance, which is the only durable and economic method of managing BCMD (Bello & Miklas, 2014). Furthermore, farmers grow varieties whose resistance to BCMNV has not been documented. This information is crucial in devising control measures.

1.5 Objectives

1.5.1. Main Objective

To determine the distribution and characterize *Bean Common Mosaic Necrosis Virus* (BCMNV) isolates from western Kenya.

1.5.2. Specific Objectives

- **i.** To determine the distribution, incidence and severity of Bean Common Mosaic Disease in selected counties of western Kenya.
- **ii.** To determine the genetic diversity of *Bean Common Mosaic Necrosis Virus* in western Kenya.
- **iii.** To screen common bean varieties and other host legume germplasm for resistance to *Bean Common Mosaic Necrosis Virus.*

1.5.3 Hypotheses

$H0_1$: Bean common mosaic disease is not widespread in western Kenya.

$H0_2$: BCMNV strains in western Kenya are not different from those found elsewhere.

$H0_3$: The popularly grown legumes in western Kenya do not react differently to BCMNV infection.

CHAPTER TWO

LITERATURE REVIEW

2.1 Bean Common Mosaic Dissease distribution

Bean common mosaic disease originated in the Americas and is one of the earliest reported virus diseases of plants (Mukeshimana *et al*., 2005). BCMD was first reported in *P. vulgaris* in USA in 1917 it is now distributed worldwide (Klein *et al*., 1988; Jeyandandarajah & Brunt, 1993; Sáiz *et al*., 1995; Omunyin *et al*, 1995; Mavrič *et al*., 2002; 2003). The disease causes a big economic damage of common bean, by reducing yield (as much as 80%) and quality of harvested product (Drijfhout, 1991). BCMD has been found to occur in certain regions of the world, particularly in East Africa, Europe, North America and Asia where it has caused considerable damage in common bean (Kelly, 1997).

In Kenya, the first case of BCMD was reported by Kulkarni (1973). The distribution of BCMD and identification of pathogenicity groups occurring in Kenya by using the Drijfhout differentials by Omunyin (1995) revealed presence of the virus in 18 out of 22 locations surveyed. Incidence of 20-63 % was observed on the farms in Kakamega and Kisii, low incidences below 20 % being recorded in south Nyanza whereas disease was least observed in Machakos, Kitui and Embu in eastern areas of Kenya (Omunyin *et al*., 1995).

2.2 Symptoms caused by *Bean Common Mosaic Necrosis Virus*

In *Phaseolus* spp. BCMNV produce several distinct symptoms. The type of symptom depends on type of infection (either seed-borne or vector transmitted), cultivar type, strain of the virus and age of the plant at infection. In susceptible genotypes at typical growing temperatures (26-28˚C), leaf curling, mosaic, vein banding, malformation of pods and leaf mottling are observed (Bos, 1971) whereas at elevated temperatures (above 30˚C) systemic necrosis appears (Drijfhout, 1991).

Tolerant varieties can become systemically infected but show only a mild deformation or narrowing of the leaves (Vetten *et al*., 1992). Some genotypes show an extreme resistance (ER) against the type strain at typical growing temperatures that manifests no visible symptoms (Bos, 1971). At higher temperatures (above 30˚C) the spreading vascular necrosis appears and often death, typical of "black root" (Bos, 1971). Local necrotic lesions that extend into the veins causing systemic necrosis in the vascular system characterize black root. This symptom only occurs in cultivars possessing the dominant resistance gene *I* (Kelly, 1997; Mukeshimana *et al*., 2005). This necrosis can extend into the roots, stem and meristem and may result in plant death if the plant is infected at an early stage. BCMNV induces necrosis not only in cultivars possessing dominant *I* gene but also those having recessive resistant genes (Bello & Miklas, 2014).

Mottling and malformation of the primary leaves is an indication that the primary infection occurred through seed (Bos, 1971). Systemically infected plants may have smaller and fewer pods and infected pods may sometimes be covered with small, dark green spots and mature later compared to uninfected pods (Mckern *et al*., 1992).

2.3 Genome organization and structure of *Bean Common Mosaic Necrosis Virus*

BCMNV are flexuous rod-shaped virions, monopartite, single stranded positive-sense RNA viruses (Hull, 2014). Virions are 750 nm long, 11–13 nm in diameter and contain a genomic RNA molecule that is approximately 10 kb long that has a 3^0 -terminal polyA tail (Fang *et al.,* 1995; El-Sawy *et al.,* 2013). Virions are constructed principally from CP molecules that are helically arranged around the genomic RNA. The VPg (viral protein genome) is an additional protein covalently linked to the 5^0-terminus of the RNA and projects out at the tip of the virus particle. VPg serves a similar purpose to the cap structure found on most cellular mRNAs (Worall *et al.,* 2015). The potyviral genomic RNA has a long open reading frame (ORF) that can be translated directly by host ribosomes to produce a polyprotein that self-cleaves to produce 10 proteins. Moreover, frame-shifting allows yield of an additional protein. Self-processing of the polyprotein is a reaction catalyzed by the proteolytic activities of P1, helper component proteinase (HC-Pro), and nuclear inclusion protein-a proteinase (NIa-Pro). The polyprotein processing to release mature forms of these proteins and synthesis of other viral proteins including P3, cylindrical inclusion (CI), VPg, nuclear inclusion-b (NIb: the viral RNA-dependent RNA polymerase) is a self-processing function (Hull, 2014; Ivanov *et al.,* 2014). Two 6 kDa polypeptides, 6K1 and 6K2 are also produced during polyprotein processing of which only 6K2 functions as a membrane anchor for the viral replication complexes (Ivanov *et al.,* 2014). NIa-Pro Cleaves seven out of the nine polyprotein cleavages by cutting both in cis and trans conformations whereas P1 and HC-Pro cuts only once in *cis* (Adams *et al.,* 2004). A serine protease, P1 cuts only at the P1/HC-Pro border.

Discovery of BCMNV began in 1917 when its close member BCMV was identified by symptomatology and named variously as Bean virus 1, Bean mosaic virus and Phaseolus virus 1(Morales & Bos, 1988). Until 1943, it was presumed that BCMV strains were identical pathogenically since the originally identified BCMV isolate was lost (Drijfhout *et al.,* 1978). Differential symptoms on ten bean cultivars were used to identify distinct pathogenic groups of BCMV in 1943. The results were utilised in taxonomy classifying the strains into seven pathogenic groups (I–VII) (Drijfhout *et al.,* 1978). BCMV strains were later categorized to A and B serotypes based on differential responses of various bean cultivars to infection, coat protein (CP) serology and proteolytic digests of CPs analysis (Vetten *et al.,* 1992). Berger *et al.* (1997) later reclassified serotypes A and B as BCMNV and BCMV respectively.

BCMNV has five identified strains i.e. TN-1, NL-8, NL-3, NL-3K and NL-5 (Mink & Silbernagel, 1992). Strains of BCMV are many including very distinct viruses, such as *Azuki bean mosaic virus* (AzMV), *Blackeye cowpea mosaic virus* (BICMV), *Peanut stripe virus* (PStV) and (*Dendrobium mosaic virus* (DeMV) (Hu *et al.,* 1995). By 2015, there were 22 BCMV full length genome sequences and 9 BCMNV full length genome sequences available through NCBI (Worrall *et al.,* 2015). 3^0 –UTR sequence data and CP genes have been used to group BCMV isolates (Fang *et al.,* 1995; Sharma *et al.,* 2011). Some virus undergo interspecific recombination when in mixed potyvirus infections resulting later in new virus strains. Studies by Silbernagel *et al.* (2001) demonstrated experimentally by inoculating bean plants doubly with BCMV US-5 and BCMNV NL-8 on beans that were resistant to one but susceptible to the other virus producing recombinants. Similarly, Larsen *et al.* (2005) demonstrated NL-3K isolate of

BCMNV naturally occurring recombinant virus with enhanced pathogenicity being a derivative of the NL-3 D strain of BCMNV but had sequences from the BCMV RU-1 strain (Larsen *et al.,* 2005). Worrall *et al.* (2015) and Larsen *et al.* (2005) showed that interspecies recombination is likely to be the genesis of novel BCMV and BCMNV strains and of new potyviral species. Both studies highlight the importance of P1 and the P1-HC-Pro precursor protein in pathogenesis and the breakage of genetic resistance.

2.4 Transmission and host range of *Bean Common Mosaic Necrosis Virus*

BCMNV is transmitted by seeds up to 83 % in *Phaseolus vulgaris* L and by several aphid species (Barnett, 1986; Flores-Estévez *et al.,* 2003; Melgarejo, *et al.,* 2007). Aphid species including; Bean aphid (*Aphis fabae*), Cowpea aphid (*Aphis craccivora*), Pea aphid (*Acyrthosiphon pisum*), Green peach aphid (*Myzus persicae*) and Potato aphid (*Macrosiphum euphobiae*) are known to transmit the virus (Zitter, 1984). Seed transmission occurs irregularly and may majorly depend on age of the plant at the time of infection, virus strain and bean variety. Aphid transmission occurs non-persistently and spread the virus on short distances as compared to seed transmission that may spread viruses around the world (Omunyin *et al.*, 1995). Infected plants are produced even at low seed transmission and at most suitable time for vector transmission may result in spreading the virus in the field very fast (Morales, 1983). Systemically infected plants prior to flowering may produce infected seeds and this may account for the higher transmission rates (Udayashanka *et al*., 2012). Seed transmission is minimal or may not occur if the infection occurs after flowering (Drijfhout & Morales, 2005).

Phaseolus species form the natural hosts of BCMNV mainly restricted to *P. vulgaris* (Morales & Castano, 2008). However, the virus has been isolated naturally from other leguminous species including *Phaseolus lunatus, Phaseolus acutifolius, Arachis hypogaea, Cajanus cajan, Bauhinia purpurea, Centrosema pubescens, Chenopodium quinoa, Crotalaria juncea, Crotalaria incana, Crotalaria spectabilis, Cucumis sativus, Lupinus angustifolius, Glycine max, Lablab purpureus, Lupinus albus, Lupinus luteus, Macroptilium lathyroides, Macroptilium atropurpureum, Medicago sativa, Melilotus alba, Trifolium incarnatum, Pisum sativum, Vicia sativa, Vigna subterranea, Rhynchosia minima, Vigna unguiculata, Vigna vexillata , Sesbania herbacea, Trifolium pretense, Trifolium repens, Trifolium subterraneum, Trifolium hybridum, Vicia villosa and Vigna radiate* (Sengooba *et al.,* 1997) .

2.5 Control measures to Bean Common Mosaic Disease

2.5.1 Management of virus diseases in beans

Integrated disease management (IDM) approach is key to containing potyvirus epidemics in legume crops. The main possible interventions take into account open field situations in Kenya. Common phytosanitary intervention helps minimize the primary source of inoculum by eliminating cultural residues at the end of the crop cycle and by isolating beans from weeds, or volunteer plants that are known hosts for BCMNV (Massimo *et al.,* 2012). Roguing can also be effective since most potyvirus–host combinations that have very obvious symptoms.

The use of virus-free seeds is also recommended. To this purpose, healthy certified seeds planted may suppress the onset of epidemics (Coutts & Jones, 2005). Agronomic measures that can be employed include scheduling planting date to avoid aphid population peaks. Agronomic and phytosanitary measures are majorly preventive and can only limit, but not control virus epidemics since some measures may prove difficult to carry out due to the tropical climate conditions and climate change that hamper regional interventions. Other measures considered also include; the use of bean cultivars carrying resistance genes for potyviruses, chemical control with insecticides, and biocontrol of aphid vectors.

2.5.2 Genetic Resistance to *Bean Common Mosaic Necrosis Virus*

Breeding for genetic resistance to BCMNV is the most durable form of managing the virus. Bean cultivars possessing the dominant *I*-gene are susceptible to BCMNV-inducing black root disease (Worrall *et al.,* 2015). Available recessive resistance genes are virus strain-specific and therefore difficult to breed bean cultivars that have a broad resistance to the existing strains of BCMNV based on one of these genes alone. Marker-assisted selection is now possible using genetic markers linked to resistance genes for BCMNV (Morales & Kornegay, 1996). Therefore, molecular markers can be utilized to pyramid the recessive genes (*bc-u, bc-1,* $bc\text{-}1^2$*, bc-2,* $bc\text{-}2^2$ and *bc-3)*, with the dominant *I*-gene in order to provide broad spectrum possible resistance (Pasev *et al.,* 2014).

The use of cellular eIF4E/eIF(iso)4E and eIF4G type translation initiation factors has been explored in breeding for resistance (Truniger & Aranda, 2009). Bean genotypes possesing *bc-3* gene for BCMV resistance were found to have homozygous non-silent

mutations in a PveIF4E coding sequence at codons 53, 65, 76, and 111 and the mutations had a closer resemblance to a pattern of mutations which govern potyvirus resistance in other plants (Naderpour *et al.,* 2010). The *bc-3* locus can have three eIF4E genes associated with it (Hart & Griffiths, 2013). This is an indication of a high level of variation at the locus, that may be driven by coevolution with bean-infecting potyviruses.

The *I*-gene renders plants susceptible to a systemic necrosis in response to infection with BCMNV. The *I*-gene is yet to be cloned (Worall *et al.,* 2015). The *I* locus however, maps to a cluster of sequences with homology to the R genes encoding plant immune receptors of the Toll/interleukin- 1-nucleotide binding site-leucine rich repeat (TIR-NB-LRR) class (Vallejos *et al.,* 2006). Resistance genes associated with BCMNV resistance can be best explored with a good understanding of strain diversity by employing symptomatology and or the most recent technology in diagnosis such as the Next generation sequencing of virus genomes.

2.6 Detection of *Bean Common Mosaic Necrosis Virus*

2.6.1 Serological detection of *Bean Common Mosaic Necrosis Virus*

Serological methods used in virus detection are based on the principle of cross-reactivity of antisera against viral proteins (Hu *et al*., 1995). These methods include the micro-precipitin test, the first serological method used to identify viruses (Bhat *et al*., 2010). Other tests include the Ouchterlony agar double diffusion tests and ELISA methods (Bailey, 1996). All these tests use antiserum prepared against a particular pathogen. They are frequently used for classification and establishment of taxonomic relationships

among different groups of viruses (Craig *et al.,* 2004). According to the micro precipitin test is based on the principle that when the virus antigens and the homologous antibodies are mixed together in optimum proportions, they bind together at their reactive arms. This binding leads to a white precipitate. However, its sensitivity is very low to be used in routine diagnosis of plant viruses. In the double diffusion tests, agar gels are used in that preparations of viral antigens and specific antibodies are placed in adjacent wells in a plate containing agar gel. The number of antigenic components in a given antigen-antibody system correspond to the number of precipitation lines between reacting substances in opposing wells. However, the formation of precipitation lines depending on high and equivalent concentrations of specific antigens make this method a least sensitive serological test. The ELISA techniques include the direct antibody coating (DAC)-ELISA (Bashir *et al*., 1996), triple antibody sandwich (TAS)-ELISA, Nitro-cellulose membrane (NCM)-ELISA, acid phosphatase (ACP)-ELISA, protein A sandwich-ELISA (PAS-ELISA) and double antibody sandwich (DAS)-ELISA (Clark & Adams, 1977; Booonham *et al.,* 2014).

2.6.2 Molecular detection of viruses

2.6.2.1 Sanger sequencing

Sanger dideoxy sequencing (Sanger *et al.,* 1977) and its modifications (Smith *et al.,* 1986; Prober *et al.,* 1987; Madabhushi *et al.,* 1998) dominated the DNA sequencing field for nearly 30 years and in the past 10 years the length of Sanger sequence reads has increased from 450 bases to more than 1 kb (Varshney *et al.,* 2009). The limitations of

Sanger sequencing are: (i) the necessity to separate elongation products by size before scanning, requiring one capillary or gel lane per sample; and (ii) the need to produce clonal populations of DNA using *Escherichia coli*, which is labor, robotics and space intensive for large-scale operations. The latter requirement could potentially be reduced by using PCR-based methods (Varshney *et al.,* 2009). Individual reaction costs can be reduced by performing the sequencing reactions in reduced reaction volumes but the fundamental restrictions on reducing the cost of Sanger sequencing are at their technological limits (Schmitt *et al.,* 2015).

2.6.2.2 Next generation sequencing

Next-generation sequencing (NGS) technology is enabling direct detection and identification of known and unknown plant viruses without a requirement for a prior knowledge or sequence information of possible infecting viruses (Barzon *et al.,* 2011). NGS is quickly revolutionizing research into plant viruses with the focus of study expanding from viruses in resource-managed systems such as crop lands and orchards that have relatively low number of viruses to agro-ecological zones and natural ecosystems that comprise hot spots of viral diversity (Malmstrom *et al.,* 2011). Much of the current work on plant viruses has focused on viruses associated with a disease, but recent studies indicate co-existence of viruses with plants without causing any disease prompting to the hypothesis of mutualistic relationship between plants and viruses (Márquez *et al.,* 2007). There is also significant interest in the use of NGS for the detection of plant viruses as part of international import/export certification programs, and domestic clean plant programs (Barba *et al.,* 2014; Candresse *et al.,* 2014). For these

programs in particular, efficient, standardized methods that minimize cross contamination of samples for NGS analysis is of critical importance.

The ability of NGS to sequence whole genomes of known and unknown viruses and the ability to detect multiple viruses from a mixed infection is creating new opportunities for the rapid and routine detection of viruses (AlRwahnih *et al.,* 2011; Ho & Tzanetakis, 2014). Since NGS is non-specific, it can be used to detect all known viruses present in a host irrespective of their pathogenicity. Plant virus genome can be either DNA or RNA, the majority having RNA genomes (Martelli, 1993). For detection, several types of genetic material can be targeted. In the first scenario, total RNA, or total RNA depleted of ribosomal RNAs from infected plants are extracted and sequenced. Viral RNAs are then detected following bioinformatic analysis (Al Rwahnihet *et al.,* 2009; Wylie *et al.,* 2012). This approach involves the sequencing and subsequent discarding of significant amounts of host RNA sequence data relative to viral RNA and a larger number of sequence reads are required to ensure the detection of low titre viruses. A second approach involves sequencing of small RNAs generated by an anti-viral defense mechanism called RNA interference (RNAi). Both DNA and RNA viruses can be identified by sequencing these small interfering RNAs (siRNA). However, coverage of viral genomes can be uneven due to the small nucleotide fragments (21–24 nt) generated by RNAi, efficiency of the plant defense mechanism (Cao *et al.,* 2014) and the presence of small endogenous plant RNAs which can interfere with viral genome assembly (Kreuze, 2014). It is also difficult to identify multiple viral sequence variants in a sample due to the very short reads. A third approach is to sequence double stranded RNA (dsRNA). Since plants do not produce extensive high molecular weight dsRNA

structures, presence of these RNAs is generally attributed to the presence of viruses (Kesanakurti *et. al.,* 2016)

2.6.2.2.1 NGS Chemistry

DNA polymerase catalyses the incorporation fluorescently labelled deoxyribonucleotide triphosphates (dNTPS) into a DNA template strand during sequential cycles of DNA synthesis. During each cycle, at the point of incorporation, nucleotides are identified by fluorophore excitation. Instead of sequencing a single DNA fragment, NGS extends the process over a million of fragments in a massively parallel fashion.

2.6.2.2.2 NGS platforms

The platforms have emerged due to advances made in the fields of microfluidics, nanotechnology and informatics and alternative technologies to increase the rapidity and/or throughput of DNA sequencing (Varshney *et al.,* 2009).

NGS collectively describes platforms available or in development other than Sanger sequencing (Kling, 2005; Service, 2006). The platforms have potential to circumvent the limiting factors of Sanger sequencing. For example, sequencing can be multiplexed to a much greater extent by many parallel reactions at a greatly reduced cost (Hudson, 2008).

Currently, Roche/454, Solexa and AB SOLiD are the platforms that are predominantly used in crop genetics and breeding applications.

These are Novel DNA sequencing techniques that provide high speed and throughput sequencing of DNA. The platforms help determine the sequence data from amplified single DNA fragments, avoiding the need for cloning of DNA fragments. Though costly

and in some applications are short read lengths, non-uniform confidence in base calling in sequence reads, particularly deteriorating 3'sequence quality in technologies with short read lengths and generally lower reading accuracy in homopolar stretches of identical bases, these technologies are very reliable with their high throughput ability (Wilhelm, 2009). Moreover, software developers are constantly improving efficiency of computer algorithms applicable in the sequencing platforms.

The 454 Genome Sequencer FLX instrument (Roche Applied Science)

The basis of this device uses the principle of pyrophosphate detection as described by Nyren and Lundin (1985) and applied in a new method for DNA sequencing reported in 1988 (Hyman, 1988). The technique was further modified into a routinely functioning method for the analysis of 96 samples in parallel in a microtiter plate (Ronaghi *et al.,* 1996). The Genome Sequencer instrument introduced by 454 Life Sciences in 2005 is the first next-generation system (Wilhelm, 2009). DNA fragments are ligated with specific adapters that cause the binding of one fragment to a bead. Emulsion PCR is carried out for fragment amplification, with water droplets containing one bead and PCR reagents immersed in oil. The amplification is necessary to obtain sufficient light signal intensity for reliable detection in the sequencing-by-synthesis reaction steps. When PCR amplification cycles are completed and after denaturation, each bead with its one amplified fragment is placed at the top end of an etched fibre in an optical fibre chip, created from glass fibre bundles. The individual glass fibres are excellent light guides, with the other end facing a sensitive charge coupled device (CCD) camera, enabling positional detection of emitted light. Each bead thus sits on an addressable position in the light guide chip, containing several hundred thousand fibres with attached beads. In

the next step polymerase enzyme and primer are added to the beads, and one unlabeled nucleotide only is supplied to the reaction mixture to all beads on the chip, so that synthesis of the complementary strand can start. Incorporation of a following base by the polymerase enzyme in the growing chain releases a pyrophosphate group, which can be detected as emitted light. Knowing the identity of the nucleotide supplied in each step, the presence of a light signal indicates the next base incorporated into the sequence of the growing DNA strand.

The method achieves reading length to the 400–500 base range, with paired-end reads.

A relatively high cost of operation and generally lower reading accuracy in homopolar stretches of identical bases are mentioned presently as the few drawbacks of the method (Schuster *et al.,* 2008).

The Illumina (Solexa) Genome Analyzer

The Solexa sequencing platform applies the principle of sequencing-by-synthesis chemistry, with novel reversible terminator nucleotides for the four bases each labelled with a different fluorescent dye, and a special DNA polymerase enzyme able to incorporate them. DNA fragments are ligated at both ends to adapters and, after denaturation, immobilised at one end on a solid support. The surface of the support is coated densely with the adapters and the complementary adapters. Each single-stranded fragment, immobilised at one end on the surface, creates a 'bridge' structure by hybridising with its free end to the complementary adapter on the surface of the support. In the mixture containing the PCR amplification reagents, the adapters on the surface act as primers for the following PCR amplification. After several PCR cycles, random

clusters of about 1000 copies of single-stranded DNA fragments (DNA ‘polonies’, resembling cell colonies after polymerase amplification) are created on the surface. The reaction mixture for the sequencing reactions and DNA synthesis is supplied onto the surface and contains primers, four reversible terminator nucleotides each labelled with a different fluorescent dye and the DNA polymerase. After incorporation into the DNA strand, the terminator nucleotide, as well as its position on the support surface, is detected and identified via its fluorescent dye by the CCD camera.

The terminator group at the 30-end of the base and the fluorescent dye are then removed from the base and the synthesis cycle is repeated. The sequence read length achieved in the repetitive reactions is about 35 nucleotides. The sequence of at least 40 million polonies can be simultaneously determined in parallel, resulting in a very high sequence throughput, on the order of Gigabases per support.

The Illumina upgrade, the Genome Analyzer II triples output compared to the previous Genome Analyzer instrument. A paired-end module for the sequencer and new optics and camera components allow the system to image DNA clusters more efficiently over larger areas, thus the instrument triples the output per paired-end run from 1 to 3 Gb. The system generates at least 1.5 Gb of single-read data per run, at least 3 Gb of data in a paired-end run, recording data from more than 50 million reads per flow cell. The run time for a 36- cycle run is done in two days for a single-read run, and four days for a paired-end run (Schuster *et al.,* 2008).

The Applied Biosystems ABI SOLiD system

The ABI SOLiD sequencing system, is a platform that is chemistry based upon ligation. In this technique, DNA fragments are ligated to adapters then bound to beads. A water droplet in oil emulsion contains the amplification reagents and only one fragment bound per bead; the emulsion PCR amplifies DNA fragments on the beads. After DNA denaturation, the beads are deposited onto a glass support surface.

In a first step, a primer is hybridized to the adapter. Next, a mixture of oligonucleotide octamers is also hybridized to the DNA fragments and ligation mixture added. In these octamers, the doublet of fourth and fifth bases is characterized by one of four fluorescent labels at the end of the octamer. After the detection of the fluorescence from the label, bases 4 and 5 in the sequence are thus determined. The ligated octamer oligonucleotides are cleaved off after the fifth base, removing the fluorescent label, then hybridization and ligation cycles are repeated, this time determining bases 9 and 10 in the sequence; in the subsequent cycle bases 14 and 15 are determined, and so on. The sequencing process may be continued in the same way with another primer, shorter by one base than the previous one, allowing one to determine, in the successive cycles, bases 3 and 4, 8 and 9, 13 and 14. This can achieve sequence reading length is of about 35 bases. Because each base is determined with a different fluorescent label, error rate is reduced. Sequences can be determined in parallel for more than 50 million bead clusters, resulting in a very high throughput of the order of Gigabases per run. The SOLiD 2.0 platform Applied Biosystems updated version increases the output of the instrument from 3 to 10 Gb per run. This change reduces the overall run time of a fragment library on the new system to 4.5 days from 8.5 days on the existing machine (Schuster *et al.,* 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Survey and sample collection

In October 2016 and May 2017, two diagnostic surveys for BCMD were carried out in 7 counties of Western Kenya namely Bungoma, Busia, Homa bay, Nandi, Vihiga, Kakamega and Siaya. Two hundred and seventy farms were surveyed, 150 in the long rain season and 120 in the short rain season. The survey covered areas as low as 1164m above sea level, a farm in Busia county, to areas as high as 1600m above sea level, a farm in Kakamega county. The southernmost farm (S00.70061) surveyed was in Homa Bay County while the Northernmost (N00.69718) was in Bungoma county. A total of 270 farms were surveyed (One hundred and fifty in the long rain season and one hundred and twenty in the short rain season). The Westernmost farm surveyed (E034.10503) was in Busia county while the Eastern most (E034.82533) was in Kakamega county (Appendix 1). A stratified random sampling procedure was adopted to determine BCMD symptom incidence and symptom severity. The incidence of BCMD was assessed according to Were *et al.* (2004; 2014) as a percentage of disease symptomatic plants in an area. Incidence was scored as presence or absence of virus disease symptoms using a rating scale where: 1-20 % (low Incidence); 21-49 % (moderate incidence) and 50-100 % (high incidence). Symptom severity was determined as the amount of disease on individual plants and scored on a scale (0-3) (Odu *et al.,* 2004) where: 0 = Absence of virus disease symptoms on plants, 1 = mild virus disease

symptoms, 2 = moderate virus disease symptoms, 3 = severe virus disease symptoms. At least five to fifteen samples based on field size were examined along a diagonal. One trifoliate leaf sample with disease symptoms was collected from the sampled plant and stored in polythene bags in a cool box prior to be taken to the laboratory for serological and molecular analysis. Sample location, common bean variety and symptoms were recorded. The household questionnaire (Appendix 2) was used to gather information on: household socio-economic characteristics, common bean agronomic practices, bean variety preferences, bean seed sources and farmers' awareness of pests and diseases through face-to-face interviews.

3.1.1 Survey data analysis

Data obtained from the survey was averaged to obtain means and percentages by each of the explanatory parameters recorded (incidence and severity). Analysis of variance (ANOVA) for the differences in the incidences and severity in the counties was done. Least significant difference values were used to separate the means at $p = 0.05$. Analyses were conducted using statistical analysis software, SAS to obtain correlation between the incidence and severity of BCMD ($r=0.843$; $p<0.001$).

3.2 Enzyme-Linked Immunosorbent Assay

Greiner Microlon medium binding microtitre plates were utilized for ELISA reactions with 100 µl/well volume used generally for each reactant.

Three intensive washing steps (3 min each) with a washing buffer for 4 min were carried out between incubations.

Antibodies and reagents used were bought from DSMZ, Germany (Appendix 3) .

3.2.1 Detection of BCMNV by DAS ELISA and BCMV by TAS ELISA

Leaf tissues of virus-infected plants were ground 1:10 (w/v) in sample extraction buffer. To detect BCMNV, Double Antibody Sandwich (DAS)-ELISA was carried out essentially as described (Were *et al.,* 2004) following manufacturer's instructions. Microtiter plates were coated with BCMNV IgG diluted 1:1000 (v/v) in coating buffer and incubated for 2h at 37 °C. To block, 2 % skimmed milk in PBST (200µl/well) was added and incubated for 30 min at 37 °C. The extracts of sap prepared from ground leaf tissues of virus-infected plants 1:10 (w/v) in sample extraction buffer (PBST + 2 % PVP) were added and incubated overnight at 4 °C. Extracts from healthy and of BCMNV infected plants were used as negative and positive controls, respectively. Alkaline phosphatase labelled Rabbit-anti-mouse RaM-AP, (DSMZ, Germany) diluted 1:1000 v/v in conjugate buffer was added and the plates incubated for 45 min at 37 °C. The substrate, *p*-Nitrophenyl phosphate diluted 1 mg/ml in substrate buffer (DEA+H_2O +NaN_3) was added and incubated for 1 h at 37 °C or until there was colour change. Quantitative measurements of the p-nitrophenol substrate conversion resulting in yellow color were made by determining the absorbance at 405 nm (A405) in a Biotek® model spectrophotometer (Labsystems Co., Finland). Twice the mean absorbance readings of healthy controls were used as the positive thresholds.

To detect BCMV, Triple Antibody Sandwich (TAS) ELISA was conducted as described (Were *et al.,* 2004) following manufacturer's instructions. Microtitre plates (96 wells) were coated with BCMV IgG diluted 1:1000 (v/v) in a coating buffer and incubated for 2 h at 37 °C. Blocking was carried out as described above. Sap extracts prepared as described above were added and incubated overnight at 4 °C. Hundred µl/well of MAbs raised against BCMV and diluted 1:100 (v/v) in conjugate buffer added and incubated for 2 h at 37°C were used for detection. Extracts from healthy and of BCMV infected plants were used as negative and positive controls, respectively. IgG alkaline phosphatase conjugate, diluted 1:1000 (v/v) in conjugate buffer, was added and incubated for 2h at 37°C. Substrate addition, incubation and absorbance readings were done as described above.

3.3 Determination of molecular diversity of *Bean Common Mosaic Necrosis Virus*

3.3.1 RNA extraction

Total RNAs from infected leaves of common bean were extracted using the Qiagen RNeasy Plant Mini Kit (Qiagen, Germany) following manufacturers' instructions. Ten microliters Mercaptoethanol was added to Buffer RLT. Forty-four milliliters ethanol was added to RPE concentrate buffer. A hundred milligrams plant leaf was crushed in a sample bag and 450-1000 µl RLT added and crushed again. Four hundred and fifty microliters of the lysate was then transfered to a Qiashredder centrifugation at full speed for 2 min. The flow-through then transferred to a new tube. Two hundred and twenty five microliters ethanol was then added and mixed by pipetting. The sample including

any precipitate (650 µl) was transferred to RNeasy spin column for centrifugation at 10000 rpm for 15 s and the flow-through discarded. Seven hundred microliters RWE buffer was added and centrifuged at 10000 rpm for 15 s to washing the membrane of spin column. The collection tube was reused after discarding the flow through. Five hundred microliters RPE buffer was added and centrifuged at 10000 rpm for 15 s and discarded the flow-through. Five hundred microliters RPE buffer was added again and centrifuged at 10000 rpm for 2 min followed by discarding the flow-through. The spin column was transferred into a new 1.5 ml tube, 50 µl RNase free water was added directly to the membrane and then centrifuged at 10000 rpm for 1 min. The final eluate contained purified RNA.

3.3.2 Quantification of isolated RNA

i) Spectrophotometric quantification

A spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and Qubit were used to measure total RNA concentration and purity. Extracted RNA was quantified by Nanodrop at spectrophotometric wavelengths of 260 nm with a conversion factor of 1 A260 unit ssRNA = 40 µg. The results were based on "A260 unit" used for nucleic acids as a quantity measure. For sample purity, the ratio for pure RNA $A_{260/280}$ is ~2.0. The samples were then subjected to a secondary nucleic acid purity check at 260/230 where values between 2.0-2.2 were regarded to be pure since the ratio appreciably lower than the range may indicate presence of contaminants. Contaminants such as EDTA, Carbohydrates and phenols have absorbance near 230

while a reagent such as guanidine isothiocyanate used for RNA isolations will absorb at ~260 nm.

3.3.3 First and Second Strand cDNA synthesis

First and second strands were prepared using the Invitrogen kit (Invitrogen) as described by Parkhomchuk *et. al.* (2009) following the manufacturers' instructions.

First strand synthesis (FSS)

FSS reaction was prepared by mixing 0.5 μg of polyA$^+$ RNA, 40 ng of $(dN)_6$ primers (Invitrogen) and 25 pmol of oligo(dT) primer (Invitrogen) in 8.5 μl of 1× reverse transcription buffer (Invitrogen), 0.5 mM dNTPs, 5 mM $MgCl_2$ and 10 mM DTT. The mixture was incubated at 98°C for 1 min to melt RNA secondary structures, then at 70°C for 5 min and was cooled to 15°C at 0.1°C/s. Slow temperature cooling was used to make annealing of secondary RNA structures and primers as reproducible as possible. At 15°C 0.5 μl of actinomycin D solution (120 ng/μl), 0.5 μl of RNase OUT (40 units/μl, Invitrogen) and 0.5 μl of SuperScript III polymerase (200 units/μl, Invitrogen) were added to the reaction. Temperature of reverse transcription reaction was increased gradually as a compromise between survival of the enzyme, stability of the primers and denaturation of RNA secondary structures: heating from 15 to 25°C at 0.1°C/s; incubation at 25°C for 10 min; heating from 25 to 42°C at 0.1°C/s; incubation at 42°C for 45 min; heating from 42 to 50°C at 0.1°C/s; incubation at 50°C for 25 min. SuperScript III polymerase was finally inactivated at 75°C for 15 min.

Removal of dNTPs

EB (20 µl) (10 mM Tris–Cl, pH 8.5, Qiagen) was added to the reaction. dNTPs were removed by purification of the first strand mixture on a self-made 200 µl G-50 gel filtration spin-column equilibrated with 1 mM Tris–Cl, pH 7.0.

Second strand synthesis (SSS)

Since the Invitrogen kit was used for the SSS, the FSS buffer had to be restored after gel filtration. Water was added to the purified FSS reaction to bring the final volume to 52.5 µl. The mixture was cooled on ice. Then, 22.5 µl of the 'second strand mixture' [1 µl of 10× reverse transcription buffer (Invitrogen); 0.5 µl of 100 mM $MgCl_2$; 1 µl of 0.1 M DTT; 2 µl of 10 mM mixture of each: dATP, dGTP, dCTP, dUTP; 15 µl of 5× SSS buffer (Invitrogen); 0.5 µl of *Escherichia coli* ligase (10 units/µl, NEB); 2 µl of DNA polymerase I (10 units/µl, NEB); and 0.5 µl RNase H (2 units/µl, Invitrogen)] were added. SSS reactions were incubated at 16°C for 2 h. ds cDNA was purified on QIAquick columns (Qiagen) according to the manufacturer's instructions.

DNA fragmentation

About 250 ng of ds cDNA was fragmented by sonication with a UTR200 (Hielscher Ultrasonics GmbH, Germany) under the following conditions: 1 h, 50% pulse, 100% power and continuous cooling by 0°C water flow-through.

3.3.4 Library preparation and Sequencing

The cDNA was processed with the transposon-based chemistry library preparation kit (Nextera XT, Illumina) following manufacturer's instructions. The Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA) was used to assess the fragment size structure of the DNA library. The indexed denatured DNA library was sequenced (200-bp paired-end sequencing) on the Illumina MiSeq platform (Illumina) to generate single end (SE) reads of 50 nt. The libraries were normalized, pooled and diluted to a final concentration of 6.5 pM. Pooled libraries were then run on the Illumina MiSeq System utilizing 12 pM of 1% PhiX as control. Paired-end sequencing was performed (2 × 300 bp). Sequencing was conducted at the BecA-ILRI Hub Nairobi Kenya.

3.3.5 Sequence data analysis

Sequence analysis was performed using CLC Genomics Workbench 9: first, adapters were trimmed from the reads, then reads were filtered by length (only reads 20-24 nucleotides long, corresponding to the size of small interfering RNAs, were retained). Mapping of reads was done on to the *Phaseolus vulgaris* genome to remove the bean sequences using Bowtie2V 2.2.8 (Langmead and Steven, 2012). Trimmed and size-selected reads were then mapped (parameters given in Appendix 5) to the NCBI Viral RefSeq database, containing representatives of all viral genomes with completely sequenced genomes. Results of the mapping were manually inspected. The remaining reads of each sample were assembled *de novo* using metaSPAdesV 3.10.1 (Nurk *et al.,* 2017) with default settings (Appendix 6). The resulting contigs were submitted to

BLAST for comparison against a local download of NCBI GenBank nucleotide database of plant viruses using BLASTn (Camacho *et al.,* 2009).

3.3.6 Phylogenetic analysis

A phylogenetic analysis was carried out using MEGA software version 7.0 with the maximum likelihood model at 1,000 bootstrap replicates (Tamura *et al.,* 2016). The sequences obtained were aligned with 17 BCMNV complete genome sequences retrieved from Genbank. Final sequences were submitted to the DNA Data Bank of Japan (**DDBJ**).

Two primers were designed using Primer3Plus (-http://primer3plus.com/cgi-bin/dev/primer3plus.cgi) using consensus sequences from this study (section 3.3.5). The primers were validated by polymerase chain reaction on five ELISA positive samples from section 3.2.1.

3.3.7 Polymerase chain reaction (PCR)

PCR was carried out as essentially described by Naidu *et al*. (1998). BCMNV isolate cDNA molecules were amplified by PCR using Primer pair (Forward_GTTCCAGCCAGCAAGGAGAT; Reverse Primer_ GAATCTGTCGCCGGCAAATC) producing amplicons (549 base pair; bp) located at position 1524 bp – 2072 bp. Amplification was done using a primer set according to Potter *et al.* (2003). Two-step RT-PCR was done using one Taqman master mix. Five microliter of cDNA was then used in the amplification step. The amplification mixture

was composed of 12 µl one Taqman master mix, 4 µl forward and reverse primers, 4 µl cDNA and water. Amplifications were carried out in a Eppendorf cycler using the following temperature regime: a denaturation phase at 94 °C for 2 min followed by 35 cycles of amplification (94 °C for 1 min, 55 °C for 1 min, and 2 min at 72 °C) and a final extension at 72 °C for 10 min.

3.3.7.1 Agarose gel electrophoresis

Ten microliter of PCR products were analyzed using 2 % agarose gel electrophoresis in 1X TAE buffer, stained with ethidium bromide and finally visualized under UV light. Agarose powder was added to a TAE buffer (2 % w/v) and microwaved for 2 min to dissolve the powder. To the cooling solution, 0.005 % Ethidium bromide was added and subsequently poured into a tray in which a comb was inserted to form sample slots. The agarose gel was allowed to solidify for approximately 30 min before the comb was removed and the gel immersed in the electrophoresis tank containing TAE buffer. To 10µl of DNA sample, 3 µl of sample buffer were added and the total volume, 13 µl loaded into a slot in the gel. Invitrogen 100 bp DNA Ladder (100–2o00 bp) was used. The gel was run at 120 volts and maximum current for 45 min before being viewed under UV light and photographed.

3.4 Biological characterization of BCMNV

3.4.1. Seed germination and mechanical inoculation

Five seeds from each of the 16 popularly grown bean varieties, cowpea, soybean, green grams and groundnut were sown in three replicates in pots containing super-mix soil with humus rich soil and 1 g of di-ammonium phosphate fertilizer applied per plant in the greenhouse at Kenya Agricultural and Livestock Research Organization (KALRO), Kakamega. Inoculation was done following Mandal *et al.* (2008). The BCMNV isolate used in inoculation had been maintained in infected bean in a greenhouse at Kenya Agricultural and Livestock research institute (KALRO)-Kakamega. The inoculum was made by crushing BCMNV infected leaves (1.0 g leaf tissue and 10 ml of 0.1M phosphate buffer, pH7.0 with 0.2% sodium sulfite) by a chilled mortar and pestle. The test plants were inoculated at the 3 leaf stage with BCMNV isolate from western Kenya beside a healthy control.

Data was taken on type of symptoms expressed by plants and the number of plants showing symptoms weekly for 3 weeks. Systemic infection was determined at the end of second week post inoculation by DAS ELISA.

CHAPTER FOUR

RESULTS

4.1 Incidence and severity of BCMD

Maps Fig 1 and Fig. 2 shows sampling points (red points) during the long and short rain seasons respectively.

Typical BCMD and other virus-like symptoms of mosaic, leaf distortion, downward curling, mottling, vein necrosis, local lesions, stunted growth or a combination of these were observed during both surveys (Fig. 3). The disease symptoms were found distributed in farms across all the counties under study.

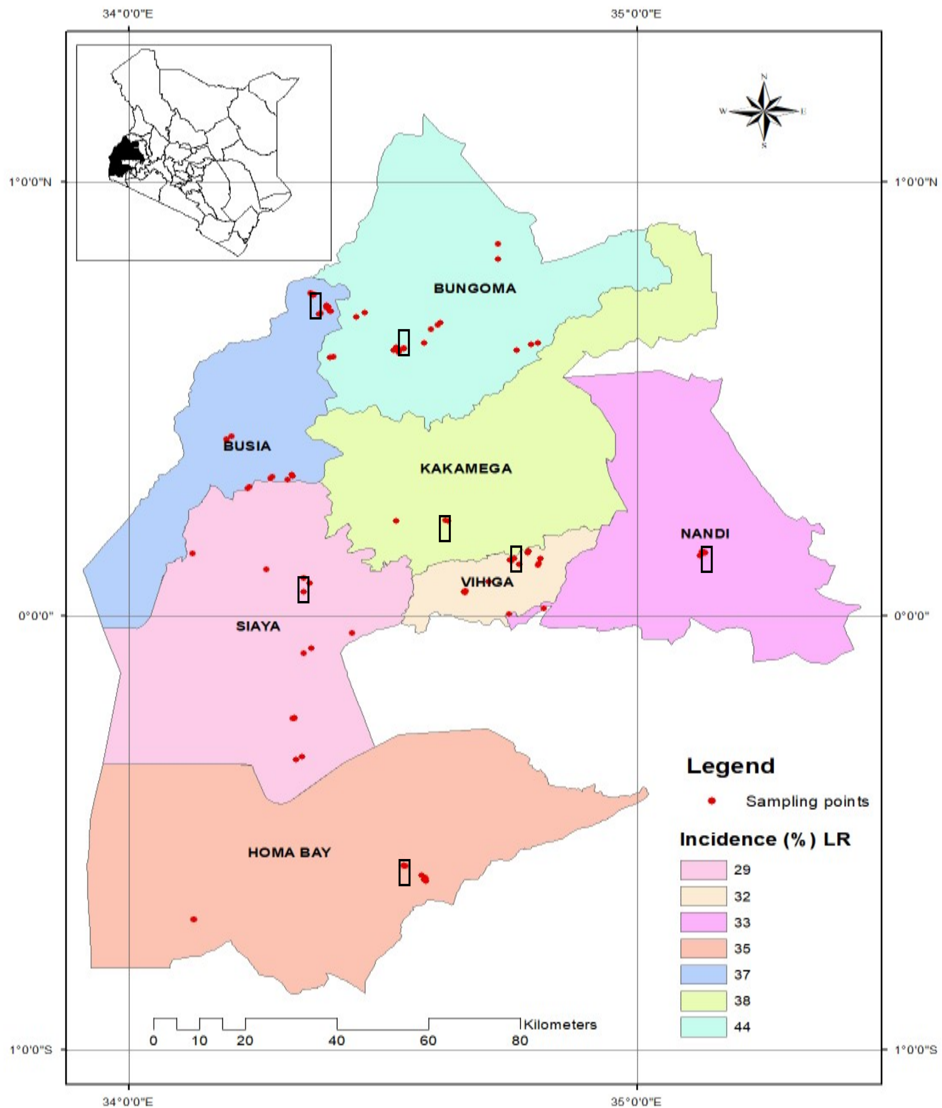

Figure 1: Map of western Kenya showing areas of virus disease incidence during long rains season. Red spots indicate farms whose bean samples were ELISA positive for BCMNV. Black spots indicate areas with plants with mixed infection (BCMNV and BCMV).

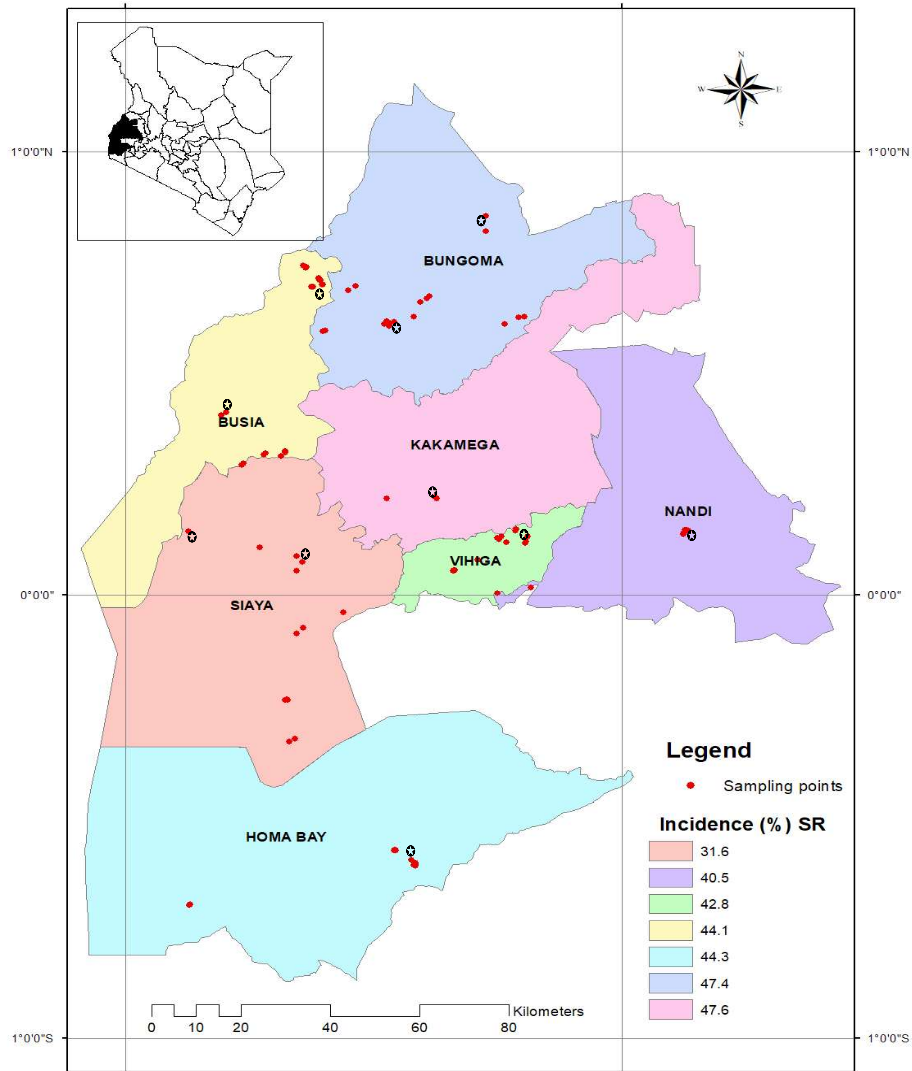

Figure 2: Map of western Kenya showing virus disease mean incidence during the short rains season. Red spots indicate farms whose bean samples were ELISA positive for BCMNV. Black spots indicate areas with plants with mixed infection (BCMNV and BCMV).

(a) Busia, 1181m asl
var Yellow

(b) Bungoma, 1432m asl
var Rosecoco

(c) Kakamega, 1592 m asl
var Rosecoco

Figure 3: Some virus-like symptoms observed on bean plants in the field during survey that were found positive for BCMNV. Above: (a) Shrivelled leaves with mosaic on variety Yellow in Busia county at 1181 meters above sea level (m asl); (b): leaves of Rosecoco variety showing yellow-net vein banding in Bungoma county and 1432 m asl and (c) : Leaves of Rosecoco variety in Kakamega county showing vein banding and curling downwards at 1592 m asl.

The average temperature and rainfall during may were 22.5 °C and 525 mm in the western areas of Kenya (Min Env. and Mineral Res. 2018). The results of visual viral disease symptoms show mean virus incidence higher during the short rain season (41.8 %) as compared to the long rain season (35.6 %). Across counties (Table 1), Kakamega county had the highest mean virus incidence (47.6 %) while Siaya had the lowest (31.6 %) during the short rain season. The highest disease mean incidence was recorded in Bungoma (44.3 %) during the long rain season and lowest in Siaya (29.4 %). Disease incidence among the counties varied significantly (p=0.05).

Table 1: Mean Bean Common Mosaic Disease incidence and severity observed during the short and long rain seasons in Western Kenya

County	Season	Number of fields	Mean incidence	Standard Error	Mean Severity
Busia	LR	25	33.6[a]	2.01	0.5
	SR	20	44.1[b]	2.24	0.2
Bungoma	LR	25	44.3[b]	3.11	1.5
	SR	20	47.4[d]	4.44	0.2
Homabay	LR	20	35.4[a]	3.56	1.2
	SR	15	44.3[b]	4.12	1.7
Kakamega	LR	20	38.4[c]	3.90	1.0
	SR	20	47.6[d]	5.34	0.3
Siaya	LR	20	29.4[e]	2.46	0.5
	SR	15	31.6[a]	3.86	1.0
Vihiga	LR	20	32.0[a]	2.14	0.5
	SR	15	42.8[b]	4.01	1.2
Nandi	LR	20	33.0[a]	3.90	1.3
	SR	15	40.5[b]	3.88	0.6
Total	**LR**	**150**	**35.6[a]**	**2.14**	**1.0**
	SR	**120**	**41.8[b]**	**3.89**	**1.5**

LR: Long rain season, SR: Short rain season * Means with the same letter(s) within a column are not significantly different at 0.05 level. **Disease Incidence**- Proportion of diseased plants per field. **Disease Severity**- Amount of disease on individual plants.

Table 2: BCMD ELISA results of samples from short and long rain seasons

Samples	Season (N)	BCMV (positive)	BCMNV(positive)	Total
Beans	Short rain (80)	15	21	36
	Long rain (100)	23	31	54
Groundnut	Short rain (20)	6	2	8
	Long rain (20)	2	4	6
Cowpea	Short rain(10)	2	1	3
	Long rain (10)	4	0	4

From Table 2, the number of samples collected from the short rain season that were positive for BCMV was 23 and 24 for BCMNV while during the long rain season, 29 samples were BCMV positive and 35 BCMNV positive (Appendix 4). Most samples from across the counties were found having mixed infections of both BCMV and BCMNV as detected by antibodies for the two viruses. Mixed infection with the two viruses causing BCMD was found in samples from all counties surveyed (Fig. 1 and Fig. 2) indicated in black spots on the maps.

Viral disease severity varied within and between fields and in counties. There was a strong positive correlation between viral disease incidence and severity ($r=0.843$; $p<0.001$) and therefore severity increased with increase in disease incidence. The mean BCMD severity in the long rains season was highest (1.5) in Bungoma county and lowest (0.5) in Busia, Siaya and Vihiga while during the long rain season it was highest in Homabay county (1.7) and lowest (0.2) in Busia and Bungoma counties (Table 1 and Appendix 2).

4.1.2 Socio-economic characteristics and BCMD management

4.1.2.1 Common bean cropping system

Row planting and intercropping were very common bean cropping systems. Common bean was intercropped majorly with maize, however in some farms it was found mixed with other legumes (Fig. 4). Soybean was mostly planted in pure stand-alone.

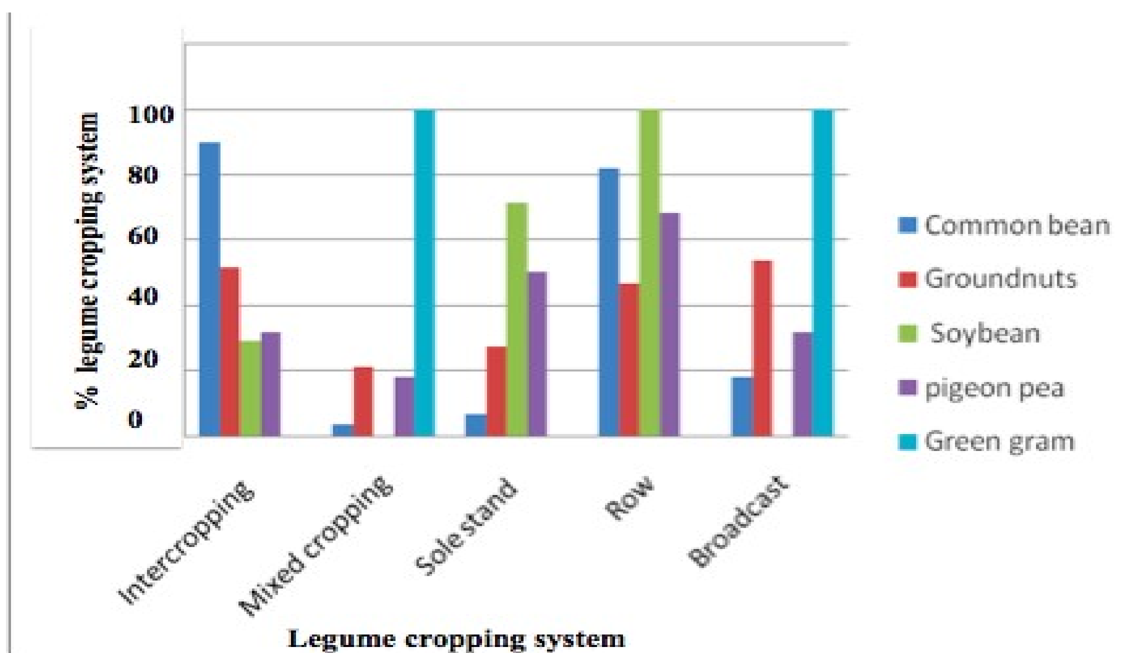

Figure 4: Cropping pattern of beans in western Kenya.

4.1.2.2 Soil management

Organic manure and fertilizers were applied in bean farms by nearly half of the farmers. Di-ammonium Phosphate (DAP) was used during planting time. However, majority of farmers who practiced soil management on common bean were those who participated in community-based organizations involved in legume production.

4.1.2.3 Common bean varieties grown in western Kenya

Most popular common bean varieties found on the farms were *Rosecoco* (GLP 2 purple mottled, medium seed (152 farms), *Wairimu* (GLP 585 red haricot, small seed) (64 farms), Yellow (29 farms), KK8 (18 farms), Punda (5 farms) and Tulu (2 farms). Punda had the highest mean viral incidence observed (56.3 %) followed by KK8 (48.2 %), Wairimu (42.7 %), Rosecocco (40.5 %), Tulu (40.0 %) while Yellow had the lowest (39.3 %) during the short rains season. Rosecoco had highest mean incidence (44.1 %) while yellow the lowest (35.0 %) during the long rains season.

Local and or improved bean varieties across the counties surveyed were found to refereed to by different names majorly depending on the area of reference or language under consideration. Farmers often describe the varietal names based on definite key noticeable variety characteristics such as grain color, growth habit, appearance and perceived point source.

4.1.2.4 Sources of common bean seed

Seed plays a crucial role in agricultural production. The results in Fig. 5 show that the main source of seed was local market (40%) followed by own save seed (48%).

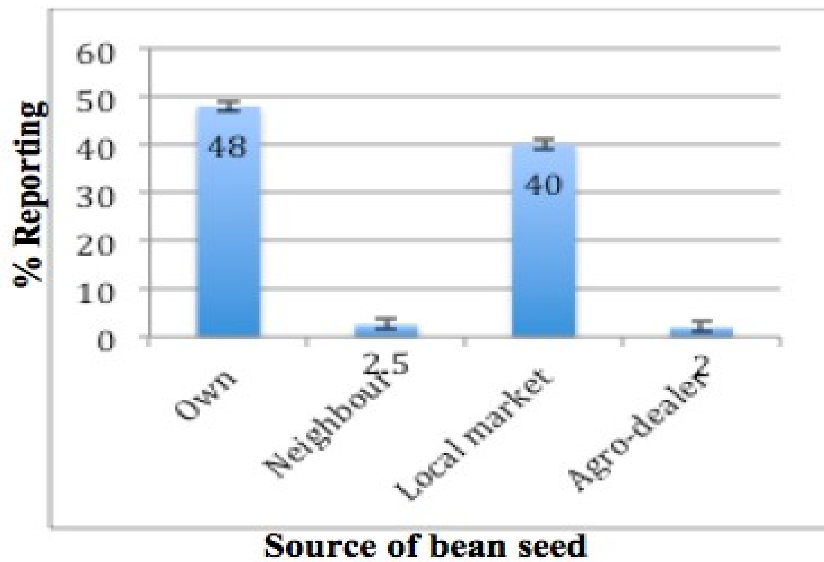

Figure 5: Sources of bean seed in western Kenya.

4.2 Molecular diversity of *Bean Common Mosaic Necrosis Virus*.

4.2.1 RNA quality and quantity determination

Quantification of extracted RNA by Nanodrop at spectrophotometric wavelengths of 260 nm with a conversion factor of 1 A260 unit ssRNA = 40 µg gave the following results shown in Table 3.

Table 3: RNA analysis using a spectrophotometer

Sample No.	Sample ID	Nucleic Acid (ng/µl)	A260 (Abs)	A280 (Abs)	260/280	260/230
1	24A	60.8	1.52	0.782	1.94	1.3
2	28	479.9	11.997	5.743	2.09	1.86
3**	42	428.9	10.722	5.014	2.14	2.08
4	41	167.7	4.193	2.065	2.03	1.32
5	24B	369.9	9.247	4.436	2.08	1.78
6	43	727.9	18.198	8.187	**2.22***	**2.09***
7	13	173.9	4.347	2.113	2.06	1.52
8	21	98.5	2.463	1.195	2.06	1.56
9**	23	599.3	14.983	7.041	2.13	2.06
10**	14	808.1	20.202	9.337	**2.16***	**2.29***
11	2	578	14.45	7.26	1.99	1.62
12	1	154.9	3.873	1.892	2.05	1.42
13	17	25.7	0.642	0.345	1.86	0.62
14**	44	1230	30.751	14.252	2.16	2.16
15**	29	856	21.401	9.82	2.18	2.2
16**	15	261.9	6.548	3.069	2.13	1.76
17**	45	708	17.7	8.021	**2.21***	**2.13***
18**	16	630.4	15.761	7.147	**2.21***	**2.17***
19**	11	409.3	10.232	4.858	2.11	2.11
20**	3	672.4	16.811	7.461	**2.25***	**2.03***

4.2.2 Sequence data

Ten samples (sample number marked **) subjected to sequencing gave varied read lengths after trimming (Appendix 7).

The genome sequence (9584 nt) of BCMNV BG12 isolate collected from a symptomatic bean in Bungoma county was revealed by Illumina Miseq platform. The BCMNV BG12 isolate sequence was 96 - 97% identical to the 17 BCMNV complete genomes available in Genbank, confirming a close phylogenetic relationship and a limited diversity in the BCMNV species (Fig. 6). Phylogenetic analysis of full length sequences available through the Genbank show that isolate BCMNV BG 12 was clustered with the Tanzanian isolate TN-1 and an isolate Oregon and TN1a both from USA (Fig. 6). The comparison of this Kenyan isolate with seventeen complete genomes from the GenBank shows a small variation of less than 10 %.

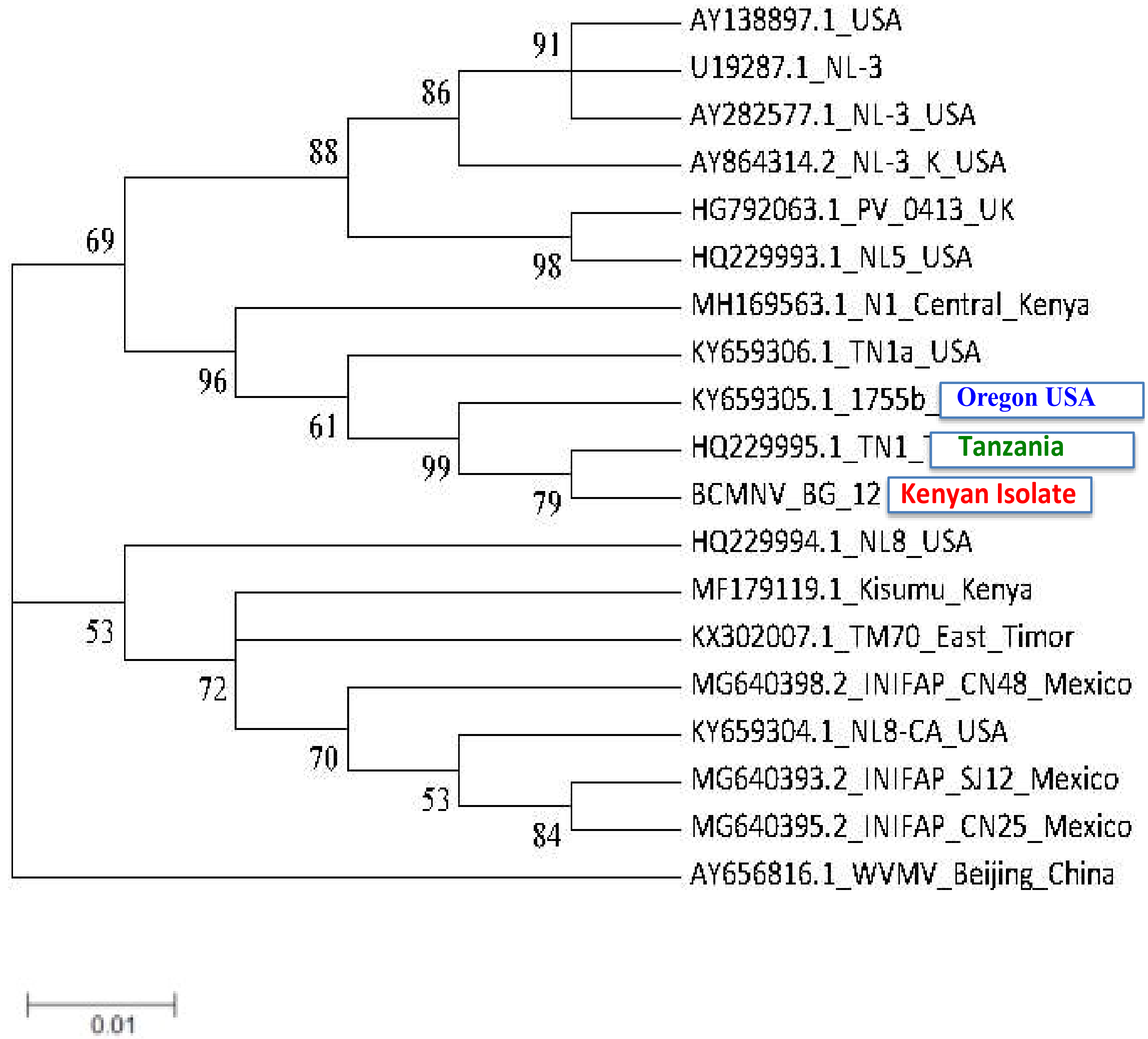

Figure 6: A maximum likelihood phylogenetic tree of BCMNV isolate BCMNV BG2. Generated using Mega 7 (Tamura-Nei default settings) from the alignment of seventeen full-length genomic sequences for BCMNV and an outgroup *Wisteria vein mosaic virus* (WVMV).

4.2.3 RT-PCR to validate primers developed from the sequence.

BCMNV presence in leaf tissues was established by specificity of the primer set by detection of a positive (+ve) control for BCMNV and infected plant samples. ~549bp fragments were amplified with the primer set for samples (1, 2 3, 4 and 5; samples whose RNA $A_{260/280}$ values in Table 3 are marked (*)) and positive (+ve) control except negative (-ve) control indicating no specificity for the primer set (Fig. 7).

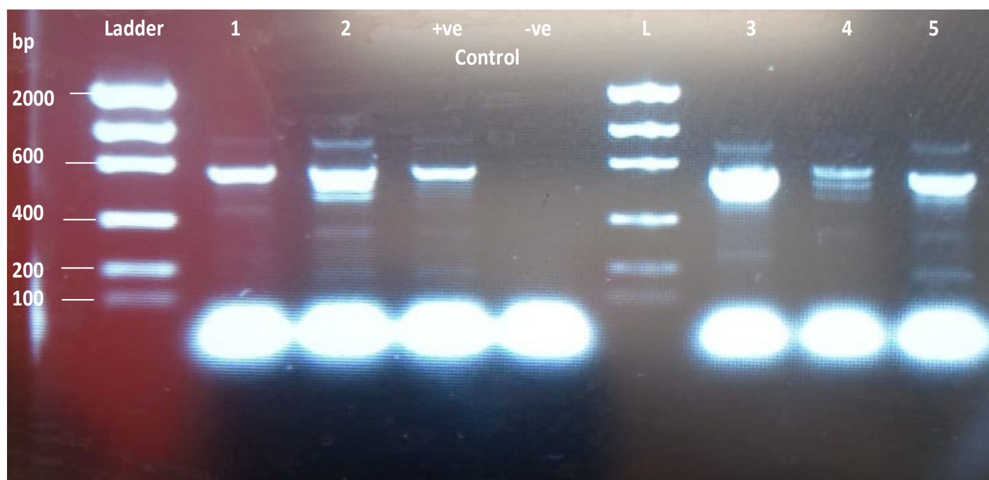

Figure 7: RT-PCR products of samples with BCMNV after electrophoretic separation in agarose gel (2 %). Lanes 1,2,3,4 and 5 indicate products of amplification with BCMNV primer (expected band size 549 bp). Positive (+ve) control was established with an isolate of BCMNV. Negative (-ve) control was obtained from a healthy bean leaf tissue. Invitrogen 100 bp DNA Ladder (L) bands are indicated in base pairs in the left margin.

4.3 Screening legume germplasm for resistance to Bean Common Mosaic Necrosis Virus

Sixteen popularly grown common bean varieties in western Kenya inoculated with BCMNV BG12 isolate from western Kenya in a greenhouse exhibited typical virus disease symptoms such as leaf mosaic, downward leaf curl and yellowing as shown on popular variety GLP 2 (Fig. 8).

Leaf mosaic, downward leaf curl and yellowing on GLP2 Control

Figure 8: Symptoms expressed on varietal screening for resistance to BCMNV BG12 isolate. ELISA Spectrophometric absorbance value at wavelength of 405nm for bean variety GLP2 was 0.777 while the negative control had 0.180.

Popularly grown legumes, Groundnut var Red Valencia expressed leaf mosaic, Soybean var 'Nyala' showed leaf yellowing, Greengrams var 'Local' expressed leaf mosaic. All cowpea varieties expressed leaf deformation except Cowpea var 'Local black' which had both leaf deformation and leaf yellowing (Fig. 9).

Figure 9: Popular legumes inoculated with BCMNV BG 12. A, Groundnut (Var Red valencia) with yellowing; B, Soybean with yellowing and leaf deformation; C, greengrams with leaf deformation and stunted growth; D, Control(cowpea).

From Table 4 below, Ten bean varieties were symptomatic and positive for BCMNV by DAS ELISA, four bean varieties (Imbeko, KK/RIL5/Red 13, Okwoto, RIL05/CAL 194) were symptomless with BCMNV BG 12 isolate from western Kenya however tested positive for BCMNV by DAS ELISA. Two bean varieties (KK RIL05 and KK 072) were symptomless and negative for BCMNV by DAS ELISA.

Table 4: Reaction of test plants to BCMNV isolate

Test plant	Variety	BCMD symptoms	Number of plants inoculated with BCMNV	Number of symptomatic plants on the 3rd week after inoculation	Number of ELISA positive plants
Bean	GLP 2	ST, D	5	5	5
Bean	RIL 05	D,M	5	4	4
Bean	KK20	ST,M	5	5	5
Bean	**KK RIL05**	**Symptomless**	**5**	**0**	**0**
Bean	Imbeko	Symptomless	5	0	5
Bean	Yellow	M,D	5	5	5
Bean	Rosecoco	Y,M	5	5	5
Bean	Wairimu	M	5	5	5
Bean	KK 8	M,Y	5	4	3
Bean	Punda	M	5	5	5
Bean	GLPX92	Y,M	5	5	5
Bean	KK15	Y	5	4	4
Bean	KK/RIL 5/Red 13	Symptomless	5	0	5
Bean	**KK072**	**Symptomless**	**5**	**0**	**0**
Bean	Okwoto	Symptomless	5	0	4
Bean	KK RIL05/CAL 194	Symptomless	5	0	3
Groundnut	Red valencia	M	5	2	2
Soybean	Nyala	Y	5	5	3
Green Grams	Local	M	5	4	4
Cowpea	Local Cream	D	5	3	3
Cowpea	Local Red	D	5	4	3
Cowpea	Local Black	D, Y	5	4	3
Cowpea	K-80	D	5	3	3
Cowpea	KVU 270-1	D	5	4	4
Cowpea	M66	D	5	3	3

Key: D, deformed leaves; M, mosaic; Y, yellowing; ST, stunted growth.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Bean Common Mosaic Disease incidence and severity

The results of BCMD surveys in western Kenya demonstrate a marked increase in its incidence and severity. A disease incidence as high as 100% was observed and recorded in many individual fields where severely affected bean plants (Score 3) prevailed. BCMD increase the risks of farming as a livelihood strategy or a commercial enterprise by decreasing agricultural yields, raising production costs and limiting marketability of food and feed legumes (Nicaise, 2014; Akinyemi *et al.,* 2016). Despite the importance of beans, virus effects are largely unrecognised by most farmers from western Kenya. In this study, higher disease incidence was observed in the short rain season than in the long rain season, a finding that concurs with previous studies by Mangeni *et al.* (2014) who found high virus incidence in common bean fields. This may be attributed to the following: Firstly, there is more rain in the long rain season, which negatively interferes with insect vector populations and hence their ability to transmit viruses; secondly, it has been said that most farmers buy seed from local market in the long rain season and use home saved seed for the short rain season. Lack of a functional formal seed system for beans in Kenya and probably limited interest in production of bean seed by commercial seed sector could be attributable reasons to the current scenario causing farmers to recycle bean seeds for a long time. This action coupled by the fact that there are more aphids transmitting and spreading the virus faster in fields. Thirdly, poor agronomic and

cultural practices favour the spread of the virus. This is supported by the fact that most farmers do not recognise this virus problem and so they unknowingly spread the virus by farm implements, planting seed with high virus load, very minimal crop rotation and inadequate weed and pest control measures as was observed in some fields.

BCMD presence in western Kenya as detected by serology concurs with earlier studies by Mutuku *et al.* (2018). ELISA, PCR and NGS detected BCMNV in bean samples collected from different parts of Western Kenya. It appears that BCMNV is the most predominant virus in common bean in this region. This could be attributed to evolutionary tendencies of RNA viruses as reported by Worobey and Holmes (1999). Previous studies by Mangeni *et al.* (2014) found both BCMNV and BCMV infecting bean plants in Western Kenya. Bean losses are made worse by infection occurring at the same time with two or more viruses. This is because multiple virus-infected plants show severe symptoms of stunted growth and low yield in quantity and quality (Hobbs et al. 2003). However, co-infection can, in at least some cases, attenuate the effects of individual viruses on plant–vector interactions to the extent that such effects are adaptive for the virus, and therefore have harmful effects on disease spread (Penaflor *et al.,* 2016). Most viruses infecting beans were not expected to be found by ELISA because the antisera was limited to detection of BCMNV and BCMV the causative agents of BCMD. Therefore, under agricultural intensification system of farming, mostly used in the region, there is a mixture of two or more crops per season per plot or in adjacent plots. Since, the vectors of these viruses are polyphagous, they may probe on all of the legumes indiscriminately thereby picking and spreading the viruses. With evolution and the effects of climate change, many vectors and the virus, BCMNV refuge

in bean and probably, other legume plants as well and vice versa. High incidence of BCMD is an indication that not much care is taken to control them, probably because most farmers do not recognise virus diseases and link symptoms to other causes such as mineral deficiency of poor soils. This observation is supported by the fact that most farmers plant their own seed (Opole *et al.,* 2003), which have been selected not based on viral disease considerations. For this farmers need awareness on virus diseases and how they can be controlled. It is therefore important note that in order to know the extensive nature of quasi-species for select viruses, a high percentage of sequence coverage is important (Kehoe *et al.,* 2014; Jo *et al.,* 2016). The detection of viruses using the conventional methods such as PCR succeeded by Sanger sequencing is relatively low and the technique is time consuming while NGS approaches give out numerous reads that are large enough to detect the target virus's quasi-species. Moreover, prior knowledge of the virus sequence is not to reveal the sequence of a virus or viroid, which hitherto may have not been known. Hence, several studies associated with virus and viroid quasi-species, prefer to use diverse NGS techniques instead of those based on the conventional PCR (Kutnjak *et al.,* 2015).

5.2 Molecular characterisation of Bean Common Mosaic Necrosis Virus

Complete nucleotide sequence analysis of 10 BCMNV isolates from western Kenya revealed a complete genome sequence of BCMNV strain present in the region. It is clear that BCMNV isolates collected are strains of BCMNV. Molecular detection for BCMNV being (+) ssRNA genome requires extraction of total RNA. The detection of

BCMNV from samples together with information on bean varieties is important to define actions necessary to manage the virus in western Kenya.

In this study, a full genome sequence of BCMNV was detected by NGS technology via Illumina Miseq platform in a bean sample from Bungoma county.

Phylogenetic analysis of BCMNV BG 12 isolate nucleotide sequences conducted using MEGA7 and the maximum likelihood algorithm to investigate the relationship between the isolate and 18 other BCMNV sequences in the GenBank as of October 2018. This analysis placed BCMNV BG 12 isolate alongside an isolate from Oregon and that from Tanzania, an indication that the three isolates are closely related and all belong to strain TN-1 (Tanzania). This can only be possible if the Oregon isolate was either taken from East Africa or the East African ones came from there most likely in bean or other legume seeds for planting.

5.3 Screening local germplasm for resistance against Bean Common Mosaic Virus

Ten popular bean varieties were susceptible to BCMNV while four were tolerant to the virus infection. In addition, the other legume species such as cowpea, greengrams, soybean and groundnuts were all susceptible to BCMNV infection.

Several virus control measures have been examined and are in use but host plant resistance seems the most practical, economical and environmentally friendly method (Wagara and Kimani, 2007). Because BCMNV and BCMV detected by serology were in mixed infections, breeding for single virus resistance may not be of much help. It is therefore worth an effort to breed for multiple-virus resistance to counter this problem as

suggested by Orawu (2013). Resistant bean varieties (KK 072 and KK RIL 05) observed in this study presents a potential source of resistance in management of BCMNV. The varieties could possess the right combinations of resistance genes against BCMNV. Previous studies have shown these two varieties contain SCAR DNA markers SW15 linked to BCMV dominant resistance *I*- gene though they have not been probed for recessive resistance genes required for pyramiding with the *I* gene that induces hypersensitive black rot symptom of BCMV (Mangeni *et al.,* 2014). The dominant *I* gene is known to inhibit all known strains of BCMV but can be overcome by necrosis-inducing strains, the BCMNV (Miklas *et al*., 2000). The dominant *I* gene, however can be combined with appropriate recessive resistance genes in order to protect it. These combinations can restrict, prevent or delay extreme hypersensitive response in plants infected with BCMNV (Bello *et al.,* 2014)

Incidence levels of viral diseases have been revealed by our studies in bean crops in all growing counties of Western Kenya. This is because, most of the viruses are seedborne and the climate favours virus vector insects (aphids) coupled by the fact that farmers plant their own seen not certified for virus freedom. The results indicate that seeds are the major source of virus infection, a finding supported by earlier reports (Demiski, 1975; Johansen *et al.,* 1994; Sastry, 2013) that observed that infected seed increased virus incidence by 25 % in groundnuts compared to certified seed. Disease free seed is laudable because insects spread the virus from some source, which if absent there is a likelihood of aphids infected with persistently-transmitted plant virus, becoming a source of inoculum. Despite the absence of infected plants in the vicinity, if aphids travel far (or carried on clothing/farm equipment) or aphids residing on other plants –

they can be a valid source for virus inoculum. Identification of BCMD resistant legume genotypes is very much essential and screening to identify stable resistance source. However, the nature of disease resistance being complex makes the identification of resistant and susceptible lines cumbersome through conventional screening techniques and therefore DNA based molecular markers such as RAPD, RFLP, AFLP and SSR will be useful to assess genetic diversity of genotypes (Manjunatha *et al.,* 2016).

5.4 Conclusion

BCMD is a widespread disease across the counties surveyed in western Kenya and that the virus is still a major disease in common bean. Viral symptom incidence was found to be high and widely distributed in counties. Symptoms of BCMV and BCMNV are indistinguishable in the field especially in western Kenya and hence the two viruses can be distinguished through serology and molecular means. Severity of viral symptoms could be due to mixed infection by two viruses, different strains and abiotic factors in counties or a combination of these. Diversity of BCMNV is less varied even with the geographical distance.

Most of the popular bean cultivars grown in the region are susceptible to BCMNV when inoculated mechanically. Other popular legume varieties such as those of cowpea, groundnut, soybean and green grams popularly grown in western Kenya are hosts of BCMNV. Use of virus resistant variety is the best alternative and durable method to alleviate occurrence of BCMD.

5.5 Recommendations

In view of these findings, farmers should be made aware of the potential risks of spread of viruses through planting virus contaminated seed and also practise good agronomic practises for legume production. To improve yields of common bean and other legume crops sustainably, farmers should always be encouraged to plant seeds certified to be virus free.

Resistance genes introgression in popular bean varieties can be explored for better resistance to the virus. Markers linked to specific resistance genes may be useful in selection for breeding and therefore make genetic diagnostics for resistance to BCMNV faster and efficient.

Planting of common bean as pure stand (as the sole legume) or intercrop with non legume plants that are non hosts for BCMNV is recommended in western Kenya. Moreover, tolerant varieties such as Imbeko, KK/RIL5/Red 13, Okwoto, RIL05/CAL 194 are therefore recommended for common bean production in high disease severity areas as varieties with compound resistance to varieties are explored through breeding.

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Appendix

Appendix 1: Survey area farms

COUNTY	AEZONE	ALTITUDE	LONGTUDE	LATITUDE	VARIETY	Season
Busia	LM1	1296	E034.31286	N00.31286	Rosecoco	Long rain
Busia	LM1	1296	E034.31286	N00.31286	Rosecoco	Long rain
Busia	LM1	1296	E034.31286	N00.31286	Rosecoco	Long rain
Busia	LM1	1257	E034.23760	N00.29552	wairimu	Long rain
Busia	LM1	1257	E034.23760	N00.29552	wairimu	Long rain
Busia	LM1	1257	E034.23760	N00.29552	yellow	Long rain
Busia	LM1	1257	E034.23760	N00.29552	rosecoco	Long rain
Busia	LM1	1264	E034.23464	N00.29288	tulu	Long rain
Busia	LM1	1264	E034.23464	N00.29288	punda	Long rain
Busia	LM1	1264	E034.23464	N00.29288	Tulu	Long rain
Busia	LM1	1264	E034.23464	N00.29288	yellow	Long rain
Busia	LM1	1310	E034.28268	N00.31942	Rosecoco (Purple)	Long rain
Busia	LM1	1310	E034.28268	N00.31942	Rosecoco	Long rain
Busia	LM1	1310	E034.28268	N00.31942	kk8	Long rain
Busia	LM1	1284	E034.32163	N00.32496	rosecoco	Long rain
Busia	LM1	1284	E034.32163	N00.32496	rosecoco	Long rain
Busia	LM1	1284	E034.32163	N00.32496	yellow	Long rain
Busia	LM1	1280	E034.32230	N00.32128	wairimu	Long rain

Busia	LM1	1280	E034.32230	N00.32128	wairimu	Long rain
Busia	LM1	1280	E034.32230	N00.32128	rosecoco	Long rain
Busia	LM2	1185	E034.19242	N00.40588	rosecoco	Long rain
Busia	LM2	1193	E034.20306	N00.41242	rosecoco	Long rain
Busia	LM2	1193	E034.20306	N00.41242	rosecoco	Long rain
Busia	LM2	1193	E034.20306	N00.41242	rosecoco	Long rain
Busia	LM2	1193	E034.20306	N00.41242	Yellow	Long rain
Busia	LM3	1382	E034.38951	N00.71284	Rosecoco	Short rain
Busia	LM3	1379	E034.38913	N00.71270	Rosecoco	Short rain
Busia	LM3	1390	E034.39028	N00.71010	Rosecoco	Short rain
Busia	LM3	1395	E034.39230	N00.71068	Yellow	Short rain
Busia	LM3	1395	E034.39230	N00.71068	Yellow	Short rain
Busia	LM3	1336	E034.35728	N00.74348	Rosecoco	Short rain
Busia	LM3	1379	E034.38913	N00.71270	Rosecoco	Short rain
Busia	LM3	1385	E034.38935	N00.71435	Rosecoco	Short rain
Busia	LM3	1440	E034.37812	N00.69597	Yellow	Short rain
Busia	LM3	1430	E034.37445	N00.69515	Rosecoco	Short rain
Busia	LM3	1385	E034.38935	N00.71435	Rosecoco	Short rain
Busia	LM3	1382	E034.38951	N00.71284	Rosecoco	Short rain
Busia	LM3	1430	E034.37445	N00.69515	Rosecoco	Short rain
Busia	LM3	1395	E034.39273	N00.71085	Rosecoco	Short rain
Busia	LM3	1407	E034.39782	N00.70042	Rosecoco	Short rain
Busia	LM2	1410	E034.39635	N00.70070	Rosecoco	Short rain

Busia	LM3	1361	E034.36237	N00.73834	Rosecoco (Purple)	Short rain
Busia	LM3	1395	E034.39273	N00.71085	Rosecoco	Short rain
Busia	LM3	1364	E034.36440	N00.74005	wairimu	Short rain
Busia	LM3	1363	E034.36406	N00.74013	Rosecoco	Short rain
Siaya	LM3	1340	E034.35617	N00.07417	Rosecoco	Long rain
Siaya	LM3	1340	E034.35617	N00.07417	Rosecoco	Long rain
Siaya	LM3	1340	E034.35617	N00.07417	Rosecoco	Long rain
Siaya	LM3	1340	E034.35617	N00.07417	Rosecoco	Long rain
Siaya	LM3	1367	E034.34445	N00.05377	wairimu	Long rain
Siaya	LM3	1367	E034.34445	N00.05377	Rosecoco	Long rain
Siaya	LM3	1367	E034.34445	N00.05377	Rosecoco	Long rain
Siaya	LM3	1290	E034.43913	S00.04043	Rosecoco	Long rain
Siaya	LM3	1290	E034.43913	S00.04043	Rosecoco	Long rain
Siaya	LM3	1176	E034.32233	S00.23762	Punda	Long rain
Siaya	LM3	1176	E034.32233	S00.23762	Punda	Long rain
Siaya	LM3	1176	E034.32233	S00.23762	Punda	Long rain
Siaya	LM3	1191	E034.32590	S00.23710	KK8	Long rain
Siaya	LM3	1191	E034.32590	S00.23710	yellow	Long rain
Siaya	LM3	1191	E034.32590	S00.23710	KK8	Long rain
Siaya	LM3	1182	E034.32560	S00.23590	Rosecoco	Long rain
Siaya	LM3	1182	E034.32560	S00.23590	Rosecoco	Long rain
Siaya	LM3	1182	E034.32560	S00.23590	Rosecoco	Long rain
Siaya	LM3	1291	E034.27107	N00.10636	Rosecoco	Long rain

Siaya	LM3	1291	E034.27107	N00.10636	Rosecoco	Long rain
Siaya	LM3	1303	E034.34433	S00.08766	Yellow	Short rain
Siaya	LM3	1303	E034.34433	S00.08766	rosecoco	Short rain
Siaya	LM4	1223	E034.33007	S00.33214	rosecoco	Short rain
Siaya	LM4	1223	E034.33007	S00.33214	Punda	Short rain
Siaya	LM4	1197	E034.34181	S00.32585	Rosecoco	Short rain
Siaya	LM4	1197	E034.34181	S00.32585	Rosecoco	Short rain
Siaya	LM4	1223	E034.33007	S00.33214	Yellow	Short rain
Siaya	LM3	1336	E034.35868	S00.07524	Rosecoco	Short rain
Siaya	LM3	1336	E034.35868	S00.07524	Rosecoco	Short rain
Siaya	LM3	1336	E034.35868	S00.07524	Rosecoco	Short rain
Siaya	LM3	1336	E034.35868	S00.07524	Rosecoco	Short rain
Busia	LM1	1286	E034.27865	N00.31569	Rosecoco	Short rain
Siaya	LM3	1301	E034.34427	S00.08644	Yellow	Short rain
Siaya	LM3	1301	E034.34427	S00.08644	Punda	Short rain
Siaya	LM3	1301	E034.34427	S00.08644	Yellow	Short rain
Bungoma	LM2	1476	E034.54118	N00.61600	Rosecoco	Long rain
Bungoma	LM2	1476	E034.54118	N00.61600	Rosecoco	Long rain
Bungoma	LM2	1476	E034.54118	N00.61600	Rosecoco	Long rain
Bungoma	LM2	1465	E034.54144	N00.61531	Rosecoco	Long rain
Bungoma	LM2	1465	E034.54144	N00.61531	Rosecoco	Long rain
Bungoma	LM2	1465	E034.54144	N00.61531	Rosecoco	Long rain
Bungoma	LM2	1465	E034.54144	N00.61531	Rosecoco	Long rain

Bungoma	LM2	1404	E034.46411	N00.69718	Rosecoco	Long rain
Bungoma	LM2	1404	E034.46411	N00.69718	Rosecoco	Long rain
Bungoma	LM2	1404	E034.46411	N00.69718	Rosecoco	Long rain
Bungoma	LM2	1404	E034.46411	N00.69718	Rosecoco	Long rain
Bungoma	LM2	1350	E034.44774	N00.68771	wairimu	Long rain
Bungoma	LM2	1350	E034.44774	N00.68771	Rosecoco	Long rain
Bungoma	LM2	1350	E034.44774	N00.68771	wairimu	Long rain
Bungoma	LM2	1350	E034.44774	N00.68771	Rosecoco	Long rain
Bungoma	LM2	1535	E034.76328	N00.61117	Rosecoco	Long rain
Bungoma	LM2	1535	E034.76328	N00.61117	Rosecoco	Long rain
Bungoma	LM2	1535	E034.76328	N00.61117	Rosecoco	Long rain
Bungoma	LM2	1591	E034.79065	N00.62522	wairimu	Long rain
Bungoma	LM2	1591	E034.79065	N00.62522	wairimu	Long rain
Bungoma	LM2	1591	E034.79065	N00.62522	Rosecoco	Long rain
Bungoma	LM2	1591	E034.79065	N00.62522	wairimu	Long rain
Bungoma	LM2	1591	E034.79065	N00.62522	Rosecoco	Long rain
Bungoma	LM2	1560	E034.80379	N00.62807	Rosecoco	Long rain
Bungoma	LM2	1560	E034.80379	N00.62807	Rosecoco	Long rain
Bungoma	LM2	1271	E034.40218	N00.59605	Rosecoco	Short rain
Bungoma	LM2	1271	E034.40218	N00.59605	Rosecoco	Short rain
Bungoma	LM2	1271	E034.40218	N00.59605	Rosecoco	Short rain
Bungoma	LM2	1271	E034.40218	N00.59605	Rosecoco	Short rain
Bungoma	LM2	1271	E034.40218	N00.59605	Rosecoco	Short rain

Bungoma	LM2	1283	E034.39667	N00.59429	Rosecoco	Short rain
Bungoma	LM2	1283	E034.39667	N00.59429	Rosecoco	Short rain
Bungoma	LM2	1283	E034.39667	N00.59429	Rosecoco	Short rain
Bungoma	LM2	1283	E034.39667	N00.59429	Rosecoco	Short rain
Bungoma	LM2	1283	E034.39667	N00.59429	Rosecoco	Short rain
Bungoma	LM2	1481	E034.53045	N00.60687	Rosecoco	Short rain
Bungoma	LM2	1514	E034.533119	N00.61361	Rosecoco	Short rain
Bungoma	LM2	1479	E034.52184	N00.61094	Rosecoco	Short rain
Bungoma	LM2	1490	E034.526390	N00.617222	Rosecoco	Short rain
Bungoma	LM2	1509	E034.58068	N00.62845	wairimu	Short rain
Bungoma	LM2	1557	E034.59395	N00.66004	Rosecoco	Short rain
Bungoma	LM2	1515	E034.60737	N00.66895	wairimu	Short rain
Bungoma	LM2	1538	E034.61226	N00.67426	wairimu	Short rain
Bungoma	UM2	1747	E034.72624	N00.82073	Rosecoco	Short rain
Bungoma	UM2	1935	E034.72564	N00.85590	Rosecoco	Short rain
Kakamega	UM1	1520	E034.78662	N00.14787	KK8	Long rain
Kakamega	UM1	1520	E034.78662	N00.14787	KK8	Long rain
Kakamega	UM1	1530	E034.66257	N00.05551	Rosecoco	Long rain
Kakamega	UM1	1530	E034.66257	N00.05551	Rosecoco	Long rain
Kakamega	UM1	1530	E034.66257	N00.05551	Rosecoco	Long rain
Kakamega	UM1	1519	E034.66122	N00.05523	Rosecoco	Long rain
Kakamega	UM1	1519	E034.66122	N00.05523	Rosecoco	Long rain
Kakamega	UM1	1519	E034.66122	N00.05523	Rosecoco	Long rain

Kakamega	UM1	1513	E034.66036	N00.05441	KK8	Long rain
Kakamega	UM1	1513	E034.66036	N00.05441	Rosecoco	Long rain
Kakamega	UM1	1513	E034.66036	N00.05441	Rosecoco	Long rain
Kakamega	UM1	1513	E034.66036	N00.05441	KK8	Long rain
Kakamega	UM1	1558	E034.74823	N00.00325	Rosecoco	Long rain
Kakamega	UM1	1558	E034.74823	N00.00325	Rosecoco	Long rain
Kakamega	UM1	1558	E034.74823	N00.00325	Rosecoco	Long rain
Kakamega	UM1	1558	E034.74823	N00.00325	Rosecoco	Long rain
Kakamega	UM1	1558	E034.74823	N00.00325	Rosecoco	Long rain
Kakamega	UM1	1600	E034.81551	N00.01565	wairimu	Long rain
Kakamega	UM1	1600	E034.81551	N00.01565	wairimu	Long rain
Kakamega	UM1	1600	E034.81551	N00.01565	wairimu	Long rain
Kakamega	UM1	1498	E034.66174	N00.05348	KK8	Short rain
Kakamega	UM1	1498	E034.66174	N00.05348	KK8	Short rain
Kakamega	UM1	1498	E034.66068	N00.05534	Rosecoco	Short rain
Kakamega	UM1	1498	E034.66068	N00.05534	Rosecoco	Short rain
Kakamega	UM1	1498	E034.66068	N00.05534	KK8	Short rain
Kakamega	UM1	1469	E034.62708	N00.21789	Rosecoco	Short rain
Kakamega	UM1	1469	E034.62708	N00.21789	Rosecoco	Short rain
Kakamega	UM1	1469	E034.62708	N00.21789	Rosecoco	Short rain
Kakamega	UM1	1469	E034.52639	N00.21789	Rosecoco	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	Rosecoco	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	Rosecoco	Short rain

Kakamega	UM1	1490	E034.62357	N00.22022	Rosecoco	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	Rosecoco	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	Rosecoco	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	wairimu	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	wairimu	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	Rosecoco	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	Rosecoco	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	wairimu	Short rain
Kakamega	UM1	1490	E034.62357	N00.22022	Rosecoco	Short rain
Homabay	LM2	1313	E034.57562	S0059917	Yellow	Long rain
Homabay	LM2	1313	E034.57562	S0059917	wairimu	Long rain
Homabay	LM2	1313	E034.57562	S0059917	Yellow	Long rain
Homabay	LM2	1313	E034.57562	S0059917	Rosecoco	Long rain
Homabay	LM2	1313	E034.57562	S0059917	wairimu	Long rain
Homabay	LM2	1338	E034.58366	S00.60474	KK8	Long rain
Homabay	LM2	1338	E034.58366	S00.60474	KK8	Long rain
Homabay	LM2	1338	E034.58366	S00.60474	KK8	Long rain
Homabay	LM2	1339	E034.58286	S00.60896	Rosecoco	Long rain
Homabay	LM2	1339	E034.58286	S00.60896	wairimu	Long rain
Homabay	LM2	1339	E034.58286	S00.60896	wairimu	Long rain
Homabay	LM2	1339	E034.58286	S00.60896	Rosecoco	Long rain
Homabay	LM2	1343	E034.58385	S00.61199	wairimu	Long rain
Homabay	LM2	1343	E034.58385	S00.61199	Rosecoco	Long rain

Homabay	LM2	1343	E034.58385	S00.61199	Yellow	Long rain
Homabay	LM2	1343	E034.58385	S00.61199	Rosecoco	Long rain
Homabay	LM4	1329	E034.12975	S00.70017	Rosecoco	Long rain
Homabay	LM4	1329	E034.12975	S00.70017	Rosecoco	Long rain
Homabay	LM4	1329	E034.12975	S00.70017	Rosecoco	Long rain
Homabay	LM4	1339	E034.12822	S00.70061	Rosecoco	Long rain
Homabay	LM2	1374	E034.54052	S00.57785	KK8	Short rain
Homabay	LM2	1327	E034.58371	S00.60816	Yellow	Short rain
Homabay	LM2	1327	E034.58371	S00.60816	Yellow	Short rain
Homabay	LM2	1327	E034.58371	S00.60816	Yellow	Short rain
Homabay	LM2	1327	E034.58371	S00.60816	Yellow	Short rain
Homabay	LM2	1327	E034.58371	S00.60816	Yellow	Short rain
Homabay	LM2	1345	E034.58086	S00.60979	Yellow	Short rain
Homabay	LM2	1337	E034.54174	S00.57543	KK8	Short rain
Homabay	LM2	1337	E034.54174	S00.57543	KK8	Short rain
Homabay	LM2	1337	E034.54174	S00.57543	KK8	Short rain
Homabay	LM2	1374	E034.54052	S00.57785	KK8	Short rain
Homabay	LM2	1362	E034.54165	S00.57765	wairimu	Short rain
Homabay	LM2	1374	E034.54052	S00.57785	wairimu	Short rain
Homabay	LM2	1345	E034.58086	S00.60979	Yellow	Short rain
Homabay	LM2	1336	E034.54420	S00.57751	Rosecoco	Short rain
Nandi	LH1	1952	E035.12769	N00.14391	Rosecoco	Long rain
Nandi	LH1	1952	E035.12769	N00.14391	Rosecoco	Long rain

Nandi	LH1	1952	E035.12769	N00.14391	Rosecoco	Long rain
Nandi	LH1	1950	E035.12580	N00.14555	Rosecoco	Long rain
Nandi	LH1	1950	E035.12580	N00.14555	Rosecoco	Long rain
Nandi	LH1	1950	E035.12580	N00.14555	Rosecoco	Long rain
Nandi	LH1	1950	E035.12580	N00.14555	Rosecoco	Long rain
Nandi	LH1	1950	E035.12580	N00.14555	Rosecoco	Long rain
Vihiga	UM1	1645	E034.80617	N00.11914	Rosecoco	Long rain
Nandi	LH1	1943	E034.12677	N00.14330	Rosecoco	Long rain
Nandi	LH1	1943	E034.12677	N00.14330	Rosecoco	Long rain
Nandi	LH1	1943	E034.12677	N00.14330	Rosecoco	Long rain
Nandi	LH1	1943	E034.12677	N00.14330	Rosecoco	Long rain
Nandi	LH1	1916	E035.12365	N00.13736	Rosecoco	Long rain
Nandi	LH1	1916	E035.12365	N00.13736	Rosecoco	Long rain
Nandi	LH1	1916	E035.12365	N00.13736	Rosecoco	Long rain
Nandi	LH1	1915	E035.12316	N00.13692	Rosecoco	Long rain
Nandi	LH1	1915	E035.12316	N00.13692	Rosecoco	Long rain
Nandi	LH1	1915	E035.12316	N00.13692	Rosecoco	Long rain
Nandi	LH1	1915	E035.12316	N00.13692	Rosecoco	Long rain
Nandi	LH1	1953	E035.13235	N00.14475	Rosecoco	Short rain
Nandi	LH1	1953	E035.13235	N00.14475	Rosecoco	Short rain
Nandi	LH1	1953	E035.13235	N00.14475	Rosecoco	Short rain
Nandi	LH1	1953	E035.13235	N00.14475	Rosecoco	Short rain
Nandi	LH1	1959	E035.13184	N00.14506	Rosecoco	Short rain

Nandi	LH1	1959	E035.13184	N00.14506	Rosecoco	Short rain
Nandi	LH1	1959	E035.13184	N00.14506	Rosecoco	Short rain
Nandi	LH1	1957	E035.13268	N00.14514	Rosecoco	Short rain
Nandi	LH1	1959	E035.13184	N00.14506	Rosecoco	Short rain
Nandi	LH1	1957	E035.13268	N00.14514	Rosecoco	Short rain
Nandi	LH1	1975	E035.13074	N00.14532	Rosecoco	Short rain
Nandi	LH1	1975	E035.13074	N00.14532	Rosecoco	Short rain
Nandi	LH1	1975	E035.13074	N00.14532	Rosecoco	Short rain
Nandi	LH1	1970	E035.12898	N00.14635	Rosecoco	Short rain
Nandi	LH1	1970	E035.12898	N00.14635	Rosecoco	Short rain
Vihiga	UM1	1572	E034.74918	N00.12742	Rosecoco	Long rain
Vihiga	UM1	1572	E034.74918	N00.12742	Rosecoco	Long rain
Vihiga	UM1	1572	E034.74918	N00.12742	Rosecoco	Long rain
Vihiga	UM1	1572	E034.74918	N00.12742	Rosecoco	Long rain
Vihiga	UM1	1572	E034.74918	N00.12742	Rosecoco	Long rain
Vihiga	UM1	1572	E034.74918	N00.12742	Rosecoco	Long rain
Vihiga	UM1	1572	E034.74918	N00.12742	Rosecoco	Long rain
Vihiga	UM1	1572	E034.74918	N00.12742	Rosecoco	Long rain
Vihiga	UM1	1577	E034.75221	N00.12560	Rosecoco	Long rain
Vihiga	UM1	1577	E034.75221	N00.12560	Rosecoco	Long rain
Vihiga	UM1	1577	E034.75221	N00.12560	wairimu	Long rain
Vihiga	UM1	1577	E034.75221	N00.12560	Yellow	Long rain
Vihiga	UM1	1577	E034.75221	N00.12560	wairimu	Long rain

Vihiga	UM1	1577	E034.75221	N00.12560	wairimu	Long rain
Vihiga	UM1	1587	E034.70854	N00.07833	Yellow	Long rain
Vihiga	UM1	1548	E034.75764	N00.13129	Rosecoco	Long rain
Vihiga	UM1	1606	E034.76754	N00.11822	Wairimu	Long rain
Vihiga	UM1	1548	E034.75764	N00.13129	Rosecoco	Long rain
Vihiga	UM1	1548	E034.75764	N00.13129	Rosecoco	Long rain
Vihiga	UM1	1548	E034.75764	N00.13129	Rosecoco	Long rain
Vihiga	UM1	1523	E034.78485	N00.14465	Rosecoco	Short rain
Vihiga	UM1	1523	E034.78485	N00.14465	Rosecoco	Short rain
Vihiga	UM1	1523	E034.78485	N00.14465	Yellow	Short rain
Vihiga	UM1	1523	E034.78485	N00.14465	wairimu	Short rain
Vihiga	UM1	1523	E034.78485	N00.14465	Yellow	Short rain
Vihiga	UM1	1523	E034.78485	N00.14465	Yellow	Short rain
Vihiga	UM1	1523	E034.78485	N00.14465	Yellow	Short rain
Vihiga	UM1	1606	E034.81022	N00.13064	Rosecoco	Short rain
Vihiga	UM1	1606	E034.76754	N00.11822	Wairimu	Short rain
Vihiga	UM1	1606	E034.81022	N00.13064	Wairimu	Short rain
Vihiga	UM1	1606	E034.81022	N00.13064	Yellow	Short rain
Vihiga	UM1	1650	E034.80540	N00.11670	Rosecoco	Short rain
Vihiga	UM1	1650	E034.80540	N00.11670	Rosecoco	Short rain
Vihiga	UM1	1650	E034.80540	N00.11670	Rosecoco	Short rain
Vihiga	UM1	1650	E034.80540	N00.11670	Rosecoco	Short rain

Appendix 2: Plant disease score sheet

SURVEY ON BEAN COMMON MOSAIC DISEASE ON *Phaseolus vulgaris* IN WESTERN KENYA

Farmer ID………………… Date of interview: ……….……………… day/month/year
Enumerator's name ………………………………………………….……………………
County……………………. …...Sub county………………… AEZ…………...…………
Location………………………. Sub location ……………Village……………………..
GPS readings ……………………… /………..……………… / …..………………
Altitude (meters) Longitude (East) Latitude (N or S)

Household Characteristics

1. Respondent's name …………………………… Farmer's name ……………………
2. Farmer's gender (tick): Male [ ] Female [ ]
3. Household head (tick): Male [ ] Female [ ]
4. Age of farmer (years, tick) 15-24 [ ] 25-34 [ ] 35-44 [ ] 45-54 [ ] >55yrs [ ]
5. Education level (tick): Primary [ ] Secondary [ ] University [ ] others [ ]
6. Land ownership: Hired [ ] owned [ ] communal [ ] family [ ]
7. Farm size ………… (Ha/acres)
8. Mode of harvesting (once or piecemeal?) ……………………………………
9. What was the yield of bean last season …………………. (bags per ha).
10. How much was sold?.......….. (bags) Price per bag (KShs.)………..…
11. Do all the members of your family work on the farm? Yes [ ], No [ ]
12. Who spends more time in the farm? Males [ ], Females [ ], Both [ ]
13. Crop mixture………………………. fertility amendment………………………..

Common bean Characteristics

13. Is the current crop for food or cash? ____________________
14. Varieties grown _________________, ____________________, ______________
15. Age of the current bean crop in month's _________ planting date indication _____
16. Up to how many generations do you grow before sourcing for new seed? _________

Farming practices and constraints

17. Where do you get bean seed for planting?
Buy from KARI [ ] Neighbour [ ] own seed [ ] Market [ ] Others …………………
18. What is your view with regard to bean seed production? ____________________
____
19. What is your view with regard to bean seed availability and price?
____
20. What pests do you encounter in bean production?
……………………………………………………………………………………………
………………………………………….
21. What type of bean diseases do you know?
……………………………………………………………………………………………

22. Are there any diseases that can be spread through bean seed? Yes [ ] No [ ] I do not know [ ]
If yes, which ones do you know of?
....
23. Have you ever obtained any information on beans from extension or research persons/organizations? Yes [ ] No [ ]
If yes, what kind of information did you get? Sources of bean seed [ ]; planting method [ ]; Disease management [ ];Pest management [ ]; Harvesting [ ]; Utilization [ ]; Marketing [ ]other ……….……..
24. Please give information about farming technologies introduced to you.
___________________ ____
____

Bean common mosaic virus incidence and severity record

Number of plants affected/ Number of plants per 10 M^2	Plant part affected (Root, stem, leaves, pods)	Distribution (whole field, spots)	Severity* 0, 1, 2, 3
Mean			

*Severity: 0= No disease; 1= Mild; 2= Moderate; 3= Severe

Number of plants affected per M^2 – Select area most affected, 10 steps sq., count infected and total plants, (e.g. 20/50 indicates 20 plants infected out of 50 plants in the 10 x 10 steps square

***Disease Incidence**- Proportion of diseased plants per field or the proportion of diseased leaves per plant.

***Disease Severity**- Amount of disease on individual plants

Appendix 3: ELISA buffers

Coating buffer (pH 9.6)

1.59 g sodium carbonate (Na_2CO_3)

2.93 g sodium bicarbonate ($NaHCO_3$)

0.20 g sodium azide (NaN_3)

Dissolved in 900 ml H_2O, adjusted pH to 9.6 with HCl and made up to 1l

PBS (pH 7.4) phosphate buffer saline

8.0 g sodium chloride (NaCl)

0.2 g monobasic potassium phosphate (KH_2PO_4)

1.15 g dibasic sodium phosphate (Na_2HPO_4)

0.2 g potassium chloride (KCl)

0.2 g sodium azide (NaN_3)

Dissolved in 900 ml H_2O, adjusted pH to 7.4 with NaOH or HCI and made up to 1 l

PBS-Tween (PBST)

PBS + 0.5 ml Tween 20 per liter

Sample extraction buffer (pH 7.4)

PBST + 2% PVP (Serva PVP-1S polyvinyl pyrrolidone)

Conjugate buffer

PBST + 2% PVP + 0.2% egg albumin (Sigma A-S253)

Substrate buffer

97 ml diethanolamine

600 ml H_2O

0.2 g sodium azide (NaN_3)

Adjusted to pH 9.8 with HCI and made up to 1 liter with H_2O

Appendix 4: ELISA results of survey Bean (Phaseolus vulgaris L) samples from the short and long rain seasons in western Kenya.

Sample ID	Season	Virus	Spectrophometric absorbance value at wavelength of 405nm	Result
157	Short Rain	BCMV	0.207	-
154	Short Rain	BCMV	0.209	-
153	Short Rain	BCMV	0.137	-
152	Short Rain	BCMV	0.659	+
94	Short Rain	BCMV	0.175	-
95	Short Rain	BCMV	0.209	-
96	Short Rain	BCMV	0.757	+
97	Short Rain	BCMV	0.150	-
98	Short Rain	BCMV	0.171	-
99	Short Rain	BCMV	0.179	-
100	Short Rain	BCMV	0.130	-
101	Short Rain	BCMV	0.652	+
155	Short Rain	BCMV	0.173	-
89	Short Rain	BCMV	0.151	-
88	Short Rain	BCMV	0.689	+
87	Short Rain	BCMV	0.523	+
159	Short Rain	BCMV	0.170	-
162	Short Rain	BCMV	0.527	+
59	Short Rain	BCMV	0.187	-
163	Short Rain	BCMV	0.657	+
165	Short Rain	BCMV	0.148	-
227	Short Rain	BCMV	0.209	-

228	Short Rain	BCMV	0.594	+
231	Short Rain	BCMV	0.156	-
122	Short Rain	BCMV	0.202	-
218	Short Rain	BCMV	0.622	+
123	Short Rain	BCMV	0.154	-
126	Short Rain	BCMV	0.246	-
127	Short Rain	BCMV	0.711	+
128	Short Rain	BCMV	0.209	-
219	Short Rain	BCMV	0.568	+
32	Short Rain	BCMV	0.180	-
33	Short Rain	BCMV	0.203	-
34	Short Rain	BCMV	0.547	+
35	Short Rain	BCMV	0.256	-
36	Short Rain	BCMV	0.777	+
37	Short Rain	BCMV	0.164	-
38	Short Rain	BCMV	0.184	-
143	Short Rain	BCMV	0.587	+
142	Short Rain	BCMV	0.518	+
140	Short Rain	BCMNV	0.751	+
141	Short Rain	BCMNV	0.380	-
145	Short Rain	BCMNV	0.337	-
44	Short Rain	BCMNV	0.178	-
45	Short Rain	BCMNV	0.581	+
46	Short Rain	BCMNV	0.226	-
47	Short Rain	BCMNV	0.949	+
48	Short Rain	BCMNV	0.165	-
49	Short Rain	BCMNV	1.460	+

220	Short Rain	BCMNV	0.190	-
222	Short Rain	BCMNV	0.181	-
224	Short Rain	BCMNV	0.248	-
79	Short Rain	BCMNV	0.797	+
225	Short Rain	BCMNV	0.523	+
226	Short Rain	BCMNV	0.206	-
56	Short Rain	BCMNV	0.872	+
57	Short Rain	BCMNV	0.162	-
432	Short Rain	BCMNV	0.519	+
429	Short Rain	BCMNV	0.470	+
404	Short Rain	BCMNV	0.216	-
61-398g*	Short Rain	BCMNV	1.205	+
399	Short Rain	BCMNV	0.521	+
237	Short Rain	BCMNV	0.599	+
185	Short Rain	BCMNV	0.195	-
177	Short Rain	BCMNV	0.180	-
182	Short Rain	BCMNV	0.461	+
180	Short Rain	BCMNV	0.647	+
178	Short Rain	BCMNV	0.243	-
173	Short Rain	BCMNV	0.621	+
17-	Short Rain	BCMNV	0.323	-
177	Short Rain	BCMNV	0.573	+
175	Short Rain	BCMNV	0.426	+
181	Short Rain	BCMNV	0.454	+
235	Short Rain	BCMNV	0.194	-
231	Short Rain	BCMNV	0.599	+
232	Short Rain	BCMNV	0.159	-

77-231*	Short Rain	BCMNV	0.453	+
78	Short Rain	BCMNV	0.137	-
157	Short Rain	BCMNV	0.279	-
80-157*	Short Rain	BCMNV	0.437	+
156	Long rain	BCMV	0.136	-
155	Long rain	BCMV	0.968	+
163	Long rain	BCMV	0.171	-
160	Long rain	BCMV	0.617	+
163	Long rain	BCMV	0.188	-
164	Long rain	BCMV	0.497	+
319	Long rain	BCMV	0.136	-
314	Long rain	BCMV	0.743	+
329	Long rain	BCMV	0.421	-
165	Long rain	BCMV	0.487	+
91	Long rain	BCMV	0.586	+
166	Long rain	BCMV	0.626	+
93-166*	Long rain	BCMV	0.253	-
161	Long rain	BCMV	0.157	-
95-161*	Long rain	BCMV	0.867	+
96	Long rain	BCMV	0.196	-
318	Long rain	BCMV	0.544	+
320	Long rain	BCMV	0.545	+
151	Long rain	BCMV	0.294	-
321	Long rain	BCMV	0.661	+
101	Long rain	BCMV	0.143	-
102	Long rain	BCMV	2.290	+
323	Long rain	BCMV	0.123	-
228	Long rain	BCMV	1.124	+
191	Long rain	BCMV	2.172	+
190	Long rain	BCMV	0.303	-
189	Long rain	BCMV	0.123	-
187	Long rain	BCMV	0.453	+
109	Long rain	BCMV	0.176	-
351	Long rain	BCMV	0.405	+
372	Long rain	BCMV	0.413	-
374	Long rain	BCMV	0.379	-
375	Long rain	BCMV	0.973	+
377	Long rain	BCMV	0.128	-
378	Long rain	BCMV	0.236	-
379	Long rain	BCMV	0.625	+
380	Long rain	BCMV	0.138	-
385	Long rain	BCMV	0.335	-

94	Long rain	BCMV	0.422	+
120	Long rain	BCMV	1.391	+
121	Long rain	BCMV	1.587	+
191	Long rain	BCMV	0.128	-
198	Long rain	BCMV	0.117	-
187	Long rain	BCMV	0.526	+
186	Long rain	BCMV	0.137	-
184	Long rain	BCMV	0.731	+
185	Long rain	BCMV	0.528	-
194	Long rain	BCMV	0.122	-
195	Long rain	BCMV	0.538	+
97	Long rain	BCMV	0.130	-
99	Long rain	BCMNV	0.266	-
133-99*	Long rain	BCMNV	0.927	+
98	Long rain	BCMNV	0.832	+
135	Long rain	BCMNV	0.708	+
91	Long rain	BCMNV	0.861	+
92	Long rain	BCMNV	0.146	-
293	Long rain	BCMNV	0.459	+
287	Long rain	BCMNV	0.177	-
289	Long rain	BCMNV	0.998	+
264	Long rain	BCMNV	0.488	+
265	Long rain	BCMNV	0.198	-
294	Long rain	BCMNV	0.577	+
291	Long rain	BCMNV	0.452	+
252	Long rain	BCMNV	0.428	+
146-252*	Long rain	BCMNV	0.626	+
250	Long rain	BCMNV	0.131	-
253	Long rain	BCMNV	0.531	+
11	Long rain	BCMNV	0.125	-
255	Long rain	BCMNV	0.163	-
257	Long rain	BCMNV	0.531	+
256	Long rain	BCMNV	0.134	-
258	Long rain	BCMNV	0.126	-
123	Long rain	BCMNV	0.642	+
129	Long rain	BCMNV	0.619	+
259	Long rain	BCMNV	0.138	-
40	Long rain	BCMNV	0.545	+
36	Long rain	BCMNV	0.649	+
31	Long rain	BCMNV	0.422	+
55	Long rain	BCMNV	0.140	-
54	Long rain	BCMNV	0.450	+
45	Long rain	BCMNV	0.134	-
33	Long rain	BCMNV	0.416	+

51	Long rain	BCMNV	0.410	+
53	Long rain	BCMNV	0.135	-
167-51*	Long rain	BCMNV	0.625	+
29	Long rain	BCMNV	0.944	+
31	Long rain	BCMNV	1.124	+
15	Long rain	BCMNV	1.394	+
171	Long rain	BCMNV	0.121	-
63	Long rain	BCMNV	0.523	+
58	Long rain	BCMNV	2.127	+
65	Long rain	BCMNV	0.625	+
60	Long rain	BCMNV	0.124	-
66	Long rain	BCMNV	0.123	-
67	Long rain	BCMNV	0.437	+
70	Long rain	BCMNV	0.530	+
68	Long rain	BCMNV	0.491	+
57	Long rain	BCMNV	0.166	-
384	Long rain	BCMNV	0.530	+
465	Long rain	BCMNV	0.130	-
Positive control BCMNV		DSMZ	0.492	+
Negative Control		BUFFER	0.181	-
Positive control BCMV		DSMZ positive	0.531	+

*ID's labeled in two numbers were used to differentiate samples picked from different plants showing virus like symptoms on the same farm.

Appendix 5: Parameters used in CLC Genomic Workbench 9 for mapping reads to consensus viral/viroid genomes

Parameter	Parameter value
References	consensus viral/viroid genomes
Masking mode	No masking
Masking track	
Match score	1
Mismatch cost	1
Cost of insertions and deletions	Linear gap cost
Insertion cost	2
Deletion cost	2
Insertion open cost	6
Insertion extend cost	1
Deletion open cost	6
Deletion extend cost	1
Length fraction	*
Similarity fraction	**
Global alignment	false
Color space alignment	false
Color error cost	3
Auto-detect paired distances	true
Non-specific match handling	Map randomly

Appendix 6: Parameters used in CLC Genomic Workbench 9 for *de novo* assembly

Parameter	Parameter value
Mapping mode	Create simple contig sequences (fast)
Update contigs	true
Mismatch cost	2
Insertion cost	3
Deletion cost	3
Colorspace error cost	3
Length fraction	0.5
Similarity fraction	0.8
Colorspace alignment	true
Alignment mode	local
Match mode	random
Create list of un-mapped reads	false
Automatic bubble size	true
Bubble size	50
Automatic word size	true
Word size	20
Minimum contig length	50
Guidance only reads	
Perform scaffolding	true
Auto-detect paired distances	true
Create report	true

Appendix 7: Number of raw reads, trimmed reads and average length of trimmed reads for every sample sequenced.

Sample no.	rRNA depleted totRNA reads		
	Reads count before trimming	Reads count after trimming	Average reads length after trimming
I	4,771,226	3,253,734	270
II	2,558,334	1,082,434	261
III	3,246,366	1,251,158	232
IV	2,337,222	1,008,158	247
V	1,178,074	1,000,600	243
VI	2,741,564	1,063,102	232
VII	2,938,320	1,026,814	211
VIII	2,714,728	2,233,224	257
IX	1,104,234	1,000,012	210
X	2,098,115	1,987,441	223
XI	3,353,958	2,625,022	283