EVALUATION OF BEAN VARIETIES (*phaseolus vulgaris* L) FOR RESISTANCE TO *bean common mosaic virus* (BCMV) and *bean common mosaic necrotic virus* (BCMNV) ACROSS A SOIL FERTILITY GRADIENT IN WESTERN KENYA

BY

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A thesis submitted to the University of Eldoret in partial fulfillment of the Requirements for the degree of Master of Science in Plant Breeding and biotechnology

DECLARATION

This thesis is entirely my original work and has not been submitted or examined in this or any other University for award of a degree.

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KARI- Kibos

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DEDICATION

To my Father Jason Mudaki Kivati with your meagre income gave me a base to build on to higher heights and my late mother Miriam Minayo Mudaki who cherished education. Mother, your famous statement “never give up in life you will always make it” has forever inspired me.

ABSTRACT

Bean Common Mosaic and Bean Common Mosaic Necrosis are some of the most devastating diseases of common beans (*Phaseolus vulgaris* L) in Kenya. These viral diseases cause more than 80% yield losses. Control by chemical method is difficult once they have set in. Use of resistant varieties offers a sustainable and economical solution. In this study, observational survey was carried out in 20 farms in Nandi South. A field study was conducted for one season in four sites across soil fertility gradient in Nandi south. Alphar lattice design was adapted for the experiment. The bean genotypes screened for BCMV and BCMNV included twenty three bean lines developed for resistance to bean root rot (BRR) and three phosphorus efficient bean lines selected from an earlier field screening of fifty Phosphorus efficiency lines. Two control varieties, RWR 719 resistance to BCMV and GLP-585 a commercial variety were included in the trial as control lines. Parameters scored were BCMV and BCMNV incidence, Aphid counts, stand counts and yield. The data was subjected to ANOVA using SAS 8.2 Statistical software and LSD at 5% level of significance was used to compare the means of the genotypes for all variables. Further twelve genotypes that did not show the viral symptoms in the field were selected and artificially inoculate in the screen house to confirm this resistance. This was done in a Completely Randomised Design. The highest incidences of BCMV (61.5%) and BCMNV (8.2%) were in Koibem. The bean root rot resistant lines showed significant differences in resistance to both BCMV and BCMNV. Six lines were resistance to BCMV while 14 were resistant to BCMNV. The genotype BCO-05/18 and BCO-05/07 were particularly resistant to both viruses. The P efficient lines were completely susceptible to BCMNV, but resistant to BCMV. These results indicate that both BRR and P efficient lines can be good sources of resistance to transfer BCMV and BCMNV resistance to popular but susceptible bean varieties.

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LIST OF ACRONYMS

ECABREN	Eastern and Central Africa Research Network
AL	Aluminium
ALS	Angular Leaf Spot
ANTH	Anthracnose
BCMV	Bean Common Mosaic Virus
BCMNV	Bean Common Mosaic Necrotic Virus
BNF	Biological Nitrogen Fixation
BRR	Bean Root Rot
CRSP	Collaborative Research Support Project
FAO	Food Agricultural Organization
FWL-1	1st Flowering
FLW-2	50% Flowering
KARI	Kenya Agriculture Research Institute
KIPPRA	Kenya Institute of Public Policy Research and Analysis
LM	Low Midland
Mg	Magnesium
MTR	Maturity
PE	Phosphate Efficient
RR-C	Resistant to BCMV
STC-1	Stand Count at Emergency
STC-2	Stand Count at Harvesting
UM	Upper Midland
NPP	Number of Pods per Plant
NSD	Number of Seed per Pod
YLD	Yield

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CHAPTER ONE
INTRODUCTION

1.0 Background Information

Common Beans (*Phaseoulus vulgaris* L) belongs to the Leguminacea family and the genus Phaseolus comprises of 30 known species. However, only five species are domesticated with the common bean being the most widely grown specie globally (Singh, 2001). It is the most important pulse ranking second to maize as a food crop in Kenya (Republic of Kenya, 2012).

Beans play a significant role in human nutrition by providing more than 45% of total protein consumed and contain high levels of lysine, which is low in cereal crops like maize making it a good complement in the diet (Allen and Edje, 1990). In Kenya, bean consumption per capita is estimated about 50 kg/year (FAO, 2007) but can be as high as 66 kg/yr in western Kenya (Buruchara, 2007). Regular consumption of common bean and other pulses are now promoted by health organizations, due to low fats that are cholesterol free, resulting in reduced risk of diseases such as cancer, diabetes and coronary heart diseases (Leterme and Munoz, 2002). The crop also restores soil fertility through Biological Nitrogen Fixation (BNF) (Kannaiyan, 1999). Further, it has a high industrial potential for citric acid production. (Torodivic *et al*., 2008)

1.1 Bean production Status in Kenya

The total area under bean cultivation in Kenya is estimated at 500,000 ha-1 (GOK, 1998) with yield at 250 kg ha-1 under mixed cropping. In pure stands, yields of 700kg ha-1 has been reported, this yield is low compared to a potential yield of up to 5000kg ha-1

(Muasya, 2001). The national yearly consumption is estimated at 500,000 metric tonnes, against an annual production range of 125,000- 380,000 metric tonnes (Muasya, 2001; MOA & KIPPRA, 2010). Production has been declining over the years with Kenya being a net importer of beans (Mutwoki et al. 2009, MOA, 2008).

1.1.1 Beans Production Constraints in Kenya

Common bean production in Kenya is adversely constrained by several factors such as low soil fertility, diseases and insect pests. Sustained and enhanced production and productivity of beans is threatened by two major viral diseases, Bean Common Mosaic Virus (BCMV) and Bean Common Mosaic Necrotic Virus (BCMNV) diseases (Bock et al., 1976; Rheene et al., 1985). The bean varieties grown in Kenya are all susceptible to these viruses (Omnyin 1995; Wagara and Kimani, 2007). Bean common mosaic virus (BCMV) and Bean common mosaic necrotic virus (BCMNV) are economically important diseases globally (Mavric et al., 2003). BCMV usually causes mosaic symptoms on susceptible bean cultivars while BCMNV cause systemic lethal necrosis on bean genotypes carrying dominant I gene (Silbernagel et al., 2001). Both viruses (BCMV) and (BCMNV) are seed borne and are transmitted by aphid species and mechanical inoculation up to 83% by seed (Movric and Susta-vozlic, 2004). The diseases (BCMV and BCMNV) can cause up to 6-98% and 100% bean yield losses respectively (Mukeshimana et al, 2003). Both viruses can be found in the same area and infecting the same plant (Silbernagel et al., 2001), combined infection cause severe damage. Bean genotypes with the I gene only are resistant to BCMV, but susceptible to the necrotic virus strain (BCMNV). But genotypes carrying the recessive gene (bc-u and bc-3) in the

presence of the dominant I gene confers resistance to all known strains of BCMV and BCMNV. Cultivars with I,bc-3 and bc-u,bc-3 combinations are immune to all strains of BCMV and BCMNV while i, bc-3 genotypes are resistant to all strains of BCMNV but susceptible to some strains of BCMV (Larsen et al., 2008; Larsen and Miklas, 2010).

Previous efforts to control BCMV focused on routinely introgressing the I gene into the Andean and Mesoamerican gene pools (Kelly, 1997). Some of the varieties were introduced to Kenya as a control measure to bean root rot (BRR), one of the constraints limiting bean production in western Kenya. There was an anticipated necrotic reaction with the varieties that were introduced as a means of managing BRR. To mitigate the necrotic disease, new bean varieties were introduced to the region to provide a solution to the problem. The introduced varieties were developed from parents carrying resistance to both BCMV and BCMNV diseases and the Kenyan commercial varieties. Before the varieties are introduced to the farmers to use there is need to evaluate them in the hot spots for the two diseases and this necessitated these study..

1.2 Problem Statement

Bean production in Kenya is on the decline, this is exacerbated by, Bean common mosaic virus (BCMV) and Bean common mosaic necrotic virus (BCMNV) (Otsyula and Echaire, 2008; Otsyula *et. al,* 2009). Further small scale farmers produce beans under degraded soils deficient in P and they hardly apply sufficient fertilizers. Development of bean technologies that can translate into sustainable yields and food security must address these viral diseases and phosphorus deficiency complex

1.3 Objectives of the Study

1.3.1 Main objective

To enhance bean production in Western Kenya through selection of high yielding bean varieties with P use efficiency and resistance to BCMV and BCMNV diseases.

1.3.2 Specific Objectives

To determine the prevalence of BCMV and BCMNV diseases across soil fertility gradient in Nandi South

To characterize bean genotypes for resistance to BCMV and BCMNV

1.4 Justification of the Study

Despite the efforts to control BCMV by routinely introgressing I gene into the Andean and Mesoamerican gene pools (Kelly, 1997), which have since been introduced in Kenya,. BCMV and BCMNV still pose a challenge to common bean productivity in Kenya because the improved varieties are necrotic to the Kenyan virus strain. Further the preferred commercial varieties are susceptible to BCMV. Chemical control of the vector that spread the viruses is infective due to high costs and also being environmental unfriendly. The aphids spread the virus in a nonpersistent manner hence insecticides provide little protection against virus spread during the season. Host plant resistance (HPR) combining resistance to the diseases is an attractive solution to increasing bean productivity among small scale farmers.

The BRR resistant varieties introduced in Kenya carry the I gene that confers resistance to BCMV. These varieties together with others carrying the recessive gene have been

used to introduce resistance for the two diseases into the commercial varieties in Kenya through KARI bean breeding programme in collaboration with CIAT. Characterizing the developed population for the two diseases will have great impact on bean variety adoption and improved rural food security in Kenya.

1.5 Hypothesis

1. H0: BCMV and BCMNV prevalence is the same across soil fertility gradient of Nandi South.

   Ha: BCMV and BCMNV prevalence vary across soil fertility gradient of Nandi South

2. H0: Resistance of bean genotype to BCMV and BCMNV is the same.

   Ha: Resistance of bean genotype to BCMV and BCMNV differ.

CHAPTER TWO
LITERATURE REVIEW

2.1 Agronomic Requirements

Common bean is a day neutral plant it grows well in temperatures between 15^{o}C and 30^{o}C, with higher temperatures resulting in poor pod set (Norman, 1992). Soils suitable for growth of common beans are deep, well-drained, loamy soils, with a pH of 5.5 to 7.0. If the soil pH is below 5.5, liming is required, because beans are sensitive to high concentrations of aluminium and manganese (Norman, 1992). Common beans grow under an annual rainfall range of 700-1000 mm (Van Schoonhoven & Voysest, 1993).

2.2 Importance of Beans

Common bean is the most important edible food legume in the world representing 50% of grain legumes for direct human consumption (Mcclean *et al.* 2004). In Kenya, it's the most important pulse, and second to maize among important food crops (FAO, 2005). It is a source of dietary protein for human with relatively high amounts of amino acids, lysine, tryptophane and methionine. A single serving of beans provides at least half of the recommended daily allowance of folic acid, which is a vitamin B that is important for pregnant women (Lanza, *et al*., 2006). It also supplies 25% to 30% of the recommended levels of iron, and meets 25% of the daily requirement of magnesium and copper, as well as 15% of the potassium and zinc (Wortmann *et al.,* 1998). The properties of the carbohydrates found in common beans, along with their fiber content, make them ideal foods for the management of abnormalities associated with insulin resistance, diabetes and hyperlipidemia (Raatz, 2013). Common beans are rich in both soluble and insoluble fibbers that provide nutritional benefits. The soluble fibre in beans dissolves in water,

trapping bile which helps to lower blood levels of cholesterol, especially if cholesterol levels were high to begin with, without compromising the level of protective cholesterol. Insoluble fibers in common beans attract water to the stool and enhance transit time of waste through the colon. This may help to combat constipation, colon cancer and other conditions that afflict the digestive tract (Raatz, 2013). Although dry beans vary considerably in flavor, size, color, and shape, their nutritional composition is remarkably similar. (Table 1 provides an example of the nutrient content of cooked dry beans)

Table 1: Nutritional value of cooked black beans (g=grams, gm=milligrams)

Nutrient available	**Quantity in 86gm**
Calorie	1g
Saturated Far	1g
Cholesterol	0g
Carbohydrates	20g
Protein	8g
Dietary Fibre	8mg
Sodium	1mg
Thiamine	1mg
Folic acid	12gm
Copper	1mg
Iron	2mg
Magnesium	60mg
Manganese	1mg
Phosphorus	120m
Potassium	306mg

Source: Nutritional value of dry beans (Raatz, 2013)

2.3 Beans Virus Disease

Bean common mosaic disease is caused by two related but distinct viruses. Usually referred to as, Bean common mosaic virus (BCMV) and Bean common mosaic necrotic virus (BCMNV). This is an economically important disease globally (Mavric et al., 2003). While sequence data are the major criteria for discriminating between different viruses, biological and biophysical properties are the major criteria for discriminating between virus strains (Movric and Susta-vozlic, 2004). BCMV usually causes mosaic symptoms on bean cultivars and only some strains can cause systemic lethal necrosis on sensitive cultivars at higher temperatures (Movric and Susta-vozlic, 2004). BCMNV cause systemic lethal necrosis on bean genotypes possessing dominant resistance gene *I* at lower and higher temperatures (Silbernagel *et al*., 2001). However, all known BCMV and BCMNV strains cause similar symptoms in bean genotypes lacking resistance genes (Morales, 2003). The occurrence of either type of symptoms on beans therefore depends on the virus species strain, bean cultivar and temperature (Brunt *et al*., 1996)

Both viruses (BCMV) and (BCMNV) are seed borne, transmitted by aphid species and mechanical inoculation up to 83% by seed (Movric and Susta-vozlic, 2004)., causing 6-98% and 100% bean yield losses respectively (Gálvez and Morales, 1989; Mukeshimana *et al*, 2003). Cultivars susceptible to BCMV and BCMNV show vein banding, leaf distortion, downward curling and mosaic or mottle symptoms on leaves (Plate 1), and may allow seed transmission (Morales, 1989).. For some BCMV isolates at temperatures greater than 30^0C and for all BCMNV susceptible cultivars infected with BCMNV and held at any growing temperature, plants will first exhibit pinpoint necrotic local lesions on inoculated primary leaves, followed by veinal necrosis and eventually top necrosis in

the trifoliate leaves which eventually kills the plant (Plate 2). Cross section of stems and pods reveals a red-brown streaking in the vascular tissue, a distinct symptom of the disease (Davis and Frate, 2007).Both viruses can be found in the same area and infecting the same plant (Silbernagel *et al*., 2001).

Plate 1: **Alternating areas of light and dark green characteristic and downward curling expressed by bean varieties susceptible to BCMV and BCMNV (Source Mukeshimana, *et al*. 2003)**

Plate 2. **Pinpoint lesions and vein necrosis on primary leaves of plants with *I* gene that were inoculated with a strain of BCMNV. Emerging trifoliate leaves start dying and finally the whole plant dies (Miklas *et al*. 2002).**

2.4 Prevalence and Distribution of BCMNV and BCMV

BCMNV strains originated in Africa but have spread to Europe, United States and Canada (Kornegay, 1992). The strains NL-3, NL-5, NL-8 and TN-1 are the widest spread in east Africa and represents 53% of all BCMNV strains in the region (Larsen et al, 2011).

Both viruses are transmitted naturally by aphids (in a non-persistent manner) and by seed, this explains their worldwide distribution. Domesticated crops and wild plants act as reservoirs for both the viruses and aphid vectors. Lima and Gonslaves, (1988) identified *Cassia occidentalis* as a reservoir of BCMV infecting cowpea. Spence and Walkey, 1995 isolated the pathogen from several wild legume species in Kenya, hence concluded that natural occurrence of BCMV in wild legume species in Africa is probably a significant factor in the ecology, epidemiology of the virus and possibly the evolution of bean common mosaic necrosis virus, which induce necrotic reactions in cultivars carrying the *I* gene for resistance to BCMV. Buruchara, (1979) isolated a severe strain of BCMV from the *P. vulgaris* cultivar Canadian Wonder in Kenya. The strain was designated K-BCMV and was shown to be similar, but not identical, to a known strain (NL3). However, the isolate caused mosaic symptoms in cultivars without the dominant resistance.

Isolates with a similar pathogenic range on differential hosts to NL-3 and NL-8 were identified in a survey of beans in Kenya (Omunyin, 1988), however, there were differences in the reactions of cultivars with dominant resistance to these isolates. Omunyin, (1995) found that BCMV incidences in Kenya varies between 0-60%. Virus isolates from infected beans have been differentiated into four pathogenetic groups (PGs),

including; KNI (PG-VI) and KN3 (PG-V), the majority of the isolates being the necrosis inducing type (Omunyin, 1995). Bock *et al.* (1976) isolated BCMV from beans in several important bean growing areas in Kenya: Thika, Muguga, Naivasha and Kakamega. The isolates were tested on the range of differentials (Drijfhout, 1978) and produced necrotic symptom. Vale *et al.,* 1994, concluded that, mixed virus infections can result into severe infections and recombination has been observed between strains of BCMV and BCMNV (Silbernagel et al., 2001).

The levels of infection of BCMV in *P. vulgaris* crops are related to both the initial levels of virus in seed and the presence of aphid vectors. The disease can be transmitted in a non-persistent manner by several aphid species (Miklas *et al.,* 2000). The Aphids normally transmit BCMV as winged migrants, especially *Acyrthosiphon pisum, Aphis fabae* and *Myzus persicae* (Kennedy *et al.*, 1962; Zettler and Wilkinson, 1966). Vectors provide a means of secondary spread of BCMV within a crop or primary infection of a healthy crop.

Studies by Klein *et al*., 1989 recorded that BCMV spread in the field were by the non-colonising aphids. More than 60% BCMV incidence were recorded in Kenya on *Phaseolus vulgaris* L, and it was concluded that, infection levels were associated with distribution of the vector *Aphis fabae* and seed selection practices of farmers (Omunyin *et al.*, 1995). BCMV is also transmitted by other cereal aphids namely *Metapolophium dirhodum, Rhopalosiphum padi*, and *Schizaphis graminum* (Halbert *et al.*, 1994). In an experiment, three Aphids transmitted 24-55 % BCMV disease effectively (Bashir and Hampton, 1994). The warm and wet weather conditions in the study area favours Aphid activity. The tropical forest surrounding the study area with divers' wild legume plants

provides alternative host for the viruses. Thus with the two factors combined there is high risk of increased virus epidemics in the region.

2.5 Genetic Control

Pathotypes (Isolates), of BCMV and BCMNV are assigned to one of the eight different pathogenicity groups based on a differential bean host reaction (Drijfhout, 1978). Each pathotype within a pathogenicity group corresponds to a known set of resistance genes. The form of resistance to the viruses include, recessive isolate specific genes and the dominant I gene. The recessive genes provide resistance to some pathotypes of the two viruses while *I* gene gives immunity to BCMV isolates at temperatures less than 30^0C (Mikilas, 2002)

The recessive isolate-specific genes consisting of five alleles *bc-1*, *bc-1*$_2$, *bc-2*, *bc-2*$_2$, and *bc-3* at three loci that require *bc-u* for expression, and the dominant *I* gene, except *bc-3*. The *bc-3* gene confers resistance to all known strains of BCMV and BCMNV in the presence of the dominant resistance *I* gene. In the presence of the recessive *i* gene, *bc-3* requires the presence of *bc-u* to be fully expressed. Cultivars with *I,bc-3* and *bc-u,bc-3* combinations are immune to all strains of BCMV and BCMNV while *i, bc-3* genotypes are resistant to all strains of BCMNV but susceptible to some strains of BCMV (Miklas *et al.*, 1998). Moreover, the *I, bc-1*$_2$, and *bc-3* gene loci have been associated with resistance to other potyviruses (Fisher and Kelly, 1994; Larsen *et al.*, 2008; Larsen and Miklas, 2010).

Cultivars susceptible to BCMV and BCMNV show vein banding and mosaic or mottle symptoms on leaves, and may allow seed transmission (Mills and Silbernagel, 1992).

Plants with resistance to a specific isolate generally remain symptomless, although some host – pathogen combinations will produce necrotic local lesions after mechanical inoculation with the virus (Milkas, *et al*., 2002). The *I* gene gives immunity to BCMV isolates at temperatures less than 30^0C (Drijfhout, 1978). For some BCMV isolates at temperatures greater than 30^0C and for all BCMNV susceptible cultivars infected with BCMNV and held at any growing temperature, plants will first exhibit pinpoint necrotic local lesions on inoculated primary leaves, followed by venial necrosis and eventually top necrosis in the trifoliolate leaves which eventually kills the plant (Milkas, *et al*., 2002), this symptom is called "black root", this is different from the black root rot fungal disease caused by *Thielaviopsis basicola*). Black root caused by BCMNV is distinct due to the blackened necrotic veins appearance when stems, pods and roots are cross-sectioned (Morales, 1989). The viruses are never seed-transmitted in cultivars possessing the *I* gene, but fields of an unprotected *I* gene cultivar may suffer total crop failure if seed of a susceptible cultivar contaminated with any strain of BCMNV is planted in close enough proximity for aphids to transport the virus from the susceptible to another unprotected *I* gene plant (Coyne *et al*., 2003). Releasing of new cultivars with *I* dominant resistance but lacking the recessive gene to protect the *I* gene is a liability to the breeding program (Allen 1992). Genotypic and phenotypic data comparison shows that genotypic selection is not superior to phenotypic selection (Kell *et al.,* 1995). However, due to epistatic effect of the recessive bc-3 gene on the dominant I gene, the use of markers is necessary to track presence of both genes (Kell *et al.,* 1995)

2.6 Phosphorus Deficiency in an Environmental Degradation contributing to a Biotic and Biotic Complexes

Mineral stress in soils occurs throughout the world in common bean production regions (Singh, 2003). Soil acidity, generally characterised by low pH, toxic levels of aluminium (Al) and manganese (Mn), deficiencies of Calcium (Ca), magnesium (Mg) and Phosphorus (P), is an important cause of low fertility in tropical soils (Sanchez, 1977). In Kenya acid soils cover 13% of arable land (Kanyanjua *et al.*, 2003)

Production of beans in Kenya is primarily by small scale farmers who use little or no fertilizer or soil amendment (Wotman *et al*., 1995). Phosphorus availability is a major limiting factor for plant growth, particularly in tropical soils. P deficiency has a stronger effect on legumes than on other plants because of the high energy costs of N_2 fixation, which requires a greater quantity of inorganic P (Vadez *et al*., 1999). Small holder farmers in the East African highlands are no longer self-sufficient in beans (David *et al*., 2000). Changes that have occurred in agricultural practices have contributed to increased insect pests and disease pressure in the ecosystem (Miguel and Nicholls, 2003). The usage of organic fertilizers and pesticides has increased rapidly in the recent past and evidence suggests that such excessive use of agrochemicals in conjunction with expanding monocultures has exacerbated pest problems (Conway and Pretty, 1991)

Research has shown that the ability of a crop plant to resist or tolerate insect pests and diseases is tied to optimal physical, chemical and mainly biological properties of the soil. Soils with high organic matter and active soil biology generally exhibit good soil fertility as well as complex food webs and beneficial organisms that prevent infection (Miguel and Nicholls, 2003). On the other hand, farming practices that cause nutrition imbalances

can lower resistance of plants to pests (Magdoff and van Es, 2000). Nutrient deficiency or toxicity in common bean may cause symptoms such as; poor emergence, reduced growth rate, seedling and adult plant stunting, leaf yellowing, chlorosis, early seedling death, delayed and prolonged flowering and maturity; excessive flower and pod abortion (Sigh, 2003). Plant resistance to pests is linked directly to the physiology of the plant and thus any may lead to changes in resistance (Miguel and Nicholls, 2003).

Due to continuous cropping on ever reducing land coupled with limited capital, land degradation and soil infertility has increased greatly; further, there is a concomitant decline in crop vigor, pest and disease tolerance and overall system productivity (Ojiem *et al.,* 2008). Although legumes are clearly important in rebuilding soil N fertility (Ojiem *et al*., 2007), the lower P fertilizer inputs applied by farmers are rapidly rendered ineffective on these P-fixing soils which, when combined with other pH-related effects, reduces legume productivity, pest and disease tolerance. The green manure (Gachene *et al.,* 1999) and legume fallow (Niang *et al.,* 2002) technologies for amending degraded soils are poorly adopted, because they do not address farmer's immediate food need and income generation. Strategies to improve crop growth under P deficiency can be managed through use of crop varieties with efficient uptake of P, and effective use of available P (Vadez and Drevon, 2001). Bean cultivars that are efficient in uptake and utilisation of available nutrients are needed to give good performance in case of low nutrient supply or utilize supplied nutrients efficiently

In crop production situations, disease stresses and soil nutrient deficiency may occur simultaneously. Further there can be interaction among soil minerals and other abiotic and biotic factors (Bache and Crooke, 1981). Therefore, it is important to promote

common bean technologies that are suitable for low-input and fitting in disease prone environments (Nickel, 1987). Bean varieties like RWR-719 are suitable in phosphate deficient soils but because it is susceptible to BCMNV, farmers in the region are selecting against it.

2.7 Management of BCMV & BCMNV

There is considerable variability in pathotypes of BCMV and BCMNV in the Great Lakes Region (Spence & Walkey, 1995; Sengooba *et al.*, 1997) but interaction of root rot resistant material with these viruses require investigation to ensure durable resistance to both fungal and viral pathogens.

In an effort to control the BCMV, resistant genes were incorporated into bean population developed in Latin America (Drijfhout, 1991; Kelly, 1997). When the genotypes were brought to Africa they expressed lethal reaction with the strains in Africa that produced black root resulting into necrosis that kills the plant.

Control of plant viruses using direct chemical application is ineffective (Mukeshimana *et al*., 2003). The common control measures focus on preventing infection, delaying time of infection or minimizing the effect of infection once it has occurred. Such measures include control of vector, phytosanitary and agricultural management and plant host resistance (Nduguru, 2003).

Phytosanitary and agricultural management involve use of certified seeds and rouging of all infected plants and weed control. Use of disease-free seeds can reduce disease incidence significantly and minimize crop loss by up to 50%, unfortunately most farmers in Kenya use bean seed from the previous season crop (Opole, *et al.* 2003). Though

Nduguru (2003) reported that rouging is effective, other people have observed that it doesn't work, because the transmission of virus is by several aphid species such as *Myzus persicae* and *Aphis fabae* (Marales, 1989). The transmition is non-persistent, thus the virus can be acquired and transmitted from infected to healthy bean plants within a few seconds (Marales, 1989). *Aphis fabae* is a serious bean pest in Kenya, (Omnyin, 1995)., therefore, BCMV can efficiently be transmitted within bean plantings and sometimes disease incidence is high. Use of resistant or tolerant cultivars is the most sustainable way of stopping virus infection and spread (William *et al.*, 1997; Mukeshimana *et al*., 2003). In real farming situation both abiotic and biotic stress may occur simultaneously therefore, holistic approach is necessary. Bean varieties that are high yielding with good nutrient use efficiency would be more useful in virus prone environments

CHAPTER THREE
MATERIALS AND METHODS

3.1 Objective 1: To collect base line information on Disease (BCMV and BCMNV) occurrence across sites in Nandi South.

3.1.1 Survey Sites in Western Kenya

Observational method survey to establish the occurrence of BCMV and BCMNV in beans was conducted in Nandi south district in four sites; Koibem (UM_2, UM_3), Bonjoge (UM_3, UM_4)) Kiptaruswo (UM_1 and Kapkerer (LM_2) representing mid (1550-1800 m) and low (700-1200 m. a. s l). altitude zones (Jaetzold and Schmidt, 2009) , High, Medium high, Medium Low and Low soil fertility (Table 1) respectively in western Kenya. The survey was conducted between November and October 2009, 50-60 days after planting. Twenty farms were randomly selected at each site from which beans were sampled for BCMV, BCMNV and aphid infestations. The procedure used to identify the farms was to select one farm after every four farms along the main and village access roads. Combinations of purposive and simple random sampling methods were used to select the bean fields and sampling sites. The main criteria for identifying BCMV in the field were the mosaic symptoms associated with stunting and Leaf malformation. BCMNV identification were in reference to symptoms associated with necrosis of apex leaves and vain necrosis, while aphid infestation was rated on a scale of 1-9, where, 1= no aphid, 3=one to five aphids, 5=five to ten aphids, 7=ten to fifteen aphids and 9=more than fifteen aphids found. The survey data was used to select bean evaluation experimental sites.

3.1.2 Data collection

Four sites were sampled per farm and 20 plants examined for disease incidence. Disease incidence was recorded as the number and expressed as a percentage of the total number of plants observed, while disease prevalence was determined as the percentage of the number of the bean fields in which the disease was observed in each site.

3.1.3 Data Analysis

Data was analyzed using SAS 8.2 Statistical software and separation of means was by Least Significance Difference (LSD) test at $\rho \leq 0.05$.

3.2 Objective ll: Evaluation of new bean genotypes resistant advanced lines for Resistance to BCMV and BCMNV

Two experiments were carried out field screening at four sites and screen house at KARI Kakamega. The relevant characteristics of these sites are shown below (Table 1)

Table 2: Geographical and climatic information of the study site

SITES	KOIBEM	BONJOGE	KIPTARUSWO	KAPKERER
LATITUDE	00° 09’ 28.2’’N	00° 06’ 52.2’’N	00° 02’ 28.1’’N	00° 00’ 31.9"N
LONGITUDE	034° 48’ 14.6" E	034° 48’ 14.6" E	034° 48’ 14.6" E	034° 48’ 14.6" E
ELEVATION	1770 MASL	1674 MASL	1582 MASL	1530 MASL
ANNUAL MEAN TEMP.	180C	18.50C	200C	210C
AEZS	UM1	UM2	UM3	UM3
SOIL FERTILITY CLUSTER	HIGH	MEDIUM HIGH	MEDIUM LOW	LOW

Source: Jaetzold, 2009

3.2.1 Experiment 1: Field Screening of Genotypes

3.2.1.1 Experimental Site and characterization

The experiments were carried out in four sites (Koibem, Bonjoge, Kiptaruswo and Kapkerer). Sites selection was done in reference to the observational survey in objective one. Before planting soil analysis was done to characterize the study sites.

3.2.1.2 Soil sampling and analysis

Soil sampling was by randomized grid method. Auguring was done to a depth of 30 cm and the soil put in a clean plastic bucket for thorough mix. A sub sample of 500g was taken. The soil was then air dried by spreading it out in a shallow tray in a well-ventilated place protected from rain and contamination. The soil lumps were crushed gently to separate gravel and roots from the mineral soil.

The soil was then sieved through a 2 mm sieve for pH (determined with water 2.5:1 H2O). Particle size analysis and extractable P determined calorimetrically (Murphy and Riley, 1962) and exchangeable bases analysis and 60 mesh soils for organic carbon was done according to Okalebo et al., 2002).

3.2.1.3 Genotypes

Twenty six bean genotypes from two gene pools (twenty three bean genotypes bred for bean root rot resistance and advanced bean lines selected for Phosphorous efficient) were evaluated. Two varieties GLP-585 a commercial variety susceptible to BCMV disease and RWR719 bean variety with specific gene resistance to BCMV were used as controls.

The 26 genotypes were subjected to field evaluation for viral disease reaction. The experiment was planted during the long rain of March 2010. The natural virus innoculum

was promoted by two approaches; Planting the experiments two weeks later after the surrounding farmer had planted, planting the susceptible variety GLP-585 round the experimental three weeks early before planting the experiments.. Prior to planting, the taste bean were treated with FansanD (20% w/w Thiram) at recommended rates (3g of chemical per 1kg seed).The aim of seed treatment was to protect the seed from bean root rot (BRR) fungi and bean stem maggot (BSM) that could set in due to late planting. Alpha lattice design was adopted for this experiment (Nguyen, 2002). Each variety was planted in a plot size of (1.5 x 2) meter square, with inter-row and intra-row spacing of 0.5 metres and 0.1metres respectively, one seed per hill. At each site, there were four genotypes in each block of 7meters square. There were a total of seven blocks replicated four times per site (total of 112 plots). No chemical spray was applied after emergence, to encourage insect vector population. Experimental fields were kept free of weeds throughout the experimental period.

Table 3: Characteristics of bean (Phaseolus vulgaris L) genotypes evaluated for BCMV and BCMNV across sites in Nandi South

Sno	Line	Special Attributes	Status of BCMV/BCMNV	Seed size	Seed colour
1	SCAM-80/5	RR	Unknown	Medium	Red Calima
2	BCO-05/10	RR	Unknown	Medium	Red Calima
3	BCO-05/32	RR	Unknown	Medium	Red Calima
4	BCO-05/18	RR	Unknown	Medium	Red Calima
5	BCO-05/37	RR	Unknown	Medium	Red Calima
6	BCO-05/07	RR	Unknown	Medium	Red Calima
7	BCO-05/43	RR	Unknown	Medium	Red Calima
8	BCO-05/25	RR	Unknown	Medium	Red Calima
9	BCO-05/03	RR	Unknown	Medium	Red Calima
10	BCO-05/09	RR	Unknown	Medium	Red Calima
11	BCO-05/35	RR	Unknown	Medium	Red Calima
12	BCO-05/162	RR	Unknown	Medium	Red Calima
13	RWR 719 (Control)	RR	RR-C	Small	Red
14	KK-RRB05/31	RR	Unknown	Small	Red
15	KK-RR-B0/21	RR	Unknown	Small	Red
16	MHR-314	P-E	Unknown	Small	Red
17	KK-RRB05/34	RR	Unknown	Small	Red
18	KK-RRB05/25	RR	Unknown	Small	Red
19	KK-RRB05/23	RR	Unknown	Small	Red
20	XRAV-187-3	RR	Unknown	Small	Black
21	ME-2221-34	P-E	Unknown	Small	Red
22	KK-RR B05 / 20	RR	Unknown	Small	Red
23	KK-RCAL-27/A	P-E	Unknown	Small	Black
24	KK-RCAL-194	RR	Unknown	Small	Black Calima
25	KK-RCAL-288	RR	Unknown	Small	Black Calima
26	KK-RCAL-70	RR	Unknown	Small	Black Calima
27	KK-RCAL-147	RR	Unknown	Small	Black Calima
28	GLP-585 (Control)	SS	SS-C	Small	Red

RR- Bean Root Rot Resistant, SS- Susceptible to Bean Root Rot disease RR- C-Resistant to BCMV, SS-C-Susceptible to BCMV and P-E = Phosphorus efficient

3.2.1.4 Field Data Collected

The parameters measured were, stand count seven days after emergence by counting the number of seedlings that had emerged and stand count at harvesting, Incidences of BCMV and BCMNV (determined 50-60 days after emergence corresponding to podding stage). The procedure of scoring was by marking of the first bean plant followed by scoring every second plant within the row to avoid biasness, a total of 20 plants were sampled in each plot. The virus symptoms were rated on a 1-9 CIAT scale (Van Schoonhoven and Pastor-Corrales, 1987; Mills and Silbernagel, 1992), where, 1= not, 3= slightly, 5= moderately, 7= severely and 9= completely diseased. Aphid colonies were assessed by counting the number of aphids present on a score scale of 1-9, where 1=no aphids), 3=1-5 aphids, 5=5-10 aphids, 7=10-15 aphids and 9= (more than 15 aphids). Yield Data was obtained by sampling mature beans per plot and threshed separately. The grains were obtained, weighed using an electronic balance and plot grain yield was determined for each genotype. The Grain Yield (GY) was calculated in tonnes per hectare using the following formula:

$$\text{Yield} = \mathbf{GW} \times \frac{(100-mc)}{87} \times \frac{(10000)}{\text{Plot size}} \times (0.85) \quad \ldots\ldots(1)$$

Where;

Y=grain yield (tonns/ha); **GW**=plot grain yield in kilograms; **mc** = moisture content of beans at harvest, **87** = is a constant, **0.85** = the shelling percentage and **Plot size**= Net plot Area

3.2.1.5 Data analysis

Data analyses were done using the statistical software GenStat (12th Edition, VSN International Ltd, 2010). Means were separated using Turkey's range test. A correlation analysis was done to check the effect and significance of the bean viruses on other dependent variables. These included stand count at emergency (ST1), Stand count at harvest (ST2), first flower (FL1), 50% flower (FL2), pods per plant (PP), seeds per pod (SP), days to maturity (MD) and yield (YLD).. Analyses of variance (ANOVA) were performed for all measured parameters for bean under virus stress at individual site and across sites. T-test was used to check the significance of the genotypes and environmental effects, as well as the genotypes by environment (G x E) interactions.

Statistical model

$$Yijkl = \mu + \alpha i + \beta j + \tau ki + \tau kj + \rho l + \tau \rho kl + \varepsilon ijkl \quad (2)$$

Where:

- Yijkl- The individual observed aspects in each plot;
- μ- Overall mean;
- αi- Estimation of ith effect of replication
- βj- Estimate of jth effect of block;
- τk -Estimate of the kth level of genotypes in the rep ith and site lth;
- τk -Estimate of the kth level of genotypes in the block jth and site lth;
- ρl- Estimate of the site effect;
- τρkl- Estimate of the genotype x site interaction effect;

3.2.2 Screen House Evaluations

3.2.2.1 Experimental Site

The Screen house evaluations were carried out at KARI Kakamega to confirm field results.

3.2.2.2 Materials and Methods

The bean genotypes that showed no symptoms for BCMV and BCMNV in the field were further tested under screen house (Table-3). RWR 719 bean variety with resistance to BCMV was included as a control. GLP-585 was also included as local check which is susceptible to BCMV. The test Beans were planted in polythene pots of 20cm diameter by 25cm height. The planting was forest Soil, Gravel previously washed and farmyard manure in the ratio of 3:1:1. The experiment was laid as a Completely Randomized Design (CRD) with 3 replicates and a pot representing a plot. Pots were filled, arranged in the screen house and watered. Bean varieties assigned random numbers were planted five seeds per pot, later thinned to three after emergence. Three treatments were applied; BCMV, BCMNV and None inoculated control plants. The pots were watered twice per day. The minimum and maximum screen house daily temperature was scored.

Table 4: **Bean genotypes evaluated in the screen house for resistance to BCMV and BCMNV Resistance**

Sno	Cultivar	Special attributes	Seed size	Seed colour
1	BCO 05/03	RR	Medium	Calima
2	RWR-719 (control)	RR	Small	Red
3	KKRCAL-288	RR	Medium	Black Calima
4	BCO-05/09	RR	Medium	Calima
5	KKRCAL-70	RR	Medium	Black Calima
6	BCO-05/25	RR	Medium	Calima
7	BCO-05/43	RR	Medium	Calima
8	BCO-05/18	RR	Medium	Calima
9	GLP-585 (control)	SS	Small	Red
10	BCO-05/07	RR	Medium	Calima
11	BCO-05/37	RR	Medium	Calima
12	KKRCAL/147	RR	Small	Black Calima

SS-susceptible to Bean root rot, RR- Resistant to Bean root rot

3.2.2.3 Inoculum and Inoculation

The BCMV and BCMNV inoculum were obtained from diseased plants in the field experiment, collected separately and used to pre-infect Umbano a bean variety susceptible to the virus. The first and second trifoliate leaves of 15 days old inoculated umbano showing disease symptoms were harvested, insuring that plants infect with different virus were handled separately. The leaves were ground in a mortar and the paste was filtrated through two layers of blotting paper and plant sap extracted. The extracted sap was diluted ten times using 0.02 M KPO4 buffer pH (7.5) to obtain the inoculum (Mill and Silbernagel, 1992, Chiumia and Msuku, 2001). The diluted sap containing the virus was maintained at -4^0C. Seven days after emergence the inoculum was applied. The leaves of the beans were dusted with carborundum and gently rubbed with cotton swap previously dipped into the suspension of the virus inoculum. Two bean plants were inoculated and one served as a control. Dimethoate was applied on two weeks interval to

prevent spread of virus from one plant to another by aphids. Reaction to bean virus was recorded from the 5th day after inoculation recommended by Chiumia and Msuku, 2001 and continued for the next 28 days, a 1 to 9 CIAT scale was used to determine the resistance,

where,1 = no visible virus symptoms on both the inoculated and un-inoculated leaves, while 9 = severely diseased or dead plants.

Statistical Analysis was by application of the PROC MIXED procedure in SAS (SAS institute 2001) version 8.2. Separation of means was done by least significant difference (LSD) at 5% probability.

The following model was used for the screen house experiments.

$Yij = \mu + \beta i + \varepsilon ij$

Where:

- Yij- the individual observation in screen house
- μ- overall mean
- βi- estimate of the ith treatment effect (innoculum: i=1, 2,……n)
- εij- estimate of experimental error
- Genotype classification was done according to CIAT recommendations (Table 5)

Table 5: Genotypes classified cording to CIAT classification

Rating	Description	Classification
1	No visible symptoms	**Resistant variety**- useful as commercial variety or parent
2-3	Visible and conspicuous symptoms resulting in only limited economic damage	**Tolelant variety**- Germplasm can be used as commercial varieties or as sources of resistance to certain diseases
4-9	Severe to very severe symptoms causing considerable yield loss or plant death	**Susceptible variety**- Germplasm in most cases is not useful as parents or commercial varieties

CHAPTER FOUR
RESULTS

4.1 Objective 1: Base line study to determine prevalence of BCMV and BCMNV diseases across soil fertility gradient.

4.1.1 Occurrence of BCMV and BCMNV in Nandi South

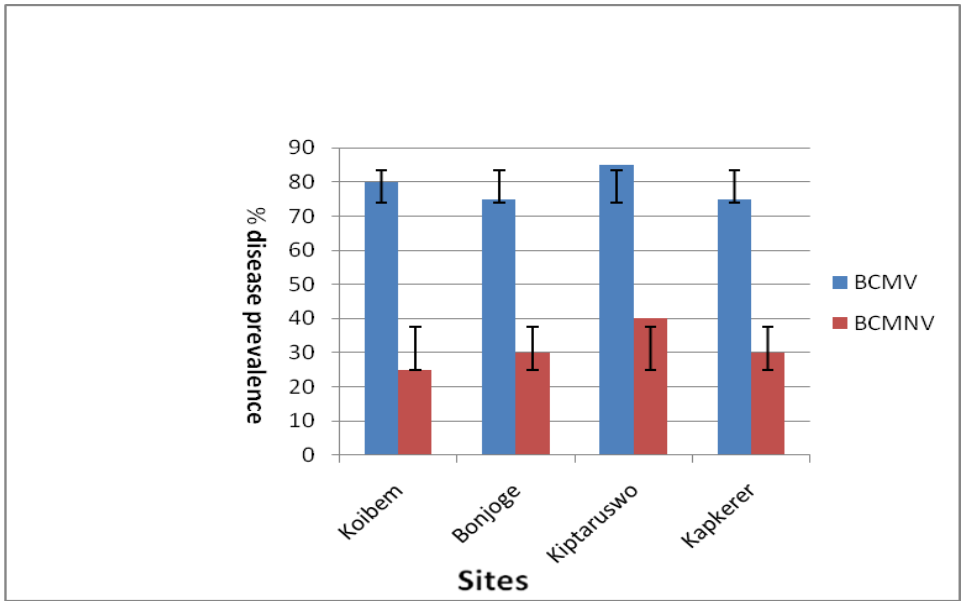

Figure1. Occurrence of BCMV and BCMNV in Nandi South. (The bars represent the standard error of differences (SED))

Bean Common Mosaic Virus was observed in all the four sites surveyed and was recorded in 78.8% farms visited. The disease was prevalent in 75% of the bean fields surveyed in Bonjoge and Kapkerer (figure 1). In Koibem and Kiptaruswo the disease prevalence was 80% and 85% respectively. BCMV was recorded highest in Kiptaruswo. In Kiptaruswo and Kapkerer three farms were free of the disease while Koibem and Bonjoge 4 and 7 farms were free of the disease respectively. On the other hand BCMNV occurred in all the sites surveyed and was recorded in 31.3% farms visited. In Bonjoge,

BCMNV was observed in 6 (30%) farms out of twenty and similarly in Kapkerer, while in Kiptaruswo and Koibem the disease was observed in 8 (40%) and 5 (25%) farms out of twenty visited in each site. However BCMV was more prevalent than BCMNV in all the four sites surveyed but both diseases were highest in Kiptaruswo (Figure 1). These results are in agreement with (Rheneen, 1984) who reported the incidence of both BCMV and BCMNV in bean growing regions in Western Kenya. The necrotic symptoms were evident in the study site (plate 3), an indicator that farmers are growing beans that carry the I gene that express such symptoms when infected with BCMNV virus

Plate 3: Necrotic symptoms on beans intercropped with maize in BonjogeMenings farm (Source: Author 2010)

4.1.2 Incidence of BCMV and BCMNV) in Nandi South

The incidence of BCMV varied among the sites (Table 6). Eighty percent of the farms visited had disease incidences. The disease incidence was highest (61.50%) in

Kiptaruswo, whereas Bonjoge had the lowest incidence (35.25%). Seventy percent of the farms in Kiptaruswo had disease incidence of more than 60%. In Bonjoge, 70% of the total farms visited, had disease incidence of less than 50%. The disease incidence BCMV in all sites was not significantly different except in Bonjoge the incidence was significantly different to Kiptaruswo and Koibem. Consequently, the disease (BCMNV) was recorded with lowest percentage incidences a cross the sites (Table 6). Ninety Eight percent of the farms had a disease incidence of less than 50%. The disease incidence was lowest (1.9%) in Kapkerer, whereas Koibem had the highest incidence (8.2%). Twenty five percent of the farms in Kiptaruswo had a disease incidence of more than 3%. In Koibem, 20% of the farms had disease incidence of more than 10%

Table 6. Incidence of BCMV and BCMNV in Nandi South

Site	BCMV Incidence	Percent Farms with BCMV	Site	BCMNV incidence	Percent Farms with BCMNV
Kiptaruswo	61.50a	70	Koibem	8.2a	25
Koibem	55.30a	35	Kiptaruswo	3.8ab	25
Kapkerer	43.60ac	40	Bonjoge	2.5b	5
Bonjoge	35.25bc	70	Kapkerer	1.9b	5
Means	48.91	53.75	Means	4.08	16.25
Lsd=0.05	17.81	-	Lsd-0.05	5.46	-
S.E	6	-	S.E	2	-

Means in each column followed by the same letters are not significantly different at $p = 0.05$

4.2 Objective II: Evaluations of Bean genotypes for resistance to BCMV and BCMNV

4.2.1 Field screening

4.2.1.1 Site characterization

Table 7: Soil physical and chemical characteristics of soils at the experimental sites

Soil parameters	Experimental sites			
	Kapkerer	Kiptaruswo	Bonjoge	Koibem
pH	5.5	5.4	5.6	6.1
Organic carbon (%)	0.3	1.0	1.6	2.4
Total nitrogen (%)	0.16	0.22	0.27	0.26
Available phosphorous (ppm)	7.59	8.12	9.25	9.38
Available potassium (ppm)	283	336	470	552
Available calcium (ppm)	521	454	480	600
Soil Type	Clay	Sandy clay loam	Clay loam	Clay loam

Table: 8 Soil classification and element critical levels

Levels	Elements					
	% Carbon	Available Phosphorous	Calcium levels (mg/kg)	% Nitrogen	Magnesium levels (mg/kg)	Nitrate Levels
High	>3.0	>13	1600-2400	>0.25	>180	15-24
Moderate	1.5-3.0	8-13	1000-1600	0.12-0.25	80-180	9-15
Low	0.5-1.5		500-1000	0.05-0.12	20-40	3-9
Very low	< 0.5	< 8	<500	<0.05	<20	<3

Source: Okalebo *et al*., 2002

In reference to Okalebo *et al*., Most of the soil elements analysed was low in Kapkerer followed by Kiptaruswo While in Bonjoge and Koibem the elements were fairly high.

This was exceptional for available calcium that was high in Kapkerer compared to the other three sites. In Kapkerer organic carbon is very low, available phosphorous is low, available calcium is very low and available nitrogen is low. Available calcium is low for all sites. This results show some soil fertility gradient in the sites.

4.2.1.2 Rainfall distribution throughout the cropping season

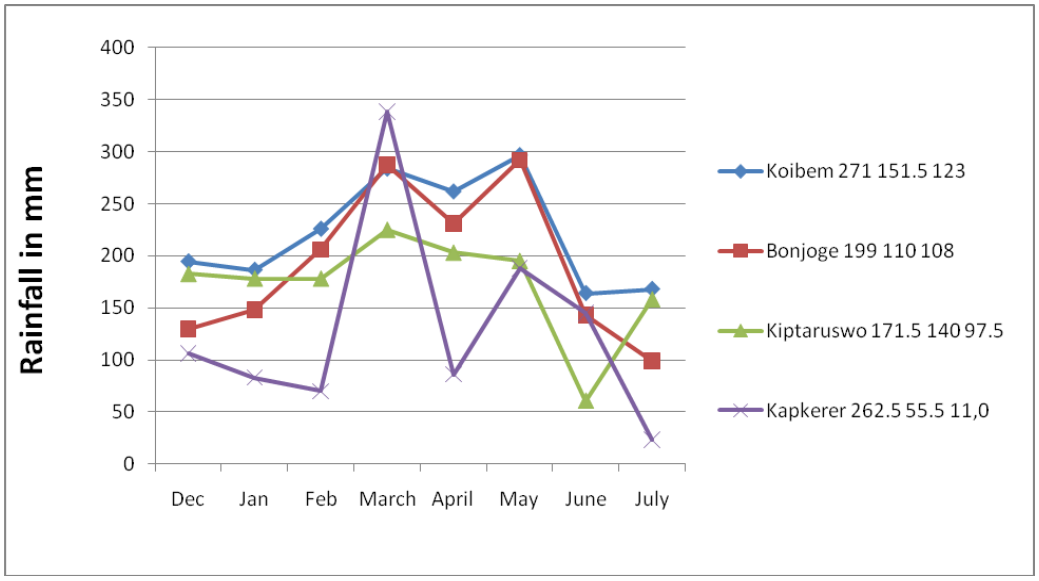

Figure 2 Rainfall distributions during cropping season 2010 across sites

Rain was distributed through out the cropping seasons in all the sites. There were two rain picks in the season; march and may in all the sites. The two picks coincided with planting and flowering of the beans. The highest rainfall (340mm) was recorded in March at Kapkerer. The highest rainfall throughout the season was recorded in Koibem, Bonjoge, Kiptaruswo and lowest in Kapkerer.

4.2.1.3 Bean Genotypes Resistance to BCMV and BCMNV and Aphid infestation

Table: 9 Genotype reactions to BCMN and BCMNV virus stress in Nandi South-Long rain 20110

Genotypes	BCMV	BCMNV	APHID
KK-RRB05/21	4.81[a]	1.00a	2.31a
KK-RRB05/31	4.75a	1.00a	2.18a
GLP-585 (conrol)	4.75a	1.37a	2.37a
KK-RRB05/34	4.62ab	1.62a	2.31a
KK-RRB05/35	4.37ab	1.62a	2.06a
KK-RR B-05 / 20	3.66b	1.37a	1.93a
KK-RRB05/23	3.55b	1.13a	2.52b
BCO-05/25	1.50bc	1.00a	1.93a
RWR 719 (Control)	1.26bc	8.20b	3.06b
BCO-05/35	1.26bc	5.00d	2.18a
BCO-05/07	1.25bc	1.00a	2.00a
KK-RCAL-27/A	1.11bc	6.06c	2.23a
MHR-34	1.1bc	6.1c	2.50a
XRAV-187	1.0bc	6.07c	3.00b
ME-2221-314	1.09bc	6.01c	2.04a
LSD	1.2	0.87	0.66

BCMV= bean common mosaic virus, BCMNV= bean common mosaic necrotic virus, APHID=Aphid. Virus data scored on a scale of 1-9, where 1= resistant and 9 = susceptible. Aphids data scored on scale of 1-9, where 1= a few aphids present and 9 = many aphid present Means followed by similar letters in the column are not significantly different

The diseases BCMV and BCMNV and aphid infection were significantly different among genotypes in (table 8). The genotypes with highest score for BCMV had lowest score for BCMNV. Genotype **BCO-05/25, BCO-05/07** showered resistance to both viruses scores 1. The phosphorous efficient genotypes showed complete resistance to BCMV and

susceptibility to BCMNV (score 6.0-9 table 8 Appendix 3 table 4). High aphid infection did not necessarily result into high disease infection (table 8)

There were highly significant differences ($\rho < 0.001$) for all measured parameters in experimental sites (Appendix 3, table 2). Pods per Plant and the diseases (BCMV and BCMNV) were not significant ($\rho < 0.001$) between replications (Appendix 3, table 2). Plant stand after 2 weeks and the diseases (BCMV and BCMNV) were highly significant among blocks. The diseases (BCMV and BCMNV) were highly significant among genotypes

The Bean common mosaic virus (BCMV) showed highly significant differences ($\rho < 0.001$) in the sites and genotypes (Appendix 3, table 2). BCMV reaction grand mean was score 5 and the individual genotype means ranged from score 1-7(Appendix 3, table 3).

The Bean common mosaic necrotic virus (BCMNV) showed highly significant differences ($\rho < 0.001$) in the sites, genotypes and site genotype interaction (Appendix 3, table 3). The individual genotype means for BCMNV ranged from score 1-9 (Appendix 3, table 4)

Table 10 Bean genotypes, reaction to BCMV, BCMNV and Aphid infestation across sites.
Numbers represents sites where, 1=Koibem, 2=Bonjoge, 3=Kiptaruswo and 4=Kapkerer

Genotype	BCMV				BCMNV				APHID			
	1	2	3	4	1	2	3	4	1	2	3	4
SCAM-80/5	1a	2a	3b	2a	1a	1a	1a	1a	1a	3b	1a	1a
BCO-05/10	4c	3b	1a	2a	1a	1a	1a	1a	3b	2a	2a	1a
BCO-05/32	3b	2a	2a	1a	1a	1a	1a	1a	2a	2a	1a	3b
BCO-05/18	2a	1a	1a	1a	1a	2a	1a	1a	2a	1a	1a	1a
BCO-05/37	1a	1a	4c	1a	2a	3b	1a	4c	2a	2a	1a	2a
BCO-05/07	1a	1a	1a	1a	1a	1a	1a	1a	2a	2a	2a	1a
BCO-05/43	1a	2a	1a	1a	1a	1a	1a	1a	3b	1a	1a	1a
BCO-05/25	3b	4c	1a	1a	1a	1a	1a	1a	2a	1a	2a	2a
BCO-05/03	3b	1a	2a	2a	1a	1a	2a	1a	2a	2a	2a	1a
BCO-05/09	2a	2a	2a	2a	1a	1a	1a	1a	3b	2a	1a	1a
BCO-05/35	1a	2a	1a	1a	7f	3b	5d	5d	2a	2a	2a	2a
BCO-05/162	3b	1a	1a	2a	1a	1a	1a	1a	2a	1a	1a	1a
RWR 719 (Control)	1a	2a	1a	1a	9g	6e	9h	8f	3b	3b	3b	3b
KK-RRB05/31	5d	4c	5d	7f	1a	1a	1a	1a	3b	2a	1a	1a
KK-RRB05/21	4c	5d	3b	6e	1a	1a	1a	1a	3b	2a	2a	1a
MHR-314	1a	1a	1a	1a	9g	8f	8g	9g	1a	2a	2a	2a
KK-RRB05/34	5d	4c	4c	5c	1a	2a	2a	1a	3b	2a	1a	1a
KK-RRB05/25	1a	1a	1a	1a	5d	4c	4c	6e	2a	2a	1a	3b
KK-RRB05/23	1a	4c	4c	1a	1a	1a	1a	2a	3b	2a	2a	1a
KK-RRB05/35	5d	4c	2a	5d	1a	1a	2a	1a	3b	2a	1a	3b
ME-2221-314	1a	1a	1a	1a	9g	6e	7f	6e	2a	1a	1a	1a
KK-RR B05 / 20	3b	3b	3b	5d	1a	1a	8g	1a	2a	2a	1a	3b
XRAV-187	1a	1a	1a	1a	6e	5d	5d	4c	2a	1a	3b	1a
KK-RCAL-194	2a	1a	1a	4c	1a	1a	1a	1a	2a	1a	1a	1a
KK-RCAL-288	4c	3b	2a	1a	1a	1a	1a	1a	2a	2a	1a	1a
KK-RCAL-70	1a	4c	3b	1a	7f	1a	1a	1a	2a	2a	1a	1a
KK-RCAL-147	4c	2a	2a	2a	1a	1a	1a	1a	2a	2a	2a	1a
GLP-585 (Control)	4c	5d	4c	6e	1a	1a	1a	2a	3b	2a	2a	1a
Means	2.60a	2.61a	2.37a	2.56a	2.92a	2.05b	1.96b	2.02b	2.60a	2.25b	1.75c	1.60c

Virus data scores (scale of 1-9); 1= resistant and 9 = susceptible. Aphids data scored on scale of 1=9, where, (1= a few aphids and 9 = many aphid) Means followed by similar letter within a column are not significantly different at 5% level of significance.

There were significant difference $p \leq 0.05$ in resistance to BCMV among bean genotypes (Table 10). In Koibem nine (9) genotypes were tolerant to BCMV score 2-3 while eleven genotypes showed resistances to BCMV score 1. Likewise fourteen (14) genotypes were tolerant to BCMV in Bonjoge score 2-3, and eight (8) genotypes showed resistances to BCMV score 1. Similarly in Kiptaruswo twelve (12) genotypes were Tolerant to BCMV score 2-3 and eleven (11) resistant to BCMV score 1. In Kapkerer, seven (7) genotypes were tolerant to BCMV score 2-3 while thirteen genotypes were resistant to BCMV. The resistant check RWR-719 showed consistent resistance score 1.0 to the disease in all the four sites while the susceptible local cultivar GLP-585 also showed susceptibility to BCMV in all the four sites (score 4-6). The resistance of the genotypes to BCMV varied across sites, one genotype was resistant in one site and susceptibility in another. Like in the case of BCO-05/23 and KKRR-CAL-70 that were resistance to the disease in Koibem and Kapkerer, but susceptible to the same in Bonjoge and Kiptaruswo. BCO-05/25 was susceptible to BCMV in Koibem and Bonjoge and not in Kiptaruswo and Kapkerer. The most common symptom displayed with the susceptible genotypes was leaf curling an example is shown on KK-RR-B05/31 at Kapkerer (plate 4).

Plate 4: Bean common mosaic virus symptoms on KK-RR-B05/3 in Kapkerer Nandi South (Source Author 2010)

As for BCMNV, symptoms were expressed on nine (9) genotypes in Koibem, four (4) in Bonjoge, seven (7) in Kiptaruswo and six (6) in Kapkerer. The BCMNV symptoms were expressed as shown in plates 5a and 5b. Disease scores varied between 2 and 9 on a CIAT scale. Plate 5a shows necrotic symptoms of BCMNV on KK-RR-B-05/25 in all the four sites. However, SCAM80/5, BCO-05/25, BCO-05/09, BCO-05/32, BCO-05/43, KKRR-05/31, KKRR-05/21, BCO-05/10, KKRRCAL-194, KKRCAL-288, KKRCAL-147, KK-RCAL-70 and BCO-05/07 showed resistance to BCMNV consistently across all the four sites.

The check RWR-719 was highly susceptible (score 8- 9) to BCMNV. This suggests that less than half of the bean genotypes carry the resistant *I* gene. The local cultivar GLP-585 showed no symptoms for BCMNV it is most likely that the local cultivar do not carry the I gene that reacts with the necrotic virus.

Plate 5a: Advanced symptoms of BCMNV on susceptible bean variety (BCO-05/35) in Kapkerer- 2010 (Source Author 2010)

Plate 5b: Severe necrosis symptoms on susceptible bean variety RWR-719 in Nandi south (Source Author 2010)

4.2.1.4 Correlation of Disease (BCMV, BCMNV) with Aphid, dependent and independent

Table: 11 Correlation of disease (BCMV, BCMNV) with Aphid, dependent and independent variables

	STC	Flw1	NPP	NSP	YLD	APHID	BCMV	BCMNV
STC	1.000							
FLW1	-0.403***	1.000						
NPP	-0.055	0.136**	1.000					
NSP	-0.057	0.0180	0.025	1.000				
YLD	0.440***	0.0719ns	0.098	0.098***	1.000			
APHID	-0.240***	-0.415***	-0.076	0.004	-0.200***	1.000		
BCMV	-0.003NS	-0.304***	-0.009	-0.15***	-0.009*	0.15**	1.000	
BCMNV	-0.330***	-0.120**	-0.144**	-0.011*	-0.09**	0.111**	-0.34***	1.000

STC=Stand count at harvest, 1STflower, NPP=number of pods per plant,

NSP= number of seeds per plant, APHD=aphids and YLD=yield, BCMV=Bean common virus,

BCMNV=Bean common mosaic necrotic virus, *, **, ***, ns →Significant at P =0.05, P=0.01 or P=0.001 and not significant respectively.

Negative and significant correlation was observed between BCMV and FW1 (r = -0.304***), NSP (r = -0.15***) and YLD (r = -0.009*), while positive and significant correlation was observed between number of aphids and BCMV (r = 0.15**) (Table 11). However, negative and non significant correlation was observed between BCMV and STC

Negative and significant correlation was observed between BCMNV and STC (r = -0.330***), FW1 (0.120**), NPP (-0.144**), NSP (-0.011*), YLD (-0.09**) and BCMV (-0.34***) while positive and significant correlation was observed between BCMNV and APHID (r= 0.111**)

4.2.2 Screen House Evaluations

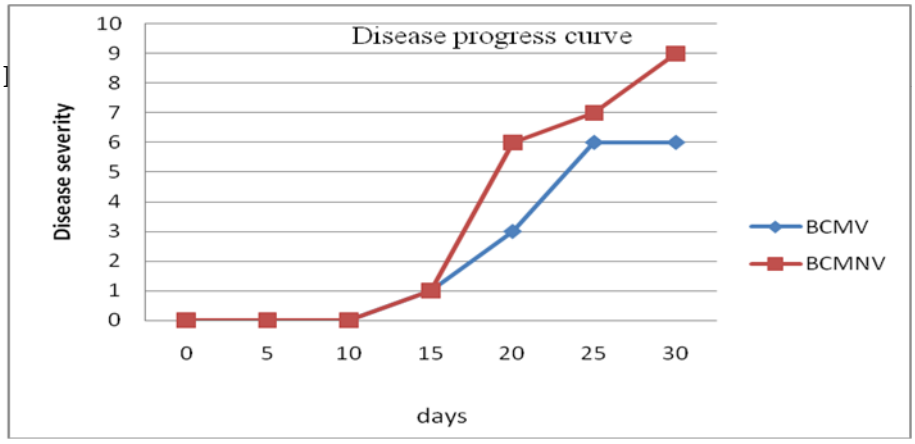

Figure 3: BCMV and BCMNV disease progress in the screen house in all tested line

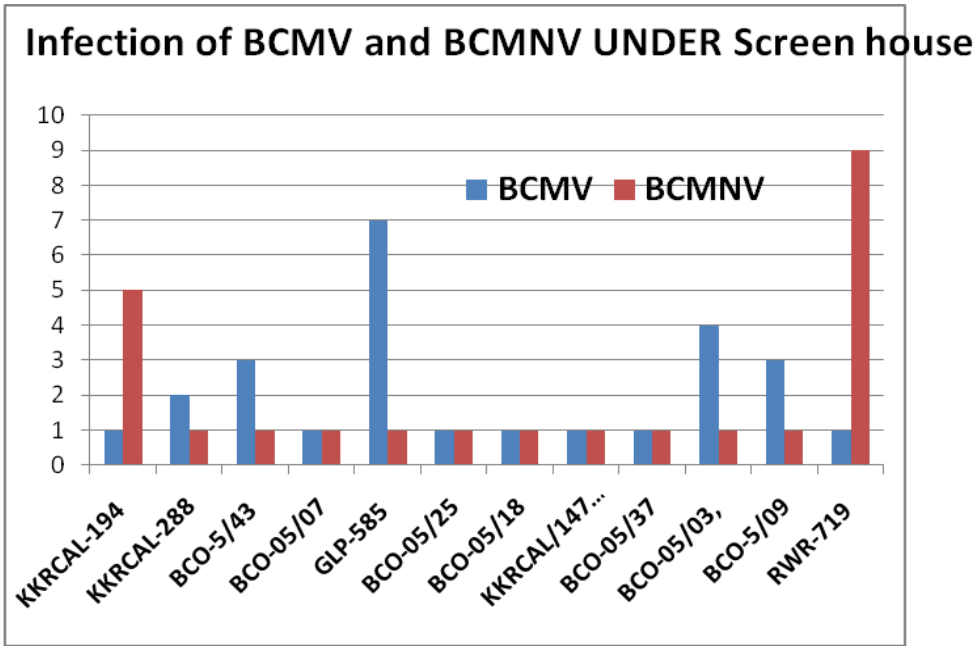

Figure 4: BCMV and BCMNV diseases scores in the screen house for all the tested lines

The bean lines artificially inoculated with BCM and BCMN virus in the screen house were infected with disease over time. The observed results showed significant difference

($p< 0.05$) among the genotypes disease resistance. The BCMV symptoms were observed on; BCO-5/09, KKRCAL-288, BCO-5/43 and BCO-5/03 and GLP-585 (Figure 3). The highest infection (score 7) was in the check line GLP-2 (Figure 3). Infection occurred from the 10^{th} day after inoculation (Figure 2). BCO-05/43 had shown resistance to BCMV under natural infection (Table 9) but expressed the viral symptoms under artificial infection (Figure 3) it is most likely that the genotype escaped Aphid infection in the field

BCMNV symptoms occurred on genotypes KKRCAL-194 under screen house. The check RWR-719 showed higher infection of BCMNV (score-9) (Figure-3) under screen house evaluation. KKRCAL 194 had shown resistance to BCMNV in the field. There were no disease symptoms on the non inoculated plants

The virus treatment induced a range of symptoms in infected bean genotypes including; severe standing and leaf distortion (Plate 6 and 7). These symptoms confirm absence of resistance to BCMV in the infected genotypes. There were some restricted local lesions on the inoculated primary leaves of KK-R –CAL-288, KK-R-CAL-27A genotypes, this would likely be due to the presence of dual resistance (combination of dominant I with bc2 recessive gene) in the genotypes. Genotypes with dominant I gene alone, will progress from necrotic local lesions to spreading veinal necrosis on the primary leaf. This spreading necrosis soon goes systemic into the vascular tissue of the midribs, petioles and stems, followed by systemic veinal necrosis and vascular discoloration from top leaves to roots, then death usually within 10-14 days (silbernagel *et al.,* 2001)

Plate 6: Sever standing after BCMV inoculation (Source Author 2010)

Plate 7: Showing pinpoint necrosis and distorted leaves (Source Author 2010)

Table 12: Classification of the BRR bean genotypes for resistance to BCMV and BCMNV

Genotype	BCMV	Classification	Genotype	BCMNV	Classification
KK-RCAL-27/A	1	Resistant	BCO-05/10	1	Resistant
BCO-05/07	1	Resistant	BCO-05/32	1	Resistant
BCO-05/35	1	Resistant	BCO-05/25	1	Resistant
RWR 719	1	Resistant	BCO-05/09	1	Resistant
ME-2221-34	1	Resistant	KK-RRB05/31	1	Resistant
BCO-05/43	1	Resistant	KK-RRB05/21	1	Resistant
MHR-314	1	Resistant	KK-RR B05 / 20	1	Resistant
KK-RCAL-70	1	Resistant	KK-RCAL-27/A	1	Resistant
BCO-05/37	1	Resistant	BC-05-/162	1	Resistant
BCO-05/162.	1	Resistant	KK-RCAL-288	1	Resistant
KK-RRB05/25	1	Resistant	KK-RCAL-147	1	Resistant
SCAM-80/5	1	Resistant	SCAM-80/5	1	Resistant
XRAV-187-3	1	Resistant	BCO-05/18	1	Resistant
BCO-05/18	1	Resistant	BCO-05/03	1	Resistant
BCO-05/03	2	Resistant	BCO-05/07	1	Resistant
BCO-05/09	2	Tolerant	GLP-585	1	Resistant
KK-RCAL-194	2	Tolerant	KKRR-B-05/25	2	Tolerant
KK-RCAL-288	2	Tolerant	KK-RRB05/23	2	Tolerant
BCO-05/25	2	Tolerant	KK-RRB05/34	2	Tolerant
BCO-05/10	3	Tolerant	BCO-05/43	2	Tolerant
BCO-05/32	3	Tolerant	KK-RCAL-70	2	Tolerant
KK-RR B05 / 20	4	Susceptible	BCO-05/37	2	Tolerant
KK-RRB05/23	5	Susceptible	KK-RCAL-194	2	Tolerant
KK-RRB05/34	5	Susceptible	BCO-05/35	4	Tolerant
KK-RRB05/31	5	Susceptible	XRAV-187-3	5	Susceptible
KK-RCAL-147	5	Susceptible	ME-2221-34	6	Susceptible
GLP-585	7	Susceptible	MHR-314	7	Susceptible
KK-RRB05/21	7	Susceptible	RWR 719	9	Susceptible

The bean genotypes tested were classified using the CIAT scale and the varieties were put into three groups for both Bean common mosaic virus and Bean common mosaic necrotic virus. The groupings were Resistant, Tolerant and Susceptible for both BCMV and BCMNV (Table 12)

CHAPTER FIVE
DISCUSSION

5.1 Disease (BCMV and BCMNV) Occurrence across Sites in Nandi South

The occurrence of BCMV and BCMNV This study showed that, BCMV was more prevalent in all the sites than BCMNV irrespective of the number of aphids per site. Further it was observed that BCMV incidence was higher than BCMNV incidence. These results show that the two strains of bean mosaic virus are present in all the four sites. It is evident that the bean varieties grown by the farmers in Nandi South are susceptible to BCMV and BCMNV. This result is in agreement with Omnyin 1995, who reported that, the bean varieties grown in the western region of Kenya are susceptible to bean common mosaic virus. The average number of aphids varied in sites but was not necessarily directly correlating to the disease symptoms this means as long as the inoculum for the disease is in the environment a single aphid will successfully inoculate the health susceptible varieties and they will show the disease symptoms. Further, the BCMNV symptoms observed (plate 3), could be associated to the fact that some farmers could be planting the BRR resistant varieties that were disseminated in the area (Odendo et al., 2001) which are known to have been developed from varieties carrying the I gene that is hyper sensitive to necrotic virus.

5.2 Field evaluations of Bean Genotypes for Resistance to BCMV and BCMNV

This study was set out with the aim of assessing the resistance of the improved bean genotypes to BCMV and BCMNV viruses. The tested genotypes showed varied reaction to both BCMV and BCMNV viruses. Some of the genotypes showed resistance while

others showed susceptibility to the viruses. Some of the tested genotypes were infected by the virus across the sites. This is a clear indication that the viruses are prevalent in all the site, just like it was reported in the first question of this research work. This implies that some genotypes were carrying the resistant genes to the viruses while others were not. In reviewing the literature, it was found that, the genotypes used in this study were developed from parents that carried resistant genes to either of the viruses. This was evident in the reaction expressed by the genotypes. `

A strong relation between the viruses and the aphid as a vector has been reported in the literature. This was evident in the positive strong correlation between the viruses and aphids in the results of the experiment. There was negative correlation between BCMV and BCMNV; this means there is a relationship between the viruses. As mentioned in the literature review that the genotypes with *I* gene that confers resistance to BCMV causes necrosis in such genotypes when exposed to environments with the necrotic virus strain. Further, the genotypes that have combined resistance of the dominant *I* and recessive gene will be immune to both viruses. Such a situation was expressed in the genotype BCO-05/07 that showed resistance to both viruses in all the sites. Aphid number varied from one site to another, this could be due to varied weather conditions in terms of temperature and rainfall intensity. The diseases pressure was not necessarily high where the vector for the disease was high. The primary aspect expressed is that as long as the disease inoculum and the susceptible varieties are available then the feeding activity of the aphid results in the infection of the susceptible bean genotype. . From the observation it is clear that the number of Aphids does not increases the bean virus disease incidence, but the inoculum in the environment, this is in agreement with Bashir and Hampton,

1994, who reported that as few as three Aphids transmitted 24-55 % BCMV disease effectively in bean crop.

There was no information found in the literature about the influence of soil fertility to bean virus. These results did not show such influence either.

5.3 Screen House Bean Genotype Evaluation

The range of symptoms induced on the bean genotype under screen house inoculation shows the variability in the bean genotypes. The commercial variety GLP-2 expressed mosaic symptoms on bean leaf infected with virus. This shows that; the genotype is susceptible to bean mosaic virus, and that the innoculum sourced from the infected beans in the field carried both BCMV.trains (Strasbaugh, 2003).

The local lesion on variety, KKRCAL-288, that was seen after mechanical inoculation with the virus, is an indication that there was pathogen combinations in the virus inoculum used (Miklas *et al*., 2002).

The high score of BCMNV on P use efficient beans genotypes indicates high susceptibility to the virus. This is a typical reaction of the bean genotypes that have the *I* gene (Candace, 2000), the resistant factor to BCMV but hypersensitive to BCMNV, this genotypes can be good sources of resistance to BCMV.

CHAPTER SIX
CONCLUSION AND RECOMMENDATIONS

6.0 Conclusion and Recommendation

Attaining high yield is the ultimate objective for bean breeding programme, but its evaluation and improvement are complicated with the complex bean biology, environmental interaction and multiple diseases

6.1 Conclusion

Both BCMV and BCMNV are prevalent in the Nandi South. Therefore bean varieties recommended for the region should carry the resistance to both viruses.

The control varieties GLP-585 used in the experiment showed high susceptibility to BCMV. Most of the BRR resistant beans genotypes showed susceptibility to BCMV while BCO-05/43, BCO-05/35, KKRR-05/25, BCO-05,18, KKRRCAL-27/A and BCO-05/07 were resistant to the virus.

Some of the BRR resistant bean genotypes showed resistance to BCMNV, this included SCAM80/5, BCO-05/25, BCO-05/09, BCO-05/16, KKRR-05/31,KKRR-05/21, KKRR-05/35, KKRR-05/20, BCO-05/10, KKRCAL-288, BCO-05/32, KKRCAL-147, BCO-05/18 and BCO-05/07

All the p use efficient bean genotypes were resistant to BCMV but susceptible to BCMNV except XRAV-187-3.

This study has identified 2 introduced bean root rot resistant genotypes (BCO-05/18 and BCO-05/07) that are resistant to both BCMV and BCMNV viruses

6.2 Recommendation

1. The bean varieties recommended for the region should carry the resistance to both viruses
2. The bean genotypes BCO-05/18 and BCO-05/07 that showed resistance to both BCMV and BCMNV should be considerer for dissemination to farmer.
3. The varieties BCO-05/43, BCO-05/35, KKRR-05/25, KKRRCAL-27/A, XRAV-187-3 that were resistant to BCMV should be considered for incorporation of the recessive gene for resistance to BCMNV to make them more suitable for the farmers.
4. The P use efficient bean genotypes MHR-314, ME-2221-34 and XRAN-187-3 be should be improved for resistance to BCMNV to suit the environment prone to bean virus
5. The varieties KK-R –CAL -27A and KK-R-CAL-288 be investigated further to confirm the resistant gene present in the genotypes for use

REFERENCES

Allen D.J., Edje, O.T. (1990). Common beans in African farming system. In: Smithson J.B., ed. Progress in improvement of common bean in East Africa. CIAT, Arusha, Tanzania. P 20-31 (Bean Research 5, Network on Bean Research in Africa work shop series no 12).

Atei E., and A. S. Tekeli, (2005). Heritability and variance components of some morphological and agronomic trais in alfalfa (medicago satival L) proc. Pak.Acad. scci, 42: 1-5.

Barshir, M. and R. O. Hapton, (1994). Seed and aphid transmission of some isolates of black eye cowpeas andcowpeaaphid-bornemosaicpotyviruses.

Bernal, G. and Graham, P.H. (2001). Diversity of the *Rhizobia* associated with *Phaseolus vulgaris* L in Equador and comparisons with Mexican bean *Rhizobia.* University of Minnesota, USA. *Journal of microbiology,* 2001. 47 (6): 526 – 534.

Bock, K. R. E. J. Guthrie, G. E Meredith and J. M. Njuguna, (1976). Virus of food legumes. Page 138-139 in East Africa Agriculture and Forest Research Organization. (EAAFRO) Annual Report 1976, Nairobi Kenya.

Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., Vanderleyden, J. (2003).Beans (*Phaseolus ssp*.)- Model food legumes. *Plant Soil*.252:55- 128.

Buruchara R. A. (1979). Identification of severe strain of bean common mosaic virus isolates from beans *Phaseolus vulgaris* L. Msc. Diss. University of Nairobi, Kenya.

Buruchara, R. (2007). Background information on Common Beans (*Phaseolus vulgaris* L.) in Biotechnology,Breeding & Seed Systems for African Crops. http://www.africancrops.net/rockefeller/crops/beans/index.htm. -15/03/2012

Candace W. C., M. F. Marston, J. C. Taylor, Molly J. , 2000. The I Gene of Bean: A Dosage-Dependent Allele Conferring Extreme Resistance, Hypersensitive Resistance,or Spreading Vascular Necrosis in Response to the Potyvirus Bean common mosaic virus MPMI Vol. 13, No. 11, 2000, pp. 1266–1270. Publication no. M-2000-0908-01N. © 2000 The American Phytopathological Society

Conway, G.R., J. Pretty, (1991). Unwelcome Harvest: Agriculture and Pollution. Earthscan, London.

Coyne, D.P., J.R. Steadman, G. Godoy-Lutz, R. Gilbertson, E. Arnaud-Santana, J.S. Beaver & J.R. Myers, (2003). Contribution of the Bean/Cowpea CRSP to management of bean disease. Field Crops Res 82: 87–102.

Davis R. M. and C. A. Frate, (2007). IPM Pest management Guideline: Dry Beans

David, S., R. Kirkby and S. Kasozi, 2000. Assessing the Impact of Bush Bean Varieties on Poverty Reduction in Sub-Saharan Africa: Evidence from Uganda. Network on Bean Research in Africa, *Occasional Publications Series, No. 31, CIAT, Kampala, Uganda.*

Drijfhout, E., (1978). Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identificationand breeding resistance. Agriculture Research Report 872: 1-98. Centre for agriculture Publishing and documentation, Wageningen, Netherlands.

Evans, A.M. (1980). Structure, variation, evolution, and classification in *Phaseolus*. In: SummerWeld RJ, Bunting AH (eds.) Advances in legume science. Royal Botanic Gardens, Kew, pp 337–347.

FAO. (2005). Food and Agriculture Organization of the United Nations. FAOSTAT statistical database. FAO. http://faostat.fao.org/default.htm.Accessed 20/10'2012

FAO. (2007). Food and Agriculture Organization of the United Nations. FAO Stat - statistical database. FAO. http://faostat.fao.org/default.htm. Accessed 02/11/2012

FAO,(2010. FAOSTAT (Food and Agriculture Organization of the United Nations) (2010). Statistics Division 2010. [Online] Available at http://faostat. Accessed 20/06/2010.

Fang, G. W., Allison, R. F., Zambolim, E. M., Maxwell, D. P. and Gilbertson, R. I. (1995). The complete nucleotide sequence and genome organization of bean common mosaic virus (NL3 strain). Virus research 39: 13-23.

Fisher, M.L., and M.M. Kyle. (1994). Inheritance of resistance to potyviruses in *Phaseolus*

vulgaris L. *III. Cosegregation of phenotypically similar dominant responses to nine potyviruses. Theor. Appl. Genet. 89:818-823.*

Florets, E. N.,J. A. Acosta-Gallegos, L. Silva-Rosales,(2003). Bean Common Mosaic Virus and Bean Common Necrotic Virus in Mexico. Plant Disease / vol. No. 1, 87:21-25

Galvez, G. E., and F.J. Morales, (1989). Aphid-transmitted viruses. p.333-361. In H. F. Schwartz and M. A, Pastor-Corrales (ed.) Bean production problems in the Tropics, 2nd. Ed. Cent. Int. Agric. Trop. (CIAT), Cali, Colombia.

Gachene, C., C. A.Palm, J. G. Mureithi., (1999). Legume cover crops for soil fertility improvement in the East African Region. AHI Soils Working Groups Report No.1. TSBF, Nairobi.

Gepts, P., T. C. Osborn, K. Rashka, F. A. Bliss, (1986). Phaseolin rotein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidencefor multiple centers of domestication. Econ. Bot. 40:451-468.

Gomez, O., (2004). Evaluation of Nicaraguan common beans (*Phaseolus Vulgaries*)

Jaetzold, R., H. Schmidt, B. Hornets and C. Shisanya (2009). Natural condition and farm management information Vol. 2, 2nd edition of farm management handbook of Kenya. Ministry of Agriculture, Kenya and the German Agency for Technical Cooperation (GTZ), Nairobi, Kenya.

Juma, R. U., J. Ojiem, M. Wambulwa, (2009). Evaluation of phosphate efficient Bean genotypes for adaptability in Western Kenya. Pages 80-81 in KARI Kakamega Annual report 2009.

Kanyanjua S.M, L. Irer , S. Wambua S. M. Nandwa, (2002). Acidic Soils in Kenya. Constraints and remedial options. KARI Technical Note No. 11.June 2002.

Klein, R. E., S. D Wyatt, W. J. Kaiser, (1989). Influence of propagation on incidence of seed borne bean common mosaic virus in the USDA Phaseolus germ plasm collection.

Kelly, J. D, L. Afanador , S.D. Haley, (1995). Pyramiding genes for resistance to bean common mosaic virus. Euphytica 82: 207–212.

Kelly, J.D. (1997). A review of varietal response to bean common mosaic potyvirus in *Phaseolus vulgaris*. Plant Varieties and Seeds 10:1-6.

Kelly, J.D., P. Gepts, P.N. Miklas & D.P. Coyne, (2003).Tagging and mapping of genes and QTL cowpea. Field Crops Res 82: 135–154.

Kornegay, J. (1992). BCMN: CIAT's Point of view. Annual Report of the Bean Improvement Cooparative 35: 56-57.

Kumar,J. H., T. Singh, L. Singh, D. S. Tonk and R. Lal, (2002). Correlation of analysis of yield and its components in summer among *vigna radiate* L. Crop Research, 24: 374-377.

Larsen, R.C., N. P. Miklas, K. L. Druffel, and S. D. Wyatt.(2005).NL-3K strain is a stable and natural occurring interspecific recombinant derived from Bean common mosaic necrotic virus and Bean common mosaic virus. Phytopathology 95:1037-1042.

Larsen, R.C and P.N. Miklas. 92010). Mapping resistance to *Peanut mottle* virus in common bean. Annu. Rep. Bean Improv. Coop. 53:

Leterme, P. and C. Munoz, (2002). Factors influencing pulse consumption in Latin America. British Journal ofNutrition88.Suppl.3,s251-s254.

Lima, J. A. A.M. F. B. Gonsalves (1988). *Cassia occidentalis*, a potential reservoir of potyviruses Infecting cowpea.

Mbugwa G. W., S. M. Wachiuri, J. I. Karoga, J. K. Kimamira, A. M. Ndegwa, and M.M. Waiganjo, (2005). Farmer participatory evaluation of dry bean varieties with multiple constraints resistance in central Kenya. *The 10th KARI Conference proceedings.*

Magdoff, F., H. van Es, (2000). Building Soils for Better Crops. SARE, Washington, DC.

Mckern, N.M.,G. L. Mink , O.W.Barnett, A. Mishra , L. A. Whittaker , M. J. Silbernagel C.W.Ward D. D. Shukla , (1992). Isolates of bean common mosaic virus comprising two distinct potyvirus. Phytopathology 82, 923-929.

Miguel A. A. and C. I. Nicholls, (2003). Soil fertility management and insect pests: harmonizing soil and plant health in agro ecosystems. Soil & Tillage Research 72 (2003) 203–211

Miklas, P.N., S. Lambert, G. Mink & M. Silbernagel, (1998). Many beans with *bc-3* resistance to BCMNV are susceptible to BCMV. Annual Report Bean Improvement Coop. 41: 33–34.

Miklas, P.N., R.C. Larsen, R. Riley & J.D. Kelly, (2000). Potential marker assisted selection for *bc-1*2 resistance to bean common mosaic potyvirus in common bean. Euphytica 116: 211–219.

Miklas, P.N., A.N. Hang, J.D. Kelly, C.A. Strausbaugh, and R.L. Forster. (2002). Registration of three kidney bean germplasm lines resistant to bean common mosaic and necrosis potyviruses: USLK-2 light red kidney, USDK-4 dark red kidney, and USWK-6 white kidney. *Crop Sci.42:674-675.*

Mills, L.J. and M.J. Silbernagel. (1992). A rapid screening technique to combine resistance to haloblight and bean common mosaic virus in Phaseolus vulgaris L. Euphytica 58:201-208.8

Mink, G. I., and M. J. Silbernagel, (1992) Serological and biological relationships among viru Ministry of Agriculture (MOA) and KIPPRA 2010. *The Kenya Agricultural Sector Data Compendium, Crops Statistics, Volume 2.*

MOA (2008) Food security reports.

Morales, F. J. (1989). Bean common mosaic: screening for disease resistance. (CIAT) publication No.33. Cali, Columbia.

Morales, F. J. (2003). Common bean. Pages 425-445 In G. Loebenstein and G. Thottappillly (EDS.) Virus and viruslike diseases of major crops in developing countries. Kluwer Academic Publishers

Muasya, R. M. (2001). Crop Physiological analysis of seed quality variation *in* common beans (*Phaseolus vulgaris* L.). Ph.D. Thesis,Wageningen University. The Netherlands.

Mukeshimana G., L. Patrick Hart and J. D. Kelly, (2003) Extension Bulletin E-2894 • September 2003 (Major Rev. of E-1561)

Niang, A., B. Amadalo, J. de Wolf, and S. Gathumbi. (2002). Species screening for short-term planted fallows in the highlands of western Kenya. Agrofor. Syst. 56,145-154.

Njau, P. J. R and Lyimo, H. F. J. (2000).Incidence of bean common mosaic virus and bean common mosaic necrosis virus in bean (*Phaseolus vulgaris* L) and wild legume seed lots in Tanzania. *Seed Sci and Technol 28: 85-92.*

Nduguru, J., (2003) Plant virus Understanding the basics © 2000 Timoth Standing

Nekesa, P. J. H.Nderitu, and R. M. Otsyula, (1998). Bean research in western Kenya: Lessons and experiences. In Farrell, G. and G.N. Kibata. Crop Protection research in Kenya. Proc. of the Second Biennial Crop Protection Conference, 16-17 September 1998. KARI/DFID Nairobi, Kenya. Pp 237-244.

Odendo, M., David S., and Otsyula R.M., (2001). Impact of root rot resistant bean varieties in western Kenya: Application of impact diagramming. Proceedings of PABRA Millenium Synthesis: A Workshop on Bean Research and Development in Africa over the Last Decade, Novotel Mount Meru, Arusha, Tanzania 28 May – 1 June 2001.

Ojiem, J. O.,J. Lauren, B. Medvecky, M. Odendo, C. Njeru, J. Nyongesa, and S. Kamwana, (2008). Effect of phosphorus and seed priming on growth and production of bean and lablab across a pp70-73

Omnyin, M. E., E. M. Gathuru, D. M Mukunya, (1988). Reaction of cultivars of beans (*Phaseolus vulgaries* L) to bean common mosaic virus (BCMV) Tropical . Agriculture. (Trinidad) 65: 166-168.

Omunyin, M. E., E. M. Gathuru, D. M. Mukunya, (1995). Pathogenicity groups of bean common mosaic virus isolates in Kenya. Plant Disease 79. 985-989.

Opole R. A., 1, P. W. Mathenge, E. O. Auma, H. A. Van rheenen, C. J.M. Almekinders, (2003). Onfarm seed production practices of common bean (Phaseolus vulgaris L.). African Crop science conference proceedings, vol. 6, 722-725.

Otsyula, R., Rubaihayo, P., Buruchara, R. (2003). Inheritance to Pythium Root rots in Beans (*Phaseolus Vulgaris*) genotypes, *African crop science society*, Vol. 6. 295 – 298. 6:61-67.

Otsyula R. M. and H. Echaire, (2008). Grain legumes. KARI Kakameg annual report 2008 KARI Kakamega pp 41-45

Otsyula R. M. R. U. Juma and M. Wambulwa, 2009. Grain legumes. KARI Kakameg annual report 2009 KARIKakamega pp 30-37

Ozlem, A. and H. Geren, 2007. Evaluation of heritability and correlation for seed yield and yield components in Faba bean L. *Journal of Agronomy, 6: 484-487. http://scialert.net/abstract/?doi=ja.2007.484.487*

Pilbeam, C.J., P.D. Hebblethwaite, H.E. Ricketts and T.E. Nyongesa, (1991). Effects of plant population density on determinate and indeterminate forms of winter field beans (*Vicia faba* L.) Part 1: Yield and yield components. *J. Agric. Sci., 116: 375-383.*

Raatz, S., Nutritional value of field beans.(2013). Retrieved from-http://beaninstitute.com/health-benefits/nutritional-value-of-dry-beans/-2/7/2013

Republic of Kenya (2006). Economic Review of Agriculture. Central Planning Unit, Ministry of agriculture, Government Printer, Nairobi.

Rheenen, H. A. Van and S. G. S. Muigai, (1985) control of bean common mosaic virus by deployement of the dominant I gene. Neth. J. plant pathol. 90: 85-94

Sanchez P.A., (1977). Advances in management of Oxisols and Ultisoils in tropical South America. Pp 535-566. In: K. Kawaguchi (ed) Int. seminar on soil, environment, and fertility management in intensive agriculture. *Tokyo Soc. Of soil sci. and manure, Tokyo. Japan.*

Sengooba, T.N., N.J. Spence, D.G.A.Walkey, D.J. Allen & A. Femi Lana, (1997). The occurrence of bean common mosaic necrosis virus in wild and forage legumes in Uganda. *Plant Patholology 46: 95–103.*

Singh SP (2001). Broadening the Genetic Base of Common Bean Cultivars: A review. *Crop Sci. 41: 1659-1675.*

Singh,S. P., (2003). Low soil fertility tolerance in landraces and improved common bean genotypes. Crop science p 4090/ January -2003

Silbernagel, M.J., G.I. Mink, R.-L. Zhao & G.-Y. Zheng, (2001). Phenotypic recombination between bean common mosaic and bean common mosaic necrosis potyviruses in vivo. Arch Virol 146:

Spence, N. J. and D. G.A. Walkey, (1995). Variation for pathogenicity among isolates of bean common mosaic virus in Africa and a reinterpretation of the genetic relationship between cultivars of Phaseolus vulgaris and pathotypes of BCMV.

Todorović, J., M. Vasić, V. Todorović, (2008). Pasulj I boranija. Institut za ratastvo i povrtarstvo, Novi Sad, Poljoprivredni fakultet, Banja Luka.

Vale C. C., J. A. Lima, C. C. Vale, (1994). Effects of isolated and mixed infections by viruses from distinct groups in cowpea.

Van Schoonhoven and Pastor-Corrales, M.A. (1987). Standard System for the Evaluation of bean Germplasm. Centro Internacional de Agricultura tropical, cali, Colombia.

Vetten H.J., D. E Lesemann, E.Maiss , (19920. Serotype A and B strains of bean common mosaic virus are two distinct potyviruses. *Arch. Virol., Suppl. 5, 415-431*.

Wiliam C., Johnson, P. Guzman, D.Madala, A. B. C. Mkandawire, S. Temple, (1997). Molecalu tagging of the bc3 Gene for introgressing into Andean common beans. *Crop Science 37: 248-254*

Wagara N. and P.M. Kimani, (2007). Resistance of nutrient-rich bean varieties to major biotic constraints in Kenya. African Crop Science Conference Proceedings Vol. 8. pp. 2087-http/www.acss.ws/upload/xmv/Research/541.pdf-10/2/2010

Wortmann C. S., D. J. Allen, (1994). Arfrica bean environment: their definition, characteristics and control. Network on bean Research in Africa, occasional papervseries 5 No. 11. Dareslaam Tanzania.

Wortmann C. S., L.Lunze, V. A. Ochwoh, J. Lynch, (1995). Bean Improvement for low fertility soil in Africa. *Africa Crop Science Journal, Vol 3. No. 4, pp 469-477,1995*

Wortmann, C. S., R. A. Kirby, C. A. Eledu and D.J. Allen (1998).Atlas of common bean production Africa, CIAT publication No. 297.

APPENDICES

Appendix I:Field Layout for Experiment Two

1,T_{17}	2,T_{2}	3,T_{11}	4,T_{21}
5,T_{8}	6,T_{26}	7,T_{25}	8,T_{5}
8,T_{14}	9,T_{22}	10,T_{13}	11,T_{23}
12,T_{15}	1,T_{24}	1,T_{19}	1,T_{9}
1,T_{1}	1,T_{4}	1,T_{7}	1,T_{12}
1,T_{18}	1,T_{20}	1,T_{10}	1,T_{3}
1,T_{16}	1,T_{6}	1,T_{27}	1,T_{28}

1,T_{13}	2,T_{26}	3,T_{19}	4,T_{17}
5,T_{14}	6,T_{9}	7,T_{25}	8,T_{22}
8,T_{27}	9,T_{11}	10,T_{15}	11,T_{4}
12,T_{6}	1,T_{24}	1,T_{3}	1,T_{2}
1,T_{21}	1,T_{5}	1,T_{20}	1,T_{1}
1,T_{16}	1,T_{23}	1,T_{28}	1,T_{10}
1,T_{12}	1,T_{7}	1,T_{18}	1,T_{8}

Rep-3

1,T_{14}	2,T_{25}	3,T_{21}	4,T_{4}
5,T_{9}	6,T_{3}	7,T_{11}	8,T_{15}
8,T_{20}	9,T_{13}	10,T_{6}	11,T_{19}
12,T_{7}	1,T_{23}	1,T_{12}	1,T_{24}
1,T_{5}	1,T_{16}	1,T_{26}	1,T_{10}
1,T_{18}	1,T_{17}	1,T_{22}	1,T_{8}
1,T_{1}	1,T_{27}	1,T_{2}	1,T_{28}

Rep-4

1,T_{24}	2,T_{17}	3,T_{19}	4,T_{18}
5,T_{12}	6,T_{5}	7,T_{21}	8,T_{22}
8,T_{16}	9,T_{11}	10,T_{1}	11,T_{7}
12,T_{27}	1,T_{4}	1,T_{3}	1,T_{25}
1,T_{6}	1,T_{23}	1,T_{10}	1,T_{2}
1,T_{14}	1,T_{9}	1,T_{8}	1,T_{15}
1,T_{20}	1,T_{13}	1,T_{26}	1,T_{28}

Appendix I1: Disease BCMV and BCMNV symptoms (Source Author 2010)

Appendix III: Project Estimated Budget for Period1/3/2010-1/12/2010

Sno.	Activity	Budget	Total
1.	**Student travel and field expenses**		
	Site selection	4775	
	Planting	12,000	
	Data score	8,100	
	Monitoring	13,200	
		Subtotal	**38,000**
2.	Student Allowance	50,000	**50,000**
3.	**Labour cost**		
	Farm hirer,	2000 x 4	8000
	Land preparation (2),	3000 x 4	12000
	Planting,	2000 x 4	8000
	weeding	1200 x 4	4800
	Harvesting	500 x 4	2000
		Sub total	**34,800**
4.	**Materials and supplies**		
	TSP	50 kgs @ 3000	3000
	CAN	50 kgs @ 2000	2000
	Fansan-D	120 gms@ 200	200
	Diazinon	1 ltr @ 1500	1500
	Screen house pots	50 @ 20	1000
	Lebel tags	1000 @ 1500	1500
	Manila paper	10@ 25	250
	Thread	5 @ 20	100
	Brown paper	2 kg @ 250	500
	Forceps	2 @ 100	200
	Cellotape	2 @ 100	200
	Scissors	1 @ 50	50
		Subtotal	**11,450**
5.	**Non-expendable equipment**		
	Flash discs	1 @ 4000	4000
	Digital Camera	1 @ 20,000	20,000
	Lab Top	1 @ 38,000	38,000
		Subtotal	**62,000**
6.	**Stationery**		
	Photocopying paper	4 @ 500	2500
	Catriridge	4 @ 1500	6000
	Pens	4 @20	80
	Pecncils	2 @20	40
	Rubber	50	50
		Subtotal	**8,670**

7.	**Literature** Subject relevant materials	5000	**5,000**
8.	**Printing and publications** Publishing	 25000	 **25,000**
9.	**Seminars/ workshops** Moi University Conferences and Seminars	 1500 1500 **Subtotal**	 **3000**
10.	**Screen house attendant**	10 md x4mnth @271.05	 **16,263**
11.	**Communication** Telephone and correspondense	 **15,000**	 **15,000**
12.	Supervisor expenses Transport Supervisor Per diem (I cnl. Of Driver) Supervisor Allowance-(2)	 **14,000** **12,000** **70,000**	 **14,000** **12,000** **70,000**
13.	**Contingency**		**36,438.3**
Grand Total			**KES 400,821.30**

Appendix 4: Result Tables

Table:1 Genotype Site Interaction

SOV	BCMV	BCMNV	APHD
SITE	1.33ns	22.7***	23.6***
REP	2.69ns	0.15ns	12.5***
SITE X REP	4.0ns	1.04ns	2.2**
GEN	23***	56.19***	1.5**
SITE x GEN	3.5*	5.36***	0.7ns
CV	36.94	25.55	24.58
Error	3.11	1.56	0.8

Table: 2 Anova Table

Source of Variation	Df	ST1	FL2	PP	SP	DM	BCMV	BCMNV	APHID	YLD
Site	3	39.4*	822***	188.6***	10.5***	245.56***	0.22***	0.31***	70.4***	5.5***
Rep(site)	12	47.5***	6.1***	26.8	3.7***	20.57***	0.08ns	0.01ns	10.66**	0.527*
Blocks(rep)	21	15.9***	2.42ns	6.07ns	2.02ns	2’64ns	0.06*	0.09***	1.01ns	3.16ns
Genotype	27	23.3*	4.17***	58.3	5.05**	8.33***	0.43***	1.16***	1.77**	1.1ns
Site*Genotype	81	14.4ns	3.5**	117.6	5.3	2.42ns	0.05*	0.06***	0.95ns	1.9ns
Error	309	13.9	2.3	14.13	2.44	13.4	2.6ns	0.5ns	1.02	0.01
CV %		10.6	5.6	17.6	4.5	9.9	15.8	6.9	23	4.9

* Significant at 5% ** Significant at 1%, *** Significant at 0.1% and ns- not significant

ST1	-plant stand after 2 weeks	**PP**	-number of pods per plant	**BCMV**	-Bean common mosaic virus
STC2	-plant stand at harvest	**SP**	- number grains	**BCMNV**	-Bean common mosaic necrotic virus
FL1	-first flower,	**DM**	- days to maturity		
FL2	-50% flowering	**YLD**	-grain yield	**APHID**	-Aphids infestation

Table: 3 Means for Bean genotypes resistance to bean virus stresses

Genotypes	BCMV	BCMNV	APD
SCAM-80/5	2.40 ± 1.7	1.3 ± 1	1 ± 1.3
BCO-05/10	2.80 ±1 .9	1 ± 0	2 ± 0,8
BCO-05/32	2.3 ± 0.6	1 ± 0	2± 0.9
BCO-05/18	1.5± 2.0	1.01± 0	1± 0.8
BCO-05/37	1.82 ± 0.6	4.25± 2.9	2± 0.9
BCO-05/07	1.12 ± 0.8	1.4 ± 1.5	2± 0-9
BCO-05/43	1.18 ± 1.	2.1 ± 2.4	2± 0.9
BCO-05/25	1.6 ± 2.1	1 ± 0.2	2± 1.1
BCO-05/03	2.12± 1.8	1.3 ± 1	2± 1.0
BCO-05/09	1.8 ± 1	1 ± 0.01	2± 0.2
BCO-05/35	1.2 ± 1.6	5 ± 1.9	2± 1.1
BCO-05/16	2.37± 1	1.0± 0	1± 0.8
RWR 719	1.00 ± 1.9	8.3 ± 2.0	3± 1.0
KK-RRB05/31	4.7 ± 2	1 ± 0	2± 1.4
KK-RRB05/21	4.81 ± 2	1 ± 0	2± 0.9
KK-RRB05/29	2.80 ± 2	3.3 ± 2.8	2± 0.9
KK-RRB05/34	4.60 ± 2	1.6 ± 1.7	2± 1.4
KK-RRB05/25	1,3 ± 2	4.1 ± 2.3	2± 0.9
KK-RRB05/23	3.80 ± 2	1.5 ± 1.3	3± 1.1
KK-RRB05/35	4.75 ± 2	1.6 ± 1.7	2± 0.9
KK-RRB05/47	1.31 ±1.0	6.1 ± 1.6	2± 1
KK-RR B05 / 20	3.75 ± 2.3	1.4 ± 0	2± 1.2
KK-RCAL-27/A	1.0±0.5	5.3 ± 1.9	2± 0.9
KK-RCAL-194	2.43±1.7	1 0 ± 0	2± 0.9
KK-RCAL-288	1.81±1.9	1.00 ± 0	1± 1
KK-RCAL-70	1.62±1.0	2.5 ± 2.7	2± 0.9
KK-RCAL-47	2.5±1.8	1 ± 0	2±0.9
GLP-585	5±2.4	1.4 ± 1.5	2±0.9

BCMV -Bean common mosaic virus,
BCMNV -Bean common mosaic necrotic virus,
APD -Aphids. The –ve or +ve value is the standard error