INCIDENCE AND MOLECULAR CHARACTERIZATION OF COMMON BEAN (*Phaseolus vulgaris* L.) VIRUSES IN TANZANIA

BEATRICE MWAIPOPO

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EXTENDED ABSTRACT

Common bean (*Phaseolus vulgaris* L.) is an important crop grown worldwide. It serves as a main source of protein and starch for over 300 million people in East Africa and Latin America. Despite its importance, production of common bean is constrained by viruses which cause important diseases of common bean. It is also known that different common bean genotypes respond differently to different viruses. In order to ascertain this information, the following specific objectives were established: (1) to characterize common bean viruses isolated from common bean using sequencing molecular techniques, (2) to determine the incidence and distribution of major viruses of common beans in Tanzania, (3) to characterize at molecular level and identify the wild plants harbouring the viruses infecting common beans in Tanzania, (4) to determine the suitable sizes of reads from deep sequenced small RNAs data for VirusDetect software-based detection of common bean viruses using low capability computers, (5) to determine genetic diversity of common bean cultivars and landraces using diversity array technology (DArT) in Tanzania, and (6) to evaluate the response of selected common bean genotypes to four common bean viruses in Tanzania. A total of 7756 common bean samples were collected during survey from five agricultural research zones, while 1340 wild plants samples were collected in four zones except western zone. Total RNAs were extracted using Cetyl trimethyl ammonium bromide method (CTAB). The symptomatic and asymptomatic common bean and wild plant samples were selected and pooled according to their respective zone. Nine and 10 pooled common bean and wild plants, respectively, including the wild plant samples (AIVN-1, AIVN-2 and AIVN-3) that were used in virus mechanical transmission study were sent to Fasteris SA (Switzerland) sequencing company, where small RNAs were sequenced using Illumina HiSeq 2500 platform. However, in wild plants was done on Illumina HiSeq 3000/4000 or Illumina NextSeq platform. Analysis of NGS sequences using VirusDetect Software revealed, 15 viruses, belonging to 11 genera, in the nine pooled common beans RNA samples. Two viruses namely, SBMV and *Tomato leaf curl Uganda virus*-

related *Begomovirus* were detected for the first time in common bean in Tanzania. In wild plants, NGS detected 122 viruse species in 20 genera. Out of these 122 viruses, 23 viruses from 12 genera were related to viruses known to infect common beans. *Peanut mottle virus* (PeMoV) and *Yam bean mosaic virus* (YBMV) were some of the viruses that were detected by RT-PCR in *Senna occidentalis* and *Senna hirsuta*, respectively. In mechanical inoculation study, out of 25 symptomatic wild plants samples only four wild plants which belonged to two plant species: *Ocimum basilicum* L. and *Bolusafra bituminosa* (L.) Kuntze, were able to infect common beans with *Cucumber mosaic virus* and a bromovirus closely related *Cowpea chlorotic mottle virus*, respectively. The wild plants RNA (collected from zones and those used fro mechanical inoculation), were identified by DNA barcoding. However, attempts to sequence 134 PCR products were only successful in only 89 (66.4% success rate). The DNA barcoded plants (89) belonged to 50 plant species. Using RT-PCR, detection of BCMV, BCMNV, CPMMV and SBMV viruses in common bean samples was done. The amplicon were scored to determine the incidence of viruses. Visually assessed field incidence of common bean viral diseases was as high 98%, in Missenyi district. The highest RT-PCR based incidence of BCMV and BCMNV were 36.7% and 76.7%, respectively. The incidence of SBMV ranged from 0 to 90.9%. In northern zone, the highest RT-PCR based SBMV incidence was 10%. The RT-PCR-based CPMMV incidence was highest in eastern zone where the incidence was as high as 46.7%. Also, using primers designed to NGS-based sequences, incidence of five viruses from wild plant (BCMV, BCMNV, CPMMV, YBMV and PeMoV) was determined in 1 430 wild plant samples by RT-PCR. Contrary to NGS results, BCMV, BCMNV and CPMMV were not detected in any wild plant samples. On the other hand, YBMV and PeMoV were detected in three and one wild plant samples, respectively. Genetic diversity of isolates of BCMV, BCMNV and CPMMV from common bean RNA samples was achieved through Sanger sequencing. The obtained nucleotide sequences encoding coat proteins of BCMV, BCMNV and CPMMV isolates revealed they were 90.2 to 100%, 97.1 to 100% and 82.9 to 99.1% similar to

each other, respectively. Some isolates, e.g., TZ:Mor 533:2015, had hallmarks of recombination events. In separate study a total of 360 common bean genotypes were grown in screenhouse and DNAs extracted using a CTAB method for genetic diversity analysis using the Diversity array technology (DArT). A total of 35 047 markers were identified of which 558 (1.6%) markers were highly informative. The genetic diversity dendrogram showed that, 278 and 82 common bean genotypes grouped in the Andean and Mesoamerican gene pools, respectively. Principal component analysis (PCA) based on genetic similarity confirmed that the genotypes belonged to two groups (252 genotypes) and their variation was 82.2%. When PCA was determined separately for the Andean and Mesoamerican gene pools, the within similarities were 82.94% and 84.60%, respectively. The response of common bean genotypes to BCMV, BCMNV, SBMV and CPMMV was studied in screen house using a Complete Randomized Design (CRD). Data on disease severity and area under disease progress curve (AUDPC) were subjected to one-way analysis of variance (ANOVA) and *post-hoc* analysis was done by using Tukey's test. Depending on the common bean genotype assessed, the symptoms appeared between 7^{th} and 12^{th} days post inoculation for all four viruses. Across all viruses used, disease severity was less than 50% in most common bean genotypes. The AUDPC ranged from 414 – 2 667, 0 – 1 586.7, 105.6 – 1 561.7 and 506 – 2 037 for BCMNV, BCMV, CPMMV and SBMV, respectively. Resistance to all four viruses ranged from susceptible to moderate resistance in inoculated common bean genotypes. However, Fibea and Selian 05 did not develop any symptoms when were inoculated with BCMV (AUDPC = 0). This work represents the first comprehensive surveys of common bean viruses in Tanzania using the of state-of-the-art next generation sequencing technique to simultaneously detect all viruses in common bean samples from five agricultural research zones in Tanzania. Using molecular information, primers were developed, optimized and used to detect viruses – including BCMV, BCMNV, CPMMV, and SBMV – in common bean and wild plants. The incidence of different viruses was determined and the distribution of common bean viruses was mapped.

DECLARATION

I, BEATRICE MWAIPOPO, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own .original work and that it has neither been submitted nor being concurrently submitted for a degree award in any other institution.

……. ……..

Beatrice Mwaipopo Date

(PhD Candidate)

The above declaration is confirmed by;

…… …….

Prof. Susan Nchimbi-Msolla Date

(Supervisor)

…… ……

Dr. P. J. Njau Date

(Supervisor)

…… ….……

Dr. Deusdedith R. Mbanzibwa Date

(Supervisor)

PUBLICATIONS

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A. Chapter one is the General Introduction.

B. Chapters two to seven are the six manuscripts out of each specific objective:

i. To characterize common bean viruses isolated from common bean (*P. vulgaris* L.) using sequencing molecular techniques
ii. To determine the incidence and distribution of major viruses of common beans (*P. vulgaris* L.) in Tanzania
iii. To characterize at molecular level the viruses of common bean in wild plants and identify the wild plants hosts of viruses in Tanzania
iv. To determine the suitable sizes of reads from deep sequenced small RNAs data for VirusDetect software-based detection of common bean (*P. vulgaris* L.) viruses using low capability computers
v. To determine genetic diversity of common bean (*P. vulgaris* L.) cultivars and landraces using diversity array technology (DArT) in Tanzania, and
vi. To evaluate the response of selected common bean genotypes to four viruses of common bean (*P. vulgaris* L.) in Tanzania

C. Chapter eight is the general conclusions and recommendations.

LIST OF ABBREVIATIONS AND ACRONYMS

+ssRNA	Positive sense single stranded ribonucleic acid
^{0}C	Degree Celsius
6K1	First 6 kilodalton protein
6K2	Second 6 kilodalton protein
ALS	Angular leaf spot
AMV	Alfalfa mosaic virus
ATF	Aphid transmission factor
BCMNV	Bean common mosaic necrosis virus
BCMV	Bean common mosaic virus
BDMV	Bean dwarf mosaic virus
BGMV	Bean golden mosaic virus
BGYMV	Bean golden yellow mosaic virus
BnYDV	Bean yellow disorder virus
BYMV	Bean yellow mosaic virus
CABMV	Cowpea aphid borne mosaic virus
$CaCl_2$	Calcium chloride
CaMV	Cauliflower mosaic virus
CBB	Common bacterial blight
cDNA	Complimentary DNA
CERV	Carnation etched ring virus
CI	Cytoplasm inclusion
CLP	Chlorotic line pattern
CLV	Carnation latent virus
CMoMV	Carrot mottle mimic virus

CMoV	Carrot mottle virus
CP	Coat protein
CPm	Minor capsid protein
CPMMV	Cowpea mild mottle virus
CPS	Capsular polysaccharide sythesis
CTAB	Cetyl trimethyl ammonium bromide
CuVCV	Cucumber vein clearing
DCL	Dicer like
DMV	Dahlia mosaic virus
DNA	Deoxyribonucleic acid
DNTPs	Deoxyribonucleotide triphosphate
EDTA	Ethylenediamine tetraacetic acid
elF4E	Eukaryotic translation initiation factor 4E
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ETBTV	Ethiopian tobacco bushy top virus
EtOH	Ethanol
EVCV	Eupatorium vein clearing virus
FAO	Food and Agriculture Organization
FMV	Figwort mosaic virus
G	Group
GIS	Geographic information system
GRV	Groundnut rosette virus
HC-Pro	Helper component proteinase
HEL	Helicase

HPLC	High performance liquid chromatograph
HRLV	Horseradish latent virus
ICTV	International committee on taxonomy of viruses
IPM	Integrated pest management
Kb	Kilobase
KDa	Kilodalton
Kg/ha	Kilogram per hectare
LSMV	Lettuce speckles mottle virus
MaYSV	Macroptilium yellow spot virus
MEGA	Molecular Evolutionary Genetics Analysis
M-MuLV	Moloney Murine Leukemia Virus
MMV	Mirabilis mosaic virus
MP	Movement protein
MTR	Methyltransferase
MUSCLE	Multiple Sequence Comparison by Log- Expectation
MYaV	Melon yellowing associated virus
NABP	Nucleic acid binding protein
NCMV	Northern cereal mosaic virus
NGS	Next generation sequencing
NIa	Nuclear inclusion a protease
Nib	Nuclear inclusion b
OPMV	Opium poppy mosaic virus
ORF	Open reading frame
P1	First protein
P3	Third protein

PCR	Polymerase chain reaction
PeMOV	Peanut mottle virus
PEMV	Pea enation mosaic virus
PIPO	Pretty Interesting *Potyviridae* ORF
PISPO	Pretty Interesting Sweet potato Potyvirus ORF
P-Pro	Papain-like protease
PvEV-1	Phaseolus vulgaris endornavirus 1
PvEV-2	Phaseolus vulgaris endornavirus 2
RdRP	RNA-dependent RNA polymerase
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RT	Dominant gene in *P. vulgaris* offers resistance to CMV
RT	Reverse transcriptase
RuFDV	Rudbeckia flower distortion virus
SbCMV	Soybean chlorotic mottle virus
SBMV	Southern bean mosaic virus
SG	Subgroup
SimMV	Sida micrantha mosaic virus
SPSS	Statistical package for the social sciences
SPuV	Soybean Putnam virus
SRDS	Small RNA Deep Sequencing
SVBV	Strawberry vein banding virus
TAV	Transactivator
TAV	Tomato Aspermy virus
TBTV	Tobacco bushy top virus

TGB	Triple gene block
TMoV	Tobacco mottle virus
ToLCArV	Tomato leaf curl Arusha virus
ToLCUV	Tomato leaf curl Uganda virus
ToLCYTV	Tomato leaf curl Mayotte virus
ToYLCV	Tomato yellow leaf curl virus
ToYLCV	Tomato yellow leaf curl virus
Tris-HCL	Tris Hydrochloride
TSV	Tobacco streak virus
Ty	Dominant gene controlling ToYLCV in tomato
UDP	Uridine 5’-diphosphate
UGT	UDP-glycosyltransferase
UTR	Untranslated viral region
VAP	Virion associated protein
VPg	Viral genomic protein
YBMV	Yam bean mosaic virus

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Common Bean as a Crop and its Origin

Common bean (*Phaseolus vulgaris* L.) is a very important crop in the world as it is regarded as the grain of hope (Pathania *et al*., 2014). It is a very important crop in supplying the nutrients in our bodies. It is also grown in a large area of the world and feeding many people in tropics and Africa (Zargar *et al*., 2017; Pathania *et al*., 2014). It is found in the family *Fabaceae.* It is an herbaceous annual plant. Its temperature requirement ranges from 15 ^{0}C to 27 ^{0}C (Salcedo, 2008). The genus *Phaseolus* contains many species, which differ in terms of growth habit, reproductive systems and their adaptations (Gept, 2001). There are two types of habits for common bean plant: erect herbaceous bushes (determinate) and climbing vines (indeterminate) (Ecocrop, 2013).

Common bean is a diploid (2n = 2x = 22) self-pollinating species that can also out-cross, albeit at very low rates (Gepts, 2001). The crop is thought to have originated in the Mesoamerica and Andean regions (Bitocchi *et al*., 2012; Gept, 1998). Marker-based and genome-wide analyses indicate that, the species falls into two main gene pools, namely the Andean and Mesoamerican gene pools (Gepts 1998; Kwak and Gepts, 2009; Schmutz *et al*., 2014). The Mesoamerican populations emerged much earlier than the Andean populations (Bitocchi *et al*., 2012). The divergence of the two gene pools resulted from separate domestication in the Andean (Peru, Bolivia and Argentina) and Mesoamerican populations (Central America and Mexico) (Gepts, 1998). From its center of origin, common bean is now grown in many areas of the world except in Antarctica (Pathania *et al*., 2014).

The crop is grown in the temperate and widely grown in subtropical regions. The bean was introduced in coastal areas of East Africa, especially Tanzania, in the 16th century by the Portuguese sailors and that further spread in inland areas occurred through the Arab traders (Wortmann *et al*., 2004). Its higher nutritious status makes the common bean even more important crop especially in the developing world. It is a source of cheap proteins and nutrients for over 500 million people in Africa, Latin America and the Caribbean (Cortés *et al*., 2013). The crop is also economically important especially in Sub-Saharan Africa as source of income and food security (Wortman *et al*., 2004; Ganesan and Xu, 2017).

The beans contain the compounds which are very important in preventing diseases and promoting health (Zargar *et al*., 2017). Apart from the supply of nutrients and cash income, common beans have a lot of uses: the straws are used as mulch or fodder for livestock, while the tender leaves can be cooked and eaten as vegetables. In addition, common bean roots are also known to form symbiotic association with a bacterium (Rhizobium) which has the ability to fix nitrogen in soils. As a result, the bean is commonly used in crop rotation or intercropping especially with cereal crops such as sorghum and maize (Sozer *et al*., 2016; Sharasia *et al*., 2017; Ojiem *et al*., 2014).

1.2 Common Bean Production in Africa

Common bean is an important food crop as it provides protein to poor people in Africa, Latin America and Caribbean (Cortés *et al*., 2013). It contains a lot of vitamins and minerals that are important for human consumption (Ganesan and Xu, 2017). Worldwide, 120 countries produce common beans. Africa produces about 17% of the

world's total, with 70% of the production occurring in Eastern Africa. Tanzania is the largest common bean producer in Africa with current production quantity estimated at 1 158 039 tons per annum. However, the yield of common bean (<1 035 kg/ha) is still low when compared with other African countries viz. Burundi (1 783.5 kg/ha; and production quantity is 371 892 tons per annum), Ethiopia (1 667.5kg/ha; 483 923 tons per annum), Uganda (1 503.4kg/ha; 1 008 410 tons per annum) and Cameroon (1 302.1 kg/ha; 390 816 tons per annum). Other countries which also common beans are produced are Rwanda (852.9kg/ha; 437 673 tons per annum) and Kenya (621.5 kg/ha; 728 168 tons per annum) (FAO, 2016). Based on this information and in a corresponding order, common bean yields are highest produced in Burundi, Ethiopia, Uganda, Cameroon, Tanzania, Rwanda and Kenya (FAO, 2016). African countries with the highest per capita consumption of common beans are Burundi, Kenya and Rwanda (Blair *et al*., 2010).

1.3 Common Bean Production in Tanzania

In Tanzania, the common beans are grown for their dry seeds and leaves. The leaves are eaten as vegetables in some parts of Tanzania while dry seeds are commonly eaten as the main or side dishes or sold on the local market for cash income (Hillocks *et al*., 2006; Ronner and Giller, 2013).

Tanzania is among the world’s leading producers of common bean (FAO, 2016). The area under common bean production has been increasing annually since 1961 to 2016 (Fig. 1.1), and so is the total production. The data in Fig. 1.2 shows that production of common beans in Tanzania was 1 158 039 tons in 2016, which represented more than

half of the total pulses produced in the country (FAO, 2016). Beans account for 71% protein of leguminous source in the country (Binagwa *et al*., 2016).

The crop is grown in medium and high altitude areas of the country where there is reliable rainfall and moderate temperature (Hillocks *et al*., 2006; Ronner and Giller, 2013). The most suitable areas for bean cultivation in Tanzania are Arusha, Manyara, Tanga and Kilimanjaro in the northern zone. Kagera, Mwanza and Mara in the lake zone, Kigoma in the western zone, and Iringa, Njombe and Songea in the southern highlands zone (Hillocks *et al*., 2006). The common bean varieties grown in Tanzania differ in terms of their seed coat colour and seed size and are grown depending on the geographical area and farmers preferences (Hillocks *et al*., 2006). There are small, medium and large seeded varieties in Tanzania (Fivawo and Msolla, 2012). There are also different ways in which common bean dishes are prepared but mainly depend on seed sizes (Fivawo and Msolla, 2012).

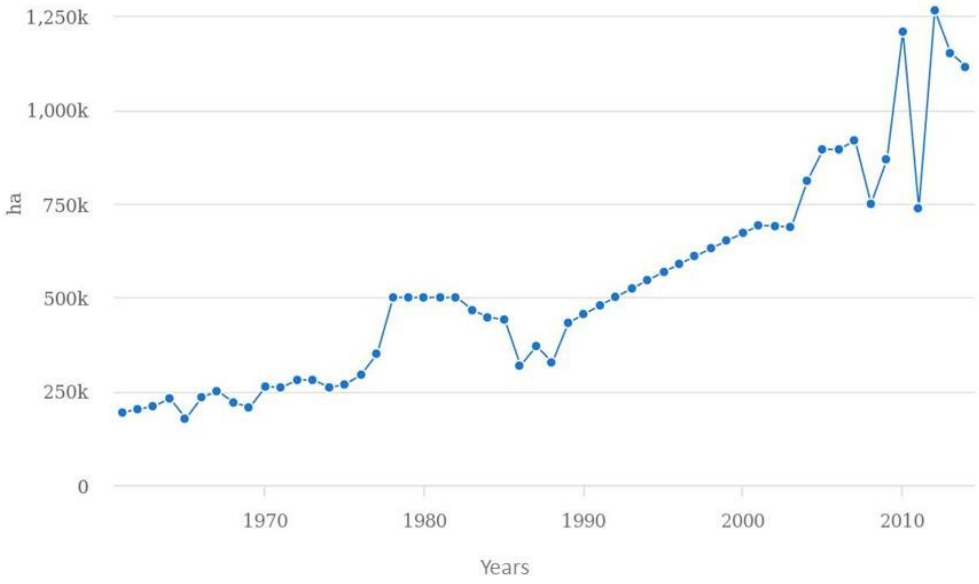

Figure 1. 1: Trend of harvest area of common bean in Tanzania from 1960 to 2016

Source: FAO (2016)

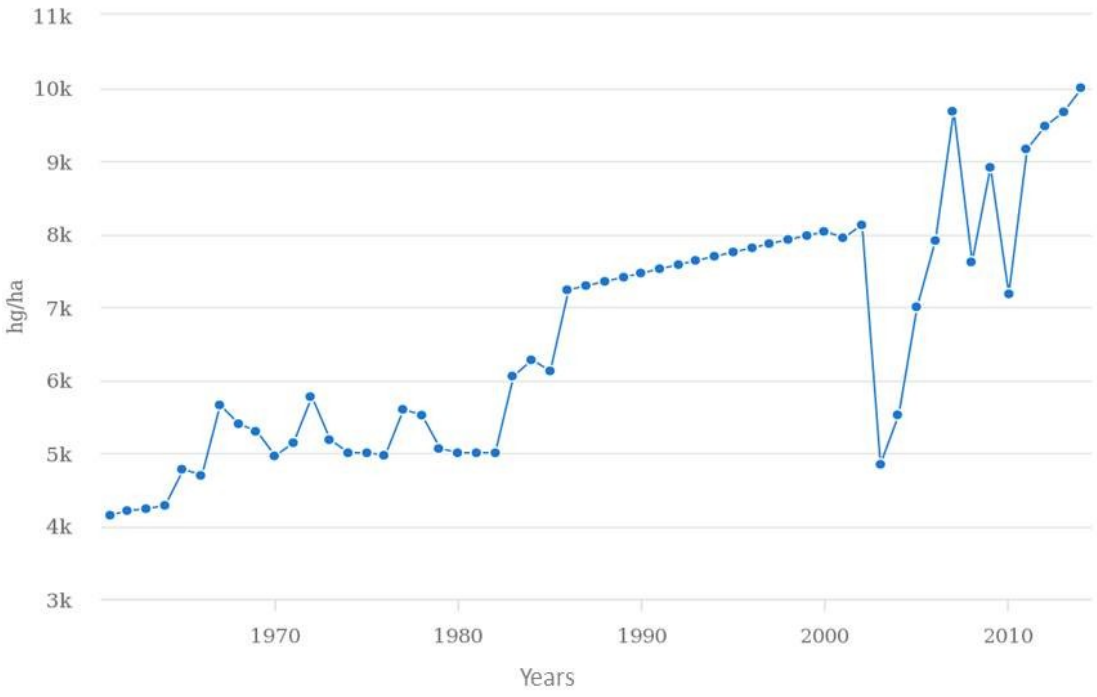

Figure 1. 2: Trend of common bean production in Tanzania from 1960 to 2016

Source: FAO (2016)

1.4 Common Beans Production Constraints

The common bean yields in Tanzania are very low when compared with the potential yields (Hillocks *et al*., 2006; Mwaipopo *et al*., 2017). The estimated average dry weight yield of common bean in Tanzania is less than 1 035 kg/ha which is low when compared with countries such as Uganda, Burundi and Ethiopia (FAO, 2016) where the potential yield is >1 500 kg/ha (Nchimbi-Msolla, 2013). The low yields of common bean are attributed to many biotic and abiotic factors. Among the abiotic factors are poor agronomic practices and extremes of environmental conditions such as low soil fertility especially deficiencies of nitrogen, phosphorus and potassium (Wortman and Allen, 1994).

The biotic factors limiting common bean production in Tanzania include insect pests and diseases caused by bacteria, fungi and viruses (Hillocks *et al*., 2006). These pathogens have ability to cause severe diseases on roots, foliage, stem, pods and seeds, which result in reduced quantities and qualities of common beans (Terán *et al*., 2013). The main insect pests of common beans both in the field and storage are thrips (*Magalurothrips sjostedii*), brown bug (*Clavigralla* spp.), pod borer (*Maruca testualis* and *Helicoverpa armigera)*, leaf beetles (*Ootheca* spp.), aphids (*Aphis* spp.), bruchid (*A. obtectus* and *Z. subfasciatus)* and stem maggot (*Ophiomyia* spp.) (Hillocks *et al*., 2006).

The most important non-virus diseases of common bean in Tanzania are bacterial brown spot (*Pseudomonas syringae* pv. *syringae* van Hall.), bacterial wilt (*Pseudomonas solanacearum* (Smith), bacterial wilt of bean (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens)*, common bacterial blight of bean (*Xanthomonas campestris* pv. *phaseoli*)**,** halo blight of bean (*Pseudomonas syringae* pv. *phaseolicola)*, angular leaf spot (*Phaeoisariopsis griseola* (Sacc.), bean anthracnose (*Colletotrichum lindemuthianum*), soybean root and stem rot (*Phytophthora megasperma*), web blight (*Thanatephorus cucumeris*), root rot (*Pythium* spp. and *Fusarium* spp.), rust (*Uromyces appendiculatus*), white mould (*Sclerotinia sclerotiorum*) and powdery mildew (*Erysiphe polygoni*) (Hillocks *et al*., 2006; Tryphone *et al*., 2013).

The viruses that have been reported to infect common bean in Tanzania are *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) (Njau and Lyimo, 2000), *Cowpea aphid-borne mosaic virus* (CABMV) and *Cowpea*

mosaic virus (Patel and Kuwite, 1982; Bock, 1973), *Cowpea mild mottle virus* (CPMMV) (Mink and Keswani, 1987), *Cucumber mosaic virus* (CMV) (Njau *et al*., 2006), *Peanut mottle virus* (PeMoV) (Bock, 1973), *Phaseolus vulgaris endornavirus* 1 (PvEV-1) and *Phaseolus vulgaris endornavirus* 2 (PvEV-2) (Nordenstedt *et al*., 2017). A comprehensive review on common bean viruses in Tanzania was published recently (Mwaipopo *et al*., 2017).

1.5 Common Bean Viruses: Occurrence and Genome Structures

1.5.1 *Potyvirus*

Potyvirus is a genus of viruses in the family *Potyviridae*. This family is the second largest group with a huge number of plant viruses that are recognized by the International Committee on Taxonomy of Viruses (ICTV). However, the family with the largest number of plant viruses is the *Geminiviridae,* which also contains notorious viruses of cassava. The genus *potyvirus* has many virus species that infect a wide range of plants (Garcia *et al*., 2014). The genomes of the viruses in the family *Potyviridae* are made up of a monopartite, positive sense, single-stranded RNA (+ssRNA), however, in nature, bipartite genomes are known to exist for viruses in the genus *Bymovirus* (Ivanov *et al*., 2014). The genomic molecule of potyviruses is approximately 10 kb long (Fang *et al*., 1995).

The potyviral genome has a long open reading frame (ORF), known as polyprotein, that is cleaved by the first protein (P1) protease, helper component protease (HC-Pro) and the nuclear inclusion a protease (NIa-Pro) into ten mature proteins (Adam *et al*., 2005). The potyviral proteins include first protein (P1), helper component proteinase (HC-Pro), third protein (P3), first 6 kilodalton protein (6K1), cytoplasmic inclusion

(CI), second 6 kilodalton protein (6K2), genome-linked viral protein (VPg), nuclear inclusion a protease (NIa), nuclear inclusion b (NIb) and major coat protein, also known as capsid protein (CP). These proteins perform different roles such as virus transmission, movement, infection and replication in plants (Table 1.1).

The potyviral genomes have been shown to have additional short open reading frames such as Pretty Interesting Potyvirus ORF (PIPO) and a recently discovered Pretty Interesting Sweet potato Potyvirus ORF (PISPO) (Chung *et al*., 2008; Untiveros *et al*., 2016). The PIPO is embedded within the P3 cistron, and is expressed as the trans-frame P3N-PIPO protein by a polymerase slippage mechanism at a conserved GA6 sequence (Chung *et al*., 2008; Rodamilans *et al*., 2015). Thus, PIPO is essential for virus intercellular movement. At the 5' end, the PIPO is linked to the virus-encode protein viral genome- linked protein (VPg) while at the 3' is polyadenylated (Ivanov *et al*., 2014).

Some potyviruses such as *Euphorbia ringspot virus* (EuRSV) have unusually large genomes (approximately 10.5 kb) whose polyprotein is cleaved into 11 proteins because HAM1h like sequence is recombined between the NIb and CP (Knierim *et al.*, 2017). This additional gene (*HAM1h*) was first observed in EuRSV and *Cassava brown streak virus* and is speculated to protect viruses against mutations (Mbanzibwa *et al.*, 2009; Tomlinson *et al*., 2019).

Table 1. 1: Virus polyproteins and their function

No.	Protein	Function
1	P1	A serine proteinase catalyses proteolysis between itself and HC-Pro[1], virus replication[2], involved in suppression of gene silencing[3]
2	HC-Pro	Viral cell to cell and long distance movement[4], cleavage activities[5], genome replication[6], gene silencing suppressor[7], vector transmission[8], viral synergism[9], Inhibit endonuclease activity[10]
3	P3	Viral replication and symptoms development[11.]
4	6K1	Induces the endoplasmic reticulum (ER) derived replication vesicles that target the chloroplast for robust the viral replication[12]
5	CI	RNA helicase and cell to cell movement [13], assist the viral genome amplification[14]
6	6K2	Involved in replication complex to the endoplasmic reticulum (ER) [15], long distance movement and symptoms induction independently[16]
7	VPg	Regulation of gene expression by boosting the viral RNA amounts[17], Role in potyviral movement[18], viral infection cycle (replication and translation) and interacting with eIF4E translation initiation factor[18]
8	NIa	Cleavage of most sites in the polyprotein[10]
9	NIb	Catalyzes the replication of RNA from RNA template[20]
10	HAM1h	Unknown function but is thought to protect viruses against mutation[3]
11	CP	Cell to cell and long-distance movement of the virus[21], vector transmission[22] and viral replication[23]

[1]Verchot and Carrington (1995), [2]Kasschau *et al*. (2003), [3]Mbanzibwa *et al*. (2009), [4]Rojas *et al*. (1997), [5]Carrington and Herdon (1992), [6]Kasschau and Carrington (1995), [7]Anandalakshmi *et al*. (1998), [8]Govier *et al*. (1977), [9]Pruss *et al*. (1997), [10]Ballut *et al*. (2005), [11]Rodriguez-Cerezo *et al*. (1993), [12]Wei *et al*. (2008), [13]Carrington *et al*. (1998), [14]Fernandez *et al*. (1997), [15]Schaad *et al*. (1997), [16]Spetz and Valkonen (2004), [17]Eskelin *et al*. (2010), [18]Keller *et al*. (1998), [19]Anindya *et al*. (2004), [20]Haldeman-Cahill *et al*. (1998), [21]Dolja *et al*. (1995), [22]Blanc *et al*. (1998), [23]Merits *et al*. (1998)

1.5.1.1 Bean common mosaic virus (BCMV) and Bean common mosaic necrosis virus (BCMNV)

Bean common mosaic virus and Bean common mosaic necrosis virus are the most important viruses of common bean that occur all over the world, wherever the crop is grown (Worrall *et al*., 2015). The mosaic disease of bean was first reported in 1897 in Russia by Ivanowski, and the cause of the disease was described as bean mosaic virus

1 by Stewart and Reddick (1917). Both BCMV and BCMNV belong to the genus *Potyvirus* in the family *Potyviridae.* These viruses can cause up to 80% bean yield losses (Drijfhout, 1991).

The two viruses are genetically related and both infect common bean plants. Previously only BCMV was known and was referred to as serotype A and serotype B, but now are known to belong to two distinct species (BCMV and BCMNV) in the same family. This is due to their differences in serology, high-performance liquid chromatography (HPLC) (Mckern *et al*., 1992) and variation in their reactions on bean differential hosts *(*Mink and Silbernagel, 1992). In bean cultivars possessing the dominant *I* gene, BCMNV causes the lethal systemic vascular necrosis (Silbernagel *et al*., 1986). Also, coat protein gene sequences revealed variability between BCMV and BCMNV (Berger *et al*., 1997).

Both BCMV and BCMNV are transmitted through seeds and by several species of aphids in a non-persistent manner (Omunyin, 1984; Mukeshimana *et al*., 2003; Zettler, 1969). Their genome size is approximately 10 kb, which cleaves into 10 functional proteins (Bravo *et al.*, 2008). Both viruses exist as a population of strains. The BCMV strains include: NL-1, NL-2, NL-4, NL-7, NL-6, NWA-1, NY 15, PR 1, RU-1, US1, US2, US, US4, US5, US6, US7 and US 10 while TN1, NL3, NL5 and NL8 belong to BCMNV (Melgarejo *et al*., 2007; Worrall *et al*., 2015; McKern *et al*., 1992; Larsen *et al*., 2011).

The BCMV and BCMNV have a wide host range including plants in the families *Amaranthaceae, Chenopodiaceae, Leguminosae - Caesalpinioideae, Leguminosae-Papilionoideae, Solanaceae and Tetragoniaceae* (Bos and Gibbs, 1995*).*

In Tanzania, mechanical inoculation of isolates of BCMNV and BCMV onto wild legume seedlings caused infections in five of the six wild legumes inoculated. The infected legumes were *Senna occidentalis*, *Senna obtusifolia*, *Cassia floribunda*, *Crotalaria spp*. and *Rhynchosia minima* (Njau and Lyimo, 2000).

1.5.1.2 Bean yellow mosaic virus (BYMV)

Bean yellow mosaic virus is another *potyvirus* that infects common beans. It is transmitted by many aphids' species in a non-persistent manner. Some of the aphid species known to transmit this virus are *Myzus persicae* (Sulzer), *Acyrthosiphon pisum* (Harris), and *Aphis fabae* (Scopoli). The virus can also be transmitted mechanically (Hampton, 2005). Its genome size is approximately 10 kb, and cleaved into the proteins P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg , NIa-Pro, NIb, and CP (Guyatt *et al*., 1996). BYMV can infect a wide range of plant species, as it has been detected in *Freesia spp*, *Gladiolus hortulanus*, *Lathyrus odoratus*, *Lupinus albus*, *Viola odoratus* (Gorter, 1977) and *Pisum sativum* (Jooste *et al*., 2001). Based on symptoms, Wallace and Wallace (1944) suggested BYMV exists in Tanzania. This virus has been confirmed to occur in common bean plants in Kenya by serology method (Vetten and Allen, 1991).

1.5.1.3 Peanut mottle virus (PeMoV)

Peanut mottle virus is a *Potyvirus* that was discovered for the first time in groundnuts (*Arachis. hypogea*) (Beikzadeh *et al*., 2015). It is transmitted in a non-persistent

manner by a number of aphid species including *Aphis craccivora*, *A. gossypii*, *Hyperomyzus lactucae*, *Myzus persicae* and *Rhopalosiphum padi* (Behncken, 1970). It is also known to be seed transmitted in host crops, which is the reason for its worldwide spread (Beikzadeh *et al*., 2015).

The virus infects many legume species such as soybeans (*Glycine max*), French beans, peas (*Pisum sativum*) and various weed and wild legume species including *Cassia* (Bock and Kuhn, 1975; Behncken, 1970). The symptoms of PeMoV in common bean include systemic necrosis on the leaves, petioles, stems and Pods (Silbernagel and Mills, 1991). There are several strains of PeMoV, named after the symptoms they cause in groundnut plants. These strains are referred to as: M-1 and M-2 (mild mottle), N (necrosis), S (severe strain) and CLP (chlorotic line pattern) (http://www. dpvweb.net/ dpv/showdpv.php?dpvno=141). Strains M-1 and M-2 cause similar symptoms on groundnut but their symptoms are different on pea; their local lesions on bean are of different sizes (Paguio and Kuhn, 1973). The symptoms caused by other strains are distinguishable in groundnut plants (Paguio and Kuhn, 1973).

The PeMoV is widely spread in the world. It has been reported to infect groundnuts and other legumes in Burkina Faso, Egypt, Niger, Sudan, South Africa, Uganda, Asia, India, Indonesia, Israel, Japan, Malaysia, Philippines, Taiwan, Australia, South America, North America, the Caribbean and Cuba (PTF, 2014). In Zambia, the PeMoV has been detected by serology in common bean (Vetten and Allen, 1991), while in Tanzania and Kenya, the virus was detected in *Cassia bicapsularis* and *Phaseolus lunatus*, respectively and these were serologically indistinguishable (Bock,

1978). The virus is mainly spread through international sharing of seeds, as it is known to be seed transmitted (PTF, 2014).

1.5.1.4 Cowpea aphid-borne mosaic virus (CABMV)

Cowpea aphid-borne mosaic virus is the virus whose main hosts are cowpea and passion fruit (Nascimento *et al*., 2006). The other hosts of the virus are in the family *Fabaceae* including common beans and most CABMV strains also infect members of the *Amaranthaceae, Chenopodiaceae, Cucurbitaceae, Laminaceae, Passifloraceae and Solanaceae* (Lovisolo and Conti, 1966).

The virus causes very severe symptoms in the susceptible plants; the symptoms include mosaics, mottling, vein chlorosis and vein-banding. It is transmitted in a non-persistent manner by various species of aphids including *Aphis craccivora, Aphis gossypii, Aphis spiraecola, Aphis fabae, Aphis sesbaniae, Macrosiphum euphorbiae, M. persicae, Rhopalosiphum maidis and Acyrthosiphon pisum* (Atiri, 1982; Bock, 1973; Bashir and Hampton, 1994). CABMV has linear, positive sense single stranded RNA (+ssRNA) (CABI, 2017).

The virus is widely distributed in Sub-Saharan Africa – including Tanzania – and other parts of the world. In Tanzania it was detected in common bean in the 1980s (Patel and Kuwite, 1982). The genome of this virus is about 9.5 kb with the P1 and P3 being the most variable regions. The CI, NIb and CP are its most conserved genomic regions. It has the covalently linked 5' terminal VPg and a poly A tail at the 3' terminal end (Mlotshwa *et al*., 2002).

1.5.2 *Sobemovirus*

The genus *Sobemovirus* consists of RNA viruses with *Southern bean mosaic virus* as a type member. Currently, the genus consists of fifteen virus species including *Velvet tobacco mottle virus*, *Turnip rosette virus*, *Soybean yellow common mosaic virus*, *Sowbane mosaic virus* (SoMV), *Southern cowpea mosaic virus*, *Southern bean mosaic virus* (SBMV) *and Rice yellow mottle virus* (Tamm and Truve, 2000). Some of sobemoviruses are not known to infect common bean.

Their genomes are approximately 4.0 to 4.5 kb (Tamm and Truve, 2000). The genomes of viruses in this genus have four ORFs (ORF 1, 2a and 2b, ORF 3). The ORF 1, 2a and 2b are translated from genomic RNA while the ORF 3 is translated from sub genomic RNA and it encodes a coat protein (Ling *et al*., 2013).

According to Sõmera *et al*. (2015) different ORFs encode different proteins, which in turn perform different functions: ORF 1 encodes P1, which is essential for viral movement, and act as the RNA silencing suppressor; ORF 2a and 2b encode P2a and P2ab proteins, respectively, these are the replication polyproteins; ORF 3 encodes coat protein which is involved in long distance movement. The Sobemoviruses have fifth ORF called ORFx, which is the most conserved region in the genome of these viruses. According to Ling *et al*. (2013), ORFx overlaps with the 5′ end of ORF2a in the +2 reading frame and extends some distance upstream of ORF2a.

Southern bean mosaic virus (SBMV) is the type member of the genus *Sobemovirus*. The viral genome is made up of +ssRNA of about 4.1 kb (Othman and Hull, 1995).

The virus infects *Glycine max* (soybean), *P. vulgaris* L., *Vigna mungo* (black gram), *Vigna unguiculata* (cowpea) and cause different symptoms (Sehgal, 1980). The strains of this virus are named depending on their ability to infect common bean and cowpea. The strain SBMV-B infects bean but not cowpea while SBMV-C strain infects cowpea but not bean (Othman and Hull, 1995). SBMV is transmitted by some species of leaf beetles (*Chrysomelidae*) (Wang *et al*., 1992). SBMV is transmitted through seed embryos (Zaumeyer and Harter, 1943; Uyemoto and Grogan, 1977). According to the plantwise map, the virus is widely distributed in the world. In Africa, SBMV has been found in West Africa (Allen *et al*., 1981) where it infects crops such as cowpea in Togo (Gumedzoe *et al*., 1996). SBMV has not been reported in East Africa (Allen *et al.,* 1989).

1.5.3 *Alphaendornavirus*

This genus consists of viruses with double stranded RNA genomes which are 9.8 to 17.6 kb in size (Okada *et al*., 2013). These viruses belong to the family *Endornaviridae* (Carstens and Ball, 2009). Two Endornaviruses that have been detected in common beans are *Phaseolus vulgaris alphaendornavirus* 1 (PvEV-1) and *Phaseolus vulgaris alphaendornavirus* 2 (PvEV-2) (Khalifa *et al*., 2016). Alphaendornaviruses are seed borne plant viruses, which are also known to infect fungi and oomycetes. They are persistence viruses that contain non-capsidated RNA (Khanrhum *et al*., 2016). Endornaviruses encode a single polyprotein that is processed into different functional proteins (Okada *et al.,* 2013). The encoded polyprotein contains conserved domains for RNA helicase, glycosyltransferase and RNA-dependent RNA polymerase (RdRp) (Nordenstedt *et al.,* 2017).

Vertical transmission of endornaviruses occurs at a high rate through seeds, pollen or fungal spores but horizontal transmission by contact or vectors is not known to occur (Nordenstedt *et al*., 2017). Its genome contains conserved motifs of RNA-dependent RNA polymerase (RdRp) similar to the alpha-like virus of positive-stranded RNA viruses (Roossinck *et al*., 2011; Okada *et al*., 2013). Viruses in the genus *Endornavirus* lack both cell to cell movement and virions as a result the viruses are present in every tissue of the plant (Fukuhara *et al*., 2006; Villanueva *et al*., 2012).

Different proteins perform different functions. The alphaendornaviral methyltransferase (MTR), is involved in enhancing the stability of messenger RNA; viral helicase 1 (Hel-1) that is involved in viral replication; capsular polysaccharide synthase (CPS), UDP-glycosyltransferase (UGT) helps the virus to avoid host anti-viral mechanisms (Markine *et al*., 2004); viral RNA dependent RNA polymerase (RdRp) is the RNA replicon (Horiuchi *et al*., 2001). The PvEV-1 and PvEV-2 differ in that, the MTR is only present in PvEV-2 and absent in PvEV-1 while CPS is found in PvEV-1 but not in PvEV-2 (Okada *et al.*, 2013). Alphaendornaviruses have been reported in common beans in Brazil (Alves-Freitas *et al*., 2015). Recently, PvEV-1 and PvEV-2 were detected in common beans in Tanzania and Nicaragua (Nordenstedt *et al*., 2017).

1.5.4 *Carlavirus*

Carlavirus is the genus of viruses found in the family *Betaflexiviridae* with *Carnation latent virus* (CLV) as a type member. Viruses in this genus have a positive-sense, single stranded RNA genome which is 5.9 to 9.5 kb in size (King *et al*., 2011; Adam

and Kreuze, 2016). There are 52 virus species in the genus *Carlavirus.* Majority member of Carlaviruses are aphid-transmitted but CPMMV, *Cucumber vein - clearing virus* (CuVCV) and *Melon yellowing associated virus* (MYaV) are transmitted by whiteflies (Menzel *et al*., 2011). The complete nucleotide sequence of the viruses found in this genus has six ORFs. ORF 1 encodes viral replicase. ORF 2, ORF 3, and ORF 4 encode triple gene block (TGB), which is a specialized evolutionarily conserved gene module involved in cell-to-cell and long-distance movement of viruses (Morozov and Solovyev, 2003). ORF 5 encodes for CP and overlaps with ORF 6 that encodes a cysteine-rich protein (King *et al*., 2012).

Cowpea mild mottle virus belongs to the family *Betaflexiviridae* and genus *Carlavirus*. It is has a positive sense single stranded RNA genome (Adam and Kreuze, 2016). The virus is transmitted in a non-persistent manner by the whitefly, *Bemisia tabacci*. The virus can be transmitted through mechanical inoculation and by seed (Brito *et al*., 2012). The virus principally infects cowpeas although it can infect *P. vulgaris* L., *A. hypogaea*, *P. lunatus*, winged bean (*Psophocarpus tetragonolobus*), *Glycine max*, tomato (*Lycopersicon esculentum*), and black gram (*Vigna mungo*) (Chang *et al*., 2013). Its genome size is approximately 8194 nt (e.g., accession number KC884249.1).

The genome of CPMMV of consists of six ORFs. ORF 1 encodes RdRp, which is a genomic RNA. ORF 2 encodes the TGB 1, ORF 3 encodes TGB2, and ORF4 encode TGB3. ORF 2, ORF 3 and ORF 3 are putative subgenomic RNAs. ORF 5 encodes for coat protein (CP) and ORF 6 encodes for nucleic acid-binding protein (NABP) (King

et al., 2012). The virus has been reported in tropical areas of Africa (Brunt and Philips, 1981; Thouvenel *et al*., 1982), Asia (Reddy, 1991), Brazil and Argentine (Laguna *et al*., 2006). In Africa, the virus has been detected in many leguminous and solanaceous plant species in Ivory Coast (Hartman *et al*., 1999). In East Africa, CPMMV was detected in common bean plants in the 1980s in Tanzania (Mink and Keswani, 1987; Vetten and Allen, 1991), Malawi, Mozambique, Uganda and Sudan (Vetten and Allen, 1991).

1.5.5 *Cucumovirus*

The genus cucumovirus comprises four viruses: CMV, *Peanut stunt virus*, *Tomato aspermy virus* and *Gayfeather mild mottle virus* (Choi *et al*., 1999; ICTV, 2017). It is found in the *Bromoviridae* family. The type species, *Cucumber mosaic virus*, has a +ssRNA genome (White *et al*., 1995). The genome of cucumoviruses is divided into three single-stranded positive-sense RNAs called RNAs 1, 2, and 3. It also contains a fourth sub-genomic RNA called RNA 4, which is generated from RNA 3 (Pita and Roossinck, 2013).

Cucumber mosaic virus has a wide host range compared to all known viruses. It infects more than 1000 plant species (Van Regenmortel *et al*., 2000). The virus was first discovered in the cucumber plants and hence the name *cucumber mosaic virus*. The virus is now known to infect vegetables such as squash, melons, peppers, beans, tomatoes, carrots, celery, lettuce, spinach, beets, some ornamentals and legumes including *P. vulgaris* L. (Davis and Hampton, 1986; Zitter and Murphy, 2009; Amayo *et al*., 2012; Mwaipopo *et al*., 2017; Vetten and Allen, 1991). In Tanzania, the virus was detected in common bean (Njau *et al*., 2006) and *Vigna unguiculata*, *Cucumis*

sativus, *Citrullus lanatus*, *Cucurbita pepo*, *Cucumis hystrix* and *Luffa aegyptiaca* (Sydänmetsä and Mbanzibwa, 2016). It is transmitted by aphids, seeds and parasitic weeds. Over 60 aphid species transmit CMV in a non-persistent manner (Francki *et al*., 1979).

Cucumber mosaic virus is divided into three RNAs has two sub-genomic RNA. The RNA 1 encodes ORF 1a that contain putative helicase and methlytransferase for virus movement. RNA 2 encodes ORF 2a and 2b proteins, which are involved in spread and virulence of the virus. RNA 3 encodes ORF 3a which functions as the movement protein and ORF 3b (CP) which encapsidates the virus. RNA 3 contains subgenomic RNA 4 from which the CP is translated (Zitter and Murphy, 2009).

The common beans infected with CMV develop symptoms including leaf curling, green mottle and blistering, and a zipper like roughness along the main veins. The symptoms can as well be observed on the pods when diseased become curved, mottled and reduced in size. The symptoms of the infected plant can be confused with *Bean common mosaic virus* (Zitter and Murphy, 2009).

1.5.6 *Umbravirus*

The genus *Umbravirus*, in the family *Luteoviridae*, comprises nine viruses: *Carrot mottle virus* (CMoV), *Carrot mottle mimic virus* (CMoMV), *Groundnut rosette virus* (GRV), *Lettuce speckles mottle virus* (LSMV), *Pea enation mosaic virus* 2 (PEMV-2), *Tobacco mottle virus* (TMoV), *Tobacco bushy top virus* (TBTV), *Opium poppy mosaic virus* **(**OPMV) *and Ethiopian Tobacco bushy top virus* (ETBTV) (Taliansky *et*

al., 2003; ICTV, 2017). The viruses in this genus can be transmitted mechanically and by aphids in a persistent manner (Li *et al*., 2006).

The umbraviral genome consists of a single, linear, positive-sense RNA (+ssRNA), which is about 4.0 to 4.2 kb in length. Their genomic RNAs are not polyadenylated at their 3′ end. The *Umbravirus* genome differs from those of other viruses. Umbraviruses do not encode a conventional capsid protein which is required for both short and long-distance movement. The absence of the conventional CP doesn't restrict the virus from spreading from cell to cell (Ryabov *et al*., 2001). According to Taliansky *et al*. (2003), the lack of a CP in umbraviruses is compensated for by the ORF 3 protein. The viruses in the genus *Umbravirus* have four RNA open reading frames, namely ORF 1, ORF 2, ORF 3 and ORF 4. ORF 1 and ORF 2 are translated as a single polyprotein, the RdRp (Taliansky *et al.*, 1996). The ORF 3 encodes protein that functions as the viral RNA protector and ORF 4 encodes a 27 to 29 KDa protein, which is the movement protein that helps the virus to move from cell to cell (Ryabov *et al*., 2011; Taliansky *et al*., 2003).

Umbraviruses have restricted host range in nature (Li *et al*., 2006). The umbraviruses have been reported in Mauritius (Gungoosingh-Bunwaree *et al*., 2009), China (Mo *et al*., 2002), Ethiopia and Zimbabwe (Abraham *et al*., 2014), but they have never been reported in common beans in Tanzania.

1.5.7 *Crinivirus*

Crinivirus is a genus of viruses found in the family *Closteroviridae*. Viruses in this genus have single stranded positive sense RNA genomes. Their genomes vary in size from 15 to 20 kb (Martelli *et al*., 2012). The genomic organization is almost the same for all viruses in this group. Their genomes have RNA 1 and RNA 2. These viruses are transmitted by whiteflies which are *Bemisia tabacci* and *Trialeurodes* sp. (Martelli *et al*., 2012).

There are presently fourteen viruses in this genus including *Bean yellow disorder virus* (BnYDV), *Lettuce infectious yellows virus* **(**LIYV), *Abutilon yellows virus* **(**AbYV), *Beet pseudo yellows virus* (BPYV), *Blackberry yellow vein-associated virus* (BYVaV), *Cucurbit yellow stunting disorder virus* **(**CYSDV), *Diodia vein chlorosis virus* (DVCV), *Lettuce chlorosis virus* (LCV), *Potato yellow vein virus* (PYVV), *Strawberry pallidosis-associated virus* (SPaV), *Sweet potato chlorotic stunt virus* (SPCSV), *Tomato chlorosis virus* (ToCV), *Tomato infectious chlorosis virus* (TICV) (Tzanetakis *et al*., 2013).

These viruses have bipartite genomes consisting of RNA 1 and RNA 2. RNA 1 and RNA 2 have several ORFs, which ecode such proteins/domains as papain-like protease (P-Pro), methyl-transferase (MTR), helicase (HEL), RNA-dependent RNApolymerase (RdRp), heat shock protein homologue (HSp70h), major capsid protein (CP) and minor capsid proteins (CPm). Members of the family *Closteroviridae*, to which the genus *crinivirus* belongs, are known for having largest genomes of all plant positive-strand RNA viruses approaching 20 kb (Tzanetakis *et al*., 2005). The criniviruses differ in number of ORFs. For example, BnYDV has 12 ORFs (Martín *et al*., 2008) while *Lettuce infectious yellows virus*, SPCSV, *Lettuce*

chlorosis virus, *Cucurbit yellow stunting disorder virus*, and *Blackberry yellow vein associated virus* have ten ORFs (Kiss *et al*., 2013). BnYDVis known to infect common bean and has been reported in Spain in common bean (Tzanetakis *et al*., 2013) but not in Tanzania.

1.5.8 *Begomovirus*

The genus Begomovirus belongs to the family *Geminiviridae.* Geminiviruses have small circular single stranded DNA genomes (Sobrinho *et al*., 2014). There are presently 388 begomoviruses, which are recognized by ICTV (2017). These viruses have a wide host range and have been reported in cultivated and non-cultivated crops. The geminiviruses have either bipartite or monopartite genomes. The bipartite genomes have two DNA components, namely DNA-A and DNA-B. On the other hand, monopartite geminiviruses have only DNA-A component (Roshan *et al*., 2017).

Examples of bipartite geminiviruses, which infect common beans are *Bean golden mosaic virus* (BGMV), *Bean golden yellow mosaic virus* (BGYMV) (Rivera-Vargas *et al*., 2001) and *Bean dwarf mosaic virus* (BDMV) (Levy *et al*., 2010), while the monopartite begomovirus is *Tomato yellow leaf curl virus* (ToYLCV) (Ji *et al*., 2012; Mwaipopo *et al*., 2018). Others are *Macroptilium yellow spot virus* (MaYSV) and *Sida micrantha mosaic virus* (SimMV). They have been reported to naturally infect common bean (Sobrinho *et al*., 2014). Each DNA component is approximately 2.6 kb in size.

DNA-A encodes six ORFs, two in the viral sense and four in the complementary sense. ORFs AV1 and AV2 are translated into coat and movement proteins, respectively. ORF AC1 is a DNA helicase required for replication; AC2 and AC3 function as transcriptional activator and replication enhancer, respectively. AC4 acts as a silencing inhibitor and symptom determinant (Lecoq and Desbiez, 2012). DNA-B encodes two ORFs (BV1 and BC1), which function as nuclear shuttle and movement proteins, respectively (Nawaz-ul-Rehman and Fauquet, 2009). Begomoviruses have been reported mostly in the Caribbean, Florida, Central America and Latin America (Morales, 2000). They have not been detected in common bean plants in East Africa.

1.5.9 *Cytorhabdovirus*

Cytorhabdovirus is one of the genera in the family *Rhabdoviridae*. The viruses have negative sense single stranded RNA genome, which is approximately 11 to 14 kb in size (Lima *et al*., 2017). The viruses in this genus have genome, which contains *N* gene that encodes the nucleoprotein, *P* gene that encodes the phosphoprotein (polymerase co-factor), *M* gene that encodes the matrix protein, *G* gene that encodes for glycoprotein and *L* gene that encodes for RNA polymerase (Willie *et al*., 2017). Virus species in the genus *Cytorhabdovirus* (acromny and accession numbers are shown in parenthesis) include *Alfa alfa dwarf cytorhabdovirus* (ADV; KP205452), *Barley yellow striate mosaic virus* (BYSMV; KM213865), *Colocasia bobone disease-associated virus* (CBDaV; KT381973), *Lettuce necrotic yellows virus* (LNYV; AJ867584), *Lettuce yellow mottle virus* (LYMoV; EF687738), *Northern cereal mosaic virus* (NCMV; AB030277), *Strawberry crinkle virus* (SCV; AY331389). Viruses from the genus *Cytorhabdovirus* have been reported to infect common beans and are transmitted by whitefly, aphids and leafhoppers (Lima *et al*., 2017).

1.5.10 *Caulimovirus*

This is a genus of viruses that belong to the family *Caulimoviridae*. A famous virus in genetic engineering and whose sequence has been used as promoter sequence (35s promoter) in gene expression studies. *Cauliflower mosaic virus* (CaMV) is a type species of this genus. Viruses in this genus include a legume infecting *Soybean Putnam virus* **(**SPuV) (Han *et al*., 2012). Others are *Carnation etched ring virus* (CERV), *Cauliflower mosaic virus* (CaMV), *Dahlia mosaic virus* (DMV), *Figwort mosaic virus* (FMV), *Horseradish latent virus* (HRLV) (Accession No. JX429923), *Lamium leaf distortion virus* (LLDV), *Mirabilis mosaic virus* (MMV), *Strawberry vein banding virus (*SVBV*)* and *Thistle mottle virus* **(**ThMoV).

Caulimoviruses are associated with vein clearing or banding mosaic disease symptoms. The viruses in this genus have a double stranded DNA genome and their replications occur through RNA intermediates, and for this reason they are termed as reverse transcribing viruses (Hull *et al*., 1987). The caulimoviruses consist of seven ORFs; ORF 1 encodes movement protein (MP), ORF 2 encode for aphid transmission factor (ATF), ORF 3 encodes virion associated protein (VAP), ORF 4 encodes coat protein (CP), ORF 5 encodes polymerase polyprotein (aspartic protease, reverse transcriptase and Rnase H), ORF 6 encodes transactivator (TAV) and the last one, ORF 7, performs unknown function(s) (Bousalem *et al*., 2010; Mushegian *et al*., 1995). Caulimoviruses have a wide host range (Hull, 1984).

1.5.11 *Soymovirus*

This is another genus in the family *Caulimoviridae*. Viruses in this genus, like those in the genus *Caulimovirus*, have double stranded DNA genomes. There are four virus species in this group, which are *Blueberry red ring spot virus* (BRRV), *Cestrum yellow leaf curling virus* (CmYLCV), *Peanut chlorotic streak virus* (PClSV) and *Soybean chlorotic mottle virus* (SbCMV). Soymoviruses cause chlorotic mottling and mosaic on the leaves and stunting of the soybeans. They infect soybeans as well as other legumes. For example, SbCMV infect common bean and Dolichos lablab (Hibi and Iwaki, 1988). In common beans, SbCMV causes chlorotic local lesions, chlorotic spots, vein clearing, mottling and leaf-curling symptoms, which appear systemically on infected leaves (Hull, 1984).

The genome of soymoviruses comprises ORF 1a and ORF 1b. The ORF 1a encodes movement protein (MP) while the function of ORF 1b is unknown. Similarly, the function of ORF 2 is unknown. ORF 3 encodes virion associated protein (VAP), ORF 4 encodes coat protein, ORF 5 enccodes polymerase polyprotein, ORF 6 encodes transactivator (TAV) and ORF 7 encodes putative aspartic protease (Bousalem *et al*., 2010; Mushegian *et al*., 1995).

1.6 Common Bean Viral Disease Symptoms

The symptoms of many viral diseases of common bean are similar and it is not always easy to identify a specific virus based on symptoms alone (Seminis, 2016). Nevertheless, for well characterized viruses, there are some distinguishing symptoms that can be used to verify their infections and indeed to confirm and distinguish the strains involved in the infections (Drijfhout, 1978; Drijfhout *et al*., 1978). Disease

symptoms caused by BCMV and BCMNV depend on strain of the virus, temperature and the common bean genotype infected. The BCMV and BCMNV cause mosaic, systemic necrosis (black root) on common bean genotypes having *I* resistance gene, or local lesions or malformations in common bean plants. Other symptoms associated with mosaic disease are leaf rolling or blistering, light and dark green patches on the leaf (green mosaic), chlorosis, vein banding, yellow mosaic and dwarfing (Worrall *et al*., 2015).

Both BCMV and BCMNV can infect plants without causing any disease symptoms but such infection may still result into yield losses (Morales, 2006). *A. hypogea* plants infected with PeMoV plants develop systemic mottle and necrosis (Bock, 1975). Plants infected with SBMV infected plants exhibit different symptoms depending on the variety. Some of the symptoms caused by this virus are mosaic, or mottle, rugosity, epinasty, vein yellowing, stunting, and necrotic local lesions **(**Maritza *et al*., 2017). The BYMV infected common bean plants develop chlorotic or necrotic local lesions often extending into the veins, followed by systemic leaf yellowish mosaic, leaf curling and plant stunting (Adhab and Rakib, 2013).

The begomoviruses BGMV and BGYMV cause diseases with almost the same symptoms: mosaic, yellowing of leaves, reduced growth and malformation, distortion of leaves and pods, and low quality of seed (Bracero and Rivera, 2003). The common bean plant infected with CABMV develops severe mosaic, leaf distortion, blistering and stunting (Elbeshehy, 2013) while CPMMV cause mottling, mild mosaic and slight leaf distortion (Brito *et al*., 2012). Briefly, viruses cause almost similar symptoms in

plants (Plate. 1.1) but a distinction between the viruses and the symptoms they cause can only be made through biological or molecular characterization of their isolates in the laboratory level.

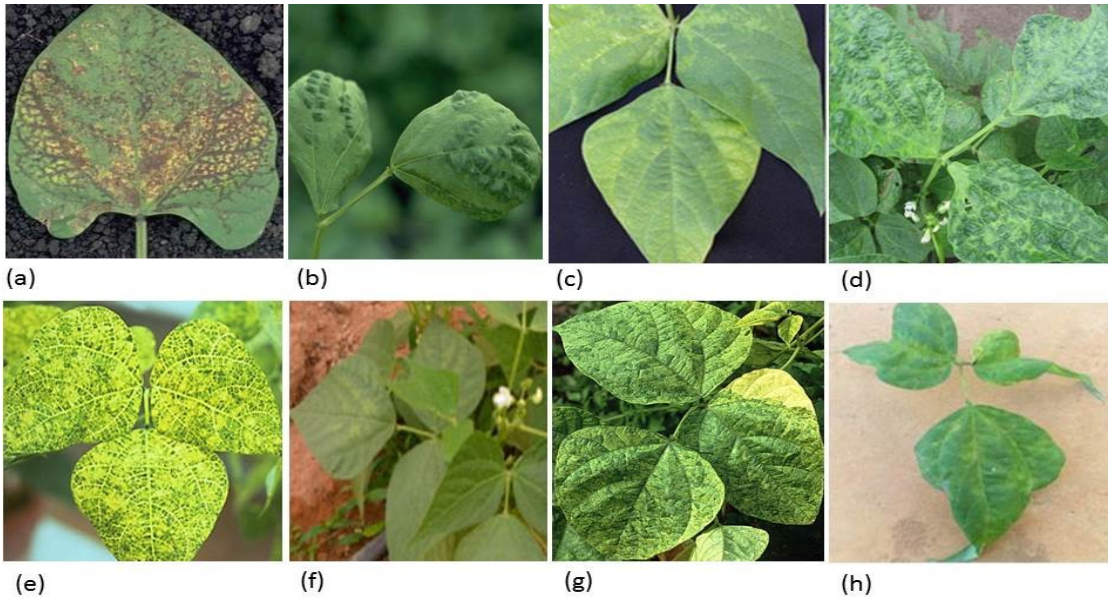

Plate 1.1: Symptoms of different viruses in common beans

Source: (a) and (b) Schwatz (2008), (c) Zanardo *et al*. (2017), (d) Draeger (2006), (e) Brown and Bird (1992), (f) Rezk (2016), (g) Catllin (2005), (h) Own photo (Screenhouse at TARI-Mikocheni). The symptoms shown in plates with letters a to h are caused by BCMV, BCMNV, CPMMV, CMV, BGMV, ToYLCV and SBMV, respectively.

1.7 Common Bean Virus Transmission

Viruses can be transmitted in many ways: mechanically, pollen, seeds, insect vectors and fungal transmission (Stevens, 1983). The most important insect vectors known to spread viral diseases in plants are aphids, thrips, leafhoppers and whiteflies (Bragard *et al*., 2013) and also beetles (Albrechtsen (2006). The virus transmission with vector is mediated by special viral proteins which are involved in the transmission mechanisms. Examples of the proteins that are involved in virus acquisition and transmission are CP and HC-Pro in potyviruses, CP in cucomoviruses and

begomoviruses, CP, P2 and P3 in caulimoviruses, and minor capsid protein (CPm) in criniviruses (Whitfield *et al*., 2015; Dietzgen *et al*., 2016).

Viruses are transmitted by vectors in a persistent, semi-persistent or non-persistent manner (Table 1.2). In the non-persistent manner, the transmission of the virus from infected plant to healthy plant takes place in a few seconds to minutes after the viral acquisition and the virus is retained in the vector stylet. In the semi-persistent manner, the virus is transmitted in hours to days and the virus is able to bind to chitin lining of the gut but they don't enter into the tissues of the vector. For persistent transmission, the vector takes several days to several weeks to transmit a virus to new plant and the route of the virus in the vector is circulative because the virus is taken by the insect and retained in the tissue and salivary gland (Dietzgen *et al*., 2016).

Persistent mode of transmission is divided into circulative propagative and circulative non-propagative. In the non-propagative circulative transmission, the virus is acquired by the vector, reach the vector tissues, and pass through the gut, hemolymph to salivary gland for transmission without replication (Dietzgen *et al*., 2016). The propagative mode of transmission involves the acquisition of the virus and replication during systematic invasion of vector tissues to salivary gland for transmission (Whitfield *et al*., 2015; Hogenhout *et al*., 2008; Dietzgen *et al*., 2016). For example, BCMV, BCMNV, BYMV, CMV, *Tomato aspermy virus* (TAV) are transmitted by several species of aphids in a non - persistent manner (Mukeshimana *et al*., 2003; Brunt *et al*., 1996; Kumar *et al*., 1994).

Table 1. 2: Types of transmission for selected viruses in eight genera

Family	Genus	[1]Virus	Vector	Type of transmission	References
Germiniviridae	*Begomovirus*	BGYMV, BGMV	Whiteflies	Persistent	Inoue - Nagata *et al*. (2016)
Luteoviridae	*Umbravirus*	GRV	Aphids	persistent	Storey and Ryland (1955)
Caulimoviridae	*Caulimovirus*	CaMV, FMV, CERV, SVBV	Aphids	Semi persistent	Mahmoudpour *et al*. (2016); Covey *et al*. (1998)
Closteroviridae	*Crinivirus*	BnYDV	Whitefly	Semi persistent	Martín *et al*. (2011)
Solemoviridae	*Sobemovirus*	SBMV	Beetles	Semi persistent	Albrechtsen (2006)
Bromoviridae	*Cucumovirus*	CMV, TAV	Aphids	Non-persistent	Kennedy *et a*l. (1962)
Betaflexiviridae	*Carlavirus*	CPMMV	whiteflies	Non-persistent	Brito *et al*. (2012)
Potyviridae	*Potyvirus*	BCMV, BCMNV	Aphids	Non-persistent	Spence and Walkey (1995)

[1]BGYMV (*Bean golden yellow mosaic virus*), BGMV (*Bean golden mosaic virus*), GRV (*Groundnut rosette virus*), CaMV (*Cauliflower mosaic virus*), FMV, CERV (*Carnation etched ring virus*), SVBV (*Strawberry vein banding* virus), BnYDV (*Bean yellow disorder virus*), SBMV (*Southern bean mosaic virus*), CMV (*Cucumber mosaic virus*), TAV (*Tomato aspermy virus*), CPMMV (*Cowpea mild mottle virus*), BCMV (*Bean common mosaic virus*), BCMNV (*Bean common mosaic necrosis virus*).

Seed transmission is very important for long distance spread of plant viral diseases. Many species of viruses are known to be transmitted through seeds of infected plants. Most seed embryos that are infected by the virus remain in the seeds for many years as the results, many viruses especially of legumes have been shown to occur all over the world through imported germplasm (Albrechtsen, 2006). For example, the larger number of legume seeds imported from other countries to Australia, their large germplasm seed bank was found to be infected with viruses when were subjected to post entry quarantine (Jones, 1987).

1.8 Role of Weeds in Virus Transmission

Weeds are the plants which are considered undesirable in a particular place. However, the weeds are very important in agriculture and many hosts can be infected by a huge number of plant viruses, which makes them potential reservoirs of crop-infecting viruses (Seal *et al.*, 2006).

Some bean infecting viruses have been reported to naturally occur in several non-*phaseolus* legume species such as *Crotalaria juncea* (Singh and Singh, 1977), *Crotalaria striata* (Sarkar and Kulshreshtha, 1978) and *Lupinus luteus* (Frencel and Pospieszny, 1979). In Uganda, the BCMNV was detected in *Centrosema pubescens*, *Crotalaria incana*, *Lablab purpureus*, *Senna bicapsularis*, *Senna sophera*, *Vigna vexillata* and other unidentified *Crotaria spp* (Sengooba *et al*., 1997). These plants are wide spread in East Africa (Sengooba *et al*., 1997). Surveys of alternative hosts of BCMNV in Tanzania in the 1990s showed that *Centrosema pubscens*, *Neonotonia wightii*, *Senna spp*, *Crotolaria spp* and *Rhynchosia zernia* were naturally infected by this virus (Myers *et al*., 2000; Mwaipopo *et al*., 2017). Moreover, Njau and Lyimo (2000) demonstrated – through mechanical inoculation – that BCMNV can infect *S. occidentalis*, *S. obtusifolia*, *Cassia floribunda*, *Crotalaria spp* and *Rhynchosia minima* and therefore these plants are potential reservoirs of the viruses.

Alfalfa is commonly grown as a forage crop but may as well grow as a weed. It has been reported to be the host of many viruses including those which infect common beans: *Bean leaf roll virus* (BLRV), BCMV, BYMV, CMV, *Lucerne transient streak virus* (LTSV), *Pea streak virus* (PeSV), *Red clover vein mosaic virus* (RCVMV), *Tobacco streak virus* (TSV), *White clover mosaic virus* (WCMV) and *Peanut stunt virus* (PSV) which were reported to infect this crop and other legumes worldwide (Guy *et al*., 2013; Rahman and Peaden, 1993; Al-Shahwan *et al*., 2017). Also, *Datura stramonium* L. (*Solanaceae*) has been reported to be the host of *Tomato yellow leaf curl virus* (ToYLCV), a begomovirus that infects tomato plants and has also been detected in common beans (Chen *et al*., 2013). According to Thomas (2001), CMV

and *Alfalfa mosaic virus* (AMV) have a wide host range and thus high possibility of the viruses to spread to crops. The occurrence of viruses in weeds and other wild hosts in Tanzania can have significant implications in management of common bean and other crops viral diseases. The elimination of these weeds and wild legume virus reservoirs is important in order to reduce the spread and evolution of new viral pathogens in common bean plants.

1.9 Management of Plant Viruses

Many crops are threatened by the plant viruses because these viruses cannot survive on their own. Viruses depend on host plants for replication, cell to cell movement and transmission. As a result, they integrate with the host machinery for their replication and other functions (Islam *et al*., 2017). It is not easy to control virus due to lack of effective control measures that can be suitable in large area. For example, chemotherapy is suitable for control of viral diseases in small area (Thresh, 2003). The potyviruses are very complex due to their high rate of seeds transmission that leads to difficulties in their management (Galves and Molares, 1989; Shukla *et al*., 1989). In order to devise proper control measures of a disease, there is a need of prior knowledge of the virus that is causing it. Most importantly, it is easier to control the spread of the virus rather than managing diseased plants (Ventura *et al.*, 2004). There are many ways of controlling the viruses including the ones shown below.

1.9.1 Use of genetic resistance materials

This is the most effective method of controlling virus diseases (Kelly *et al*., 1995). It has been used for managing diseases in many crops for increasing productivity by reducing the damages caused by either pests or pathogens (Thresh, 2003). In

determining the resistant materials, marker assisted selection (MAS) has been used successfully in identifying disease resistance gene (Miedaner and korzun, 2012). For example, it has been reported that dominant *I* gene and *bc-3* recessive gene together in the same variety of common bean gives complete resistance to BCMV and BCMNV (Drijfhout, 1978; Vallejos, 2006; Naderpour *et al*., 2010). *Ty 1* and *Ty 3* are the dominant genes controlling ToYLCV in tomato (Butterbach *et al*., 2014) while *RT4-4* dominant gene in common beans confers resistance to CMV (Seo et al., 2006). Common bean has been engineered for many different traits. In Brazil, for example, common bean has been engineered for RNA interference mediated resistance against BGMV (Bonfim *et al.*, 2007) and the product from this genetically modified common bean has been commercialized (Tollefson, 2011).

1.9.2 The use of certified disease-free seeds

The use of certified disease-free seeds is one of the best ways of controlling virus diseases because certified seeds are free from diseases compared to seeds saved from the previous harvest. Use certified disease-free seed is very important to avoid the spread of diseases caused by viruses, bacteria or fungal (Biddle *et al*., 1992). Also, the selection of best planting materials which are free from viruses is very important to avoid the spread of the pathogens not only in common beans but also in other crops (Munir, 2017). For example, bean common mosaic disease is caused by BCMV, which is transmitted mainly by vectors and through seeds. BCMV can survive in seed for more than 30 years, and can survive heat treatments hot enough to kill the seed.

1.9.3 Management of vectors

Virus diseases can also be managed through management of vectors by using chemicals. However, application of pesticides may not be rational for control of non-persistent transmitted viruses because they need relatively short inoculation times – much shorter than the time needed for insecticides to kill them (Fereres and Raccah, 2015). According to Fereres and Raccah (2015), insecticides make insects restless and thus lead to increased number of attempts to probe plants, which is not the case with calm insects. However, for persistent (and phloem-limited viruses), the use of chemicals can be effective.

1.9.4 Use of integrated pest management (IPM)

Control of viruses is most effective through use of integrated pest management (IPM), crop rotation, controlling of weeds, destroying of old crop, avoiding planting of new crop to diseased plantings and rouging are very important (Persley *et al*., 2010; Munir, 2017).

1.10 Research on Common Bean Virus Diseases in the Country

Worldwide, common bean is infected by a large number of both single and double-stranded, DNA and RNA plant viruses. There are several published reports on detection of viruses that caused disease symptoms on common bean in Tanzania in the 1980s and 1990s (Vetten and Allen, 1991; Spence and Walkey, 1994; Spence and Walkey, 1995; Myers *et al*., 2000).

Bean common mosaic virus which then existed as two distinct serotypes A and B, respectively, were detected in Tanzania (Vetten and Allen, 1991). Silbernagel *et al*. (1986) reported the presence of TN-1 strain of BCMV in Tanzania which was pathogenically and serologically related to the temperature insensitive strain (NL3). Based on HPLC obtained results, TN-1 isolate was classified together with NL-3, NL-8 and NL-5 as strains of BCMNV (McKern *et al*., 1992). This was the first published report of the occurrence of the temperature insensitive necrosis inducing strain of BCMV in Tanzania (Larsen *et al*., 2011). The TN-1 isolate was sequenced and assigned accession number HQ229995 (Larsen *et al*., 2011). Spence and Walkey (1994) also reported about the existence of NL1 (BCMV), NL8 and NL3 (BCMNV) in Tanzania.

In 1999, a comprehensive survey for the incidence of BCMV and BCMNV in seeds of common bean and other legumes aiming at studying the viral seed transmission were conducted. A total of 10 300 seeds were collected, representing 341 and 30 seed lots of common bean and wild legumes, respectively (Njau and Lyimo, 2000). The two viruses were detected only in bean seed samples collected from northern and eastern Tanzania. These viruses were not detected in wild legume seeds. The highest incidence for BCMNV was 36.6%, whereas it was only 12.4% for BCMV (Njau and Lyimo, 2000). The strains of BCMV and BCMNV known to occur in Tanzania as determined using differential cultivars are NL1, NL3, NL5, NL8, TN1, TN2 and TN3 (Vetten and Allen, 1991; Spence and Walkey, 1994; Njau and Lyimo, 2000).

Apart from BCMV and BCMNV, other viruses have been detected in common bean in Tanzania. CPMMV was detected in mung bean plants collected from around the Sokoine University of Agriculture (Mink and Keswani, 1987; Chang *et al*., 2013). CABMV was detected for the first time in Tanzania about thirty-three years ago during surveys of common bean viruses in Africa (Taiwo and Gonsalves, 1982; Taiwo *et al*., 1982; Patel and Kuwite, 1982; Sengooba, 2003; Bashir *et al*., 2002; Bock, 1973).

Other viruses detected in common bean were CMV (Davis and Hampton, 1986; Njau *et al*., 2006), PvEV-1 and PvEV-2 (Nordenstedt *et al.*, 2017). Cryptic viruses (PvEV-1 and PvEV-2) were detected in common bean seeds collected from in Tanzania and Nicaragua using molecular based technique (Nordenstedt *et al*., 2017). PeMoV was also detected in all samples from East Africa, including Tanzania where the virus was found in cowpeas, groundnuts and bambara groundnut (*Voandzeia subterranean*) in Kagera and around Lake Victoria (Bock, 1973). All the viruses were detected in Tanzania, either by using serological methods or via symptoms.

1.11 Detection Methods and Role of Next Generation Sequencing

There are many methods of virus detection: polymerase chain reaction (PCR), fluorescence in-situ hybridization, enzyme linked immunosorbent assay (ELISA), immunofluorescence, flow cytometry, thermography, fluorescence imaging, hyper spectral technique, gas chromatography, biosensor platforms based on nanomaterial, affinity biosensor, antibody-based biosensors, DNA/RNA based affinity biosensor, enzymatic electrochemical biosensors, bacteriophage biosensor (Fang *et al*., 2015).

The most famous detection methods ones are the serological (ELISA, western blotting, dot blot, immunostrip assay) and PCR (Martinelli *et al*., 2015). The ELISA has been used to detect various plant pathogens. The detection of viruses evolved when the ELISA method was adapted for the detection of plant viruses by Adam and Clark in 1977, although the antibodies shelf life is short and variation among batches have been experienced (Martinelli *et al*., 2015). Use of ELISA method requires availability of high-quality antisera for sensitivity; the method cannot be used to distinguish the strains and species of pathogen during detection (Boonham *et al*., 2014).

The PCR method involves the amplification of nucleic acid using degenerate or specific primers (Boonham *et al.,* 2014). This method is widely used and is very specific to what is being amplified/detected (Cai *et al*., 2014). The method was advanced to reverse transcriptase PCR (RT-PCR) to detect the RNA viruses (Lopez *et al*., 2003). The RNA strands of the viruses are converted to complimentary DNA before detection. However, RT-PCR has its limitations and thus continued efforts to develop more advanced methods. The efficiency of PCR depends on the quality of DNA and RNA extracted from plant samples. The detection success of PCR also depends on specificity and optimization of primers designed, concentration of buffers, dNTPs, and other reagents (Van der wolf *et al*., 2001).

The PCR can only be carried out when the sequences of the pathogen or its closest relative are available. Therefore, for a plant infected with unknown pathogen, it may prove difficult to detect it using PCR method. Following these limitations on the use of PCR to detect plant pathogens– including the need for *prior* knowledge of virus

sequences – and disadvantages of the ELISA method, and also following the limitations of other methods reviewed elsewhere (Boonham *et al*., 2014), scientists have strived to develop technologies which can rapidly, sensitively, simultaneously and effectively detect pathogens in plant and other samples.

One of the technologies that are currently being used to simultaneously detect pathogens in plant samples is the next generation sequencing technique (NGS). It is a powerful tool in molecular biology (Adam *et al*., 2012, Prabha *et al*., 2013). The NGS technique has led to many discoveries in science, which has resulted into increased and expanded genomics related studies. NGS detection technique enables generation of a lot of information of a sample without a prior knowledge of it (Raza and Ahmad, 2016). A lot of good methods for detecting plant pathogens have been described by different authors but still these methods may fail to identify unknown pathogens but NGS based detections normally overcome these limitations (Adam *et al*., 2009).

The NGS technique is used in detection and discovery of novel viruses, obtaining partial and complete sequences of genomes, investigation on viral quasispecies and antiviral defense mechanism (Prabha *et al*., 2013). NGS gives high levels of multiplexing instead of using virus specific reagents (Boonham *et al*., 2014). In Tanzania and elsewhere, NGS based detection of viruses has been done using total RNA extracted from cassava (Ndunguru *et al*., 2015) or through deep sequencing of virus-derived small RNAs isolated from sweet potato plants (Kreuze *et al*., 2009; Mbanzibwa *et al*., 2014). The small RNAs are naturally generated in plants following sequence-specific cleavage of viral double stranded RNA molecules. Viruses produce double stranded RNA molecules during their replication and these double stranded RNAs trigger plant defence mechanism known as RNA silencing. The endonucleases

(dicer like; DCL) cleave double stranded RNA, which is then incorporated into Argonaute protein to form RNA-induced silencing complex (RISC) which targets and chops homologous viral RNAs into small RNAs of sizes 21 to 25 nt (Pantaleo *et al*., 2007; Mlotshwa *et al*., 2008; Mbanzibwa *et al*., 2014; Wilson and Doudna, 2013; Unver and Budak, 2009). The small RNAs of the sizes 21, 22 and 24 nt contain small RNAs cleaved from virus strands.

It is known that there are cleavage hotspots (normally GC-rich) in the viral genomes which may cause different quantities of small RNAs from different viral genomic regions (Donaire *et al*., 2009). The small RNAs, cleaved from both DNA and RNA viruses, are targeted in deep sequencing to detect all types of viruses in plant samples (Kreuze *et al.,* 2009). There are different protocols for isolation of small RNAs and may involve purification by electrophoresis in acrylamide gel (Mbanzibwa *et al*., 2014). Complementary DNA libraries are then made using isolated small RNAs and sequenced.

There are many commercial sequencers of different companies but the most leading companies' platforms are Roche, Illumina and Life technology. Roche do pyrosequencing by using 454 FLX Titanium and 454 FLX+ platforms while Life technology company sequence by ligation using SOLiD 4, SOLiD 5 500, SOLiD 5 500xl, SOLiD 5 500W and SOLiD 5 500xlW platforms. And Illumina do sequencing by synthesis by using GAIIx, HiSeq 1 000, HiSeq 1 500, HiSeq 2 000, HiSeq 2 500, HiSeq 3 000, HiSeq 4 000, HiSeqX ten, NovaSeq5 000 and NovaSeq 6 000 platforms (Pillai *et al*., 2017). Versions of the platforms are improved from time to time.

Selection of platform depends on the genome studied, accuracy and depth coverage needed. For sequencing, Illumina and SOLiD platforms are presently the best in terms of accuracy and throughput (Radford *et al*., 2012).

The Illumina sequencing technologies vary in the output they produce, but the Ilumina Hiseq 2 500 has the lowest cost of sequencing and produces the biggest output. This platform has been used more frequently in several countries compared to other methods of sequencing (Barba *et al*., 2014). Recently Illumina has confirmed the presence of HiSeq X and HiSeq 3 000/4 000, which have been proven to be efficient in terms of their ultra-high-output (Levy and Myers, 2016).

The first procedure after having the DNA-Seq data is alignment and assembly. The alignment of the DNA-Seq reads to the reference sequence is achieved by using many different software depending on the output required by the researcher: MAQ, BWA, BWA-SW, PERM, BOWTIE, SOAPV2, MOSAIK and NOVOALIGN (Torri *et al*., 2012), while those for *De novo* assembly include Velvet, Spades, SOAP-denovo, MIRA, ALPATHS, QUAST and InGAP-SV (D'Agaro, 2017), ABYSS (Torri *et al*., 2012). There are other commercial packages like Geneious (http://www.geneious.com) and CLC-Bio (http://www.clcbio.com), VirusDetect program (Zheng *et al*., 2017). Although it is possible to assemble and map plant virus genomes using commercial packages (Kehoe *et al*., 2014), it is expensive to purchase such software as CLC genomic workbench and Geneious whose costs may be around USD 6 000 and 300, respectively, or even more depending on terms and conditions

(Smith, 2014). There are also costs associated with updating of software (Smith, 2014; Mwaipopo *et al*., 2017).

Recently, there has been an increase in number of software for NGS data analysis. Choosing software to use requires thinking on time, expertise, type and capability of computers for handling of these softwares, which are used to analyse huge data (Torri *et al*., 2012). Generally, extraction of useful information from NGS data requires availability of competent bioinformaticians and facilities including high capability computational machines and access to useful software (Mwaipopo *et al*., 2017).

1.12 Justification of the Study

Production of common beans in Tanzania is presently estimated at 1 158 039 tons annually (FAO, 2016). The average bean yield on smallholder fields in Tanzania is <1 035 kg/ha while the yield of improved varieties at research stations and commercial farms is estimated at 1 500 to 3 000 kg/ha (Hillocks *et al*., 2006). Several biotic and abiotic factors are reportedly the main cause of low common bean yields on samallholders' field (Hillocks *et al*., 2006; FAO, 2016). Among the biotic factors are the diseases caused by fungal, bacteria and viruses (Fivawo and Msolla, 2012).

Viruses are economically important pathogens that limit common beans production wherever the crop is grown (Mavric and Vozlic, 2004). Virus diseases can cause serious damages to plants thereby leading to huge crop yield losses as high as 100% (Segundo *et al*., 2008). In Tanzania several viruses of common beans and wild legumes have been reported; BCMNV (Silbernagel *et al*., 1986), BCMV (Spence and Walkey, 1994), BCMV and BCMNV (Njau and Lyimo, 2000), CPMMV (Mink and

Keswani, 1987), CMV (Njau *et al*., 2006), CABMV (Patel and Kuwite, 1982) and PeMoV (Bock, 1973).

Weeds and wild plants, whether indigenous or introduced, can be infected by a huge number of plant viruses, as a result may act as reservoirs of crop-infecting viruses (Seal *et al.*, 2006). The biotrophic nature of viruses requires the alternative host in absence of main host so that the circle of survival is insured through virus-host-vector association (Ara *et al*., 2012). For example, in nature BCMV and BCMNV principally infect *Phaseolus* species, especially common beans (Spence and David, 1994). BCMV has been reported to occur naturally in several non - *Phaseolus* legume species (Singh and Singh, 1977; Sarkar and Kulshreshtha, 1978; Frencel and Pospieszny, 1979; Meiners *et al.*, 1978; Zaumeyer and Thomas, 1957).

All past surveys of common beans viruses in Tanzania were planned in a way to meet some specific objectives. One of the comprehensive surveys to ever take place in the past was on virus transmission through common bean and legume seeds (Njau and Lymo, 2000). The others studies were conducted but never covered all places where common bean production occurs. Thus, it was not possible to get enough data to establish the levels of incidence of common bean viruses for the whole country.

Molecular information on viruses in Tanzania is scanty because there were no powerful tools to simultaneously detect viruses in samples. Previous works on common beans viruses in Tanzania was based only on ELISA, conventional PCR and differential cultivars tests (Mwaipopo *et al*., 2017). These techniques, however, have

limited sensitivity and reliability (Boonham *et al*., 2014) and, as such, were apparently unable to detect the entire range of the bean infecting viruses. In this study, NGS and Sanger sequencing techniques were used simultaneously to detect bean infecting viruses in the common bean samples collected in the country. A combination of NGS and Sanger sequencing techniques can help in generating information on genetic variability and the occurrence of any new pathogens, and hence aid in development of rapid, reliable and cost-effective diagnostic tools that can be used in integrated pest management (IPM).

Management of plant virus diseases requires a thorough understanding of the genetic diversity of causal viruses. Unfortunately, in Tanzania, common bean viruses have not been characterized at the molecular level. NCBI search revealed that only one isolate of BCMNV from Tanzania has been sequenced (Mwaipopo *et al*., 2017; Larsen *et al*., 2011). There were no complete or partial sequences of other viruses from Tanzania (Mwaipopo *et al.,* 2017). Molecular information on virus is required for a number of reasons: development of molecular based tools – e.g., PCR, understanding of selection pressure and evolution of the viruses (informs breeders on the virus hotspots and areas where resistant varieties need to be deployed for the management of the viruses).

There are many methods which can be used to control viruses including control of vectors, use of disease free seeds and cultural practices, but the use of resistance materials is the most reliable and cost effective way of reducing yield losses. In Tanzania, most of the common bean materials have been screened for tolerance or

moderate resistance against BCMV and BCMNV only (Tryphone *et al*., 2013; Kusolwa *et al*., 2016). This study sought to challenge common bean genotypes to different viruses and identify those genotypes, which can tolerate or resist virus diseases. Such materials can then be used by common bean breeders in the country. In Tanzania, more efforts have been put only on two viruses BCMV and BCMNV, whereby there is high possibility of existence of more viruses due to unusual viral symptoms which are frequently being observed in farmers' fields. Breeding for resistance to common bean viruses is very important in Tanzania, as it may result into the development of bean materials with resistance or tolerance to major viruses' occurring in Tanzania. This study was achieved through challenging of both released and local varieties with selected common bean viruses.

1.13 Objectives

1.13.1 Overall objective

To establish the incidence, distribution and molecular characterization of economically important viruses for effective and sustainable management of the common bean viral diseases in Tanzania

1.13.2 Specific objectives

i. To characterize common bean viruses isolated from common bean (*P. vulgaris* L.) using sequencing molecular techniques
ii. To determine the incidence and distribution of major viruses of common beans (*P. vulgaris* L.) in Tanzania
iii. To characterize at molecular level the viruses of common bean in wild plants and identify the wild plants of viruses hosts in Tanzania

iv. To determine the suitable sizes of reads from deep sequenced small RNAs data for VirusDetect software-based detection of common bean (*P. vulgaris* L.) viruses using low capability computers
v. To determine genetic diversity of common bean (*P. vulgaris* L.) cultivars and landraces using diversity array technology (DArT) in Tanzania, and
vi. To evaluate the response of selected common bean genotypes to four viruses of common bean (*P. vulgaris* L.) in Tanzania

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CHAPTER TWO

2.0 CHARACTERIZATION OF COMMON BEAN VIRUSES ISOLATED FROM COMMON BEAN (*Phaseolus vulgaris* L.) USING SEQUENCING MOLECULAR TECHNIQUES

Beatrice Mwaipopo[1, 3*], Susan Nchimbi-Msolla[1], Paul Njau[1], and Deusdedith Mbanzibwa[2]

[1]Department of Crop Science and Horticulture, Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Tanzania.

[2]Tanzania Agricultural Research Institute Mikocheni, P. O. Box 6226, Dar es Salaam, Tanzania.

[3]Tanzania Agricultural Research Institute Ilonga, P.O Box 33, Morogoro, Tanzania.

Corresponding author email: beatricemwaipopo@yahoo.com*

Abstract

Viral diseases constrain common bean (*Phaseolus vulgaris* L.) production in Tanzania. Worldwide at least 35 viruses are known to infect common bean naturally. In Tanzania, there is scanty information on the viruses that infect common bean and many have not been characterized at the molecular level. In this study, symptomatic and asymptomatic common bean leaf samples were collected from all 11 major common bean growing areas in Tanzania and nucleic acids were extracted. To universally detect viruses in the collected samples, next generation sequencing (NGS) was done on viral-derived small RNAs. Analysis of 21 - 24 nucleotides (nt) sized small RNAs revealed there were 15 viruses infecting common bean plants in the country. The viruses belonged to 11 genera: *Potyvirus*, *Sobemovirus*,

Alphaendornavirus, *Carlavirus*, *Cucumovirus*, *Umbravirus*, *Crinivirus*, *Begomovirus*, *Cytorhabdovirus*, *Caulimovirus* and *Soymovirus*. *De novo* assembly resulted in many contigs including complete or nearly complete sequences of *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV) and *Southern bean mosaic virus* (SBMV). Some viruses, for example SBMV and *Tomato leaf curl Uganda virus*-related begomovirus were detected for the first time in common bean plants in Tanzania. Sanger sequencing was used to confirm some of the viruses detected by NGS. The Sanger - based nucleotide sequences encoding coat proteins of BCMV, BCMNV and CPMMV isolates were 90.2 to 100%, 97.1 to 100% and 82.9 to 99.1% identical to each other, respectively. Phylogenetic analysis showed that BCMV (21) isolates were more diverse than BCMNV (12) isolates. The Russian (RU1) and NL-1 were the most common of the BCMV strains in common bean plants in the country. High genetic variation was also observed within isolates of CPMMV. For the first time, next generation sequencing was used to detect common bean viruses in Tanzania. The information generated here will be of value in the development of molecular diagnostic tools and strategies for management of viral diseases (e.g., sites for seed multiplication and their strategic deployment) in and outside the country.

Keywords: BCMV, BCMNV, Common bean viruses, Molecular detection Tanzania

2.1 Introduction

Tanzania is the largest producer (1 158 039 tons annually) of common bean (*Phaseolus vulgaris* L.) in sub-Saharan Africa (FAO, 2016). The increase in quantity of bean produced in Tanzania is due to area expansion rather than increase in

productivity (FAO, 2016). In terms of yield per unit area, Tanzania is outperformed by several East African countries: Uganda, South Sudan, Madagascar (FAO, 2016). The estimated yield of common bean for Tanzania is <1 000 kg/ha and the potential yield from 1 500 to 3 000 kg/ha (Hillocks *et al*., 2006; Nchimbi-Msolla, 2013). The poor yields of common bean are attributed to fungal, bacterial and viral diseases among other factors (Hillocks *et al.*, 2006; Mwaipopo *et al.*, 2017).

Worldwide, about 35 viruses are known to naturally infect common bean plants, causing yield losses as high as 100% (Segundo *et al*., 2008; Hagedorn and Inglis, 1986; Worrall *et al.*, 2015). But, *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) are considered the most important viruses of common bean worldwide (Grogan and Walker, 1948; Worrall *et al*., 2015). Five viruses – BCMV, BCMNV, *Cowpea mild mottle virus* (CPMMV; *Carlavirus*), *Cucumber mosaic virus* (CMV; *Cucumovirus*) and *Cowpea aphid-borne mosaic virus* (CABMV; *Potyvirus*) have been detected either using enzyme linked immunosorbent assays (ELISA) or differential cultivars in common bean plant samples collected from different parts of Tanzania (Davis and Hampton, 1986; Mink and Keswani, 1987; Njau and Lyimo, 2000; Njau *et al*., 2006).

A comprehensive review on viruses infecting common bean in Tanzania and the status of their characterization at the molecular level showed that there is scant information about viruses infecting common bean plants in Tanzania (Mwaipopo *et al*., 2017). However, recently, using next generation sequencing (NGS), two non-pathogenic dsRNA viruses belonging to the genus *Alphaendornavirus* (*Phaseolus vulgaris*

alphaendornavirus 1 (PvEV-1) and *Phaseolus vulgaris alphaendornavirus 2* (PvEV-2) and a pathogenic virus, CPMMV, were detected in seeds collected from farmers in three agricultural research zones of Tanzania (Nordenstedt *et al.*, 2017). *Peanut mottle virus* (PeMoV) has been detected in common bean samples in Zambia, and *Bean yellow mosaic virus* were detected in samples from Kenya (Vetten and Allen, 1991). Thus, this information suggests that common bean plants in East Africa are infected by many different viruses.

Common bean is grown in many geographically isolated parts of the country and consequently genetically distinct (known and unknown) viruses may occur. ELISA method was commonly used to detect viruses in Tanzania (Mwaipopo *et al*., 2017). Unfortunately, a given antibody can only be used to detect a single virus or a group of very closely related viruses. Thus, many plant samples may test negative despite showing typical viral disease symptoms. Next generation sequencing is presently the most robust technique for detection of viruses and has unique power to universally detect viruses of all types, thus overcoming limitations of other plant pathogen detection methods (Boonham *et al*., 2014; Kehoe *et al*., 2014; Kreuze *et al*., 2009). NGS technique is used in detection and discovery of novel viruses, obtaining of partial and complete nucleotide sequences, investigation on viral quasispecies and antiviral defense mechanism (Prabha *et al*., 2013). NGS gives high levels of multiplexing instead of using virus specific reagents (Boonham *et al*., 2014).

In Tanzania and elsewhere, NGS based detection of viruses has been done using total RNA extracted from cassava (Ndunguru *et al*., 2015) or through deep sequencing of

virus-derived small RNAs or micro RNAs isolated from sweet potato plants (Kreuze *et al*., 2009; Mbanzibwa *et al*., 2014). The small RNAs are generated in plants following sequence-specific cleavage of viral double stranded RNA molecules. Viruses produce double stranded RNA molecules during their replication and these double stranded RNAs trigger plant defence mechanism known as RNA silencing. The endonucleases (dicer like, DCL) cleave double stranded RNA into small RNAs, which are then incorporated into Argonaute protein to form RNA-induced silencing complex (RISC). The RISC is then guided by the incorporated single strand viral derived small RNA to targets and cleave homologous viral RNAs into viral small RNAs of sizes 21 to 25 nt (Pantaleo *et al*., 2007; Mlotshwa *et al*., 2008; Mbanzibwa *et al*., 2014).

Prior to this work, only one nucleotide sequence of a BCMNV isolate originating from Tanzania was available in the GenBank (Larsen *et al.*, 2011; Accession no. HQ229995) and there was no any other Tanzanian sequence of common bean viruses (Mwaipopo *et al*., 2017). Therefore, there was scanty molecular information of viruses infecting common bean in Tanzania. In this study, NGS and Sanger sequencing techniques were employed to universally and simultaneously detect viruses in common bean plant samples collected during the most comprehensive and countrywide surveys of common bean viruses. The information generated in this work will 1) enable development of rapid and cost-effective molecular based diagnostic tools, 2) inform common bean breeders on the viruses occurring in the country and their genetic makeup.

2.2 Materials and Methods

2.2.1 Survey and sampling for common beans

The surveys on the occurrence and distribution of viruses in common bean were conducted in 23 districts (Fig. 2.1) in five common bean growing Agricultural Research Zones: northern, eastern, southern highlands, lake and western zones. A total of 279 fields were surveyed in five zones and 4 to 15 common bean fields were surveyed per district depending on the availability of common bean fields in the areas. The common bean leaf samples collected per district ranged from 120 to 450. Thus, countrywide, a total of 7 756 common bean leaf samples were collected. In each zone, the selection of fields for common bean leaf samples collection was done randomly along the main and feeder roads. The distance between sampled fields was a few hundred meters to 10 kilometres or more depending on the availability of common bean fields. The sampling was done when common bean plants were at the flowering stage.

Fifty common bean plants were visually assessed for the presence or absence of the viral disease symptoms. Then using a 2 x 2 m quadrat, five points were randomly chosen in each field and observations of virus-like disease symptoms were made on 10 plants at each point. For laboratory analysis at Mikocheni Agricultural Research Institute, at each selected point, six plants were sampled and kept in herbarium (plant - press), silica gel, $CaCl_2$ and ELISA bags. Thus, a total of 30 symptomatic and asymptomatic leaf samples were collected per field. These leaf samples were collected in nylon (ELISA) bags and frozen at -80 °C.

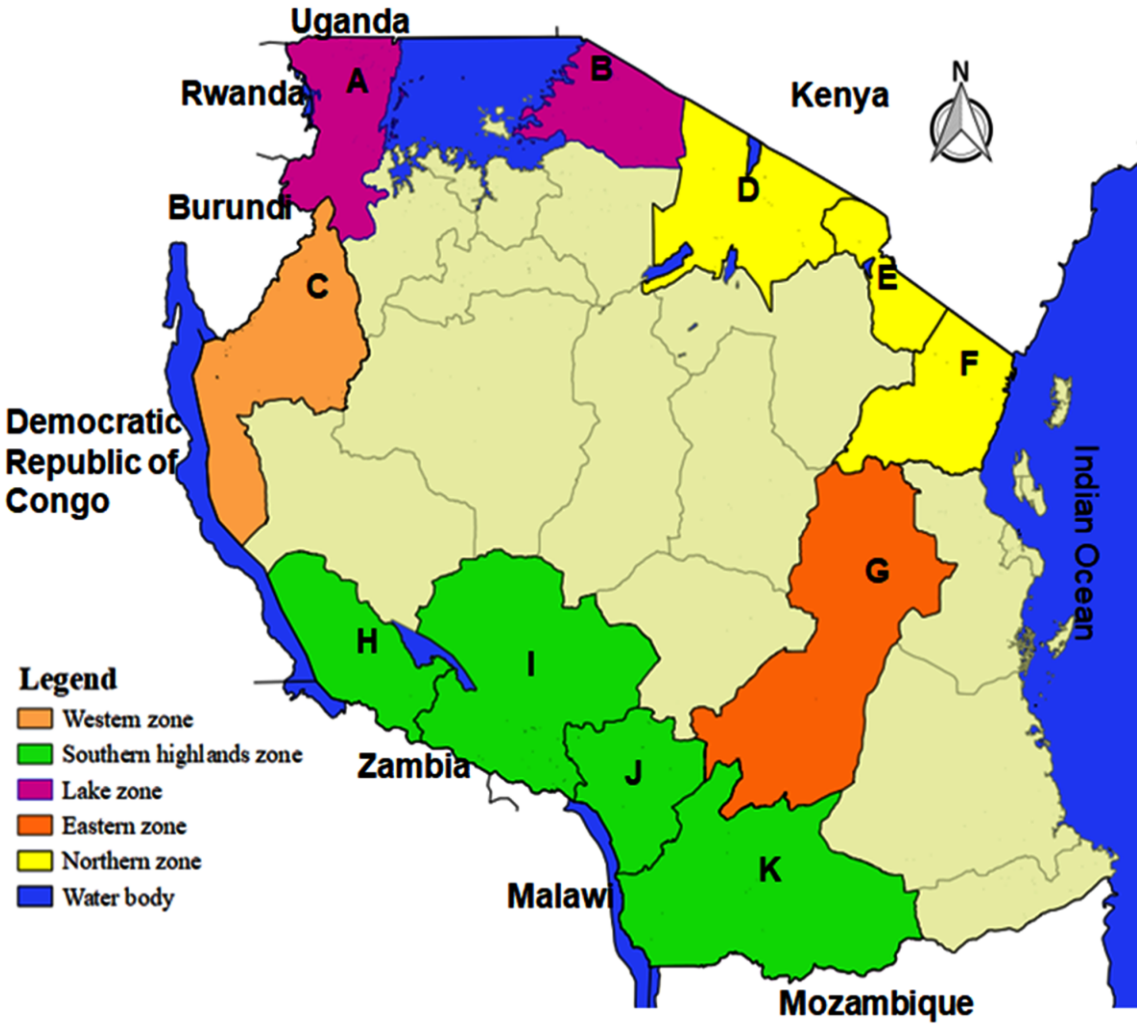

Figure 2.1: Sites surveyed for common bean viral diseases in Tanzania

Locations surveyed for common bean viral diseases in Tanzania. lake zone: **A**, Kagera region (Ngara, Biharamulo, Muleba, Missenyi and Karagwe districts) and **B**, Mara region (Tarime district). Western zone: **C**, Kigoma region (Kasulu and Kibondo districts). Northern zone: **D**, Arusha region (Karatu and Arumeru districts), **E**, Kilimanjaro region (Siha and Hai districts) and **F**, Tanga region (Lushoto district). Eastern zone: **G**, Morogoro region (Morogoro Rural, Mvomero and Gairo districts). Southern highlands zone: **H**, Rukwa region (Nkasi district), **I**, Mbeya and Songwe regions (Mbozi, Mbeya Rural districts), **J**, Njombe Region (Njombe Rural and Wanging'ombe districts) and **K**, Ruvuma region (Namtumbo and Mbinga districts).

2.2.2 Nucleic acid extraction

Nucleic acids (DNA and RNA) were extracted from a total of 7 756 common bean leaf samples from either silica gel and $CaCl_2$ desiccated or herbarium-pressed dry common bean leaf samples using a modified Cetyl trimethyl ammonium bromide

(CTAB) method (Allen *et al.*, 2006). A 0.03 g of common bean leaf samples were taken from the plant-press and the samples were put into the eppendorf tubes containing two stainless steel balls and ground into fine powder using a genogrinder (SPEX Sample prep® Genogrinder 2010, Metuchen, NJ, USA) at 1 400 rpm for 45 seconds and repeated two to three times. Then, 750 µl of CTAB (2 g CTAB, 2.5 M NaCl, 100 mM Tris-HCl and 20 mM EDTA) buffer containing 1% sodium sulphite, 2% polyvinylpyrrolidone and 2.5% mercaptoethanol was added into each Eppendorf tube. The samples were vortexed for a few seconds to dispense tissue in buffer, and then incubated in the heating block (STUART ® block heater (SBH 130D) Staffordshire, UK) at 65 ^{0}C for 30 min while mixing by inversion after every 10 min samples were subsequently removed from the water bath and left at room temperature for 10 min. Equal volume (750 µl) of chloroform: isoamyl alcohol (24: 1) was added and mixed by inversion for 10 min. The mixture was centrifuged (MIKRO 220R Hettich Zentrifugen**,** Tuttlingen Germany) at 12 000 rpm for 10 min at 4 ^{0}C. A total volume of 500 µl from the upper aqueous phase (supernatant) was transferred to a 1.5 ml clean and sterile tube. Then equal volume (500 µl) of cold isopropanol was added and inverted gently to precipitate nucleic acids. The mixture was incubated at -20 ^{0}C for 30 min. The tube was spin at 13 000 rpm for 10 min at 4 ^{0}C and then the isopropanol was decanted. Five hundred (500 µl) of 70% ethanol was added for washing the pellet. It was spin again at 13 000 rpm for 5 min at 4 ^{0}C and the ethanol was decanted. The pellets were air dried for 40 min and then re-suspended in 40 µl of nuclease-free water.

2.2.3 Deep sequencing of small RNAs (Next generation sequencing)

Equal amounts (7 µg) of total RNA extracted from 30 plants from each of the five zones were separately pooled to make six (two samples for the lake zone) zonal pooled RNA samples. The selection of the samples was based on variation in symptoms as observed among the samples. The pooled samples were HXH-1 (30) from southern highlands zone, HXH-2 (30) from eastern zone, HXH-3 (30) from northern zone, HXH-6 (20) also contained RNA from cassava plants and HXH-7 (30) both were from lake zone, and HXH-15 (30) from western zone. Moreover, there were three non-pooled RNA samples coded HXH-4, HXH-5, and HXH-13. The HXH-4 sample also contained RNA extracted from cassava, a subject for another study. For deep sequencing of small viral RNAs, total RNA was shipped on dry ice to Fasteris SA in Switzerland. Then the small RNAs were isolated (acrylamide gel size selection) and cDNA libraries prepared and sequenced using Illumina HiSeq 2 500 (Illumina Inc., San Diego, CA, USA) as described previously (Mbanzibwa *et al.*, 2014; Nordenstedt *et al.,* 2017).

2.2.4 Sanger sequencing

2.2.4.1 Complementary DNA synthesis

The first - strand complementary DNA (cDNA) synthesis was done using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MuLV RT; #M0253; New England Biolabs (NEB), Ipswich, MA, USA) following a standard protocol with some modifications. The first mix contained 1 µg of RNA, 1 µl of 100 µM oligo $(dT)_{18}$ or random hexamer (Bioneer, Seoul, South Korea), 1 µl of dNTPs and DEPC water to a volume of 10 µl. The mixture was heated at 65 °C for 5 min and spun down after

chilling on ice for 2 min. The second master mix was prepared according to NEB's standard protocol. Total volume for cDNA synthesis reaction was 20 µl. Random hexamer and oligo $(dT)_{18–21}$ primed reactions were incubated at 37 °C and 42 °C, respectively, for 90 min and enzymes were inactivated at 65 °C for 20 min.

2.2.4.2 Polymerase chain reaction (PCR) of BCMV, BCMNV and CPMMV

The total of 43 common bean representative nucleic acid samples from different zones were selected and purposely amplified for BCMV (21), BCMNV (12) and CPMMV (10). PCR reactions were run using different pairs of primers (Table 2.1). These primers were initially designed using sequences of BCMNV, BCMV and CPMMV retrieved from GenBank and later using some sequences obtained in this study. A high fidelity Phusion DNA polymerase (#M0530S; NEB) was used when PCR was run to generate PCR products for Sanger sequencing. For Phusion DNA polymerase, the 50 µl PCR reaction contained 10 µl of 5× Phusion GC buffer, 1 µl of dNTPs (10 mM), 2 µl of each forward and reverse primer (10 µM), 0.5 µl of Phusion DNA polymerase (2 U/µl) and 5 µl of cDNA template. The primer pairs used to amplify BCMV and BCMNV for sequencing were BCMVFcpF1/BCM-NVcommonR or BCMVFcpF1/ BCMVFcpR1 and BCMNVFcpF2/BCM-NVcommonR or BCMNVF1/BCMNVR1, respectively (Table 2.1). For CPMMV, CPMMV2F1/ CPMMV2R1 primer pair was used (Table 2.1).

The PCR program was the same for the four pairs of primers of BCMV and BCMNV; initial denaturation was at 98 °C for 30 sec, followed by 32 cycles at 98 °C for 5 sec, 57 °C for 20 sec and 72 °C for 30 sec. Final extension was at 72 °C for 10 min. The

annealing temperature for CPMMV was 56 °C. Two primer pairs (533-340F1/533-340R1 and 533-139F1/533-139R) were designed to amplify BCMV in order to compare sequences obtained by NGS and Sanger sequencing data for isolate TZ: Mor533:2017. These primers annealed at 60 °C. The primer pair ToLCUV-F1/ToLCUV-R1 was used to detect *Begomovirus* in common bean samples (Table 2.1). The PCR products were run in a 1% agarose gel stained with ethidium bromide. Gel images were captured using a Benchtop UV Transilluminator (UVP; Upland, CA, USA) under UV light.

Table 2.1: Primers designed and used in this study

Primer name (in pairs)	Primer sequences 5′–3′ direction	Product size (bp)	Targeted region[1]	Virus[2]
BCMVFcpF1	GCGGAGAATCTGTGCACCTACA			
BCM-NVcommonR	GTCCCKTGCAGTGTGCCT	839	CP	BCMV
BCMVFcpF1	GCGGAGAATCTGTGCACCTACA			
BCMVFcpR1	ATTGCAATGGTTCTTCCGGC	1075	CP, 3′UTR	BCMV
BCMNVFcpF2	GCTGGGGCCGATGAGAG			
BCM-NVcommonR	GTCCCKTGCAGTGTGCCT	711	CP	BCMNV
BCMNVF1	CAAAGGCCCAGCGGATAAA			
BCMNVR1	GGTGGTATAACCACACTGGAATTG	823	CP, 3′UTR	BCMNV
533-340F1	GCTGGAACAGCTCACCAA			
533-340R1	CCTTTGATTCTCTCTGCCTTT	668	P3	BCMV; TZ:Mor533:2017
533-139F1	GTCAAGCAAGCAAAGAGTGC			
533-139R1	TGTGTAATCCCTCAAATACCGC	546	CI	BCMV; TZ:Mor533:2017
ToLCUV-F1	GTGAATCCCCAATTCCTTCCTC			
ToLCUV-R1	TCCCACTATCTTCCTCTGCAA	434	C2, C3	ToLCUV
CPMMV2F1	AACAAAAACTGGCGTTCCAAA			
CPMMV2R1	GGAAAATAACTTTAAAACCGG	1300	CP	CPMMV

[1]CP, UTR, P3 and CI indicate viral coat protein, untranslated region, third protein and cytoplasmic inclusion, respectively while C2 and C3 refer to transcriptional activation and replication enhancement proteins of *Tomato leaf curl Uganda virus* (ToLCUV) - related *Begomovirus* detected in this study.
[2]TZ:Mor533:2017 is an isolate of BCMV that was collected from Morogoro region in eastern zone.

2.2.4.3 Sequencing of PCR products

A total of 43 PCR products were sequenced at three different facilities: Haartman Institute (Finland), Mbeya Zonal Referral Hospital Laboratory (Tanzania) and Bioneer (Seoul, South Korea). PCR products were purified using PCR purification kits (Bioneer) or treated with exonuclease I and calf intestinal alkaline phosphatase (NEB) following the enzyme manufacturers' instructions, and then sequenced on both strands. Sequences were submitted to the NCBI nucleotide database and assigned accession numbers (Table 2.2 and Appendix 2.1).

Table 2.2: Accession numbers assigned to viral sequences obtained in this study

No	virus	No. of sequences	Method of sequencing	Accession numbers
1	BCMV	21	Sanger	MF043409, MF043410, MF043411, MF043412, MF043413, MF043414, MF043415, MF043416, MF043417, MF043418, MF043419, MF043420, MF043421, MF043422, MF043423, MF066258, MF066259, MF066260, MF784802, MF784803, MF784804
2	BCMNV	12	Sanger	MF066261, MF066262, MF066263, MF066264, MF066265, MF066266, MF066267, MF066268, MF066269, MF066270, MF066271, MF066272
3	SBMV	3	NGS	MF784807, MF784808, MG344643
4	PeMOV	2	NGS	MF784805, MF784806

5	BCMV	3	NGS	MF405190, MF405191
6	BCMNV	4	NGS	MF078484, MF405187, MF405189, MF405192

2.3 Data Analysis

2.3.1 Next generation sequencing data analysis

Analysis of NGS data was done using the VirusDetect program v.1.6 and v.1.7 (Zheng *et al.,* 2017) on a supercomputer (https://www.csc.fi; Finland) accessed between January 2016 and October 2017. The files received from Fasteris SA (with extension '.tar'; submitted at Zenodo and assigned DOI 10.5281/zenodo.841170) were unzipped using the command 'tar -vxf filename'. Then, all reads of sizes not within 21 to 24 nucleotides (nt) were deleted. The remaining reads were analysed using two approaches: *de novo* assembly was first done on each read size separately, and later the inserts for these four sizes were combined (for simplicity herein called 'combined inserts') using the command 'cat *.fastq >filename.fastq' to obtain one fastq file and assembled using the command 'virus_detect.pl *.fastq' (for offline analysis the command was 'perl virus_detect.pl filename'). Offline analysis on desktop/laptop computers (random access memory of 8 GB; installed with virtual Linux machine) using VirusDetect (v.1.6) was possible for 'not combined' reads but failed for some combined inserts (i.e., inserts of sizes 21 to 24 nt as a single fastq file). The contigs obtained were inspected for open reading frames using the Expasy translate tool (http://www.expasy.org/). To obtain and manually edit longer nucleotide sequences, contigs obtained by analysing inserts of sizes 21 or 22 nt were aligned against identical contigs obtained through analysis of combined inserts. For the non-pooled sample HXH-4 (isolate TZ:Mor533:2015), the contigs that mapped to the same

reference sequence or to too-closely related virus sequences were assembled using the SeqMan program (v.5.03) (DNASTAR, Madison, WI, USA) and cross-checked using Sanger sequencing.

2.3.2 Sequence analysis

Phylogenetic analysis was achieved using MEGA7 software (Kumar *et al*., 2016). The coat protein (CP) - encoding nucleotide sequences of BCMV, BCMNV and CPMMV were first aligned using the MUSCLE program (Edgar, 2004) and trimmed to equal size (620 nt) for BCMV and BCMNV and (229 nt) for CPMMV. Then, the evolutionary history was inferred using the Maximum Likelihood method based on the Tamura–Nei model (Tamura and Nei, 1993). The tree with the highest log-likelihood was used. Initial tree (s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pair wise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log-likelihood value. All positions containing indels were eliminated. Nucleotide and amino acid sequence identities were determined using the BioEdit Sequence Alignment Editor (Hall, 1999). Translation of nucleotide sequences into protein sequences was achieved using the ‘translate’ option in MEGA7. Putative cleavage sites in potyviral sequences were predicted as described by Adams *et al*. (2005); for other viruses, comparisons were made to previously annotated sequences.

2.4 Results

2.4.1 Number of reads and inserts in sequenced samples

The results showed that the highest proportion of reads were in the inserts range of 18 to 26 nt although for the sample HXH-1 the highest number of reads were observed for the insert size of 27 to 44 nt. The percentages of reads were more than 45% across all samples for an insert range of 18 - 26 nt (Table 2.3; Fig. 2.2).

Table 2.3: Number of reads (small RNA) obtained through NGS on common bean RNA samples

Samples [1]		Insert range (nt)				
		0	**1-17**	**18-26**	**27-44**	**Remaining**
HXH-1	Reads	21 614	1 890 768	9 216 550	7 451 870	262 711
	% reads	0.11	10.03	48.91	39.55	1.39
	%insert	0.12	10.18	49.60	40.11	-
HXH-2	Reads	31 533	3 388 844	11 556 722	4 880 144	326 809
	% reads	0.16	16.79	57.26	24.18	1.62
	%insert	0.16	17.07	58.20	24.58	-
HXH-3	Reads	19 427	2 931 313	9 416 084	5 234 238	307 577
	% reads	0.11	16.37	52.58	29.23	1.72
	%insert	0.11	16.65	53.50	29.74	-
HXH-4	Reads	116 863	3 212 740	21 580 900	9 197 491	798 187
	% reads	0.33	9.20	61.83	26.35	2.29
	%insert	0.34	9.42	63.27	26.97	-
HXH-5	Reads	719 361	2 280 432	27 552 156	2 781 016	711 548
	% reads	2.11	6.70	80.93	8.17	2.09
	%insert	2.16	6.84	82.66	8.34	-
HXH-6	Reads	119 134	587 994	15 825 457	11 025 094	639 633
	% reads	0.42	2.09	56.12	39.10	2.27
	%insert	0.43	2.13	57.43	40.01	-
HXH-7	Reads	125 445	984 979	13 521 055	14 774 963	600 821
	% reads	0.42	3.28	45.06	49.24	2.00
	%insert	0.43	3.35	45.98	50.24	-
HXH-13	Reads	66 790	2 412 551	36 334 808	9 063 562	-
	% reads	0.14	5.04	75.89	18.93	-
HXH-15	Reads	34 878	1 161 684	39 951 679	10 153 139	-
	% reads	0.07	2.26	77.88	19.79	-

[1] Abbreviations HXH-1, HXH-2, HXH-3, HXH-6, HXH-7, HXH-15 represent samples from the southern highlands, eastern, northern, lake zone (Tarime), lake zone (Kagera) and western research zones, respectively. HXH-13 was the sample from western zone that contained only a single sample having SBMV. HXH-4 and HXH-5 represent the samples from eastern and southern highlands zones, respectively. Samples HXH-4 and HXH-5 were not pooled in order to individually target BCMV and BCMNV.

2.4.2 Insert relative abundance

The insert relative abundance describes the percentage number of reads where the small RNAs are found. The main peaks were at 21 and 24 nt sizes. Abnormally, however, there was also another peak at around 30 nt size and was especially in sample HXH-1 (Fig. 2.2). Shorter inserts were also observed at around 16 nt and do most likely correspond to degradation products (Fig. 2.2).

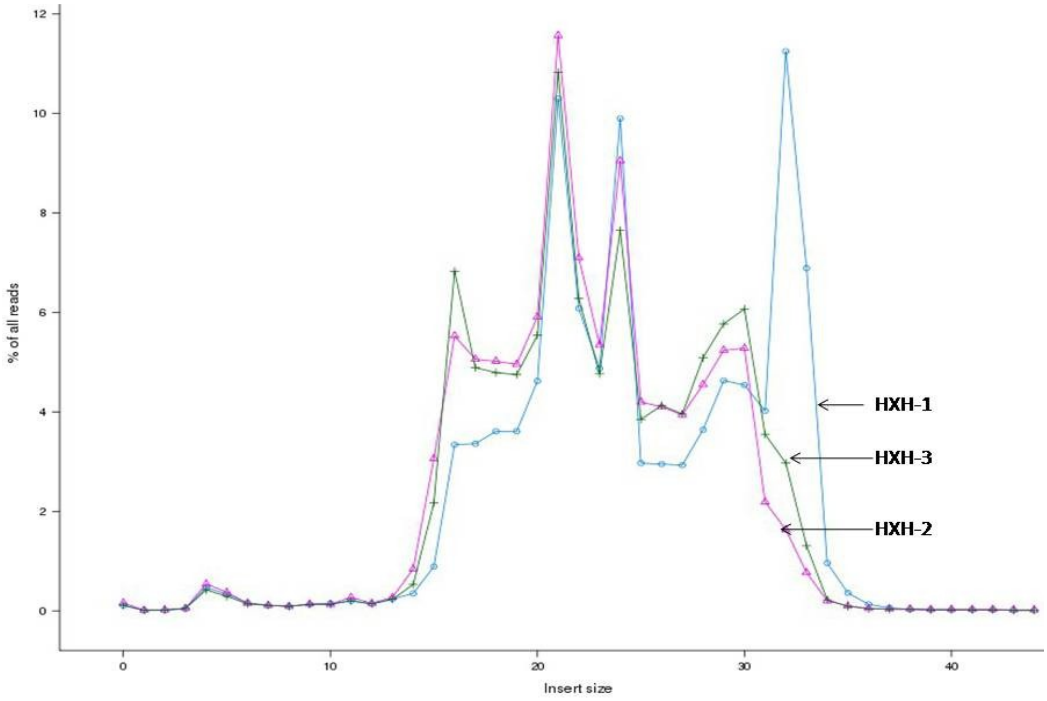

Figure 2.2: A graphical representation of the inserts for libraries

HXH-1, HXH-2, HXH-3 are the common bean samples from southern highlands, eastern, northern research zones, respectively

2.4.3 Reads aligned to reference and viruses detected by next generation sequencing

Table 2.4 shows the viruses detected in Tanzania, total reads, reads aligned to reference sequences, virus taxonomy, and accession numbers of closely related reference sequences. The reads aligned to the reference viral sequences in the sequence databases ranged from 80 846 in sample HXH-7 (from Kagera in lake Zone) to 3 793 494 in sample HXH-15 from the western zone (Table 2.4).

Blastn and Blastx revealed viruses belonging to 11 genera: *Potyvirus* (*Potyviridae*), *Sobemovirus* (*Solemoviridae*), *Alphaendornavirus* (*Endornaviridae*), *Carlavirus* (*Betaflexiviridae*), *Cucumovirus* (*Bromoviridae*), *Umbravirus* (*Tombusviridae*),

Crinivirus (*Closteroviridae*), *Begomovirus* (*Geminiviridae*), *Cytorhabdovirus* (*Rhabdoviridae*), and *Caulimovirus* and *Soymovirus* (*Caulimoviridae*) (Table 2.4; Table 2.5; Fig. 2.3). The blast searches using contig sequences obtained in this study matched sequences of over 32 viruses in the sequence database (Table 2.4). Some contig sequences obtained through de novo assembly were related to more than one virus in the sequence database (e.g., an *Umbravirus* in sample HXH-2); the most likely viruses infecting plants whose RNA was included in the sequenced samples are the ones shown in Table 2.4. Therefore, the NGS contigs revealed that common bean plants in Tanzania were infected by 15 viruses (Table 2.4).

BCMNV, PvEV-1 and PvEV-2 were the commonest viruses in samples from at least three agricultural research zones (Table 2.4; Fig. 2.4). Notably, BCMNV was detected in all but sample HXH-7 from Tarime in the lake zone agricultural research. The seed transmitted viruses; PvEV-1 and PvEV-2 were detected in pooled RNA samples HXH-1 (southern highlands zone), HXH-2 (eastern zone) and HXH-6 and HXH-7 (lake zone). PvEV-1 but not PvEV-2 was detected in the pooled RNA sample from the western zone (HXH-15). Neither PvEV-1 nor PvEV-2 was detected in sample HXH-3 (northern zone). BCMV, widely reported to infect common bean worldwide, was detected in samples from the eastern (HXH-2) and northern (HXH-3) zones but not from the southern highlands (HXH-1), lake (HXH-6 and HXH-7) and western zones.

Other viruses detected were CPMMV (HXH-2 and HXH-3), PeMoV (HXH-1; Accessions MF784805 and MF784806), SBMV (HXH-1; Accessions MF784807 and MF784807); and HXH-15, Accessions MG344643), CABMV (HXH-6), BnYDV

(HXH-3), CMV (HXH-2), unidentified umbravirus closely related to *Carrot mottle mimic virus* (CMoMV), *Carrot mottle virus* (CMoV), *Opium poppy mosaic virus* (OPMV), *Ethiopian tobacco bushy top virus* (ETBTV) and *Tobacco bushy top virus* (TBTV) (HXH-2), *Tobacco* mottle virus (TMoV) (HXH-7), *Northern cereal mosaic virus* (NCMV) (HXH-3), and a caulimovirus most closely related to *Strawberry vein banding virus* (SVBV) and other caulimoviruses (HXH-7; Table 2.4).

A sequence with some similarity to begomoviruses (*Tomato leaf curl Uganda virus*; ToLCUV) was found in sample HXH-6. A primer pair was designed and used to detect this virus in all samples that were mixed to make the pooled RNA sample HXH-6 in which ToLCUV-related begomovirus was detected. PCR results confirmed the presence of ToLCUV in one sample in the pooled RNA sample.

2.4.4 Molecular evidence for the occurrence of viruses in different RNA samples of common bean plants

Table 2.5 shows the list of viruses detected by next generation sequencing mapped to the reference sequence (accession numbers) on the data base, contig number, coverage in percent, depth and the contig length of the virus detected. The number of contigs obtained by blastn and blastx ranged from one contig for MMV, SbCMV, SVBV and SBMV to 98 contigs for PvEV-1. The genome coverage (%) ranged from 15.6% in ToLCArV to 100% in BCMV. The contigs length varied from one virus to another. For example, the longest contig of BCMNV was 9634 nt, which represented a nearly complete genome. On the contrary, the longest contig of CMoV was 143 nt (Table 2.5).

Table 2.4: Number of reads, reads aligned to reference and viruses detected by NGS of virus-derived small RNAs from common bean samples

Zone[1]	RNA pool sample	Total reads (21–24 nt)	Reads aligned to reference	Viruses detected (reference sequences and coverage in % are shown in parentheses)[2]
SHZ	HXH-1	5 869 348	370 969	BCMNV; +ssRNA; *Potyvirus* (AY864314; 95.1) PeMoV; +ssRNA; *Potyvirus* (AF023848; 98.6) SBMV; +ssRNA; *Sobemovirus* (DQ875594; 99.9) PvEV-1; dsRNA; *Endornavirus* (KT456287; 95.0) PvEV-2; dsRNA; *Endornavirus* (AB719398; 96.4)
EZ	HXH-2	6 674 109	264 061	BCMNV; +ssRNA; *Potyvirus* (AY864314; 93.4) BCMV; +ssRNA; *Potyvirus* (KT175569; 100) PvEV-1; dsRNA; *Endornavirus* (KT456287; 86.6) PvEV-2; dsRNA; *Endornavirus* (AB719398; 97.5) CPMMV; +ssRNA; *Carlavirus* (KC774020; 68.4) CMV; +ssRNA; *Cucumovirus* (KJ400004; 86.0) **CMoV; +ssRNA; *Umbravirus* (CED51824; 51.5)** **CMoMV; +ssRNA; *Umbravirus* (ACJ03575; 56.1)** **OPMV; +ssRNA; *Umbravirus* (AHZ65104; 33.0)** **ETBTV; +ssRNA; *Umbravirus* (AIL27641; 33.1)** **TBTV; +ssRNA; *Umbravirus* (TBTV; 77.9)**
NZ	HXH-3	5 286 206	203 035	BCMNV; +*ssRNA*; *Potyvirus* (AY864314; 86.1) BCMV; +*ssRNA*; *Potyvirus* (KF114860; 99.9) BnYDV; +*ssRNA*; *Crinivirus* (EU191905; 77.9) CPMMV; +ssRNA; *Carlavirus* (KJ534277; 73.6) **NCMV; -*ssRNA*; *Cytorhabdovirus* (ADE61669; 24.6)**
LZ	HXH-6	11 658 110	378 180	BCMNV; +ssRNA; *Potyvirus* (AY864314; 95.8) CABMV; +ssRNA; *Potyvirus* (DQ397527; 89.2) ToLCArV; +/–ssDNA; *Begomovirus* (DQ519575; 24) ToLCYTV; +/–ssDNA, (AJ865340; 19.3) ToLCUV; +/–ssDNA; *Begomovirus* (DQ127170; 62.8) PvEV-1; dsRNA; *Endornavirus* (KT456287; 61.8) PvEV-2; dsRNA; *Endornavirus* (AB719398; 94.2)
	HXH-7	7 989 740	80 846	TMoV; +ssRNA; *Umbravirus* (AY007231; 91.1) PvEV-1; dsRNA; *Endornavirus* (KT456287; 91.0) PvEV-2; dsRNA; *Endornavirus* (AB719398; 94.7) **RuFDV; dsDNA-RT; unassigned (ACL36982; 23.1)** **HRLV; dsDNA-RT; *Caulimovirus* (AAW56089; 14.3)** **CERV; dsDNA-RT; *Caulimovirus* (ABX80503; 23.2)** **EVCV; dsDNA-RT; unassigned (ACB69773; 13.1)** **MMV; dsDNA-RT; *Caulimovirus* (AAM53128; 22.6)** **DMV; dsDNA-RT; *Caulimovirus* (ABW80581; 24.1)** **SbCMV; dsDNA-RT; *Soymovirus* (CAA33833; 14.2)** **PEMV; +ssRNA; *Umbravirus* (AAU20330; 22.3)** **SVBV; dsDNA-RT; *Caulimovirus* (AKB94072; 36.7)** **GRV; +ssRNA; *Umbravirus* (CTQ57207; 33.7)** **SPuV; dsDNA-RT; *Caulimovirus* (AFP95350; 15.9)**
WZ	HXH-15	28 223 699	3 793 494	BCMNV; +*ssRNA*; *Potyvirus* (AY864314;77.2) SBMV; +ssRNA; *Sobemovirus* (DQ875594; 98.5) PvEV-1; dsRNA; *Endornavirus* (KT456287; 99.3)

[1] Abbreviations SHZ, EZ, NZ, LZ and WZ are as described for Table 2. [2] Database searches for bolded viruses were achieved using Blastx approach. Expanded names for abbreviations used:BCMNV '*Bean common mosaic necrosis virus*', BCMV '*Bean common mosaic virus*', PeMoV '*Peanut mottle virus*', SBMV '*Southern bean mosaic virus*', PvEV-1 '*Phaseolus vulgaris endornavirus1*', PvEV-2 '*Phaseolus vulgaris endornavirus 2*', CPMMV '*Cowpea mild mottle virus*', CMV '*Cucumber mosaic virus*', CMoV '*Carrot mottle virus*', CMoMV '*Carrot mottle mimic virus*', OPMV '*Opium poppy mosaic virus*', ETBTV '*Ethiopian tobacco bushy top virus*', TBTV '*Tobacco bushy top virus*', CABMV '*Cowpea aphid-borne mosaic virus*', BnYDV '*Bean yellow disorder virus*', NCMV '*Northern cereal mosaic virus*', ToLCArV '*Tomato leaf curl Arusha virus*', ToLCYTV '*Tomato leaf curl Mayotte virus*', ToLCUV '*Tomato leaf curl Uganda virus*', TMoV '*Tobacco mottle virus*', RuFDV '*Rudbeckia flower distortion virus*', HRLV '*Horseradish latent virus*', CERV '*Carnation etched ring virus*', EVCV '*Eupatorium vein clearing virus*', MMV '*Mirabilis mosaic virus*', DMV '*Dahlia mosaic virus*',SbCMV '*Soybean chlorotic mottle virus*', PEMV '*Pea enation mosaic virus*', GRV '*Groundnut rosette virus*', SVBV '*Strawberry vein banding virus*' and SPuV '*Soybean Putnam virus*'. For sample HXH-2 viruses CMoV, CMoMV, OPMV, ETBTV and TBTV (all belonging to genus *Umbravirus*) identified through Blastx are most likely sequences of one and the same novel virus. This Table was published in Mwaipopo *et al*. (2018);

Table 2.5: Number of contigs, coverage and contig length of viruses in different RNA samples of common bean plants

Sample[1]	Mapped reference[2]	Virus detected	Number of contigs		Coverage (%)	Depth	Contig lengths[4]	
			Blastn	Blastx[3]			Combined inserts (21–24)	Only insert size 21 nt
HXH-1 (SHZ)	AY864314	BCMNV; *Potyvirus*	2	-	95.1	614.4	45–9 412	9 634
	AF023848	PeMoV; *Potyvirus*	18	-	98.6	39.1	57 – 3 186	41 – 1 808
	DQ875594	SBMV; *Sobemovirus*	4	-	99.9	386.8	62 – 2 345	
	KT456287	PvEV-1; *Endornavirus*	68	-	95.0	14.3	41 – 825	41 – 482
	AB719398	PvEV-2; *Endornavirus*	40	-	96.4	23.4	60 – 1 106	43 – 794
HXH-2 (EZ)	AY864314	BCMNV; *Potyvirus*	9	-	93.4	189.5	41 – 6 769	41 – 2 494
	KT175569	BCMV; *Potyvirus*	28	-	100	212.8	42 – 10 061	43 – 1 868
	KT456287	PvEV-1; *Endornavirus*	87	-	86.6	10.8	41 – 537	41 – 311
	AB719398	PvEV-2; *Endornavirus*	32	-	97.5	37.9	42 – 537	42 – 1 160
	KC774020	CPMMV; *Carlavirus*	60	-	68.4	10.4	43 – 389	41 – 230
	KJ400004	CMV; *Cucumovirus*	9	-	86.0	119.0	51 – 1 209	-
	ACJ03575	CMoMV; *Umbravirus*	-	7 (10)	56.1	321.1	151 – 589	77 – 160
	CED51824	CMoV; *Umbravirus*	-	0 (2)	51.5	217.9	-	77 – 143
	AHZ65104	OPMV; *Umbravirus*		0 (5)	33.0	165.3	-	48 – 160
	AIL27641	ETBTV; *Umbravirus*	-	3 (0)	33.1	330.5	104 – 589	-
	AAN62864	TBTV; *Umbravirus*	-	3 (0)	77.9	618.9	142 – 409	-
HXH-3 (NZ)	AY864314	BCMNV; *Potyvirus*	50	-	86.1	53.5	41 – 1 007	48 - 894
	KF114860	BCMV; *Potyvirus*	15	-	99.9	305.7	45 – 7 104	45 – 7 092
	EU191905	BnYDV; *Crinivirus*	50	-	77.9	11.1	41 - 402	-
	KJ534277	CPMMV; *Carlavirus*	14	-	73.6	7.7	44 – 154	42 – 150
	ADE61669	NCMV; *Cytorhabdovirus*	-	1 (1)	24.6	131.0	348	256
HXH-6 (LZ)	DQ519575	ToLCArV; *Begomovirus*	8	-	24.0	46.7	55 – 191	-
	AJ865340	ToLCYTV; *Begomovirus*	6	-	19.3	52.8	55 – 177	-
	EF194760	ToLCArV; *Begomovirus*	6	-	15.6	30.9	47 – 177	-
	DQ127170	ToLCUV; *Begomovirus*	12	-	62.8	37.5	54 –481	44 – 140
	KT456287	PvEV-1; *Endornavirus*	98	-	61.8	18.8	41 –360	41 – 201
	AB719398	PvEV-2; *Endornavirus*	55	-	94.2	34.8	46 –1 138	43 – 727
	AY864314	BCMNV; *Potyvirus*	8	-	95.8	345.3	48 –9 419	42 – 9 161
	DQ397527	CABMV; *Potyvirus*	3	-	89.2	879.9	53 – 801	-
HXH-7 (LZ)	AY007231	TMoV; *Umbravirus*	8	-	91.1	1063.3	44 – 562	
	KT456287	PvEV-1; *Endornavirus*	75	-	91.0	11.6	43 – 849	-
	AB719398	PvEV-2; *Endornavirus*	51	-	94.7	17.4	41 – 1 519	-
	ACL36982	RuFDV; unassigned	-	5 (7)	23.1	263.0	76 – 181	51 – 187
	AAW56089	HRLV; *Caulimovirus*	-	3	14.3	226.5	83 – 129	-
	ABX80503	CERV; *Caulimovirus*	-	4	23.2	173.8	58 – 87	-
	ACB69773	EVCV; *Caulimovirus*	-	3	13.1	246.4	77 – 129	-
	AAM53129	MMV; *Caulimovirus*	-	1	14.0	1133.0	73 – 238	-
	ABW80581	DMV; *Caulimovirus*	-	5	24.1	259.1	76 – 181	-
	CAA33833	SbCMV; *Soymovirus*	-	1	14.2	271.8	188	-
	AAU20330	PEMV-2; *Umbravirus*	-	4	22.3	638.4	68 – 414	-
	AKB94072	SVBV; *Caulimovirus*	-	0 (1)	36.7	875.0	-	519
	AFP95350	SPuV; *Caulimovirus*	-	0 (3)	15.9	153.5	-	78 – 187
	CTQ57207	GRV; *Umbravirus*	-	0 (3)	33.7	443.3	-	112 – 558

HXH-15 (WZ)	AY864314	BCMNV; *Potyvirus*	54	-	77.2	13.6	40 – 806	42 – 405
	DQ875594	SBMV; *Sobemovirus*	1	-	98.5	5 632.0	4 132	120 – 3 364
	KT456287	PvEV-1; *Endornavirus*	20	-	99.3	163.8	62 – 2 022	46 – 592

[1] SHZ, EZ, NZ, LZ and WZ refer to agricultural zones in Tanzania: southern highlands, eastern, northern, lake and western zones, respectively. HXH-1, HXH-2, HXH-3, HXH-6, HXH-7 and HXH-15 are sample codes given by the sequencing company. [2] Only one representative accession no. for mapped references is shown even when there were many mapped references for the same virus in the same RNA sample; low covered references are not shown. [3] Number of contigs shown in parenthesis in column five are for Blastx, considering only inserts of size 21 nt while those not in parentheses are contigs obtained under combined inserts. [4] Combined inserts indicates reads of sizes 21, 22, 23 and 24 nt were analysed as one fastq file. When contig lengths are shown for both combined inserts and only 21-nt inserts, the coverage shown is for the combined inserts. Accession numbers for mapped reference sequences are shown for both Blastn and Blastx. Some contig sequences matched sequences of more than one virus in the same genus (for example, the contig sequence that matched SMV (HXH-3) also matched BCMV suggesting they may be contigs of BCMV; some Blastx found viruses, for example in sample HXH-3, were not shown because they are most likely sequences of BCMNV as contigs matched BCMNV sequences for which there was strong evidence of its occurrence). This Table was published in Mwaipopo *et al*., 2018 and is reproduced under conditions shown under Table 2.4.

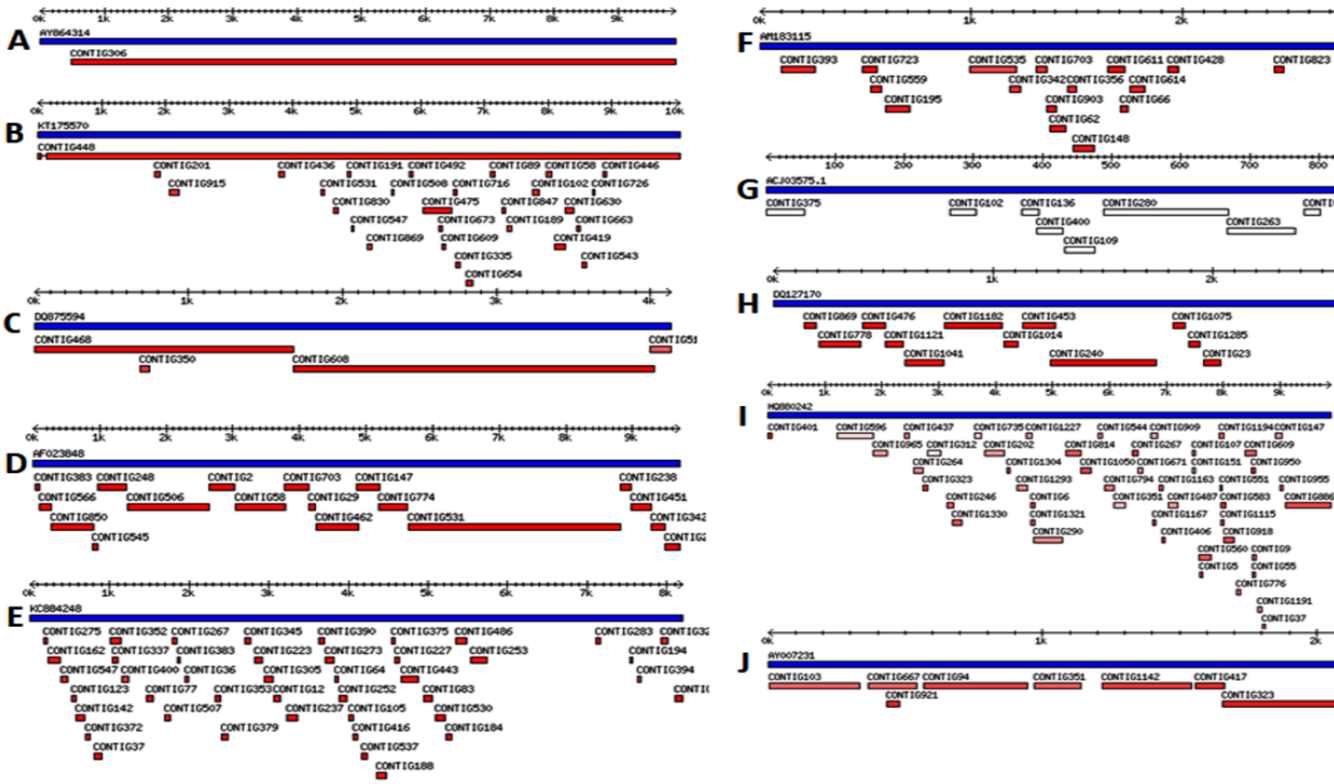

Figure 2.3: Mapping of viral contigs (obtained through *de novo* assembly of reads) to viral sequences in database

Numbers on blue and red bars are accession and contig numbers, respectively. **A,** BCMNV detected in pooled RNA sample HXH-1 by *de novo* assembly of reads of size 21 nt. **B,** BCMV detected in pooled RNA sample HXH-2 (combined inserts). **C,** SBMV detected in pooled RNA sample HXH-1(combined inserts). **D,** PeMoV in pooled RNA sample HXH-1 (combined inserts). **E,** CPMMV detected in pooled RNA HXH-3 (combined inserts). **F,** CMV in pooled RNA sample HXH-2 (combined inserts). **G,** CMoV in pooled RNA sample HXH-2 (insert size 22nt; blastx). **H,** ToLCUV-related virus in pooled RNA sample HXH-6 (combined inserts). **I,** CABMV in pooled RNA sample HXH-6 (combined inserts). **J,** *Umbravirus* related to TMoV in pooled RNA sample HXH-7 (combined inserts) (This Figure was published in Mwaipopo *et al*., 2018).

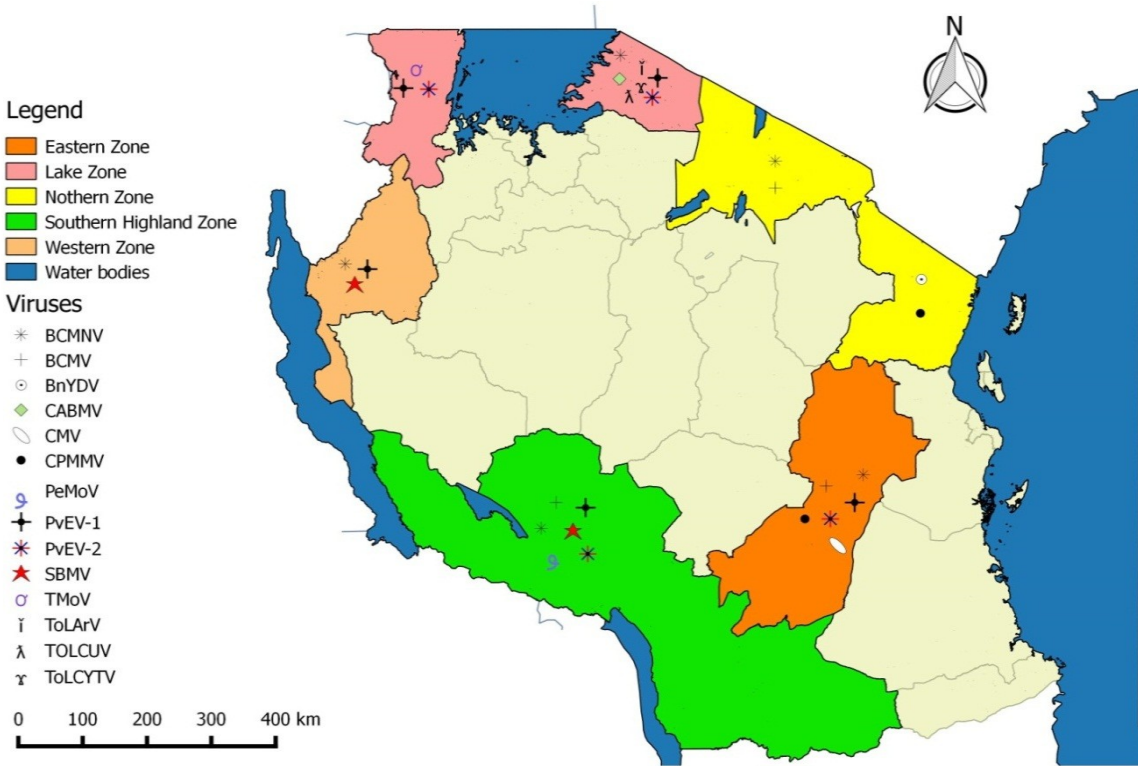

Figure 2.4: **The map of Tanzania showing zonal distribution of viruses detected by NGS and Sanger sequencing**

The green, yellow, orange, pale orange and pink colors represent areas where the comprehensive survey of common bean viruses was conducted. The symbols marked in the surveyed areas stand for different viruses that were detected through NGS and Sanger sequencing. Note that the symbols are not placed in the exact spot (e.g., field) where the viruses were detected but are used to show the zone where a particular virus was detected.

2.4.5 Partial and full sequences of BCMV and BCMNV sequences

Next generation sequencing generated full and partial sequences of BCMV and BCMNV. The sequences were obtained after the analysis of samples: HXH-1, HXH-2, HXH-3, HXH-6 and the sample collected from TZ:Mor533:2015. One complete and three nearly complete BCMNV sequences were obtained (Table 2.6). Two complete or near complete and one partial sequence of BCMV were also obtained. Among the BCMV sequences, the full genome for TZ:Mor533:2015 with accession number MF405190 were obtained through NGS-sequencing of the non-pooled sample. The details of the sequences are shown in Table 2.6.

Table 2.6: Number of Partial and full sequences of BCMV and BCMNV sequences

Isolate	Place of collection in Tanzania[1]	Virus and region sequenced[2]	Accession number	Sequencing technique[3]
BCMNV pool HXH-1		BCMNV; Complete	MF078483	NGS; reads size 21; Pooled RNA
BCMNV;HXH-2	EZ	BCMNV; Partial P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, CP	MF405187	NGS; reads size 22; Pooled RNA
BCMV;HXH-3; Pooled RNA	NZ	BCMV; P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, partial NIb-Pro	MF405188	NGS; combined inserts; pooled RNA
BCMNV;HXH-6; Pooled RNA	Tarime, LZ	BCMNV; Partial P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, CP	MF405189	NGS; combined inserts; pooled RNA
TZ:Mor533:2015	Morogoro Urban; EZ	BCMV; Complete	MF405190	NGS; reads size 21 and combined inserts; contigs assembled using SeqMan 5.03
BCMV;HXH-2-21-24; pooled RNA	EZ	BCMV; Complete or nearly complete	MF405191	NGS; combined inserts
TZ:NKS3:2015	SHZ; Nkasi district	BCMNV; Partial P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, CP	MF405192	NGS; combined inserts

[1] EZ, NZ, LZ and SHZ indicate eastern, northern, lake and southern highlands zones, respectively; [2] P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP represent potyviral proteins: first protein, helper component proteinase, third protein, first 6-kDa protein, cytoplasmic inclusion, second 6-kDa protein, genome-linked viral protein, nuclear inclusion a, nuclear inclusion b and coat protein, respectively; 3′UTR indicates 3′ untranslated region, NGS indicates next generation sequencing. [3] Combined inserts indicates that reads of sizes 21, 22, 23 and 24 nt were analysed as one fastq file.

2.4.6 Genetic variation of BCMV and BCMNV isolates from common bean

2.4.6.1 Genetic variability of BCMV isolates from common bean samples

Percent nucleotide (upper triangle) and amino acids (lower triangle) sequence similarities among Tanzanian isolates of BCMV are presented in Table 2.7. The Sanger-based nucleotide coat protein sequences similarity of BCMV isolates ranged from 90.2 to 100% while the amino acids sequences similarity ranged from 91.9 to 100%.

Table 2.7: Nucleotide (upper) and amino acid (lower triangle) sequence similarities among BCMV isolates

Seq	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
						99	99				99				99				
		100	91.5	91.0	98.3	98.39	97.89	97.8	97.8	97.6	98.29	98.8	98.3	98.3	98.09	98.2	98.3	91.0	90.2
A																			
	100		91.5	91.0	98.3	98.39	97.89	97.8	97.8	97.6	98.29	98.8	98.3	98.3	98.09	98.2	98.3	91.0	90.2
B		**																	
	93.4	93.4	**	99.3	92.0	92.09	91.89	91.8	91.8	91.6	91.99	92.0	91.9	91.9	92.09	92.1	91.9	99.3	90.2
C																			
	92.6	92.6	98.8		91.5	91.51	91.39	91.3	91.3	91.1	91.49	91.5	91.4	91.4	91.59	91.6	91.4	100	90.5
D				**															
	98.8	98.8	93.4	92.6	**	00	98.99	98.9	98.9	98.8	99.69	99.2	99.6	99.6	99.29	99.3	99.6	91.5	91.1
E																			
	98.8	98.8	93.4	92.6	100	*	98.99	98.9	98.9	98.8	99.69	99.2	99.6	99.6	99.29	99.3	99.6	91.5	91.1
F						*9													
	98.8	98.8	93.4	92.6	98.8	98.89	*	100	100	99.8	98.89	98.7	98.8	98.8	98.99	99.3	98.8	91.3	90.6
G							1												
	98.4	98.4	93.0	92.3	98.8	98.89	100.1	**	100	99.8	98.89	98.7	98.8	98.8	98.99	99.3	98.8	91.3	90.6
H																			
	98.4	98.4	93.0	92.3	98.8	98.89	100.9	100.0	**	99.8	98.89	98.7	98.8	98.8	98.99	99.3	98.8	91.3	90.6
I																			
	98.0	98.0	92.6	91.9	98.4	98.41	98.69	99.6	99.6	**	98.87	98.5	98.7	98.7	98.89	99.2	98.7	91.1	90.5
J																			
	98.8	98.8	93.4	92.6	100.4	100.09	98.89	98.8	98.8	98.4	*	98.8	99.4	99.4	99.19	99.2	99.4	91.4	91.0
K											*9								
	99.2	99.2	93.8	93.0	99.6	99.61	99.29	99.2	99.2	98.8	99.61	**	98.9	98.9	98.99	99.1	98.9	91.5	90.9
L																			
	98.8	98.8	93.4	92.6	100.4	100.09	98.89	98.8	98.8	98.4	100.9	99.6	**	100	99.19	99.2	100	91.4	90.7
M																			
	98.8	98.8	93.4	92.6	100.4	100.09	98.89	98.8	98.8	98.4	100.9	99.6	100.0	**	99.19	99.2	100	91.4	90.7
N																			
	99.2	99.2	93.8	93.0	99.6	99.69	99.29	99.2	99.2	98.8	99.69	100.0	99.6	99.6	*	99.3	99.1	91.5	90.9
O																			
P	98.8	98.8	93.4	92.6	99.4	99.69	99.29	99.2	99.2	98.8	99.69	99.2	99.4	99.4	99.9	**	99.4	91.4	90.8

						9.	9.				9.				9.				
	.8	.8	.4	.6	.2	2	6	.6	.6	.2	2	.6	.2	.2	6		.2	.6	.7
						1	9				1				9				
	98	98	93	92	10	0	8.	98	98	98	0	99	10	10	9.	99		91	90
Q	.8	.8	.4	.6	0	0	8	.8	.8	.4	0	.6	0	0	6	.2	**	.4	.7
						9	9				9				9				
	92	92	98	10	92	2.	2.	92	92	91	2.	93	92	92	3.	92	92		90
R	.6	.6	.8	0	.6	6	3	.3	.3	.9	6	.0	.6	.6	0	.6	.6	**	.5
						9	9				9				9				
	92	92	94	95	92	2.	2.	92	92	91	2.	93	92	92	3.	92	92	95	
S	.3	.3	.2	.3	.6	6	3	.3	.3	.9	6	.0	.6	.6	0	.6	.6	.3	**

The BCMV - CP nucleotide sequence length used was 782 nt (starting with the 52nd nt with reference to isolate TZ: KRG2-7:2015). The corresponding amino acids were used to determine amino acid sequence identity. The letters A–S represent the BCMV isolates sequenced: A = TZ:MBY1:2016 (MF066258), B = TZ:MBY3:2016 (MF066259), C = TZ:MSY1-1:2015 (MF066260)' D = UNKNOWN (MF043409), E = TZ:MVR15-16:2015 (MF043410), F = TZ:MVR15-23:2015 (MF043411), G = TZ:MVR14-13:2015 (MF043412), H = TZ:MVR14-17:2015 (MF043413), I = TZ:MVR14-16:2015 (MF043414), J = TZ:MVR14-15:2015 (MF043415), K = TZ:KRT7-18:2015 (MF043416), L = TZ:ARM12-19:2015 (MF043417), M = TZ:SIHA1-17:2015 (MF043418), N = TZ:SIHA1-15:2015 (MF043419), O = TZ:MVR4-3:2015 (MF043420), P = TZ:MVR3-1:2015 (MF043421), Q = TZ:KRT3-4:2015 (MF043422), R = TZ:KRG2-7:2015 (MF043423) and S = TZ:Mor533:2015 (MF784804) (published as Mwaipopo *et al*., 2018).

2.4.6.2 Genetic variability of BCMNV isolates from common bean at CP level

The Sanger-based nucleotide coat protein sequences similarities of BCMNV isolates ranged from 97.1 to 100% while at the amino acids level the similarity of BCMV isolates range from 99.1% to 100%. Most of nucleotide sequences were similar to each other by more than 98% (Table 2.8).

Table 2.8: Nucleotide (upper) and amino acid (lower triangle) sequence similarities among BCMNV isolates

Seq	A	B	C	D	E	F	G	H	I	J	K	L	M
A	**	97.9	97.7	97.4	97.9	98.0	98.0	97.9	97.9	97.1	97.1	97.1	97.6
B	99.5	**	98.2	98.8	99.3	99.5	99.5	99.0	98.4	98.5	98.5	98.5	99.0
C	99.5	100	**	97.7	98.2	98.4	98.4	98.8	97.6	98.0	98.0	98.0	97.9
D	99.0	99.5	99.5	**	98.8	99.0	99.0	99.2	98.2	98.7	98.7	98.7	98.5
E	99.0	99.5	99.5	99.0	**	99.8	99.8	99.0	98.4	98.5	98.5	98.5	99.0
F	99.5	100	100	99.5	99.5	**	100	99.2	98.5	98.7	98.7	98.7	99.2
G	99.5	100	100	99.5	99.5	100	**	99.2	98.5	98.7	98.7	98.7	98.2
H	99.5	100	100	99.5	99.5	100	100	**	98.4	98.8	98.8	98.8	97.8
I	99.5	100	100	99.5	99.5	100	100	100	**	97.6	97.6	97.6	98.0
J	99.5	100	100	99.5	99.5	100	100	100	100	**	100	100	98.2
K	99.5	100	100	99.5	99.5	100	100	100	100	100	**	100	98.2
L	99.5	100	100	99.5	99.5	100	100	100	100	100	100	**	2
M	99.0	99.5	99.5	0	99.0	99.5	99.5	99.5	99.5	99.5	99.5	99.5	**

Genetic variability among BCMNV isolates. The BCMNV-CP nucleotide sequence length used was 626nt. The letters A – M represent the BCMNV isolates sequenced: A = TZ:MSY15-1:2015 (MF066261), B = TZ:MBZ4-18:2015 (MF066262), C = TZ:MVR13-2:2015 (MF066263), D = TZ:TRM10-4:201 (MF066264), E = TZ:NKS3-19:2015 (MF066265), F = TZ:NKS3-1:2015_MF066266, G = TZ:NKS3-5:2015 (MF066267), H = TZ:MVRD:2016 (MF066268), I =

TZ:ARM7-51:2015 (MF066269), J = TZ:Maruku:2016 (MF066270), K = TZ:KRT1-3:2015 (MF066271), L = TZ:NMT1-8:201 (MF066272) and M = Strain TN1 (HQ229995) (published as Mwaipopo *et al*., 2018).

2.4.6.3 BCMV and BCMNV isolates phylogenetic tree

Fig. 2.5 shows the evolutionary tree of BCMV and BCMNV isolates. In BCMV there were three distinct clades in which evolutionary were coming from a common ancestor. In group I, the sequences varied a bit but they were closely related to the NL1 strain. Group II consisted of isolates related to RU strain. BCMV isolates in group II were obtained from Kagera region in northwestern Tanzania. Group III consisted of a virus isolate, which was collected from Morogoro. This isolate was closely related to RU strain. Its full genome (isolate TZ: Mor533:2015, accession no. MF405190) indicated it was most closely related to a recombinant isolate with accession number TX420811 in GenBank. Comparison of BCMNV sequences showed that, there was low genetic variation between isolates of this virus. However, addition of more sequences related to BCMNV isolate related to TZ: ARM7-51:2015 would probably result in formation of two distinct groups of BCMNV (Fig. 2.5).

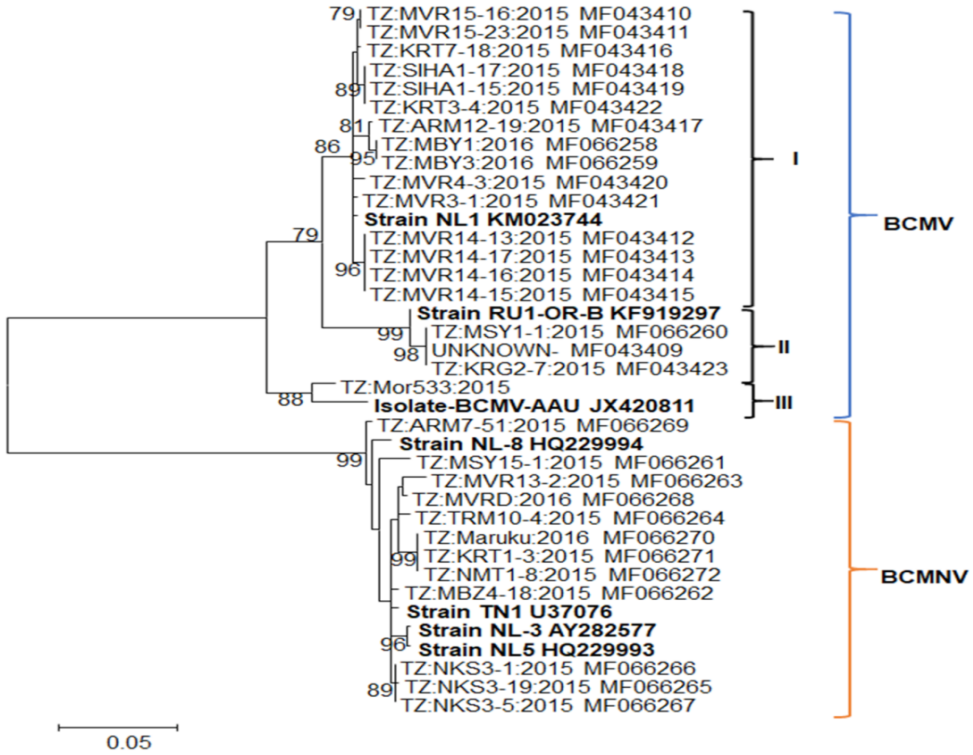

Figure 2.5: BCMV and BCMNV phylogenetic tree

Phylogenetic tree generated using the coat protein nucleotide sequences (620 nt) of BCMV and BCMNV isolates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Numbers at branches represent bootstrap values of 1 000 replicates, of which only values of >60% are shown. The isolates names are shown along with the accession numbers assigned to their sequences in this study (non-bold text) and in previous studies (bold text). This figure has been published in Mwaipopo *et al*., 2018.

2.4.7 Genetic variation of CPMMV from common beans samples

2.4.7.1 Sequence similarities among CPMMV isolates from common bean samples

The nucleotide sequence similarities among the CPMMV isolates ranged from 82.9 to 99.5%. At amino acid sequence level, the similarity of CPMMV isolates ranged from 96 to 100%. Thus, the genetic variation within isolates of CPMMV was higher at the nucleotide sequence level than at amino acids level. The nucleotide sequence of isolate (SIHA 1 - 1) from Siha district was related to sequences of isolates from Mvomero and Gairo districts by 82.9 to 88.2%. On the other hand, the isolates from

Mvomero and Gairo districts in eastern zone were 87.7 to 99.5% similar to each other at nucleotide sequence level (Table 2.9). Most isolates from Mvomero were over 98% identical to isolates from Gairo at nucleotide sequence level. At amino sequence level, CPMMV isolates were 97.3 to 100% identical to each other.

Table 2.9: Nucleotide (upper triangle) and amino acids (lower triangle) sequence similarities among CPMMV isolates

Seq	A	B	C	D	E	F	G	H	I	J	K	L	M
A	**	98.2	86.8	87.3	86.8	97.8	98.6	82.9	84.2	85.5	86.4	98.2	87.3
B	100	**	87.3	87.7	87.3	98.6	99.5	83.4	84.7	86	86.8	98.6	87.3
C	97.3	97.3	**	97.3	89.5	87.7	87.7	88.2	89.5	88.2	88.6	88.6	89
D	97.3	97.3	100	**	89.5	88.2	88.2	89	90.3	88.2	87.7	88.6	88.2
E	96	96	98.6	98.6	**	87.7	87.7	89.5	90.8	91.2	93.8	88.6	90.8
F	100	100	97.3	97.3	96	**	99.1	83.8	85.1	87.3	88.2	98.2	87.7
G	100	100	97.3	97.3	96	100	**	83.8	85.1	86.4	87.3	99.1	87.7
H	97.3	97.3	100	100	98.6	97.3	97.3	**	97.8	88.2	88.6	84.7	87.3
I	97.3	97.3	100	100	98.6	97.3	97.3	100	**	88.2	89.9	86	88.6
J	98.6	98.6	98.6	98.6	97.3	98.6	98.6	98.6	98.6	**	95.6	87.3	89
K	97.3	97.3	100	100	98.6	97.3	97.3	100	100	98.6	**	87.7	90.8
L	100	100	97.3	97.3	96	100	100	97.3	97.3	98.6	97.3	**	88.6
M	97.3	97.3	100	100	98.6	97.3	97.3	100	100	98.6	1	97.3	**

Genetic variability among CPMMV isolates. The CPMMV-CP nucleotide sequence length used was 229 nt. The letters A-M represent the CPMMV isolates sequenced: A = MVR 1-6, B = MVR 2-20, C = MVR 3-28, D = MVR16-25, E = CHANGARAWE, F = GR 9-5, G = GR 11-30, H = SIHA 1-1, I = HAI 6-23, J = HAI 6-29, K = KJ534277, L = GU191840, and M = KX534092, respectively.

2.4.7.2 CPMMV isolates phylogenetic tree

According to the phylogenetic tree generated using 229 nt of the coat protein gene (CP) in Fig. 2.6, the sequences of CPMMV formed two groups. Within each group there was distinct sub - groups. Isolates from the eastern zone (Mvomero and Gairo districts) fell into one group. Likewise, isolates from the northern zone formed one group but an isolate named CHANGARAWE which was collected from Mvomero district in eastern zone, was found to cluster with the isolates from northern zone. Thus, except for CHANGARAWE isolate, CPMMV's sequences clustered according to their geographical locations.

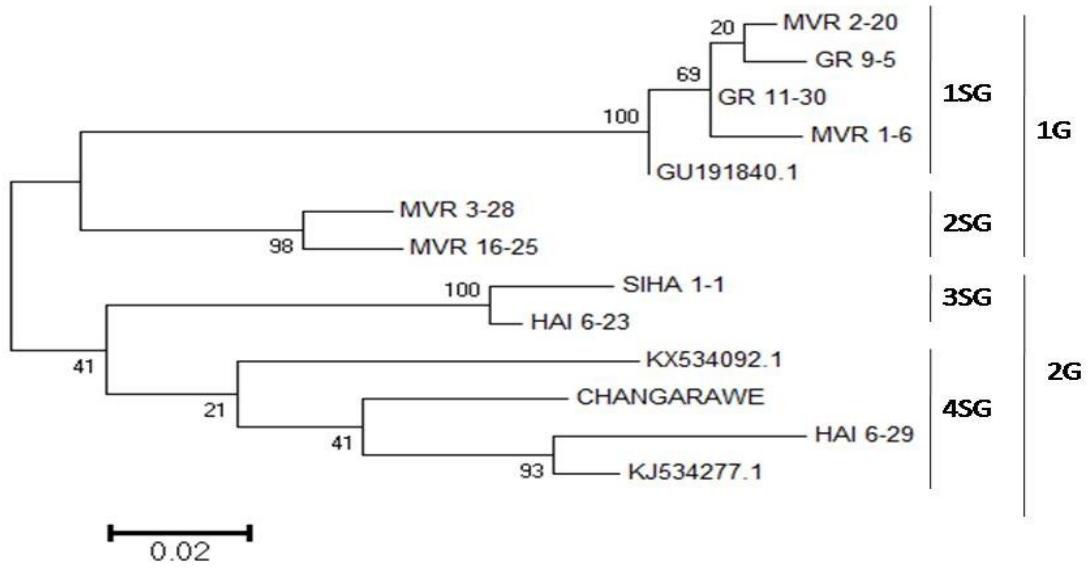

Figure 2.6: Evolutionary relationships of CPMMV isolates

The evolutionary history tree was generated by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura *et al*., 2013). All positions containing gaps and missing data were eliminated. There were a total of 229 positions in the final dataset and the analysis involved 13 nucleotide sequences of CPMMV which included three sequences retrieved from GenBank (accession numbers shown). The abbreviations MVR, GR, SIHA, HAI, G, and SG represent Mvomero district, Gairo district, Siha district, Hai district, group and subgroup, respectively.

2.5 Discussion

Using next generation sequencing technique, 15 viruses belonging to 11 genera were detected in the common bean samples collected from all major bean growing areas that included 11 regions and 23 districts in the mainland Tanzania. The viruses detected included those reported in previous surveys of common bean viral diseases in the country, namely BCMNV, BCMV, CMV and CPMMV (Mink and Keswani, 1987; Njau and Lyimo, 2000; Njau *et al*., 2006; Vetten and Allen, 1991). Also, seed transmitted endornaviruses; PvEV-1 and PvEV-2 were detected, which agreed with the findings of Nordenstedt *et al*. (2017). Other viruses detected have been reported to

infect plants other than common bean in Tanzania: CABMV (*Potyvirus*) in cowpea (*Vigna unguiculata* L. (Walp) (Patel and Kuwite, 1982) and CMV in cucurbits (Sydänmetsä and Mbanzibwa, 2016).

Results of this work showed that there were viruses that had not been previously reported in common bean plants in Tanzania. These included SBMV, *Umbravirus* (ToMV and CMoMV) and ToLCUV - related *Begomovirus.* Although these viruses were not known to infect common bean in Tanzania, they have previously been reported to infect common beans in other countries (Macedo *et al*., 2017; Abraham *et al*., 2014; Allen *et al*., 1981). ToLCUV related begomovirus, for example, has been reported in Latin America, where begomoviruses principally infect tomato but also infect common bean (Macedo *et al*., 2017).

The use of NGS not only helped reveal viruses infecting common bean in Tanzania, but also generated much more needed molecular information for development of diagnostic tools for viral disease management. Indeed, the primers were developed and used in this work and are now being used in other studies. Despite its robustness in detecting viruses, there are still limitations associated with the use of NGS technique. For instance, the discovery of novel viruses through *de novo* assembly of NGS data requires that a database contains previously submitted sequences that are related to the query contig sequences (Kreuze *et al*., 2009). Thus, it is not surprising that in this study some viruses that were detected could only be identified to the genus level. In some instances, a certain contig matched more than one viral sequence in the database. When a contig matches with more than one virus in a database, with low

coverage and similarities, it indicates that it is only related to those viruses and that it could be a sequence of a different virus or strain. Therefore, some identified viruses may not be exactly the ones infecting common bean in Tanzania, but closely or distantly related viruses or strains and for which sequences have not been deposited in the sequence databases. Examples from this study include an umbravirus (pooled RNA sample HXH-2) and a caulimovirus (pooled RNA sample HXH-7).

The NGS technology is still in its infancy, and the possibility of sequencing errors and further problems is expected during *de novo* assembly of reads. There are concerns that sequences derived from pools of plant material can be chimeras artificially assembled from pieces of multiple viruses (Roossinck *et al*., 2015; Simmonds *et al*., 2017). The possibility that chimeras did occur in this study cannot be discounted; however, some sequences obtained in this study were over 99% identical to Sanger sequences in nucleotide databases (Mwaipopo *et al*., 2018). Moreover, for one isolate (TZ:Mor533:2015), the three randomly selected genomic regions that were Sanger sequenced were identical to the NGS-based sequence. Moreover, primers designed using NGS sequences worked perfectly for specific detection of viruses e.g. CPMMV, SBMV, and ToLCUV- related begomovirus. Therefore, in this study NGS worked perfectly as it was proven by Sanger sequencing method.

Absence of BCMV in the western zone (Chapter three) could be partly due to the isolation of the area from other common bean growing areas in Tanzania. Interregional (within country) bean seed trade may not have led to virus spread as seed borne viral diseases are rare (Nordenstedt *et al*., 2017). RT-PCR results showed

that BCMV was present in all zones, except the western zone. However, NGS data indicated absence of BCMV in the southern highlands, western and lake zones, but through PCR it was detected in southern highlands zone. This discrepancy can be explained by low incidence of BCMV in common bean plants in the country as well as a small sample size (30 plants per zone) used in NGS; however, it has nothing to do with detection sensitivity of NGS and RT-PCR methods. It is, therefore, likely that the chance of detecting BCMV, and possibly other viruses, may rise with an increased number of pooled RNA samples per zone.

The results showed that isolates of BCMV were more diverse than those of BCMNV. These results are consistent with previously available information (Worrall *et al*., 2015). The Russian (RU-1) and NL-1 strains were the most common of the BCMV strains in common bean plants. High genetic variability among BCMV isolates means that common bean genotypes bred for resistance to this virus must be challenged against a wide range of isolates before being considered for commercial release. High genetic variability was also observed for CPMMV.

The results of this study indicated that, the isolates of CPMMV were largely geographically separated. The northern zone isolates formed one group while those from the eastern zone formed their own clade (except one isolate). While this may not be conclusive about their geographical isolation until full genomes are obtained for CPMMV isolates in Tanzania, it provides the very first molecular evidence that the isolates of CPMMV infecting common bean in Tanzania could be evolving separately in different geographical areas. However, one isolate from the eastern zone clustered with the isolates from the northern zone suggesting that CPMMV isolates could be

moved from one geographical area to another. This is a possibility as it was recently shown that CPMMV isolates are transmitted in seeds in Tanzania (Nordenstedt *et al*., 2017; Chilagane, 2018). There was no molecular evidence to suggest BCMNV and BCMV isolates were geographically separated.

2.6 Conclusions and Recommendations

2.6. 1 Conclusions

This work has provided molecular evidence for the occurrence of viruses previously reported in the country as well as those not reported before including the begomovirus. The occurrence of many different viruses in common bean plants in Tanzania may explain the observation that viral disease symptoms are highly variable on common bean plants. This means that researchers and breeders in Tanzania must consider all viruses of economic importance – not only BCMV and BCMNV –when breeding for resistance in common bean. The damage caused by most or all of common bean viruses detected have not been investigated in Tanzania and therefore plant pathologists in the country ought to also investigate damage and yield losses caused by each of these viruses. The information reported herein will be useful in development of molecular diagnostic tools and strategies for management of viral diseases, for example, for deciding where to set up seed multiplication sites or deploy certain planting material. The diagonostic tools are very important because apart from other use will be very usefull in seed certifying entities such as Tanzania Official seed certification institute.

2.6.2 Recommendations

i. The study has revealed many viruses infecting common bean plants as opposed to long established thinking that common bean production in Tanzania is constrained by BCMV and BCMNV. Therefore, further studies should be carried out to establish the incidences and effects of the newly reported viruses on common bean production in Tanzania.

ii. A potentially recombinant isolate (TZ: Mor533:2015) was detected in samples collected from Morogoro and Gairo district in eastern zone. There is therefore a need to establish its distribution and the damage it is causing to common bean plants.

iii. With regarding to breeding, common beans for resistance to viruses, breeders should focus on all viruses rather than BCMV and BCMNV alone, and should extend the search for sources of resistance to these viruses inside or outside the country.

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Appendices

Appendix 2.1: Accession numbers assigned to sequences obtained in this study

	Isolate	Place of collection in Tanzania[1]	Virus and region sequenced[2]	Accession number	Sequencing technique[c]
1	Unknown	Unknown	BCMV; CP	MF043409	Sanger
2	TZ:MVR15-16:2015	Mvomero – Morogoro; EZ	BCMV; CP	MF043410	Sanger
3	TZ:MVR15-23:2015	Mvomero – Morogoro; EZ	BCMV; CP	MF043411	Sanger
4	TZ:MVR14-13:2015	Mvomero – Morogoro; EZ	BCMV; CP	MF043412	Sanger
5	TZ:MVR14-17:2015	Mvomero – Morogoro; EZ	BCMV; CP, 3′UTR	MF043413	Sanger
6	TZ:MVR14-16:2015	Mvomero – Morogoro; EZ	BCMV; CP, 3′UTR	MF043414	Sanger
7	TZ:MVR14-15:2015	Mvomero – Morogoro; EZ	BCMV; CP, 3′UTR	MF043415	Sanger
8	TZ:KRT7-18:2015	Karatu – Arusha; NZ	BCMV; CP, 3′UTR	MF043416	Sanger
9	TZ:ARM12-19:2015	Arumeru – Arusha; NZ	BCMV; CP	MF043417	Sanger
10	TZ:SIHA1-17:2015	Siha – Kilimanjaro; NZ	BCMV; CP,3′UTR	MF043418	Sanger
11	TZ:SIHA1-15:2015	Siha – Kilimanjaro; NZ	BCMV; CP, 3′UTR	MF043419	Sanger
12	TZ:MVR4-3:2015	Mvomero – Morogoro; EZ	BCMV; CP, 3′UTR	MF043420	Sanger
13	TZ:MVR3-1:2015	Mvomero – Morogoro; EZ	BCMV; CP,3′UTR	MF043421	Sanger
14	TZ:KRT3-4:2015	Karatu – Arusha; NZ	BCMV; CP; 3′UTR	MF043422	Sanger
15	TZ:KRG2-7:2015	Karagwe – Kagera; LZ	BCMV; CP, 3′UTR	MF043423	Sanger
16	TZ:MBY1:2016	Mbeya DC – Mbeya; SHZ	BCMV; CP, 3′UTR	MF066258	Sanger
17	TZ:MBY3:2016	Mbeya DC – Mbeya; SHZ	BCMV; CP, 3′UTR	MF066259	Sanger
18	TZ:MSY1-1:2015	Missenyi – Kagera; LZ	BCMV; CP, 3′UTR	MF066260	Sanger
19	TZ:MSY15-1:2015	Missenyi – Kagera; LZ	BCMNV; CP	MF066261	Sanger
20	TZ:MBZ4-18:2015	Mbozi – Songwe; SHZ	BCMNV; CP	MF066262	Sanger
21	TZ:MVR13-2:2015	Mvomero – Morogoro; EZ	BCMNV; CP	MF066263	Sanger
22	TZ:TRM10-4:2015	Tarime – Tarime; LZ	BCMNV; CP	MF066264	Sanger
23	TZ:NKS3-19:2015	Nkasi – Rukwa; SHZ	BCMNV; CP	MF066265	Sanger
24	TZ:NKS3-1:2015	Nkasi – Rukwa; SHZ	BCMNV; CP	MF066266	Sanger
25	TZ:NKS3-5:2015	Nkasi – Rukwa; SHZ	BCMNV; CP	MF066267	Sanger
26	TZ:MVRD:2016	Mvomero – Morogoro; EZ	BCMNV; CP, 3′UTR	MF066268	Sanger
27	TZ:ARM7-51:2015	Arumeru – Arusha; NZ	BCMNV; CP, 3′UTR	MF066269	Sanger
28	TZ:Maruku:2016	Bukoba – Kagera; LZ	BCMNV; CP, 3′UTR	MF066270	Sanger
29	TZ:KRT1-3:2015	Karatu – Arusha; NZ	BCMNV; CP, 3′UTR	MF066271	Sanger
30	TZ:NMT1-8:2015	Namtumbo – Ruvuma; SHZ	BCMNV; CP, 3′UTR	MF066272	Sanger
31	BCMNV pool HXH-1		BCMNV; Complete	MF078483	NGS; reads size 21; Pooled RNA
32	BCMNVHXH-2	EZ	BCMNV; Partial P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, CP	MF405187	NGS; reads size 22; Pooled RNA
33	BCMVHXH-3; Pooled RNA	NZ	BCMV; P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, partial NIb-Pro	MF405188	NGS; combined inserts; pooled RNA
34	BCMNVHXH-6; Pooled RNA	Tarime, LZ	BCMNV; Partial P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, CP	MF405189	NGS; combined inserts; pooled RNA

35	TZ:Mor533:2015	Morogoro Urban; EZ	BCMV; Complete	MF405190	NGS; reads size 21 and combined inserts; contigs assembled using SeqMan 5.03
36	BCMVHXH-2-21-24; pooled RNA	EZ	BCMV; Complete or nearly complete	MF405191	NGS; combined inserts
37	TZ:NKS3:2015	SHZ; Nkasi district	BCMNV; Partial P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, CP	MF405192	NGS; combined inserts
38	TZ:Mor533:2015	Morogoro Urban	BCMV; P3	MF784802	Sanger
	TZ:Mor533:2015	Morogoro Urban	BCMV; CI	MF784803	Sanger
	TZ:Mor533:2015	Morogoro Urban	BCMV; CP	MF784804	Sanger
	HXH-1 southern zone pooled RNA inserts 21–24 contig506	SHZ	PeMoV; HC-Pro, P3	MF784805	NGS; combined inserts
39	HXH-1 southern zone pooled RNA inserts 21–24 contig 531	SHZ	PeMoV; Partial 6K2, VPg, NIa_Pro, NIb, partial CP	MF784806	NGS; combined inserts
40	HXH-1 southern zone pooled RNA inserts 21–24 contig 608	SHZ	SBMV; P2a, P2ab, CP	MF784807	NGS; combined inserts
41	HXH-1 southern zone pooled RNA inserts 21–24 contig 468	SHZ	SBMV; MP, P2a	MF784808	NGS; combined inserts
42	TZ:SBMV pooled RNA	Kigoma; WZ	SBMV; MP, P2a, P2ab, CP	MG344643	NGS; combined inserts

[1] EZ, NZ, LZ, SHZ and WZ indicate eastern, northern, lake, southern highlands and western zones, respectively; [2] P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb and CP represent potyviral proteins: first protein, helper component proteinase, third protein, first 6-kDa protein, cytoplasmic inclusion, second 6-kDa protein, genome-linked viral protein, nuclear inclusion a, nuclear inclusion b and coat protein, respectively; 3′UTR indicates 3′ untranslated region. *Southern bean mosaic virus* (SBMV) proteins are MP, P2a, P2ab and coat protein (CP), NGS indicates next generation sequencing and Sanger indicatesSanger sequencing. [c] Combined inserts indicates that reads of sizes 21, 22, 23 and 24 nt were analysed as one fastq file.

CHAPTER THREE

3.0 THE OCCURRENCE AND DISTRIBUTION OF COMMON BEANS (*Phaseolus vulgaris* L.) VIRUSES IN THE MAIN BEAN GROWING AREAS OF TANZANIA

Beatrice Mwaipopo[1, 3*], Susan Nchimbi-Msolla[1], Paul Njau[1], and Deusdedith Mbanzibwa[2]

[1]Department of Crop Science and Horticulture, Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Tanzania.

[2]Tanzania Agricultural Research Institute Mikocheni, P. O. Box 6226, Dar es Salaam, Tanzania.

[3]Tanzania Agricultural Research Institute Ilonga, P.O Box 33, Morogoro, Tanzania.

Corresponding author email: beatricemwaipopo@yahoo.com*

Abstract

Common bean viral diseases cause up to 100% yield losses or severely reduce the grain quantity and quality. Thus, the goal of this study was to determine the occurrence and distribution of common beans viruses in major bean growing areas of Tanzania. Accordingly, surveys were conducted in the northern, southern highlands, western, lake, and eastern agricultural research zones of Tanzania. A total of 7 756 common bean leaf samples were collected from 279 common bean fields in 23 districts. Disease incidence was visually assessed on 50 plants. Then, 30 of the 50 symptomatic and asymptomatic samples per field were randomly collected. The DNA and RNA were extracted from common beans samples by using the Cetyl trimethylammonium bromide method. The detection of BCMV, BCMNV, CPMMV

and SBMV was done by PCR. The incidence of BCMV and BCMNV was analysed by SPSS 16.0v. The maps of the surveyed areas were generated from the coordinates using Quantum GIS (v.2.6). Visually assessed incidence of common bean viral diseases in the fields was as high as 98% in Missenyi district in lake zone while the RT-PCR based highest incidence of BCMV and BCMNV were 36.7% and 76.7%, respectively. Most of the fields had 3.3% incidence of both BCMV and BCMNV. However, 26.1% of the districts where samples were collected, no BCMV were detected. Likewise, BCMNV was not detected in samples collected from 39.1% of the districts. There was no linear relationship between visually assessed bean viral incidence and PCR based incidence for both BCMV and BCMNV. However, there was a weak correlation between BCMV and BCMNV for RT-PCR based incidence. *Cowpea mild mottle virus* (CPMMV) incidence was highest in the eastern zone where common bean fields had incidence of up to 46.7%. In northern zone, the highest CPMMV incidence was 10%. The *Southern bean mosaic virus* (SBMV) was predominant in Kasulu district where the highest incidence was 90.9%. Based on virtual assessment, Tanzania has a lot of crop infecting viruses, although their incidence vary from one location to another and some areas have low or complete absence of viruses when considering a single virus.

Keywords: BCMV, BCMNV, CPMMV, Common beans viruses, Incidence, RT-PCR, SBMV

3.1 Introduction

Viral diseases are among the major challenges of common bean production, which lead to yield reduction worldwide (Arli-Sokman *et al*., 2016). Common bean can be

naturally infected by over thirty-four viruses (Morales and Bos, 1988). The viruses infecting common bean in Tanzania and elsewhere have been reviewed by different research groups (Mwaipopo *et al.*, 2017; Worrall *et al*., 2015; Hillocks *et al*., 2006). Common bean viruses of economic importance include *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Southern bean mosaic virus* (SBMV), *Tobacco streak virus* (TSV) and *Tomato aspermy virus* (TVA) (Rastgou and Jalali, 2017).

Many viruses affecting common beans production are transmitted through pollen or seeds and by insect vectors. Some of the insect vectors that are important in the transmission of plant viruses are aphids, whiteflies and beetles (Bragard *et al*., 2013). The seeds and insect vectors are mostly responsible for the spread of the viruses within and between fields. Seeds facilitate the long-distance dispersal of viruses and act as initial source of inoculum for the vector to start spreading the virus from one plant to another (Sastry *et al*., 2013). The epidemiology and ecology of viruses is influenced by many factors including the interaction between the viruses and hosts. Other factors which influence epidemiology of viruses are the host range, modes of transmission and human activities, especially cultural practices (Aranda and Freitas-Astúa *et al*., 2017). Knowing the virus incidence and its distribution is very important for the establishment of effective virus control measures (Jones, 2006).

The incidence and distribution of viruses that infect common bean in Tanzania have not been determined in the past seventeen years. In a few cases where spread and incidence of viruses were determined, only a few locations and specific viruses were

targeted. Moreover, the detection of viruses in common bean samples was done using the ELISA technique, which has limitations including less sensitivity when compared to PCR-based detection of pathogens. Previous studies on common bean viral diseases carried out in Tanzania were mainly focused on BCMV and BCMNV only. That not with standing, the variations of virus disease symptoms in common bean fields in Tanzania appear to be high, suggesting many different viruses could be infecting the crop (Mwaipopo *et al.*, 2018).

Thus, this study was focused on detecting and determining incidence and distribution of four viruses, namely BCMV, BCMNV, SBMV and CPMMV. BCMV and BCMNV were studied because of their historical impact on common bean production. On the other hand, CPMMV and SBMV were included in this study because CPMMV was recently shown to cause severe symptoms on common bean plants (Chilagane, 2018) while during this study SBMV caused severe symptoms on plants in preliminary experments carried out in screen house. Data generated was used in mapping the distribution of the above-mentioned viruses in Tanzania. The information generated in this work will be used to determine the best control measures including breeding for resistant varieties, selecting seed multiplication sites, and strategic deployment of planting materials. The government will use the information on virus disease incidence and distribution to develop or revise agricultural policies related to food security and restriction of spread of virus diseases

3.2 Materials and Methods

3.2.1 Leaf samples collection

Collection of common bean leaf samples was done as described in chapter two. Briefly, samples were collected from 23 administrative districts (Fig. 2.1 in Chapter two). In each district, four to 15 common bean fields, located near the main or feeder roads were randomly selected for sample collection. The distance between sampled fields was a few hundred meters to 10 kilometres or more depending on the availability of common bean fields. In each selected common bean field, 30 representative samples (both symptomatic and asymptomatic) were collected and a total of 7 756 samples were collected from the 23 districts. Samples were either pressed well for herbarium or preserved in silica gel and calcium chloride.

3.2.2 RNA extraction

RNA was extracted from either silica gel and $CaCl_2$ desiccated or herbarium-pressed dry common bean leaf samples using a modified CTAB method (Allen *et al*., 2006; Mbanzibwa *et al*., 2014). Briefly, the CTAB buffers contained 2% CTAB, 100 mM Tris–HCl, 20 mM EDTA, 2.5 M NaCl, freshly prepared 1% sodium sulphite, 2% polyvinylpyrrolidone and 2.5% 2-mercaptoethanol in nuclease-free (DEPC) water. Other procedures were as explained by Nordenstedt *et al.* (2017) and chapter two. The integrity of RNA in the samples was assessed visually by agarose gel electrophoresis after staining the gel with ethidium bromide. The RNA concentration and purity were determined with a Nanodrop 2000c UV–vis Spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

3.2.3 Reverse transcription polymerase chain reaction (RT-PCR) for BCMV and BCMNV

3.2.3.1 Complementary DNA synthesis (cDNA)

The 7 756 nucleic acids samples were reduced to 774 pooled RNA samples. Each pooled RNA samples contained 10 to 16 nucleic acids samples extracted from individual plant leaf samples. The pooled samples were mainly from the same field. The RNA was converted into cDNA whereby 10 µl master mix one composed of 5 µl of DEPC water, 1µl of 100 µM Oligo $(dT)_{18}$ or random hexamers (Bioneer, Seoul, South Korea), 1 µl of 10 mM dNTPs (New England Biolabs, NEB; Ipswich, MA, USA) and 3 µl of common bean RNA was heated at 65 ^{0}C for 5 min and chilled immediately on the ice for 2 min. The second mix (mix two) composed of 7 µl of sterile distilled water, 2 µl of 10X M-MuLV RT buffer (≠B0253), 0.3 µl of 40 U/µl RNase inhibitor, and 0.7 µl of 200 U/µl M-MuLV RT polymerase enzymes (≠MO253S). 10 µl of mix two was added to mix one to make a total of 20 µl. The mixture was incubated at 42 ^{0}C for 90 min and reaction terminated at 65 ^{0}C for 20 min. The cDNA was diluted with 20 µl DEPC water.

3.2.3.2 RT-PCR of BCMV and BCMNV for incidence and prevalence determination

The primers for detection of BCMV and BCMNV were developed using the sequences available in the data base. These primers were designed to target the coat protein (CP) gene of the viruses. During PCR amplification, primers were optimized to detect BCMV and BCMNV (Table 3.1). The primer pairs used were BCMV1F/ BCMV1R and BCMNVF1/ BCMNVR1, yield 320 bp and 823 bp PCR amplicons for BCMV and BCMNV, respectively. The AccuPower® PCR PreMix and phusion® high-fidelity DNA polymerase were used for PCR amplification. AccuPower® PCR

PreMix contains 1U TopDNA polymerase, 250 µM dNTPs, 10mM Tris-HCL (pH 9.0), 30 mM Kcl, 1.5 mM $MgCl_2$, stabilizer and a tracking dye.

The reaction buffer was in a premixed format, freeze-dried into a pellet. 15 µl of double distilled water was added to AccuPower® PCR tubes, 1 µl of 10 mM of each forward and reverse primers and 3 µl of template were added to make a volume of 20 µl. When using high fidelity Phusion DNA polymerase, the total reaction volume was 25 µl. 5 µl of 5X Buffer GC Phusion buffer (≠HB0519S), 0.25 µl of 2 U/µl phusion ®HF DNA polymerase enzyme (≠ M0530S), 0.5 µl of 10 mM DNTPs, 1 µl of 10 mM of each primer and nuclease free water were added to 22.5 µl volume, the 2.5 µl of template was added to the mix to final volume of 25 µl. For AccuPower® PCR PreMix, PCR was carried out in 2 720 thermal cycler (Applied Biosytems) and TC-412 TECHNE PCR machine with the following programme: for the primer set BCMV1F/BCMV1R, 1 cycle of 2 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 25 sec (denaturation), 50 °C for 25 sec (annealing), 72 °C for 30 sec (extension) and a final cycle (final extension) at 72 °C for 10 min. For the primer set BCMNVF1/BCMNVR1, 1 cycle of 3 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 30 sec (denaturation), 50 °C for 30 sec (annealing), 72 °C for 1 min (extension) and a final cycle (final extension) at 72 °C for 10 min. For phusion® high-fidelity DNA polymerase, PCR was carried out in TC-412 TECHNE PCR machine with the following programmed: for the BCMNVF1/BCMNVR1 primer sets, the 1 cycle of 30 sec at 98 °C (initial denaturation), then 32 cycles at 98 °C for 5 sec (denaturation), 57 °C for 20 sec (annealing), 72 °C for 30 sec (extension) and a final cycle (final extension) at 72 °C for 10 min. The amplicons were run in the 1% agarose

gel that contains of ethidium bromide. The PCR amplicons were visualized on the Benchtop UV Transilluminators (UVP). Each amplified pool was opened and the RT-PCR was done as in the pool. The electrophoresis gel images were used to determine the infection status of the samples.

Table 3.1: Primer pair used for incidence determination of viruses

Primer pair	Primer sequences 5′–3′ direction	Virus amplified	[1]Target gene	Size (bp)	Reference
BCMV1F	GTAGCACAGATGAAGGCAGCA	BCMV	CP	320	This study
BCMV1R	GGTTCTTCCGGCTTACTCATA				
BCMNVF1	CAAAGGCCCAGCGGATAAA	BCMNV	CP	823	This study
BCMNVR1	GGTGGTATAACCACACTGGAATTG				
CPMMV2F1	AACAAAAAACTGGCGTTCCAAA	CPMMV	CP	1300	This study
CPMMV2R1	GGAAAATAACTTTAAAACCGG				
SBMV 315F1	AGTCGGCTTGCAAGTTTAGA	SBMV	P8/P10	490	This study
SBMV 315R1	GGTCCACAGGGGATTTATT				

[1]CP indicate viral coat protein, P8/P10 is a C-terminal protein with ATPase and RNA binding properties

3.2.3.3 RT-PCR for CPMMV isolates from common beans samples

A total 1 020 samples from eastern zone (Mvomero, Gairo and Morogoro Rural districts) and 1 740 samples from northern zone (Arumeru, Karatu, Hai, Siha and Lushoto) districts were amplified for CPMMV. The two zones were selected because NGS results detected CPMMV in samples from those areas, and also severe CPMMV disease symptoms that were previously observed in screen house grown bean plants (Chapter two; Chilagane, 2018). The RNA was converted into cDNA as described in section 3.2.3.1 for detection of BCMV and BCMNV, except that during cDNA synthesis random hexamers were used. The primers pair used for detection of CPMMV were designed to amplify the coat protein gene (CP) of the virus (Table 3.1). The reagents used were the AccuPower® PCR PreMix and the master mix was as for BCMV and BCMNV in section 3.2.3.2. PCR was carried out using 2 720 Thermal Cycler (Applied Biosytems) and TC-412 TECHNE with the following programme: 1

cycle of 2 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 45 sec (denaturation), 56 °C for 45 sec (annealing), 72 °C for 1 min (extension) and a final cycle (final extension) at 72 °C for 10 min. The PCR products were run in 1% agarose gel that contain ethidium bromide and visualized on the Benchtop UV Transilluminators (UVP).

3.2.3.4 RT-PCR for SBMV isolates from common beans samples

A total of 316 common bean leaf samples from Kigoma (western zone) and 2 760 leaf samples from southern highlands zone, eastern zone and northern zone were used to detect SBMV. Selection of bean samples for the detection of SBMV was based on NGS results that showed the presence of the virus in two zones, namely southern highlands and, western zones. Leaf samples from northern and eastern zones were included because NGS results showed they were hotspots of many viruses although the NGS data did not reveal the presence of SBMV in these zones. The cDNA was synthesized as described for BCMV, BCMNV and CPMMV but instead of Oligo $(dT)_{18,}$ the random primer was used in this case. The PCR amplification was achieved using the AccuPower® PCR PreMix. The PCR reactions were as described for previously mentioned viruses. The primers used are indicated in Table 3.1. Amplification was carried out in a TC-412 TECHNE PCR machine using the following programme: 1 cycle of 3 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 30 sec (denaturation), 50 °C for 30 sec (annealing), 72 °C for 1 min (extension) and a final cycle (final extension) at 72 °C for 10 min. The amplicons were run in the 1% agarose gel that contain ethidium bromide and visualized on a Benchtop UV Transilluminator (UVP).

3.3 Data Analysis

The visually assessed disease incidence was obtained by scoring 1 for presence or 0 for absence of viral disease symptoms. The PCR-based virus disease incidence was determined by scoring the gel images. This was done separately for all assessed viruses. Incidence was then determined as the percent of diseased plants in a plant population (equation 1) while prevalence was a statistical concept referring to the number of cases or the probability of presence of common bean viral disease in a particular district (equation 2).

Percent (%) viral disease incidence = n/N x 100 …… **(Equation 1)**

Where n = number of diseased plants in a given common bean field, N = total number of plants on which observations were made.

Percent (%) viral disease prevalence = p/P x 100……. **(Equation 2)**

Where p = total number of common bean fields with at least one infected plant, P = total number of common bean fields surveyed in a given district.

The determined incidence of BCMV and BCMNV were analysed using SPSS version 16.0 for windows. The crosstab under the descriptive statistic was used to describe the relationship between two categorical variables which are visually and RT-PCR based. The Pearson bivariate correlation analysis of visually assessed versus PCR-based incidence was determined.

The maps of surveyed areas were generated from coordinates using the Quantum GIS (v 2.6) available online at QGIS-OSGeo4W-2.6.1-1-Setup-x86.exe**.** Since the spatial

data were initially in degree/seconds/minutes format while the software uses decimal degree format of coordinates, they were converted to decimal degree format using the formulas shown in equtions 3 and 4:

Latitude: ([Latitude Degrees]) + ([Latitude Minutes]/60) + ([Latitude Seconds]/3600) …….. **(Equation 3)**

Longitude: ([Longitude Degrees]) + ([Longitude Minutes]/60) + ([Longitude Seconds]/3600)…… **(Equation 4)**

The data were prepared in the Microsoft Excel sheets (Microsoft Office 2016) and the file was saved using the CSV file format. The file was imported in the Quantum GIS program and the data were assigned to UTM coordinates (Arc 1960, zone 37S). Then the spatial mapping to produce thematic map of BCMV and BCMNV incidences and prevalence were done.

3.4 Results

3.4.1 Symptoms observation

Various viral disease symptoms were observed in common bean plants in different common bean fields surveyed. The observed virus symptoms were yellowing of leaves, vein clearing, vein banding, vein yellowing, mosaic, stunted growth, necrosis on the leaves and stem, bristling on leaves, leaf curling, rolling and malformation (Plate. 3.1). The symptoms were observed in all areas surveyed.

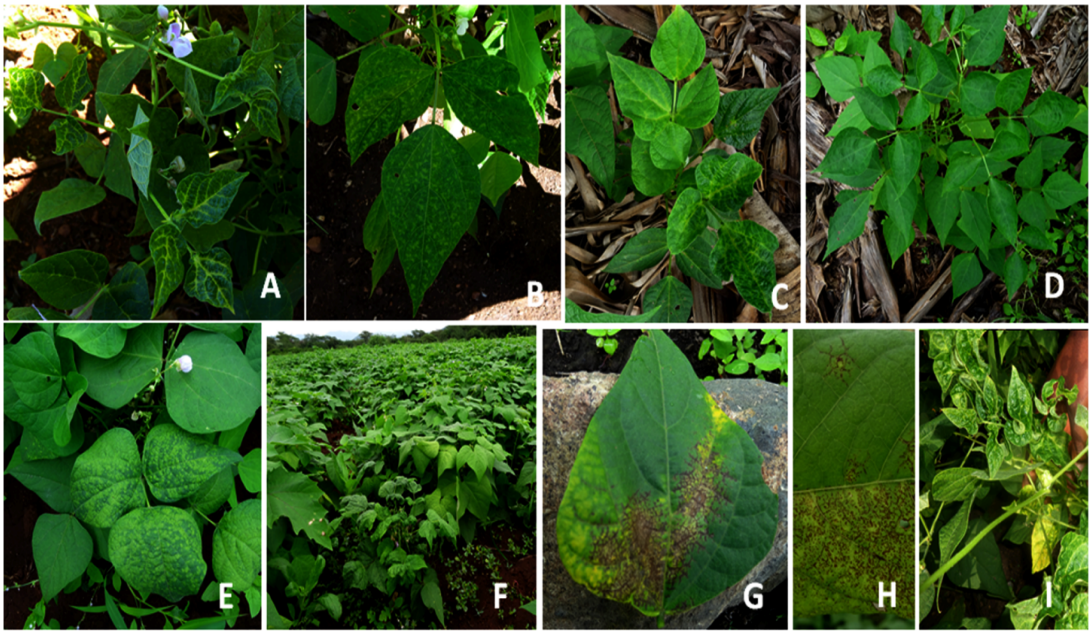

Plate 3.1: Virus symptoms observed on common bean plants in the fields

A, Vein clearing in plants infected with SBMV (Kasulu district in western zone). B, Yellow mottling (Missenyi district in lake zone). C and D, Mosaic and leaf curl (Missenyi district in lake zone). E, mosaic and leaf curling (Karatu district in northern zone). F, Mosaic and stunted growth (northern zone). G and H, leaf necrosis (Kilimanjaro Region in northern zone). I, Mosaic and severe leaf deformation (Mvomero district in eastern zone) (This photo has also been published in Mwaipopo *et al*., 2018).

3.4.2 Visually (field) assessed and RT-PCR based incidence of virus disease symptoms in common bean

The incidence of visually assessed viral disease symptoms in common bean plants countrywide for the country ranged from 0 to 98% while the incidence per zone ranged from 0 to 86%, 6 to 76%, 0 to 94%, 4 to 98%, and 0 to 80% for southern highlands, eastern, northern, lake and western zones, respectively (Table 3.2). The highest disease incidence (98%) was observed in a common bean field in Missenyi district in northwestern Tanzania (Table 3.2; Fig. 3.1).

RT-PCR based incidence for BCMV ranged from 0 to 36.7% and the highest incidence (36.7%) was observed in common bean fields in Lushoto, Mvomero and Tarime districts (Table 3.2; Fig. 3.2). On the other hand, the BCMNV incidence

ranged from 0 to 76.7%. The common bean field with the highest PCR-based BCMNV incidence was in Gairo district (Table 3.2; Fig. 3.3).

3.4.3 Incidence and prevalence of BCMV and BCMNV based on PCR at district level

The incidence of BCMV and BCMNV at the district level was computed as a percentage of infected plants in each district. The highest PCR-based incidence for BCMNV was 6.7% in samples collected from Hai district (Table 3.3). However, in most districts sampled, the incidence of BCMNV was equal to or less than 3.3%. BCMNV was not detected in common bean leaf samples from eight districts (Table 3.3). Four of the districts whose fields were free of BCMNV were from the lake zone.

In the northern zone, the district with the highest BCMV incidence was Siha (11.4%) followed by Lushoto district (6.4%). The other districts had BCMV incidence of less than or equal to 5.8% (Table 3.3). In the southern highlands, the highest incidence of BCMNV and BCMV at the district level were 1.4% (Mbeya rural district) and 5.1% (Njombe district). Contrarily, in the northern zone, the highest incidence of BCMNV and BCMV were 6.7% (Hai district) and 11.4% (Siha district). The RT-PCR based prevalence (%) for BCMV and BCMNV were highly observed in northern and eastern zones. (Table 3.3; Fig. 3.4; Fig. 3.5).

Table 3.2: Summary of visually assessed and RT-PCR based incidences of BCMNV and BCMV at field level in 23 districts of Tanzania

Zone	District[1]	RT-PCR based disease incidence range at field level (%)			Visually assessed incidence of virus-like disease symptoms in 50 plants per field (%)
		N[2]	BCMNV	BCMV	
SHZ	Nkasi (**H**)	450	0–10.0	0–13.3	0–86
	Mbozi (**I**)	420	0–3.3	0	10–60
	Mbeya Rural (**I**)	420	0–10.0	0–6.7	0–80
	Njombe (**J**)	330	0	0–26.7	0–30
	Wanging'ombe (**J**)	150	0	0	6–48
	Mbinga (**K**)	450	0	0	2–42
	Namtumbo (**K**)	300	0–3.3	0	6–44
EZ	Gairo (**G**)	450	0–76.7	0–26.7	6–54
	Mvomero (**G**)	450	0–40.0	0–36.7	12–76
	Morogoro R. (**G**)	120	0–3.3	0	18–24
NZ	Karatu (**D**)	450	0–16.7	0–20.0	2–86
	Arumeru (**D**)	420	0–3.3	0–26.7	6–94
	Siha (**E**)	240	0–23.3	0–33.3	2–34
	Hai (**E**)	210	0–36.7	0–20.0	0–52
	Lushoto (**F**)	420	0–30.0	0–36.7	2–56
LZ	Ngara (**A**)	240	0	0–3.3	12–36
	Karagwe (**A**)	450	0	0–10.0	4–54
	Missenyi (**A**)	450	0–16.7	0–16.7	8–98
	Muleba (**A**)	450	0	0	18–64
	Biharamulo (**A**)	150	0	0–10.0	4–38
	Tarime (**B**)	420	0–3.3	0–36.7	4–22
WZ	Kasulu (**C**)	163	0	0	0–80
	Kibondo (**C**)	153	0 – 3.3	0	0–60

[1]The letters A to H represent locations as shown in Fig. 2.1. [2]Indicates the number of common bean samples, collected from each district on which RT-PCR was performed. Only four common bean fields were surveyed in Morogoro Rural district. SHZ, EZ, NZ, LZ and WZ abbreviations indicate agricultural research zones in Tanzania: southern highlands, eastern, northern, lake and western zones, respectively (a modified version of this Table was published in Mwaipopo *et al*., 2018).

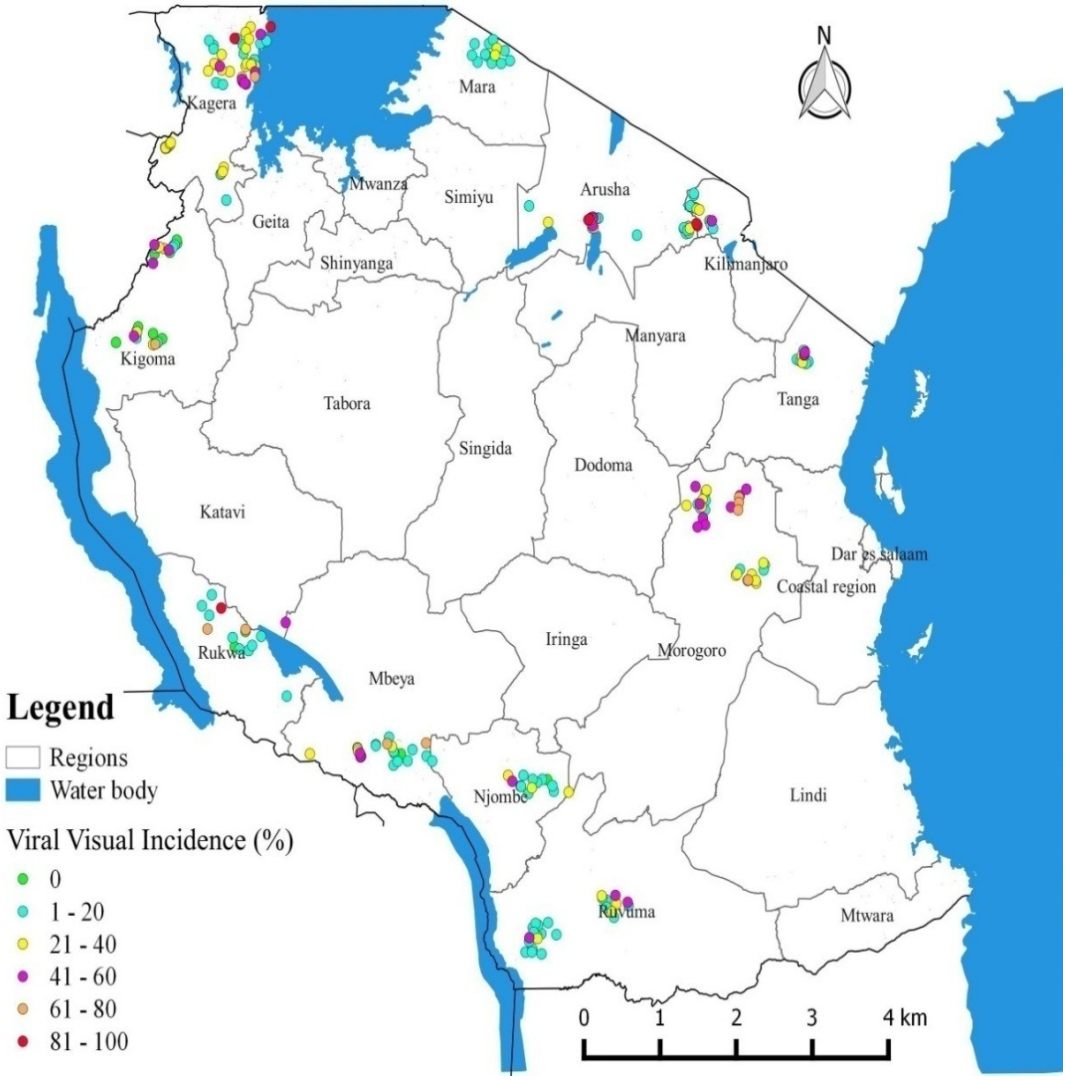

Figure 3.1: Map of Tanzania showing the visually assessed common bean viral disease incidence

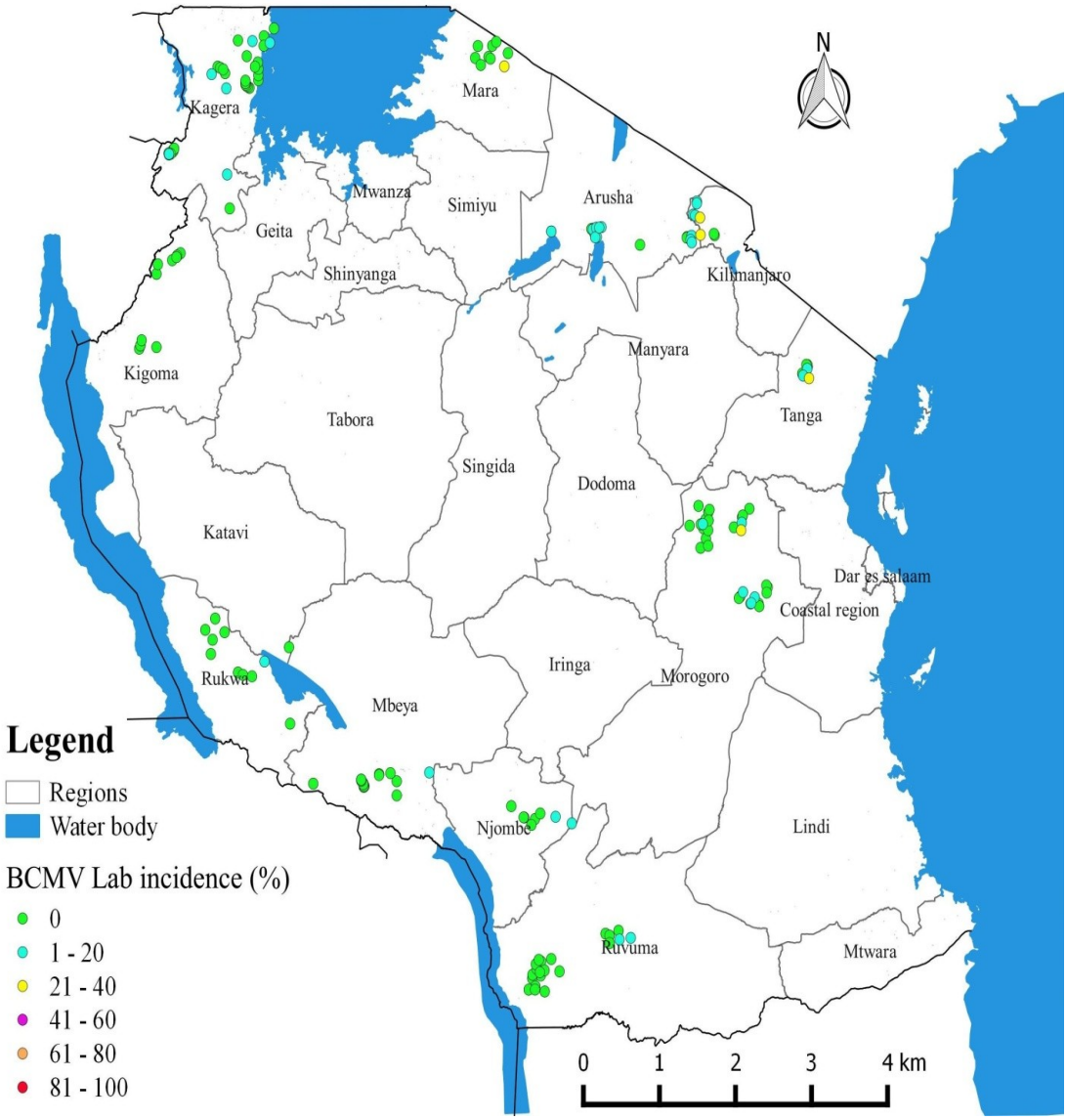

Figure 3.2: Map of Tanzania showing RT-PCR based BCMV incidence and its distribution in Tanzania

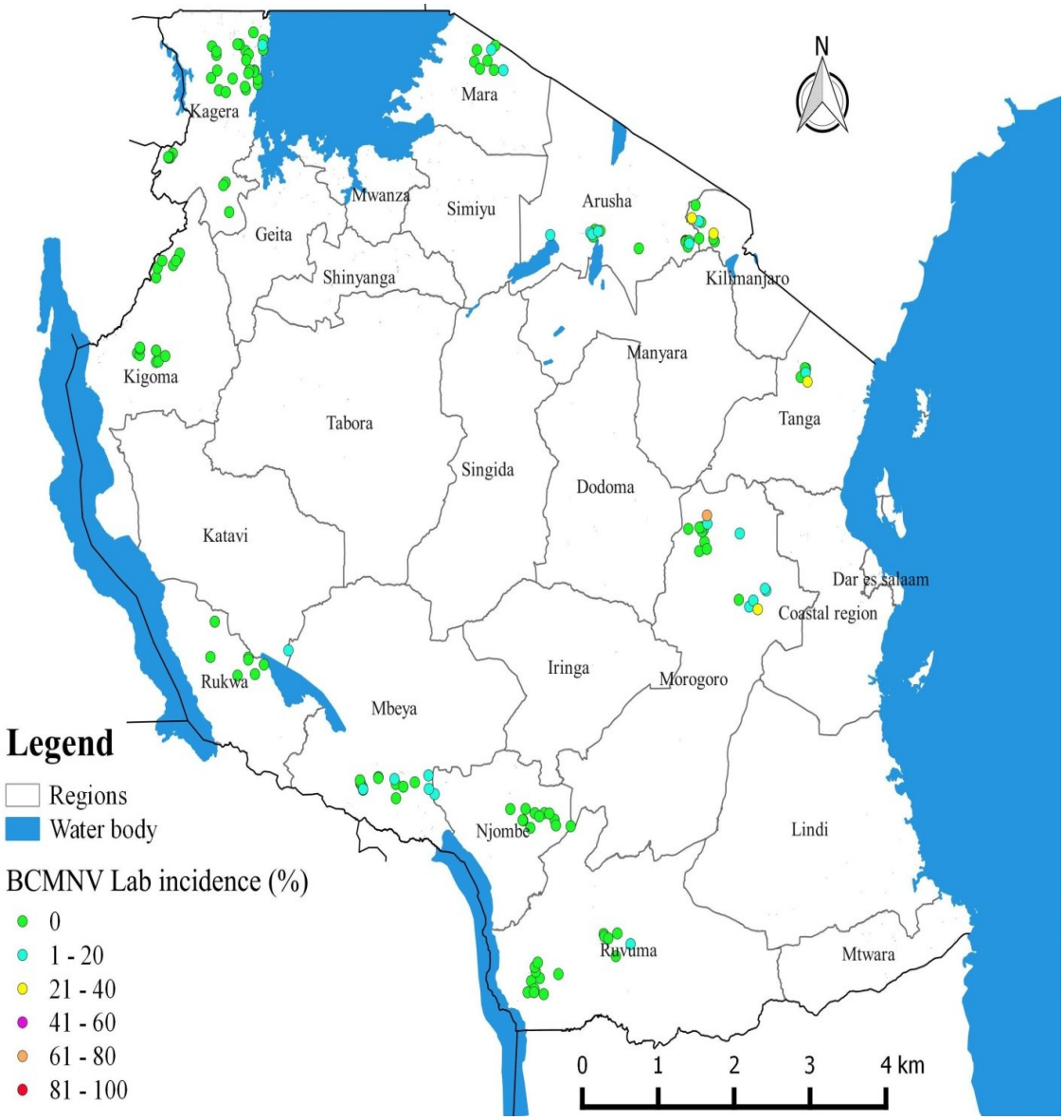

Figure 3. 3: Map of Tanzania showing the RT-PCR based incidence of BCMNV and its distribution in Tanzania

Table 3.3: RT-PCR based disease incidence and prevalence at district level

Zone	Districts [1]	RT-PCR based disease incidence at district levels (%)			RT-PCR based prevalence (%)	
		N[2]	BCMNV	BCMV	BCMNV	BCMV
SHZ	Nkasi (H)	450	0.7	1.1	6.7	13.3
	Mbozi (I)	420	0.2	0.0	7.1	0.0
	Mbeya Rural (I)	420	1.4	1.0	28.6	14.3
	Njombe (J)	330	0.0	5.1	0.0	36.4
	Wanging'ombe (J)	150	0.0	0.0	0.0	0.0
	Mbinga (K)	450	0.0	0.0	0.0	0.0
	Namtumbo (K)	300	0.3	0.0	10.0	0.0
EZ	Gairo (G)	450	5.6	2.0	13.3	13.3
	Mvomero (G)	450	6.4	5.8	33.3	33.3
	Morogoro R. (G)	120	1.7	0.0	50.0[c]	0.0
NZ	Karatu (D)	450	2.2	3.3	20.0	46.7
	Arumeru (D)	420	0.2	2.6	7.1	28.6
	Siha (E)	240	3.3	11.4	25.0	50.0
	Hai (E)	210	6.7	5.7	28.6	28.6
	Lushoto (F)	420	3.3	6.4	14.3	21.4
LZ	Ngara (A)	240	0.0	0.4	0.0	12.5
	Karagwe (A)	450	0.0	2.2	0.0	26.7
	Missenyi (A)	450	2.0	1.3	13.3	13.3
	Muleba (A)	450	0.0	0.0	0.0	0.0
	Biharamulo (A)	150	0.0	2.0	0.0	20.0
	Tarime (B)	420	0.5	2.6	14.3	7.1
WZ	Kasulu (C)	163	0.0	0.0	0.0	0.0
	Kibondo (C)	153	0.7	0.0	0.0	0.0

[1]Letters A to K refer to locations indicated in Fig. 2.1. [2]Indicates the number of common bean samples collected from each district and on which RT-PCR was performed. [c]Only four common bean fields were surveyed in Morogoro Rural district. The abbreviation SHZ, EZ, NZ, LZ and WZ means southern highland zone, eastern zone, northern zone, lake zone and western zone, respectively.

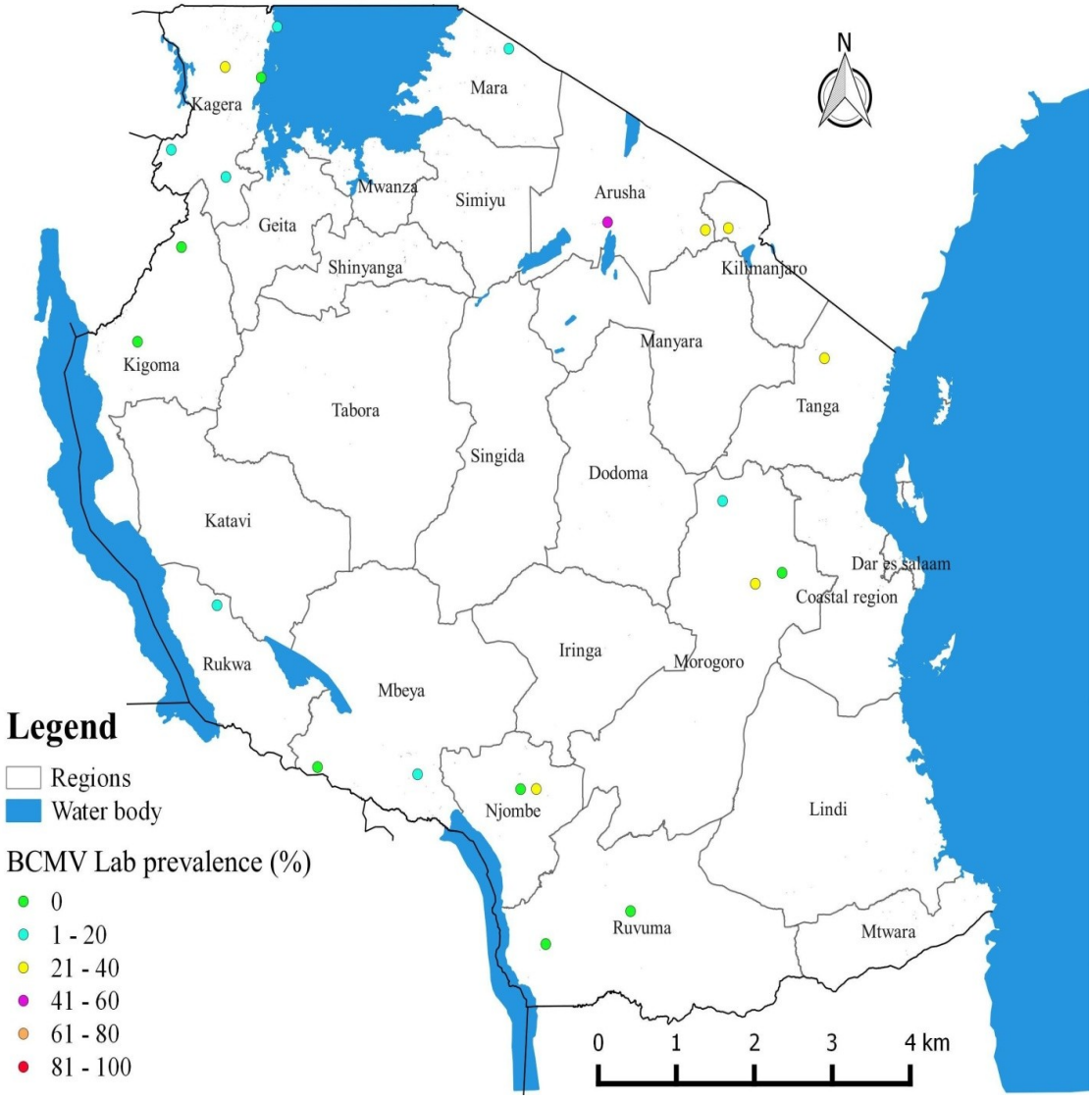

Figure 3.4: A map of Tanzania showing the RT-PCR based prevalence and distribution of BCMV in Tanzania

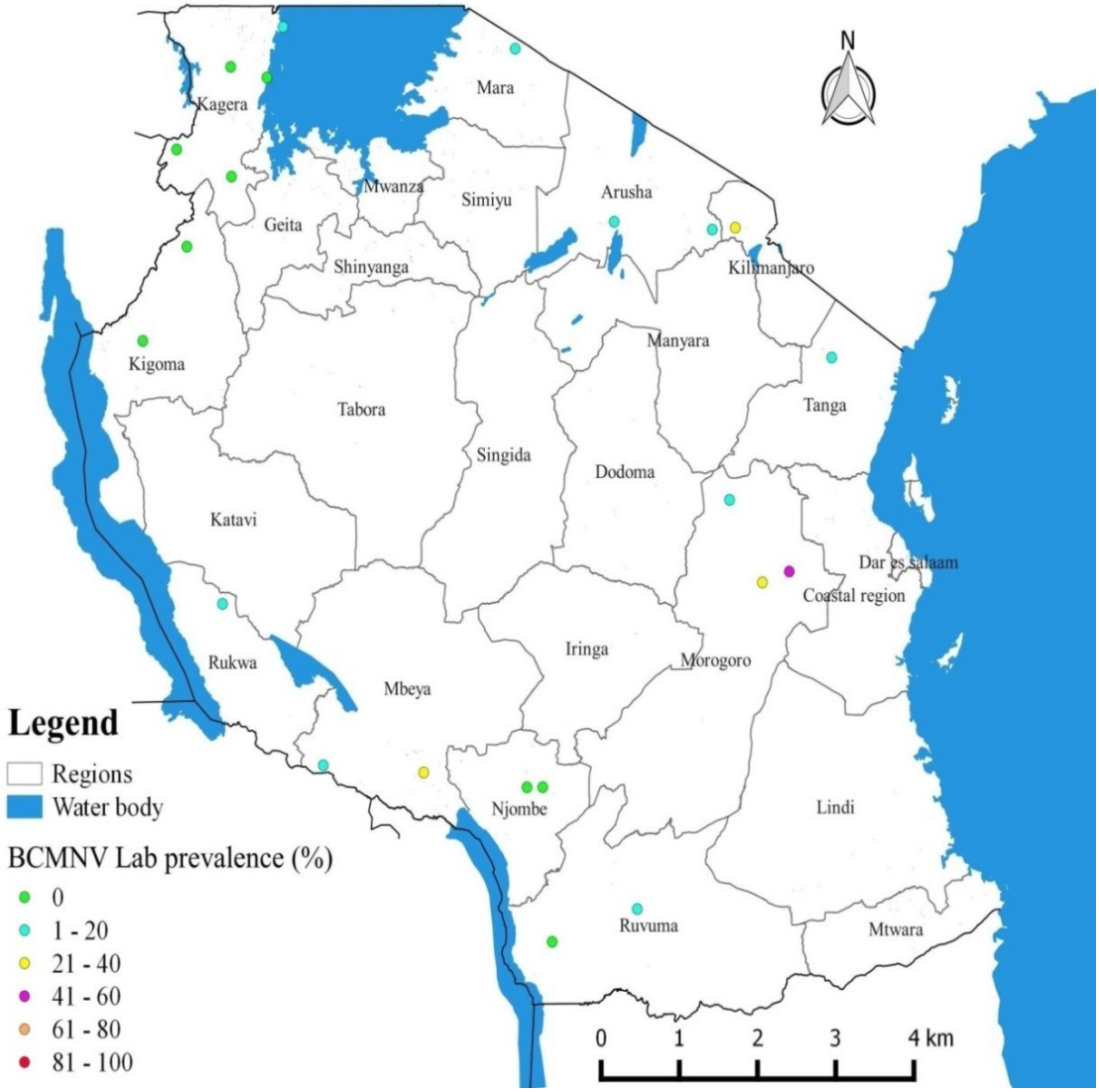

Figure 3.5: Map of Tanzania showing the RT-PCR based prevalence of BCMV in Tanzania

3.4.4 Correlation between the visually assessed and RT-PCR based viruses incidence

A correlation analysis between visually assessed and RT-PCR based incidence of BCMV and BCMNV was carried out using the SPSS program. Results presented in Table 3.4 and Fig. 3.6a showed that, there was no linear relationship between the visually assessed bean viral disease symptoms and RT-PCR based percent incidence for BCMV (r = -0.025 and R^2 linear = 6.085E-5). Likewise, there was no linear

relationship between visually assessed bean viral disease symptoms percent incidence and RT-PCR based percent incidence for BCMNV (r = 0.005 and R^2 = 2.286E-5) (Table 3.4 and Fig. 3.6b). However, there was a weak positive correlation between the BCMV and BCMNV RT-PCR-based percent incidence (r =0.121* and R^2 = 0.015) (Table 3.4 and Fig. 3.6c and d). The correlation between the visually assessed percent incidence and the RT-PCR based percent incidence of BCMV and BCMNV was not significant at P = 0.681 and P = 0.937 while the correlation between BCMV and BCMNV RT-PCR based percent incidence was significant at P = 0.042 (Table 3.4).

Table 3.4: Correlation between the viral visually assessed and the RT-PCR based Incidence

		Visually assessed incidence (%)	RT-PCR base of BCMV incidence (%)	RT-PCR base of BCMNV incidence (%)
Visually viral assessed incidence (%)		1.000	-0.025	0.005
	Pvalue		0.681	0.937
RT-PCR base of BCMV incidence (%)		-0.025	1.000	0.121*
	Pvalue	0.681		0.042
RT-PCR base of BCMNV incidence (%)		0.005	0.121*	1.000
	Pvalue	0.937	0.042	

* Correlation was significant at the 0.05 level (2-tailed), N=279

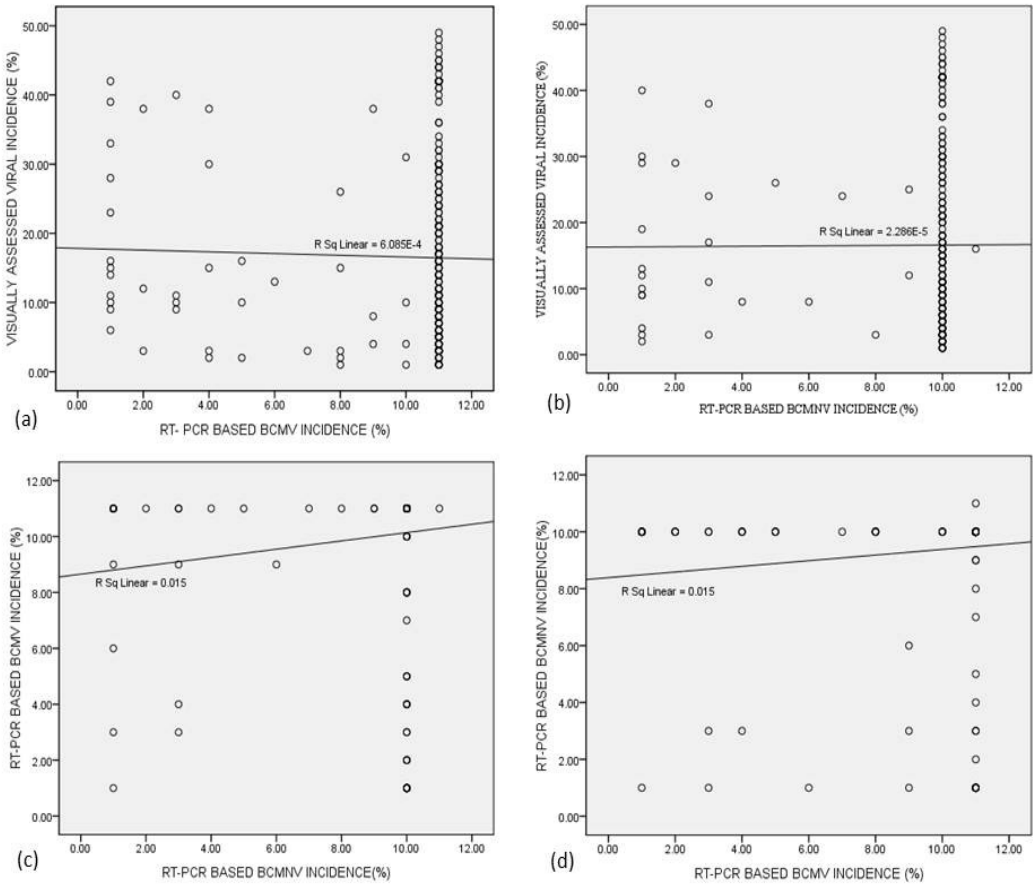

Figure 3.6: Scatter plots of virus disease incidence as determined visually and by RT-PCR

(a) Scatter plot showing the linear relationship between visually assessed viral disease incidence and RT-PCR based BCMV incidence (b) Scatter plot that show the linear relationship between visually assessed viral disease incidence and RT-PCR based BCMNV incidence (c) and (d) Scatter plot based on BCMV and BCMNV RT-PCR based incidence.

3.4.5 Fields assessed visually for virus-like disease symptoms (%)

Table 3.5 summarizes the percentage of fields with visually determined levels of virus incidence in different districts. According to the results, most fields surveyed and visually assessed for virus-like symptoms had disease incidence ranging from 1 to 20%. Most fields had disease symptoms incidence of less than 60%. There were some districts (Hai, Nkasi, Mbeya Rural, Njombe Rural, Kasulu and Kibondo) which had some common bean fields without plants with viral disease symptoms; all fields in districts had plants with viral disease symptoms (Table 3.5).

Table 3.5: Range of percentage of fields assessed visually for viral like disease symptoms

Percentage of fields at different incidence levels	0	1-20	21-40	41-60	61-80	81-100	Total
Districts	**[1]RFAVVDS (%)**						
Karatu	0	20.1	33.5	33.5	6.7	6.7	100
Arumeru	0	78.4	7.1	0	0	14.2	100
Siha	0	75	25	0	0	0	100
Hai	14.3	42.9	14.3	28.6	0	0	100
Lushoto	0	60.2	20.1	20.1	0	0	100
Nkasi	13.3	60.1	0	6.7	13.4	6.7	100
Namtumbo	0	50	10	40	0	0	100
Mbinga	0	86.8	6.7	6.7	0	0	100
Mbozi	0	28.4	35.6	35.5	0	0	100
Mbeya Rural	6.7	73.4	6.7	0	6.7	6.7	100
Wanging'ombe	0	60	20	20	0	0	100
Njombe Rural	9.1	72.8	18.2	0	0	0	100
Gairo	0	33.4	26.8	40	0	0	100
Mvomero	0	6.7	40.1	20.1	20.1	13.3	100
Morogoro Rural	0	75	25	0	0	0	100
Ngara	0	25	75	0	0	0	100
Bihalamuro	0	40	60	0	0	0	100
Muleba	0	6.7	53.4	33.3	6.7	0	100
Karagwe	0	53.3	33.5	13.4	0	0	100
Missenyi	0	20.1	40.1	26.8	0	13.3	100
Tarime	0	85.7	14.3	0	0	0	100
Kasulu	53.3	20	13.4	6.7	6.7	0	100
Kibondo	40	26.7	13.4	20.1	0	0	100

[1]RFAVVDS (%) = Range of % fields assessed visually for viral like disease symptoms

3.4.6 Comparison of RT-PCR based BCMV incidence within districts

The proportion of fields without BCMV infected common bean plants ranged from 50% to 100% (Table 3.6) suggesting that most fields in Tanzania were free of BCMV. For example, all fields sampled in Namtumbo, Mbinga, Mbozi, Wanging'ombe, Morogoro Rural, Muleba, Kasulu and Kibondo districts had 0% PCR-based BCMV incidence. Most of the BCMV affected fields had low incidence of 3.3%. A few fields had high incidence of 33.3% and 36.7%. Siha had the highest percent (50%) of fields containing plants infected with BCMV, followed by Karatu (46.8%), Hai (28.6% and Arumeru (28.5%) (Table 3.6).

Table 3.6: Percentage field with BCMV based on RT-PCR within the district

RT-PCR based BCMV incidence (%)	0	3.3	6.7	10	13.3	16.7	20	23.3	26.7	33.3	36.7	Total
Districts	**[1]FIBBP (%)**											
Karatu	53.3	26.7	6.7	0	0	6.7	6.7	0	0	0	0	100
Arumeru	71.4	21.4	0	0	0	0	0	0	7.1	0	0	100
Siha	50	0	0	0	12.5	12.5	0	12.5	0	12.5	0	100
Hai	71.4	0	0	0	0	0	28.6	0	0	0	0	100
Lushoto	80	0	0	0	6.7	0	0	0	0	0	6.7	100
Nkasi	86.7	6.7	0	0	6.7	0	0	0	0	0	0	100
Namtumbo	100	0	0	0	0	0	0	0	0	0	0	100
Mbinga	100	0	0	0	0	0	0	0	0	0	0	100
Mbozi	100	0	0	0	0	0	0	0	0	0	0	100
Mbeya Rural	86.7	0	13.3	0	0	0	0	0	0	0	0	100
Wanging'ombe	100	0	0	0	0	0	0	0	0	0	0	100
Njombe Rural	63.6	9.1	0	0	18.2	0	0	0	9.1	0	0	100
Gairo	86.7	6.7	0	0	0	0	0	0	6.7	0	0	100
Mvomero	73.3	0	6.7	0	0	6.7	6.7	0	0	0	6.7	100
Morogoro R.	100	0	0	0	0	0	0	0	0	0	0	100
Ngara	87.5	12.5	0	0	0	0	0	0	0	0	0	100
Bihalamuro	80	0	0	20	0	0	0	0	0	0	0	100
Muleba	100	0	0	0	0	0	0	0	0	0	0	100
Karagwe	80	6.7	0	13.3	0	0	0	0	0	0	0	100
Missenyi	93.3	0	0	0	0	6.7	0	0	0	0	0	100
Tarime	92.9	0	0	0	0	0	0	0	0	0	7.1	100
Kasulu	100	0	0	0	0	0	0	0	0	0	0	100
Kibondo	100	0	0	0	0	0	0	0	0	0	0	100

[1]%FIBBP = % Field affected with BCMV based on RT-PCR

3.4.7 Comparison of RT-PCR based BCMV incidence in different fields at country level

According to crosstab analysis of BCMV incidence based on RT-PCR, 85.5% of the surveyed fields in the country were having plants free from BCMV while 4.1% were having BCMV incidence of 3.3%. Only a few common bean fields had plants with BCMV incidence above 3.3% (Fig. 3.7). The highest BCMV incidence in the country was 36.7% and this was observed in only 1.1% of the common bean fields.

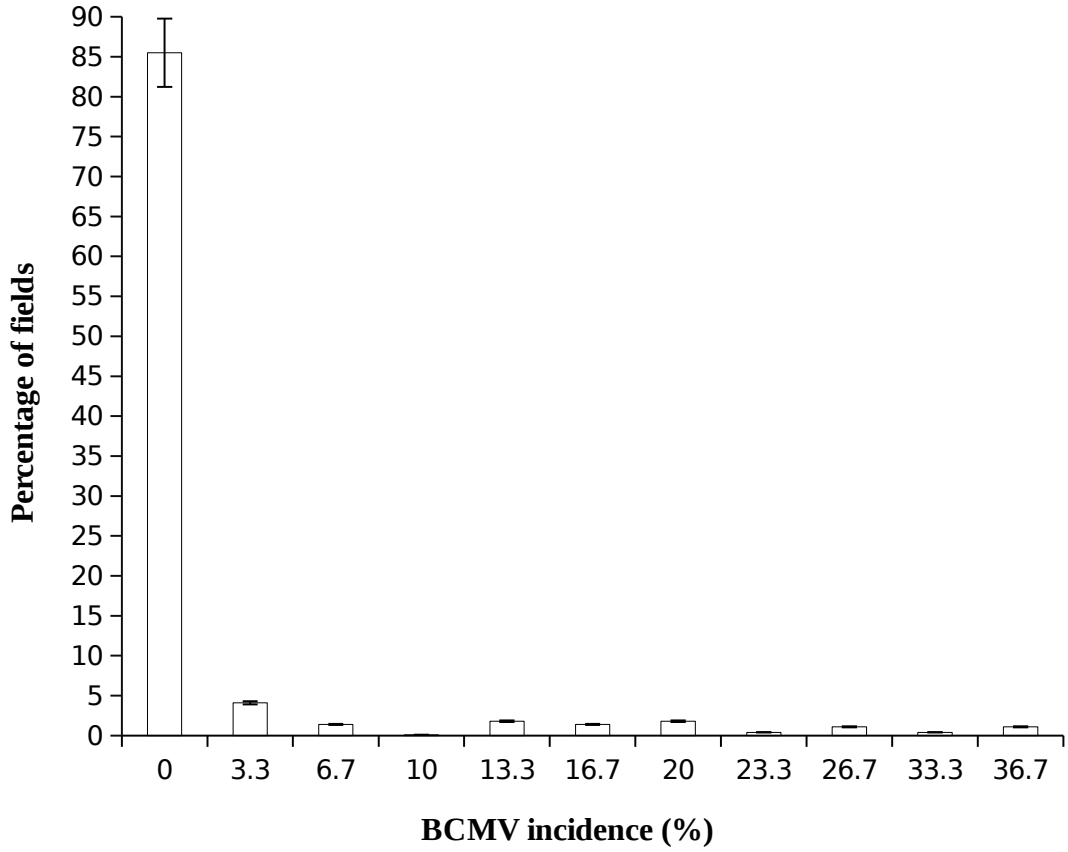

Figure 3.7: Percentage of common bean fields with RT-PCR predetermined BCMV incidence at country level

Error bars represent ±5% standard error around the fields percent affected with BCMV.

3.4.8 Comparison of RT-PCR based BCMNV incidence between different districts

Based on the RT-PCR results, all (100%) fields in Wanging'ombe, Njombe Rural, Ngara, Biharamulo, Muleba, Karagwe and Kasulu districts had no (0%) BCMNV. Most of the common bean fields surveyed in Tanzania had 3.3% BCMNV incidence. Mvomero district had the highest percent of fields with BCMNV. In both Mvomero and Gairo districts, 6.7% of common bean fields had common bean infected at 40% and 76.7% RT- PCR-based BCMNV incidence, respectively (Table 3.7).

Table 3.7: Percentage fields with predetermined BCMNV incidence for different districts

RT-PCR based BCMNV incidence	0	3.3	6.7	13.3	10	16.7	23.3	30	36.7	40	76.7	Total
Districts	**[1]FIBNBP (%)**											
Karatu	86.7	0	6.7	0	0	6.7	0	0	0	0	0	100
Arumeru	92.9	7.1	0	0	0	0	0	0	0	0	0	100
Siha	75	12.5	0	0	0	0	12.5	0	0	0	0	100
Hai	85.7	0	0	0	0	0	0	0	14.3	0	0	100
Lushoto	86.7	0	0	0	0	6.7	0	6.7	0	0	0	100
Nkasi	100	0	0	0	0	0	0	0	0	0	0	100
Namtumbo	90	10	0	0	0	0	0	0	0	0	0	100
Mbinga	100	0	0	0	0	0	0	0	0	0	0	100
Mbozi	92.9	7.1	0	0	0	0	0	0	0	0	0	100
Mbeya Rural	80	20	0	0	0	0	0	0	0	0	0	100
Wanging'ombe	100	0	0	0	0	0	0	0	0	0	0	100
Njombe Rural	100	0	0	0	0	0	0	0	0	0	0	100
Gairo	93.3	0	0	0	0	0	0	0	0	0	6.7	100
Mvomero	73.3	0	0	6.7	13.3	0	0	0	0	6.7	0	100
Morogoro r.	50	50	0	0	0	0	0	0	0	0	0	100
Ngara	100	0	0	0	0	0	0	0	0	0	0	100
Bihalamuro	100	0	0	0	0	0	0	0	0	0	0	100
Muleba	100	0	0	0	0	0	0	0	0	0	0	100
Karagwe	100	0	0	0	0	0	0	0	0	0	0	100
Missenyi	86.7	0	0	6.7	0	6.7	0	0	0	0	0	100
Tarime	85.7	14.3	0	0	0	0	0	0	0	0	0	100
Kasulu	100	0	0	0	0	0	0	0	0	0	0	100
Kibondo	93.3	6.7	0	0	0	0	0	0	0	0	0	100

[1]%FIBNBP = % Field infected with BCMNV based on RT-PCR

3.4.9 Comparison of RT-PCR based BCMNV incidence in different fields at country level

Results presented in Fig. 3.8 showed that, 90.7% of the fields surveyed in Tanzania were free from BCMNV. However, 4.3% of all surveyed fields in the country had BCMNV incidence of 3.3%. A few common bean fields had more than 3.3% incidence (Fig. 3.8).

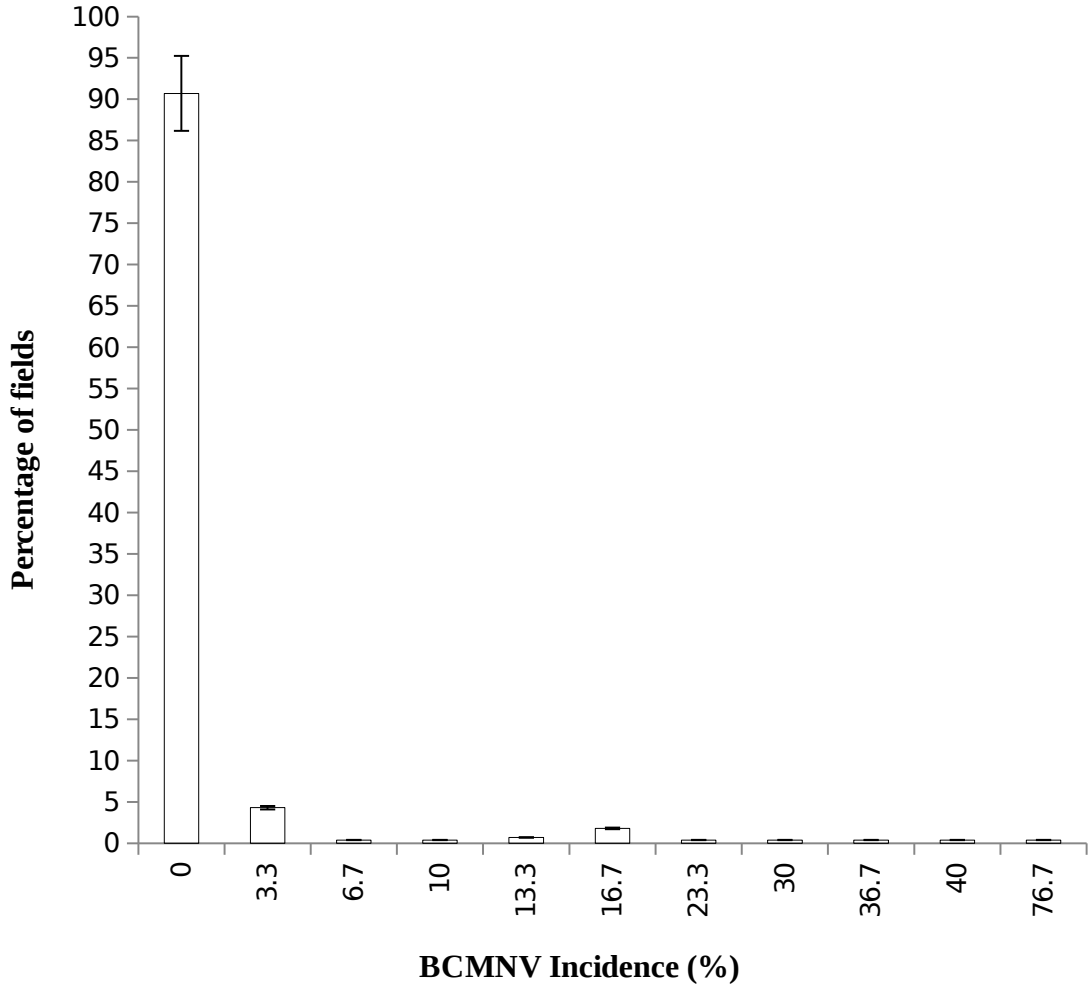

Figure 3.8: Percentage of common bean fields with RT-PCR predetermined BCMNV incidence at country level

Error bars represent ±5% standard error around the fields percent affected with BCMNV

3.4.10 Mixed infections of BCMV and BCMNV in common bean plants

Out of 7 756 common bean leaf samples analysed for BCMNV and BCMV infections, only 7 samples were found co-infected with both viruses. Therefore, co-infection was encountered in 0.09% of the samples collected in the country. Four of seven BCMV+BCMNV co-infected common bean leaf samples were collected from Siha district while the remaining one sample was from Arumeru district. Both districts are found in the northern zone. This indicates a case of the co-infection of BCMV and

BCMNV is highest in the northern zone (Table 3.8). The remaining two co-infected common bean leaf samples were collected from Mvomero district, in the eastern zone.

Table 3.8: Co-infection of BCMV and BCMNV observed in common bean plants

District	Number of co-infected samples	Sample code[1]
Mvomero	1	MVR 4-10
Mvomero	1	MVR 15-23
Arumeru	1	ARM 7-51
Siha	4	SIHA 1-12, SIHA 1-14, SIHA1-16, SIHA 1-20

[1]Code assigned at field level during surveys

3.4.11 Incidence of CPMMV in eastern and northern zone

RT-PCR was used to detect CPMMV, which was recently indicated as a potential threat to common bean cultivation in Tanzania (Chilagane, 2018). Moreover, the NGS results indicated this virus could be common in the northern and eastern parts of Tanzania (Chapter two). The RT-PCR based incidence (2 760 samples) of CPMMV in eastern and northern zones ranged from 0 to 46.7% (Fig. 3.9). The common bean field with the highest CPMMV incidence (46.7%) was from Mvomero district. This was followed by another field in the same district, which had 30% incidence of CPMMV (Fig. 3.9a). In Gairo district, the highest CPMMV incidence was 6.7% while the lowest was 3.3%. In the northern zone, the highest incidence of CPMMV was 10% in Karatu and Hai districts while the lowest incidence was 3.3% in Siha district (Fig. 3.9a and Fig. 3.9b).

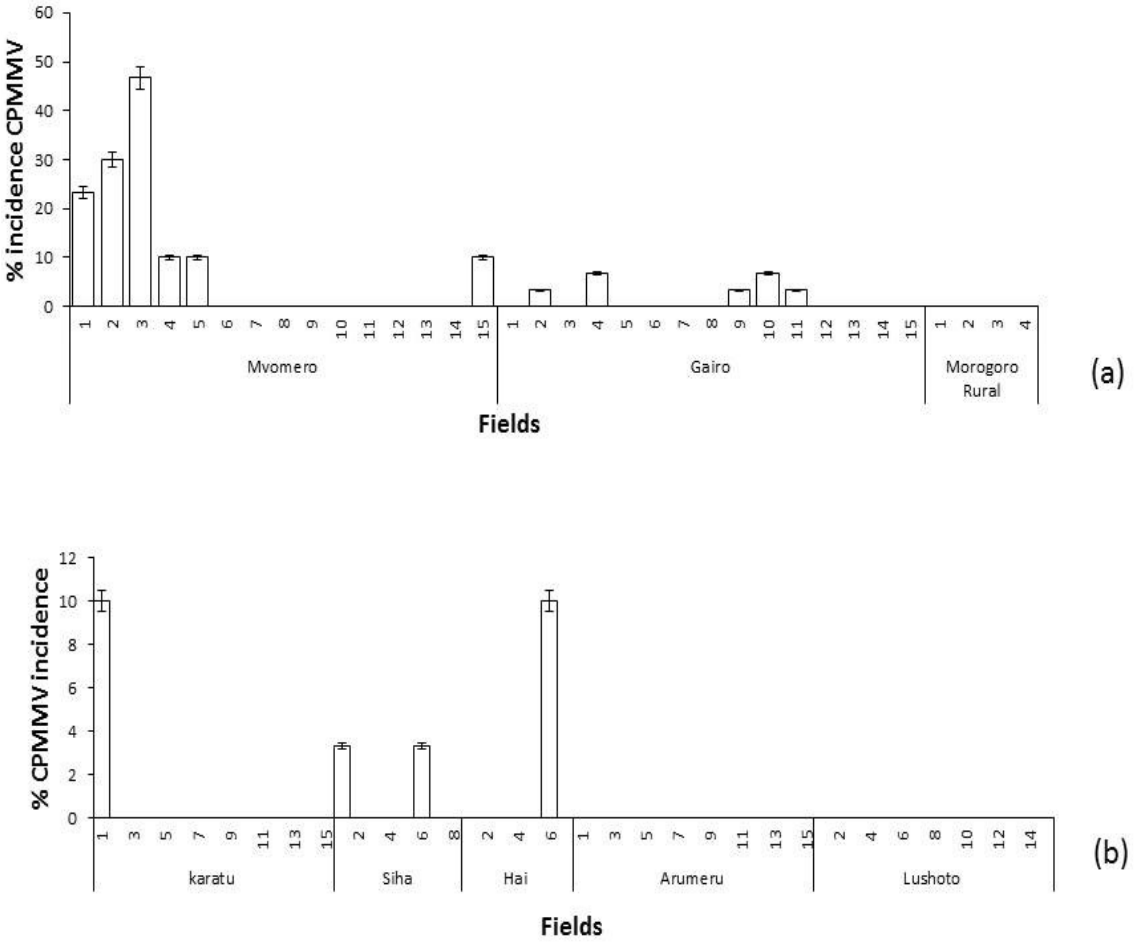

Figure 3.9: Incidence of CPMMV in common bean samples collected from eastern and northern zone of Tanzania

(a) PCR-based incidence of CPMMV in eastern zone (b) the graph of CPMMV incidence in northern zone, showing the CPMMV incidence (%) in different fields in Karatu, Siha, Hai, Arumeru and Lushoto. The areas in bar graphs with no bars mean the field was not infected with CPMMV. Error bars represent ±5% standard error around CPMMV percent incidence

3.4.12 Incidence of SBMV in samples from western zone

The NGS data indicated SBMV was common in western zone and as presented in Chapter seven, it causes severe disease symptoms in common bean plants. Thus, primers were designed and used for the detection of this virus in common bean samples collected from Kasulu and Kibondo districts in Kigoma region. The RT-PCR results revealed that the plants from Kibondo district were not infected with the virus (0% SBMV incidence in all cases) (Fig. 3.10), but the virus was common in bean

samples from Kasulu district in which the percent infections ranged from 0 to 90.9%. Out of 15 common bean fields surveyed in Kasulu districts, 10 fields were having plants infected with SBMV (Fig. 3.10).

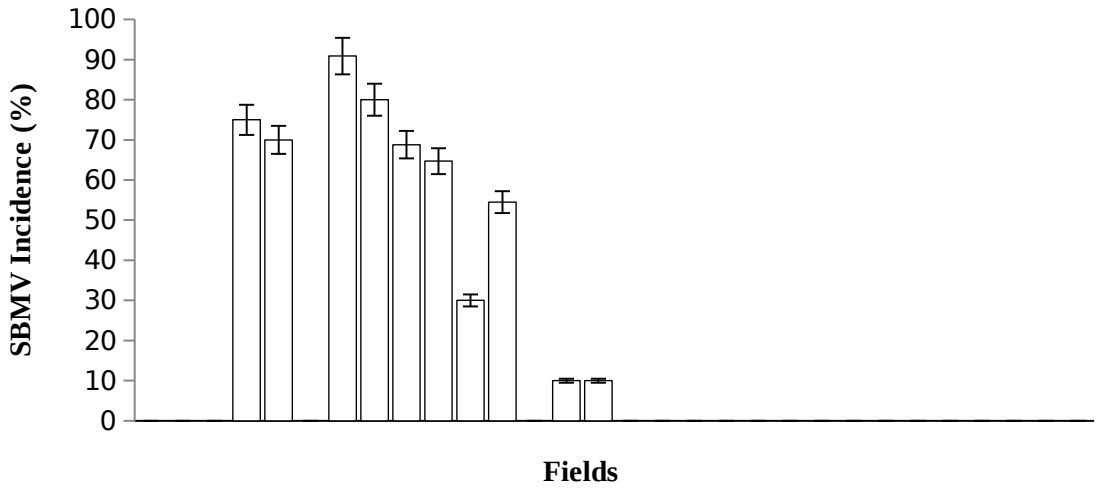

Figure 3.10: Incidence of SBMV in common bean samples collected from western zone

The areas in bar graphs with no bars mean the field was not infected with CPMMV. Error bars represent ±5% standard error around SBMV percent incidence.

3.5 Discussion

In a recent review (Mwaipopo *et al*., 2017) it was shown that there has never been a comprehensive survey for common bean viruses in Tanzania in recent years and that all past virus studies used ELISA method as a detection and identification tool of common bean viruses. The ELISA however is less sensitive when compared with the PCR based techniques (Boonham *et al*., 2014). Moreover, the previous common bean virus surveys did not cover large part of the country. Thus, this study represents the first comprehensive surveys of common bean viruses in Tanzania and for the first time the RT-PCR approach was used to detect four important viruses of common bean in the country (SBMV and CPMMV) in the country.

The results presented herein showed that BCMV and BCMNV were widely distributed in all of the agricultural research zones surveyed, and that the disease and virus incidence varied from one location to another. There were some districts whose fields were not containing plants infected with any of the detected viruses. However, and notable, all over the country and in all places that were surveyed, visual assessment revealed that virus and virus-like disease symptoms were common and occurred at high incidence. The presence of the high viral disease incidence can be attributed to many factors. As Njau and Lyimo (2000) showed, virus incidence in seeds collected from Tanzania could be as high as 36.6% and 12.4 for BCMNV and BCMV, respectively. The average incidences for both viruses were less than 8% (Njau and Lyimo, 2000). However, in a recent study it was shown that seed transmission of viruses was rare in seeds collected from Tanzania and Nicaragua (Nordenstedt *et al*., 2017). It should be noted that vectors can facilitate transmission of viruses in fields even when there were few plants infected through seed transmission.

According to the data presented herein, visually assessed disease incidence was higher than the RT-PCR based BCMNV and BCMV incidences. This may indicate that many distinct viruses infect common bean plants in Tanzania. Indeed, the results for detection of SBMV and CPMMV in the samples confirmed that the incidence of other viruses could be high as well. Further evidence for the occurrence of more viruses in bean in Tanzania was obtained using the NGS approach (Chapter two; Mwaipopo *et al*., 2018). Therefore, many district viruses are causing the disease symptoms that are observed in plants in common bean fields.

There was no positive correlation between the RT-PCR based and visually assessed disease incidence, which provided further evidence that, viral disease symptoms observed in the fields were presumably caused by viruses other than those found in this study. The observed differences in virus symptoms in the field could also be attributed to a wide range of cultivars grown in Tanzania (Fivawo and Msolla, 2011; Nordenstedt *et al*., 2017). However, for a variety of reasons that include lack of awareness and cost avoidance, lack of adoption of improved bean varieties by most common bean farmers, and use of local bean seeds instead of certified and quality declared seeds, viruses have continued to infect common bean in the country. It is worth noting here that efforts have been made in Tanzania to promote the use of improved and quality declared seeds (Mwaipopo *et al*., 2017). Other factors likely to affect the levels of viral disease incidence in the area are the availability of alternative hosts and virus vectors for viruses (Chapter four; Spence and Walkey, 1995). These factors play an important role in new virus infections in different cropping seasons. For example, in Tanzania, BCMNV, BCMV, CMV and CABMV have been detected in hosts other than common bean plants (Myers *et al*., 2000; Patel and Kuwite, 1982).

At the zonal level, the eastern and northern zones were found to be the hotspots of viral diseases, especially for BCMV, BCMNV and CPMMV. In the lake zone (Kagera region) there were many viral disease symptoms but the incidences of BCMV and BCMNV were low suggesting that, other viruses could be causing the observed viral disease symptoms. These results were supported by NGS results (Chapter two). Common bean viral disease pressure was lowest in the southern highlands of the country. This finding agreed with results obtained in previous studies, which revealed

low and high virus disease (BCMNV) incidence in the southern highlands and northern zone, respectively (Myers *et al*., 2000). These authors attributed low incidence of BCMNV in the southern highlands where the common bean are grown in the high altitude. BCMV and BCMNV were most prevalent in the northern and eastern zones, respectively probably because at high temperatures the disease development is high (Ghini *et al*., 2008). Most researchers seem to agree that warmer temperatures (>20 ^{0}C) result in various types and higher populations of insect vectors including for viral diseases transmission, although in warmer temperatures (>20 ^{0}C) the population can decrease due to predation (Petzoldt and Seaman, 2006). Low populations of insect vectors population may result into low rate of diseases spread from one plant to another. Areas which are found at low altitudes do experience high temperatures, which tend to decrease with increased altitudes (Wang *et al*., 2011). Hillocks *et al*. (1999) observed high incidences of Cassava brown streak disease at low altitude and absence of the disease at high altitudes.

Although this work did not focus on vectors studies it can be assumed that those areas with high viral disease pressure had high vector populations (Alicai *et al.*, 2007). In this study, many viral diseases were found in the eastern zone in Mvomero district where temperatures are high (21 ^{0}C – 31 ^{0}C) and the altitude is low (500 metres above sea level). Conversely, the virus disease incidences were low in southern highlands areas where average temperature is low (below 24 ^{0}C) and altitude is above 1000 m above sea level. It has to be noted that, northern Tanzania had high virus incidence despite having low temperatures in areas such as Karatu district, which suggest incidence are related to many factors; for example, a lot of research on common beans

have been conducted in Karatu for years, Therefore, it is possible that introduction of seeds from other places could have led to spread of viruses in this area.

Most common bean fields surveyed had low BCMV and BCMNV incidence of 0% and 3.3%, although some of the fields had highest incidence of up to 36% and 76%, respectively. In surveys conducted by Petrovic *et al*. (2010) in Serbia, the BCMNV and BCMV incidences were 2.67% and 30.53%, respectively. These contrasting results suggest incidence of BCMNV and BCMV vary from location to location and that incidence can be higher or lower for the same virus in different locations.

In this study, it was found out that co-infection of BCMV and BCMNV in common bean plants in Tanzania do occur, albeit at low incidence. According to Diaz-Munoz (2017), the co-infection occurance depends on host ecology, virus-virus interaction, environment and vector population, so in this case the coinfection was low in Tanzania due to failure of those factors to occur. The cases of viral mixed infection (BCMV and BCMNV) in bean plants have also been reported in Kenya (Mangeni *et al*. (2014) and Mexico (Lepe-soltero *et al*. (2012). Other combinations of viruses in mixed infections can occur as observed by Dizadji and Shahraeen *et al*. (2011) for BYMV and BCMNV in Iran. In Mexico, Chiquito-Almanza *et al*. (2017) found a 7% mixed infection in common bean samples collected from common bean fields. Mixed infections can occur when viruses are transmitted by the same vector as in the case of *Watermelon mosaic virus* and *Zucchini yellow mosaic virus* which are transmitted by aphids (Salvaudon *et al*., 2013). Mixed infections, especially of closely related viruses, can result into emergence of recombinant viruses or strains through exchange of genetic materials (Larsen *et al*., 2005). Therefore, the lower the incidence of mixed

viral infections the lowers the chances of emergence of new strains of virus which results from homologous recombination of sequences of closely related viruses.

BCMV was not detected in common bean leaf samples collected from Namtumbo, Mbinga, Mbozi, Morogoro Rural, Muleba, Kibondo and Kasulu districts. Likewise, BCMNV was not detected in common bean samples collected from Mbinga, Nkasi, Wanging'ombe, Njombe Rural, Ngara, Biharamulo, Muleba, Karagwe, Kasulu and kibondo districts. Failure to detect a virus in the common bean leaf samples could not be taken to mean that the virus does not occur in the area but it is possible that the incidence and titres of the virus were too low for the virus to be detected. It can also be that most common bean varieties grown in the country have developed resistance due to efforts invested in breeding for resistance against BCMV (Nordenstedt *et al*., 2017; Kusolwa *et al.*, 2016). Increasing the size of the samples could perhaps result into detection of viruses occurring at low incidence. However, very low viral disease pressure in some areas could be due to low population of vectors or because the farmers are using disease free seeds most of the time. Areas with low viral disease pressure can be used for seed multiplication as it is less likely that in such areas the seeds will be infected by the viruses

CPMMV incidence was determined only in common bean samples collected from the eastern and northern zones because the NGS results showed the presence of CPMMV in these two zones. The CPMMV was highly prevalent in the eastern zone but it occurred at low incidence in northern zone. In eastern zone, CPMMV is predominant in Mvomero district specifically in Ndole village where CPMMV was detected in

common bean plants collected from five common bean fields. In the northern zone, a low incidence of CPMMV was observed in common bean fields in Karatu, Siha and Hai districts. The CPMMV was reported for the first time in Tanzania in mung bean plants in Morogoro region by Mink and Keswani (1987). There were no further reports on the virus in the country. Because the research focus by then was on BCMNV and BCMV, the CPMMV and other bean infecting viruses received less attention. From this study and another recent study (Chilagane, 2018), the high incidence of CPMMV in some areas (the eastern and northern zone) is alarming and therefore there is a need to conduct further studies on this virus.

In the case of SBMV, this virus was found predominant in the western zone in Kasulu district but was virtually absent in the nearby Kibondo district where not even a single plant sample was found infected with the virus. Other areas where SBMV was found are Hai district in northern zone and Wanging'ombe district in southern highlands of Tanzania. In these areas, the SBMV was detected in only one sample in each district. This is the first time SBMV is detected in common bean plants in Tanzania despite the virus being distributed worldwide (Verhoeven *et al*., 2003). SBMV has been reported in Ivory Coast (Givord, 1981), Brazil (Cupertino *et al*., 1982) and many other parts of the world including Africa. Verhoeven *et al*., 2003 reported surveys covering Spain over one hundred greenhouses, diseased bean plants were found in approximately 10% of the greenhouses with infections rates ranging from 1% to 99% of the plants. Morales and Castano (1985) reported the SBMV to cause economically important disease with average yield losses of 56.3% determined.

3.6 Conclusions and Recommendations

3.6.1 Conclusions

This study has generated useful information about the incidence and distribution of four common bean viruses, namely BCMV, BCMNV, CPMMV, and SBMV in Tanzania. Except for CPMMV and SBMV, BCMNV and BCMV have been fairly surveyed for and studied in the past. Common bean viruses are widely distributed in the surveyed common bean growing regions of Tanzania with different percent incidence scores. The visually assessed incidence of common bean viruses was high in lake zone especially in Missenyi district in Kagera, while, at the same place the incidence of BCMV and BCMNV using RT-PCR was low compared to all surveyed areas. Northern and eastern were the hotspot of BCMV and BCMNV, while, Southern highlands zone being the area with low BCMV and BCMNV incidence. There was no relationship between viral symptoms observed in the field and the laboratory test results. Both BCMV and BCMNV occurred at low to high incidence, and were detected in bean samples collected from all the surveyed areas except in Wanging'ombe, Mbinga, Muleba and kasulu. On the other hand CPMMV was detected at low incidence in most areas tested for the virus. However, the prevalence of CPMMV was high in Gairo and Mvomero districts. SBMV incidence was high; also the prevalence was high in Kasulu. Kibondo is near Kasulu district but there were no even a single SBMV sample was detected. Overall, the Southern highlands had low incidence of virus diseases compared with all surveyed areas. Following this comprehensive survey, viruses' distribution maps were developed and will be useful in strategic deployment of planting material and in deciding areas where experiments that require high disease pressure can be setup i.e., breeders and entomologists could

use high disease pressure areas for testing their materials and conducting vector transmission studies, respectively. Furthermore, the generated information will guide plant pathologists and other agricultural stakeholders in development of strategies for management for specific viral diseases.

3.6.2 Recommendations

i. This study showed that the eastern and northern zones are the hotspots not only of BCMNV and BCMV but also of CPMMV and SBMV. This calls for the need to put more efforts in combating viral diseases in those areas.
ii. High disease pressure areas can be used for screening of genotypes for resistance to common bean viruses. For example, northern and eastern zone have high disease pressure.
iii. In the lake zone there were many common bean fields with symptomatic plants but low incidence of BCMNV and BCMV. There is, therefore, a need to investigate further into what viruses could be causing those symptoms.
iv. Some of the causal viruses of the disease symptoms observed were detected using NGS (Chapter two) but it remains undetermined how they are distributed and their economic importance with regards to common bean production.
v. It is worth noting here that there are many other viruses that infect common bean that could be causing these symptoms. Given the information obtained in this study, it is high time common bean breeders and pathologists take seriously the threat posed by SBMV and CPMMV, that is, common bean virus studies should not only be focused on BCMV and BCMNV. BCMNV and BCMV are important but it was demonstrated that other viruses may be equally important as shown in chapter seven of this work.

vi. The use of molecular tools in detetion of viruses is very important because it tells the reality of what is present in the field rather than the use of symptoms to identify the viruses.

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CHAPTER FOUR

4.0 MOLECULAR CHARACTERIZATION OF COMMON BEAN VIRUSES IN WILD PLANTS AND IDENTIFICATION OF WILD PLANT HOSTS OF VIRUSES IN TANZANIA

Beatrice Mwaipopo[1, 3*], Susan Nchimbi-Msolla[1], Paul Njau[1], and Deusdedith Mbanzibwa[2]

[1]Department of Crop Science and Horticulture, Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Tanzania.

[2]Tanzania Agricultural Research Institute Mikocheni, P. O. Box 6226, Dar es Salaam, Tanzania.

[3]Tanzania Agricultural Research Institute Ilonga, P.O Box 33, Morogoro, Tanzania.

Corresponding author email: beatricemwaipopo@yahoo.com*

Abstract

Many viruses which infect crops have weeds or wild plants as their alternative hosts. Thus, wild plants are important in the ecology and epidemiology of plant viruses. Therefore, the present study aimed to detect, characterize and identify viruses in weeds and wild plants found around common bean fields in Tanzania. A collection of wild plants with viral disease symptoms was done in four Agricultural Research Zones: southern, eastern, northern, and lake. A total of 1 430 wild plant samples were collected and total RNA was then extracted using the CTAB method. A total of 10 pooled RNA samples (JDH-1 to JDH-6, HXH-16, AIVN-1, AIVN-2 and AIVN-3 –

the last two were common bean samples) were sequenced using Illumina HiSeq 3 000/4 000 or Illumina NextSeq (Next Generation Sequencing). In total, 149 RNA samples from wild plant samples (144) and common bean (5) were sequenced. PCR was used to detect specific viruses: BCMV, PeMoV, BCMNV, and CPMMV. Using mat*K* primers, DNA barcoding was done to identify wild plants whose RNA was NGS-sequenced. Mechanical transmission study was done in screen house to establish if viruses found in the wild plants could infect common bean plants. Deep sequencing of small RNAs, which were isolated from wild plants, enabled detection of 122 viruses from 15 families and 20 genera. Out 122 viruses, 23 viruses – including PeMoV, YBMV and CMV – from 12 genera were known to infect common bean plants. CMV (from *Ocimum basilicum* L.) and CCMV related bromovirus (from *Bolusafra bituminosa* (L.) Kuntze) transmitted to different common bean genotypes. Sanger sequencing showed this CMV isolate (accession number MK330848) was 97% identical at nt sequence level to an isolate from tomato (accession no. KX525736) at nt sequence level.YBMV was detected in *Senna hirsuta* but was not transmitted to common bean plants in repeated inoculations. PeMoV was detected in *Senna occidentalis;* however, it was not used in transmission studies. Only 89 (out of 134) wild plants could be DNA barcoded (66.4% success rate). The DNA barcoded plants (89) belonged to 50 plant species. The detection of 122 viruses – including 23 common bean infecting ones – in wild plants and succesful transmission of two viruses to common bean plants is an indication that there are wild plants which serve as reservoirs of common bean viruses in Tanzania.

Keywords: Common bean plant, Next generation sequencing, Wild plants

4.1 Introduction

Viruses are obligate parasites that depend on host plants for their survival (Chen *et al*., 2013). Weeds, crops and wild plants around crop fields are important in ecology of plant viruses. During off season, the weeds turn to be the alternative hosts of plant viruses (Chen *et al*., 2013). Common beans are affected by many viruses and most of these viruses are harboured in the wild plants during off season. For example, *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) have been reported to infect plants in at least six families of weeds and wild plants (Bos and Gibbs, 1995; Worrall *et al*., 2015).

Myers *et al*. (2000) reported *Centrosema pubscens*, *Neonotonia wightii*, *Senna* spp., *Crotolaria* spp. and *Rhynchosia zernia* as hosts of BCMNV in Tanzania. Additionally, Njau and Lyimo (2000) reported six wild legumes (*S. occidentalis*, *Senna obtusifolia*, *Cassia floribunda*, *Crotalaria* spp. and *Rhynchosia minima*) as experimental host of BCMNV and BCMV. In Uganda, Sengooba *et al*. (1997) reported the natural occurrence of BCMNV in *C. pubescens*, *Crotalaria incana*, *Lablab purpureus*, *Phaseolus lunatus*, *Senna bicapsularis*, *Senna sophera*, *Vigna vexillata* and also *Crotalaria* spp. The BCMNV and BCMV have also been reported to occur naturally in *Glycine max* (Spence and Walkey, 1995; Worrall *et al*., 2015).

Alfa alfa weed is a good example of an alternative host of many plant pathogenic viruses – including *Alfa alfa mosaic virus* (AMV). Al-shahwan *et al*. (2017) reported *Bean leaf roll virus* (BLRV), BCMV, *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Tobacco streak virus* (TSV), *Peanut stunt virus* (PSV) and *Pea streak virus* (PeSV) in the Alfa alfa weeds in Saudi Arabia. Since these viruses cause

diseases in common bean plants, the presence of alfa alfa weeds within or around the bean fields can act as initial sources of infection from which the viruses spread to common bean plants.

Tomato yellow leaf curl virus (ToYLCV; *Begomovirus*) infects tomato crop but it has also been found to infect *Datura stramonium* weed in China (Chen *et al*., 2013) and common bean in Tanzania (Mwaipopo *et al*., 2018). *Bean golden yellow mosaic virus* (BGYMV) has been confirmed in *Macroptilium lathyroides* (Bracero *et al*., 2003).

Wild plants play a major role in the spread of plant viruses in the world because there is a close relationship between the main host, alternative host and vectors which facilitate the spread of the virus (Chen *et al*., 2013). Furthermore, domestication of different plants has provided conditions favourable for virus epidemics. Virus control measures act by minimizing the sources of virus. For example, weeding helps in delaying of infections (Zadocks and Schein, 1979).

Identification of wild plant hosts of plant viruses is very important in ensuring that the weed hosts of viruses in the farmer’s fields are effectively managed. In this study, next generation sequencing and normal PCR (Sanger sequencing) techniques were used to detect and identify common bean viruses in the wild hosts. In addition, transmission studies were done to verify if the viruses from wild hosts could infect the common beans. To the best of my knowledge, this was the first time NGS was used to detect viruses of common bean in wild plants.

4.2 Materials and Methods

4.2.1 Surveys and samples collection

The surveys to collect weeds and wild plant samples were conducted in four agricultural research zones. The surveys covered nine and 17 administrative regions and districts in Tanzania, respectively. The covered districts in Tanzania are shown in Fig. 2.1 in chapter two. A total of 1 430 samples were collected. Ten symptomatic and asymptomatic leaves were collected and preserved as described for common bean samples in chapter two. The leaf samples were taken from wild plants (including weeds) found in common bean fields and up to 10 m away from common bean fields. The preservation procedures were as presented in chapter two.

4.2.2 Nucleic material extraction

Total RNA was extracted from 1 430 wild plant leaf samples. The extraction was done using a modified CTAB Method (2% CTAB, 100 mM Tris-HCL, 20 mM EDTA, 2.5 M NaCl). 2% polyvinylpyrrolidone, 1% sodium sulphite and 2.5 % mercapto-ethanol were added fresh during extraction. The extraction procedures followed were as described in chapter two. The integrity of RNA samples was assessed visually by agarose gel electrophoresis after staining the gel with ethidium bromide. RNA concentration and purity were determined with a Nanodrop 2 000 c UV–vis Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The RNA was re-suspended in 40 µl of nuclease free water.

4.2.3 NGS-based detection of viruses in wild and inoculated common bean plants

From 1 430 samples extracted, 139 samples from four zones were selected for NGS. Equal amounts (7 µg) of total RNA extracted from 139 wild plants from each of the four zones were separately pooled to make seven samples. Two pooled samples were prepared for each zone except the lake zone where only one sample was prepared. The selection of samples was based on variation in symptoms and locations they were collected from. RNA extracted from asymptomatic samples was also included in the pooled RNA samples. The total number of samples in each pool varied from 10 to 30 for RNA from plants collected from the four zones and from 1 to 5 RNA samples for detection of viruses that was used in mechanical transmission studies (Table 4.1). The pooled samples were: JDH-1 and JDH-5 (eastern zone), JDH-2 and JDH-6 (northern zone), JDH-3 and JDH-4 (southern highlands zone) and HXH-16 (lake zone). A total of 25 wild plant samples (24 presented in Plate 4.1 and one not presented in the plate but contain YBMV) were collected from the eastern zone and used as sources of inocula in mechanical transmission studies (Plate. 4.1). After inoculation, viruses from four of the 25 wild plants were found to cause symptoms in common bean plants (genotypes: Mwaspenjele, Pesa, Rosenda and Rozikoko). Accordingly, RNA was extracted from each of these four wild plants and pooled for sequencing (AIVN-1). Then, RNA was extracted from each of the common bean plants that developed virus symptoms. NGS for one common bean plant showing severe symptom was done separately (AIVN-2) while the total RNA from three samples collected from common bean plants showing mild mosaic symptoms were pooled and sequenced as one sample (AIVN-3). Total RNA samples were shipped on dry ice to Fasteris SA in Switzerland where small RNAs were isolated and sequenced.

Before sequencing, the quality control (QC) of the RNA was done. The quantity of RNA was achieved by using Quibit (Picogreen) while the quality was done by loading 1 µl of diluted RNA on Bioanalyzer Nano chip. Then the small RNAs were isolated (acrylamide gel size selection) and cDNA libraries was prepared and sequenced using Illumina HiSeq 3000/4000 (Illumina Inc., San Diego, CA, USA) as described previously (Mbanzibwa *et al.*, 2014; Nordenstedt *et al.*, 2017). Kit version used was HiSeq 3000/4000 SBS Kit, and for samples AIVN-1 to AIVN-3, Illumina NextSeq was used. Bases were inferred from light intensity signals (Base calling pipeline) at HiSeq Control Software 3.376, RTA 2.7.6 and bcl2fastq2.17 v2.17.1.14. Illumina pipelines estimated the reads quality according to the percentage of bases having a base quality value greater or equal to 30 (Q30), i.e., less than 1 error in 1000 bases. The bases that corresponded to the standard illumina adapters were removed by using the trimmomatic package version 0.32 as explained by Bolger *et al*. (2014). Trimmomatic looks for seed matches (<16 bases) allowing a defined number of mismatches. In case of single end reads, whenever the seed alignment occurs, the entire alignment was scored. The trimming options: seed mismatches were set at 2 and simpleClipThreshold was at 5 (Bolger *et al*., 2014).

Table 4.1: Wild plant RNA samples sequenced by NGS method

No.	Sample[1]	Place[2]	Number of samples for NGS / pool	Type of sample	Year of collection
1	JDH-1	EZ	20	Wild plants	2016
2	JDH-2	NZ	16	Wild plants	2016
3	JDH-3	SHZ	10	Wild plants	2016
4	JDH-4	SHZ	19	Wild plants	2016
5	JDH-5	EZ	20	Wild plants	2016
6	JDH-6	NZ	24	Wild plants	2016
7	HXH-16	LZ	30	Wild plants	2016
8	AIVN-1	EZ	5	Wild plants	2018
9	AIVN-2	EZ	1	Common bean	2018
10	AIVN-3	EZ	4	Common bean	2018

[1]JDH 1 to JDH-6, HXH-6 and AIVN-1 to AIVN-3 are code names of samples given by the sequencing company. [2]In this study, unless otherwise stated, abbreviations EZ NZ, SHZ and LZ stand for eastern, northern, southern highlands and lake zones.

For samples AIVN-1 to AIVN-3, the cDNA libraries were sequenced on Illumina NextSeq (Number of cycles were 1x50+8). The illumina pipelines estimated the reads quality as described for samples JDH-1 to HXH-16. Trimmomatic package was used to remove bases that correspond to the standard illumina adapters.

4.2.4 Complementary DNA synthesis and RT-PCR

The first-strand complementary DNA (cDNA) synthesis was done using Moloney Murine Leukemia Virus Reverse Transcriptase (M-MuLV RT; #M0253; New England Biolabs (NEB), Ipswich, MA, USA) following a standard protocol with some modifications. The procedure has been described in chapter two of this thesis. RT-PCR was done to confirm some of the viruses detected in wild plants by NGS. Primers were designed to detect BCMV, BCMNV, CPMMV, CMV, *Cowpea chlorotic mottle virus* (CCMV), *Southern bean mosaic virus* (SBMV), *Yam bean mosaic virus* (YBMV), and *Peanut mottle virus* (PeMoV). The primers for detection of these viruses were developed using the sequences obtained through NGS and those which

have been published in the database NCBI (National Center for Biotechnology Information) (Table 4.2).

During PCR amplification, AccuPower® PCR PreMix and One*Taq*® DNA polymerase kits were used. For primer pairs used to detect BCMV, BCMNV, CPMMV, YBMV, PeMoV and SBMV, the AccuPower® PCR PreMix was used. The AccuPower® PCR PreMix contained 1U TopDNA polymerase, 250 µM dNTPs, 10 mM Tris-HCL (pH 9.0), 30 mM Kcl, 1.5 mM $Mgcl_2$, stabilizer and a tracking dye. 15 µl of double distilled water was added to AccuPower® PCR tubes. 1 µl of 10 µM of each forward and reverse primers and 3 µl of template were added to make a volume of 20 µl. For primer pairs designed to amplify CMV and CCMV, One*Taq*® DNA polymerase was used. The reagents mixed together for reaction were 0.5 µl of 10 mM dNTPs, 0.5 µl of 10 µM forward and reverse primer, 5 µl of 5x One*Taq* standard reaction buffer, 0.125 µl One*Taq*® DNA Polymerase, 5 µl of DNAtemplates (10 ng) and the nuclease-free water was added up to 25 µl. The PCR conditions were different for each virus and all PCR reactions were carried out in 2720 thermal cycler (Applied Biosytems) or TC-412 TECHNE PCR machine.

The PCR conditions for BCMVIF/ BCMV1R and BCMNV F1/BCMNV R1 have been explained by Mwaipopo *et al*. (2018) and Chapter two. For CPMMV :1 cycle of 3 minutes at 94 °C (initial denaturation), then 35 cycles at 94 °C for 45 sec (denaturation), 56 °C for 45 sec (annealing), 72 °C for 1 sec (extension) and a final cycle (final extension) at 72 °C for 10 minutes.

The primer set AH-CMVF1/AH-CMVR1 for CMV and primer set CCMVF1/CCMVF2 and CCMVF1/CCMVR2 for CCMV had the following PCR condition; 1 cycle of 30 sec at 94 °C (initial denaturation), then 35 cycles at 94 °C for 30 sec (denaturation), 50 °C for 30 sec (annealing), 68 °C for 45 sec (extension) and a final cycle (final extension) at 68 °C for 10 min.

For PeMoVcp1257F1/PeMoVcp1257R1, the PCR condition was 1 cycle of 3 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 45 sec (denaturation), 53 °C for 45 sec (annealing), 72 °C for 1 min (extension) and a final cycle (final extension) at 72 °C for 10 min. For the primer set BC-YBMVF1/ BC-YBMVR1 and SBMV-N315F1/ SBMV-N315R, the PCR condition was 1 cycle of 3 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 25 sec (denaturation), 50 °C for 25 sec (annealing), 72 °C for 30 min (extension) and a final cycle (final extension) at 72 °C for 10 min. PCR products were run in 1% agarose gel stained with ethidium bromide. PCR products were visualized on the Benchtop UV Transilluminators (UVP).

Table 4.2: Primer pairs used for detection of viruses in wild plants

Primer pair	Primer sequences 5′–3′ direction	Virus	Target[1]	Size (bp)	Reference
BCMV1F	GTAGCACAGATGAAGGCAGCA	BCMV	CP	320	This study
BCMV1R	GGTTCTTCCGGCTTACTCATA				
BCMNVF1	CAAAGGCCCAGCGGATAAA	BCMNV	CP	823	This study
BCMNVR1	GGTGGTATAACCACACTGGAATTG				
CPMMV2F1	AACAAAAACTGGCGTTCCAAA	CPMMV	CP	1300	This study
CPMMV2R1	GGAAAATAACTTTAAAACCGG				
PeMOVcp1257F1	TGATACAGGCTCACAAGGAATAG	PeMOV	CP	535	This study
PeMOVcp1257R1	AGCTGATGTACGCGACGTG				
BC-YBMVF1	TGCGSCARATYATGCACCATTT	YBMV	CP	350	This study
BC-YBMVR1	TGCCTTTCAGTATTCTCGCTGG				
SBMV-N315F1	AGTCGGCTTGCAAGTTTAGA	SBMV	P8/P10	490	This study
SBMV-N315R1	GGTCCACAGGGGATTTATT				
AH-CMVF1	GACCGTGGGTCTTATTATGGT	CMV	1a	650	This study
AH-CMVR1	TCTTGTGCTAGAAGTACACGGA				
CCMVF1	CTGTTATTGTTGAACCCATCGC	CCMV	RNA3-CP	333	This study
CCMVR1	CCTCTGAATACATCGCGAC				
CCMV F1	CTGTTATTGTTGAACCCATCGC	CCMV	RNA3-CP	170	This study
CCMVR2	AGCTGCTTGTTCCTTTCGGACG				

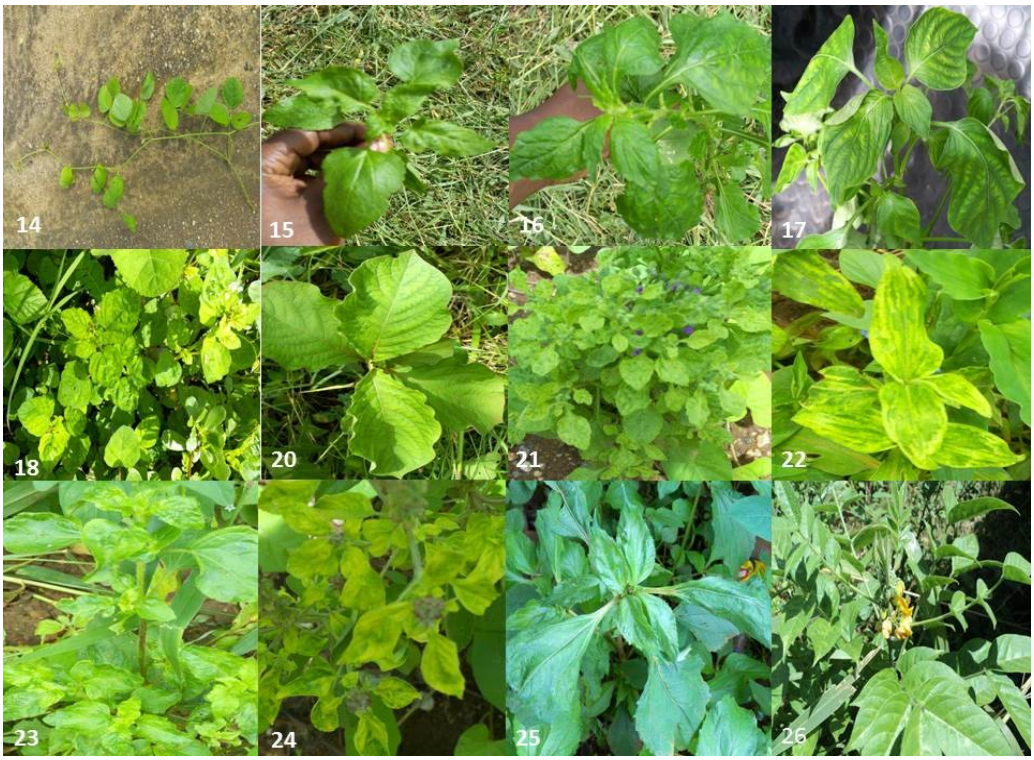
[1]CP indicate virus coat protein, P8/P10 is a C-terminal protein with ATPase and RNA binding properties, 1a is a helicase-like motifs found in the RNA 1 of *Cucumber mosaic virus*, and bp means base pair.

4.2.5 Mechanical transmission of viruses from wild to common bean plants

Two experiments were conducted in the screen house at two different times at Sokoine University of Agriculture (SUA) in Morogoro. In the first experment, two attempts were made to transmit YBMV from wild plant collected in Morogoro to eleven common bean genotypes, namely Pesa, Zawadi, Mshindi, SUA 90, Rojo, Lyamungo 90, Calima uyole, PASI, Rosenda, JESCA and Uyole 96. The wild plants (*S. hirsuta*) used showed clear viral mosaic symptoms and had been confirmed using PCR to be infected with YBMV. In the second study, attempts were made to transmit unknown viruses from 25 legume and non-legume wild plants to four common bean genotypes. The common bean genotypes used in the second study were Pesa, Mwaspenjele, Rozikoko and Rosenda. These varieties were selected because in

previous experiments, they showed to be susceptible to most of viruses tested. Four seeds were planted in each pot, which enabled inoculation of four common bean plants for each symptomatic wild plant. The inoculation and buffer preparation was done as described by Noordam (1973). Whereby, 0.01 M phosphate buffer solution pH 7.0 that contained KH_2PO_4 (MW= 136.086 g/mol) and $Na_2HPO_4.2H_2O$ (MW = 177.99 g/mol) was used for mechanical inoculation. The wild plants used as inoculum in the experiment were only from the symptomatic legume and non-legume plants (Plate. 4.1). The leaf samples were collected in a cool box a day before or on the day inoculation was done. The plants were inoculated on the 8th day post sowing. Leaf samples from 25 different wild plants with typical virus disease symptoms were separately ground in a phosphate buffer using motor and pestle. The leaf sample to phosphate buffer ratio was 1:10 (w/v). A pinch of 600 mesh caborundum was spread on the leaves and the sap from wild plants was gently rubbed on the fully opened common bean leaves using the base of the pestle. After 30 min, the plants were washed well using sterile water to remove the buffers and caborundum. Then the plants were left to grow and the inoculated common bean plants were watered three times a week. Initial symptoms started to appear on the inoculated plants on the 4th day after inoculation.

(a)



(b)

Plate 4.1: Symptomatic wild plants used in virus transmission studies

Wild plants with viral symptoms collected in Morogoro and used as a source of inoculum for inoculation of common beans. Numbers 1-26 in the picture (a) and (b) are the different species of weeds with viral symptoms found in common bean growing areas.

4.2.6 DNA Barcoding of wild plant species

To determine the identity of the wild plants that were used in this study, DNA barcoding was done. A total of 134 wild plant samples were amplified for barcoding, including those samples which were collected in Morogoro and used in mechanical transmission of viruses to common beans for viral symptoms expression. DNA extracted from each of these wild plants was amplified with PCR. The primers used were Matk-F (5'-CGTACAGTACTTTTGTGTTTACGAG-3') /Matk-R (5'-ACCCAGTCCATCTGGA AATCTTGGTTC-3') or Matk2.1af (5'-ACTCATCT GGAAATCTTAGT-3')/ Matk5r (5'-GTTCTAGCACAAGAAAGTCG-3').These primers were designed from sequences of maturase *K* gene of the chloroplast. This is the most variable gene in angiosperm which is promising candidate for barcode due to its high evolutionary rate which is important in distinction of plant species (Kar *et al*., 2015). The AccuPower® PCR PreMix was used for PCR amplification. PCR condition was 1 cycle of 3 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 30 sec (denaturation), 47 °C for 30 sec (annealing), 72 °C for 45 min (extension) and a final cycle (final extension) at 72 °C for 10 min. The amount of DNA used was 50 ng per PCR reaction at a final volume of 25µl. The PCR products were run in the 1% agarose gel that contained ethidium bromide. The amplicons were visualized on the Benchtop UV Transilluminators (UVP).

Out of 134 DNA samples from wild plants, both JDH's and AIVN's samples, amplification was achieved for only 70 DNA samples from JHD sample while further 19 samples from AIVN's samples were DNA barcoded to determine identity of wild plants whose infecting viruses were transmitted to common bean plants in the screen

house at SUA. These samples were sequenced at Bioneer sequencing company in South Korea and Mbeya Zonal Referral Hospital Laboratory (Tanzania). Prior to sequencing, PCR products were purified using PCR purification kits (Bioneer) and then sequenced on both strands using forward and reverse primers.

4.3 Analysis of NGS and Sanger Sequences

Analysis of NGS data was done using the VirusDetect program v.1.6 and v.1.7 (Zheng *et al.*, 2017) and VirusDetect chipster on a supercomputer (https://www.csc.fi; Finland). The files received from Fasteris SA (with extension '.tar') were unzipped using the command 'tar -xzvf filename'. Then, all reads of 21 to 24 nucleotides (nt) were analysed separately while for AIVN1 to AIVN-3 samples, analysis was done using the combined 21-24 nt insert size. The fastq files were assembled using the command 'virus_detect.pl *.fastq' (for offline analysis using VirusDetect (v.1.6), the command was 'perl virus_detect.pl filename'). For combined inserts 21-24 nt (AIVN-1 to AIVN-3), the analysis was done in Chipster environment (Chipster v3.13) accessed at https://www.csc.fi. The contigs obtained were checked for open reading frames using the Expasy-translate tool (http://www.expasy.org/).

The Sanger sequences of different virus isolates as well as those of chloroplast maturase *K* gene (matK) were blasted to the National Center of Biotechnology Information (NCBI) to obtain sequences which were highly similar to them for virus and plant taxonomy purpose.

4.4 Results

4.4.1 Percentage reads of wild plant samples through NGS

The highest proportion of reads was observed in the inserts range of 18 to 26 nt (Fig. 4.1; Table 4.3). The percent reads at the insert range of 18 - 26 in sample JHD-1, JHD-2, JHD-3, JHD-4, JHD-5 and JHD-7 ranged from 37.82 to 72.27%. For samples AIVN-1, AIVN-2 and AIVN-3, inserts of the size 18-26 nt ranged from 75.56 to 85.2%.

Table 4.3: Number and proportion of small RNA reads from NGS

			Insert range			
Samples	**Zone**		**0**	**1-17**	**18-26**	**27-50**
JDH- 1	EZ	reads	114 040	1 460 984	22 502 252	12 926 757
		% reads	0.31	3.95	60.81	34.94
JDH-2	NZ	reads	397 050	4 712 426	55 908 465	16 345 479
		%reads	0.51	6.09	72.27	21.13
JDH-3	SHZ	reads	120 202	2 015 205	19 095 786	29 254 435
		% reads	0.24	3.99	37.82	57.95
JDH-4	SHZ	reads	232 973	8 086 369	33 862 576	15 543 668
		% reads	0.4	14.01	58.66	26.93
JDH-5	EZ	reads	143 285	4 023 103	43 957 025	15 000 429
		% reads	0.23	6.37	69.64	23.76
JDH-6	NZ	reads	532 059	7 279 604	57 856 149	37 327 755
		% reads	0.52	7.07	56.17	36.24
HXH-16	LZ	reads	39 686	2 770 804	34 559 621	20 751 803
		% reads	0.07	4.77	59.46	35.7
AIVN-1	EZ	reads	511 024	1 976 479	44 756 129	9 038 100
		% reads	0.91	3.51	79.52	16.06
AIVN-2	EZ	reads	1 873 196	1 442 068	55 778 185	14 728 690
		% reads	2.54	1.95	75.56	19.95
AIVN-3	EZ	reads	1 523 275	1'795 929	48 380 872	5 085 746
		% reads	2.68	3.16	85.20	8.96

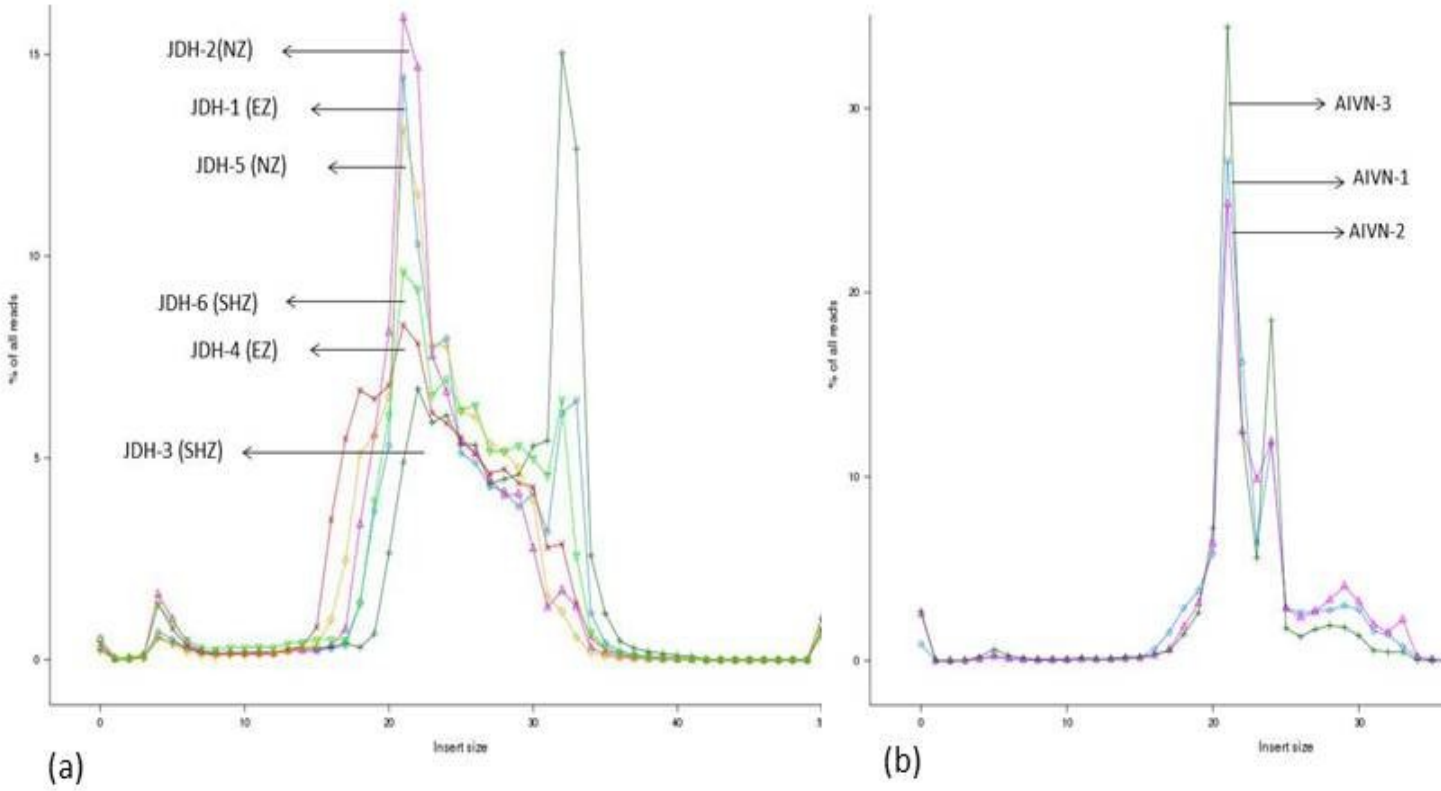


Figure 4.1: Insert relative abundance in different sequenced RNA samples

Shown in figures (a) and (b) are the graphical presentation of the inserts range for libraries of JHD-1, JHD-2, JHD-3, JHD-4, JHD-5, JHD-6, AIVN-1, AIVN-2 and AIVN-3 from wild plants.

4.4.2 Reads alignment and De *novo* assembly of the wild plant samples

The raw data obtained in this work was deposited at zenodo and assigned DOI 10.5281/zenodo.2539276. The NGS data for RNA samples JHD-1 to JDH-6, HXH-16 and AIVN-1 to AIVN-3 were analysed using VirusDetect and Chipster (Fig. 4.2 and Fig. 4.3; Table 4.4). A total of 122 viruses were detected. Some viral contigs matching several viral sequences in the database. The contigs matched each virus having different strains which leads to total of 232 viruses by blastn and 124 viruses by blastx as shown in Table 4.4. Information for total reads, reads aligned to reference sequences and number of contigs obtained after *de novo* assembly is presented in Table 4.4. For all samples JHD-1 to JDH-6, and HXH-16 the information shown was obtained following analysis of inserts of size 21 nt. However, for AIVN-1 to AIVN-3, the information shown was obtained from analysis of combined inserts (21-24 nt). Based on the blastn results and only for wild plant samples not meant for inoculation, VirusDetect software using blastn revealed highest number of viruses (27) in JDH-1 sample (EZ). JDH5 (EZ) and JDH-6 (NZ) samples contained 24 and 26 viruses, respectively. Samples JDH-3 and JDH-4, from SHZ only one virus each. Blastx identified more viruses than blastn in each sample except in SHZ where blastx did not identify any viruses in the database. The total number of reads ranged from 246 523 in JHD-3 to 12 308 028 in JHD-2. The reads aligned to reference sequences were less than 10 000 in JHD-4 and JDH-3 but higher than 30 000 in all other samples.

In samples AIVN-1 to AIVN-3, the total number of reads ranged from 34 652 368 in AIVN-1 sample to 43 590 022 in AIVN-2 sample. The highest number of aligned reads was observed in AIVN-2 (Table 4.4). Many viruses were identified in sample

AIVN-1 (a pooled sample of RNAs from wild plants) in both blastx and blastn (Table 4.4).

Table 4.4: Next generation sequencing data from wild plants samples

		Aligning reads[2]			De *novo* assembly	After removal of redundancies	Number of viruses obtained (21-24nt)	
Sample	**Zone[1]**	**Total reads**	**Reads aligned**	**Unique contigs**	**Unique contigs**	**Unique contigs**	**Blastn**	**Blastx**
JHD-1	EZ	5 342 932	249 983	246	1 321	1 285	27	37
JHD-2	NZ	12 308 028	366 565	143	2 341	2 306	11	35
JHD-3	SHZ	246 523	9 972	4	186	184	1	0
JHD-4	SHZ	4 791 256	7 768	1	228	226	1	0
JHD-5	EZ	8 309 861	278 167	182	1 030	1 103	24	37
JHD-6	NZ	9 907 472	266 755	118	384	463	26	26
HXH-16	LZ	5 344 166	30 768	46	780	821	2	26
AIVN-1	EZ	34 652 368	790 674	117	5 024	4 948	13	63
AIVN-2	EZ	43 590 022	7 597 609	260	4 003	3 972	7	2
AIVN-3	EZ	40 231 768	3 652 546	65	2 657	2 613	12	6

[1]Abbreviations EZ, NZ, SHZ and LZ stand for eastern, northern, southern highlands and lake zones, respectively. [2]For samples JHD-1 to JHD-6 and HXH-16 the reads used were of the size 21 nt insert range while for AIVN-1 to AIVN-3, the samples were analysed using combined insert of 21-24 nt insert range.

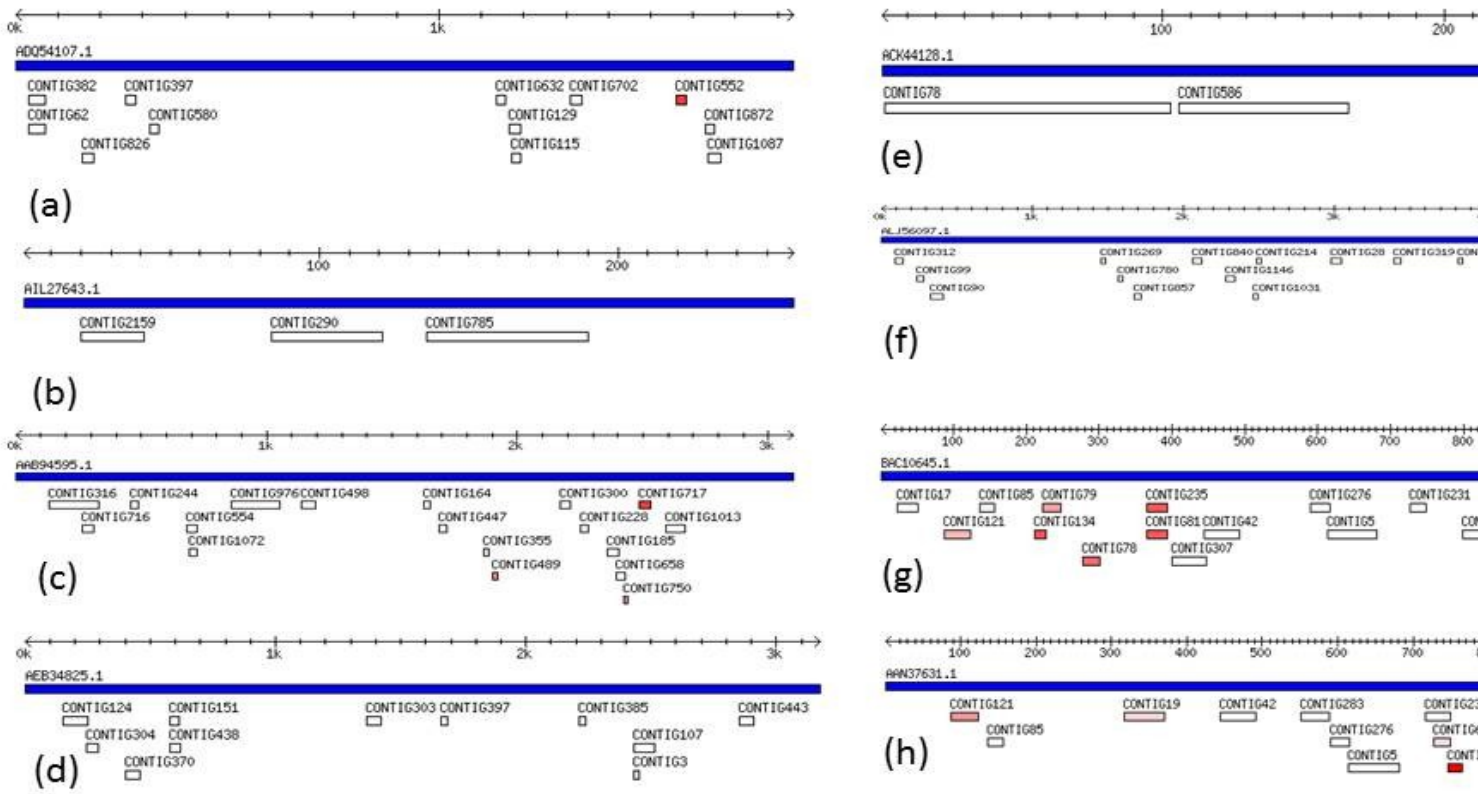


Figure 4.2: Viruses identified with blastx in the database

The viral contigs obtained through de novo assembly of reads were mapped to viral sequences in the database (numbers on blue and red/white bars are accession and contig numbers, respectively). The shown examples (reference sequences in parenthesis) represent *(a) Cowpea mild mottle virus* (ADQ54107.1; sample JHD1), (b) *Ethiopian Tobacco bushy top virus* (AIL27643.1; Sample JHD-2) (c) *Peanut mottle virus* (AAB94595.1; sample JHD-5) (d) *Cowpea aphid borne mosaic virus* (AEB34825.1; sample JHD-6) (e) *Tomato leaf curl New Delhi virus* (ACK44128.1; sample AIVN-1) (f) *Phaseolus vulgaris endornavirus 1* (ALJ56097.1; sample AIVN-3) (g) *Spring beauty latent virus* (BAC10645.1; sample AIVN-3) *(*h) *Cowpea chlorotic mottle virus* (AAN37631.1; AIVN-3).

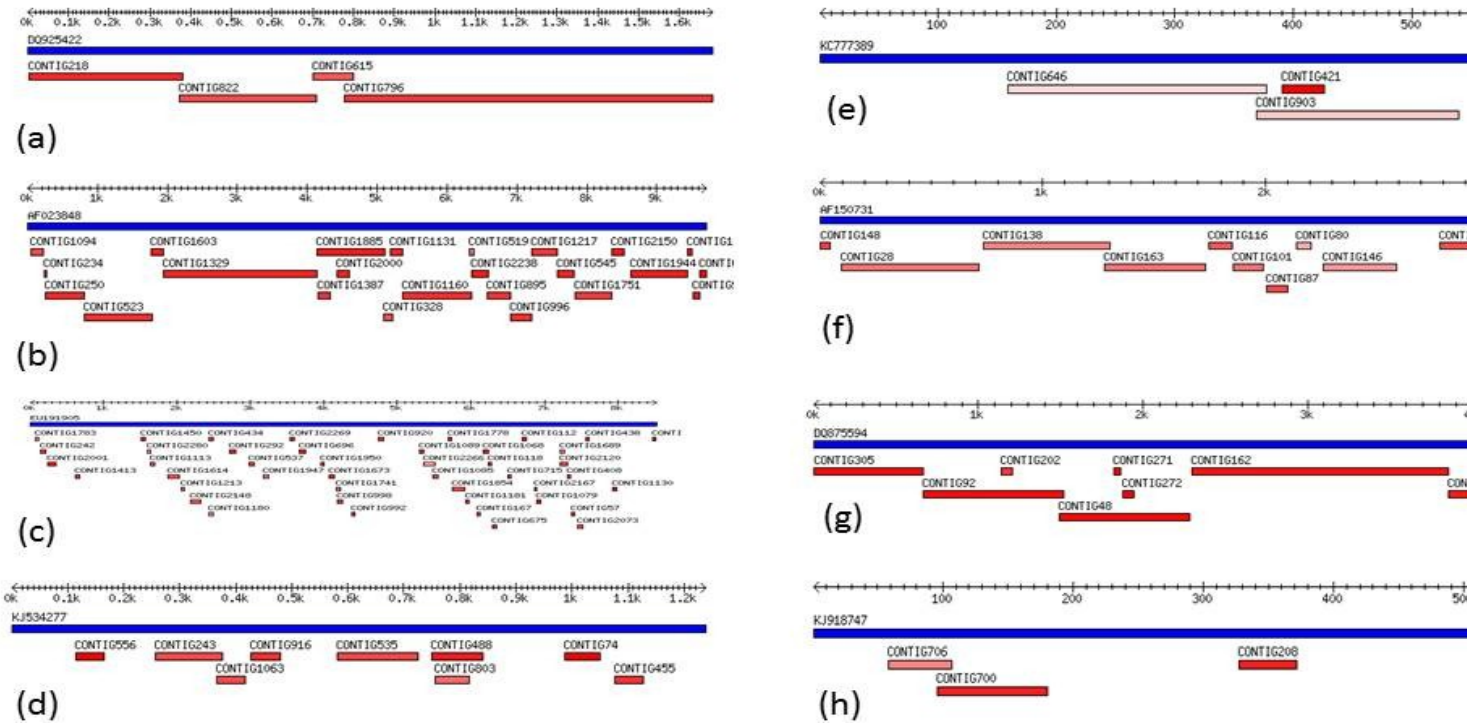


Figure 4.3: Viruses identified using blastn in the database

The viral contigs obtained through de novo assembly of reads were mapped to viral sequences in the database (numbers on blue and red bars are accession and contig numbers, respectively). The shown examples (reference sequences in parenthesis) represent *(a) Bean common mosaic virus* (DQ925422; sample JHD-1), (b) *Peanut mottle mosaic virus* (AF023848; Sample JHD-2), (c) *Bean yellow disorder virus* (EU191905; sample JHD-2*)*, (d) *Cowpea mild mottle virus* (KJ534277; sample JHD-5), (e) *Cowpea aphid borne mosaic virus* (KC777389; sample JHD-5), (f) *Cucumber mosaic virus* (AF150731; sample AIVN-2) (g) *Southern bean mosaic virus* (DQ875594); sample AIVN-3), and *(*h) *Ethiopian tobacco bushy top virus* (KJ918747; HXH-16).

4.4.3 Coverage, depth, identity and contig number and length of wild plant virus in eastern, northern, southern highlands and lake zone

4.4.3.1 Viruses detected in wild plant samples from eastern zone

Samples JHD-1 and JHD-5 represent RNA samples extracted from wild plants collected from eastern zone (Appendix 4.1). The viruses detected in these samples belonged to eight families: *Potyviridae, Geminiviridae, Betaflexiviridae, Fimoviridae, Luteoviridae, Solemoviridae, Caulimoviridae* and *Secomoviridae*. The detected viruses in this sample belonged to the genera *Potyvirus, Begomovirus, Carlavirus, Emaravirus, Polerovirus, Sobemovirus, Cavemovirus, Comovirus* and *Foveavirus*. Of the 36 detected viruses, 11 viruses in JHD-1 and JHD-5 samples have been previously detected in common beans plants. These viruses are BCMV, CPMMV, *Mungbean yellow mosaic virus* (MYMV), *Tomato leaf curl virus* (ToLCV), *Tomato yellow leaf curl virus* (ToYLCV), YBMV, CABMV, PeMoV, *Peanut stripe virus,* CPMV and *Ethiopian tobacco bushy top virus*. A summary of viruses identified through blastn and blastx is presented in Table 4.5. The begomoviruses were identified through both blastn and blastx while other viruses were identified by blastn. YBMV and BCMV shared some of the contigs (Table 4.5).

Table 4. 5: Summary of coverage, depth, identity and contig number and length of wild plant virus likely to infect common beans in eastern zone

Sample	Reference sequence	Coverage (%)	Number of contig		Contig length (nt)	Depth	%Identity	Genus	Viruses
			blastx	blastn					
JHD-1 and JHD-5	AGS77263	16.6	2	-	70-116	21.2	64.52	*Begomovirus*	*Mungbean yell*
	DQ925422	99.7	-	4	99-903	205	95.58	*Potyvirus*	*Bean common*
	AM701768	32.2	-	11	41-171	40.8	97.53	*Begomovirus*	*Tomato leaf cu*
	EF194760	16.2	-	6	44-191	121.8	89.8	*Begomovirus*	*Tomato leaf cu*
	AAB94595	28.4	18	-	59-580	1919.2	53.91	*Potyvirus*	*Peanut mottle*
	AAX82171	16.6	12	-	43-589	1381.6	57.49	*Potyvirus*	*Peanut stripe v*
	KJ918747	24.8	-	1	129	11.1	97.67	*Umbravirus*	*Ethiopian toba virus*
	DQ127170	66.9	-	16	45-282	64.9	97.03	*Begomovirus*	*Tomato leaf cu*
	KC774020	78.7	-	54	42-551	15.2	97.71	*Carlavirus*	*Cowpea mild m*
	X00729	51.8	-	18	47-340	172.9	85.41	*Comovirus*	*Cowpea mosai*
	JN190431	80.2	-	49	42-743	334.5	90.14	*Potyvirus*	*Yam bean mosa*
	KC777389	66.5	-	3	41-224	301.7	84.95	*Potyvirus*	*Cowpea aphid-virus*

[1]Only viruses, which have been reported to infect common bean plants are shown in column titled 'viruses identified'. Those which are not known to infect common bean are shown in Appendix 4.1

4.4.3.2 Viruses detected in wild plant samples from northern zone

The samples JDH-2 and JHD-6 were pooled using RNA samples extracted from wild plants/weeds collected from northern zone. The viruses detected in these two samples belonged to nine families, namely *Betaflexiviridae*, *Potyviridae*, *Closteroviridae*, *Tombusviridae*, *Secoviridae*, *Luteoviridae*, *Geminiviridae*, *Alphaflexiviridae* and *Bromoviridae*. The same viruses belonged to 12 genera: *Foveavirus*, *potyvirus*, *Crinivirus*, *Umbravirus*, *Torradovirus*, *Polerovirus*, *Begomovirus*, *Carlavirus*, *Carmovirus*, *Enamovirus*, *Potexvirus* and *Cucumovirus* (Appendix 4.1). Out of 29 viruses detected in the JHD-2 and 28 in JHD-6, 16 viruses in total have been detected in common beans in previous studies. The viruses identified in northern zone and that have been Previously reported to cause diseases in common bean were summarized in Table 4.6.

As shown in Table 4.6, in JDH-2 and JHD-6 samples from northern zone, the number of contigs associated with viruses that are known to infect common bean ranged from 1 to 11 and 6 to 45 contigs for blastx and blastn, respectively. For these samples, the similarities between contigs and reference sequences ranged from 50 to 97.74%. However, the coverage were low for some contigs (Table 4.6).

Table 4.6: Summary of coverage, depth, identity and contig number and length of wild plant virus likely to infect common beans in northern zone

Sample	Reference sequence	Coverage (%)	Number of contig		Contig length (nt)	Depth	%Identity	Genus	Viruses
			blastx	blastn					
JHD-2 and JHD-6	AAD39155	25.4	1	-	105	149.2	50	*Begomovirus*	*Tomato yellow lea*
	AAC54603	22.6	7	-	50-95	1558.8	84.72	*Carmovirus*	*Cowpea mottle vir*
	CED51824	53.3	2	-	98-120	182.4	80.56	*Umbravirus*	*Carrot mottle viru*
	CTQ57207	41.6	11	-	57-203	134	79.64	*Umbravirus*	*Groundnut rosette*
	AAN62863	32	8	-	57-203	124.6	77.46	*Umbravirus*	*Tobacco bushy top*
	ACJ03575	27.7	9	-	57-182	105.1	73.63	*Umbravirus*	*Carrot mottle mim*
	AIL27643	43.4	3	-	69-164	267.9	64.29	*Umbravirus*	*Ethiopian tobacco*
	EU191905	32.5	-	45	43-169	16.7	95.61	*Crinivirus*	*Bean yellow disor*
	AF023848	97.8	-	24	49-2193	199.9	96.86	*Potyvirus*	*Peanut mottle viru*
	JN190431	81.1	-	31	43-1110	689.1	89.27	*Potyvirus*	*Yam bean mosaic*
	DQ925422	99.8	-	6	93-653	402.4	95.27	*Potyvirus*	*Bean common mos*
	AAA7230	27	3	-	64-96	98.5	80.49	*Enamovirus*	*Pea enation mosai*
	AHZ65104	37.9	6	-	49-203	146.8	76.79	*Umbravirus*	*Opium poppy mos*
	AEB34825	15.2	11	-	67-318	2268.4	57.43	*Potyvirus*	*Cowpea aphid-bor*
	DQ127170	75.1	-	20	45-511	72.5	96.93	*Begomovirus*	*Tomato leaf curl U*
	AM701757	46.5	-	13	46-438	38.1	97.53	*Begomovirus*	*Bean leaf curl Ma*
	KJ534277	52	-	8	46-114	8.9	97.74	*Carlavirus*	*Cowpea mild mott*

[1]Only viruses, which have been reported to infect common bean plants are shown in column titled 'viruses identified'. Those which are not known to infect common bean are shown in Appendix 4.1

4.4.3.3 Viruses detected in samples from southern highlands zone

Two pooled RNA samples collected from SHZ were labelled as JHD-3 and JHD-4. In these samples, a single virus was found in both JHD-3 and JHD-4 pooled RNA sample. Virus in these samples belonged to one family – *Tombusviridae* – in genera – *Umbravirus*. The detected virus was ETBTV (Table 4.7 and Appendix 4.1). ETBTV has been detected in common beans. Only one contig releted to ETBTV was obtained in each of these two samples. The contigs length of ETBTV in JHD-3 was 128 nt while in JHD-4 was 111 nt. The contig and reference (KJ918747) sequences in both samples were 98.15% similar to each other (Table 4.7).

4.4.3.4 Viruses detected in wild plant samples from the lake zone

In a pooled RNA sample HXH-16 for wild plant samples collected from lake zone the viruses detected belonged to four families: *Germiniviridae, Tombusviridae, Virgaviridae and Potyviridae.* They belonged to four genera, namely *Begomovirus, Umbravirus, Tobamovirus* and *Potyvirus* (Appendix 4.1). Of the 24 viruses detected in this pooled RNA sample, 6 viruses have been reported to infect common beans (Table 4.8). These were BCMV, *Cowpea aphid borne mosaic virus, Ethiopian tobacco bushy top virus, Peanut mottle virus, Tomato yellow leaf curl virus* and *Tomato leaf curl virus*. For each virus detected in these samples (only refering to viruses that are known to infect common bean and presented in Table 4.8), the number of contigs identified by blastx ranged from 1 to 29 contigs. On the other hand, blastn resulted into 3 contigs for the identified virus where by,the identitities of contigs and reference sequences were in the range of 50.95 - 95.51% (Table 4.8).

Table 4.7: Summary of coverage, depth, identity and contig number and length of wild plant virus likely to infect common beans in southern highlands

Sample	Zone	Reference	Coverage (%)	Number of contig (Blastn)	Contig length	Depth	%Identity	Genus	Virus identified
JHD-3	**SHZ**	KJ918747	20.7	1	128	7.9	98.15	*Umbravirus*	Ethiopian tobacco virus
JHD-4	**SHZ**	KJ918747	24.4	1	111	7.9	98.15	*Umbravirus*	Ethiopian tobacco virus

[1]Only viruses, which have been reported to infect common bean plants are shown in column titled 'viruses identified'. Those which are not known to infect common bean are shown in Appendix 4.1

Table 4.8: Summary of coverage, depth, identity and contig number and length of wild plant virus likely to infect common beans in lake zone

Sample	Reference sequence	Coverage (%)	Number of contig		Contig length	Depth	%Identity	Genus	Viruses i
			blastx	blastn					
HXH-16	KJ918747	32.1	-	3	45-86	19.6	95.51	*Umbravirus*	*Ethiopian tobacco*
	AAO32352.1	87.9	4	-	157-258	327.5	56.25	*Begomovirus*	*Tomato leaf curl N*
	AAG27473.1	15.7	1	-	69	82.8	71.43	*Begomovirus*	*Tomato yellow lea*
	AAF04154.1	100	1	-	463	860.7	56.74	*Begomovirus*	*Tomato leaf curl I*
	AAB94595.1	25.1	29	-	57-296	95.9	65.88	*Potyvirus*	*Peanut mottle viru*
	AGZ92019.1	15.4	15	-	59-296	88.7	64.77	*Potyvirus*	*Bean common mo peanut stripe*
	AIT11627.1	15	13	-	63-328	88.3	64.83	*Potyvirus*	*Bean common mo RU*
	AIZ48757.1	10.2	9	-	76-246	134.1	50.95	*Potyvirus*	*Cowpea aphid-bo*

[1]Only viruses, which have been reported to infect common bean plants are shown in column titled 'viruses identified'. Those which are not known to infect common bean are shown in Appendix 4.1

4.4.4 RT-PCR detection of viruses in wild plants

A total of 1 430 wild plant samples were screened for presence of five viruses known to infect common bean: YBMV, PeMoV, BCMV, BCMNV and CPMMV. These viruses were previously detected in the wild plant samples using next generation sequencing technique. Although all five viruses were detected in common bean samples using NGS, RT-PCR was unable to detect BCMV, BCMNV and CPMMV (Fig. 4.4). Only YBMV and PeMoV were amplified as shown in Fig. 4.5. Three RNA samples (samples 47, 48 and 75) yielded PCR products for YBMV as shown in Fig. 4.5a and b. The wild plant samples (*S. hirsuta*) in which YBMV was detected were collected from Morogoro rural in eastern zone (samples 47 and 48) and from Arumeru in northen zone (Sample 75). Moreover, PeMoV was detected in a wild plant sample two (*S. occidentalis*) (Fig. 4.5c), which was collected from Arumeru district in northern zone. PeMoV was confirmed through sequencing as this was the first time the primer pair was used. It was found to be 99% identical at nucleotide sequence level to an isolate of the same virus (Accession no. KY350138) from groundnut (*Arachis hypogaea*) in Brazil. The PeMoV sequence was submitted in the NCBI database and was assigned accession number MK330847.

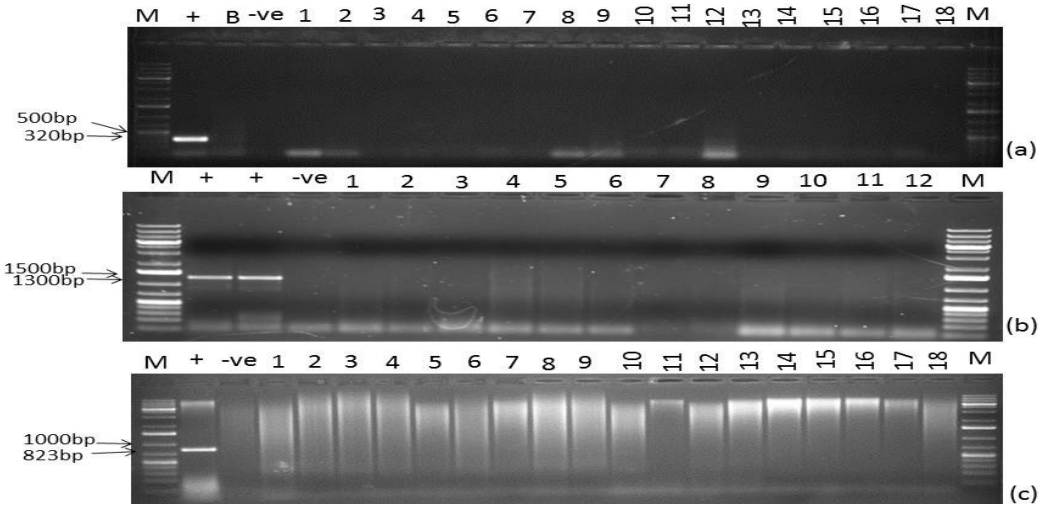


Figure 4.4 Gel picture showing the RT-PCR amplification of BCMV, BCMNV and CPMMV

Plates (a), (b) and (c) show PCR amplification results for BCMV, CPMMV and BCMNV, respectively, obtained using primers that were expected to yield PCR products of the sizes 320 bp, 1 300 bp and 823 bp, respectively (Table 4.2). The lane labelled with a letter 'M' was loaded with a marker (Thermoscientific O'GeneRuler 1kb Plus DNA Ladder); lanes labelled with '+' mark were loaded with PCR products for positive controls; lanes labelled with a '-ve' mark were loaded with PCR products for negative controls; lanes labelled with a 'B' mark were loaded with PCR products from PCR reactions where no any RNA template but free nuclease water; lanes labelled with the numbers were loaded with PCR products from RT-PCR reactions where RNA template from different wild plants were used.

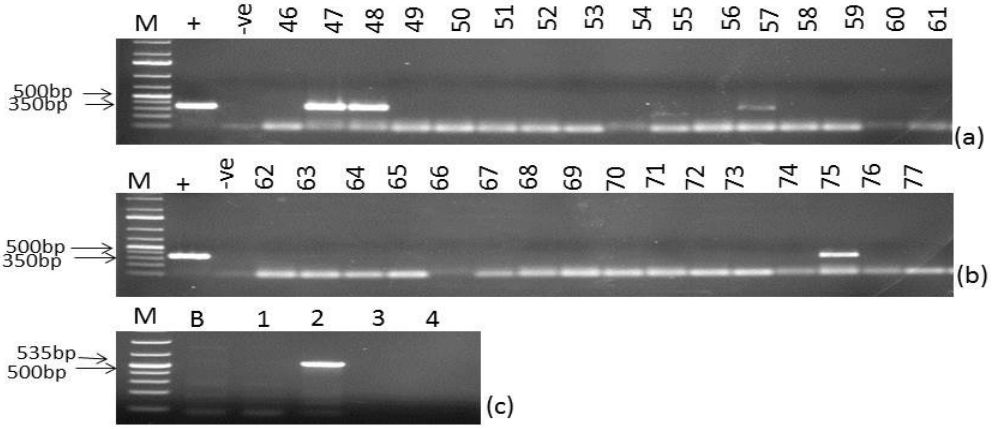


Figure 4.5: Gel picture showing the RT-PCR amplification of YBMVand PeMOV

Plates (a) and (b) are gel pictures of PCR amplification of YBMV while (c) is the gel picture of PCR amplification of PeMoV. The expected band sizes were 350 bp for YBMV and 535 bp for PeMOV (Table 4.2). The lane labelled with a letter 'M' was loaded with a marker (Thermoscientific O'GeneRuler 1kb Plus DNA Ladder); lanes labelled with '+' mark were loaded with PCR products for positive controls; lanes labelled with a '-ve' mark were loaded with PCR products for negative controls; lanes labelled with a 'B' mark were loaded with PCR products from PCR reactions where no any RNA template but free nuclease water; lanes labelled with the numbers were loaded with PCR products from RT-PCR reactions where RNA template from different wild plants were used.

4.4.5 Mechanical transmission of viruses from wild plants to common beans and confirmation of viral infections

4.4.5.1 Inoculation of common bean plants

Symptoms development was only observed in common bean plants inoculated with sap from five different wild plant samples numbered 4, 5, 6, 8, and 25. Inoculation of bean seedlings with sap from wild plant samples 4, 5, 6 and 8 were repeated twice and then inoculated common bean plants were consistently infected and showed similar symptoms. Common bean plants of four varieties – Mwaspenjele, Rozikoko, Pesa and Rosenda – inoculated with sap from wild plant samples 4, 5, and 6 caused bristles on leaves, mild mosaic and vein yellowing depending on the variety (Plate 4.2 a, b, c, d). However, the seedlings of common bean variety Pesa that were inoculated with sap from wild plant sample number 8 showed severe necrosis on different parts of the plant; mosaic, slight leaf roll, stunted growth and eventually died after 14 days. Further inoculation using sap from infected common bean plants onto the same common bean varieties (Mwaspenjele, Rozikoko and Rosenda) resulted into severe symptoms, but the plants survived (Plate 4.2 e, f, g, h).

Following inoculation of common bean with sap from wild plant number 25, symptoms were observed on a common bean plant after it had stayed in the screen house for one month. The infected common bean plants exhibited mosaic symptoms. However, symptoms development was fast when sap from infected common bean was used in mechanical transmission to other common bean plants (Mwaspenjele, Rozikoko, Pesa and Rosenda). No stunted growth or necrosis was observed on inoculated common bean plants in repeated experiments.

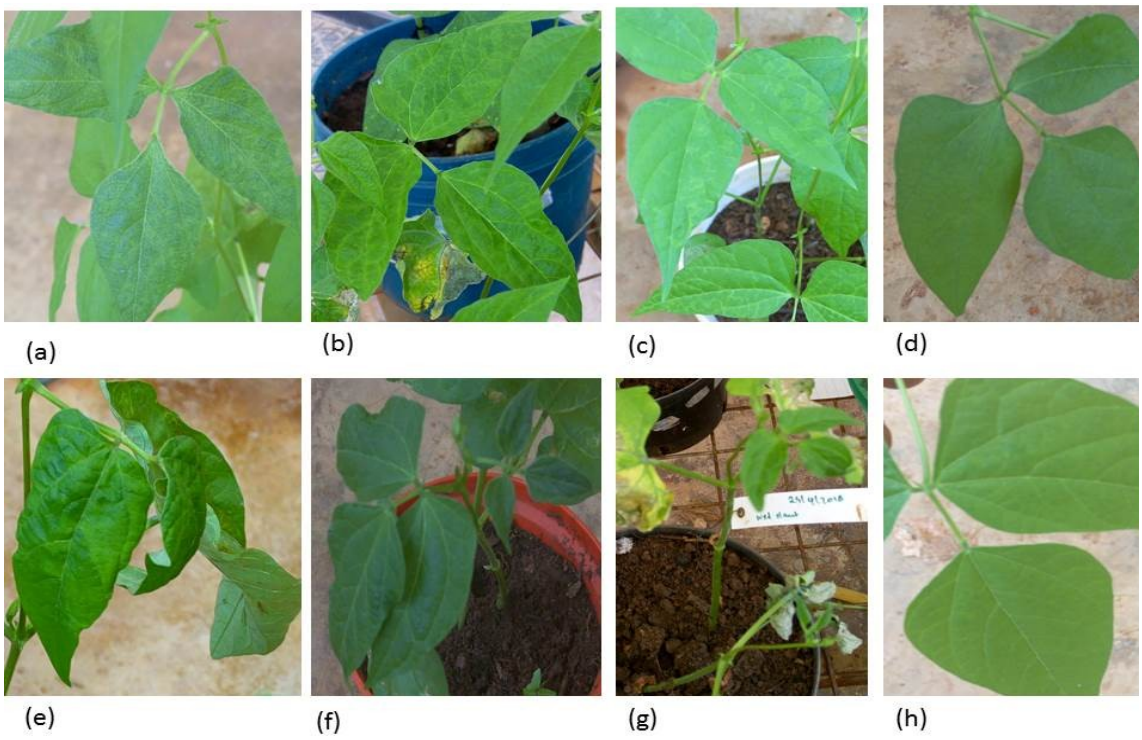


Plate 4.2: Symptoms expressed in common bean after inoculation with sap from wild plant with virus

Plates (a) to (d) represent viral disease symptoms expressed in common beans plants inoculated with sap from wild plants numbered 4, 5 and 6, where by the (a) and (b) was inoculated with virus from wild plant four. (c) with virus inoculum from plant six and (d) with virus inoculum from wild plant five.The genotypes shown are (a) Mwaspenjele (b) Rosenda (c) Pesa and (d) Rozikoko varieties. Plates (e) to (h) are pictures of the symptoms expressed in common bean plants after inoculation with sap from wild plant number 8. The genotypes shown in (e) to (h) are Mwaspenjele, Rosenda, Pesa and Rozikoko, respectively. Virus transmitted from wild plants numbered 4, 5, and 6 was a CCMV related bromovirus while CMV was the virus transmitted from wild plant number 8.

4.4.5.2 NGS and RT-PCR-based confirmation of viral infections in inoculated common bean plants

4.4.5.2.1 Next generation sequencing

As explained above, mechanical transmission of viruses to common bean plants was done. The RNA of five isolates from wild plants infected common beans and the common beans that expressed symptoms were sequenced by NGS as explained. Samples AIVN-1 represent the RNA pool of RNA samples from wild plants while

AIVN-2 and AIVN-3 represent RNA pools for RNA extracted from common beans plants infected with virus transmitted from different wild plants.

Next generation sequencing data analysis was done for samples AIVN-1, AIVN-2, and AIVN-3, whereby the sequences were aligned to reference sequences in the database (Fig. 4.2e and 4.3f). RNA - pooled sample AIVN-1 was made from five RNA samples extracted from five wild plant samples, which caused virus infections in common bean plants. Table 4.9 shows the viruses that were found in wild plant samples and which infected common bean plants. NGS revealed that the five plants (Section 4.4.5.1) were infected with viruses belonging to six genera, namely *Polerovirus*, *Begomovirus*, *Bromovirus*, *Cucumovirus*, *Endornavirus* and *Torradovirus* which belonged to five families: *Luteoviridae*, *Germinividae*, *Bromoviridae*, *Endornaviridae* and *Secoviridae*, respectively. Five of the viruses detected have been reported to infect common bean. These were CCMV, CMV, PvEV-1, *Tomato leaf curl virus*-related begomoviruses and *Tomato yellow leaf curl virus* (Table 4.9). For the above-mentioned viruses, the similarities between their contigs and database reference sequences ranged from 51.81% for *Melandrium yellow fleck virus* to 98.6% for PvEV-1 (Table 4.9). Viruses related to those known to infect common bean were mainly detected in wild plant samples using blastx option indicating that they could be novel viruses (Table 4.9). The longest contig (2 163 nt) was observed for CMV and was closely related to a sequence with accession number DQ006805 in the database (Table 4.9).

One isolate from a wild plant (called plant 8 in this study; *O. basilicum* L.) caused severe mosaic symptoms on inoculated common bean plants. The symptoms were

reproducible in repeated inoculation experiments. Interested to know the causal virus, RNA was extracted from one of the plants exhibiting severe symptoms and sent for NGS. This sample was named as sample AIVN-2.

Interested also to understand the genetic identity of this virus in AIVN-2, analysis on the combined insert (21 - 24 nt) revealed *Cucumber mosaic virus (Cucumovirus; Bromoviridae)* was the only virus in the infected common bean sample. The coverage to the reference genome was 100% while its similarity was to the most closely related contig was 93.44%. The blastn search identified 11 CMV related contigs. The longest contig was 2 196 nt. This sequence was closely related to a sequence with accession number U20219 in database (Table 4.9). Three contigs of this virus isolate, which were 1 606, 1 472, and 1 189 nt long, were submitted to NCBI and assigned accession numbers MK330844 (RNA2), MK330845 (RNA1), and MK330846 (RNA1), respectively. After the virus isolate had been transmitted to the common bean, it was isolated and Sanger sequenced using primer pair AH-CMVF1 and AH-CMVR1 (Table 4.2), which targeted RNA3, protein 3b. It was found closely related (97%) to a sequence with accession number KX525736 for CMV isolate from *Solunum lycopersicum.*

In RNA pooled sample AIVN-3 for RNA samples extracted from three common bean plants inoculated with sap from wild plant samples named as plants 4, 5 and 6 (all being of same plant species, *B. bituminosa* (L.) Kuntze), six viruses from the families *Bromoviridae (Cucumovirus and Bromovirus), Endonaviridae (Endornavirus)* and *Solemoviridae (Sobemovirus)* were identified. The detected viruses were *Phaseolus*

vulgaris alphaendornavirus 1, SBMV, CMV, *Spring beauty latent virus, Melandrium yellow flek virus* and *Cowpea chlorotic mottle virus* (CCMV). Among the viruses identified in AIVN-3, only *Melandrium yellow fleck virus* had > 51.81% similarity to those in the database (Table 4.9).

Table 4.9: Summary of coverage, depth, identity and contig number of wild plant virus infect common beans in eastern zone

Sample	Reference sequence	Coverage (%)	Number of contig		Contig length	Depth	%Identity	Genus	Virus identified (c
			Blastx	blastn					
AIVN-1	JN591385	21	-	3	66-345	1515	83.47	*Begomovirus*	*Tomato leaf curl vir*
	DQ006805	98.6	-	9	46-2163	1888.1	93.25	*Cucumovirus*	*Cucumber mosaic v*
	BAJ41520.1	33.1	10	-	53-328	3600.5	72.54	*Bromovirus*	*Brome mosaic virus*
	AAA46370.1	75.3	2	-	52-344	5714.4	83.11	*Bromovirus*	*Cowpea chlorotic m*
	BAC10645.1	68.3	12	-	63-476	3127.9	81.43	*Bromovirus*	*Spring beauty latent*
	ABF61898.1	16.4	1	-	58-261	1778.7	84.21	*Begomovirus*	*Tomato leaf curl Aru*
	AAL05296.1	20.1	1	-	84	457.7	74.07	*Begomovirus*	*Tomato yellow leaf c*
AIVN-2	U20219	100	-	11	46-2196	5752.2	93.44	*Cucumovirus*	*Cucumber mosaic v*
AIVN-3	J02052	10.7	-	1	91	5809.5	86.67	*Bromovirus*	*Cowpea chlorotic m*
	DQ412732	65.3	-	19	44-154	24.1	97.19	*Cucumovirus*	*Cucumber mosaic v*
	KT456287	99	-	24	58-1566	154.2	98.6	*Endornavirus*	*Phaseolus vulgaris e*
	DQ875594	99.1	-	2	117- 4113	5403.5	98.46	*Sobemovirus*	*Southern bean mosa*
	BAC10645.1	59.9	10	-	75-362	4560.6	75.65	*Bromovirus*	*Spring beauty latent*
	BAI40163.1	63.7	2	-	206-450	5579.9	51.81	*Bromovirus*	*Melandrium yellow*
	AAA46370.1	85.3	1	-	56-496	6720.6	79.63	*Bromovirus*	*Cowpea chlorotic m*

4.4.5.2.2 PCR amplification

After obtaining NGS results which indicated samples 4, 5 and 6 were infected with a bromovirus closely related to CCMV and *Spring beauty latent virus* and that sample 8 was infected with CMV, primers were designed to NGS - obtained contigs. The primer pairs CCMVF1/CCMVR1 and CCMVF1/CCMVR2 (Table 4.2) detected the bromovirus in samples 4, 5 and 6 (Fig. 4.6 a and b). Amplicons of the sizes, 333 bp and 170 bp were obtained as expected for the two primer pairs, respectively. Primer pair AH-CMVF1/AH-CMVF1 amplified CMV in sample 8 with the expected size of 650 bp (Fig 4.6 c). These results indicated that plant samples 4, 5, and 6 were infected by a virus in the genus *Bromovirus*. The source plant of the isolates were a leguminous wild plant, identified through DNA barcoding (MK414463) as *B. bituminosa* (L.) Kuntze (Plate 4.3a; Table 4.10). The infected *B. bituminosa* (L.) Kuntze plants were collected from three different locations in Mvomero district in eastern zone at a distance of over three kilometers from each other. The wild plant sample that is referred to herein as sample 8 was identified through DNA barcoding as a perennial *O. basilicum* L. belonging to the family *Lamiaceae* (Plate. 4.3b; Table 4.10). This plant was collected from Mvomero district.

The PCR products obtained using primer pair CCMVF1/CCMVR1 and CCMVF1/CCMVR2 (CCMV related bromovirus) and AH-CMVF1/AH-CMVF1 (CMV) were sent to Bioneer sequencing company in South Korea for sequencing. The results showed that the sequences obtained were similar to the NGS sequences and confirmed that common bean plants inoculated with saps from wild plant 4, 5, and 6 were infected by a bromovirus related to CCMV while that inoculated with a

sap from plant sample 8 was infected with a CMV. The YBMV was detected in the wild legume plant called *S.hirsuta* (Plate. 4.3 c) but despite repeated attempts, it could not be transmitted to any common bean genotypes.

Southern bean mosaic virus was detected in common bean plants,but it was not detected in wild plants. Since the screenhouse was previously containing plants infected with this virus, it was reasonable to conclude that this virus infected common bean plants through contamination.

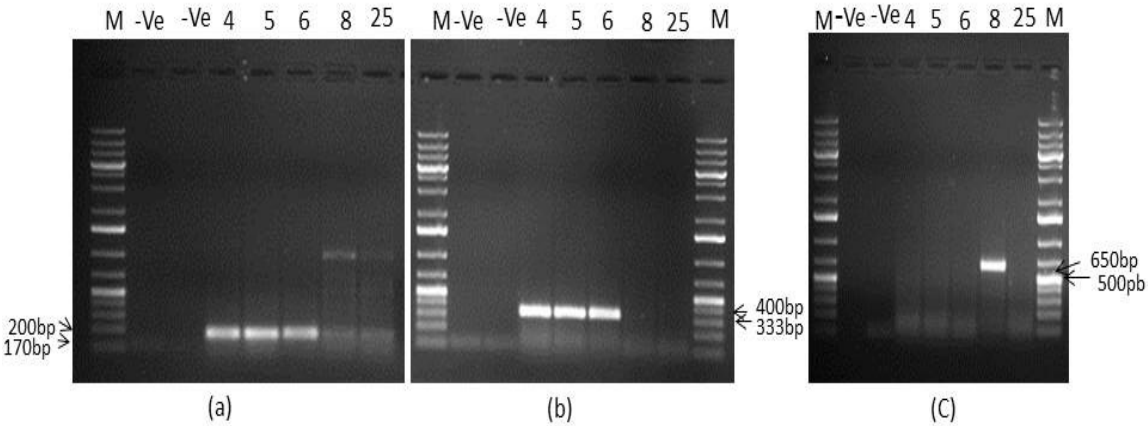


Figure 4.6: Gel picture showing the RT-PCR amplification of CMV and CCMV related bromovirus

(a) and (b) represent gel pictures of amplification of a CCMV related bromovirus detected using primers that were expected to yield PCR products of the sizes 170 bp and 333 bp, respectively. (c) A gel picture of CMV amplified using a primer pair AH-CMVF1/AH-CMVF1 that yields PCR products of size 650 bp. The lanes labelled with a letter 'M' were loaded with a DNA marker (Thermoscientific 'O'GeneRuler 1 kb Plus DNA Ladder); lanes labelled with a '-ve' mark were loaded with PCR products for negative controls; lanes labelled with the numbers 4, 5, 6, 8 and 25 were loaded with PCR products from RT-PCR on RNA extracted from common bean plants inoculated with isolates from wild plants 4,5,6, 8 and 25. No positive controls were used because this was the first time these viruses were detected and there were no known infected plant materials.

Plate 4.3: Wild plants identified as alternative hosts of common bean viruses

Viral disease symptoms on (a) *B. bituminosa* (L.) Kuntze, wild legume infected with CCMV-related bromovirus that was transmitted to common bean plants *(b) Ocimum basilicum* wild plant infected with CMV that was transmitted to common bean *(c) S. Hirsuta* is the wild legume plant, which was confirmed to be infected with YBMV but the isolates of this virus could not be mechanically transmitted to common bean plants in repeated experiments.

4.4.5.3 DNA barcoding of wild plant species

A total of 134 wild plant samples were subjected to PCR amplification in this study for DNA barcoding purpose (Fig. 4.10). Of these, 109 plant samples were from wild plant species which were used in NGS to detect viruses in wild plants growing around common bean fields. The remaining 25 wild plant species were collected for mechanical transmission of viruses from wild to common bean plants. During PCR amplification of maturase *K* gene, out of 134, only 89 DNA samples from wild plants yielded PCR products and were sequenced (Table 4.10; Table 4.11). The PCR amplified and DNA barcoded samples included the four-plant species from which viruses were transmitted to common bean plants. The results were blasted in the database (National Centre for Biotechnology Information; NCBI) and the wild plant were identified (Table 4.10; Table 4.11). According to NCBI blastn results, the 89 amplified and sequenced DNA samples were found to be from or related to 50 plant species (Table 4.10). The sequences were submitted at Zenodo and were assigned with a digital object identifier (DOI) 10.5281/zenodo.2539239.

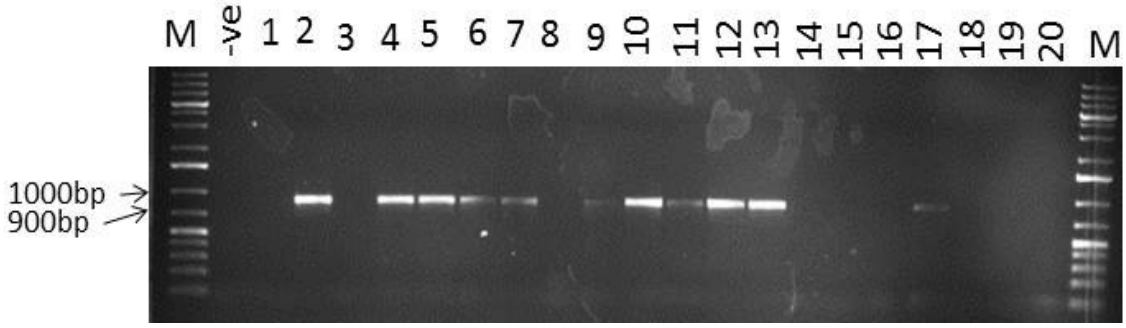


Figure 4.7: Gel picture showing the PCR amplification of maturase *K* gene of the chloroplast of wild plants

A representative gel picture of PCR amplification of maturase *K* gene of the chloroplast of wild plants collected in Tanzania for identification of alternative hosts of common bean viruses. Matk-F/ Matk-R and Matk2.1af/Matk5r primer pairs were used with the expected size of PCR products 900 bp and 790 bp, respectively. The lane labelled with a letter 'M' was loaded with a DNA marker (Thermoscientific O'GeneRuler 1 kb Plus DNA Ladder). Lane labelled with a '-ve' mark was loaded with PCR products from PCR reaction without DNA template. The lane labeled 1 to 20 were loaded with the PCR products from PCR reactions where DNA templates from different wild plant species were used.

Table 4.10: DNA barcoded wild plant species used in mechanical transmission of viruses to common bean plants

Sample number	Wild plant name[1]	Symptoms	Collection location
1	*Crotalia spp*	Vein banding and discoloration, leaf malformation	SUA
2	*B. bituminosa* (L.) Kuntze	Yellowing, mosaic	SUA
3	*S. obtusifolia.*	Vein banding, mosaic	Changarawe
4	***B. bituminosa* (L.) Kuntze**	**Mosaic and yellowing**	**Sangasanga**
5	***B. bituminosa* (L.) Kuntze**	**Mosaic, bristles, vein yellowing**	**Sanga sanga**
6	***B. bituminosa* (L.) Kuntze**	**Mosaic, bristles, yellowing**	**Sanga sanga**
7	*Synedrella nodiflora*	Mosaic	Sanga sanga
8	***O. basilicum* L.**	**Yellowing, mosaic, leaf malformation**	**Sanga sanga**
9	*S. hirsuta*	Mosaic, yellowing	Changarawe
10	*S. obtusifolia.*	Mosaic, yellowing	Mzumbe
12	*Crotalaria capensis*	Mosaic and yellowing	Kipera
13	Non-legume	Yellowing	SUA
14	*B. bituminosa* (L.) Kuntze	Vein yellowing, mosaic	SUA
15	*Agerautum haustonianum*	Mosaic, yellowing	Sanga sanga
16	*Lepidonia jonesii*	Mosaic, yellowing	Sanga sanga
17	*Dicliptera congesta*	Yellowing, mosaic	Sanga sanga
18	*Boerhavia erecta*	Mosaic, leaf malformation, yellowing	Mzumbe
20	Non-legume	Vein banding	Mzumbe
21	Non-legume	Mosaic, leaf malformation, yellowing. like kivumbasi	Mzinga
22	*Commelina spp*	Yellowing	Mzinga
23	Non-legume	Mosaic	Mzinga
24	*Ocimum spp.*	Mosaic, malformation	Mzinga
25	*Synedrella nodiflora*	Mosaic	Mzinga
26	*S. hirsuta*	Mosaic, yellowing	Kinole

[1]Wild plant species from which viruses were successfully mechanically transmitted to common bean plants are bolded. Samples 3, 4, 8, 10, 12, 15, 16, 17, and 25 were DNA barcoded and assigned NCBI's accession numbers. MK414457, MK414463, MK414465, MK414458, MK414459, MK414460, MK414461, MK414462, MK414464, respectively.

Table 4.11: Wild plants samples which were DNA barcoded to identify alternative hosts of common bean viruses used in NGS

No.	Zone[1]	Wild plant species
1	SHZ	*Desmodium illinoense, Psychotria asiatica, Ageratum houstonianum, Galinsoga quadriradiata, Vernonia sp.*
2	EZ	*Ageratum houstonianum, Momordica boivinii, B. bituminosa* (L.) Kuntze, *Mucuna pruriens, Bidens subalternans, Pycnophyllum spathulatum, Macrotyloma uniflorum, Mucuna cochinchinensis, Solanum linnaeanum, Neonotonia wightii, Vernonia sp., Bidens hintonii, Operculina macrocarpa, Ipomoea involucrate, Tephrosia heckmanniana, Desmodium illinoense, S.occidentalis, S.hirsuta.*
3	NZ	*Cleome gynandra, Vangueria edulis, Mucuna pruriens, Juanulloa Mexicana, Bidens hintonii, Okenia hypogaea, B. bituminosa* (L.) Kuntze, *Helianthus annuus, Erucastrum abyssinicum, Cajanus cajan, S. occidentalis, Datura stramonium, Sesamum indicum, Galinsoga quadriradiata, Desmodium angustifolium, Commelina benghalensis, S. obtusifolia, Richardia sp*

[1]Abbreviations SHZ, EZ and NZ represent southern highlands, eastern, and northern zones, respectively

4.5 Discussion

Small RNAs-based deep sequencing (NGS) approach was used in this study to detect viruses in wild plants collected from common bean fields or from locations that were

up to 10 m from common bean fields. These wild plants were collected from northern, eastern, southern highlands and lake zones. Following analysis using a VirusDetect software (Zheng *et al*., 2017), 122 viruses were detected and identified from reads of sizes 21 and combined inserts (21 - 24 nts). The viruses detected in the samples belonged to 20 genera in 15 families: *Tolecusatellitidae (Betasatellite), Geminiviridae (Begomovirus), Bromoviridae (Bromovirus and Cucumovirus), Betaflexiviridae (Carlavirus and Foveavirus), Tombusviridae (Carmovirus and Umbravirus), Caulimoviridae (Cavemovirus and Soymovirus), Secoviridae (Comovirus and Torradoviridae), Closteroviridae (Crinivirus), Fimoviridae (Emaravirus), Endornaviridae (Alphaendornavirus), Luteoviridae (Polerovirus), Alphaflexiviridae (Potexvirus), Potyviridae (Potyvirus), Solemoviridae (Sobemovirus), Virgavirdae (Tobamovirus)*. Out of 122 viruses detected, 23 common bean infecting viruses, belonging to 12 genera, namely *Umbaravirus, Tobamovirus, Begomovirus, Potyvirus, Carlavirus, Carmovirus, Crinivirus, Enarmovirus, Cucumovirus, Comovirus*, *Bromovirus* and *Alphaendornavirus w*ere detected. These viruses were previously detected in common bean from Tanzania and elsewhere (Mwaipopo *et al*., 2018; Worrall *et al*., 2015; Beikzadeh *et al*., 2015; Lovisolo and Conti, 1966; Khalifa *et al*., 2016).

Overall, the detected viruses (see appendix 4.1) infect many cultivated crops like okra, pepper, cotton, cassava, soybean, spinach, squash, tobacco, tomato, cowpea, cucumber, potato, figs, pigeonpea, apple, groundnut, beets, chickpea, lettuce, banana, papaya, sweetpotato, watermelon, zucchini, blueberry, carrot and pea. Most of these viruses are transmitted by insect vectors – e.g. aphids , beetles and whitefly – from

one plant to another. Insect pests feed on infected plants and spread the viruses to the health plants, irrespective of them being weeds or cultivated crops, as long as the plants can be infected by the virus (Goyal *et al*., 2012). Kucharek and purcifull (2001) reported several weeds that host viruses – some of reported viruses were identified in this study.

BCMV, a virus which is a world wide distributed and known to cause damage to common bean plants, was found in pooled RNA samples for wild plants collected from EZ, NZ and LZ. BCMV is widely spread in common bean fields in Tanzania as explained in chapter two and by Mwaipopo *et al*. (2018). Moroever, BCMV RU strain was detected in wild plant (pooled RNA sample HXH-16) from lake zone (Kagera). The BCMV-RU strain has never been found in common bean in any other parts of Tanzania other than in the lake zone (Mwaipopo *et al*. (2018). However, this related strain may be also occuring and infecting common bean in eastern zone in Morogoro. It is worthwhile to note here that the contigs that matched BCMV sequences in database also matched YBMV's sequences. Therefore, it can not be fully resolved that the isolates detected in wild plants were indeed BCMV isolates.

Detection of BCMV in wild plants has been reported for samples collected from Uganda, Malawi, Rwanda, Kenya, Tanzania and other parts of the world (Worral *et al*., 2015). Also, Spence and Walkey (1995) reported the presence of BCMV in *Crotalaria incana, Rhynchosia spp*, *Macroptilium atropurpureum*, *Cassia hirsuta, Cassia sophera, Crotalaria comanestiana, Cassia occidentalis, Gycine max* and *Vigna vexillata.*

BCMNV is a common bean virus that is widely spread in Tanzania (Mwaipopo *et al*. 2018; Njau and Lyimo, 2000; Vetten and Allen, 1991), the virus was not detected in any NGS sample. Although it was not detected in any sample in this study, BCMNV has been detected in naturally infected wild plants: *C. pubescens*, *Crotalaria incana*, *Lablab Purpureus*, *Phaseolus lunatus*, *Senna bicapsularis*, *Senna sophera*, *Vigna vexillata* (Sengooba *et al*. 1997). During 1993 surveys in Tanzania, several wild plants including *Centrosema pubscens*, *Neonotonia wightii*, *Senna* spp., *Crotolaria* spp. and *Rhynchosia zernia* were identified as hosts of BCMNV (Myers *et al*., 2000).

Tomato leaf curl virus and *Tomato yellow leaf curl virus* have been found in the wild plants and have been proven to infect common bean (Lapidot, 2002; Mwaipopo *et al*. 2018). In this work, many strains related to ToLCV were detected by NGS. They include *Tomato leaf curl virus* strain Diana, Namakely, Madagascar, Sudan, Toriala, Arusha, Uganda, Delhi and Laos. Similarly, several strains of ToYLCV were identified but not all strains were presented in Table 4.5, 4.6 and 4.8. Of these ToLCV strains, only ToLCAV and ToLCUV strains were identified in common beans (Mwaipopo *et al*., 2018).

BnYDV, CPMMV and CABMV cause economically important viral diseases of common bean. All these viruses were detected in at least one pooled RNA samples of RNA extracted from wild plants. The CABMV was detected by NGS. This virus is known to infect *Amaranthus hybridus*, *Crotalaria incana, C. spectabilis, C. juncea, Arachis hypogaea and Vigna unguiculata* (Gonzalez-Segnana *et al*., 2013). Rodrigues

et al. (2014) repoted *Ludwigia octovalvis* (Jacq.), *Triantheme portulacastrom* L., *Ipomea sp. Boerhavia erecta* L., *Argemone Mexicana* L., *Macroptilium lathyroides* (L.) to be host species of CPMMV. Therefore, many viruses that infect crops, particulary common bean, have more than one host for their life cycles.

In the pooled RNA samples, YBMV and PeMoV were traced to know the specific wild plants which hosted them. YBMV was detected in the legume plant known as *S. hirsuta* while PeMoV was detected in *S. occidentalis*. Therefore, this is not the first time viruses are detected in weeds and wild plants in Tanzania. However, this work represents the first comprehensive surveys of viruses in wild plants in Tanzania. Also, it is the first time a robust and highly sensitive technique, NGS (targeting small RNAs) was used to detect viruses in wild plants collected from Tanzania.

PCR confirmation of viruses detected by NGS did not succeed for BCMV, BCMNV and CPMMV but was successful for PeMoV and YBMV. PeMoV was detected in *S. occidentalis*, a wild legume sample collected from Arumeru district in the northern zone. The PeMoV found in this study was closely related to PeMoV isolate from groundnut in Brazil. Failure to detect BCMV and BCMNV shows that these viruses could have occurred at low titres in wild plants such that only sensitive techniques such as NGS could detect them. Alternatively, and as argued already, the contigs matching these viruses could be from YBMV, a virus closely related to BCMV and BCMNV.

YBMV was detected in *S. hirsuta*, a plant species that is widely spread around common bean fields in Morogoro in the eastern zone. Thus, attempts were made to transmit YBMV from *S. hirsuta* to different genotypes of common bean. Despite repeated attempts to transmit it, inoculated common bean plants never developed any viral disease symptoms. Detection of YBMV using RT-PCR did not yield any positive results for any sample from inoculated common bean plants suggesting the virus can not be transmitted to common bean plants by mechanical means or was just unstable in the phosphate buffer used. There is limited information on transmission of YBMV. But according to Yarwood (1957), some plant viruses are not mechanically transmitted to secondary hosts. For example, the viruses causing aster yellows, potato leaf roll, phony peach, tristeza, and many others have rarely or never been transmitted mechanically, though insect transmission methods are successful (Yarwood, 1957). Therefore, future studies may focus on vector transmission of YBMV from *S. hirsuta* to common bean. *S. hirsuta* plants observed around common bean fields were symptomatic. However, YBMV was not detected in common bean plants in eastern zone but was detected in common bean samples collected from northern zone.

The *B. bituminosa* (L.) Kuntze, which was found to host a bromovirus related to CCMV is the leguminous plant found in the family *Fabaceae* – the family to which common bean belongs. *B. bituminosa* (L.) Kuntze was wide spread in Mvomero district. There was no published accounts of *B. bituminosa* (L.) Kuntze being a host of bromovirus closely related to CCMV. However, CCMV was previously detected in *P. vulgaris* in Illinois, United States of America (Bancroft, 1971). CCMV can be transmitted mechanically and by beetles (*Coleoptera*) to many plants; for example *Nicotiana clevelandii* (Mello *et al*., 2010). It infects plants in over 27 cultivated and

uncultivated plant species, but some plant species have been reported to be resistant to CCMV (Lane, 1981).

Another virus that was transmitted to common bean plants was CMV. The CMV, a virus that infects over 1 300 plant species (Azizi and Shams-bakhsh *et al*., 2014), was detected and transmitted from *O. basillicum* L., a non-leguminous wild plant belonging to the family *Lamiaceae*. During surveys conducted from 2016 to 2018, this weed was found widely spread in Tanzania, especially in eastern and northern zones. This wild plant species resembles other *Ocimum* species. *Ocimum* spp. are known to be hosts of many viruses (Wintermantel and Natwick, 2012; Davino *et al*., 2009; Sanz *et al*., 2001). The CMV which was detected in *O. basillicum* L. in this study, was previosly known to infect *Ocimum sanctum* (Khan *et al*., 2011). CMV was reported for the first time in *Ocimum sanctum* from Italy (Marini, 1955). Other viruses which have been reported in *Ocimum spp* are AMV (Feldman and Garcia, 1970), *Tomato spotted wilt virus* (TSWV) (Holcomb *et al*., 1999), *Broad bean wilt virus* (BBWV) (Sanz *et al*., 2001) and *Pepino mosaic virus* (PepMV) (Davino *et al*. 2009). But *Tomato spotted wilt tospovirus* has been reported in *O. basilicum* L. (Holcomb *et al*., 1999), it caused leaf distortion and severe mosaic disease symptoms.

In this study, it was revealed that 122 viruses belonging to 20 genera infect wild plants growing in the vicinity of common bean fields in four agricultural research zones in Tanzania. Of these, 23 viruses were related to viruses known to infect common bean plants. In chapter two and Mwaipopo *et al*. (2018), it was shown that common bean plants in Tanzania are infected by 15 viruses belonging to 11 genera. Therefore, considering information presented in chapter two, more viruses were detected in wild

plants than in common bean, which indicates that there are barriers in the transmission of viruses from wild hosts to common bean plants. Evidently, mechanical transmission of viruses from wild to common bean plants was inefficient except for two viruses, CMV and CCMV related bromovirus. However, it could be that some of the viruses found in wild plants are infecting cultivated crops other than common bean. An example is SPFMV virus, detected in sample JHD-1, which is known to infect *Ipomea batatas* (Sivparsad and Gubba, 2013). Therefore, the information generated in this work is not only useful in management of common bean virus diseases in common bean but it will also be useful in developing strategies for management of virus diseases of other crops.

Viruses are widely scattered in many plants including both cultivated and non-cultivated plants (Van Etten and Meints, 1999). Plant viruses co-evolve with wild plants and the human activities facilitate the interaction between viruses and plants. World trade and other human activities, especially agriculture, facilitate the interaction between viruses and plants worldwide (Cooper and Jones, 2006). The identification of wild plants/weeds, which host viruses of different crops, especially of common beans fields is very important. Knowledge of alternative hosts of viruses is important in development of virus disease management strategies.

In the past, no attempts were made to identify and characterize alternative hosts of common bean viruses using DNA barcoding technique in Tanzania. In this study, all plants from which RNA was extracted for NGS were subjected to DNA barcoding. Out of 134 wild plant species, DNA amplification and sequencing were successfull on 89 samples. This was despite use of two different primer pairs. Previous studies have

shown that DNA barcoding using maturase *K* gene primers fail for some plant species (Bafeel *et al*., 2011). The efficacy of *matK* gene regions in discriminating species has been reported to deliver the highest species resolution of 45 - 80% (Braukmann *et al*., 2017) and 69% (Bafeel *et al*., 2011). Therefore, the success rate (66.4%) was comparable to that observed in previous studies (Braukmann *et al*., 2017; Bafeel *et al*., 2011). Low amplification rate indicates that while DNA barcoding is useful for identification of plant species, there is still a need to use taxonomic keys in identifying some plants when PCR amplification can not be achieved. Fortunately, in this study, wild plants used in mechanical transmission were amplified and identified.

4.6 Conclusions and Recommendations

4.6.1 Conclusions

This study has generated useful information on the presence of viruses that infect common bean and other crops in Tanzania. Although most of the viruses were not mechanically transmitted to common bean plants, wild plants species *B. bituminosa* (L.) Kuntze and *O. basillicum* L. were identified as potential alternative hosts of viruses that infect common beans in Tanzania. Management of virus diseases of common bean in Tanzania should involve avoiding these plants around common bean fields. A few plants that may not be accepted around common bean fields, based on results of this study, are *B. bituminosa* (L.) Kuntze, *O. basillicum* L., *S. hirsuta* and *S. occidentalis*. Generally, from results of this and past studies, *Senna spp.* seem to harbour viruses that can infect common bean plants. There are many viruses in wild plant growing around common bean fields. However, it seems only few viruses are able to infect common bean plants.

4.6.2 Recommendations

i. Although no vector mediated transmission of plant viruses was studied, viruses were however mechanically transmitted from some wild plant species to common bean plants suggesting that the wild plants are alternative hosts of common bean viruses in Tanzania. Therefore, management of common bean viral diseases should take into account of these alternative hosts.

ii. Vector transmission studies should be conducted to see whether the viruses found in wild plants can be transmitted to common beans and from common bean to wild plants naturally by vectors.

iii. Mechanical transmission was done using fresh wild plant samples from Morogoro region only. Future studies should include wild plants from all Agricultural Research zones.

iv. There are questions which remain unanswered: 1) Are CMV and CCMV (or a related bromovirus) naturally infecting common bean in Tanzania? 2) failed of some viruses to be mechanically transmited on common beans from wild plant has to do with buffer conditions, low virus titer or prsence of inhibitors? 3) are the viruses detected in this study genetically similar to ICTV recognized viruses or are just novel viruses? These questions can be answered through focused studies and because of the scope of this study, these questions were not addressed in the present comprehesive study.

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Appendices

Appendix 4.1: List of viruses detected using next generation sequencing in wild plant samples collected from four agricultural research zones

JDH1	JDH2	JDH3	JDH4	JDH5	JDH6	HXH-16	AIVN-1	AIVN-2	AIVN-3
Bean common mosaic virus, Bhendi yellow vein India virus, Butterbur mosaic virus, Butterbur mosaic virus, Butterbur mosaic virus, Chilli veinal mottle virus, Chrysanthemum virus B, Cotton leaf curl Gezira virus, Cowpea mild mottle virus, Cucumber vein-clearing virus, Datur East African cassava mosaic Cameroon virus a leaf distortion virus, Euphorbia leaf curl Guangxi virus, Fig mosaic virus, Hedyotis uncinella yellow mosaic virus, Helleborus net necrosis virus, Hippeastrum latent virus, Ipomoea yellow vein virus, Lettuce yellows virus, Luffa aphid-borne yellows virus, Malvastrum leaf curl Guangdong virus, Mungbean yellow mosaic virus, Okra leaf curl Mali virus, Pepper veinal mottle virus, Pigeonpea sterility mosaic virus, Potato virus M, Rubus chlorotic mottle virus, Shallot latent virus, Sinaloa tomato leaf curl virus, Sowbane mosaic virus, Soybean yellow common mosaic virus, Sweet potato feathery mottle virus, Sweet potato leaf curl Georgia virus, Sweet potato virus C, Tobacco leaf curl virus, Tobacco leaf curl virus, Tobacco vein-clearing virus, Tomato leaf curl Arusha virus, Tomato leaf curl Sudan virus,	Apple stem pitting virus, Bean common mosaic virus, Bean yellow disorder virus, Carrot mottle mimic virus, Carrot mottle virus, Chickpea chlorotic stunt virus, Chilli Leaf curl Vellanad virus, Clerodendron leaf curl virus, Cowpea mild mottle virus, Cowpea mottle virus, Cucumber vein-clearing virus, Cucurbit chlorotic yellows virus, Elderberry virus A, Ethiopian tobacco bushy top virus, Eupatorium yellow vein virus,Groundnut rosette assistor virus, Groundnut rosette virus, Jatropha mosaic Nigerian virus, Opium poppy mosaic virus, Pea enation mosaic virus, Peanut mottle virus, Soybean chlorotic blotch virus, Soybean yellow mottle mosaic	Ethiopian tobacco bushy top virus	Ethiopian tobacco bushy top virus	American hop latent virus, Apple stem pitting virus, Basella rugose mosaic virus, Bean common mosaic virus, Beet mosaic virus, Cowpea aphid-borne mosaic virus, Cowpea aphid-borne mosaic virus, Cowpea mild mottle virus, Cowpea mosaic virus, Crotalaria mosaic virus, Cucumber vein-clearing virus, Ethiopian tobacco bushy top virus, Fig mosaic virus, Freesia mosaic virus, Hippeastrum latent virus, Hollyhock leaf crumple virus, Kalanchoe latent virus, Keunjorong mosaic virus, Nerine latent virus, Ornamental onion stripe mosaic virus, Ornithogalum mosaic virus, Passiflora latent carlavirus, Passion fruit woodiness virus, Peanut mottle virus, Peanut stripe virus, Pigeonpea sterility mosaic virus, Potato virus S, Rhynchosai mild mosaic virus, Sarcochilus virus, Sida yellow mosaic virus, Sweet potato mild speckling virus, Sweet	Bean common mosaic virus, Bean leaf curl Madagascar virus, Bhendi yellow vein mosaic virus, Cotton leaf curl Gezira virus, Cowpea mild mottle virus, Cucumber mosaic virus, Ethiopian tobacco bushy top virus, Tomato leaf curl Arusha virus, Tomato leaf curl Diana virus, Tomato leaf curl Uganda virus	Angelica virus Y, Banana bract mosaic virus, Basella rugose mosaic virus, Bean common mosaic virus, Beet mosaic virus, Blue squill virus A, Clerodendron leaf curl virus, Cowpea aphid-borne mosaic virus, Dasheen mosaic virus, East Asian Passiflora virus, Ethiopian tobacco bushy top virus, Freesia mosaic virus, Gossypium punctatum mild leaf curl virus, Keunjorong mosaic virus, Okra yellow crinkle virus, Peanut mottle virus, Soybean chlorotic blotch virus, Spinach yellow vein Sikar virus, Sweet potato mild speckling virus, Tomato leaf curl Laos virus, Tomato leaf curl New Delhi virus, Tomato mosaic virus, Tomato yellow leaf curl China virus, Watermelon mosaic virus, Wisteria vein mosaic virus, Zucchini yellow mosaic virus	Beet mild yellowing virus, Blueberry red ringspot virus, Brome mosaic virus, Carrot torrado virus, Clerodendrum golden mosaic Jiangsu virus, Cowpea chlorotic mottle virus, Cucumber mosaic virus, Jatropha mosaic virus, Lagenaria siceraria endornavirus, Luffa aphid-borne yellows virus, Melandrium yellow fleck virus, Melandrium yellow fleck virus, Okra yellow crinkle virus, Persea americana endornavirus, Phaseolus vulgaris endornavirus 1, Soybean chlorotic blotch virus, Spring beauty latent virus, Squash leaf curl China virus, Tomato leaf curl New Delhi virus, Tomato necrotic dwarf virus, Tomato torrado virus, Tomato yellow leaf curl Kanchanaburi virus, Tomato yellow leaf curl Sardinia virus, Turnip yellows virus, Yerba mate endornavirus	Cucumber mosaic virus	Cucumber mosaic virus, Phaseolus vulgaris endornavirus 1, Southern bean mosaic virus, Spring beauty latent virus, Melandrium yellow fleck virus, Cowpea chlorotic mottle virus

JDH1	JDH2	JDH3	JDH4	JDH5	JDH6	HXH-16	AIVN-1	AIVN-2	AIVN-3
Tomato leaf curl Toliara virus, Yam bean mosaic virus	virus, Tobacco bushy top virus, Tomato chocolate virus, Tomato leaf curl Sudan virus, Tomato leaf curl Uganda virus, Tomato marchitez virus, Tomato yellow leaf curl Thailand virus, Yam bean mosaic virus			potato virus, Tomato leaf curl Arusha virus, Tomato leaf curl Madagascar virus, Tomato leaf curl Mayotte virus, Tomato leaf curl Namakely virus, Tomato leaf curl Uganda virus, Tomato yellow leaf curl virus, Ugandan Passiflora virus, Watermelon mosaic virus, Wild tomato mosaic virus, Yam bean mosaic virus, Zucchini yellow mosaic virus					

CHAPTER FIVE

5.0 DETERMINING SUITABLE SIZES OF READS FROM DEEP SEQUENCED SMALL RNA DATA FOR VIRUSDETECT SOFTWARE-BASED DETECTION OF COMMON BEAN (*Phaseolus vulgaris* L.) VIRUSES USING LOW CAPABILITY COMPUTERS

Beatrice Mwaipopo[1, 3*], Susan Nchimbi-Msolla[1], Paul Njau[1], and Deusdedith Mbanzibwa[2]

[1]Department of Crop Science and Horticulture, Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Tanzania.

[2]Tanzania Agricultural Research Institute Mikocheni, P. O. Box 6226, Dar es Salaam, Tanzania.

[3]Tanzania Agricultural Research Institute Ilonga, P.O Box 33, Morogoro, Tanzania.

Corresponding author email: beatricemwaipopo@yahoo.com*

Abstract

Analysis of small RNAs based next generation sequencing data for detection of plant viruses is commonly based on fastq files of combined reads of sizes 21 – 24 nt. However, these files are in most cases heavy (bytes) and cannot be analysed offline using small capability computers. Thus, high capability supercomputers are needed to analyse small RNA data. This is a challenge in developing countries, including Tanzania. In this study, using VirusDetect software developed recently for detection of plant viruses in deep sequenced small RNAs data, it was shown that viruses such as *Southern bean mosaic virus* (SBMV; *Sobemovirus*) and a virus closely related to *Cowpea chlorotic mottle virus* (CCMV; *Bromovirus*) could be detected using reads of

sizes > 24 nt but < 35 nt. It was also found out that, viral sequences of all viruses are represented in inserts of size 20 nt. The fastq files containing reads of sizes < 21 nt and > 24 nt are normally smaller in size (bytes) compared to the fastq files containing reads of sizes 21 – 24 nt owing to the RNA interference mediated mechanism of plant defence against plant viruses. The results of this work also indicated that double stranded RNAs of some viruses are cleaved into small RNAs in a range outside 21 – 24 nt. The findings that files containing reads of sizes other than 21 - 24 nt are important because the offline analysis of small RNAs - based NGS data (big files of 21 - 24nt) normally terminate abruptly when computers run out of memories. Since potyviruses, the most important common bean viruses are not represented in reads of sizes > 24 nt, it is recommended that reads of size 20 nt be used for rapid identification of viruses when low capability computers are used but reads of sizes 21 – 24 nt remain the best for obtaining long viral contigs and confirming viral infections in common bean samples.

Keywords: Contig, Next Generation Sequencing, Nucleotide, Small RNA, Viruses, Wild plant

5.1 Introduction

Viruses are important organisms that cause infections in both plants and animals. The plant infecting viruses have been reported by Wang *et al*. (2012). Through co-evolution, plants have developed the defence mechanisms against pathogens including viruses; the mechanisms are the RNA silencing, hormonal mediated defence, immune receptor signalling protein degradation and regulation of metabolism (Calil and Fontes, 2016).

The RNA silencing plays a big role in virus defence and regulation of gene expression as induced by the siRNA (small interfering RNA) (Pumplin and Voinnet, 2013). Plants have evolved three basic RNA silencing pathways, which are represented by the miRNA (Micro RNA) pathway, the siRNA-directed RNA degradation pathway, and the siRNA-directed DNA methylation (RdDM) pathway (Wang and Smith, 2016). Small RNA is the non-coding RNAs that regulate gene expression. It involves the degradation of messenger RNA that leads to loss of gene activity. The mechanism target both host and viral genomes as long as the RNA is in double stranded (ds) structure. The dsRNA act as substrate for Dicer like (DCL) structure to produce two types of small RNA which are microRNA and siRNA, which are cleaved into 21 - 25 nt (Liu *et al*., 2017; Pantaleo *et al.*, 2007; Mlotshwa *et al.*, 2008). One strand of the small dsRNA is loaded into Argonaute protein (Ago) complexes and combined with other proteins to form the RNA-induced silencing complex (RISC). The guide siRNA within RISC forms base pairs with viral RNA to elicit RNA silencing via RNA degradation (Li *et al*., 2016; Vaucheret, 2008).

The generated small RNAs can be targeted for detection of plant viruses (Kreuze *et al*., 2009; Mbanzibwa *et al*., 2014). Detection of plant viruses using such software such as VirusDetect has been based on viral genome derived sRNAs of the sizes 21, 22, and 24 nt. Normally, these small RNAs are combined and analysed as one fastq file (Kreuze *et al*., 2009). However, analysis of small RNAs of sizes other than 21-24 nt has shown that, there are viruses that can be detected in insert of 25 nt and above. For instance, Mwaipopo *et al.* (2018) reported detection of *Southern bean mosaic virus* (SBMV) in inserts sizes 21 - 34 nt. It was speculated that there could be a

different mechanism involved in defending against some viruses including SBMV (Mwaipopo *et al*., 2018). It could as well be that the reads are from degraded RNAs. Moreover, NGS data files of sizes 21 to 24 nt are in most cases heavy (bytes) and cannot be analysed offline using small capability laptops – e.g., those with random access memory of 8 GB. Thus, high capability laptops or supercomputers are needed to analyse small RNA- based deep sequencing data. Thus, the present study aimed at investigating if viruses other than SBMV can be detected in insert sizes other than 21-24 nt.

5.2 Materials and Methods

5.2.1 RNA extraction

The extraction of RNA from common bean samples known to contain SBMV, Bromoviruses and potyviruses was described in chapter two.

5.2.2 Sequencing of small RNAs

For the purposes of investigating detection of viruses in insert sizes other than 21 - 24 nt, samples HXH-1 and AIVN-3 were selected. Sample HXH-1 contained viruses BCMNV, PeMoV, SBMV, PvEV-1 and PvEV-2 (Mwaipopo *et al*., 2018; Chapter two). On the other hand, sample AIVN-3, contained viruses PvEV-1, an unidentified bromovirus closely related to *Cowpea chlorotic mottle virus* (CCMV) (Chapter four) and SBMV (Chapter two; chapter four). HXH-1 and AIVN-3 samples were prepared and sequenced as described in chapter two and chapter four, respectively. Deep sequencing of small RNAs was also as described in Chapters two and Chapter three.

5.3 Data Analysis

Analysis of NGS data was done using the VirusDetect program (v.1.6). This analysis was done offline using a laptop computer with random access memory of 8 GB and installed with virtual Linux machine. The analysis was done for individual 11 to 36 nt insert fastq files. The files received from Fasteris SA (with extension '.tar'; submitted at Zenodo and assigned DOI 10.5281/zenodo.841170) were unzipped using the command 'tar -xzvf filename'. Then, all reads of sizes not within 11 to 36 nt were deleted. Detection of viruses in inserts of each size was done separately. *De novo* assembly of reads was done using the command 'perl virus_detect.pl filename' (Mwaipopo *et al*., 2018).

5.4 Results

5.4.1 Virus detection limit in sample HXH-1

A summary of reads, contigs and viruses detected in sample HXH-1 is presented in Table 5.1 and Appendix 5.1. Highest number of reads per insert size was observed at 21 to 23 nt reads size. A total of five viruses were expected in sample HXH-1 (Mwaipopo *et al*., 2018). Indeed, five viruses, namely BCMNV, PeMoV, SBMV, PvEV-1 and PvEV-2 were detected in this sample (Appendix 5.1). The small RNA reads evaluated were from 15-36 nt. SBMV was detected in reads of all sizes (15 – 35 nt). BCMV was detected in reads of size 16 to 23 nt but not reads of size 24 and above. PeMoV was not detected in reads of < 20 nt but was detected in reads 20 - 22 nt. The remaining two viruses, PvEV-1 and PvEV-2 were detected in reads of sizes 21 – 22 nt. Thus, it was observed that the number of viruses detected in sample HXH-1 increased with increased size of reads from 16 to 22 nt and reduced for reads of larger

sizes. A total of five viruses were observed at 21-22 nt reads size and this was the highest number of viruses observed in sample HXH-1. Only one virus was detected by blastx at 20 nt reads size (Table 5.1).

Table 5.1: Number of reads and viruses detected by NGS in sample HXH-1 at 15 - 36 nt small RNA

		Aligned reads			De *novo* assembly	After removal of redundancies	Number of viruses obtained	
Sample	**Small RNA (nt)**	**Total reads**	**Reads aligned**	**Unique contigs**	**Unique contigs**	**Unique contigs**	**Blastn**	**Blastx**
HXH-1	15	167 212	143 578	25	47	68	1	0
	16	628 785	324 997	102	112	207	2	0
	17	633 855	112 892	124	147	254	2	0
	18	680 719	36 726	116	155	243	2	0
	19	679 333	23 063	160	113	267	2	0
	20	870 547	25 458	194	115	289	2	1
	21	1 940 055	195 863	210	190	334	5	0
	22	1 146 000	150 450	239	381	465	5	0
	23	918 223	10 146	121	200	271	2	0
	24	1 865 070	14 510	81	789	800	1	0
	25	560 258	2 566	49	67	113	1	0
	26	556 345	2 065	57	79	133	1	0
	27	551 251	2 011	59	93	141	1	0
	28	686 506	1 735	54	117	158	1	0
	29	872 925	1 651	53	73	119	1	0
	30	855 608	1 612	61	52	110	1	0
	31	757 030	1 548	56	50	104	1	0
	32	2 120 756	1 483	58	80	129	1	0
	33	1 298 090	936	23	78	94	1	0
	34	181 657	298	27	54	77	1	0
	35	66 965	111	15	65	80	1	0
	36	23 889	48	0	0	0	0	0

5.4.2 Virus detection limit in sample AIVN-3

The viruses which were expected in sample AIVN-3 were CMV (*Cucumovirus*), SBMV (*Sobemovirus*), CCMV (*Bromovirus*), and PvEV-1 (*Alphaendornavirus*) (Chapter four). These viruses were detected as expected (Table 5.2; Appendix 5.2). SBMV was detected in all read sizes (15 -33 nt). A bromovirus closely related to CCMV was found in read of sizes 17 to 33 nt. CMV was only assembled in reads of sizes 20 to 22 nt. PvEV-1 was found reads of sizes 19 to 26 nt. All viruses in four genera were detected in reads of sizes 20 – 22 nt (Table 5.2; Appendix 5.2).

Table 5.2: Number of reads and viruses detected by NGS in sample AINV- 3 at 12 - 34 nt small RNA

		Aligned reads			De *novo* assembly	After removal of redundancies	Number of viruses obtained	
Sample	**Small RNA (nt)**	**Total reads**	**Reads aligned**	**Unique contigs**	**Unique contigs**	**Unique contigs**	**Blastn**	**Blastx**
AIVN-3	15	111 046	90 865	14	4	18	1	0
	16	206 484	141 277	32	25	53	1	0
	17	313 731	79 125	24	92	100	1	1
	18	818 544	70 210	48	181	184	1	4
	19	1 487 947	125 954	77	224	243	2	9
	20	4 099 433	224 643	111	231	309	3	5
	21	19 540 190	926 924	90	-	-	4	5
	22	7 014 439	1 496 423	62	362	296	4	7
	23	3 170 363	276 357	130	386	483	2	7
	24	1 050 6776	952 842	108	-	-	-	-
	25	1 000 477	46 100	59	418	429	2	6
	26	742 703	14 621	51	144	169	2	3
	27	971 822	10 447	56	160	189	2	6
	28	1 088 467	8 257	51	252	267	1	7
	29	1 027 596	7 777	51	242	258	2	7
	30	7 73 941	6 059	38	116	130	2	6
	31	317 487	2 947	20	212	189	1	5
	32	273 836	969	34	130	145	1	1
	33	270 271	4 442	16	66	69	1	0
	34	21 298	97	0	40	37	0	0

- no value, the analysis of 21 nt and 24 nt failed to be completed.

5.5 Discussion

In this study viruses of common bean (HXH-1) and wild plants (AIVN-3) were identified from Tanzania, through this work, it was rational to also optimize data analysis. Analysis of NGS data can be challenging as it requires the use of high capability computers, which are few in Africa. Fortunately, the offline software, VirusDetect was recently developed which is very important for scientist who do not have access to supercomputers (Zheng *et al*., 2017). *De novo* assembly for detection of plant viruses is normally done on reads of sizes 21 to 24 nt contained in one fastq file. In the present study, combined and separate read sizes (21 to 24 nt) were analysed. The viruses detected following analysis of combined inserts or separate reads of sizes 21 to 24 nt were the same (especially for sizes 21 and 22 nt) but the contigs obtained differed in size. Interestingly, when the contigs overlapped they were identical to each other. In plant defence, viral genomes are normally cleaved into small RNAs of sizes 21, 22 and 24 nt (Baulcombe, 2004; Mlotshwa *et al*., 2008).

As it was introduced earlier, most African countries have less access to supercomputers for data analysis. The remaining solution is the use of offline analysis using the VirusDetect tool. Due to that, this study aimed at establishing detection limit of viruses in reads of different sizes. This followed an observation that contigs of SBMV were obtained in read sizes of up to 35 nt (Mwaipopo *et al*., 2018). The results have shown that sequences of most viruses are obtained in reads of sizes 15 to 24 nt. All potyviruses were detectable in this range of insert sizes. SBMV was assembled into contigs when inserts sizes in the range of 15 to 35 (HXH-1) and 15 to 33 nt (AIVN-3) were used. A novel bromovirus closely related CCMV was detected in the

range 17 to 33 nt in sample AIVN-3. These results therefore showed that reads mapping to different viruses have size limits, which are virus specific. As expected, all viruses were found in 21 and 22 nt size reads. This agrees with data obtained in previous studies which showed DCL endonucleases cleave double stranded RNAs into nucleotides of sizes 21 - 25 (Pantaleo *et al*. 2007; Zhu and Guo, 2012).

Using these two samples and SBMV as a control virus – because it was in both samples – it was shown that the cleavage of the genome of this virus and that of a bromovirus may be leading to generation of reads of sizes 15 to 35 nt. The detection of PvEV-1 at 26 nt is not surprising as close to the size (21 - 25 nt) of small RNAs generated in plants following RNA interference mediated resistance. However, degradation of RNA could not be discounted as a cause of the reads with sizes out of the normal range (21 - 25 nt). But the difficult question to answer was why small RNAs of 25 nt and above were not observed for some viruses like BCMNV, PeMov, and CMV. Moreover, CMV, a bromovirus related to CCMV and SBMV are highly mechanically transmitted to common bean samples suggesting they are relatively stable viruses. A possible explanation for occurrence of small RNAs > 24 nt could be due to a different mechanism e.g., a different endonuclease involved in plant defence.

It was shown in this study that sRNAs of greater than 24 nt are found in infected common bean plants but only for some viruses, SBMV and bromoviruses.

The aim of this study was to establish if small (in term of bytes) fastq files of sequence reads, which are normally of small RNA sizes < 21 nt and > 24 nt could be used to detect plant viruses in common bean. Since only a few viruses can be detected

in sequence reads of size > 24 nt, many viruses (including BCMNV) would be missed if fastq files of reads of sizes > 24 nt were used. However, in this work, all viruses detected in the small RNAs of sizes 21 – 24 nt were also detected in a fastq file of reads of size 20 nt. In most cases, fastq files containing reads of size 20 nt are small (in terms of bytes) and can be analysed offline using virtual machines installed in laptops with random access memory of at least 8 GB. This would eliminate the need of supercomputers which may not be available at the time results are needed urgently, for example, when dealing with outbreaks.

5.6 Conclusions and recommendations

5.6.1 Conclusions

This work has demonstrated that only a few viruses are represented in sequences reads of sizes >24 nt. In this work, all viruses detected in the small RNAs of sizes 21 – 24 nt were also detected in a fastq file of reads of size 20 nt. Most of viruses vary, some were found at less than 20 nt while others will never found at that range. This approach can be used to detect and enforce quarantine measures at the country entry points.

5.6.2 Recommendations

The sequence reads of 20 nt, which are smaller in size (bytes) compared to files of reads 21 - 24 nt, can be used in rapid identification of viruses in a common bean sample.

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Appendices

Appendix 5. 2: Number of viruses detected by NGS in sample HXH-1 from 15 - 35 nt reads

Sample	Small RNA (nt)	Reference sequence	Coverage (%)	Number of contig		Contig length	Depth	%Identity	Genus	Virus identified
				blastx	blastn					
HXH-1	15	DQ875594	30	-	21	42-114	24.3	99.11	*Sobemovirus*	*Southern bean mosaic virus*
	16	AY864314	36.2	-	61	41-129	6.1	99.03	*Potyvirus*	*Bean common mosaic necrosis virus*
		DQ875594	81.5	-	31	43-255	9.9	98.75	*Sobemovirus*	*Southern bean mosaic virus*
	17	AY864314	70.5	-	83	41-215	6.0	98.84	*Potyvirus*	*Bean common mosaic necrosis virus*
		DQ875594	91.6	-	21	41-303	12.9	98.71	*Sobemovirus*	*Southern bean mosaic virus*
	18	AY864314	79	-	64	41-352	6.9	98.77	*Potyvirus*	*Bean common mosaic necrosis virus*
		DQ875594	91.9	-	17	44-659	12.8	98.6	*Sobemovirus*	*Southern bean mosaic virus*
	19	AY864314	85.5	-	49	42-309	8.9	98.79	*Potyvirus*	*Bean common mosaic necrosis virus*
		DQ875594	92.5	-	16	42-843	14.6	98.63	*Sobemovirus*	*Southern bean mosaic virus*
	20	AY864314	94.1	-	14	44-2775	19.2	98.72	*Potyvirus*	*Bean common mosaic necrosis virus*
		DQ875594	95.5	-	10	89-1232	61	98.58	*Sobemovirus*	*Southern bean mosaic virus*
		AAB94595.1	14.3	8	-	113-252	4.7	98.81	*Potyvirus*	*Peanut mottle virus*
	21	AB719398	90.7	-	73	43-663	13.6	96.15	*Endornavirus*	*Phaseolus vulgaris endornavirus 2*
		KT456287	80.1	-	101	41-482	7.9	95.54	*Endornavirus*	*Phaseolus vulgaris endornavirus 1*
		AY864314	95.1	-	1	9634	349.4	98.59	*Potyvirus*	*Bean common mosaic necrosis virus*
		AF023848	96.8	-	25	41-1808	26.8	97.02	*Potyvirus*	*Peanut mottle virus*
		DQ875594	98.3	-	7	41-1923	69.9	96	*Sobemovirus*	*Southern bean mosaic virus*

Appendix 5. 3: Number of viruses detected by NGS in sample AIVN-3 from 15 – 33 nt reads

Sample	Small RNA (nt)	Reference sequence	Coverage (%)	Number of contig		Contig length	Depth	%Identity	Genus	Virus identified
				blastx	blastn					
AIVN-3	15	DQ875594	23.5	-	14	51-85	7.3	99.07	*Sobemovirus*	*Southern bean mosaic virus*
	16	DQ875594	56.4	-	32	41-3.3	8.9	98.71	*Sobemovirus*	*Southern bean mosaic virus*
	17	DQ875594	82.5	-	24	45-343	25.5	98.83	*Sobemovirus*	*Southern bean mosaic virus*
		AAA46370.1	19.5	2	-	56-61	167.6	81.01	*Bromovirus*	*Cowpea chlorotic mottle virus*
	18	DQ875594	95.9	-	14	41-657	88.4	98.81	*Sobemovirus*	*Southern bean mosaic virus*
		AAA46370.1	41.1	3	-	52-131	228	74.36	*Bromovirus*	*Cowpea chlorotic mottle virus*
		BAI40163.1	24.7	2	-	68-155	137.4	60.27	*Bromovirus*	*Melandrium yellow fleck virus*
		BAC10646.1	21.7	6	-	51-126	48.5	73.33	*Bromovirus*	*Spring beauty latent virus*
	19	KT456287	27.9	-	64	41-158	5.3	98.55	*Endornavirus*	*Phaseolus vulgaris endornavirus 1*
		DQ875594	96.8	-	10	51-1616	159.3	98.69	*Sobemovirus*	*Southern bean mosaic virus*
		BAJ41520.1	22.1	7	-	51-277	174.9	79.63	*Bromovirus*	*Brome mosaic virus*
		BAI49161.1	24.9	7	-	62-277	163	80.93	*Bromovirus*	*Melandrium yellow fleck virus*
		AEI54608.1	29.7	11	-	63-277	180.7	81.43	*Bromovirus*	*Cowpea chlorotic mottle virus*
		BAC10645.1	35.7	12	-	51-227	172.3	78.95	*Bromovirus*	*Spring beauty latent virus*
	20	DQ302717	11.7	-	2	43-49	7.4	95.68	*Cucumovirus*	*Cucumber mosaic virus*
		KT456287	49.8	-	98	44-150	8.4	95.35	*Endornavirus*	*Phaseolus vulgaris endornavirus 1*
		DQ875594	98	-	9	58-1559	383.1	92.16	*Sobemovirus*	*Southern bean mosaic virus*
		BAC10645.1	49.4	15	-	47-205	545.9	*73.58*	*Bromovirus*	*Spring beauty latent virus*
		BAI40163.1	46.1	3	-	74-223	1856.7	*63.97*	*Bromovirus*	*Melandrium yellow fleck virus*
		AAA46370.1	60	1	-	70-129	2501	*78.95*	*Bromovirus*	*Cowpea chlorotic mottle virus*
	21	J02052	11.7	-	1	97	6164	*87.76*	*Bromovirus*	*Cowpea chlorotic mottle virus*
		HM015286	56.5	-	6	81-122	23.1	99.25	*Cucumovirus*	*Cucumber mosaic virus*
		KT456287	94.3	-	64	77-803	49.7	98.65	*Endornavirus*	*Phaseolus vulgaris endornavirus 1*
		DQ875594	99.1	-	2	101-4030	2387.3	98.4	*Sobemovirus*	*Southern bean mosaic virus*
		BAC10645.1	73.7	10	-	70-889	3542.8	74.48	*Bromovirus*	*Spring beauty latent virus*
		BAJ41521.1	44.4	5	-	59-688	3270.5	56.22	*Bromovirus*	*Brome mosaic virus*
		BAI40161.1	13.1	3	-	93-402	4474.1	78.23	*Bromovirus*	*Melandrium yellow fleck virus*
		AAA42740.1	12.1	3	-	81-138	3147.4	70.59	*Bromovirus*	*Broad bean mottle virus*
		AAA46370.1	56.3	1	-	70-889	6257.7	78.5	*Bromovirus*	*Cowpea chlorotic mottle virus*
	22	JO2052	13.6	-	1	126	4362.2	87.72	*Bromovirus*	*Cowpea chlorotic mottle virus*
		AJ237850	23.2	-	7	42-116	10.7	96.89	*Cucumovirus*	*Cucumber mosaic virus*
		KT456287	98.3	-	46	52-1067	92.1	98.50	*Endornavirus*	*Phaseolus vulgaris endornavirus 1*
		DQ875594	99.1	-	2	105-4109	8318.7	98.48	*Sobemovirus*	*Southern bean mosaic virus*
		BAC10645.1	67	16	-	79-442	2195.4	81.2	*Bromovirus*	*Spring beauty latent virus*
		AEI54608.1	54.3	16	-	62-283	2266.3	80.48	*Bromovirus*	*Cowpea chlorotic mottle virus*
		BAI40161.1	13.8	3	-	117-206	2559.4	74.81	*Bromovirus*	*Melandrium yellow fleck virus*
	23	KT456287	69.9	-	123	41-266	11.9	98.77	*Endornavirus*	*Phaseolus vulgaris endornavirus 1*
		DQ875594	99	-	4	67-3438	1298.8	98.87	*Sobemovirus*	*Southern bean mosaic virus*
		BAC10645.1	51	15	-	51-220	184.7	78.42	*Bromovirus*	*Spring beauty latent virus*
		AAA46370.1	96.3	2	-	47-532	1070.2	80.75	*Bromovirus*	*Cowpea chlorotic mottle virus*
		BAI40161.1	56.9	2	-	63-188	1175.1	60.36	*Bromovirus*	*Melandrium yellow fleck virus*
	25	KT456287	25.3	-	55	41-132	5.7	98.68	*Endornavirus*	*Phaseolus vulgaris endornavirus 1*

CHAPTER SIX

6.0 DETERMINATION OF GENETIC DIVERSITY OF COMMON BEANS (*Phaseolus vulgaris* L.) CULTIVARS AND LANDRACES USING DIVERSITY ARRAY TECHNOLOGY (DArT) IN THE MAJOR BEAN GROWING AREAS OF TANZANIA

Beatrice Mwaipopo[1, 3*], Susan Nchimbi-Msolla[1], Paul Njau[1], and Deusdedith Mbanzibwa[2]

[1]Department of Crop Science and Horticulture, Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Tanzania.

[2]Tanzania Agricultural Research Institute Mikocheni, P. O. Box 6226, Dar es Salaam, Tanzania.

[3]Tanzania Agricultural Research Institute Ilonga, P.O Box 33, Morogoro, Tanzania.

Corresponding author email: beatricemwaipopo@yahoo.com*

Abstract

Common bean (*Phaseolus vulgaris* L.) is genetically very diverse owing to evolution driven by natural factors and breeding activities. Based on the response to fungal pathogens, uptake of nutrients and morphological traits, it has been suggested that in Tanzania there are many genetically distinct common bean genotypes. Hence, this study was initiated to use molecular markers (DArT) to determine genetic diversity of common bean cultivars and landraces (genotypes) from farmers. A total of 584 of common bean seed samples were collected from farmers in southern highlands, eastern, northern, lake zones and Agricultural Research institutes in Tanzania. The common bean samples were sorted according to their colours and sizes and reduced to

360 genotypes, which were planted in screen house. DNA was extracted using a CTAB method. The 360 samples were packed on MicroAmp®96-Well plates and sequenced using Diversity array technology (DArT) at Canberra University in Australia. Analysis was done using DArTsoft version 7.3 and Numerical Taxonomy and Multivariate Analysis System (NTSYSpc version 2.10t) generated 35 047 markers. The average call rate was 94.1% and reproducibility ranged from 90 to 100%. The polymorphic information content (PIC) ranged from 0.007 to 0.5. Out of 35 047 markers, 558 (1.6%) markers were highly informative, 13 751 (39.2%) markers were of intermediate informative and 20 738 (59.2%) markers were of low genetic diversity. Moreover, out of 35 047 markers, 24 158 (68.9%) were mapped to chromosomes with the remaining mapping to scalfolds and non-chromosomal materials. The genetic diversity dendrogram was developed using 252 common bean samples, the maximum number the software used could analyse. Two clusters corresponding to Mesoamerican and Andean gene pools were identified. A total of 278 and 82 common bean genotypes were grouped in Andean gene pool and Mesoamerican gene pool, respectively. Principal component analysis (PCA) based on genetic similarity supported occurrence of these two groups. The overall variation within the common bean genotypes was 82.2%. When PCA was determined separately for the Andean and Mesoamerican gene pools, the within similarities were 82.94% and 84.60%, respectively. The results indicated occurrence of large gene pool, which will help in planning and implementing breeding programs in Tanzania.

Keywords: Common bean, Diversity array technology, Genotypes, Markers

6.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is a 2n =2x= 22 crop that is grown at 15 to 25 °C mean growing temperatures (Singh and Schwartz, 2010). There are two types of common bean plant habits: the climbing and bush types *(*Gichangi *et al*., 2012).

The crop originated from Central and South America Andean culture, and then from there it spread all over the world. Among pulses, common bean is the largest group that comprises of many species: *P. vulgaris, P. acutifolius, P. dumosus, P. coccineus and P. lunatus* (Delgado-salinas *et al*., 1999). There are two common bean gene pools, namely Mesoamerican and the Andean gene pools (Bitocchi *et al*., 2012). These two gene pools have been distinguished using botanical, archaeological, biochemical traits (Becerra *et al*., 2010), morphological traits (Gepts and Debouck, 1991; Singh *et al*., 1991), and agronomic traits (Singh *et al.*, 1991). They have also been distinguished based on seed proteins (Gepts *et al*., 1986), allozymes (Koenig and Gepts, 1989), and different types of molecular markers (Kwak and Gepts, 2009). The two gene pools have partial reproductive isolation, and thus they have low chances of crossing with each other (Gepts and Bliss, 1985).

There are different molecular markers, which are used in genotyping of common bean. The molecular based methods for plants genotyping include amplified fragment length polymorphism (AFLP) (Andrade *et al*., 2016; Tohme *et al*., 1996), random amplified polymorphic DNA (RAPD) (Bukhari *et al*., 2015; Ravz *et al*., 2013) and simple sequence repeats (SSR) (Gyang *et al*., 2017; Blair *et al*., 2011). The SSR marker is the most frequently used molecular marker in common bean genotyping. Another molecular marker that is used in the genetic diversity studies is the single

nucleotide polymorphisms (SNPs). This technique has been used with great success by different researchers including Cortés *et al*. (2011) and Goretti *et al*. (2014).

The above mentioned markers have limitations including inability to cover whole genome, a need for knowledge of sequence information in the database, and labour intensive and thus time consuming (scoring of gels). To overcome these limitations, diversity array technology (DArT) method was developed (Huttner *et al*., 2005). DArT method is a sequence-based technology that gives a high throughput and enables discovery of a lot of markers in a single run. According to DArT service provider (Diversity Arrays Technology Pty Ltd, University of Canberra, Australia), many methods have been developed to reduce genome complexity for genomic characterization. However, the DArT methods provide a significant advantage via an intelligent selection of genome fraction corresponding predominantly to active genes. According to them (DArT service provider), the use of a combination of restriction enzymes makes it possible to separate low copy sequence from the repetitive fraction of the genome (https://www.diversityarrays.com/ index.php/techno logy- and-resources /dartseq/). Also, the DArTseq has been developed for some crops including common beans (Huttner *et al*., 2005; Brinez *et al*., 2012).

Conventional and marker assisted breeding has been done in order to improve common bean in Tanzania and elsewhere (Kusolwa *et al*., 2016). The aims have been to develop varieties with resistance to pest and diseases as well as drought tolerance (Beaver and Osorno, 2009; Kusolwa *et al*., 2016).

In the next chapter (chapter seven) of this work data is presented on response of common bean to selected common bean viruses. Carrying out experiments on response of selected common bean genotypes to common bean viruses depended on the successful implementation and generation of data on genetic diversity of common bean cultivars in Tanzania. This enabled choosing of the cultivars that were included in the response experiments. The aim of this objective is to determine genetic diversity of common bean cultivars and landraces using diversity array technology (DArT) in Tanzania.

6.2 Materials and Methods

6.2.1 Collection and planting of common bean seed samples

A total number of 584 of common bean seed samples, each weighing 150 g, were collected from farmers in southern highlands zone (Nkasi, Mbeya rural, Mbozi, Iringa rural, Wanging'ombe, Namtumbo and Mbinga), eastern zone (Gairo, Morogoro rural and Mvomero), northern zone (Hai, Siha, Karatu and Arumeru) and lake zone (Missenyi, Karagwe, Bukoba rural, Muleba and Tarime). The common bean seeds were also collected from Sokoine University of Agriculture (SUA) in Morogoro and from Tanzania Agricultural Research Institute (TARI) – TARI-Uyole and TARI-Selian. Initially, the common bean samples were sorted according to their colours and sizes (Plate. 6.1) and reduced to 360 genotypes. These genotypes were planted in an insect-proof screen house at TARI-Mikocheni (Plate 6.2). The potting (forest) soil was collected from TARI-Mikocheni sub-station called Chambezi and was heat sterilized. The one-litre plastic pots were filled with the sterilized soil and three bean seeds were planted per pot at 3 cm depth. The plants were watered once per day and when the

plants were at the 3rd trifoliate leaf stage (three weeks from planting); the leaves were collected from plants of each genotype.

(a) (b)

Plate 6.1: Initial sorting of common bean seeds based on morphological traits and names given by farmers

Plate (a) Sorting of common bean seed samples according to their relatedness (b) this is the yellow variety from farmers which have already been sorted

(a) (b) (c)

Plate 6.2: Common bean planted in screen house for DArTseq

(a) Sowing of the sorted common bean common bean seeds collected from farmers, (b) The sample of the germinated common bean seeds when was still young, and (c) Collection of the common bean leaves in the screen house for DNA extraction.

6.2.2 DNA extraction from common bean leaves

Genomic DNA was extracted from common bean leaf samples using the CTAB method (Allen *et al.*, 2006) with some modifications. The buffer contained 2% CTAB, 100 mM Tris-HCL, 20 mM EDTA, and 2.5 M NaCl. The four components were mixed well using a magnetic stirrer and then autoclaved at 120 ^{0}C for 20 min. 2% polyvinylpyrrolidone, 1% sodium sulphite and 2.5% Mercapto-ethanol were added to the CTAB buffer a few minutes before extraction. The procedure for extraction has

been described in Chapter two. The electrophoresis was done using 1% agarose gel, which was stained with ethidium bromide. The gel image was visualized and captured using the Benchtop UV Transilluminators (UVP) (Fig. 6.1).

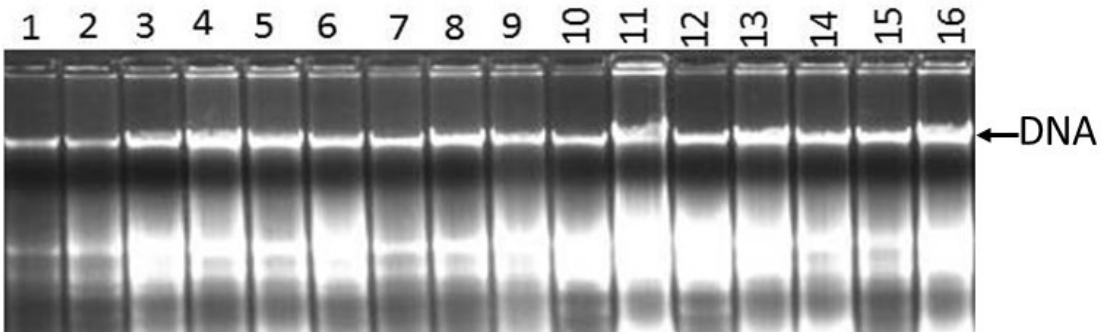

Figure 6.1: Gel picture showing the quality of DNA extracted from common bean genotypes

6.2.3 Preparation of samples and location for genotyping

The DNA extracted from 360 common bean genotypes were placed in the MicroAmp®96-well plates (Thermo Fisher Scientific, MA, the USA) and packed according to the instructions provided by the sequencing company before sent for sequencing. The genotyping was done at Diversity Arrays Technology Pty Ltd, Canberra, Bruce in Australia.

6.2.4 Genotyping

6.2.4.1 Enzyme digestions of the samples

A common bean DArT system has been developed (Brinez *et al*., 2012). The DArTseq service provider referred to this system as the one followed in digesting the samples that were sent. According to the protocol shown by Brinez *et al.* (2011), rare cutter (*Pst*I) and frequent cutters (*BstN*I, *Taq*I, *Alu*I, *Bam*II, *Mse*I, *Hae*III and *Msp*I) were used. The methodology involved the combinations of the rare-cutting and frequent

cutter restriction enzymes: (*Pst*I/*BstN*I, *Pst*I/*Taq*I, *Pst*I/*Alu*I, *Pst*I/*Bam*II, *Pst*I/*Mse*I, *Pst*I/*Hae*III and *Pst*I/*Msp*I) (New England Biolabs, Beverly, MA, USA). 20 U/µl of enzymes was used except for *BstN*I where 10 U/ µl were used. 100 ng of DNA was mixed with 9 µl of the digestion/ligation mix containing 0.2 µl of restriction enzymes, but for *Taq*I and *Msp*I 0.1 µl of enzyme was used. The mixtures were digested at 37 ^{0}C for 1 hour. The digestion profile was analysed and the best restriction enzyme combinations for beans DNA were identified and these were *Pst*I/*BstN*I and *PstI*/*Taq*I. The two restriction products were then ligated to *Pst*I adapters (5´-CACGATGGATCCAGTGCA-3´ annealed with 5´-CTGGATCCATCGTGCA-3´) with 2 U of T4 DNA ligase (New England Biolabs). 1 µl of restriction and ligation mixture were amplified by the PCR in 50 µl total mixture. The primer (5´-GATGGATCCAGTGCAG-3´) which was used is complimentary to adapters. The PCR conditions were 94 ^{0}C for 1 min, followed by 30 cycles at 94 ^{0}C for 20 sec, 58 ^{0}C for 40 sec, 72 ^{0}C for 1 min and 72 ^{0}C for 7 min.

6.2.4.2 Library preparation for the DArT array

According to Xie *et al*. (2006), the best two combinations (*PstI*/*BstN*I and *Pst*I/*Taq*I) were involved in enzyme digestion, library construction and bacterial insert amplification. The PCR product of each sample were combined and ligated by using the PCR® 2.1 TOPO vector (TOPO cloning kit from invitrogen life technologies corporation, Carlsband, CA, USA). The ligated vector was transformed by using the TOP 10F *E. coli* competent cells. This was subjected to heat shock according to Invitrogen company protocol. The transformed cells were screened on medium containing ampicillin (100 µg/ml) and X-gal (40 µg/ml). The white colonies were

picked and transferred to 96-well plate were by each well contained LB medium which had 50 µg/ml ampicillin and then was incubated at 37 ^{0}C for 22 hours.

6.2.4.3 Generation of DArT^{TM} arrays

The PCR amplification of 1 µl of the insert was done from direct LB media. M13 forward and reverse universal primers were used. The reactions were performed according to Xie *et al*. (2006) with the following PCR profile: initial denaturation at 95 °C for 3 min, denaturation at 94 °C for 3 sec, annealing at 52 °C for 30 sec, and extension at 72°C for 1 min, 35 cycles, followed by a final extension at 72 °C for 5 min. PCR products were precipitated with isopropanol, washed with ethanol and resuspended in spotting buffer 2 (DArT Spotter solution) (Brinez *et al.*, 2011). The PCR products were subsequently printed onto polylysine-coated slides using a MicroGrid II arrayer (Genomic Solutions, Lincoln, NE, USA). After arraying, the slides were placed in a water bath at 95 $^{\circ}$C for 2 min to denature the DNA and then immersed in a solution containing 0.1 mM DTT and 0.1 mM EDTA and dried by centrifugation (500 g; 7 min; at room temperature).

6.2.4.4 DArT genotyping

Genotyping diversity panel has three procedures which are fluorescent labelling of presentation, hybridization and washing and scanning image analysis and data manipulation. Fluorescent labelling of presentation involved the two best genomic representations and was labelled with the cy3-dUTP and cy5-dUTP fluorescent nucleotides. This was done in a primer extension reaction with random decamers and the exo-Klenow fragment of *E. coli* DNA polymerase, respectively. Probes were

labelled with 6 - FAM and used as a reference to determine the amount of DNA from each clone spotted on the array (Jaccoud *et al*., 2001). The hybridization mix was prepared as described in Jaccoud *et al*. (2001). The hybridization mix was denatured on a Corbett PCR machine at 95 ˚C for 3 min, followed by 56 ˚C for 5 min and 55 ˚C for unlimited period of time until hybridization with the microarray printed with the DArT clones. The hybridization reaction was incubated overnight at 65 ˚C, after which the slides were well washed and scanned using a Tecan LS300 confocal laser scanner (Grödig, Salzburg, Austria).

6.3 Genotyping Data Analysis

The resulting TIF images were analysed using DArTsoft version 7.3 developed by DArT P/L. This software identifies and scores polymorphic markers, with each marker being scored as 0 (absence), 1 (presence) or - (unable to score). The three principal parameters used for marker selection were: the quality parameter (Q value) that measured the fraction of the total variation across all individuals attributable to bimodality. The reproducibility (R) that derived from replicate individuals that were supposed to give identical results, and the call rate (CR) that represented the number of scored spots versus the maximum number of potential scores. The polymorphism information content (PIC) was used to assess how the marker scores were distributed between the clusters (scored as 0 or 1).

The scores were analysed by using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc version 2.10t, (c) 2000 - 2001, Applied Biostatistics Inc). The file was prepared as per NTSYSpc protocol. The file was converted to Excel 1997 - 2003

workbook and saved. The saved excel file was loaded in the NTEdit which is within the program, then saved as an output file named file name.NTS. The file name.NTS was converted to file name.SIM in qualitative data which is under similarity icon. The SIM file was the one which was used for generation of dendrogram, Principle component analysis and 3 - Dimension analysis. The sequential, agglomerative, hierarchical, and nested clustering methods were performed as defined by Sneath and Sokal (1973). The groups of genotypes observed in PCA were named as Andean or Mesoamerican based on clusters observed as well as their seed sizes.

6.4 Results

6.4.1 Polymorphic information content (PIC), call rate and reproducibility

According to Table 6.1, a total of 35 047 markers were generated in common bean among three hundred sixty (360) genotypes collected in four zone of Tanzania. The call rate ranged from 74.1 to 100% with an average of 94.1% call rate. The scoring reproducibility was 90 to 100%. The marker variation was determined using the PIC values, which were calculated using DArTsoft version 7.3. The PIC was a good index for genetic diversity evaluation. Botstein *et al*. (1980) reported that PIC index can be used to evaluate the level of gene variation; when PIC is > 0.5, the locus is of high diversity; when PIC is < 0.25, the locus is of low diversity and the locus is of intermediate diversity when the PIC is between 0.25 and 0.5. Out of 35 047 markers generated, 558 (1.6%) markers were highly informative, 13 751 (39.2%) markers were of intermediate diversity and 20 738 (59.2%) markers were of low genetic diversity (Table 6.1).

Table 6.1: Polymorphic information content (PIC) values, number and percentage of polymorphic markers generated from common beans

PIC value range	Number of markers	% markers
0.007 - 0.240	20 738	59.20
0.250 - 0.490	13 751	39.20
0.500	558	1.60
Total	**35 047**	**100**

6.4.2 Markers in the chromosome

Of 35 047 markers generated, 24 158 (68.9%) markers were mapped to the chromosomes that make up the common bean genome (Table 6.2). The remaining markers were mapped to the scalfolds and non-chromosomal materials. In chromosomes, the highest number of markers was found in chromosome 2 while the lowest numbers of marker were found in chromosome 10 (Table 6.2).

Table 6.2: Total number of markers and marker percentage mapped in chromosomes

Chromosome	Total markers	%Markers
1	2 065	5.9
2	2 853	8.1
3	2 610	7.4
4	1 735	5.0
5	1 809	5.2
6	1 810	5.2
7	2 307	6.6
8	2 666	7.6
9	2 142	6.1
10	1 729	4.9
11	2 432	6.9
Scalfold	172	0.5
Not found in any chromosome	10 717	30.6
Total	35 047	**100**

6.4.3 Genetic diversity of common bean landrace

A total of 35 047 markers generated in this study were used to construct a dendrogram. Since the NTSYS program used in this study could not handle data for all 360 common bean genotypes at once, the genotypes were divided into four groups – each with 100 genotypes except one which had 60 genotypes; data for each group was analysed separately. This allowed identifying genotypes which were the same and thus enabled reducing the number of genotypes from 360 to a software manageable number (252 genotypes) through removing genotypes that appeared as duplicates. Therefore, the final dataset used in analysis contained 252 common bean genotypes. These were then used to construct a dendrogram presented in Fig. 6.2.

The dendrogram revealed there were two major groups of common bean genotypes in Tanzania. One group represented the Mesoamerican gene pool while the other represented the Andean gene pool. The bean seeds of the Mesoamerican gene pool composed of mostly small seeded (1-24g) common bean genotypes while the Andean pool consisted of genotypes with large sized seeds (40g above). Of 360 common bean genotypes, 278 were placed in Andean gene pool and 82 common bean genotypes were placed in Mesoamerican gene pool. The main clusters also had sub-clusters both in Mesoamerican and Andean gene pools. According to genetic diversity tree of seed samples collected from farmers in Tanzania, the Andean group of genotypes exhibited higher genetic variation than the Mesoamerican gene pool (Fig. 6.2). Using the same number of markers, the individual two groups were analysed separately. The dendrogram was constructed by using Mesoamerican gene pool that consisted of 82 common bean genotypes (Fig. 6.3). The Andean gene pool formed the largest group in

which the analysis was done twice to remove the genotypes that appeared duplicates. And the final dataset that could be accommodated in the program was 252 (Fig. 6.4). Individual analysis of the Andean group revealed four diverse subgroups (Fig. 6.3). On the other hand two subgroups containing closely related genotypes were observed in the mesoamerican group. However, within the subgroups the genotypes appeared to be very close related (Fig. 6.4).

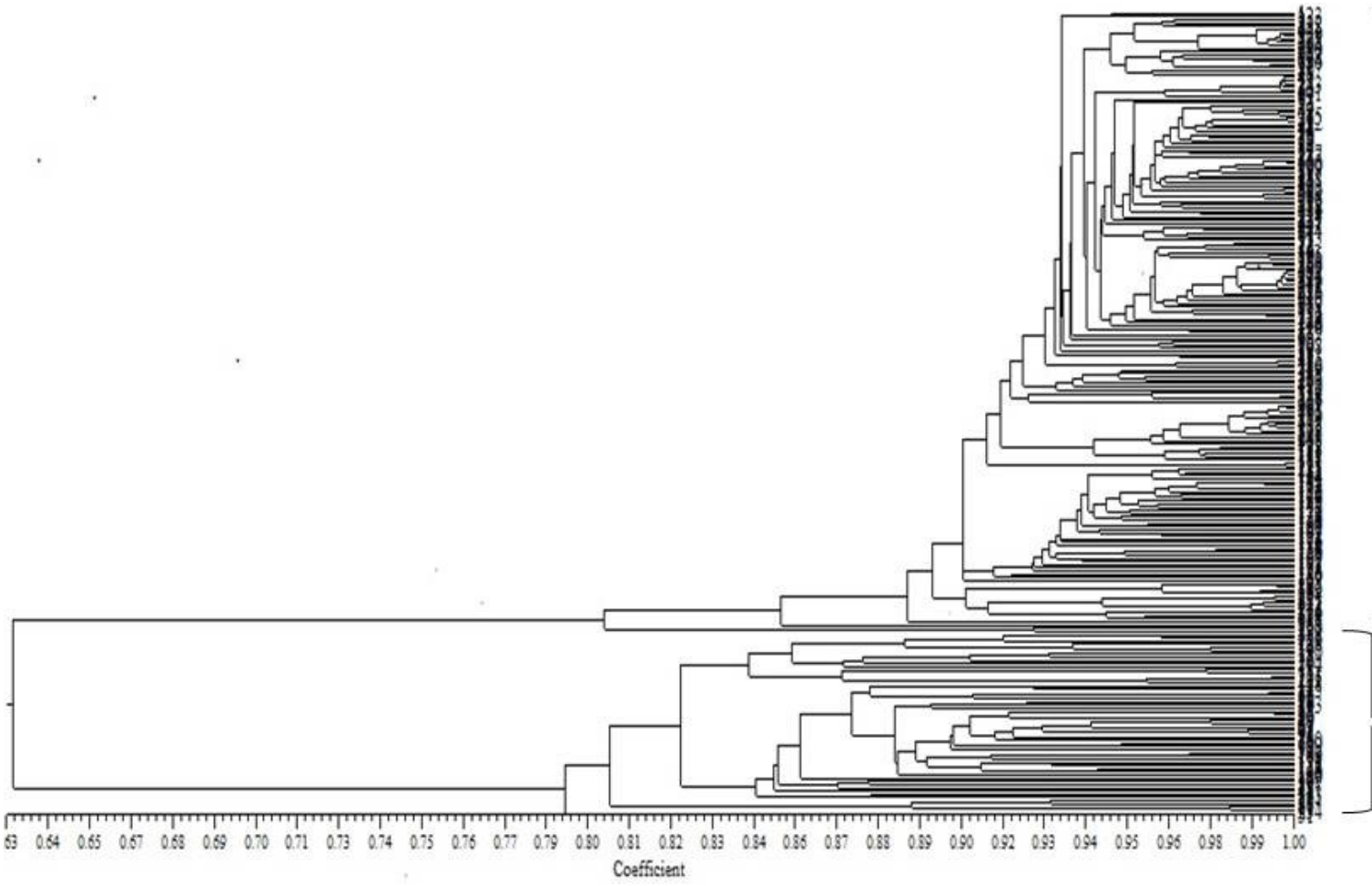

Figure 6.2: The phylogenetic tree of common bean genotypes (252) collected in Tanzania developed by NTSYS program

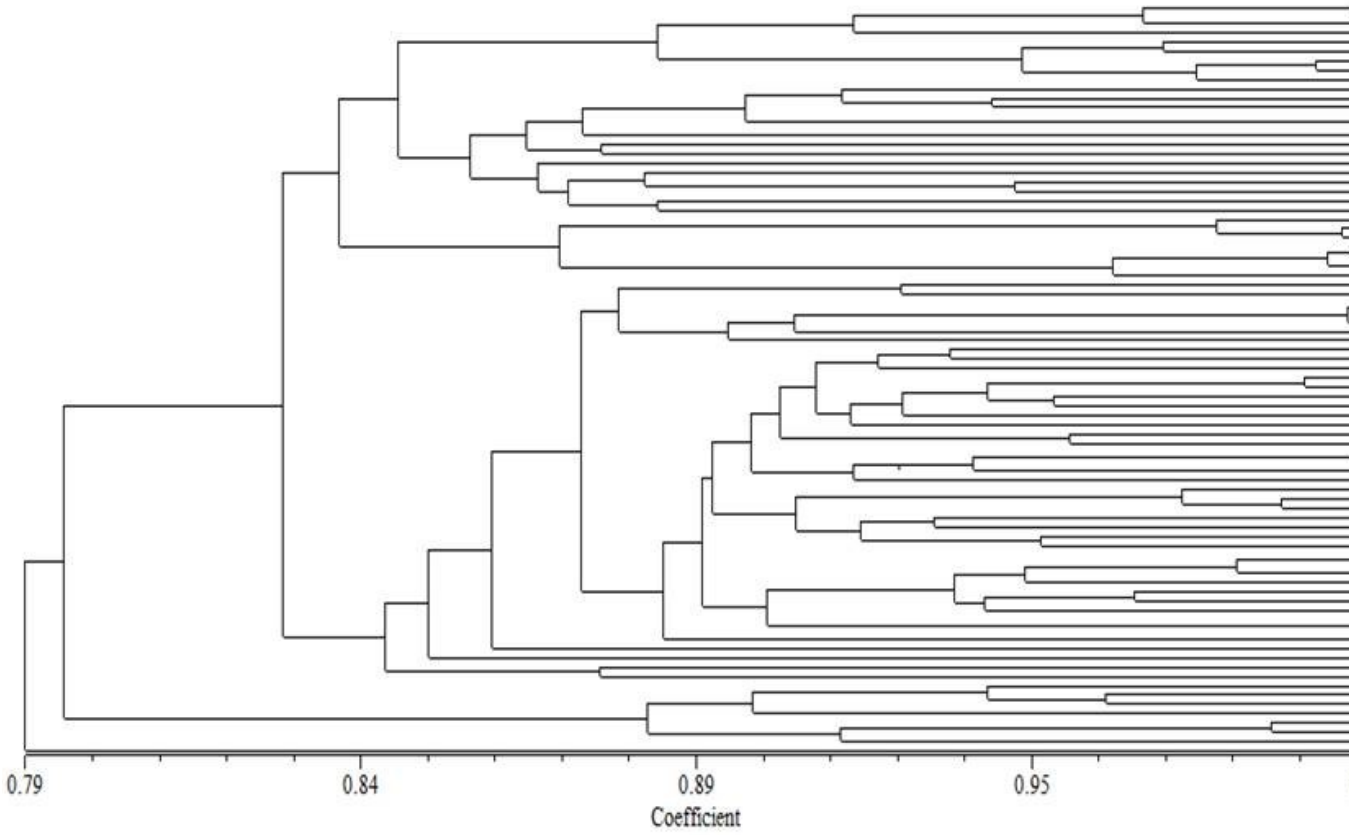

Figure 6.3: The phylogenetic tree for Mesoamerican common bean genotypes (82) collected in Tanzania developed by NTSYS program

SG in the figure refers to subgroup

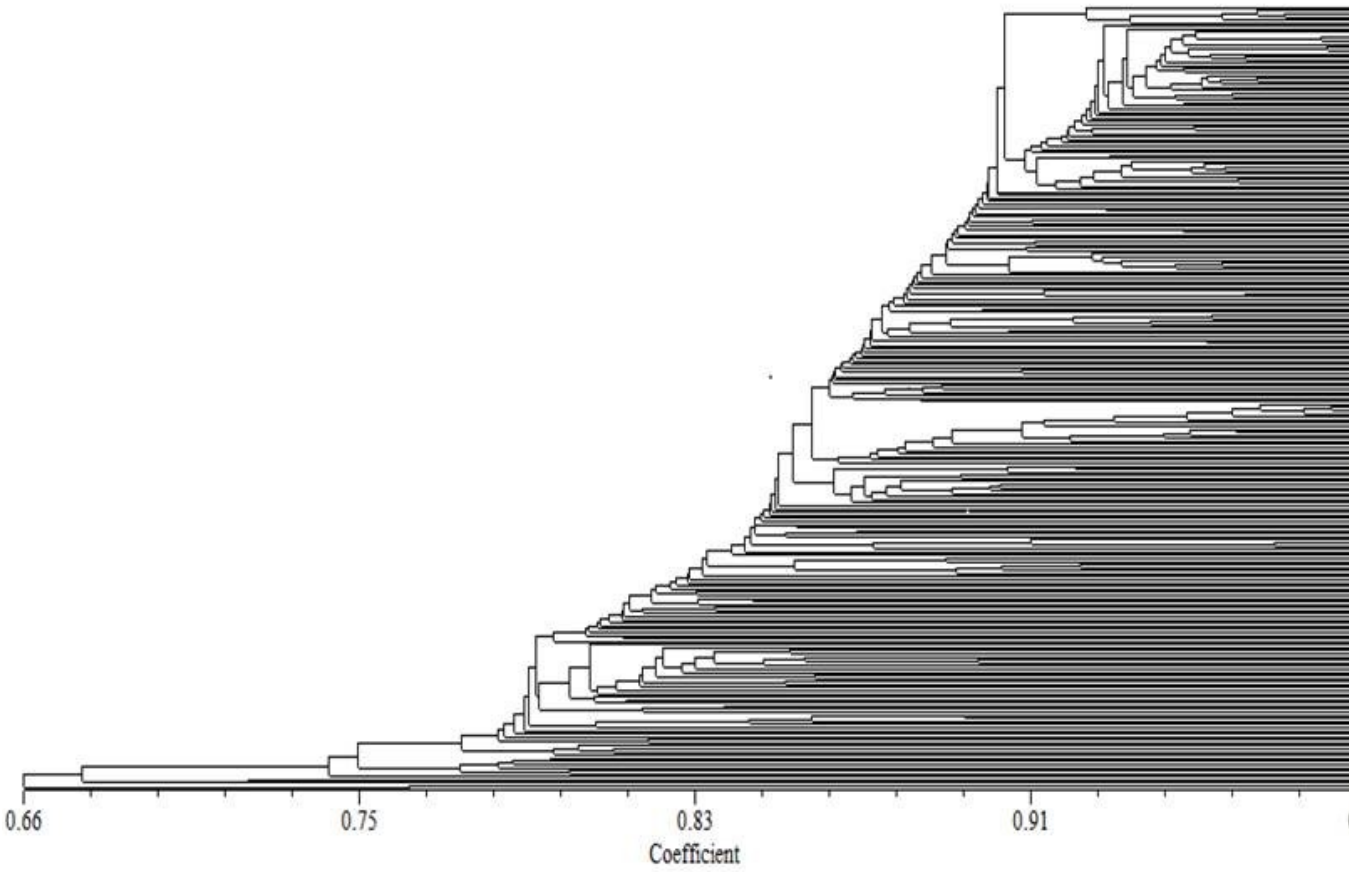

Figure 6.4: The phylogenetic tree of Andean common bean genotypes (252) collected in Tanzania developed by NTSYS program

SG in the figure refers to subgroup

6.4.4 Principal component analysis (PCA)

Principal component analysis (PCA) based on genetic similarity was used to visualize groups of the common bean genotypes collected from Tanzania in two and three-dimensions (Fig. 6.5a and b). The PCA was also conducted on individual groups which are Mesoamerican (Fig. 6.6) and Andean groups (Fig. 6.7). In agreement with results obtained using a dendrogram, the PCA placed the common bean genotypes into two major gene pools represented by the Andean and Mesoamerican groups (Fig.6.5). According to the results, the overall similarities of both Mesoamerican and Andean genotypes were 82.2% across the three axes as it was determined by eigenvectors matrix. In this analysis, some of the genotypes deviated appreciably from the main groups. This deviation was remarkable in the Mesoamerican gene pool; the good examples being the common bean genotypes numbered 227 and 51 (Fig. 6.5). Principal component analysis revealed the within similarity of 82.94% among Andean genotypes. For the Mesoamerican gene pool, the within similarity was 84.60%.

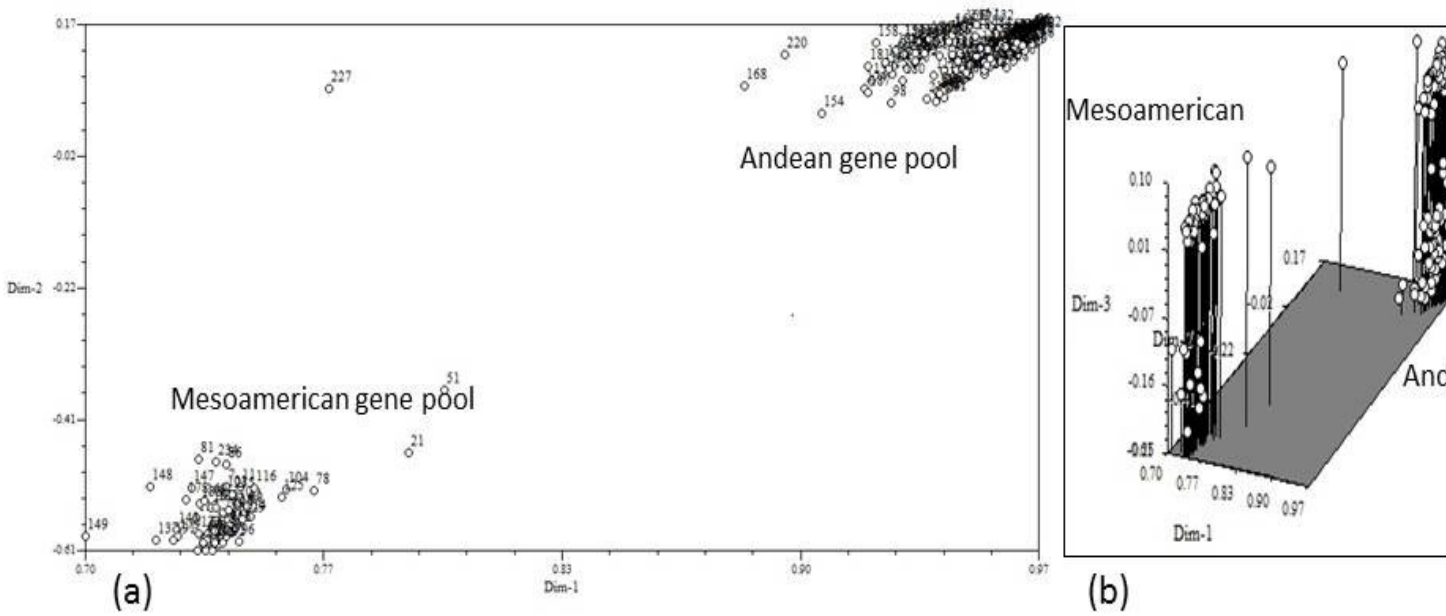

Figure 6.5: Principal component analysis based on the analysis of 252 common bean genotypes

The genotypes were collected from four agricultural research zones of Tanzania. Plates (a) and (b) show analysis in two and three dimensions, respectively. Both Plates (a) and (b) indicate the two groups of genotypes (Mesoamerican and Andean groups). Sub clusters are more observable in (b).

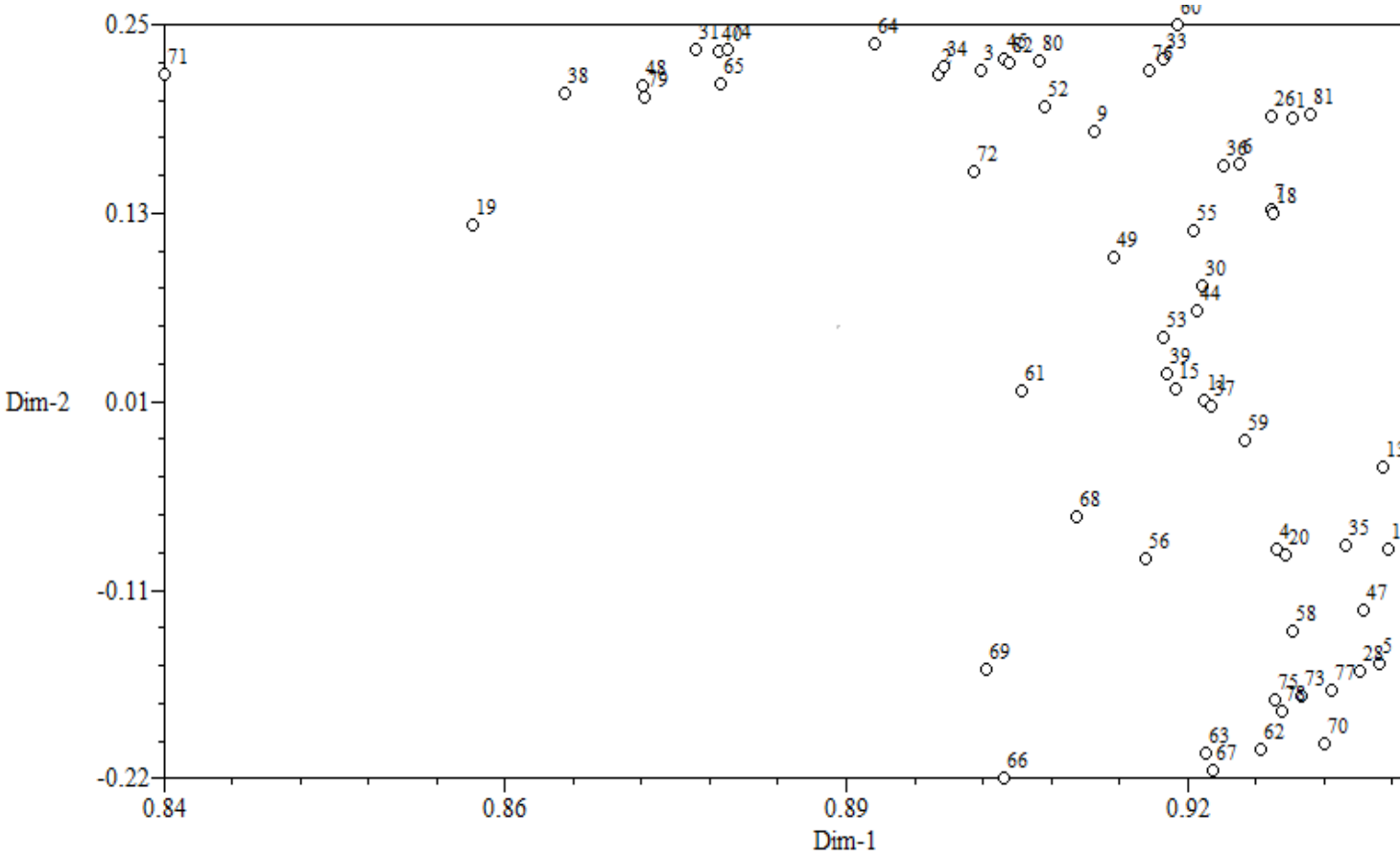

Figure 6.6: **Principal component analysis based on 82 Mesoamerican common bean genotypes**

The genotypes were collected from four agricultural research zones of Tanzania.

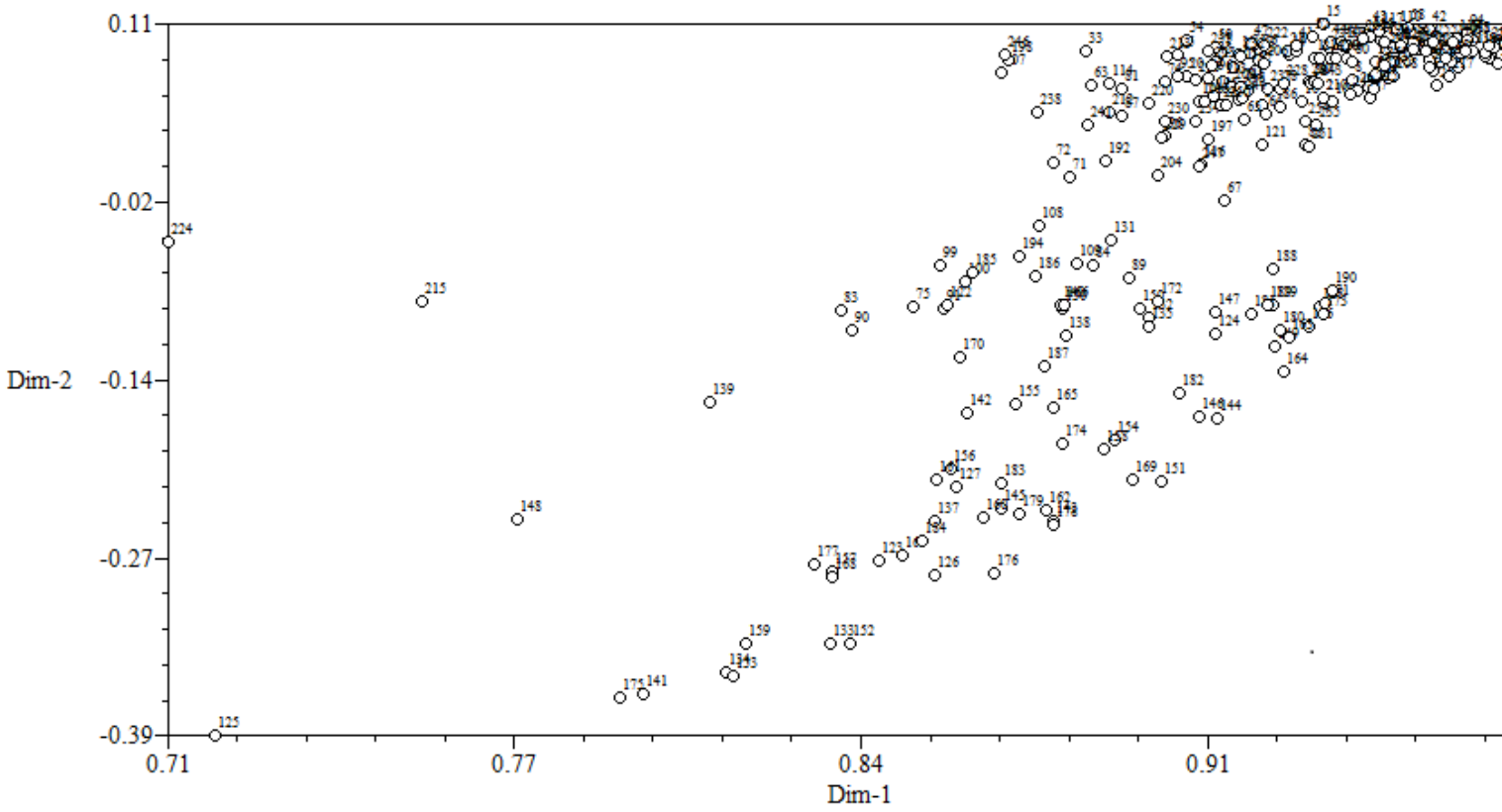

Figure 6.7: **Principal component analysis based on 252 Andean common bean genotypes**

The genotypes were collected from four agricultural research zones of Tanzania.

6.5 Discussion

Knowledge of genetic diversity of common beans is very important in the utilization of genotypes or germplasm in the research institutes that are dealing with agriculture. For example, if the common beans studied have high genetic diversity, that information can be useful in breeding strategies especially in incorporating or pyramiding of gene depending on the goal of a breeder, for example, disease resistance or drought tolerance (Gyang *et al*., 2017). Diversity array technology was applied for the first time to elucidate the genetic diversity of common bean in Tanzania. The results of the diversity analysis showed that the samples collected in four agricultural research zones clustered into two major groups, namely Andean and Mesoamerican gene pools. These results are the same as those reported by Blair *et al*. (2006) and Gill-Langarica *et al*. (2011) who used simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP), respectively, to distinguish common bean genotypes. The DArT is a molecular marker technology with the ability to detect the variation among genotypes in any plant and produce several hundred genomic loci in parallel without depending on sequence from the database (Wenzl *et al*., 2004). DArT has been proven to be efficiency in many studies: rice (Jaccoud *et al*., 2001), cassava (Xia *et al*., 2005), pigeonpea (Yang *et al*., 2006), tomato (Van Schalkwyk *et al*., 2008) and barley (Wenzl *et al*., 2004; Wittenberg *et al*., 2005), wheat (Akbari *et al*., 2006).

The PIC for genetic diversity evaluation in this study, ranged from 0.007 - 0.5 for common bean genotypes in Tanzania. According to Botstein *et al*. (1980), PIC index can be used to evaluate the level of gene variation; when PIC is > 0.5, the locus is of

high diversity; when PIC is < 0.25, the locus is of low diversity and the locus is of intermediate diversity when the PIC ranges between 0.25 and 0.5. Also, Hildebrand *et al*. (1994) showed that the PIC ranges from 0 to 1, and that a PIC of 0 indicates that the marker has only one allele while at the PIC value of 1, the marker would have infinitive number of allelles. According to results of this study, most markers generated were from low to intermediately informative. Approximately 1.6% and 39.2% of the markers were highly and moderately polymorphic, respectively, which indicated that most of the genotypes from Tanzania were closely related as it was also reported by Blair *et al*., (2011). Also these varieties can be genetically close related but when it comes to specific trait, they are are not related.

In Tanzania, the Andean gene pool is larger than the Mesoamerican gene pool. This contrasts the results obtained by Brinez *et al*. (2012), which showed that the Mesoamerican gene pool was larger than the Andean for common bean genotypes in Brazil. This disparity is due to preference of farmers and consumers in Tanzania, whereby more preference is on large seeded beans indirectly selecting for Andean gene pool.

According to the dendrogram developed from markers of 360 common bean genotypes from Tanzania, the varieties genotyped were a bit genetically diverse, especially within the Mesoamerican gene pool. The Mesoamerican seeds are small in size while the Andean genotypes are characterized by large seeds. The 100 seed weight of few varieties of each group was determined and the results generally supported criterion for distinguishing the two gene pools based on their seed sizes (Appendix 6.1). According to Fivawo and Msolla (2012), the 100 seed weight of 1 -

24 g, 25 - 35 g, and > 40 g indicates small, medium and large seeds, respectively. However, it was found out that some genotypes with 100 seeds weight of 19 g and up to 32 g were unexpectedly classified as Andean (normally large seeded) and Mesoamerican (normally small seeded), respectively. Thus, while generally the size of the seed is useful in categorizing genotypes into the gene pools, caution must be exercised as there could be mis-identification of a gene pool when this criterion is used.

According to data of this work, most of Mesoamerican genotypes are grown in Kagera region in north western Tanzania (Appendix 6.2). This is the region where the small seeded common bean varieties are predominantly grown. Other agricultural research zones seem to prefer growing large seeded common bean varieties although in southern highlands of Tanzania there are also few small seeded varieties. Many factors can drive variety preferences but commercial and consumption reasons are probably the most important factors in deciding which common bean genotypes to grow. Most common bean genotypes grown for commercial purposes are known to be large seeded.

The results of this study showed that farmers prefer to use their local names instead of those assigned by breeders. Most of the common bean genotypes collected from farmers and research institutions had morphological resemblance and most of them were clustered together suggesting they were the same despite bearing different local names. For example, Lyamungo series released by TARI-Selian are popularly called Rozikoko in many places. Also, farmers have renamed released JESCA variety as Kablanketi, Soya or Kombati depending on locations it is adopted.

6.6 Conclusions and Recommendations

6.6.1 Conclusions

In Tanzania there are many common bean genotypes with moderate genetic variation. This variation can be capitalized on by the breeders to improve agronomic and disease resistance of common bean in Tanzania. The Mesoamerican group was larger than the Andean group.

6.2.2 Recommendations

i. The usuall way of classifying bean genotypes into gene pool by seed size should be complimented by molecular marker analysis since sometime the seed size does not work.
ii. Studies and breeding programs should capitalize on the genetic variability in common bean genotypes to improve the performance of the genotypes.
iii. More hybridization should be performed to increase the variability of within the gene pools, where possible use of genotypes from other sources and interspecific hybridazation should also be performed.

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Appendices

Appendix 6.1: Description of common bean genotypes collected from different farmers in Tanzania

Genotyping ID	Planting ID	Field number	Field name	District	Variety name	Variety type	Size	Colour	Group	100 seed weight(g)
276	372	682	TARIME 4	Tarime	Unknown		small	red	Mesoamerican	12.9
70	82	648	BKR/Miss 3	Missenyi					Mesoamerican	13.7
74	87	678	MLB 9-6	Muleba	Temaekibila		small	white	Mesoamerican	13.8
56	60	646	KRGS 12	Karagwe	kasukari	Landrace	small	brown	Mesoamerican	14.3
255	**325**	**606**	**Miss 14-2**	**Missenyi**	**Unknown**		**small**	**brown**	**Andean**	**14.5**
116	134	689	MLBS 8-2	Muleba	Wifi nyehegera		small	white to brown	Mesoamerican	14.8
254	324	680	MISS 12-1	Missenyi	Rukululana		small	purple	Mesoamerican	14.8
11	13	604	Miss 7	Missenyi	Kagondo	Landrace	small	"kaki"with red stripes	Mesoamerican	16.5
264	343	677	MLBS 9-3	Muleba	KyaKaragwe		small		Mesoamerican	16.7
24	20	607	BKR/Miss2-3	Missenyi	Unknown		small	brown	Mesoamerican	17.4
113	131	616	Missenyi	Missenyi	Shonaigunia		small		Mesoamerican	17.5
274	370	656	Tarime 16	Tarime	Onyege(luo)		small		Mesoamerican	17.6
39	48	651	MLBS 8	Muleba	KAEMAP	landrace	small		Mesoamerican	17.8
153	177	686	KRGS 5-1	Karagwe	White	released/ landrace	small	white	Mesoamerican	17.8
13	26	676	MLBS 8-3	Muleba					Mesoamerican	17.8
118	**136**	**628**	**Miss 9-2**	**Missenyi**	**Temaekibila**		**small**	**white**	**Andean**	**19.1**
145	169	692	MISS6-3	Missenyi	Kakalakale		small		Mesoamerican	19.2
42	51	693	MLBS 10-3	Muleba			medium	red	Mesoamerican	19.7
273	**369**	**643**	**Tarime 14**	**Tarime**	**Kombati**	**Landrace**	**large**	**brown and whitish strips**	**Mesoamerican**	**20**
15	16	603	Miss6	Missenyi	Mali yahinda		small	red	Mesoamerican	20.7
23	19	621	KRG 5-2	Karagwe	Unknown		small	pale brown and red strips	Mesoamerican	21.1
51	63	126	126	Nkasi	Local	local			Mesoamerican	21.6
182	220	612	KRG 13	Karagwe	Mwanamwana		medium	red	Mesoamerican	21.8
10	12	627	Miss 2-2	Missenyi	Rushala	Landrace	medium	yellow	Mesoamerican	22.2
267	353	202	202	Mbozi	Cheupe	relased/landrace	small	white	Mesoamerican	22.3
115	133	675	MLBS 5-2	Muleba	Wifi nyehegera		small	white to brown	Mesoamerican	22.8
14	15	641	Miss 10-2	Missenyi	Unknown		large	white to brown with black strips	Andean	23.2
52	59	622	Miss 9-1	Missenyi	Rukelelana		small	red	Mesoamerican	23.4
12	14	694	MLBS 9-4	Muleba	Rushala/Njano	Landrace	large	yellow	Andean	23.7
16	17	679	MLBS 9-1	Muleba	Bulushu		small	pale brown and red strips	Mesoamerican	23.7
198	237	672	MLBS 11	Muleba	KyaKaragwe		small	like brown	Mesoamerican	23.8
112	130	644	mlbs 1-3	Muleba	Shereka/kabaune		small	white	Mesoamerican	23.9
8	10	609	Miss 5-4	Missenyi	Soya ndogo	Landrace	medium	grey	Andean	24.7
168	197	230	230	Mbinga	Choroko	Landrace	medium		Mesoamerican	25.1
248	314	623	Tarime3	Tarime	Soya njano	Landrace	medium	yellow	Mesoamerican	25.3
216	257	280	280	Mbinga	Kigoma		large		Andean	25.7
212	253	658	MLB 3	Muleba	Rushala		medium	yellow	Andean	26.2
249	315	640	Tarime 7	Tarime	Soya njano	Landrace	medium	yellow	Andean	26.4

60	70	659	KRGS 9-2	Karagwe	Chileakikale		small	pale brown and red strips	Mesoamerican	26.4
229	276	190	190	Mbozi	Rosenda	realesed	large	brown and pale brown	Mesoamerican	26.6
43	52	657	MLBS 5-3	Muleba	Kashukari	Landrace	small	white	Mesoamerican	26.9
211	252	665	MLBS 10	Muleba	Golori	Landrace	large	yellow	Andean	27.6
159	185	205	205	Mbozi	Njano	Landrace	large	yellow	Andean	27.7
127	145	386	386	Iringa	JKT	Landrace			Mesoamerican	28.5
282	385	653	Miss 5-1	Missenyi	maziwa		small	red	Andean	29.8
344	626	626	Miss 15	Missenyi	Njano ya Izimbya	Landrace	large	yellow	Andean	30.4
296	420	256	256	Mbinga	Kinogasene		medium		Andean	30.5
199	239	496							Andean	30.6
221	263	464	464	Morogoro rural	Njano	Landrace	large	yellow	Andean	30.8
215	256	229	229	Mbinga	Kinogasene				Andean	30.8
225	268	688	KRG 7	Karagwe	CAEMP /Njano	Landrace	medium	yellow	Andean	30.9
260	334	282	282	Nkasi	Lusaka				Andean	31.2
65	75	392	392	Iringa	Rozi koko	Landrace	large		Andean	31.4
94	109	385	385	Namtumbo	Maasai red	Landrace	medium	red	Andean	31.4
5	7	Njano ciat	Njano ciat	Bukoba	Njano ciat	released	large	yellow	Andean	31.5
205	245	681	KRG 14-2	Karagwe	chakuponza like Kablanketi		small	gray	Andean	31.5
297	421	383	383	Iringa	Njano	Landrace	medium	yellow	Andean	31.7
128	**147**	**414**	**414**	**Iringa**	**Maasai red**	**Landrace**	**small**	**red**	**Andean**	**31.9**
33	45	685	MLBS 1-2	Muleba	Kisapuli	Landrace	medium	black	Andean	32
31	39	399	399	Iringa	Salundi	Landrace	large	cream	Andean	33
195	234	701							Andean	33.1
69	81	250	250	Mbinga	Mkonge				Andean	33.8
247	313	639	KRG 14	Karagwe	Rushala	Landrace	large	yellow	Andean	33.9
191	229	652	Miss 11-2	Missenyi	Shonaigunia		medium	red	Andean	33.9
161	187	443	443	Mvomero	Local	local			Andean	33.9
208	248	16	16	Mbeya	Nshamanzi				Andean	34.3
272	364	234	234	Mbinga	Karanga	Landrace	medium	red mottled	Andean	34.7
203	243	629	KRG 1-3	Karagwe	Soya/ ruondela	Landrace	medium	gray	Andean	34.8
213	254	660	KRG 9-3	Karagwe	Njano	Landrace	large	yellow	Andean	34.9
30	37	93	93	Njombe	Soya	Landrace	medium	gray	Andean	35.2
279	375	192	192	Mbozi	Kasukanywele	Landrace	large	cream green strips	Andean	35.5
7	9	JESCA	JESCA	Bukoba	JESCA	Released	large	pink mottled	Andean	35.6
220	262	364	364	Siha	Lyamungo 85	Lyamungu	large		Andean	35.6
257	327	664	TARIME 11	Tarime	Kombati	Landrace	large		Andean	35.6
53	64	654	Tarime 14	Tarime	Kombati	Landrace	large	brown with khaki strips	Andean	35.9
90	105	47	47	Wanging'ombe	Kipapi	landrace	large		Andean	36.6
190	228	671	MLBS 1-1	Muleba	Rushala		large	yellow	Andean	36.6
262	338	684	MLBSS 7	Muleba	Rushala		large	yellow	Andean	37
37	46	650	KRG 4	Karagwe	Rozikoko	landrace	large	red with white strips	Andean	37.1
163	192	232	232	Mbinga	Mkonge				Andean	37.2
183	221	498							Andean	37.6
152	176	257	257	Mbinga	Njano	Landrace	Large	yellow	Andean	37.7
125	143	478	478	Morogoro rural	Kablanketi	Landrace	large		Andean	37.8
142	165	56	56	Wanging'ombe	Njano	Landrace	large	yellow	Andean	37.8
286	396	8	8	Mbeya	Mwaspenjele	Landrace	large	cream red strips	Andean	38

206	246	642	Tarime 16	Tarime	Soya	Landrace	medium	gray	Andean	38.2
278	374	411	411	Iringa	Maasai red	Landrace	medium	red	Andean	38.4
186	224	4	4	Mbeya	Masusu	landrace		Dark green	Andean	38.4
36	44	262	262	Mbinga	Local	local			Andean	39
214	255	618	KRG 7	Karagwe	CAEMP /Njano	Landrace	large	yellow	Andean	39.8
244	308	655	Tarime 5	Tarime	Kombati	Landrace	large	red and white strips	Andean	39.8
38	47	690	MLBS 2-1	Muleba	Kisapuli	landrace	medium	black	Andean	39.8
235	285	673	MISS16-1	Missenyi					Andean	39.8
98	114	378	378	Iringa	Salundi	Landrace	large	cream	Andean	39.9
154	179	663	MLBS 9-5	Muleba	Kisapuri	Landrace	large	red	Andean	39.9
177	214	263	263	Mbinga	Choroko	Landrace			Andean	40.5
239	293	308	308	Namtumbo	Kasukanywele	landrace	large		Andean	40.8
318	457	413	413	Iringa	Kablanketi	Landrace	large	gray	Andean	40.8
256	326	630	Tarime 1	Tarime	Kombati	Landrace	large	red and white strips	Andean	42.1
40	49	631	Tarime 9	Tarime	Rozikoko	landrace	large	pale brown and red strips	Andean	42.5
284	393	44	44	Wanging'ombe	Rozi koko	Landrace	large	pale brown and red strips	Andean	43
121	140	667	MLB 10-12	Muleba					Andean	43.8
306	435	462	462	Morogoro rural	Rozi koko	Landrace	large	pale brown and red strips	Andean	44.8
29	36	107	107	Njombe	Soya ndefu	Landrace	large	gray	Andean	45.6
271	363	406	406	Iringa	Salundi	Landrace	large	cream	Andean	45.6
172	204	301	301	Nkasi	Mwaspenjele	Landrace	large	cream red strips	Andean	48.4
176	212	160	160	Gairo	Mbalawala				Andean	48.6
196	235	395	395	Iringa	Kipapi	Landrace	large		Andean	49.8
79	92	367	367	Siha	Nyeupe	realesed/landrace	large		Andean	51.4
6	8	Lyamungo 90	Lyamungo 90	Bukoba	Lyamungo 90	Lyamungu	large		Andean	53.8
77	90	231	231	Mbinga	Choroko	Landrace			Andean	54.8
41	50	610	KRGS 2-2	Karagwe	Rozikoko	landrace	large	pale brown and red strips	Andean	59.2

Bolded rows are unusual expectations in the sample in terms of the 100 seed weight

Appendix 6.2: List of Tanzanian common bean varieties genotyped and their groups (Andean or Mesoamerican)

Genotyping ID	Planting ID	Field number	Field name	District	Variety	size	colour	group
1	2	464	464	Morogoro rural	Njano	large	yellow	Andean
2	3	166	166	Mbozi	Kigoma	medium	yellow	Andean
3	5	391	391	Iringa	Salundi	large	cream	Andean
4	6	122	122	Nkasi	E 36			Andean
5	7	Njano ciat	Njano ciat	Bukoba	Njano ciat	large	yellow	Andean
6	8	Lyamungo 90	Lyamungo 90	Bukoba	Lyamungo 90	large		Andean
7	9	JESCA	JESCA	Bukoba	JESCA	large	deep gray	Andean
8	10	609	Miss 5-4	Missenyi	Soya ndogo	medium	grey	Andean
9	11	647	Tarime 15	Tarime	Unknown	large	red	Andean
10	**12**	**627**	**Miss 2-2**	**Missenyi**	**Rushala**	**large**	**yellow**	**Mesoamerican**
11	13	604	Miss 7	Missenyi	Kagondo	small	"kaki"with red stripes	Mesoamerican
12	14	694	MLBS 9-4	Muleba	Rushala/Njano	large	yellow	Andean
13	26	676	MLBS 8-3	Muleba				Mesoamerican
14	15	641	Miss 10-2	Missenyi	Unknown	large	white to brown with black strips	Andean
15	16	603	Miss6	Missenyi	Mali yahinda	small	red	Mesoamerican
16	17	679	MLBS 9-1	Muleba	Bulushu	small	pale brown and red strips	Mesoamerican
17	18	373	373	Iringa	Kigoma	medium	yellow	Andean
18	27	77	77	Wanging'ombe	Kipapi	large	deep gray	Andean
19	28	355	355	Arumeru	Bukoba			Mesoamerican
20	31	209	209	Mbozi	Kigoma	medium	yellow	Andean
21	32	304	304	Nkasi	Local			Mesoamerican
22	33	196	196	Mbozi	Local			Andean
23	19	621	KRG 5-2	Karagwe	Unknown	small	pale brown and red strips	Mesoamerican
24	20	607	BKR/Miss2-3	Missenyi	Unknown	small	brown	Mesoamerican
25	35	170	170	Mbozi	Local			Mesoamerican
26	**22**	**649**	**Miss16-2**	**Missenyi**	**Matomatagwa**	**large**		**Mesoamerican**
27	40	103	103	Njombe	Salundi	large	cream	Andean
28	25	483	483	Morogoro	Maasai red	medium	red	Andean
29	36	107	107	Njombe	Soya ndefu	large	gray	Andean
30	37	93	93	Njombe	Soya	medium	gray	Andean
31	39	399	399	Iringa	Salundi	large	cream	Andean
32	41	258	258	Mbinga	Wanja	large	deep yellow	Andean
33	45	685	MLBS 1-2	Muleba	Kisapuli	medium	black	Andean
34	42	284	284	Nkasi	Kigoma	yellow		Andean
35	43	24	24	Mbeya	Local			Andean
36	44	262	262	Mbinga	Local			Andean
37	46	650	KRG 4	Karagwe	Rozikoko	large	red with white strips	Andean
38	47	690	MLBS 2-1	Muleba	Kisapuli	medium	black	Andean
39	48	651	MLBS 8	Muleba	KAEMAP	small		Mesoamerican
40	49	631	Tarime 9	Tarime	Rozikoko	large	pale brown and red strips	Andean
41	50	610	KRGS 2-2	Karagwe	Rozikoko	large	pale brown and red strips	Andean
42	51	693	MLBS 10-3	Muleba		medium	red	Mesoamerican

43	52	657	MLBS 5-3	Muleba	kashukari	small	white	Mesoamerican
44	54	384	384	Iringa	Njano	medium		Andean
45	55	97	97	Njombe	Tanseed	large		Andean
46	57	467	467	Morogoro	Mexico			Mesoamerican
47	56	220	220	Mbinga	Kinogasene			Andean
48	58	358	358	Siha	Njano	large	yellow	Andean
49	61	30	30	Mbeya	Uyole 03	large		Andean
50	62	608	Miss 8-2	Missenyi	Rozikoko	medium	brown with red strips	Mesoamerican
51	63	126	126	Nkasi	Local			Mesoamerican
52	59	622	Miss 9-1	Missenyi	Rukelelana	small	red	Mesoamerican
53	64	654	Tarime 14	Tarime	Kombati	large	brown with kaki strips	Andean
54	**65**	**18**	**18**	**Mbeya**	**Kasukanywele**	**small**	**khaki with red**	**Andean**
55	66	62	62	Wanging'ombe	Mhabuka			Andean
56	60	646	KRGS 12	Karagwe	kasukari	small	brown	Mesoamerican
57	67	124	124	Nkasi	Utafiti	small		Mesoamerican
58	**68**	**37**	**37**	**Wanging'ombe**	**Salundi**	**large**	**cream**	**Mesoamerican**
59	69	33	33	Mbeya	Wanja	large	deep yellow	Andean
60	70	659	KRGS 9-2	Karagwe	Chileakikale	small	pale brown and red strips	Mesoamerican
61	71	613	Tarime 2	Tarime	Kitenge	large	pale brown and red strips	Andean
62	72	246	246	Mbinga	Karanga			Andean
63	73	433	433	Mvomero	Maini	large	yellow	Andean
64	74	333	333	Karatu	JESCA	large		Andean
65	75	392	392	Iringa	Rozikoko	large		Andean
66	77	492	492		Bukoba			Andean
67	78	468	468	Morogoro	Mshindi	large		Andean
68	**80**	**495**	**495**			**large**		**Mesoamerican**
69	81	250	250	Mbinga	Mkonge			Andean
70	82	648	BKR/Miss 3	Missenyi				Mesoamerican
71	83	215	215	Mbozi	Maini	medium	yellow	Mesoamerican
72	84	167	167	Mbozi	Meupe			Mesoamerican
73	86	198	198	Mbozi	Maini	medium	yellow	Andean
74	87	678	MLB 9-6	Muleba	Temaekibila	small	white	Mesoamerican
75	88	228	228	Mbinga	Masunga			Andean
76	89	283	283	Nkasi	Kabanima			Andean
77	90	231	231	Mbinga	Choroko			Andean
78	91	273	273	Mbinga	Kinogasene			Andean
79	**92**	**367**	**367**	**Siha**	**Cheupe**	**small**		**Andean**
80	93	65	65	Wanging'ombe	Mhabuka			Andean
81	94	199	199	Mbozi	Maini	medium	yellow	Mesoamerican
82	95	108	108	Njombe	Salundi	large	cream	Andean
83	97	75	75	Wanging'ombe	Local			Andean
84	**98**	**118**	**118**	**Nkasi**	**Kigoma**	**large**		**Mesoamerican**
85	99	293	293	Nkasi	Maasai red	medium	red	Andean
86	100	687	KRGS 14-3	Karagwe	Unknown	small	white	Mesoamerican
87	101	147	147	Gairo	Maasai red	small	red	Mesoamerican
88	103	131	131	Nkasi	Utafiti			Mesoamerican
89	104	52	52	Wanging'ombe	Samhabuka			Andean
90	105	47	47	Wanging'ombe	Kipapi	large		Andean
91	106	365	365	Karatu	JESCA	large	deep grey	Andean

92	**107**	**254**	**254**	**Mbinga**	**Maasai red**	**small**	**red**	**Andean**
93	108	458	458	Morogoro	Local			Andean
94	**109**	**385**	**385**	**Namtumbo**	**Maasai red**	**small**	**red**	**Andean**
95	111	341	341	Karatu	Bukoba			Mesoamerican
96	112	72	72	Wanging'ombe	Mhabuka			Andean
97	113	245	245	Mbinga	Maasai red	large		Andean
98	114	378	378	Iringa	Salundi	large	cream	Andean
99	115	428	428	Mvomero	Local			Mesoamerican
100	116	460	460	Morogoro	Local	medium		Andean
101		92	92	Njombe	Salundi	large	cream	Andean
102	119	115	115	Njombe	Salundi	large	cream	Andean
103	121	356	356	Arumeru	Bukoba			Mesoamerican
104	122	100	100	Njombe	Local			Mesoamerican
105	123	489	492		Bukoba			Andean
106	124	25	25	Mbeya	Kigoma	large		Andean
107	125	268	268	Mbinga	Mkonge	small		Mesoamerican
108	126	427	427	Mvomero	Local			Mesoamerican
109	127	335	335	Arumeru	Kariasee			Mesoamerican
110	128	222	222	Mbinga	Choroko			Mesoamerican
111	129	178	178	Mbozi	Nzelu			Mesoamerican
112	130	644	MLBS 1-3	Muleba	Shereka/kabaune	small	white	Mesoamerican
113	131	616	Missenyi	Missenyi	Shonaigunia	small		Mesoamerican
114	**132**	**668**	**KRGS 2-1**	**Karagwe**	**Canada**	**large**	**red**	**Mesoamerican**
115	133	675	MLBS 5-2	Muleba	Wifi nyehegera	small	white to brown	Mesoamerican
116	134	689	MLBS 8-2	Muleba	Wifi nyehegera	small	white to brown	Mesoamerican
117	135	615	KRG 9-4	Karagwe	Rutelanaabatani	small	white	Mesoamerican
118	**136**	**628**	**Miss 9-2**	**Missenyi**	**Temaekibila**	**small**	**white**	**Andean**
119	139	639	KRG 14	Karagwe	Rushala	large	yellow	Andean
120	137	617	KRG 1-2	Karagwe	Chibamukundile	medium	black with brown strips	Andean
121	140	667	MLB 10-12	Muleba				Andean
122	138	643	Tarime 13	Tarime	Unknown	medium	red	Mesoamerican
123	141	683	MLBS 9-2	Muleba				Andean
124	142	17	17	Mbeya	Masusu	large	kahawia	Andean
125	143	478	478	Morogoro	Kablanketi	large		Andean
126	144	41	41	Wanging'ombe	Mhanga			Andean
127	145	386	386	Iringa	JKT			Mesoamerican
128	**147**	**414**	**414**	**Iringa**	**Maasai red**	**small**	**red**	**Andean**
129	148	227	227	Mbinga	Mkonge			Andean
130	149	253	253	Mbinga	Local			Mesoamerican
131	150	488	488		Kablanketi	large	grey	Andean
132	151	225	225	Mbinga	Makyete			Andean
133	152	376	376	Iringa	Bukoba			Andean
134	153	305	305	Nkasi	DRK			Andean
135	155	174	174	Mbozi	Msafiri			Andean
136	156	465	465	Morogoro	Local			Andean
137	**157**	**437**	**437**	**Mvomero**	**Maasai red**	**small**	**red**	**Andean**
138	158	99	99	Njombe	Local			Mesoamerican
139	160	15	15	Mbeya	Mwaspenjele			Andean
140	161	470	470	Morogoro	SUA 90			Andean
141	162	177	177	Mbozi	Kalima Uyole			Andean

142	165	56	56	Wanging'ombe	Njano	large		Andean
143	166	288	288	Nkasi	Lusaka			Andean
144	167	9	9	Mbeya	Maini	medium		Mesoamerican
145	**169**	**692**	**MISS6-3**	**Missenyi**	**kakalakale**	**small**		**Andean**
146	**170**	**153**	**153**	**Gairo**	**Nyeupe**	**large**		**Mesoamerican**
147	171	397	397	Iringa	Kablanketi			Andean
148	173	191	191	Mbozi	Fibea	medium	kaki	Andean
149	174	480	480	Morogoro	Kablanketi	large		Andean
150	159	430	430	Mvomero	Kombati	large		Andean
151	175	417	417	Namtumbo	Maasai red	large	red	Andean
152	176	257	257	Mbinga	Njano	Large		Andean
153	177	686	KRGS 5-1	Karagwe	white	small	white	Mesoamerican
154	179	663	MLBS 9-5	Muleba	Kisapuri	large	red	Andean
155	180	408	408	Namtumbo	Kablanketi	large		Andean
156	181	59	59	Wanging'ombe	Kipapi	large		Andean
157	184	303	303	Iringa	Maasai red	small	red	Mesoamerican
158	182	236	236	Mbinga	Kasusuli			Mesoamerican
159	185	205	205	Mbozi	Njano			Andean
160	186	184	184	Mbozi	Mwaspenjele			Andean
161	187	443	443	Mvomero	Local			Andean
162	188	139	139	Gairo	Rozi koko	large		Andean
163	192	232	232	Mbinga	Mkonge			Andean
164	193	294	294	Nkasi	Urambo			Andean
165	194	285	285	Nkasi	Mwaspenjele	large		Andean
166	195	151	151	Gairo	Rozi koko	large		Andean
167	196	98	98	Njombe	Local			Mesoamerican
168	197	230	230	Mbinga	Choroko	medium		Mesoamerican
169	198	136	136	Gairo	Rozikoko	large		Andean
170	200	374	374	Namtumbo	Soya			Andean
171	202	137	137	Gairo	Chitemo			Andean
172	204	301	301	Nkasi	Mwaspenjele	large		Andean
173	208	173	173	Mbozi	Local			Andean
175	211	19	19	Mbeya	Mwaspenjele	large		Andean
176	212	160	160	Gairo	Mbalawala			Andean
177	214	263	263	Mbinga	Choroko			Andean
178	**215**	**683**	**MLBS 9-3**	**Muleba**	**kaKaragwe**	**small**	**white**	**Andean**
179	216	156	156	Gairo	Maasai red	medium	red	Andean
180	218	450	450	Mvomero	Rozi koko			Andean
181	219	422	422	Mvomero	Kigoma			Andean
182	220	612	KRG 13	Karagwe	Mwanamwana	medium	red	Mesoamerican
183	221	498						Andean
184	222	221	221	Mbinga	Kinogasene	medium		Andean
185	223	402	402	Iringa	Local			Mesoamerican
186	224	4	4	Mbeya	Masusu			Andean
187	225	10	10	Mbeya	Masusu			Mesoamerican
188	226	186	186	Mbozi	Meupe	large		Mesoamerican
189	227	217	217	Mbozi	Msafiri	large		Andean
190	228	671	MLBS 1-1	Muleba	Rushala	large	yellow	Andean
191	**229**	**652**	**Miss 11-2**	**Missenyi**	**Shonaigunia**	**small**	**red**	**Andean**
192	230	8	8	Mbeya	Mwaspenjele	large		Andean

193	232	361	361	Arumeru	Bwanashamba	large		Andean
194	233	201	201	Mbozi	Maini	medium	yellow	Andean
195	234	701						Andean
196	235	395	395	Iringa	Kipapi	large		Andean
197	236	113	113	Njombe	Local			Andean
198	237	672	MLBS 11	Muleba	kyaKaragwe	small	like brown	Mesoamerican
199	239	496						Andean
200	240	123	123	Nkasi	Msafiri	large		Andean
201	241	125	125	Nkasi	Njano	large	yellow	Andean
202	242	480	480	Morogoro	Kablanketi		gray	Andean
203	243	629	KRG 1-3	Karagwe	Soya/ ruondela	medium	gray	Andean
204	244	688	KRG 6	Karagwe	Chakuponza like Kablanketi	medium	gray	Mesoamerican
205	245	681	KRG 14-2	Karagwe	Chakuponza like Kablanketi	small	gray	Andean
206	246	642	Tarime 16	Tarime	Soya	medium	gray	Andean
207	247	206	206	Mbozi	Kabanima	large		Andean
208	248	16	16	Mbeya	Nshamanzi			Andean
209	249	158	158	Gairo	Kablanketi			Andean
210	251	88	88	Njombe	Kalima Uyole	large		Andean
211	252	665	MLBS 10	Muleba	Golori	large	yellow	Andean
212	253	658	MLB 3	Muleba	Rushala	medium	yellow	Andean
213	254	660	KRG 9-3	Karagwe	Njano	large	yellow	Andean
214	255	618	KRG 7	Karagwe	CAEMP /Njano	large	yellow	Andean
215	256	229	229	Mbinga	Kinogasene			Andean
216	257	280	280	Mbinga	Kigoma	large		Andean
217	259	86	86	Njombe	Local			Mesoamerican
218	260	306	306	Namtumbo	Mwaya			Andean
219	261	292	292	Nkasi	Lusaka	large		Andean
220	262	364	364	Siha	Lyamungo 85	large		Andean
221	263	464	464	Morogoro	Njano	large		Andean
222	264	637	Tarime 10	Tarime	Rozikoko	large	pale brown and red strips	Andean
223	**265**	**424**	**424**	**Mvomero**	**Maasai red**	**small**	**red**	**Andean**
224	267	486	486		Maini	medium	yellow	Andean
225	268	688	KRG 7	Karagwe	CAEMP /Njano	medium	yellow	Andean
226	269	601	Miss 12-2	Missenyi	Njano/ Rushala	large	yellow	Andean
227	270	600	Miss 11-1	Missenyi	Njano/ Rushala	large	yellow	Andean
228	272	270	270	Mbinga	Njano	large		Andean
229	276	190	190	Mbozi	Rosenda	large	brown and pale brown	Mesoamerican
230	278	750						Mesoamerican
231	279	200	200	Mbozi	Msafiri			Andean
232	281	278	278	Mbinga	Choroko			Andean
233	282	704						Andean
234	284	619	KRG 3	Karagwe	Matama	small	black with brown strips	Andean
235	285	673	MISS16-1	Missenyi				Andean
236	286	145	145	Gairo	Kablanketi			Andean
237	287	36	36	Wanging'ombe	Rozikoko	large		Andean
238	291	670	KRGS 8	Karagwe		medium	red	Mesoamerican
239	293	308	308	Namtumbo	Kasukanywele			Andean
240	294	213	213	Mbozi	Kigoma	large		Andean

241	295	366	366	Hai	Lyamungo 85			Andean
242	303	466	466	Morogoro	Njano	large	yellow	Andean
243	304	260	260	Mbinga	Manyoni			Andean
244	308	655	Tarime 5	Tarime	Kombati	large	red and white strips	Andean
245	309	362	362	Siha	Lyamungo 90	large		Andean
246	312	307	307	Nkasi	Kalima Uyole	large		Andean
247	313	639	KRG 14	Karagwe	Rushala	large	yellow	Andean
248	314	623	Tarime3	Tarime	Soya njano	medium	yellow	Mesoamerican
249	315	640	Tarime 7	Tarime	Soya njano	medium	yellow	Andean
250	319	132	132	Nkasi	Kalima Uyole	large		Andean
251	320	11	11	Mbeya	Rozikoko	large	pale brown and red strips	Andean
252	321	181	181	Mbozi	Mwaspenjele	large		Andean
253	322	182	182	Mbozi	Mwaspenjele	large		Andean
254	324	680	MISS 12-1	Missenyi	Rukululana	small	purple	Mesoamerican
255	**325**	**606**	**Miss 14-2**	**Missenyi**	**Unknown**	**small**	**brown**	**Andean**
256	326	630	Tarime 1	Tarime	Kombati	large	red and white strips	Andean
257	327	664	Tarime 11	Tarime	Kombati	large		Andean
258	330	632	Tarime 8	Tarime	Rozikoko	medium	pale brown and red strips	Andean
259	332	286	286	Nkasi	Lusaka	large		Andean
260	334	282	282	Nkasi	Lusaka	large		Andean
261	335	78	78	Wanging'ombe	Njano	large		Andean
262	338	684	MLBSS 7	Muleba	Rushala	large	yellow	Andean
263	**340**	**435**	**435**	**Mvomero**	**Maasai red**	**medium**	**red**	**Andean**
264	343	677	MLBS 9-3	Muleba	kyaKaragwe	small		Mesoamerican
265	345	138	138	Gairo	Kablanketi	large		Andean
266	352	180	180	Mbozi	Nzelu			Mesoamerican
267	**353**	**202**	**202**	**Mbozi**	**Nyeupe**	**large**		**Mesoamerican**
268	360	389	389	Iringa	Maini	medium	yellow	Mesoamerican
269	361	219	219	Mbinga	Karanga			Andean
270	362	379	379	Iringa	Salundi	large	cream	Andean
271	363	406	406	Iringa	Salundi	large	cream	Andean
272	364	234	234	Mbinga	Karanga			Andean
273	**369**	**643**	**Tarime 14**	**Tarime**	**Kombati**	**large**	**brown and whitish strips**	**Mesoamerican**
274	370	656	Tarime 16	Tarime	Onyege(luo)	small		Mesoamerican
275	371	633	Tarime 12	Tarime	Unknown	medium	red	Mesoamerican
276	372	682	Tarime 4	Tarime	Unknown	small	red	Mesoamerican
277	359	302	302	Namtumbo	Soya			Mesoamerican
278	374	411	411	Iringa	Maasai red	medium	red	Andean
279	375	192	192	Mbozi	Kasukanywele	large		Andean
280	382	13	13	Mbeya	Masusu	large	kahawia	Andean
281	384	415	415	Iringa	Nambalala			Andean
282	385	653	Miss 5-1	Missenyi	maziwa	small	red	Andean
283	391	274	274	Mbinga	Maasai red	medium	red	Andean
284	393	44	44	Wanging'ombe	Rozi koko	large		Andean
285	394	74	74	Wanging'ombe	Rozi koko	large		Andean
286	396	8	8	Mbeya	Mwaspenjele	large		Andean
287	400	456	456	Morogoro	Maasai red	medium	red	Andean
288	403	459	459	Morogoro	Rozi koko	large		Andean
289	397	37	37	Wanging'ombe	Salundi	large	cream	Andean

290	398	62	62	Wanging'ombe	Mhabuka			Andean
291	408	471	471	Morogoro	Njano	large		Andean
292	409	269	269	Mbinga	Maasai red	medium	red	Andean
293	399	479	479	Morogoro	Local			Andean
294	413	194	194	Mbozi	Rosenda	large		Andean
295	416	363	363	Hai	Njano	large		Andean
296	420	256	256	Mbinga	Kinogasene	medium		Andean
297	421	383	383	Iringa	Njano	medium		Andean
298	422	32	32	Mbeya	Njano	medium		Andean
299	426	372	372	Siha	Njano	medium		Andean
300	427	238	238	Mbinga	Kigoma	medium		Andean
301	428	208	208	Mbozi	Njano			Andean
302	429	139	139	Gairo	Rozi koko	large		Andean
303	432	14	14	Mbeya	Maini	medium	yellow	Andean
304	433	49	49	Wanging'ombe	Samwelu			Andean
305	434	216	216	Mbozi	Mwaspenjele	large		Andean
306	435	462	462	Morogoro	Rozi koko	large		Andean
307	438	85	85	Wanging'ombe	Melu			Andean
308	441	53	53	Wanging'ombe	Sewolo ndefu			Andean
309	443	299	299	Nkasi	Mwaspenjele	large		Andean
310	444	22	22	Mbeya	Maini	medium	yellow	Andean
311	445	67	67	Wanging'ombe	Njano	large		Andean
312	446	120	120	Nkasi	Lusaka	medium		Andean
313	448	164	164	Mbozi	Nzelu			Mesoamerican
314	451	129	129	Nkasi	Nyeupe	large		Andean
315	452	134	134	Gairo	Calima Uyole	large		Andean
316	453	12	12	Mbeya	Kablanketi	large		Andean
318	457	413	413	Iringa	Kablanketi	large		Andean
319	458	760	760					Andean
320	459	298	298	Nkasi	Kablanketi	large		Andean
321	461	26	26	Mbeya	Kablanketi	large		Andean
322	462	472	472	Morogoro	Kablanketi	large		Andean
323	463	463	463	Morogoro	Kablanketi	large		Andean
324	464	436	436	Mvomero	Kipapi	large		Andean
325	465	453	453	Mvomero	Kipapi	large		Andean
326	466	448	448	Mvomero	Kablanketi	large		Andean
327	467	421	421	Mvomero	Kipapi	large		Andean
328	468	444	444	Mvomero	Kablanketi	large		Andean
329	469	434	434	Mvomero	Kablanketi	large		Andean
330	470	449	449	Mvomero	Kablanketi	large		Andean
331	571	475	475	Morogoro	Kablanketi	large		Andean
332	472	438	438	Mvomero	Kablanketi	large		Andean
333	473	419	419	Mvomero	Kablanketi	large		Andean
334	474	432	432	Mvomero	Kipapi	large		Andean
335	475	104	104	Njombe	Local			Mesoamerican
336	**476**	**133**	**133**	**Nkasi**	**Mnyarwanda**	**small**		**Andean**
337	477	625	Miss 14-1	Missenyi	Njano	large	yellow	Andean
338	313	639	KRG 14	Karagwe	Rushala	large	yellow	Andean
339	**53**	**614**	**Tarime 6**	**Tarime**	**unk nown black**	**small**	**black**	**Andean**
340	602	602	Miss 5-2	Missenyi	Soya njano	large	yellow	Andean

341	141	683	MLBS 9-4	Muleba	Njano	large	yellow	Andean
342	477	625	Miss 14-1	Missenyi	Njano	large	yellow	Andean
343	**292**	**662**	**MLBS 5-1**	**Muleba**	**Unknown**	**large**	**red**	**Mesoamerican**
344	626	626	Miss 15	Missenyi	Njano ya Izimbya	large	yellow	Andean
345	**290**	**645**	**Miss 13-2**	**Missenyi**	**Canada**	**large**	**red**	**Mesoamerican**
346	Kablanketi	Kablanketi	Kablanketi		Kablanketi	large	gray	Andean
347	Mshindi	Mshindi	Mshindi	Morogoro SUA	Mshindi	large	red	Andean
348	Kitenge	Kitenge	Kitenge	Bukoba	Kitenge	large	red with white strips	Andean
349	JESCA	JESCA	JESCA	Bukoba	JESCA	large	deep gray	Andean
350	Maini	Maini	Maini	Market Dar	Maini	medium	yellow	Andean
351	SUA 90	SUA 90	SUA 90	Morogoro SUA	SUA 90	small	khaki green	Andean
352	Njano	Njano	Njano	Bukoba	Njano	large	yellow	Andean
353	Pesa	Pesa	Pesa	Morogoro SUA	Pesa	large	red	Andean
354	Lyamungo 90	Lyamungo 90	Lyamungo 90	Bukoba	Lyamungo 90	large		Andean
355	Kablanketi	Kablanketi	Kablanketi		Kablanketi	large	gray	Andean
356	Mshindi	Mshindi	Mshindi	Morogoro SUA	Mshindi	large	red	Andean
357	Kitenge	Kitenge	Kitenge	Bukoba	Kitenge	large	red with white strips	Andean
358	JESCA	JESCA	JESCA	Bukoba	JESCA	large	deep gray	Andean
359	Maini	Maini	Maini	Market Dar	Maini	medium	yellow	Andean
360	SUA 90	SUA 90	SUA 90	Morogoro SUA	SUA 90	medium	khaki green	Andean

Bolded rows are unusual expectations in the group that the sample were placed

CHAPTER SEVEN

7.0 RESPONSE OF SELECTED COMMON BEAN GENOTYPES TO FOUR VIRUSES OF COMMON BEANS (*Phaseolus vulgaris* L.) IN TANZANIA

Beatrice Mwaipopo[1, 3*], Susan Nchimbi-Msolla[1], Paul Njau[1], and Deusdedith Mbanzibwa[2]

[1]Department of Crop Science and Horticulture, Sokoine University of Agriculture, P. O. Box 3005, Morogoro, Tanzania.

[2]Tanzania Agricultural Research Institute Mikocheni, P. O. Box 6226, Dar es Salaam, Tanzania.

[3]Tanzania Agricultural Research Institute Ilonga, P.O Box 33, Morogoro, Tanzania.

Corresponding author email: beatricemwaipopo@yahoo.com*

Abstract

There are many common bean genotypes, which are produced by smallscale holder-farmers in major bean growing areas in Tanzania. Virus diseases are known to constrain common bean production in the country but there has not been comprehensive screening for disease resistance for specific viruses. The aim of this study was therefore to evaluate the response of selected bean genotypes to the common bean viruses: BCMNV, BCMV, SBMV and CPMMV. A total of 43, 25, 22 and 23 common bean genotypes were sap inoculated with BCMNV, BCMV, SBMV and CPMMV, respectively. The experiments were set under Complete Randomized Design (CRD). The four viruses were inoculated on common bean plants by using the phosphate buffer (pH 7.0). Scoring for disease symptoms was achieved using a

modified scale. The percentage disease severity and AUDPC were determined. The data were subjected to One-way analysis of variance (ANOVA) using the GenStat 15th edition and *post hoc* were done by using Tukey's test. For each virus inoculated, there was significance differences (P = 0.01) in disease severity and AUDPCs between different common bean genotypes. However, there was no significant difference in CPMMV disease severity on different genotypes when observations were made at 7th day (P = 0.588) and 12th day (P =0.336) for the experiment that was set up in Morogoro. Depending on the common bean genotype assessed, the symptoms appeared between 17th and 12th days post inoculation for all four viruses. However, delayed symptoms (17th day) were observed in Calima genotype when it was challenged with CPMMV. Across all virus isolates used in this study, most of common bean disease severity was less than 50% while the AUDPC ranged from 414 - 2 667, 0 - 1 586.7, 105.6 -1 561.7 and 506 – 2 037 in BCMNV, BCMV, CPMMV and SBMV, respectively. Thus, for all inoculated common bean genotypes the disease resistance ranged from moderate resistance to susceptible. However, Fibea and Selian 05 common bean genotypes when challenged with BCMV suggesting to have complete resistance. From the findings of this study, it was concluded that common bean genotypes respond differently to different virus isolates and therefore yield losses may be different for different common bean genotypes when infected with different viruses.

Keywords: Common beans, Disease severity, Genotyping, Inoculation, Virus,

7.1 Introduction

Common bean (*Phaseolus vulgaris* L.) is the major legume crop grown in many parts of Africa, particularly in eastern Africa. However, the crop is highly susceptible to infection caused by fungi, bacteria and viruses (Hillocks *et al*., 2006).

Viral diseases affect common beans production worldwide; hence the reduction of common beans yields. Common bean plants are infected with many types of viruses; common beans usually express different symptoms depending on the virus involved in infection (Rastgou and Jalali, 2017). There are at least 30 reported viruses of common bean; For example, these are some of the genus where the viruses are found: *Cucumovirus, Begomovirus, Illavirus, Luteovirus, Potyvirus, Sobemovirus, Tobamovirus*, and *Tospovirus* (Ghorbani *et al*., 2010). Hema *et al*. (2014) reported that yield losses associated with viral disease depends on the virus that infect common bean.

Several common bean genotypes with tolerance or resistance to BCMV and BCMNV have been released in Tanzania (Tryphone *et al*., 2013). Njau *et al*. (1994) screened for resistance against many different common bean viruses in Tanzania and observed that some common bean genotypes did not express any symptoms. The same authors also observed that some common bean cultivars were having resistance genes, which protected them from virus infections. Genetic studies have established the *R*-gene (dominant gene) that works in a gene for gene manner. The interaction between effector proteins (*R*-gene) and cognate pathogen elicitor (*Avr* gene) leads to resistance, whereby the virus become confined at initial stage of infection through a phenomenon known as hypersensitive reaction (HR) (Seo *et al*., 2006).

Screening for resistance is very important as it helps in planning for crop improvement. Development of plant genotypes with high levels of resistance to pest and diseases is important especially for common bean farmers (Beaver and Osorno, 2009).

The aim of this work was to evaluate the response of selected common bean genotypes to the common bean viruses: SBMV, CPMMV, BCMV and BCMNV. *Bean common mosaic virus* and BCMNV are known to cause viral diseases in Tanzania (Mwaipopo *et al.,* 2017). On the other hand, CPMMV was detected in common bean plants in the country by Mink and Keswani (1987). Preliminary studies, using nine common bean genotypes, indicated CPMMV may cause yield loss and severe symptoms on common bean plants (Chilagane, 2018). SBMV was recently detected in common bean plant samples collected from different parts of the country (Mwaipopo *et al*., 2018) and appeared to cause severe symptoms in common bean plants. Based on the above facts, selected common bean genotypes were challenged with these four viruses. Common bean genotypes were collected from different areas of Tanzania. The common bean genotypes used in this study included farmer's preferred ones.

7.2 Materials and Methods

7.2.1 Seed collection

The seeds used in this study were collected from farmers in southern highlands, eastern, lake and northern agricultural research zones and from research centres under Tanzania Agricultural Research Institute (TARI). The centres from which the seeds were obtained; TARI - Selian and TARI - Uyole. Seeds were also collected from

Sokoine University of Agriculture. The seeds names recorded were those given by farmers or breeders at research centres (Table 7.1).

7.2.2 Virus isolates used in this study

The viruses used to inoculate common bean plants in this study were BCMV and BCMNV (Genus *Potyvirus*), CPMMV (*Carlavirus*) and SBMV *(Sobemovirus*). The BCMV isolate TZ: SUA1:2017 used in this study was related to sequenced strain NL1 (Accession number KM023744). This isolate was collected from the Sokoine University of Agriculture (SUA) crop museum. The BCMNV isolate (Accession number MF066270) used was closely related to a sequenced isolate (Accession number Z17203). It was collected in 2016 from TARI - Maruku farm in lake zone Tanzania. The CPMMV isolate TZ: CHANGARAWE: 2016 was used in this study was 92% similar to the sequence of CPMMV in database (Accession number KJ534277; isolated from *Vigna mungo* (L.) Hepper). This CPMMV isolate was collected from a common bean plant in Changarawe area in Mvomero district in Morogoro region. The sequence of SBMV isolate used to challenge common bean plants was 99% similar to a sequence of SBMV (Sao Paulo) with accession number DQ875594. This SBMV isolate was collected from western Tanzania (Kasulu district; Kigoma region) (Table 7.1). These isolates were maintained in virus free seedlings of bean cv Pesa, PASI and Cheupe genotypes in screenhouse at TARI-Mikocheni and SUA before inoculation.

Table 7.1: List of common bean genotypes with their respective viruses inoculated

No.	Genotypes	Genotype type	Virus tested	Experiment location	Gene pool
1	Calima Uyole	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
2	JESCA	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
3	Kablanketi fupi	Landrace	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
4	Kablanketi ndefu	Landrace	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
5	Lyamungo 85	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
6	Mshindi	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
7	Njano uyole	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA /TARI-Selian	Andean[1]
8	PASI	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
9	Pesa	improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[2]
10	Rojo	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
11	Rosenda	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
12	Rozikoko	Landrace	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
13	Selian 05	Improved	BCMV/BCMNV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
14	SUA90	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
15	Urafiki	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[2]
16	Zawadi	Improved	BCMNV/BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[2]
17	Choroko	Landrace	BCMNV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
18	kigoma	Landrace	BCMNV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
19	Lyamungo 90	Improved	BCMV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
20	Maasai red	landrace	BCMNV/CPMMV/SBMV	SUA/TARI-Selian	Andean[1]
21	Mwaspenjele	Landrace	BCMNV/CPMMV/SBMV	SUA/ TARI-Selian	Andean[1]
22	Selundo	Landrace	BCMNV/CPMMV/SBMV	SUA/TARI-Selian	Andean[2]
23	Uyole 04	Improved	BCMNV/CPMMV/SBMV	SUA/TARI-Selian	Andean[2]
24	Uyole 96	Improved	BCMV/CPMMV/SBMV	SUA /TARI-Selian	Andean[2]
25	Cheupe	Improved	CPMMV/SBMV	SUA/TARI-Selian	Mesoamerican[1]
26	Fibea	Improved	BCMNV/BCMV	SUA	Andean[1]
27	Kitenge	Landrace	BCMNV/BCMV	SUA	Andean[1]
28	Kombati	Landrace	BCMNV/BCMV	SUA	Andean[1]
29	Nyeupe	Improved	BCMNV/BCMV	SUA	Andean[1]
30	Selian 15	Improved	BCMNV/BCMV	SUA	Andean[2]
31	Selian 94	Improved	BCMNV/BCMV	SUA	Andean[2]
32	Selian 97	Improved	BCMNV/BCMV	SUA	Andean[2]
33	Uyole 16	Improved	BCMNV/BCMV	SUA	Andean[2]
34	Canada	Landrace	BCMNV	SUA	Mesoamerican[1]
35	KAEMAP	Landrace	BCMNV	SUA	Mesoamerican[1]
36	Kariasee	Landrace	BCMNV	SUA	Mesoamerican[1]
37	Kasukanywele	Landrace	BCMNV	SUA	Andean[1]
38	Kisapuli	Landrace	BCMNV	SUA	Andean[1]
39	Local	Local	BCMNV	SUA	Andean[1]
40	Wifi nyehegera	Landrace	BCMNV	SUA	Mesoamerican[2]
41	Uyole 03	Improved	BCMNV	SUA	Andean[1]
42	Soya Kombati	Landrace	BCMNV	SUA	Andean[1]
43	Soyakijivu	Landrace	BCMNV	SUA	Andean[1]
44	Selian 14	Improved	BCMNV	SUA	Andean[2]
45	Onyege	landrace	BCMNV	SUA	Mesoamerican[1]
46	Masunga	Landrace	BCMNV	SUA	Andean[1]
47	Matama	Landrace	BCMNV	SUA	Andean[1]

[1]The gene pools were determined through genotyping as presented in Chapter six; [2]gene pool was determined based on the seed size

7.2.3 Planting and mechanical inoculation

Common bean genotypes to be inoculated were planted in screen houses at Sokoine University of Agriculture (SUA; Morogoro) and Tanzania Agricultural Research Institute - Selian (TARI-Selian; Arusha). For BCMV and BCMNV, the experiments were set only at SUA while for SBMV and CPMMV; experiments were set at both research stations. A total number of 43, 25, 23 and 22 common bean genotypes were used to study response of common bean genotypes to BCMNV, BCMV, CPMMV and SBMV, respectively. The different number of genotypes was screened for different viruses because for the first year all collected samples were viable thus there was many genotypes for BCMNV, but other experiments in the next season there was reduction of number of genotypes due to low viability, although the criteria for choosing genotypes for BCMV, CPMMV and SBMV were based on results from BCMNV, where by susceptible and resistant genotypes were choosen. The experiments were established under Completely Randomized Design (CRD).

In the first experiment, forty-three common bean genotypes were used to study response of plants to BCMNV. There were two treatments (Mock inoculation and BCMNV inoculation), which were replicated three times. In the second experiment, a total of 25 common bean genotypes were challenged with BCMV. As was for BCMNV, there were two treatments, which were mock inoculation and BCMV inoculation. CRD design was used and treatments were replicated three times. The third experiment was set in two places, namely TARI-Selian and SUA. In this case, 21 common bean genotypes were planted at SUA for studying their response to CPMMV and SBMV. Only twenty two genotypes were planted at TARI- Selian to study their

response to SBMV and CPMMV. In this experiment there were three treatments (mock inoculation, SBMV inoculation and CPMMV inoculation).

In the first experiment that involved BCMNV inoculation, two seeds were planted per pot while in the second and third experiment for CPMMV, BCMV and SBMV; three seeds were planted per pot. In all experments, the plants were watered three times a week or when it was observed that there was a need for watering.

The plants were inoculated when they were 7 days old. The inoculation buffer was prepared as described by Noordam, (1973). Whereby, 0.01 M phosphate buffer solution pH 7.0 that contained KH_2PO_4 (MW = 136.086 g/mol) and $Na_2HPO_4.2H_2O$ (MW = 177.99 g/mol) was used for mechanical inoculation. Infected leaf samples were used as sources of inocula. The leaf sample to phosphate buffer ratio was 1:10 (w/v). Infected leaf samples were carefully macerated using sterilized motors and pestles. Caborundum powder was well spread on the first two fully opened common bean leaves. Then, sap from infected leaf was gently rubbed on the leaves. The inoculated leaves were sprayed with distilled water after 30 min.

7.2.4 Confirmation of virus infection using RT-PCR

Sytematically infected leaf samples were taken from all common bean plants inoculated with different viruses at 15 days post inoculation. They were preserved in nylon bags and immediately stored in cool boxes and later freezed at -80 °C. The nucleic acids (RNA and DNA) were extracted from collected leaf samples using facilities at TARI - Mikocheni. The CTAB method was used as described in chapter two. The cDNA was synthesized as described previously (Mwaipopo *et al*., 2018). The PCR amplification was done using the primers designed for specific virus (Table

7.2). For the primer set BCMV1F/BCMV1R, thermocycling conditions were 1 cycle for 2 min at 94 °C (initial denaturation), 35 cycles at 94 °C for 25 sec (denaturation), 50 °C for 25 sec (annealing), 72 °C for 30 sec (extension) and a final extension was done at 72 °C for 10 min. For the primer set BCMNVF1/BCMNVR1, thermocycling conditions were 1 cycle of 3 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 30 sec (denaturation), 50 °C for 30 sec (annealing), 72 °C for 1 min (extension) and a final cycle (final extension) at 72 °C for 10 min. For CPMMV2F1/ CPMMV2R1; 1 cycle of 2 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 45 sec (denaturation), 56 °C for 45 sec (annealing), 72 °C for 1 min (extension) and a final cycle (final extension) at 72 °C for 10 min. For SBMV 315F1/ SBMV 315R1; 1 cycle of 3 min at 94 °C (initial denaturation), then 35 cycles at 94 °C for 30 sec (denaturation), 50 °C for 30 sec (annealing), 72 °C for 1min (extension) and a final cycle (final extension) at 72 °C for 10 min. The amplicons were run in 1% agarose gel that contains ethidium bromide. The amplicons were visualized on the Benchtop UV Transilluminator (UVP).

Table 7.2: Primers used for detection of BCMV, BCMNV, CPMMV and SBMV in inoculated plants

Primer pair	Primer sequences 5′–3′ direction	Virus amplified	Amplicon[1]	Size (bp)	Reference[2]
BCMV1F	GTAGCACAGATGAAGGCAGCA	BCMV	CP	320	Mwaipopo *et al*., 2018
BCMV1R	GGTTCTTCCGGCTTACTCATA				
BCMNVF1	CAAAGGCCCAGCGGATAAA	BCMNV	CP	823	Mwaipopo *et al*., 2018
BCMNVR1	GGTGGTATAACCACACTGGAATTG				
CPMMV2F1	AACAAAAACTGGCGTTCCAAA	CPMMV	CP	1300	This study
CPMMV2R1	GGAAAATAACTTTAAAACCGG				
SBMV 315F1	GTAGCACAGATGAAGGCAGCA	SBMV	P8/P10	490	This study
SBMV 315R1	GGTTCTTCCGGCTTACTCATA				

[1]CP indicate virus coat protein, P8/P10 is a C-terminal protein with ATPase and RNA binding properties. [2]These primers are also shown in Chapter two.

7.3 Data collection and analysis

7.3.1 Scoring for disease symptoms development and severity

Assessments for disease symptoms development and severity were initiated at four days post inoculation. The scoring key developed by Spence and Walkey (1994) was used to score for viral disease symptoms development with some modifications. For BCMV and BCMNV the description of the scale was; 0 - no visible symptoms on any part of the plant; 1 - leaf yellowing/vein yellowing; 2 - mild mosaic, vein greening, mild systemic necrotic lesions, vein clearing, no stunted growth, plant recovery; 3 - leaf rolling/malformation, severe mosaic, deep green bristles, leaf dying and detouching from plant, stunted or without stunted growths; 4 - very severe mosaic, very severe necrosis on plant, plant stunted, very severe leaf malformation; 5 - plant death. For SBMV, 0 - no visible symptoms on any part of the common bean plant; 1 - leaf yellowing, faint mosaic; 2 - mild mosaic, mild necrosis (Mild symptoms); 3 - severe mosaic, severe necrosis on leaves and stem, leaf rolling, leaf malformation

stunting or no stunting growth (severe symptoms); 4 - leaves death, plant dieback, very severe necrosis on the stem and leaves, very severe leaf malformation and leaf rolling, plant stunting (very severe symptoms) and; 5 = plant death. For CPMMV, 0 = no visible symptoms; 1 = yellowing; 2 = mild mosaic, vein clearing; 3 = severe mosaic symptoms and bristles on the leaves; 4 = very severe symptoms and; 5 = plant death.

7.3.2 Data analysis

The percentage disease severity and area under disease progress curve (AUDPC) were determined as described by Campbell and Madden (1990). Accordingly, percentage disease severity was determined using formulae as shown in equation (1)

% Disease severity = ∑n*v*100/N*V …… (**Equation 1**)

Where by n = number of leaves infected with the virus, v = value score of each category attack, N = number of leaves observed and V = value of the highest score

The AUDPC was determined using formulae as shown in equation (2)

$$AUDPC = \sum_{i=1}^{n-1} (¿0.5(Ti+1)-Ti) \quad \frac{(Yi+1)+Yi}{} \quad \text{…… (\textbf{Equation 2})}$$

Where by AUDPC = area under disease progress curve, Yi = disease severity on the i^{th} date, Ti = Date on which the disease was scored and n = number of dates on which the disease was scored.

Severity and AUDPC data were subjected to one-way analysis of variance (ANOVA) at P = 0.05 using the GenStat 15^{th} edition and *post hoc* analysis (means separation) were done by using Tukey's range test. To compare response of different genotypes,

the graphs for disease symptom severity and AUDPCs were developed using Microsoft Office Excel version 2010.

7.4 Results

7.4.1 BCMNV disease symptoms expressed in different common bean genotypes

Starting from four days post inoculation with BCMNV (isolate TZ: Maruku:2016; Accession no MF066270), common bean plants were monitored for disease symptoms development. Different symptoms were observed in 43 different common bean genotypes, which were inoculated. The symptoms observed were stunted growth of the common bean plants, necrosis on leaves, mosaic, leaf rolling or malformation, vein yellowing, vein greening and bristles on leaves (deep green/rugosity) (Plate. 7.1; Table 7.3). The common viral disease symptoms that were expressed in many common bean genotypes were stunted growth, mosaic, leaf rolling/ malformation and vein green banding (Table 7.3). Selian 14, SUA 90 and Kigoma genotypes appeared to recover from disease symptoms caused by BCMNV (Table 7.3).

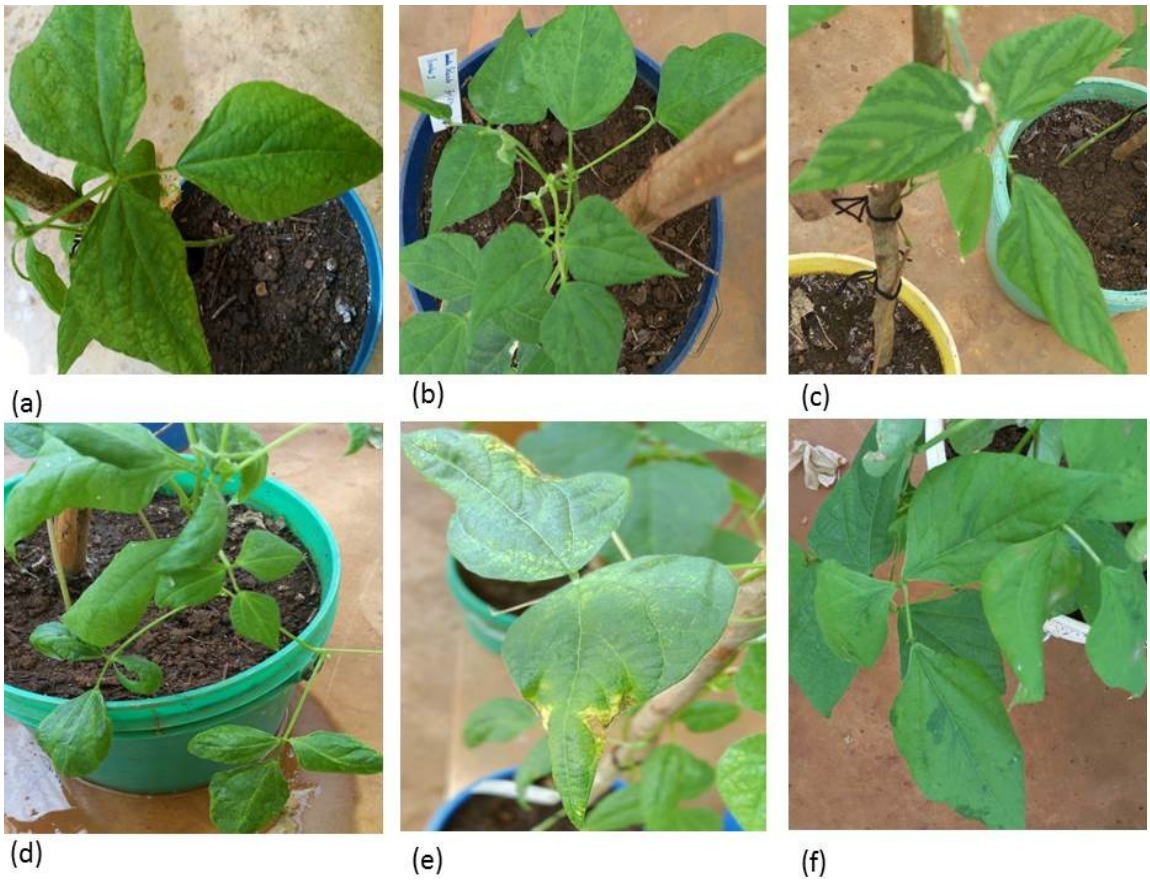

Plate 7.1: Some of symptoms observed in common bean plants infected with BCMNV isolate TZ: Maruku:2016

Shown using letters (a) to (f) are viral disease symptoms expressed in Kitenge, Selundo, Uyole 04, Kablanketi, Rosenda and Lyamungo 85 common bean genotypes, respectively. Symptoms shown in (a) are mosaic and vein greening; (b) mosaic; (c) green vein banding; (d) leaf malformation, leaf rolling and mosaic; (e) yellowing and necrosis on leaves; and (f) green patches and bristles on leaves.

Table 7.3: Symptoms expressed in each common bean genotype after BCMNV inoculation

Genotypes	Status of genotypes	Stunted	Necrosis on leaves	Mosaic	Leaf rolling/malformation	Vein yellowing	Vein greening	Recovery upper leaves	Deep green bristles	Leaf dying and detaching	Plant death
Calima	Released	+	-	+	-	-	+	-	-	-	
Canada	Landrace	+	-	+	-	-	-	-	-	-	-
Choroko	Landrace	+	-	-	+	-	-	-	+	-	-
Fibea	Released	+	-	+	-	+	+	-	-	-	-
JESCA	Released	+	-	+	-	-	+	-	-	-	-
Kablanketi fupi	Landrace	-	-	+	-	-	-	-	-	-	-
Kablanketi ndefu	Landrace	+	-	+	+	-	+	-	-	-	-
KAEMAP	Landrace	+	-	+	-	-	+	-	-	-	-
Kariasee	Landrace	-	-	+	-	+	+	-	-	-	-
kasukanywele	Landrace	+	-	+	+	-	-	-	-	-	-
kigoma	Landrace	-	-	+	+	-	-	-	-	-	-
Kisapuli	Landrace	+	-	+	-	-	+	-	-	-	-
Kitenge	Landrace	-	-	+	+	-	+	-	+	-	-
Kombati	Landrace	-	-	-	+	-	-	-	-	-	-
Local	Landrace	-	-	-	-	-	-	-	-	-	-
Lyamungo 85	Released	-	-	-	+	+	-	-	+	-	-
Selian 14	Released	-	-	+	-	-	+	-	-	-	-
Maasai red	landrace	+	-	+	+	-	+	-	-	-	-
Masunga	Landrace	+	-	+	+	-	+	-	-	-	-
Matama	Landrace	+	-	+	-	-	+	-	-	-	-
Mshindi	Released	-	-	+	-	-	+	-	-	-	-
Mwaspenjele	Landrace	-	-	-	-	-	-	-	-	-	-
Njano uyole	Released	+	-	+	+	-	+	-	-	-	-
Nyeupe	Released	+	-	+	+	+	-	-	-	-	-
Onyege	Landrace	+	-	+	-	-	-	-	+	-	-
PASI	Released	+	+	-	-	-	-	-	-	+	+
Pesa	Released	+	-	+	+	+	-	-	+	-	-
Rojo	Released	-	-	+	+	-	+	-	-	-	-
Rosenda	Released	+	+	+	+	+	-	-	-	+	-
Rozikoko	Landrace	+	-	-	+	-	+	-	+	-	-
Selian 15	Released	+	+	-	-	-	-	-	-	-	-
Selian 94	Released	-	-	-	-	-	+	-	-	-	-
Selian 97	Released	+	-	+	-	-	-	-	+	-	-
Selundo	Landrace	+	-	+	-	-	-	-	-	-	-
Soya Kombati	Landrace	-	-	+	+	-	+	-	-	-	-
Soya kijivu	Landrace	-	-	+	-	-	+	-	+	-	-
SUA90	Released	-	-	-	-	-	+	-	-	-	-
Urafiki	Released	-	-	-	+	-	+	-	-	-	-
Uyole 03	Released	+	-	+	+	-	+	-	-		-
Uyole 04	Landrace	+	-	-	-	-	+	-	-	-	-
Uyole 16	Released	-	-	+	-	-	+	-	-	-	-
Wifi nyehegera	Landrace	-	-	+	-	-	-	-	-	-	-
Zawadi	Released	+	-	+	-	-	-	-	-	-	-

+ Presence of the symptom and – is absence of the symptom

7.4.2 BCMNV disease severity and AUDPC

Results of disease severity and area under disease progress curve (AUDPC) following inoculation with BCMNV are shown in Fig. 7.1 and Fig. 7.2, respectively. The AUDPC and disease severity values were significantly different ($P < 0.001$) between different common bean genotypes (Appendix 7.1).

The disease symptoms in common bean plants appeared from the 5th day after inoculation but most of symptoms were observed from the 7th day (Fig. 7; Appendix 7.1). The disease severity recorded over time was high for PASI, Pesa, Rosenda, Kombati and Rojo genotypes. For most common bean genotypes the disease severity reached the peak at around 22nd days and decreased thereafter. However, for three common bean genotypes, namely PASI, Rosenda and Pesa, the disease severity remained over 60% from 22 days to the end of data collection (42 days). At 37 days post inoculation (dpi), lower disease severity values (< 21%) were observed in SUA 90, Kablanketi fupi, Selian 14 and Selian 05 genotypes. Remarkably, in SUA 90, disease severity was below 15% by 27 dpi.

The AUDPC obtained after inoculation with BCMNV ranged from 414 to 2 667. None of the common genotypes showed complete resistance to BCMNV (Fig 7.2; Appendix 7.1). Moderate resistance to BCMNV (Farooq *et al.*, 2018) was observed in SUA 90, Kablanketi fupi, Selundo, Selian 05, Wifi nyehegera, Canada, Lyamungo 85, Kisapuli, Uyole 04, Soya Kombati, Zawadi, Selian 14 and Kasukanywele common bean genotypes, which exhibited AUDPC values ranging from 414 to 1 000. A total of 13 common bean genotypes had AUDPC ranging from 1 000 to 1 200 and these were moderate susceptible. The common bean genotypes that had AUDPC value greater than 1 200 were susceptible to BCMNV (Fig 7.2; Appendix 7.1).

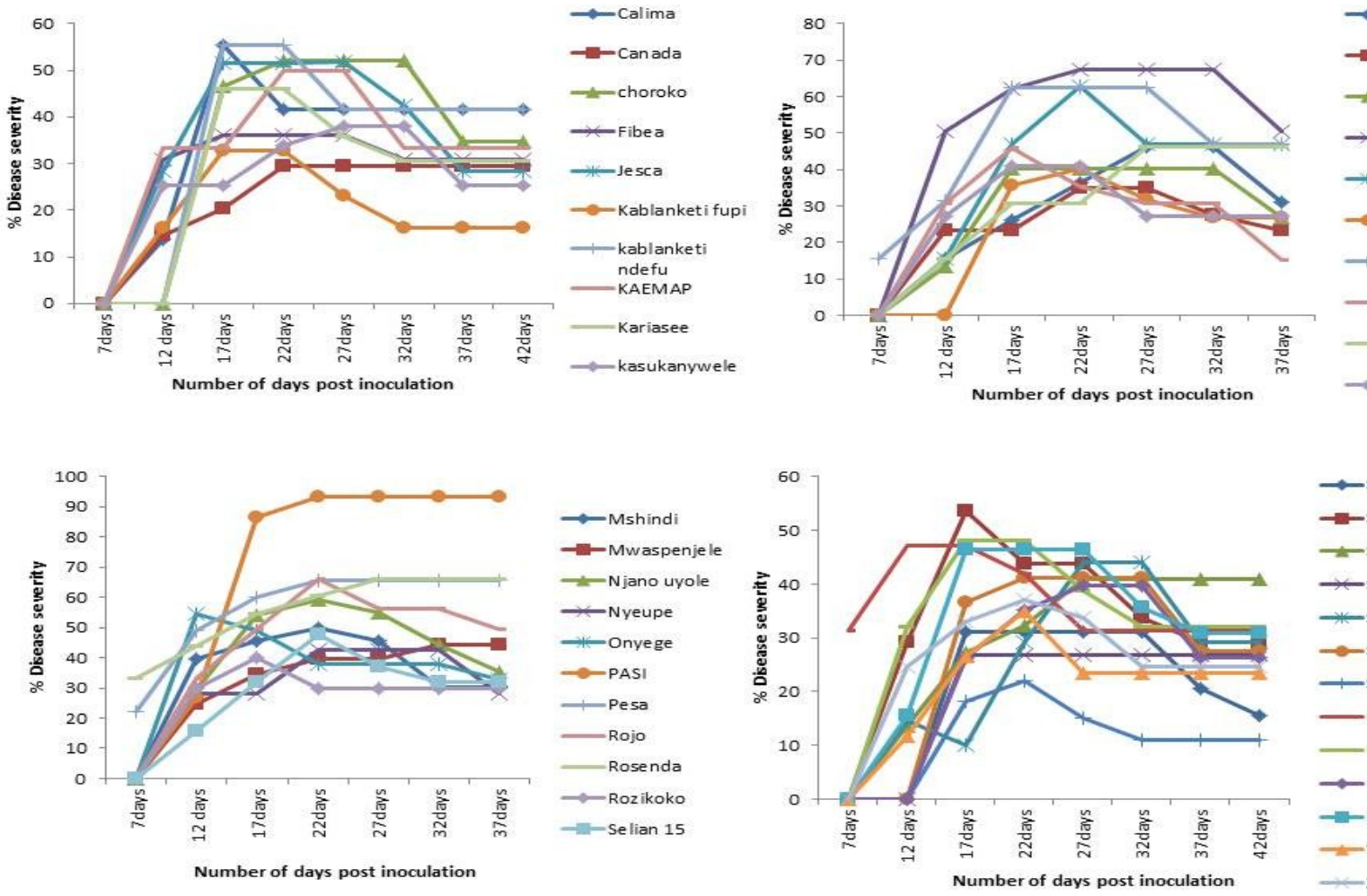

Figure 7.1: Disease severity observed on the common bean genotypes inoculated with BCMNV

For clarity, the severity data of common genotypes were plotted on different graphs. The data for these graphs are also shown in Appendix 7.1.

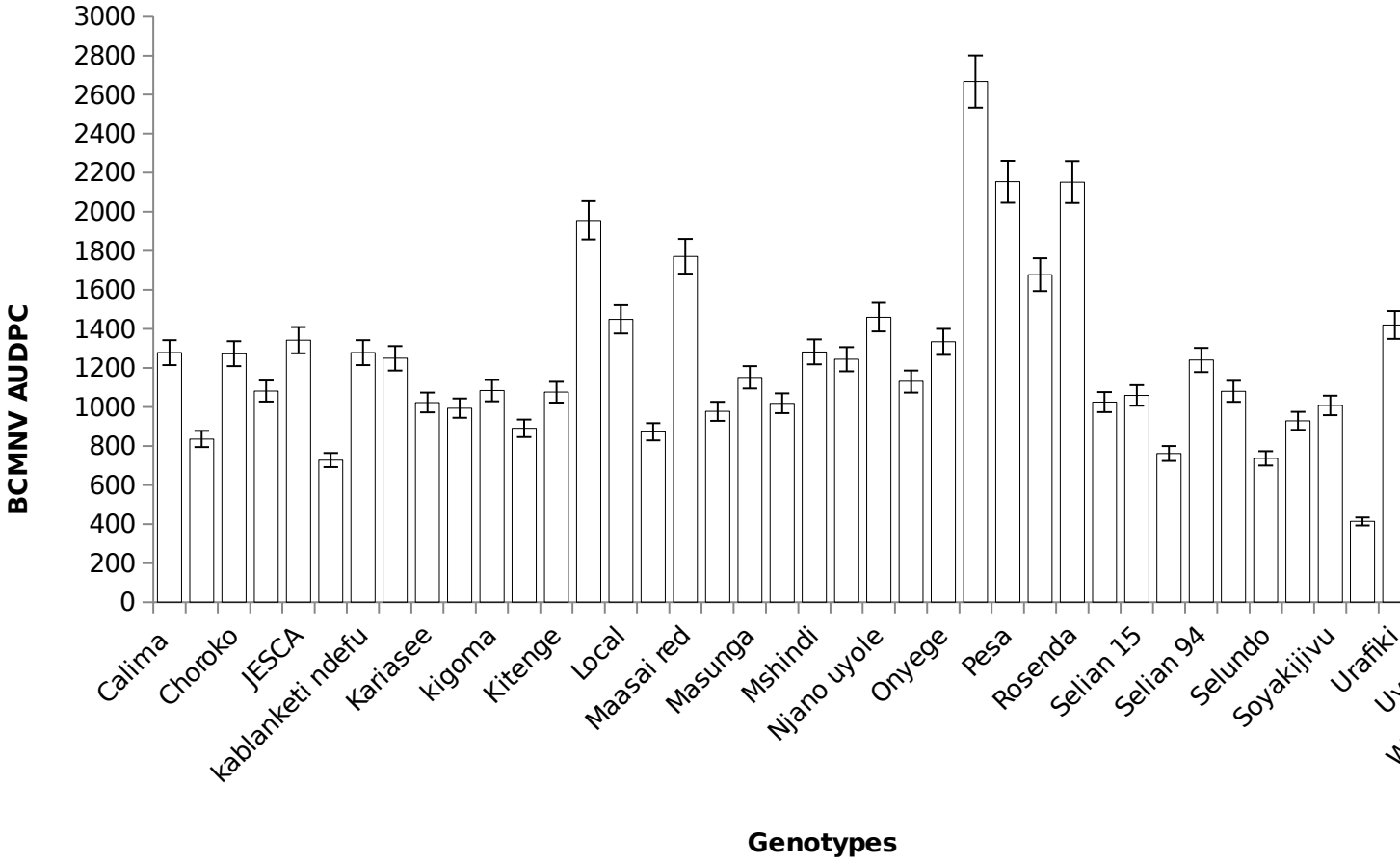

Figure 7. 2: Area under disease progress curve of common bean genotypes infected with BCMNV

Error bars represent ±5% standard error among common bean genotypes infected with BCMNV

7.4.3 BCMV disease symptoms expressed in different common bean genotypes

A total of 25 common bean genotypes were inoculated with BCMV. According to results shown in Plate 7.2 and Table 7.4, the common bean genotypes that were inoculated with BCMV (Isolate TZ:SUA1:2017), expressed different symptoms in different common bean genotypes. The symptoms observed were mosaic, vein banding, vein chlorosis, leaf rolling, leaf bristles, yellowing and chlorotic patches. Some common bean genotypes showed signs of recovery from BCMV disease symptoms (reduction in virus titres in plant tissues was not confirmed). Examples of the common bean genotypes, which recovered from BCMV disease symptoms, were Rosenda, Zawadi, Pesa, SUA 90 and Mshindi. Two common bean genotypes did not express any symptoms and these were Fibea and Selian 05. The most common symptom expressed on common bean genotypes inoculated with BCMV was mosaic, which occurred in plants of 22 out 25 common bean genotypes inoculated with this virus.

Plate 7.2: Some of symptoms observed on common bean plants infected with BCMV

The common bean genotypes represented by letters a, b and c are Rozikoko, Nyeupe and Calima, respectively. They expressing different symptoms upon BCMV infection: (a) and (b) mosaic and yellowing symptoms, and (c) vein banding symptom.

4: Symptoms expressed in each of twenty-five common bean genotypes after BCMV inoculation

Genotype	Mosaic	Systemic mosaic	Severe mosaic	Vein banding	Vein chlorosis	Leaf rolling	Leaf bristles	Plant recovery	no symptoms	Chlorotic patches	Yellowing
Rozikoko	+	-	-	+	+	+	-	-	-	-	-
Kitenge	+	-	-	-	-	-	-	-	-	-	-
Kombati	+	-	-	-	-	-	-	-	-	-	+
Uyole 96	+	-	-	+	-	-	-	-	-	-	-
Urafiki	+	-	-	+	-	+	-	-	-	-	-
Fibea	-	-	-	-	-	-	-	-	+	-	-
Rosenda	+	-	-	-	-	-	-	+	-	-	-
PASI	+	+	+	-	-	-	-	-	-	-	+
Nyeupe	+	+	-	+	-	-	+	-	-	-	+
Calima	+	-	-	+	+	-	-	-	-	-	-
Njano uyole	+	-	-	-	+	+	-	-	-	-	-
Selian 15	+	-	-	-	-	+	-	-	-	-	-
Uyole 16	+	-	-	-	+	-	-	-	-	-	-
JESCA	+	-	-	-	-	+	-	-	-	-	-
Selian 94	+	+	+	-	+	-	-	-	-	-	-
Selian 97	+	-	-	+	+	-	-	-	-	-	-
Rojo	-	-	-	+	-	-	-	-	-	+	-
Zawadi	+	-	-	+	-	-	-	+	-	-	-
Selian 05	-	-	-	-	-	-	-	-	+	-	-
Lyamungo 85	+	-	-	-	+	+	+	-	-	-	-
Lyamungo 90	+	-	-	-	-	-	-	-	-	+	-
Pesa	+	-	-	-	-	-	-	+	-	-	-
SUA 90	+	-	-	-	-	-	-	+	-	-	+
Mshindi	+	-	-	-	-	-	-	+	-	-	+
Kablanketi fupi	+	-	-	-	+	-	-	-	-	-	-

+ Presence of symptom and – absence of symptom

7.4.4 BCMV disease severity and AUDPC

The % disease severity and AUDPC results are shown in Fig. 7.3; Fig. 7.4; Appendix 7.2. These results showed that there was significant difference at (P = 0.001) between common bean genotypes at all time points the disease assessment was done. No disease development was observed in the Fibea and Selian 05 genotypes (Fig. 7.3; Appendix 7.2). Most of common bean genotypes developed symptoms within one-week post inoculation. For example, in PASI and Selian 94 common bean genotypes, the % disease severity was above 38% at 7dpi (Fig. 7.3). It took 12 days for three genotypes – SUA 90, Selian 15 and Lyamungo 85 to express visible symptoms. Some common bean genotypes developed severe disease symptoms at early days from inoculation but later the plants showed signs of recovery from the disease. For example, at early stage, Rosenda genotype had the highest peak of disease severity (44%) but later the plant fully recovered from disease symptoms (Fig.7.3). However, in some of common bean genotypes, for example SUA 90, the rate of disease increase was low from the beginning and the infected plants eventually recovered from disease symptoms (Fig. 7.3; Appendix 7.2). Recovery from disease symptoms (that is, 0% disease severity) occurred from 27 to 37 dpi for Rosenda, Selian 05, SUA 90, Pesa and Zawadi common bean genotypes. For all other common bean genotypes, disease severity remained above 6%. Njano uyole had highest % disease severity (45%) at the end of data taking (37 dpi).

In this experiment, AUDPC ranged from 0 to 1 586.7. Out of 25 common bean genotypes studied, two genotypes (Fibea and Selian 05) had an AUDPC of 0, that is, did not develop any visible symptoms throughout the experiment. Fifteen common

bean genotypes were moderately resistant to BCMV since their AUDPC ranged from 124 to 953.7 (Farooq *et al*., 2018). The genotypes with AUDPC values in this range were SUA 90, Lyamungo 85, Selian 15, Lyamungo 90, Mshindi, Zawadi, Pesa, Rosenda, Rojo, JESCA, Kablanketi fupi, Nyeupe, Uyole 16, Uyole 96 and Njano uyole. The genotypes Calima, Rozikoko and Urafiki were moderately susceptible while Kitenge, Kombati, Selian 97, PASI and Selian 94 were susceptible to BCMV (Fig 7.4; Appendix 7.2).

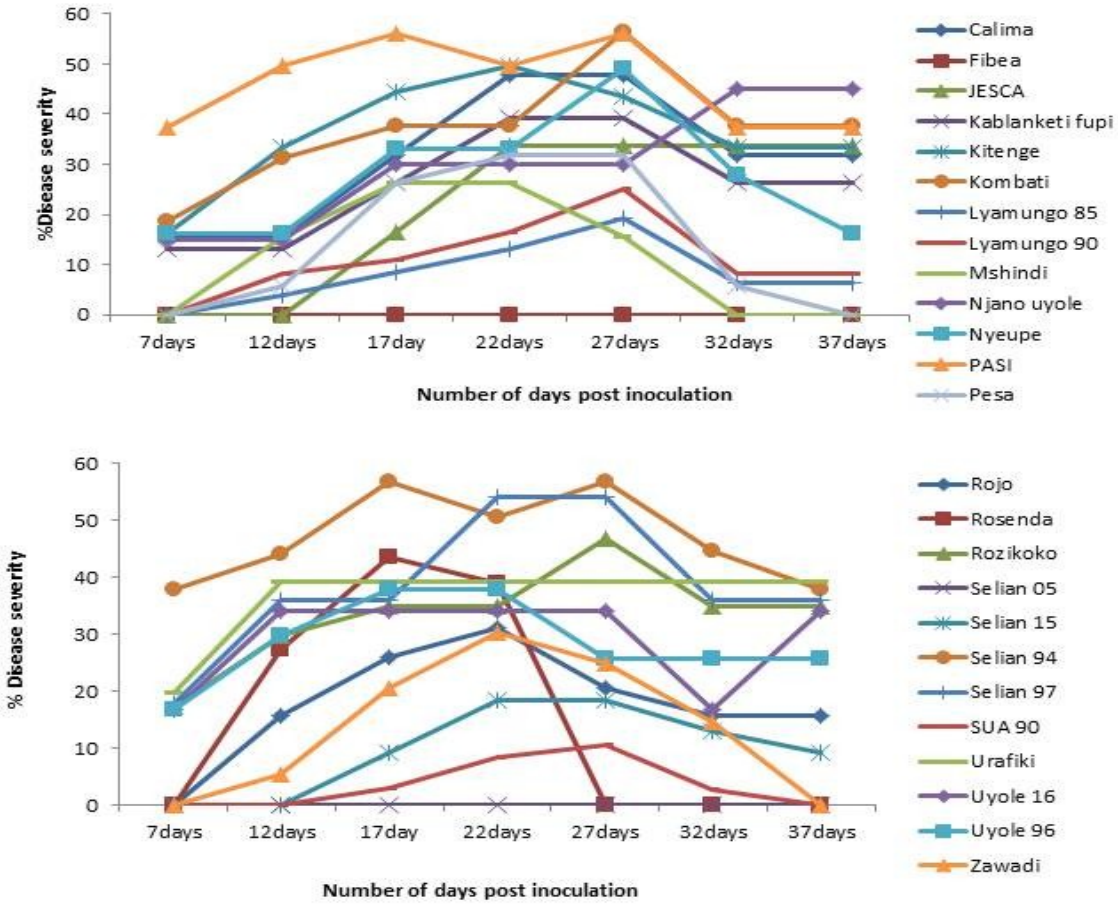

Figure 7.3: Disease severity observed on the common bean genotypes inoculated with BCMV

For clarity, the data was presented in two graphs

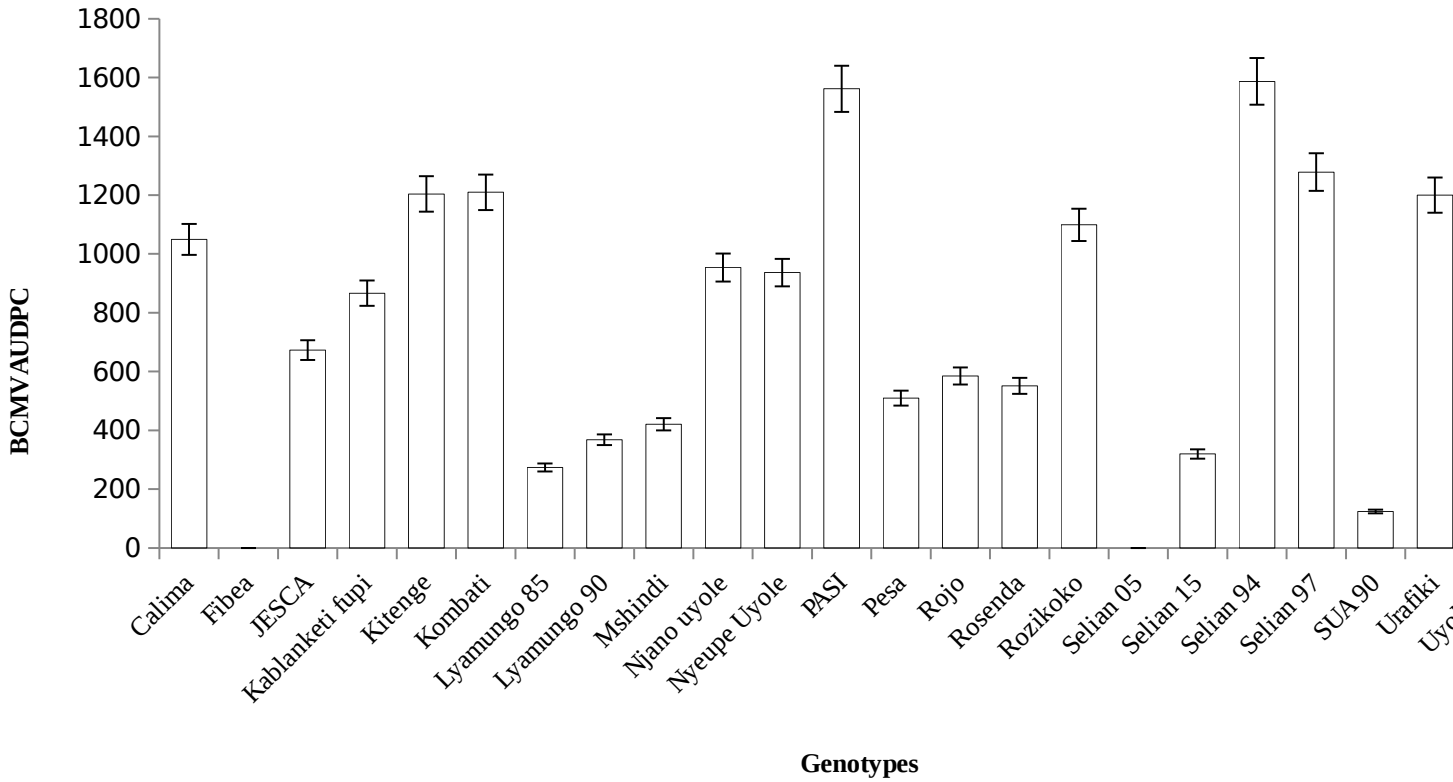

Figure 7.4: Area under disease progress curve of common bean genotypes infected with BCMV

Error bars represent ±5% standard error around sample means, the Tukey's test $_{(p=0.05)}$ was used for means separation

7.4.5 CPMMV disease symptoms expressed in different common bean genotypes

Experiments for studying response of common bean genotypes to CPMMV were set up at SUA in Morogoro (Location G in Fig.2.1) and TARI-Selian in Arusha (Location D in Fig 2.1). Disease symptoms observed in plants of different common bean genotypes following inoculation with CPMMV isolate TZ:CHANGARAWE: 2016 are presented in Plate 7.3 and also summarized in Table 7.5. The symptoms observed were mosaic, yellowing, necrosis, stunted growth, vein greening, vein yellowing and leaf malformation. The most distinguishing symptom of CPMMV was vein clearing (Plate 7.3c). Sometime there was tissue clearing. Vein clearing was observed in all plants and all genotypes (Table 7.5). The second commonest disease symptom of CPMMV was mosaic. It occurred in 23 out of 26 common bean genotypes. The symptoms observed in CPMMV infected plants were the same in all genotypes at both stations (Arusha and Morogoro).

Plate 7.3: Some of symptoms observed on common bean plants infected with CPMMV

The common bean genotypes shown in a, b and c are Mshindi, Pesa and Rozikoko, respectively. All plants show mosaic and vein clearing. Leaf rugosity was observed in plate b only.

Table 7.5: Symptoms expressed in each common bean genotype after CPMMV inoculation

Common bean Genotypes	Mosaic	Yellowing	Necrosis	Vein clearing	Stunted growth	Vein greening	Vein yellowing	Leaf Malformation	Recovery from disease
Calima	-	+	-	+	-	-	-	-	-
JESCA	+	-	-	+	-	-	-	-	-
Kablanketi fupi	-	+	-	+	-	-	-	-	-
Kablanketi ndefu	+	-	-	+	-	-	-	-	-
Kigoma	+	-	+	+	-	-	+	-	-
Lyamungo 85	+	-	-	+	-	-	-	-	-
Lyamungo 90	-	+	-	+	-	-	-	-	-
Maasai red	+	-	-	+	-	-	-	-	-
Kasukanywele	+	-	-	+	-	-	-	-	-
Mshindi	+	-	-	+	-	-	-	-	-
Mwaspenjele	+	-	+	+	-	-	-	-	-
Njano uyole	+	+	-	+	-	-	-	-	-
PASI	+	+	-	+	-	-	-	-	-
Pesa	+	+	-	+	-	-	-	-	-
Rojo	+	-	-	+	-	-	-	-	-
Rosenda	+	+	-	+	+	-	-	-	-
Rozikoko	+	+	+	+	-	-	-	-	-
Selian 05	+	+	+	+	-	-	-	-	-
Selundo	+	-	+	+	+	-	-	+	-
SUA 90	+	-	-	+	-	-	-	-	-
Uyole 04	+	-	-	+	-	+	-	-	-
Uyole 96	+	+	-	+	-	-	-	-	-
Zawadi	+	-	-	+	-	-	-	-	-
Cheupe	+	+	-	+	-	-	-	-	-
Choroko	+	-	-	+	-	-	-	-	-
Urafiki	+	+	-	+	-	-	-	-	-

+ means presence of symptom and – is absence of symptoms.

7.4.6 CPMMV disease severity and area under disease progress

According to results presented in Fig 7.5; Fig 7.6; Fig.7.7; Appendix 7.3 and Appendix 7.4, there was statistically significant difference (P = 0.001) in disease severity and area under disease progress (AUDPC) between common bean genotypes inoculated with CPMMV, both in Arusha and Morogoro. However, there no significant difference was observed on % disease severity of genotypes at 7^{th} day (P = 0.588) and 12^{th} day (P = 0.336) when the experiment was set up in Morogoro (Appendix 7.3). The disease development in common bean genotypes were assessed after every five days. For trials conducted at the two stations (Arusha and Morogoro), there was no any common bean genotype with complete resistance to CPMMV; most

of the common bean genotypes expressed disease symptoms between seven- and twelve-days post inoculation (Fig.7.5and 7.6).

In Arusha, disease severity reached up to 77% (Rosenda) but in most genotypes it was less than 52% (Fig. 7.5). The common bean genotypes which had higher than 50% disease severity were Rosenda, Kasukanywele, Uyole 04 and Selundo. The common bean genotypes with less than 30% were Calima, SUA 90 and Rozikoko (Fig. 7.5). In Morogoro, disease severity for CPMMV was less than 50%, whereby the common bean genotypes with more than 30% disease severity were Lyamungo 85, Uyole 04, Cheupe and Urafiki. The common bean genotypes with less than 30% disease severity were SUA 90, JESCA, Rozikoko, Maasai red and Mwaspenjele (Fig. 7.6).

The AUDPC for common bean genotypes grown in Arusha ranged from 105.6 to 1 561.7 (Fig 7.7a) while for experiment set up in Morogoro it ranged from 412.2 to 1 106.5 (Fig. 7.7b). Calima, SUA 90, Pesa, Kablanketi fupi, Mshindi, Maasai red, Rozikoko, Kablanketi ndefu, Choroko, Uyole 96, Kigoma, Mwaspenjele, JESCA, PASI, Lyamungo 90, Selian 05, Njano uyole, Rojo, Urafiki and Lyamungo 85 were moderatly resistant to CPMMV.

Performance of some common bean genotypes differed when they were grown in two different locations. For example, Kasukanywele genotype exhibited moderate susceptibility in Arusha but was moderately resistant to CPMMV when was grown in Morogoro region. Also, Rosenda, Uyole 04, and Selundo common bean genotypes were very susceptible to CPMMV when planted in Arusha but the same genotypes were moderately susceptible (Uyole 04) to moderately resistant (Selundo and Rosenda) when they were planted in Morogoro (Fig. 7.7).

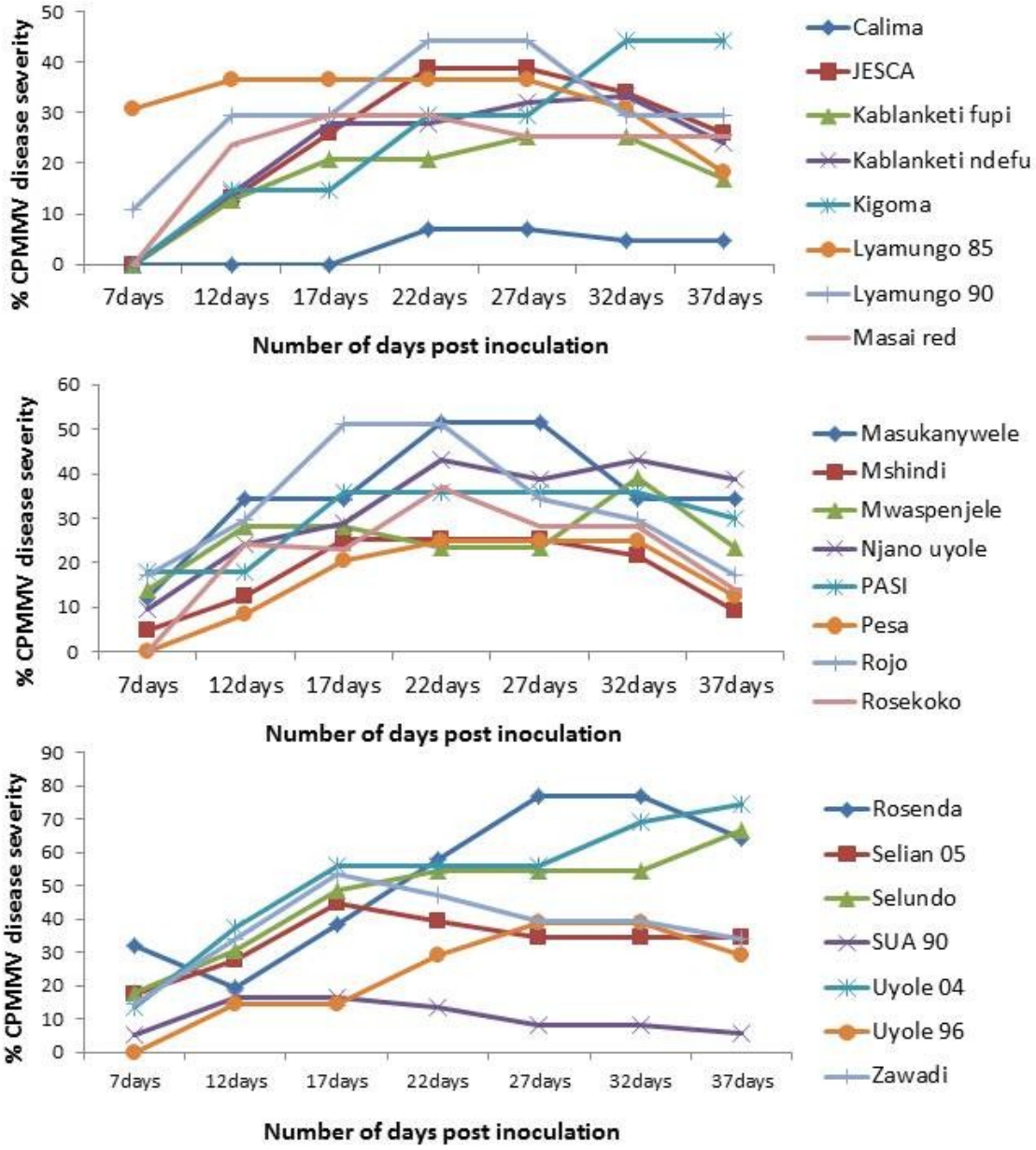

Figure 7.5: Disease severity of common bean genotypes infected with CPMMV as observed at TARI-Selian in Arusha

The data is split into three graphs for clarity. (a) and (b) are the line graphs drawn from experiments set at TARI – Selian screen house in Arusha

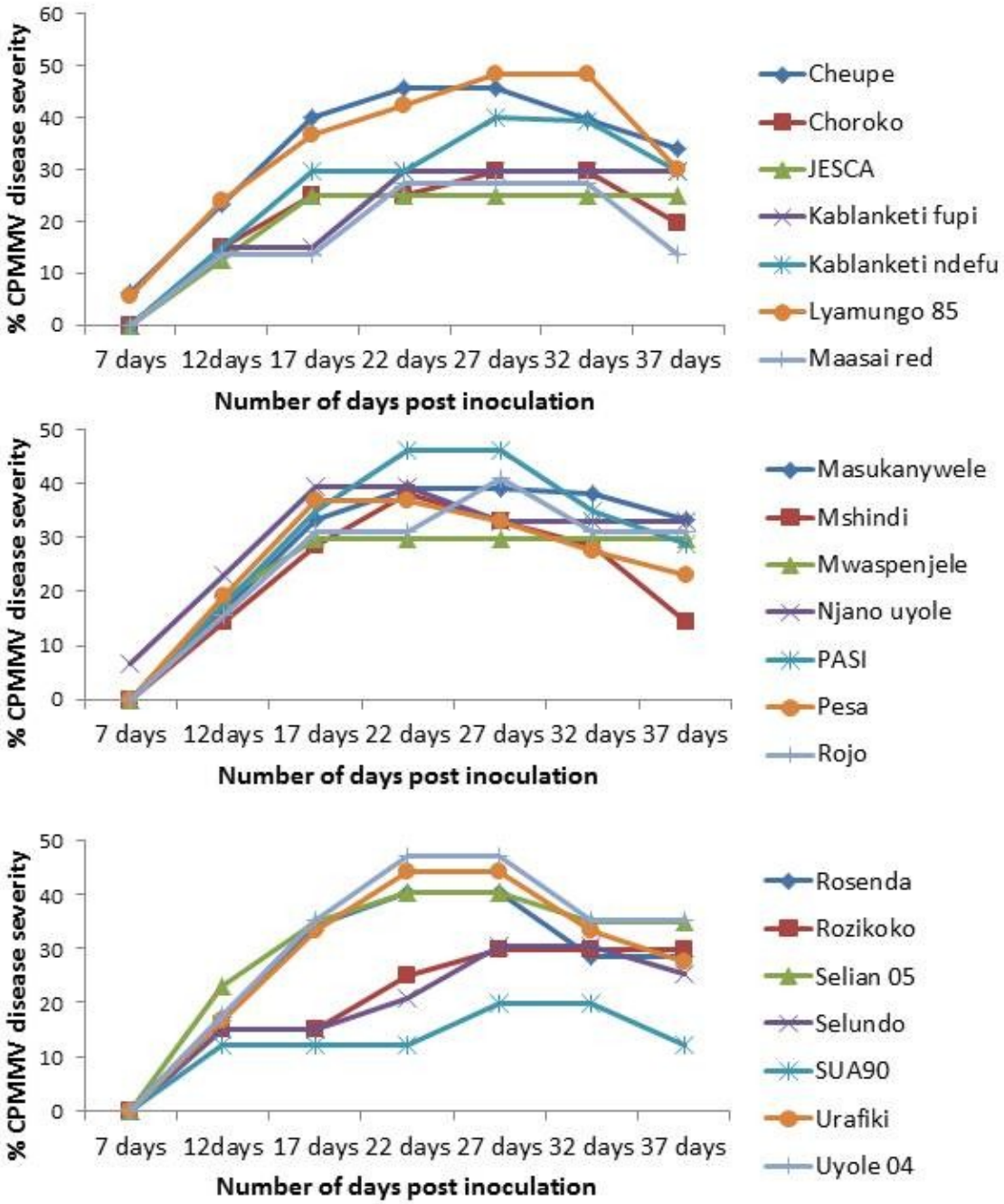

Figure 7.6: Disease severity of common bean genotypes infected with CPMMV as observed at SUA in Morogoro

(a) and (b) are the line graphs drawn from experiments set at Sokoine University of Agriculture in Morogoro respectively.

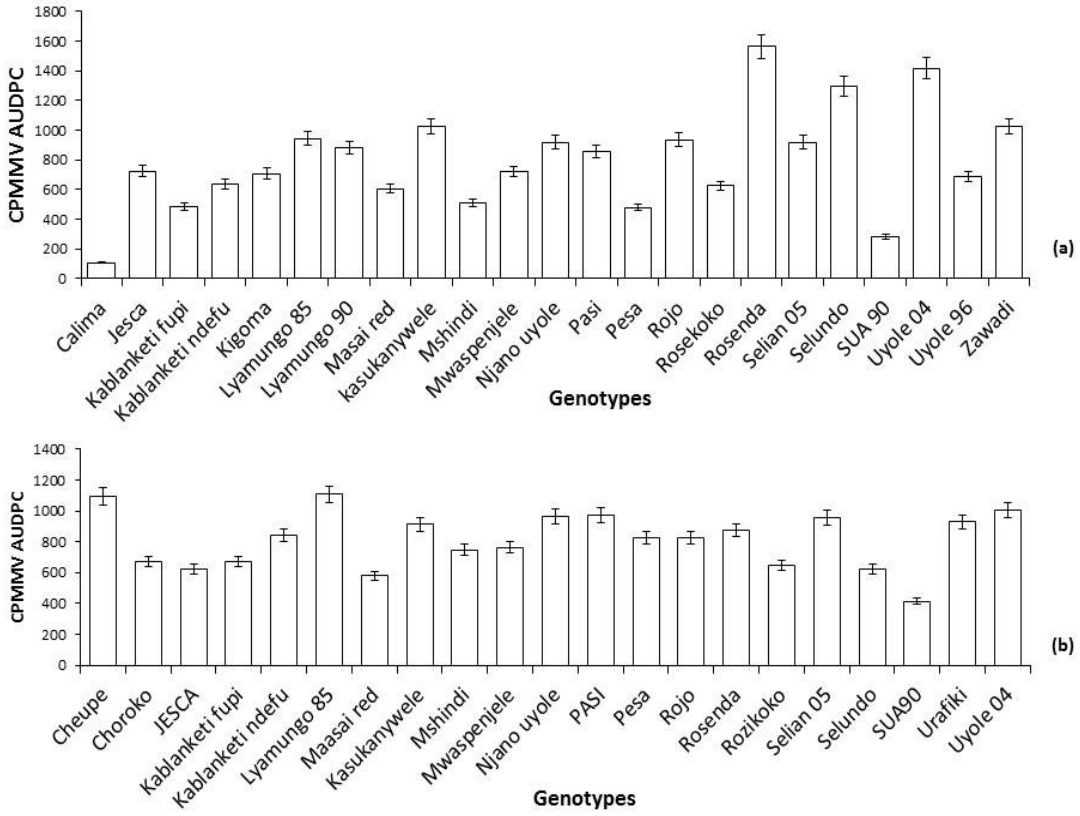

Figure 7.7: Area under disease progress curve of common bean genotypes infected with CPMMV

(a) and (b) are the bar graphs drawn from experiments set at TARI – Selian screen house in Arusha and Sokoine university of agriculture in Morogoro respectively. Error bars represent ±5% standard error around sample means, the turkey $_{(p=0.05)}$ was used for means separation.

7.4.7 SBMV disease symptoms expressed in different common genotypes

According to results presented in Plate 7.4 and Table 7.6, SBMV caused different viral disease symptoms in plants of different common bean genotypes: mosaic, chlorosis on leaves, stunted growth, leaf and pod malformation, leaf death, plant death, and necrosis on leaves, stem and pods. Necrosis mainly occurred along mid ribs or veins and in some common bean genotypes it was manifest as necrotic spots. Necrosis on tips of young growing plants caused die back symptoms. SUA 90 and Lyamungo 90 genotypes showed signs of recovery from the SBMV disease symptoms. Stunted growth (22 genotypes), necrosis (15 genotypes), mosaic (18 genotypes) and necrosis on leaves were the commonest SBMV disease symptoms in

plants of many common bean genotypes. Necrosis was also observed on pods especially in Njano uyole, Pesa, PASI and Rosenda genotypes (Plate 7.4). SBMV showed profound effect on pod formation. For example, plants of PASI genotype were severely affected and very few pods were formed and some of the pods of plants of this genotype did not form any seeds (Plate 7.5).

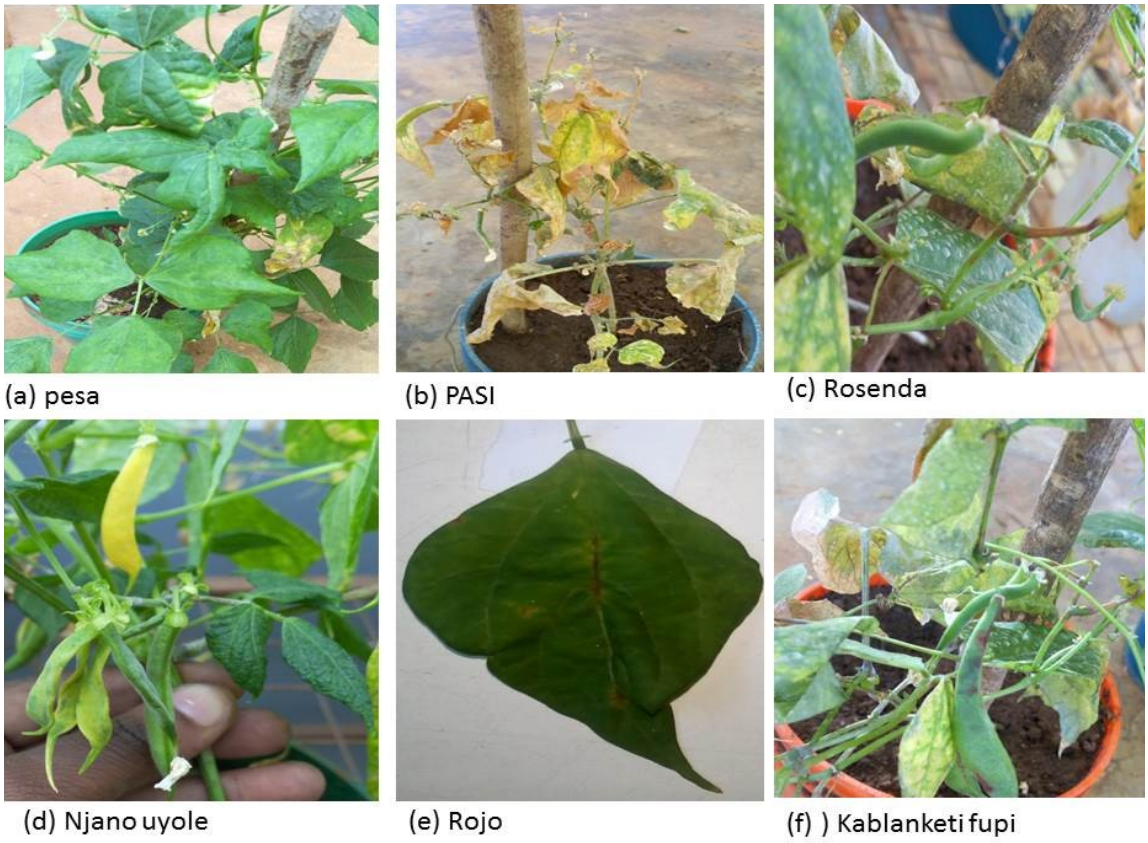

Plate 7.4: Symptoms observed on common bean plants infected with SBMV
Inoculated common bean genotypes represented with letters a to f are Pesa, PASI, Rosenda, Njano uyole, Rojo and Kablanketi fupi, respectively. The symptoms observed were (a) leaf necrosis which also caused leaf rolling; (b) plant death following necrosis and stunted growth; (c) yellowing (chlorotic patches) and stem necrosis on leaf petiole; (d) weakened pod formation; (e) necrosis and yellowing on leaves while (f) expressed necrosis on pods and yellowing of the plant.

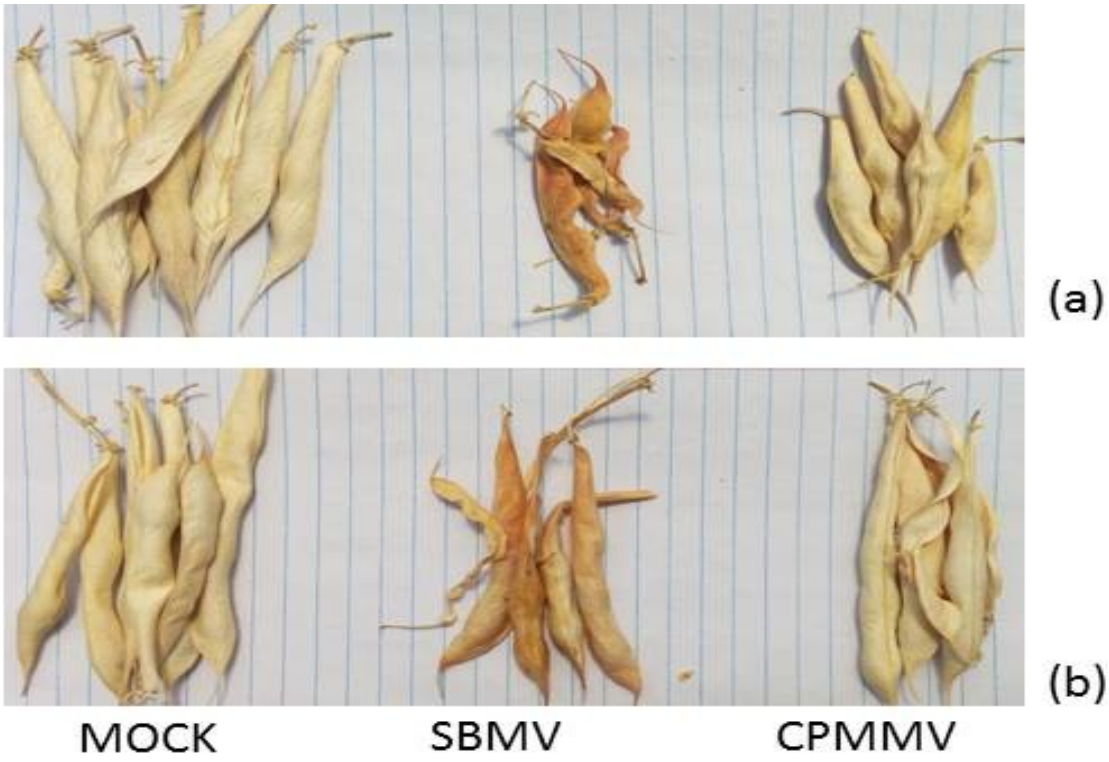

Plate 7.5: The effects of SBMV and CPMMV infections on common bean pods formation

(a) Pods harvested from PASI common bean genotype at TARI-Selian screen house and (b) pods harvested from Njano uyole common bean varieties. In (a) and (b), pods taken from mock inoculated common bean plants are shown in the left-hand sides and are labelled.

Table 7.6: Symptoms expressed in each common bean genotype after inoculation with SBMV

Genotypes	Mosaic	Yellowing	Necrosis			Stunted growth	Malformation		Leaf death	Plant death	Recovery from disease symptoms
			leaves	Stem	Pod		leaves	pods			
Calima	+	+	-	-	-	-	-	-	-	-	-
JESCA	+	+	+	+	-	+	+	-	-	-	-
Kablanketi fupi	+	-	-	-	-	+	-	-	-	-	-
Kablanketi ndefu	+	-	+	-	-	+	+	-	-	-	-
Kigoma	-	+	+	-	-	+	-	-	-	-	-
Lyamungo 85	+	-	-	-	-	-	-	-	-	-	-
Lyamungo 90	+	-	+	-	-	+	+	-	-	-	-
Maasai red	+	-	-	-	-	+	+	-	-	-	-
Kasukanywele	-	-	-	-	-	+		-	-	-	-
Mshindi	+	+	+	-	-	+	+	-	-	-	-
Mwaspenjele	+	-	+	+	-	+		-	+	-	-
Njano uyole	-	-	+	-	+	+	+	+	-	-	-
PASI	-	+	+	+	+	+	+	+	+	+	-
Pesa	+	+	+	+	+	-	+	+	-	-	-
Rojo	+	-	+	+		-		-	-	-	-
Rosenda	+	-	+	+	+	+	+	+	-	-	-
Rozikoko	+	+	+	-	-	+		-	-	-	-
Selian 05	+	-	-	-	-	+	+	-	-	-	-
Selundo	-	-	-	-	-	+	-	-	-	-	-
SUA 90	+	-	-	-	-	+	-	-	-	-	-
Uyole 04	+	+	-	-	-	+	-	-	-	-	-
Uyole 96	-	-	+	+	-	+	-	-	-	-	-
Zawadi	-	+	+	-	-	+	-	-	-	-	-
Cheupe	+	-	-	-	-	+	-	-	-	-	-
Choroko	-	-	+	-	-	+	-	-	-	-	-
Urafiki	+	-	-	-	-	+	-	-	-	-	-

+ Presence of symptom and – absence of symptom

7.4.8 SBMV disease severity and area under disease progress

According to analysis, the disease severity and AUDPC in common bean genotypes caused by SBMV at both experimental sites (TARI-Selian and SUA) were significantly different (P = 0.001) (Appendix 7.5; Appendix 7.6). In this study, no any common bean genotype showed complete resistant to SBMV. All genotypes developed SBMV disease symptoms (Fig 7.8; Fig 7.9 and Fig.7.10). Disease symptom development reached a peak starting from around day 17 for plants evaluated in experiment that was set up in Arusha. The highest peak (77%) was

observed in plants of Mwaspenjele and Rosenda genotypes (Fig. 7.8). In Morogoro, the disease severity peaks were observed starting from 22 dpi.

In Arusha, the highest disease severity was high as 77%, but most genotypes had less than 50% disease severity. The common bean genotypes which had more than 50% disease severity were Rosenda, PASI, Pesa, JESCA and Njano uyole. The genotypes with low disease severity of less than 20% were SUA 90, Uyole 04 and Lyamungo 85 (Fig. 7.8; Appendix 7.5). Morogoro, the disease severity reached up to 100% in PASI genotype but all other common bean genotypes had less than 64% disease severity. The common bean genotypes with more than 50% disease severity were Pesa, Cheupe Uyole Rosenda, Rojo and Urafiki. The genotypes with less than 30% disease severity were Choroko, Selian 05, Selundo and Lyamungo 85 (Fig.7.9; Appendix 7.6)

The AUDPC ranged from 513 to 1 808 for genotypes planted in Arusha (Fig. 7.10a; Appendix 7.5) while for genotypes planted in Morogoro it ranged from 506 to 2 037 (Fig. 7.10b; Appendix 7.6). For experiment set up in Arusha, out of 22 common bean genotypes, nine genotypes showed moderate resistant to SBMV; these genotypes were SUA 90, Lyamungo 85, Kablanketi fupi, Rozikoko, Uyole 04, Selundo, Zawadi and Maasai red. Eight genotypes were moderately susceptible. These were Kablanketi ndefu, Selian 05, Mshindi, Calima, Kigoma, Kasukanywele, Rojo and JESCA. On the other hand, six genotypes (Uyole 96, Njano uyole, Pesa, Mwaspenjele, PASI and Rosenda) were susceptible to SBMV (Fig. 7.10a; Appendix 7.5). In Morogoro, out of 21 genotypes inoculated with SBMV, eight genotypes showed moderate resistance to this virus. The genotypes with moderate resistance to SBMV were Choroko, Selian 05, Lyamungo 85, Selundo, SUA 90, Maasai red, Kablanketi fupi and Kasukanywele.

The rest of common bean genotypes were susceptible to SBMV (Fig. 7.10b; Appendix 7.6). However, some genotypes, for example, Selian 05 were moderately resistant in the experiment that was conducted in Morogoro but were moderately susceptible in Arusha. PASI was the most susceptible common bean genotype to SBMV in Morogoro environment.

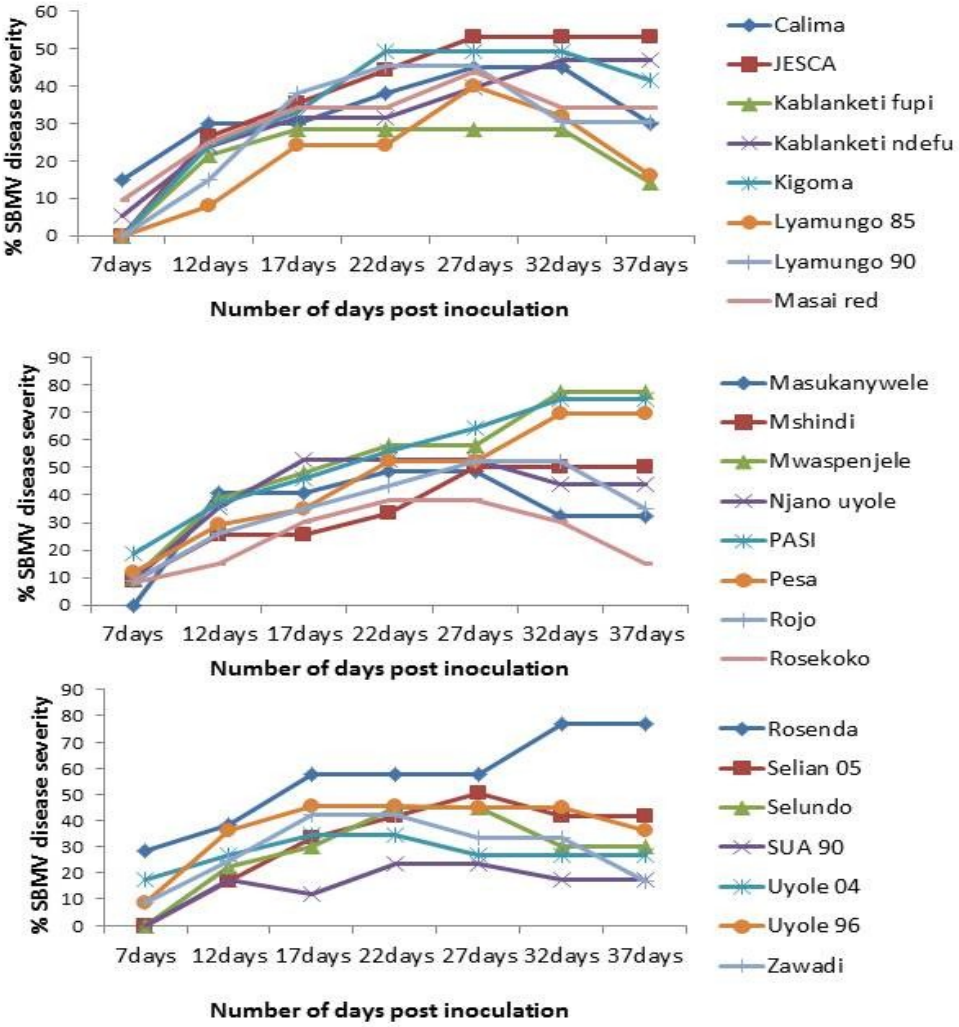

Figure 7.8: Disease severity on common bean genotypes inoculated with SBMV at TARI-Selian in Arusha

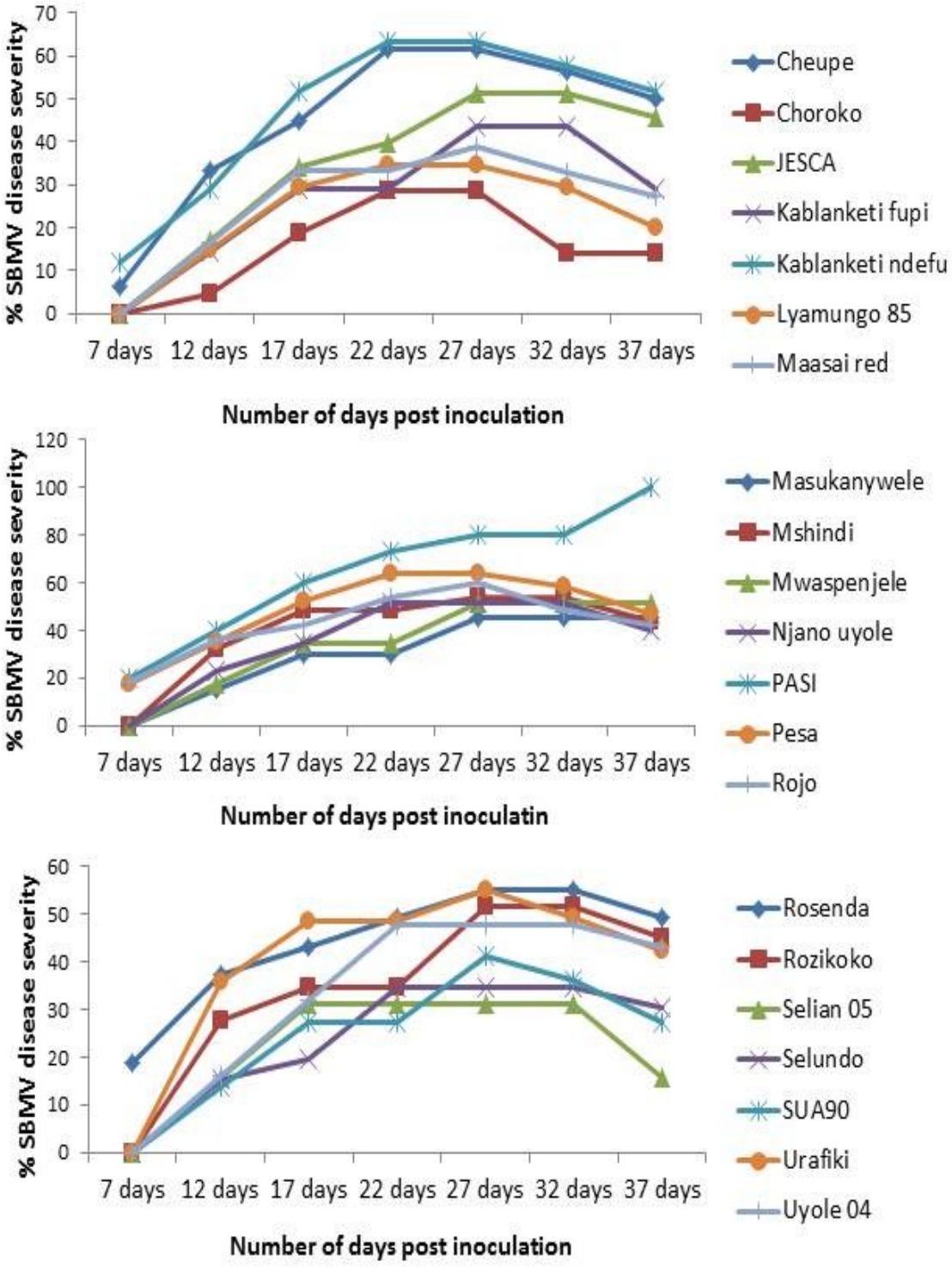

Figure 7.8: Disease severity on common bean genotypes inoculated with SBMV at SUA in Morogoro

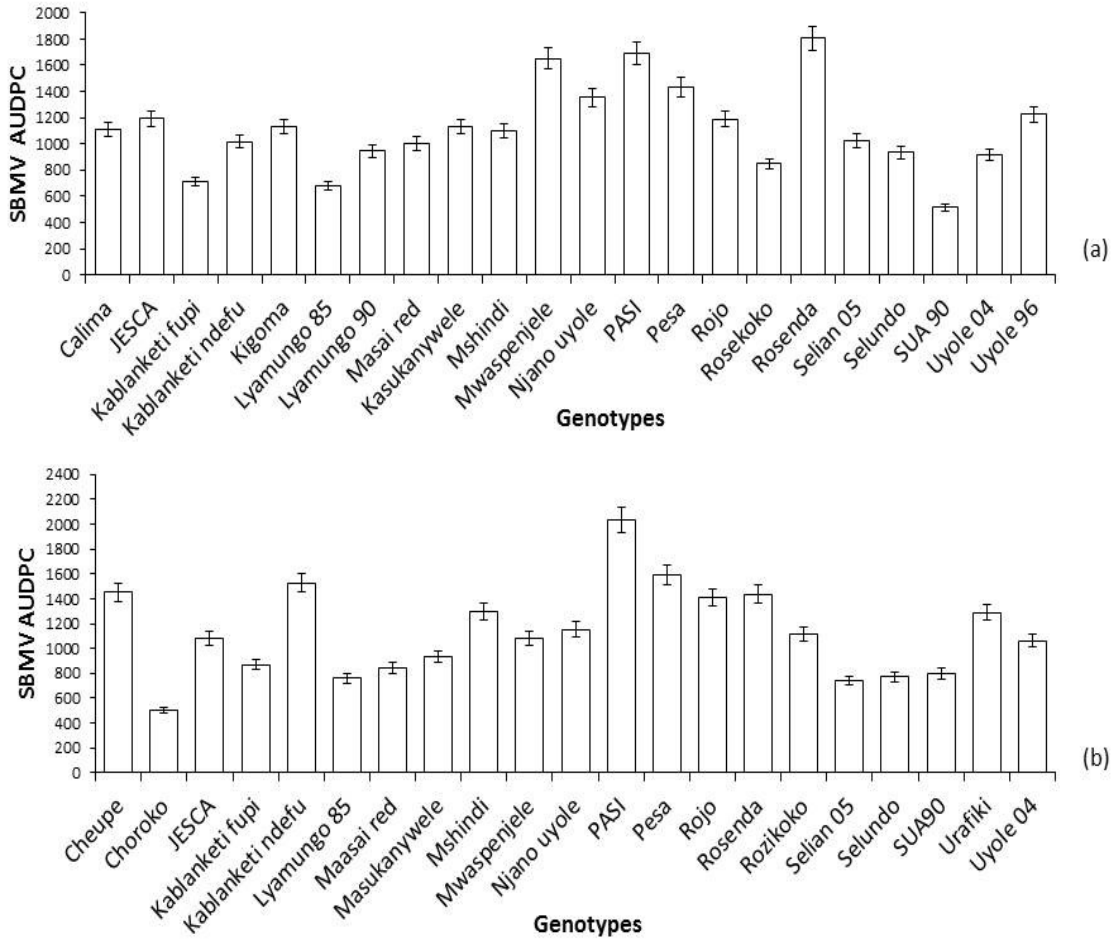

Figure 7.9: Area under disease progress curve of common bean genotypes infected with SBMV

(a) and (b) are the bar graphs obtained from analysis of data from experiments set up at TARI – Selian in Arusha and Sokoine University of Agriculture in Morogoro, respectively. Error bars represent ±5% standard error around sample means, the Tukey's test $_{(p=0.05)}$ was used for means separation.

7.4.9 RT-PCR based confirmation of viral infections

Reverse transcription polymerase chain reaction (RT-PCR) was used to confirm BCMNV, BCMV, SBMV and CPMMV in inoculated plants (Fig. 7.11). RNA was extracted from common bean leaf samples of all plants that were inoculated. In all plants that showed symptoms, all four viruses were detected. On the other hand, and as expected, BCMV was not detected in RNA extracted from leaf samples collected from plants of two genotypes (Selian 05 and Fibea), which never showed any viral symptoms. (Table 7.7 and Fig 7.11).

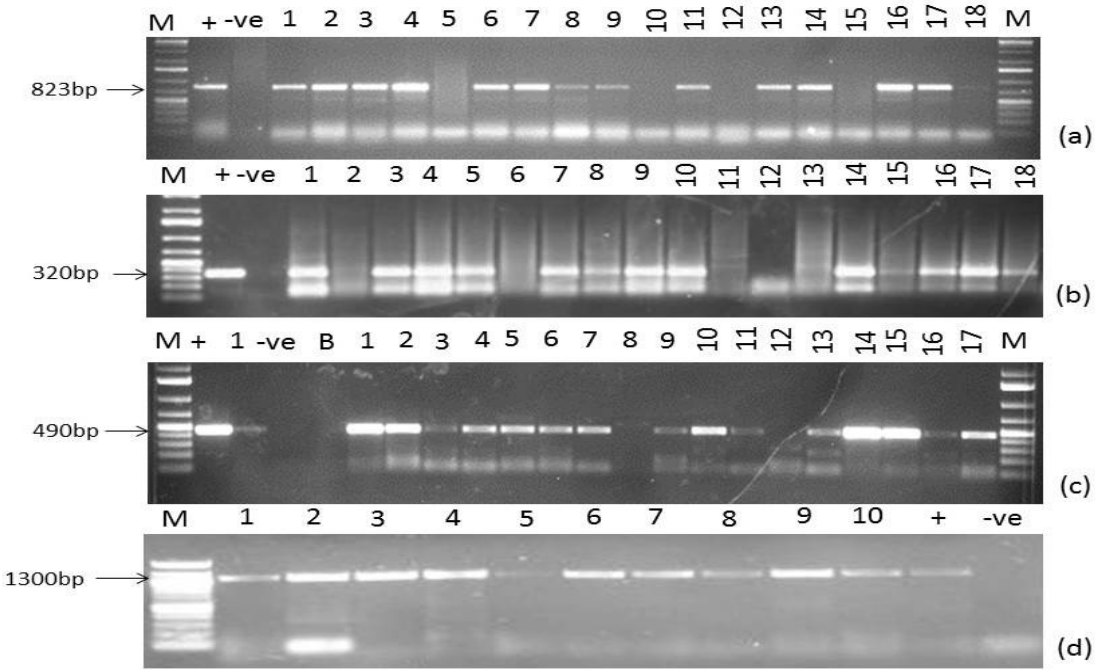

Figure 7.10: Gel picture showing the RT-PCR amplification of BCMV, BCMNV, SBMV and CPMMV

Letters (a), (b), (c) and (d) represent gel pictures obtained after electrophoresis of PCR products obtained from RT-PCR on on samples infected with BCMNV, BCMV, SBMV and CPMMV, respectively. PCR products sizes expected were 823bp for BCMNV (primer pair BCMNVF1/BCMNVR1), 320bp for BCMV (BCMV1F/BCMV1R), 490bp for SBMV (SBMV 315F1/SBMV 315R1) and 1 300bp for CPMMV (CPMMV2F1/CPMMV2R1). The primers shown in brackets are also shown in Table 7.1. The lane labelled with a letter 'M' was loaded with a marker (Thermoscientific O'GeneRuler 1 kb Plus DNA Ladder except for gel image (d) in which 100bp DNA ladder (NEB) was used; lanes labelled with a '-ve' mark were loaded with PCR products for negative controls (RNA template used was from healthy plants) and lanes labelled with '+' were the positive control (known infected samples); lanes labelled with the numbers were loaded with PCR products of common bean viruses (BCMNV, BCMV, SBMV and CPMMV).

Table 7.7: RT-PCR amplification score of BCMV, BCMNV, SBMV and CPMMV in inoculated common bean samples

No.	Genotype	Type	Virus inoculated	RT-PCR amplification			
				BCMV	BCMNV	SBMV	CPMMV
1	Calima Uyole	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
2	JESCA	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
3	Kablanketi fupi	Landrace	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
4	Kablanketi ndefu	Landrace	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
5	Lyamungo 85	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
6	Mshindi	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
7	Njano uyole	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
8	PASI	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
9	Pesa	improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
10	Rojo	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
11	Rosenda	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
12	Rozikoko	Landrace	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
13	Selian 05	Improved	BCMV/BCMNV/CPMMV/SBMV	-	+	+	+
14	SUA90	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
15	Urafiki	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
16	Zawadi	Improved	BCMV/BCMNV/CPMMV/SBMV	+	+	+	+
17	Choroko	Landrace	BCMNV/CPMMV/SBMV	NT	+	+	+
18	kigoma	Landrace	BCMNV/CPMMV/SBMV	NT	+	+	+
19	Lyamungo 90	Improved	BCMV/CPMMV/SBMV	+	NT	+	+
20	Maasai red	landrace	BCMNV/CPMMV/SBMV	NT	+	+	+
21	Masuka nywele	Landrace	BCMNV/CPMMV/SBMV	NT	+	+	+
22	Mwaspenjele	Landrace	BCMNV/CPMMV/SBMV	NT	+	+	+
23	Selundo	Landrace	BCMNV/CPMMV/SBMV	NT	+	+	+
24	Uyole 04	Improved	BCMNV/CPMMV/SBMV	NT	+	+	+
25	Uyole 96	Improved	BCMV/CPMMV/SBMV	+	NT	+	+
26	Cheupe	Improved	CPMMV/SBMV	NT	NT	+	+
27	Kitenge	Landrace	BCMV/BCMNV	+	+	NT	NT
28	Kombati	Landrace	BCMV/BCMNV	+	+	NT	NT
29	Nyeupe	Improved	BCMV/BCMNV	+	+	NT	NT
30	Selian 15	Improved	BCMV/BCMNV	+	+	NT	NT
31	Selian 94	Improved	BCMV/BCMNV	+	+	NT	NT
32	Selian 97	Improved	BCMV/BCMNV	+	+	NT	NT
33	Uyole 16	Improved	BCMV/BCMNV	+	+	NT	NT
34	Fibea	Improved	BCMV/BCMNV	-	+	NT	NT
35	Canada	Landrace	BCMNV	+	+	NT	NT
36	KAEMAP	Landrace	BCMNV	NT	+	NT	NT
37	Kariasee	Landrace	BCMNV	NT	+	NT	NT
38	Kasukanywele	Landrace	BCMNV	NT	+	NT	NT
39	Kisapuli	Landrace	BCMNV	NT	+	NT	NT
40	Local	Local	BCMNV	NT	+	NT	NT
41	Masunga	Landrace	BCMNV	NT	+	NT	NT
42	Matama	Landrace	BCMNV	NT	+	NT	NT
43	Onyege	landrace	BCMNV	NT	+	NT	NT
44	Selian 14	Improved	BCMNV	NT	+	NT	NT
45	Soya Kombati	Landrace	BCMNV	NT	+	NT	NT
46	Soyakijivu	Landrace	BCMNV	NT	+	NT	NT
47	Uyole 03	Improved	BCMNV	NT	+	NT	NT
48	Wifi nyehegera	Landrace	BCMNV	NT	+	NT	NT

‘+’ stands for the positive results following RT-PCR amplification while ‘-’ stands for negative results of RT-PCR in the respective samples. ‘NT’ stands for not tested to mean that the plants were not inoculated with the respective virus and therefore RT-PCR was not done.

7.5 Discussion

This study aimed at evaluating resistance in selected common bean genotypes to four viruses, namely BCMV, BCMNV, SBMV and CPMMV. These viruses were selected for this study because they are known to be of economic importance worldwide (Mwaipopo *et al.,* 2017; Mink and keswani, 1987; Othman and Hull, 1995; Kehoe *et al*., 2014) and were detected in common bean plants during comprehensive surveys conducted in Tanzania from 2015 to 2016 (Chapter two; Mwaipopo *et al.*, 2018). Response of Tanzanian common bean genotypes to these viruses was assessed through determination of disease severity and area under disease progress curve (AUDPC). The viral infections were confirmed using RT-PCR method. Disease severity was considered as the percentage of relevant host tissue covered by symptoms and is very important in predicting yield losses and for determining plant resistance or susceptibility (Bock *et al*., 2010). On the other hand, AUDPC is a measure of injury intensity caused by pathogens (e.g., viruses) over time (Sparks *et al*., 2008).

Plant response mechanism involves hypersensitive reactions in genotypes with resistance to viruses (Feng *et al*., 2018). Therefore, in this study all symptoms were carefully observed and evidence of hypersensitive reaction (e.g., necrotic lesions) was recorded for each genotype following inoculation with a specified virus. BCMV did not cause necrosis in any common bean genotype whereas BCMNV caused it in three genotypes (PASI, Rosenda and Selian 15). Death of plants resulting from necrosis caused by BCMNV was observed only in PASI. In Rosenda and Selian 15, necrosis

was systemic and caused stunted growth. The reaction (e.g., symptoms) of common genotypes to pathogen infections depends on the presence or absence of genes of resistance. For example, in accordance with Drijfhout (1978) and Feng *et al*. (2018), resistance to BCMV and BCMNV in common bean is governed by one dominant *I* gene, and four recessive genes: *bc-u*, *bc-1*, *bc-2*, and *bc-3*. The dominant *I* gene confers extreme resistance or immunity against all strains of BCMV when the temperature stays below 30 °C, and variable types of local and systemic necrosis when temperature exceeds 30 °C. It is therefore possible that Rosenda, PASI and Selian 15, which expressed necrosis, contain dominant *I* gene that triggered hypersensitive reaction in these common bean genotypes. It is worth noting that these experiments were not conducted under controlled environmental conditions and it is known that resistance in plants is affected by several environmental factors including abiotic stresses (Robert-Seilaniantz *et al.,* 2009). Variability in temperature, humidity, light, virus titre, virus strains and genetic backgrounds of the plant can also lead to expression of different phenotypes following infection with a given virus (Hinrichs-Berger *et al.,* 1999; Drijfhout, 1978).

Both CPMMV and SBMV caused mild to severe virus disease symptoms depending on common bean genotypes. CPMMV did not cause necrosis in any common bean genotypes whereas SBMV caused it in most of the inoculated genotypes. Depending on common bean genotype, necrosis caused by SBMV appeared on one or more parts – leaves, stems and pods – of the infected plants. Necrosis mainly occurred on leaves. SBMV caused necrosis on pods of plants of four common bean genotypes – Njano uyole, PASI, Pesa, and Rosenda. Furthermore, infection with SBMV led to reduction in number of pods; the pods that formed were of reduced sizes (Fig. 7.12). Reduction

in pod size was also observed when plants were infected with CPMMV which was in agreement with results obtained in a recent study that used only nine common bean genotypes (Chilagane, 2018). Since it was not possible to obtain pure lines of the common bean genotypes, the effect of these viruses on yield parameters was not determined but the observed symptom severity and reduction in pods number and size indicated there could significant reduction in yields following infection with these viruses.

CPMMV and SBMV were used to inoculate common bean plants grown in Arusha where the temperature is low at an average of 14 – 25 ℃ and in Morogoro where the temperature is high ranging from 18 to 30 ℃. There appeared to be an influence of environmental conditions in the response of different common bean genotypes to SBMV and CPMMV. For instance, for SBMV the AUDPC was highest for genotypes Rosenda, PASI and Mwaspenjele in Arusha while in Morogoro the highest AUDPC was observed in PASI, Kablanketi and Pesa. Moreover, for CPMMV the AUDPC was highest for Rosenda, Selundo, and Uyole 04 when they were grown in Arusha but in Morogoro Region the highest AUDPC was observed for Cheupe, Lyamungo 85 and Uyole 04. Since the seeds planted at the two locations were from the same batches, genetic differences (purity of lines) of the seeds within the same genotypes may have not contributed appreciale ly to the observed differences in their performance at the two different locations. For instance, PASI and Uyole 04 genotypes were having high AUDPC at both locations when infected with SBMV and CPMMV, respectively. Overall, for CPMMV, the AUDPC values were higher in Morogoro than in Arusha.

According to Jones and Barbetti (2012), increasing the temperature of already infected plants usually increases the rate of virus multiplication and systemic movement in the plants. The viral disease severity is high in areas with high temperature compared with low temperature areas. Also, Canto and Palukaitis (2002) showed increased mean temperature decreases the effectiveness of single dominant gene resistances that have temperature dependency and become ineffective when temperatures exceed a threshold that leads to severity. Therefore, higher severity of CPMMV disease symptoms in Morogoro than in Arusha can be attributed to differences in temperatures in the two locations.

This study revealed that within the same common bean genotype the symptoms expression differed from plant to plant. Disease severity differed between plants of the same genotype thereby leading to different scores in disease assessment within genotypes. These results were in agreement with previous observations by Jones and Barbetti (2012) that there is often a correlation between severity of disease symptoms and virus titres in plants. In this study the differences observed between plants of the same genotypes inoculated with the same virus could be because 1) the amount of initial concentration of the virus that was used in mechanical inoculation was different between plants, 2) although the seeds provided by farmers and research institutes were from the same batches, there could have been small genetic differences (segregation) as they were probably from different mother plants, 3) common bean plants differed in their growth vigour, which has implication on symptoms expression, and 4) the virus exist in plants as quasispecies and indeed two closely related strains of the same virus may be infecting the plant used as a source of inoculum. Thus, reliable results could be obtained through use of viral infectious clones or after serial

passaging in plants where necrotic lesions are formed. However, in this study there were four viruses to deal with and there were no plants known to develop necrotic lesions upon infection with each of the four viruses. Attempts were made to ensure that only one virus was infecting plants, which were used as sources of inoculum.

The results of this work revealed the AUDPC in the range of 414 – 2 667, 0 – 1 586.7, 105.6 – 1 561.7, and 506 – 2 037 for BCMNV, BCMV, CPMMV and SBMV, respectively. For BCMNV and SBMV, most genotypes had AUDPC of over 1 000 which suggested increased disease severity over time and susceptibility. According Farooq *et al*. (2018), the AUDPC of 1 000 and above, represent moderate to high susceptibility to virus diseases. Therefore, BCMNV and SBMV are likely to cause high yield losses in affected plants. However, it is noted that the economic importance of given virus may highly depend on the genotype cultivated and prevailing environmental conditions. It is unclear if the response of the studied common bean genotypes would be the same if they were grown under field conditions and allowed to be naturally infected by the viruses used in this work. In Tanzania, as demonstrated in a recent work (Nordenstedt *et al*., 2017), seed transmission of common bean viruses is rare. Therefore, the economic importance of each studied virus would very much depend on the availability of insect vectors and their effectiveness in transmitting the viruses to different genotypes.

Some genotypes inoculated with BCMV showed signs of recovery from disease symptoms. The genotypes which recovered from BCMV disease symptoms were Rosenda, Pesa, Zawadi, SUA 90, and Mshindi. The recovery occurred between 26- and 37-days post inoculations. Reversion has been reported for viruses infecting sweet potato in East Africa but not in common bean plants (Wasswa, 2012). However,

recovery of common bean plants from virus disease symptoms is a known phenomenon, for example, it was reported in common bean plants infected with *Cucumber mosaic virus* (http:// vegetablemdonline .ppath.cornell. edu/factsheets/ Virus_Beans.htm). The recovery from BCMV observed in five genotypes indicates these genotypes could be containing resistance genes. The resistance shown by most common bean genotypes to BCMV is not surprising because, for many years, efforts have been geared towards introgressing BCMV resistant genes into common bean genotypes (e.g., Kusolwa *et al*., 2016).

BCMNV, BCMV, CPMMV and SBMV infections in all inoculated common bean plants were confirmed using RT-PCR. All inoculated plants were found infected with respective viruses except for Fibea and Selian 05 genotypes, which were inoculated but BCMV could not be detected. It is possible that, in these two genotypes, BCMV did not infect plants at all or the titre of the virus was too low to be detected using RT-PCR. In previous works (Njau *et al*., 1994) it was observed that some common bean genotypes in Tanzania contained resistance genes, which protected them from viral infections. Also, Tryphone *et al.* (2013) showed that there were common bean genotypes – including Selian 05 – with moderate resistance. Taken together, it is therefore possible that Fibea and Selian 05 have resistance genes which act against BCMV infection.

7.6 Conclusions and Recommendations

7.6.1 Conclusions

This work has demonstrated that four of economically important viruses (BCMV, BCMNV, CPMMV and SBMV) do infect and cause severe symptoms on common bean genotypes in Tanzania. All plants of all genotypes (except two genotypes which could not be infected with BCMV) were sensitive to all four viruses through mechanical inoculation. The results have shown further that the levels of severity are genotypes dependent. Also, the results have shown that different genotypes respond differently to different viruses. BCMNV and SBMV caused more severe symptoms than CPMMV. However, severe reduction in pod sizes and number of pods per plant was caused by SBMV and CPMMV and not by BCMNV and BCMV. Stunted growth, which has an implication on yield performance of the common bean genotypes, was observed in plants infected with BCMNV, CPMMV and SBMV but not BCMV. While no BCMV infections were detected by RT-PCR in Fibea and Selian 05, conclusion on complete resistance cannot be made at this point until the results are confirmed through repeating experiments.

7.6.2 Recommendations

Based on the findings of this work the following recommendations can be made:

i. Since different genotypes responded differently to viruses, recommendations for genotypes to be planted by farmers in different geographical areas should be guided by the distribution of common bean viruses as mapped in Chapter three.
ii. The common bean genotypes which showed resistance to BCMV can be used as parents in breeding for resistance against the virus but only after their resistance has been confirmed through further testing.
iii. Although this study did not look into the effect of temperature on response of common bean genotypes to viruses it is, however recommended that future studies should take into account environmental conditions or else find ways to

study response of common bean genotypes to viral infections under field conditions without introducing new viruses into new environment.

iv. SBMV and CPMMV (also see Chilagane, 2018) are two emerging viruses in Tanzania and there is a need to consider them in breeding programmes in Tanzania.

v. Carefully planned experiments to confirm resistance in some genotypes (Fibea and Selian 05) identified in this study should be done using viral infectious clones or virus inoculum obtained after a serial passaging in plants where necrotic lesions are developed.

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Appendicies

Appendix 7.1: Disease severity and AUDPC of common bean genotypes inoculated with BCMNV at SUA

Genotype	7Days	12Days	17Days	22Days	27Days	32Days	37Days	42Days	AUDPC
Calima	0a	8.33ab	39.93b-j	39.93a-g	39.93a-g	39.93c-g	39.93e-l	39.93f-k	1140b-g
Calima uyole	0a	13.82a-c	55.29g-j	41.47a-h	41.47a-h	41.47c-g	41.47f-l	41.47g-k	1279b-h
Canada	0a	14.68a-c	20.4a-c	29.37a-c	29.37a-d	29.37a-e	29.37b-i	29.37b-h	836a-e
Choroko	0a	0a	46.67c-j	52c-j	52c-h	52f-i	34.67d-l	34.67e-j	1273b-h
Fibea	0a	30.97b-f	35.97a-i	35.97a-e	35.97a-e	30.97a-e	30.97b-j	30.97d-h	1082b-g
JESCA	0a	28.3b-f	51.6 e-j	51.6c-j	51.9c-h	42.45c-g	28.3a-g	28.3b-g	1342d-h
Kablanketi fupi	0a	16.33a-c	32.67a-g	32.67a-d	23ab	16.33ab	16.33a-c	16.33a-d	728ab
kablanketi ndefu	0a	0a	55.29g-j	55.29d-j	41.47a-h	41.47c-g	41.47f-l	41.47g-k	1279b-h
KAEMAP	0a	33.33b-f	33.33a-g	50b-j	50b-h	33.33b-f	33.33c-l	33.33e-i	1250b-h
Kariasee	0a	0a	46.03c-j	46.03a-j	35.87a-e	30.69a-e	30.69b-j	30.69c-h	1023b-f
kasukanywele	0a	25.37a-e	25.37a-e	33.82a-d	38.06a-e	38.06c-g	25.37a-f	25.37a-f	994a-f
kigoma	0a	15.46a-c	25.92a-e	36.26a-f	46.38b-h	46.38e-h	30.92b-j	30.92d-h	1084b-g
Kisapuli	0a	23.17a-d	23.17a-b	34.76a-d	34.76a-d	27.62a-e	23.17a-e	23.17a-e	891a-f
Kitenge	0a	13.45a-c	40.35b-j	40.35a-g	40.35a-g	40.35c-g	26.9a-g	26.9b-g	1076b-g
Kombati	0a	50.62ef	62.16i-k	67.49j	67.49hi	67.49i	50.62lm	50.62kl	1956ij
local	0a	15.67a-c	47d-j	62.67g-j	47b-h	47e-h	47j-l	47i-k	1449f-i
Lyamungo 85	0a	0a	35.52a-h	40.29a-g	31.62a-d	26.86a-e	26.86a-g	26.86b-g	873a-f
Maasai red	15.6b	31.23b-f	62.45jk	62.45g-j	62.45e-h	46.84e-h	46.84i-l	46.84i-k	1772h-j
Masunga	0a	15.36a-c	30.72a-g	30.72a-c	46.08b-h	46.08e-h	46.08h-l	30.72c-h	1152b-g
Matama	0a	27.17b-e	40.76b-j	40.76a-g	27.17a-c	27.17a-e	27.17a-g	27.17b-g	1019b-f
Mshindi	0a	39.76c-f	45.48c-j	49.92b-j	45.48b-h	30.32a-e	30.32b-j	30.32b-h	1282b-h
Mwaspenjele	0a	24.76a-e	34.52a-h	39.52a-g	39.52a-g	44.29d-g	44.29g-l	44.29h-k	1245b-h
Njano uyole	0a	29.54b-f	54.19f-j	59.08e-j	54.91d-h	44.31d-g	35.25d-l	29.54b-h	1460f-i
Nyeupe	0a	28.29b-f	28.29a-f	42.43a-i	42.43b-h	42.43c-g	28.29a-g	28.29b-g	1131b-g
Onyege	0a	54.59f	49.03d-j	38.02a-f	38.02a-e	38.02c-g	32.69b-k	32.69e-i	1334c-h
PASI	0a	26.67a-e	86.67k	93.33k	93.33i	93.33j	93.33n	93.33n	2667k
Pesa	22c	49.19d-f	60.04h-j	65.59h-j	65.59f-h	65.59hi	65.59m	65.59lm	2154jk

Rojo	0a	33.06b-f	49.58e-j	66.11ij	56.25d-h	56.25g-i	49.58k-m	49.58jk	1678g-j
Rosenda	33.1d	43.93d-f	54.64f-j	60.36f-j	66.19gh	66.19hi	66.19m	66.19m	2152jk
rozikoko	0a	30b-f	40b-j	30a-c	30a-d	30a-e	30b-j	30b-h	1025b-f
selian 05	0a	0.00a	31.04a-g	31.04a-c	31.04a-d	31.04a-e	20.52a-d	15.52a-c	762a-d
Selian 14	0a	30.52b-f	45.78c-j	35.37a-e	30.52a-d	30.52a-e	15.26ab	15.26ab	978a-f
Selian 15	0a	15.89a-c	31.78a-g	47.67b-j	37.11a-e	31.78b-f	31.78b-j	31.78e-i	1059b-f
Selian 94	0a	29.25b-f	53.66f-j	43.88a-j	43.88b-h	33.7b-f	29.25b-h	29.25b-h	1241b-h
Selian 97	0a	13.62a-c	27.23a-e	32.23a-d	40.85a-h	40.85c-g	40.85f-l	40.85g-k	1080b-g
Selundo	0a	0a	26.79a-e	26.79ab	26.79a-c	26.79a-e	26.79a-f	26.79b-g	737a-c
Soya kombati	0a	14.65a-c	10a	29.3a-c	43.95b-h	43.95d-g	29.3b-h	29.3b-h	929a-f
Soyakijivu	0a	0a	36.67b-j	41.25a-g	41.25a-h	41.25c-g	27.5a-g	27.5b-g	1008a-f
SUA 90	0a	0a	18.18ab	22.1a	14.97a	11.05a	11.05a	11.05a	414a
Urafiki	31.4d	47.14d-f	47.14d-j	41.96a-i	31.43a-d	31.43a-e	31.43b-j	31.43d-h	1420e-i
Uyole 03	0a	32.14b-f	48.21d-j	48.21b-j	38.81a-f	32.14b-f	32.14b-k	32.14e-i	1239b-h
Uyole 04	0a	0a	26.41a-e	35.17a-e	39.62a-g	39.62c-g	26.41a-f	26.41b-g	902a-f
Uyole 16	0a	15.45a-c	46.34c-j	46.34b-j	46.34b-h	35.65b-f	30.89b-j	30.89d-h	1182b-h
Wifi nyehegera	0a	11.67ab	26.67a-e	35a-e	23.33ab	23.33a-c	23.33a-e	23.33a-e	775a-d
Zawadi	0a	24.67a-e	33a-g	37a-f	33.67a-d	24.67a-d	24.67a-f	24.67a-f	950a-f
Fpr	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
S.E	±1.6	±8.1	±7.9	±7.2	±8.1	±6.1	±5.2	±4.6	±180.8

Appendix 7.2: Disease severity and AUDPC of common bean genotypes inoculated with BCMV at SUA in Morogoro

Genotype	7 Days	12 Days	17 Days	22 Days	27 Days	32 Days	37 Days	AUDPC
Calima	15.67bc	15.67a-d	32d-h	47.67f-i	47.67fg	32d-i	32d-f	1049.7g-i
Fibea	0a	0a	0a	0a	0a	0a	0a	0a
JESCA	0a	0a	16.67a-d	33.67d-h	33.67c-f	33.67e-i	33.67d-f	672.7d-f
Kablanketi fupi	13b	13ab	26.33c-g	39.33e-i	39.33d-g	26.33b-h	26.33de	866.3e-g
Kitenge	16.33bc	33.33c-f	44.33hi	49.67g-i	43.67e-g	33.33e-i	33.33d-f	1204g-i
Kombati	18.67bc	31.33d-f	37.67e-h	37.67e-i	56.33g	37.67g-i	37.67fg	1209.7hi
Lyamungo 85	0a	4a	8.67ab	13a-c	19.33bc	6.33	6.33ab	273.7a-c
Lyamungo 90	0a	8.33a	11a-c	16.67a-d	25b-e	8.33	8.33a-c	368b-d
Mshindi	0a	15.67a-d	26.33c-g	26.33b-e	15.67a-c	0a	0a	421b-d
Njano uyole	15bc	15a-c	30d-h	30c-f	30c-f	45i	45g	953.7f-i
Nyeupe	16.33bc	16.33a-d	33d-h	33d-h	49f-g	28c-i	16.33c	936.7f-h
PASI	37.33d	49.67f	56i	49.67g-i	56g	37.33g-i	37.33fg	1561.7j
Pesa	0a	5.67a	26.33c-g	32d-g	32c-f	5.67a	0a	509.7cd
Rojo	0a	15.67a-d	26b-f	31c-f	20.67b-d	15.67a-e	15.67c	585c-e
Rosenda	0a	27.33b-e	43.67g-i	39e-i	0a	0a	0a	551.3c-e
Rozikoko	17.33bc	29.67b-e	35e-h	35d-h	46.67fg	35f-i	35ef	1099g-i
Selian 05	0a	0a	0a	0a	0a	0a	0a	0a
Selian 15	0a	0a	9.33a-c	18.33a-d	18.33a-c	13a-c	9.33bc	319.7a-c
Selian 94	38d	44ef	56.67i	50.67hi	56.67g	44.67h-i	38fg	1586.7j
Selian 97	18bc	36ef	36e-h	54i	54g	36g-i	36f	1278.3ij
SUA 90	0a	0a	3a	8.33ab	10.67ab	2.67a	0a	124ab
Urafiki	19.67c	39.33ef	39.33f-i	39.33e-i	39.33d-g	39.33g-i	39.33fg	1200g-i
Uyole 16	16.67bc	34d-f	34d-h	34d-h	34c-f	16.67a-f	34d-f	949.7f-i
Uyole 96	16.67bc	29.67b-e	38eh	38e-i	25.67b-e	25.67b-g	25.67d	950f-i
Zawadi	0a	5.33a	20.67b-e	30.33	25b-e	14.67a-d	0a	480.3cd
Fpr	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
S.E	±2.049	±5.959	±5.543	±5.897	±6.039	±5.865	±2.778	±107.9

Appendix 7.3: Disease severity and AUDPC of common bean genotypes inoculated with CPMMV at SUA in Morogoro

Genotypes	7 Days	12 Days	17 Days	22 Days	27 Days	32 Days	37 Days	AUDPC
Cheupe	6.19a	23.15a	40.11c	45.75b	45.75b	39.56bc	33.92c	1093.6c
Choroko	0a	14.85a	24.85a-c	24.85ab	29.7ab	29.7ab	19.52a-c	668.5a-c
JESCA	0a	12.47a	24.93a-c	24.93ab	24.93ab	24.93ab	24.93a-c	623.3a-c
Kablanketi	0a	14.85a	14.85ab	29.7ab	29.7ab	29.7ab	29.7a-c	668.3a-c
Kablanketi ndefu	0a	14.85a	29.7a-c	29.7ab	39.88ab	39.21bc	29.7a-c	840.9a-c
Lyamungo 85	5.71a	24.02a	36.61c	42.33b	48.25b	48.25c	29.95a-c	1106.5c
Maasai red	0a	13.67a	13.67a	27.33ab	27.33ab	27.33ab	13.67ab	580.8ab
Kasukanywele	0a	16.61a	33.22bc	39.05ab	39.05ab	38.06bc	33.22c	913bc
Mshindi	0a	14.21a	28.41a-c	38.17ab	33.17ab	28.41ab	14.21ab	747.4a-c
Mwaspenjele	0a	19a	29.67a-c	29.67ab	29.67ab	29.67ab	29.67a-c	762.5a-c
Njano uyole	6.67a	23.11a	39.56c	39.56ab	32.89ab	32.89ab	32.89c	962.2bc
PASI	0a	17.43a	34.86c	46.29b	46.29b	34.86bc	28.86a-c	970.7bc
Pesa	0a	19.04a	36.89c	36.89ab	32.89ab	27.7ab	23.04a-c	824.6a-c
Rojo	0a	15.52a	31.04a-c	31.04ab	41.22ab	31.04ab	31.04bc	826.9a-c
Rosenda	0a	17.19a	34.37c	40.3ab	40.3ab	28.44ab	28.44a-c	874.1a-c
Rozikoko	0a	14.85a	14.85ab	25.03ab	29.7ab	29.7ab	29.7a-c	644.8a-c
Selian 05	0a	23.06a	34.83c	40.47ab	40.47ab	34.83bc	34.83c	955.4bc
Selundo	0a	15.16a	15.16ab	20.87ab	30.32ab	30.32ab	25.32a-c	622.4a-c
SUA90	0a	12.26a	12.26a	12.26a	19.76a	19.76a	12.26a	412.2a
Urafiki	0a	16.65a	33.3bc	44.31b	44.31ab	33.3a-c	27.66a-c	928.6bc
Uyole 04	0a	17.69a	35.37c	47.13b	47.13b	35.37bc	35.37c	1001.9bc
Fpr	0.588	0.336	0.001	0.001	0.002	0.001	0.001	0.001
S.E	±4.06	±5.76	±6.06	±9.15	±8.17	±4.83	±5.76	±158.24

Appendix 7.4: Disease severity and AUDPC of common bean genotypes inoculated with CPMMV at SUA in Arusha

Genotypes	7 Days	12 Days	17 Days	22 Days	27 Days	32 Days	37 Days	AUDPC
Calima	0a	0a	0a	6.98a	6.98a	4.76a	4.76a	105.6a
JESCA	0a	12.93a-d	25.86b-e	38.79c-i	38.79b-f	34.02b-d	25.86a-d	723.5b-e
Kablanketi fupi	0a	12.61a-c	20.78a-d	20.78a-c	25.22a-c	25.22a-c	16.89a-d	485.1a-c

Kablanketi ndefu	0a	14a-d	28b-f	28a-e	32bc	33.33b-d	24a-d	635.9b-e
Kigoma	0a	14.81a-d	14.81ab	29.63b-g	29.63bc	44.44c-e	44.44d-f	706.7b-e
Lyamungo 85	30.90bc	36.61e	36.61c-h	36.61c-i	36.61b-f	30.9a-d	18.31a-d	942.2c-g
Lyamungo 90	10.926ab	29.47b-e	29.47b-f	44.21d-i	44.21c-f	29.47a-d	29.47a-d	881.9c-f
Maasai red	0a	23.54b-e	29.54b-f	29.54b-g	25.44a-c	25.44a-c	25.44a-d	603.6a-e
Kasukanywele	12a-c	34.37de	34.37b-g	51.56g-i	51.56d-f	34.37b-d	34.37b-d	1024.1d-g
Mshindi	4.762a	12.54a-c	25.08b-e	25.08a-e	25.08a-c	21.75a-c	9.21ab	508.9a-d
Mwaspenjele	14.10a-c	28.19b-e	28.19b-f	23.43a-d	23.43ab	38.95cd	23.43a-d	718.8b-e
Njano uyole	9.667a	24.19b-e	28.86b-f	43.29d-i	38.62b-f	43.29c-e	38.62c-e	918.4c-g
PASI	17.835a-c	17.84a-e	35.67b-h	35.67c-h	35.67b-e	35.67cd	29.96a-d	856.1c-f
Pesa	0a	8.44ab	20.44a-d	24.89a-d	24.89a-c	24.89a-c	12.44a-c	479.6a-c
Rojo	17.115a-c	29.62b-e	51.35gh	51.35f-i	34.23b-d	29.62a-d	17.12a-d	931.9c-g
Rosekoko	0a	24.1b-e	22.87b-d	36.97c-i	28.21bc	28.21a-d	14.1a-c	624.3b-e
Rosenda	31.852c	19.26a-e	38.52d-h	57.78i	77.04g	77.04f	64.44e-g	1561.7h
Selian 05	17.128a-c	27.59b-e	44.72e-h	39.38c-i	34.26b-d	34.26a-d	34.26b-d	915.9c-g
Selundo	18.095a-c	30.48c-e	48.57f-h	54.29hi	54.29ef	54.29d-f	66.67fg	1295f-h
SUA 90	5.36a	16.43a-e	16.43a-c	13.57ab	8.21a	8.21ab	5.71a	281.9ab
Uyole 04	13.33a-c	37.33e	56h	56hi	56f	69.33ef	74.67g	1416gh
Uyole 96	0a	14.67a-d	14.67ab	29.33b-f	38.67b-f	38.67cd	29.33a-d	682.9b-e
Zawadi	14.44a-c	34.07c-e	53.7gh	47.04e-i	39.26b-f	39.26cd	34.07b-d	1027.9e-g
Fpr	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
S.E	±6.43	±6.9	±6.9	±7.04	±6.24	±8.75	±8.92	±165.46

Appendix 7.5: Disease severity and AUDPC of common bean genotypes inoculated with SBMV at SUA in Arusha

Genotypes	**7 Days**	**12 Days**	**17 Days**	**22 Days**	**27 Days**	**32 Days**	**37 Days**	**AUDPC**
Calima	15.079a b	30.16b-e	30.16a-d	38.1a-e	45.24a-f	45.24b-e	30.16a-e	1110b-f
JESCA	0a	26.5a-e	35.33b-e	44.17a-e	53d-f	53ef	53gh	1192c-h
Kablanketi fupi	0a	21.37a-e	28.37a-d	28.37ab	28.37a-c	28.37ab	14.19a	710a-c
Kablanketi ndefu	5.333	23.9a-e	31.43a-d	31.43a-c	39.62a-e	47.14c-e	47.14e-g	1017b-f
Kigoma	0a	24.37a-e	32.89b-e	49.33b-e	49.33c-f	49.33de	41.41d-g	1130b-f
Lyamungo 85	0a	8.1a	24.12ab	24.12a	40.14a-e	32.04a-d	16.02ab	683ab
Lyamungo 90	0a	15.09ab	38.15b-f	45.28a-e	45.28a-f	30.19a-c	30.19a-e	945a-f
Masai red	9.39ab	24.95a-e	34.34b-e	34.34a-d	43.74a-f	34.34a-e	34.34b-f	1001a-f
Kasukanywele	0a	40.56e	40.56b-f	48.56a-e	48.56b-f	32.37a-d	32.37a-f	1134b-f
Mshindi	8.69a	25.42a-e	25.42a-c	33.47a-d	50.2c-f	50.2de	50.2fg	1101b-f
Mwaspenjele	10ab	38.67de	48d-f	58e	58ef	77.33g	77.33i	1653g-i

Njano uyole	9.33ab	35.33b-e	53ef	53c-e	53d-f	43.67b-e	43.67d-g	1355e-i
PASI	18.67ab	37.33c-e	46c-f	56de	64.67f	74.67g	74.67i	1692hi
Pesa	11.67ab	29.05a-e	34.76b-e	52.14c-e	52.14d-f	69.52fg	69.52hi	1432f-i
Rojo	8.67a	26.24a-e	34.91b-e	43.58a-e	52.36d-f	52.36ef	34.91c-g	1187c-g
Rosekoko	8.19a	15.07ab	30.15a-d	38.33a-e	38.33a-e	30.15a-c	15.07a	847a-d
Rosenda	28.52b	38.52de	57.78f	57.78e	57.78ef	77.04g	77.04i	1808i
Selian 05	0a	16.75a-c	33.5b-e	41.92a-e	50.26c-f	41.84b-e	41.84d-g	1026b-f
Selundo	0a	22.22a-e	30a-d	45a--e	45a-f	30a-c	30a-e	936a-f
SUA 90	0a	17.5a-d	11.79a	23.57a	23.57a	17.5a	17.5a-c	513a
Uyole 04	17.35ab	26.61a-e	34.71b-e	34.71a-e	26.61ab	26.61ab	26.61a-d	917a-e
Uyole 96	8.67a	36.12b-e	45.52c-f	45.52a-e	44.79a-f	44.79b-e	36.12d-g	1226d-h
Zawadi	8.69a	24.78a-e	42.16b-f	42.16a-e	33.47a-d	33.47a-d	16.73a-c	974a-f
Fpr	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
S.E	±6.19	±6.94	±6.6	±7.46	±7.03	±5.98	±5.92	±159.63

Appendix 7.6: Disease severity and AUDPC of common bean genotypes inoculated with SBMV at SUA in Morogoro

Genotypes	7 Day	12 Days	17 Days	22 Days	27 Days	32 Days	37 Days	AUDPC
Cheupe	6.19ab	33.3ef	44.83b-g	61.48cd	61.48b-e	56.35bc	50.16d	1450de
Choroko	0a	4.44a	18.63a	28.38a	28.38a	14.19a	14.19a	506a
JESCA	0a	17.12a-d	34.24a-f	39.7a-c	51.36a-d	51.36bc	45.53cd	1083a-e
Kablanketi fupi	0a	14.52a-c	29.04ab	29.04ab	43.56a-d	43.56a-c	29.04a-d	871a-d
Kablanketi ndefu	11.83bc	29.12d-f	51.86e-g	63.32cd	63.32c-e	57.86bc	51.86d	1528ef
Lyamungo 85	0a	14.63a-c	29.26ab	34.44ab	34.44ab	29.26ab	19.81a-c	760ab
Maasai red	0a	16.71a-d	33.43a-e	33.43ab	38.76a-d	32.84ab	27.38a-d	844a-c
Kasukanywele	0a	15a-c	30a-c	30ab	45a-d	45a-c	45cd	938a-d
Mshindi	0a	32.47ef	48.7d-g	48.7a-d	53.88a-e	53.88bc	43.37b-d	1297b-e
Mwaspenjele	0a	17.26a-d	34.52a-f	34.52ab	51.78a-e	51.78bc	51.78d	1079a-e
Njano uyole	0a	22.93b-e	34.19a-f	51.28a-d	51.28a-d	51.28a-c	40.11a-d	1155b-e
PASI	20c	40f	60g	73.33d	80e	80c	100e	2037f
Pesa	17.5c	35ef	52.5fg	64.17cd	64.17de	58.33bc	46.67cd	1592ef
Rojo	17.98c	35.97ef	42.03b-g	53.96b-d	60.02b-e	48.09a-c	42.03a-d	1413c-e
Rosenda	18.65c	37.31f	43.23b-g	49.16a-d	55.22a-e	55.22bc	49.29d	1436de
Rozikoko	0a	27.85c-f	34.52a-f	34.52ab	51.78a-e	51.78bc	45.11cd	1115a-e
Selian 05	0a	15.64a-d	31.28a-d	31.28ab	31.28a	31.28ab	15.64ab	743ab
Selundo	0a	15.19a-c	19.63a	34.81ab	34.81a-c	34.81ab	30.37a-d	772ab
SUA90	0a	13.69ab	27.38ab	27.38a	41.07a-d	36.31ab	27.38a-d	798ab
Urafiki	0a	35.85ef	48.44c-g	48.44a-d	55.11a-e	49.19a-c	42.52a-d	1291b-e
Uyole 04	0a	15.93a-d	31.86a-d	47.79a-d	47.79a-d	47.79a-c	43.02a-d	1063a-e
Fpr	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
S.E	±3.25	±4.37	±6	±8.23	±9.17	±11.96	±9.3	±187.17

CHAPTER EIGHT

8.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

8.1 General Conclusions

i. Both BCMV and BCMNV are predominant and widely spread in Tanzania compared to other viruses. CPMMV is common in eastern and northern zones while SBMV is widespread in the western zone.
ii. Common bean in Tanzania is infected by at least 15 viruses belonging to eleven genera; they include pathogenic and cryptic viruses.
iii. In screen house settings, SBMV and CPMMV caused more severe symptoms than BCMNVand BCMV.
iv. Over 23 viruses that are known to infect common bean plants were found infecting wild plants within and around common bean fields.
v. Common bean infecting viruses can be reliably detected in NGS reads of size 20 nt when an offline analysis is conducted. SBMV and bromovirus can be detected in reads of size >25 nt.
vi. Many viruses detected in wild plants could not be mechanically transmitted to common bean plants suggesting that the viruses are either transmitted to common bean crop via insect vectors or are not infectious to common bean plants. CMV and a CCMV related bromovirus were the only viruses readly transmitted by mechanical inoculation to common bean plants.
vii. Begomoviruses, which until now are known to infect common bean only in the new world may be infecting common bean in Tanzania as exemplified by *Tomato leaf curl virus* detected in this study.
viii. Two wild plants, *O. basilicum* L. and *B. bituminosa* (L.) Kuntze were found to host CMV and CCMV related virus, respectively.
ix. The Tanzanian isolates of BCMV are more genetically variable than BCMNV isolates
x. The common bean genotypes in Tanzania belong to two gene pools: the Andean gene pool and the Mesoamerican gene pool.

8.2 General Recommendations

From the findings of this work, the following recommendations were made:

i. Development of management strategies for common bean viral diseases should take into account the occurrence of 15 viruses in common bean plants in Tanzania. SBMV and CPMMV are likely to be as economically important as BCMV and BCMNV.
ii. Because of high genetic diversity within and between isolates of different viruses – especially in BCMV – any study that aim at determining resistance in common bean genotypes should challenge common bean plants to as many isolates as possible.
iii. There is high virus disease pressure in Morogoro when compared to other places. Also, many different viruses are found in Morogoro. Thus Morogoro (Mvomero district) can serve as good place for screening for common bean viral disease resistance.
iv. Low disease pressure in the southern highlands of Tanzania means that the location can serve as good location for commercial seed production.
v. There is a need to conduct surveys for establishing incidence and distribution of viruses detected using NGS. This should go hand in hand with controlled experiments to establish the economic importance of these viruses.
vi. Wild plants within and around common bean fields with virus should be weeded/removed to prevent movement of viruses between wild and common bean plants
vii. It is apparent from this and recent studies that vectors may be playing crucial role in transmitting viruses from wild plants to common bean plants. Therefore, vector transmission study should be conducted for the wild plant viruses which were or could not be mechanically transmitted to common bean in this study.

viii. No attempts were made to associate viral disease incidence and severity with weather conditions. Future studies should attempt to, for example, study correlation of altitude and temperature, with incidence, distribution and severity of common bean viral diseases in the country.

ix. While many different viral disease symptoms were observed in plants in the Lake Victoria basin (lake zone), the incidence of BCMV, BCMNV and CPMMV were low. This means there could be other viruses there which cause severe viral disease symptoms and these should be investigated.

x. Bean genotypes which showed resistance to different viral diseases should be evaluated further both under screen house and field conditions.