MARKER-ASSISTED SELECTION FOR RESISTANCE TO BEAN COMMON MOSAIC NECROSIS VIRUS IN FRENCH BEAN CULTIVARS IN KENYA

GRACE WAMBUI WATARE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PLANT BREEDING AND BIOTECHNOLOGY OF THE UNIVERSITY OF EMBU

AUGUST, 2023

DECLARATION

This thesis is my original work and has not been presented elsewhere for a degree or any other award.

Signature…… Date……

Grace Wambui Watare
Department of Water and Agricultural Resource Management
A506/1171/2017

This thesis has been submitted for examination with our approval as the University Supervisors

Signature…… Date……

Dr. Edith Esther Arunga
Department of Water and Agricultural Resource Management
University of Embu

Signature…… Date……

Dr. Bernard Mukiri Gichimu
Department of Water and Agricultural Resource Management
University of Embu

DEDICATION

To my beloved mother Jane Watare Kariuki, you will forever be my inspiration.

ACKNOWLEDGEMENT

I would like to express my sincere appreciation to my esteemed supervisors, Dr. Esther Arunga and Dr. Bernard Gichimu, for their invaluable guidance, unwavering support and expert advice. I am deeply grateful to the Kirkhouse Trust for their invaluable financial support in funding this research and providing necessary trainings. I would also like to extend my heartfelt gratitude to the Kirkhouse trust team at the University of Embu; Serah Njau, Brian Wekesa, and Nancy Munubi who have been a source of inspiration and motivation. Additionally, my gratitude goes to the KALRO Kakamega team, especially Dr. Reuben Otsyula, Shamir, Shadrack, and Yona for their assistance throughout the research process.

TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF ABBREVIATIONS

BC	Backcross
BCMV	Bean Common Mosaic Virus
BCMNV	Bean Common Mosaic Necrosis Virus
BGMV	Bean Golden Mosaic Virus
CIAT	Centro Internacional de Agricultural Tropical / International Centre for Tropical Agriculture
CMV	Cucumber Mosaic Virus
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide Triphosphate
EtBr	Ethidium Bromide
EDTA	Ethylene Diamine Tetra-acetic acid
FAO	Food and Agricultural Organization
F_1	First filial generation
HCD	Horticultural Crops Directorate
KALRO	Kenya Agricultural and Livestock Research Organization
LSD	Least Significant Difference
MAB	Marker Assisted Breeding
MAS	Marker Assisted Selection
M	Molar
mM	Millimolar
MT	Metric Tones
PCR	Polymerase Chain Reaction
RCBD	Randomized Complete Block Design
SCAR	Sequence Characterized Amplified Region
UV	Ultraviolet Radiation

LIST OF SYMBOLS AND CHEMICAL FORMULA

%	Percentage
°C	Degree celsius
µg/ml	Microgram per milliliter
µl	Microliters
KPO_4	Potassium phosphate
$MgCl_2$	Magnesium chloride
ng	Nanogram

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

ABSTRACT

Worldwide, commercial production of French bean (*Phaseolus vulgaris L.*) is constrained by diseases, key among them being the bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV). These potyviruses are the most devastating to common bean farmers and can cause total yield loss under heavy infestation. The objective of this study was to characterize the BCMNV resistance in commercial French bean cultivars and initiate a breeding program against the disease. A set of 32 entries, comprising 27 French bean genotypes together with 5 dry bean varieties were evaluated for resistance under field conditions. All the 29 French bean genotypes showed susceptibility to BCMNV but the 3 dry bean resistant checks (MCM 2001, MCM 5001 and MCM 1015) were resistant to the disease. The French bean cultivars displayed varied reactions to BCMNV pathotypes III and VI under greenhouse conditions ranging from top necrosis, mosaics, mottling, and deformed leaves to stunted growth. In this study, molecular marker SW13 and SBD5 were used to detect the presence of the *I* gene and the *bc-1*^{2} gene, respectively. ROC11 and CAPS elF4E that are linked to BCMNV *bc-3* gene, were also used to detect specific resistance genes. Molecular analyses showed that only SW13 and elF4E markers were consistent in identifying the presence/absence of the *I* and *bc-3* gene, respectively. The two molecular markers and ROC-11 were successfully utilized in the introgression of *bc-3* gene into three commercial French bean cultivars (Amy, Serengeti and Vanilla) in a backcross breeding program involving MCM 2001, MCM 5001 and MCM 1015 as donor parents. From the breeding program, 19 breeding lines combining field resistance against both BCMNV and BCMV were developed. The selected lines were further phenotypically evaluated for quality and yield traits such as pod length, pod quality, pod suture string, pod wall fiber, pod yield and the number of pods per plant. The results confirmed significant maintenance of the yields and quality traits among the 19 selections. The developed breeding lines will be further screened and improved for release as new French bean cultivars thus enabling the production of this valuable vegetable in areas where these potyviruses are prevalent. In addition, the developed lines can be utilized as sources of BCMNV resistance in future breeding programs. Availability of reliable sources of BCMNV resistance within French bean gene pool will simplify the future breeding programs.

CHAPTER ONE

INTRODUCTION

1.1 Background information

French bean (*Phaseolus vulgaris* L.) is among the leading vegetables in Kenya that is mainly grown for export to the European market. This group of common beans is mainly grown for thin tender pods with small seeds which are either for fresh consumption or processing (Hargety *et al.,* 2016). The crop is also referred by other different names such as snap bean, green beans and haricot bean (Singh & Singh, 2015). The vegetable is rich in vitamins such as A, C, K, B6 and folic acid. It's also a good source of minerals such as calcium, iron, manganese, potassium and copper (Juma, 2012). In addition, the beans also possess some medicinal attributes as they are used in the treatment of diabetes, certain cardiovascular problems, bladder burn, dysentery, eczema, hiccups and tenesmus (Yadav *et al.,* 2015). French beans are produced worldwide with the leading producer being China (17.96 MT) followed by Indonesia (0.89 MT) and India (0.64 MT) (FAOSTAT, 2020). Kenya is the leading producer of French beans by area and volume in East Africa followed closely by Tanzania. The crop is mainly exported from Kenya to major markets like European Union, United States, France, South Africa, India, China and Russia (USAID- KAVIS, 2015) but there is gradual increase in local consumption (HCDA, 2020).

The cropping of French beans in Kenya is highly favored by ample climatic conditions making the country a seasonal counter-season supplier to the European market (Fulano *et al*., 2021). The crop is majorly grown for export by contracted small-scale growers and has gained popularity due to its short life cycle of 45-65 days after sowing depending on the cultivar (NAFIS, 2017). The major producing counties include Nakuru, Kajiado, Kirinyaga, Embu and Machakos, contributing 74% of the total annual production (HCD, 2020). Because of its intensive production nature, French bean offers employment to about 60,000 small to medium-scale farmers in Kenya (Kimani *et al.,* 2016). Additionally, with proper management, French beans can be a source of income year around. However, the export market of French beans is highly regulated in terms of pesticide residues which

affect the growers who are constantly constrained by pests and diseases. The most important pests of the French bean include thrips (*Callothrips fasciatus*), pod borers (*Maruta vitrata*), spidermites (*Tetranuchus spp*) bean fly (*Ophiomyia phaseoli*), and cutworms (*Striacosta albicosta*) (Ogala, 2013; Fulano *et al*., 2021). On the other hand, major challenging diseases affecting French bean production in Kenya include rust, caused by *Uromyces appendiculatus*, anthracnose (*Colletotrichum lindemuthianum*), angular leaf spot (*Pseudocercospora griseola)* and viral diseases bean including bean common mosaic virus (BCMV), bean common mosaic necrosis virus (BCMNV), bean yellow mosaic virus (BYMV) and bean curly top virus (BCTV) among others (Mangeni *et al*., 2014).

In Kenya, French bean research and breeding for disease resistance have mainly focused on rust, anthracnose and angular leaf spot (Wasonga *et al.,* 2010; Arunga *et al.,* 2012; Wahome *et al.,* 2013; Kamiri *et al.,* 2021). However, BCMV and BCMNV are the two potyviruses devastating common bean farmers and can cause total yield loss under heavy infestation (Mukeshimana & Kelly, 2003; Morales, 2006; Mangeni *et al*., 2014). Most of the BCMNV work in Kenya has solely focused on dry beans compared with French beans (Mangeni *et al.,* 2014; Njuguna, 2014; Mutuku *et al.,* 2018). In an attempt to control the BCMV (serotype B), bean breeders across the world developed cultivars possessing the dominant *I* gene that confers resistance to the strain. However, the dominant *I* gene was overcome by BCMNV (serotype A) (Miklas *et al*., 2006) and the latter strain has become endemic and thus presents a major problem in common bean production in East Africa. It triggers systemic necrosis in susceptible genotypes leading to the death of the entire crop (Worrall *et al*., 2015).

Control of BCMNV presents a major problem since most control measures focus on preventing infection through control of aphids that act as vectors, early planting or minimizing the effect of infection by use of clean seeds and rouging (Ndunguru & Kapinga 2007). However, these measures have been reported to be ineffective because BCMNV is a systemic disease hence, rouging may not be effective as asymptomatic plants may be overlooked and act as a source of inoculum spread by the aphids (Juma, 2012). Moreover, due to its non-persistent nature, the virus can be swiftly acquired and

transmitted from infected to healthy bean plants within seconds (Mukeshimana *et al*., 2003). Recycling of seeds presents a major challenge leading to the accumulation of the virus over time given the weak seed systems in Sub-Saharan Africa (Kelly & Vallejo, 2004; Ferreira *et al*., 2013). Therefore, the most sustainable way to control this virus is the development of resistant cultivars and their adoption by small and large-scale farmers. The BCMNV is endemic in Africa, where it survives on non-domesticated legume species, so the means to control it differ from those adopted outside the continent (Coyne *et al*., 2003). While the supply of virus-free seed has proven to be an effective control measure in North America this is unlikely to be valuable in Africa as a result of contamination from alternate hosts (Worrall *et al*., 2015). Therefore, host resistance is the only known virus control method that is both effective and durable (Tang & Feng, 2022).

The protection afforded by genetic resistance is not only more environmentally sustainable than a reliance on chemical inputs but also is consistent with the market's preferred pod quality and regulatory requirements. Resistance to BCMNV is conferred by four recessive genes *bc-u, bc-1, bc-2*^{1}*/bc-2*^{2}, and *bc-3* (Feng *et al*., 2015). Combinations of these genes with the *I* gene have been shown to deliver durable levels of host resistance. This is attributed to the fact that resistance to BCMV and BCMNV requires the additional presence of a fourth *bc* gene (*bc-u*). Therefore, varieties carrying either *I* and *bc-3* or *bc-u* and *bc-3* are resistant to both BCMNV and BCMV (Miklas *et al*., 2006). Gene pyramiding is therefore recommended for durable resistance against both BCMV and BCMNV. Furthermore, polymerase chain reaction (PCR)-based molecular assays that are tightly linked to the genes conferring resistance to BCMV and BCMNV have been developed (Haley *et al.,* 1994; Johnson *et al*., 1997; Naderpour *et al*., 2010). These molecular markers are useful in cultivar identification, linkage mapping, gene introgression and marker-assisted selection (Chilagane *et al*., 2013). Successful stacking of resistance genes will combat viral spread through contaminated seeds as well as by aphids that are difficult to control by small-scale farmers.

1.2 Statement of the problem

Production of French bean is highly constrained by viruses among other diseases. Common bean farmers in Kenya manage BCMNV by chemical control of the vector

which is ineffective because the aphids spread the virus in a non-persistent manner. Besides, the use of chemicals is also unsustainable due to the high cost, the risk it poses to users and the environment, and the stringent export regulations. This underscores the need to pursue sustainable non-chemical control strategies such as host resistance. In the past, breeders concentrated in developing germplasm resistant to BCMV which is a lesser destructive strain compared with BCMNV. Unlike BCMV whose resistance is successfully conferred by the dominant *I* gene, the necrosis inducing strain of BCMNV has managed to overcome this resistance thus becoming a serious problem in French bean production. Although there has been an international effort to protect the commercial French bean cultivars against the BCMV using the *I* gene, BCMNV still pose a challenge in French bean production in Kenya. Consequently, production of BCMV resistant French beans in Kenya is limited in areas where BCMNV is prevalent such as western Kenya. In addition, most of the breeding efforts against BCMNV in Kenya have been directed towards dry bean cultivars. Currently the dominant *I* gene in French bean varieties in Kenya has not been pyramided with resistance genes against the BCMNV and this is a major risk against their sustainability in case of serious BCMNV outbreaks.

1.3 Justification

Use of host resistance is the most sustainable strategy of controlling virus infection and spread for both small and large-scale farmers. Most commercial French bean cultivars grown in Kenya possess the dominant *I* gene conferring resistance to BCMV which does not protect the crop against BCMNV when used alone. Combinations of these recessive genes with the dominant *I* gene have been shown to deliver durable levels of host resistance against the destructive potyviruses hence the need to pyramid them into a single cultivar. Gene pyramiding was made feasible by availability of DNA assays linked to these resistance genes which facilitates rapid and efficient introgression of the resistance gene(s). This study aimed at screening for resistance to BCMNV in commercial French bean varieties and initiating a breeding program by developing breeding lines that possess genes that confer resistance to BCMV and BCMNV. The developed breeding lines can be advanced and released to farmers as resistant varieties whose subsequent adoption would

reduce the use of chemicals in French bean production. This will in return reduce the residual levels in the beans and expand production to areas where the disease is prevalent.

1.4 Research hypotheses

The study was guided by the following hypotheses:

1. Selected French bean cultivars have no significant difference in their host resistance to BCMNV.
2. There are no suitable molecular markers that can be used to screen French bean cultivars for resistance to BCMV and BCMNV.
3. The *bc-3* gene that confers resistance to BCMNV cannot be introgressed into French bean breeding lines.

1.5 Research objectives

1.5.1 General objective

To contribute to French bean breeding in Kenya by developing breeding lines with host resistance to bean common mosaic necrosis virus in Kenya.

1.4.2 Specific objectives

1. To evaluate for host resistance to BCMNV among selected French bean cultivars under field and greenhouse conditions.
2. To determine the suitability of molecular markers in screening for resistance to BCMV and BCMNV among French bean cultivars.
3. To introgress the *bc-3* gene that confers resistance to BCMNV in selected French bean cultivars.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and domestication of French beans

French beans are believed to have been derived from common beans (*Phaseolus vulgaris* L.) of South Mexico to Mesoamerica and Ecuador origin (Gepts 1998; Vidyakar *et al.*, 2017). They were originally developed in the nineteenth century from Andean genetic resources brought from Europe and it made a household vegetable and given the name French (Kimutai, 2018). Studies suggest that French beans were developed as a result of selection for tender, low fibre and stringless cultivars from common beans rather than wild beans (Myers & Baggett, 1999; Singh, 2005). Other studies suggest that French beans were as a result of crosses from the Andean and Mesoamerican gene pools in attempt to introgress disease resistance in beans (Gepts, 1998). The first French bean cultivar had round and stringless pods which was released in the 1800s. Blue Lake Green and Tendercrop cultivars were later developed and released in the early to mid-1900s for canned and frozen bean industries (Singh & Singh, 2015). Numerous breeding and selection efforts for French beans have continued, with the primary goals being yield, pod quality and resistance to biotic and abiotic stresses (Sofkova *et al.,* 2010; Wahome *et al.,* 2013; Beshir *et al.,* 2016).

2.2 Ecological requirements and agronomic practices of French beans

French beans are adapted to altitudes of 1500-2000 m above sea level in East Africa (Kimani, 2016). They can also be grown under plain conditions provided maximum daily temperatures do not exceed 30 °C. The optimum pollination is obtained at 15-25 °C, temperatures of more than 30 °C result in poor pod set and poor flower development (Hargety *et al.,* 2016). The seedlings do not tolerate temperatures less than 10°C (Santosa *et al.,* 2017). French beans can be propagated in a wide range of soils including sandy, loam and clay. However, the crop produces best in silty loam to heavy clay soil which is well-drained and has high organic matter (FarmLink, 2018). Application of organic and inorganic fertilizers is recommended in soils that have nutrient deficiencies in order to attain optimal yield (NAFIS, 2017).

Most of the cultivars are sensitive to soil acidity and aluminum toxicity (Grubben *et al.*, 2004). French beans require soil pH of about 6.5 to 7.5 but the common bean can withstand a low pH of up to 4.5 (Messiaen *et al.*, 2004). A pH of 4.5 limits the development of the rhizobium bacteria and this reduces the amount of nitrogen fixed at the root galls (Kimani, 2006). The crop also requires a well-distributed rainfall of 900-1200 mm per annum. During the off-season, cultivation is maintained through supplementary irrigation as a reduction of moisture cause flower abortion and deformed pods (Kimani *et al*., 2016). The pods are harvested 6-8 weeks after planting and 2-3 times per week depending on the target market. Harvested pods are graded to get rid of the twisted, broken, damaged and blemished ones after which they are immediately packaged and stored at 4°C and 80% relative humidity (Fulano *et al*., 2021).

2.3 French bean production constraints

French bean production in Kenya is adversely constrained by both biotic and abiotic factors. The widest spread abiotic factors are the edaphic factors such as aluminum and magnesium toxicity, low soil fertility, element deficiency such as nitrogen and drought (Mbeke *et al.*, 2014). Biotic factors include major fungal, bacterial and viral diseases as well as insect pests. Some fungal diseases include rust, anthracnose and angular leaf spot (Otim *et al.*, 2011; Arunga *et al.*, 2012; Kamiri *et al.*, 2021). Bacterial diseases of major concern include common bacterial blight caused by *Xanthomonas axonopodis pv. phaseoli,* halo blight (*Pseudomonas syringae pv. phaseolicola*) and bacterial brown spot caused by *Pseudomonas syringae pv. syringae* (Wasonga *et al.*, 2010). Viral diseases include the bean common mosaic virus (BCMV), the bean common mosaic necrosis virus (BCMNV), bean golden mosaic virus (BGMV), clover yellow vein virus, and cucumber mosaic virus (Worrall *et al.*, 2015). The bean fly (*Ophiomyia phaseoli*) is the most devastating pest in French bean production (Otim *et al.*, 2011). The aphids and leaf hoppers not only inflict damage on the crop but also act as vectors for viral diseases (Nellist *et al*., 2022).

2.4 French bean breeding

2.4.1 Breeding for pod yield

Researchers and small-scale farmers are turning their attention towards the creation of high-yielding crops with excellent pod quality and disease resistance. The goal is to reduce production costs and maximize yields, as the quality of the product heavily relies on genetic resistance (Kimutai, 2018). Breeding French beans is challenging due to the complex nature of French bean yield, which has a low heritability (Singh & Singh, 2015). The components of pod yield include various physical traits such as plant height, growth habit, leaf number, internode length, and number of pods per plant. Meanwhile, pod quality is influenced by factors such as pod length, color, texture, and shape. The relationship between yield and pod quality traits is complex, with some traits having a positive correlation and others having a negative correlation (Checa & Blair, 2012). Due to these complex relationships and the involvement of multiple genes, breeding for both yield and pod quality in French beans cannot be disregarded (Hagerty *et al*., 2016).

The yield of French beans can be influenced by various factors including weather, diseases insect infestations, weed growth, the number of plants in an area and the type of variety grown (Wahome *et al*., 2011). Small farmers often struggle with low yields due to a lack of proper technology for post-harvest handling. French beans are grown for their green pods, which can be consumed fresh, canned, or frozen, so it's important for the pods to meet market requirements in terms of quality (Checa & Blair, 2012). The pod traits that are most important in the market include the shape, curvature, length, color, and the ability to snap. The market requirements vary, but there are some common traits that are important across different regions. French beans are marketed based on pod characteristics, whereas dry beans are marketed based on seed characteristics and horticultural traits are given less consideration. The cross-sectional shape of the pod is determined by its wall thickness and the stage of crop development and can result in round, flat, or creased-back shapes (Njau, 2016).

French bean grading in Kenya is based on the width and cross-section of the pod and is classified as extra fine, fine, or bobby (Wahome *et al*., 2011). The market requires straight pods to achieve precise cuts or for packaging whole products, while the curvature of the

pod is influenced by the specific plant type (HCD, 2020). Straight pods are produced by straight bush and climbers' lines, while the market requires long pods ranging from 9-16 cm in length. The preferred market color is light or dark green and the beans should snap easily without any fiber.

2.4.2 The role of host resistance to disease management

Disease-resistant varieties are not only environmentally sustainable but also meet the market's preferred pod quality and regulatory requirements. By offering these high-yielding, resistant crops, small-scale farmers can increase their production to meet growing domestic and international demand (Buruchara *et al*., 2011). French bean production, primarily carried out by small-scale farmers, faces challenges from diseases. Many commercial varieties, including Julia, Serengeti, Samantha, Paulista, and Morgan, are highly susceptible to some major diseases leading to up to 100% yield loss. To mitigate this, small-scale farmers rely on fungicides, which increases the risk of their produce being rejected if the level of chemicals exceeds the recommended maximum residue levels (Buruchara *et al.,* 2011). To address this, breeding for multiple disease resistance can reduce the reliance on fungicides and decrease post-harvest losses.

It is crucial to understand the dynamics of diseases and their causes, as well as the conditions that promote their development and their economic impact. The severity of certain diseases is influenced by environmental conditions, but sources of resistance have been identified. Developing disease-resistant crops is the most cost-effective method of preventing diseases, but the durability of resistance can be compromised as new pathotypes emerge, making it less effective over time (Arunga *et al*., 2010). To overcome this, it's suggested to pyramid different genes that confer resistance at different stages of the plant's growth and slow disease development, as well as introgress quantitatively inherited genes through various breeding approaches (Singh & Singh, 2015).

2.5 Pathology and management of BCMV and BCMNV

2.5.1 Biology of the BCMV and BCMNV

The BCMV and BCMNV are members of the genus Potyvirus of the family Potyviridae (Peyambari *et al.,* 2006; Mwaipopo *et al.,* 2017). The Genus potyvirus contains other

economically important viruses such as watermelon mosaic virus, soybean mosaic virus, and zucchini yellow mosaic virus among others (Mukeshimana, 2003). The BCMNV and BCMV strains have been classified into pathogenic groups based on the virulence of the isolate on differentials cultivars. The BCMV strains were grouped into seven pathogenicity groups (I to VII) based on the different reactions they exhibited on the differential cultivars (Drijfhout, 1978). Each pathotype corresponds to a different set of resistance genes within a pathogenicity group. Molecular and serological studies carried out on the pathogenicity groups described earlier by Djifhout (1978) led to the reclassification of BCMV strains after the discovery of BVMNV in East Africa. The BCMV strains were grouped into five pathogenicity groups (I, II, IV, V, and VII) (Vetten *et al*., 1992) while BCMNV strains were group into two pathotypes (III and VI) (Xu & Hampton, 1996). The BCMNV was then identified as bean common mosaic virus serotype A while the BCMV was identified as serotype B based on serological and symptomatic differences (Mangeni *et al*., 2014; Worrall *et al*., 2015).

BCMNV is a single stranded positive-sense RNA virus that form flexuous rod-shape virions (Ivanov *et al.*, 2014). The virions are 750 nm in length and 11-15 nm in diameter and its genome contains 5% nucleic acids and 95% protein (Mangeni *et al.*, 2014; Worrall *et al.*, 2015). BCMNV has a short particle and a smaller capsid protein compared to BCMV. Common bean cells that have been infected with strains of BCMNV have a specific proliferated endoplasmic reticulum which is absent in BCMV infection (Mukeshimana, 2005). BCMNV induces a lethal necrosis response in plant germplasm that possess the dominant *I* gene that confers resistance to BCMV (Silbernagel *et al.*, 2001) BCMNV is endemic and infects most wild and forage cultivars of legumes as well as legumes grown for human consumption (Jordan & Hammond, 2008).

2.5.2 Geographical distribution of BCMV and BCMNV

The BCMV is one of the earliest stated viral diseases of beans in the world (Mukeshimana *et al.*, 2003). The first incidence of BCMV was reported in the United States in 1917 when it was referred to as bean mosaic and later referred to as bean common mosaic (Mwaipopo *et al.*, 2017). The pathogen later appeared in Eastern Africa probably as a result of recombination of BCMV strains after beans were introduced into the region (Tang &

Feng, 2022). However, it has been spread to other parts of the world through contaminated bean seeds (Flores-Est´evez *et al*., 2003). The presence of bean common mosaic necrosis virus (BCNMV) varies across Africa depending on the pathotype. Pathotype IV isolates of BCMNV have been observed to be widely distributed in Africa while those of pathotype III has been found in limited regions in Kenya, Rwanda, Burundi, Tanzania, and Southern Uganda (Beaver *et al*., 2003). The first report of basic BCMNV strains NL-3, NL-5 and NL-8 was made by Djifhout (1978). Later, a Tanzanian strain was identified that was serologically similar to other BCMNV strains (Silbernagel *et al*., 1986).

Research on common bean production in East Africa revealed that the equatorial region of Africa exhibits the highest genetic diversity of BCMNV isolates (Myers *et al*., 2000, Njau & Lyimo, 2000; Chiumia & Msuka *et al*., 2001; Mutuku *et al*., 2018). The long-distance dissemination of both viruses to many regions including North America, South America as well as Africa itself has been aided by the high efficacy of seed transmission of BCMV and BCMNV (Beaver *et al.*, 2003). In Africa, BCMNV has been found to infect both wild and weed legumes which is not the case outside Africa. The first incidence of BCMNV in Kenya was reported in 1973 (Kulkarni, 1973). Later, Buruchara (1979) isolated a severe strain of BCMV from Canadian Wonder cultivar that resembled but not identical to NL-3. The isolate induced mosaic on varieties that lacked the dominant resistance. Bock *et al.* (1976) observed necrotic reactions from BCMV isolates collected from common bean growing regions such as Thika, Kakamega, Muguga and Naivasha. A survey conducted by Omunyin *et al.* (1995) revealed presence of the virus in 18 out of 22 locations surveyed in Western and Central Kenya. A disease incidence of 20-63% was observed on farms in Kisii and Kakamega. Low incidence was observed in South Nyanza, Embu, Machakos and Kitui. Mangeni *et al*. (2014) concluded that BCMNV incidence is widely distributed in the agro-ecological zones in western Kenya. The viral strains NL-3, NL-5, NL-8 and TL-1 are the widest spread in East Africa and represents 53% of all BCMNV (Larsen *et al.,* 2011). The BCMV distribution is more intensive than that of BCMNV probably because BCMNV evolved from BCMV (Worrall *et al.*, 2015). In Central and East Africa, where the greatest BCMNV strains diversity is found, both viruses are major production constraints and cause 100% crop loss (Morales, 2006).

2.5.3 Transmission of BCMV and BCMNV

Most of the potyviruses are introduced into the host by aphids, although a few are carried by whiteflies (Hull, 2013). Infected seeds and plants of susceptible bean cultivars act as the initial inoculum of BCMV and BCMNV (Mukeshimana *et al*., 2003). The stability of the virus in the embryo is a major contributing factor to the spread of these viruses throughout the globe (Beaver *et al.*, 2003). For instance, the 1977 BCMV epidemic in America and Europe was believed to have been initiated by contaminated germplasm (Worrall *et al*., 2015). Seeds of *Phaseolus vulgaris* have been found to retain the BCMV for about 3 decades (Pierce & Hungerford, 1929; Silbernagel *et al.,* 2001). According to Sastry (2013), stage of infection, virus strain, host cultivar and environment affects the rate of transmission of BCMV and BCMNV. The proportion of infected seeds varies from 0.67% to 98% (Chiumia & Msuku, 2001). Plants whose infections occur after flowering does not yield infected seeds. This is due to absence of the virus in the embryo and cotyledons (Bragard *et al.,* 2013). Infected pollen grains may cause infection to the mother plant and the seed (Westwood & Stevens, 2010). Transmission of BCMV and BCMNV has proven to be a major problem since even the certified seeds may contain 1% of the infected seeds (Morales, 2006). Plants that are grown from infected seeds or are infected early in the growing phase tend to have few pods, seeds per pod and delayed maturity (Mukeshimana *et al.,* 2003).

Aphids transmit both BCMV and BCMNV through a non-persistent manner which is a mode generally utilized by potyviruses (Miklas *et al.*, 2006). The most important aphid species are *Acythosiphon pisum*, *Aphis fabae*, *Myzus persicae* and *Aphis craccivora* (Silbernagel *et al.*, 2001). *Myzus persicae* and *Aphis fabae* are the commonly used aphid species in the transmission of BCMV and BCMNV (Melgarejo *et al.*, 2007). Studies carried out using the electrical penetration graph method showed acquisition and inoculation occur within one minute and no latent period is required (Powell, 2005). Potyviruses are retained at the stylets of the aphids for a limited time if only they do not feed (Westwood & Stevens, 2010). Aphid transmission is secondary within the crop and its incidence is high during warm and wet conditions. However, aphids do not transmit all viruses with the same efficiency and thus aphid transmission is an important factor in viral

epidemiology (Worrall *et al.*, 2015). Presently, the effect of BCMV and BCMNV on the aphid vector is not well known. The relationship among the vectors, the host plant and the viruses offer a new approach towards the control of BCMNV (Bragard *et al.*, 2013).

2.5.4 Symptoms of BCMV and BCMNV

Potyvirus infection causes dramatic changes in the plant cell membrane followed by virus replication in the host cell (Grangeon *et al*., 2012). After a potyvirus enters the host cell, its first step is to shed its outer coat through uncoating. Subsequently, it utilizes the translational machinery of the host cell to undergo translation and produce its polyprotein (Worrall *et al.,* 2015). Once the infection has occurred, the virus factories moves to periphery of the infected host and utilizes the host cell skeleton (Agbeci *et al*., 2013). BCMV and BCMNV induce distinct symptoms in common beans. The type of symptom exhibited depends on the type of strain, type cultivar, age of the plant and the stage of the plant at infection (Feng *et al.,* 2014). In bean lines that are devoid of *I* gene, BCMNV induces symptoms that are similar to BCMV which include leaf curling, dwarfing, mosaic and chlorosis (Flores-Est´evez *et al.,* 2003). BCMNV strains induce systemic hypertensive reactions in bean cultivars that possess the *I* gene which results into plant death. This phenomenon is commonly referred to as black root rot (Mukeshimana *et al.,* 2003). The symptoms first appear at the trifoliate or primary leaves as small red-brown spots (Agbeci *et al.,* 2013). At these spots the veins become brown-black and this spreads to the phloem tissues. This is later followed by wilting and the whole plant eventually dies (systematic necrosis) (Silbernagel *et al.,* 2001). Some strains of BCMV (NL-2 and NL-6) may cause black root at temperatures above 30 °C in *I* gene bean line. This type of black root is termed as temperature-dependent (Worrall *et al*., 2015). The death of plant as a result of BCMNV hinders the plant from being the source of transmission (Mukeshimana *et al.,* 2003). Necrotic symptoms of BCMNV may be confused with those of Fusarium wilt. However, the absence of necrosis in the vascular tissues of the pods is the diagnostic for Fusarium wilt (Larsen *et al.,* 2011). Systematically infected plants may have small pods.

2.5.5 Control of BCMV and BCMNV

Resistance of BCMNV at the crop and population levels can be achieved by inhibiting virus transmission by hindering spread of the aphids (Westwood *et al.*, 2010), minimizing the virus spread by controlling aphids (Mwaipopo *et al.*, 2017), planting virus-free materials and incorporating the host plant resistance to BCMNV (Worrall *et al.*, 2015). Chemical control has been shown to have a slight effect on the spread of aphids but on the other hand, it encourages their movement and hence transmission of BCMV and BCMNV to other uninfected crops (Mukeshimana *et al.*, 2003). Besides, the continuous use chemicals have led to the rejection of Kenyans produce in the European market due to the high chemical residual levels. Mechanical control methods such as, rouging out of symptomatic plants have been used in some cases but has proved to be ineffective since it is possible that systematically infected plants with subtle symptoms will remain to act as the source of inoculum for secondary infection by aphids (Jordan & Hammond, 2008). The aphids also acquire and transmit BCMNV in a non-persistent manner thus infection can occur within a few minutes. In Eastern Africa, where BCMNV has been identified to infect wild and weedy leguminous plants, these plants can act as a source of inoculum to healthy materials growing in adjacent fields. As a result, BCMNV management in Africa differs from that of the other parts of the world.

Cultural control methods such as early planting, use of certified seeds and field sanitation have also been applied. Early planting has been used to escape the high incidence of aphid vectors (Buruchara *et al.*, 2011; Adams *et al.*, 2013). Plants that are infected at a later growing season tend to have less yield loss and the harvested seeds may have traces of the virus (Mukeshimana *et al.*, 2003). The use of certified seeds has substantially reduced the rate of disease incidence by 50% (Worrall *et al.*, 2015). However, since even a low percentage of infected seeds can lead to an epidemic, small traces of the virus harbored in these seeds have proven to be problematic. Therefore, the only safe method is to avoid recycling of seeds (Mukeshimana *et al.*, 2003; Opole *et al.*, 2003). The use of host resistance remains as the best remedy for controlling the spread of BCMV and BCMNV through planting of resistant cultivars (Strausbaugh *et al*., 2003). Cultivars have been

developed that possess the dominant *I* gene and the *bc-3* gene therefore conferring resistance to both BCMV and BCMNV (CIAT, 2001).

2.5.6 Host interaction with BCMV and BCMNV

The genome of Bean common mosaic necrosis virus is characterized by a single, lengthy open reading frame (ORF) and two small sequences located at the 5' and 3' ends. This ORF is responsible for encoding a polyprotein that is subsequently converted into nine proteins (Flores-Est´evez *et al.,* 2003). Bean common mosaic necrosis virus movement and replication has not been extensively studied at the molecular level therefore its host interaction is based on other portyvirus plant interactions (Worrall *et al.,* 2015). The BCMV is controlled by dominant resistance gene while BCMNV is controlled by recessive resistance genes (Mukeshimana *et al.,* 2014). The dominant unspecific *I* gene is known to confer resistance of all strains of BCMV (Mangeni *et al*., 2020). It was mapped on linkage group B2 and was found to be tightly linked to the seed coat intensifying *B* gene (Freyre *et al.,* 1998). BCMNV is controlled by recessive *bc* genes. The recessive isolate-specific genes consist of 5 alleles; *bc-1, bc-1*^{2}*, bc-2, bc-2*^{2} and *bc-3* which are independently inherited except for the pairs *bc-1: bc-1*^{2} *and bc-2: bc-2*^{2} (Mangeni *et al*., 2014). Recessive genes *bc-1*^{2}*, bc-2*^{2} and *bc-3* have been proven to act constitutively by restricting the virus movement in the plant (Kelly *et al.,* 2003). In the absence of *I* gene, *bc-u* is required for the expression of the other recessive genes except *bc-3* gene.

The dominant *I* gene alone is not sufficient to protect against systematic infection of BCMNV (Singh & Singh, 2015). Resistance to all known strains of BCMV and BCMNV is conferred by the dominant *I* gene in the presence of the recessive *bc-3* gene (Silbernagel *et al.,* 2001; Mavric & Vozlic, 2004; Morales, 2006). Genotypes that possess the recessive *i* and *bc-3* gene confers resistant to all BCMNV strains but vulnerable to some strains of BCMV (Larsen *et al.,* 2008). Cultivars that possess the dominant *I* gene and the recessive *bc-3* such as MCM 5001, MCM 2001, BRB191, BRB29, BRB32, BelNeb RR-1 and BelNeb RR-2 are resistant to all the strains of BCMV and BCMNV (CIAT, 2001; Kelly *et al.,* 2003; Mukeshimana *et al.,* 2005). Epistatic interactions have been reported in certain strains of BCMNV where *bc-3* and *bc-2*^{2} have been observed to mask the *I, bc-2*^{2}

and *bc-1*^{2} genes respectively (Kelly *et al.,* 2003). As a result of the masking effect, the *I* gene cannot be detected phenotypically (Mangeni *et al.*, 2014) and hence the need for marker-assisted selection (MAS).

2.5.7 Molecular markers linked to BCMV and BCMNV resistance genes

Marker assisted selection (MAS) has been combined with convectional breeding in order to intensify the process of selection (Njuguna, 2014). The fundamentals for a classical procedure of MAS involve the use of DNA markers and linkage analysis. This approach aims to identify molecular markers that are closely associated with the genes responsible for controlling specific traits of interest. These molecular markers enable the detection of genetic variations between organisms (Jiang, 2013). Marker assisted selection (MAS) has been exploited in common bean breeding program (Kelly *et al.*, 2003; Miklas *et al.*, 2006). Different molecular markers have been developed and utilized in bean breeding to accurately determine the location of the *bc-3* gene on bean linkage group B6 (Mukeshimana *et al*., 2005). These include amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), sequence characterized amplified regions (SCAR), and sequence-tagged sequences (STS). Johnson *et al*. (1997) converted RAPD markers linked to the *bc-3* gene to SCAR marker ROC11 which is linked to the *bc-3* gene in the repulsion phase in order to improve their efficiency in selection. Additionally, Mukeshimana *et al*. (2005) suggested SEACAMCGG-134/137 STS and RAPD markers that are linked to the *bc-3* gene. Previous studies have shown that the resistance of potyviruses is highly influenced by the translation initiation factors (TlFs) (Perez-vega *et al.,* 2010). These translation initiation factors act by restricting the replication of potyviruses in the plant. Based on this fact, the *bc-3* gene locus in beans has been found to be associated with a mutation in a sequence encoding elF4E protein, hence a stable cleaved amplified polymorphic sequence (CAPS) marker was developed. It is a dominant marker that is converted into a co-dominant marker upon digestion by the *Rsal* restriction enzyme.

The SCARs are extended specific sequences of RAPD with approximately of 20 nucleotides and are highly reproducible. In addition, they are dominant but some can be converted into codominant by digesting them with restriction enzymes (Collards *et al.,*

2005; Tryphone *et al.,* 2013). The SCAR markers have been broadly used in the development of disease resistance controlled by dominant gene. While incorporating resistance to angular leaf spot and BCMNV in adapted common bean genotype, Chilagane *et al*. (2013) used SCAR markers SNO2, ROC11 and SW-13 linked to *Phg-2, bc-3* and *I* gene respectively. Other SCAR markers that are linked to recessive genes controlling common bean diseases have also been identified (Miklas *et al.,* 2009). Melleto *et al.* (1998) developed a SCAR marker tightly linked to the *I* gene which has been used to select and develop germplasm with resistance to BCMV. The SW-13 SCAR marker is one of the most widely used, and has been employed to confirm the presence of *I* gene for BCMV resistance (Pastor-Corrales *et al.,* 2007; Miklas *et al.,* 2009). Larsen & Miklas (2004) recommended the use of SCAR markers in the selection for bean curly top virus resistance in French beans. Miklas *et al*. (2000) identified a SCAR marker tightly linked to the recessive gene *bc-1*^{2} in the French bean population. However, the marker was found to be unreliable in both cranberry and kidney beans. A single nucleotide polymorphisms (SNP) marker was also developed that is tightly linked to recessive loci *bc-u* and *bc-1*(Soler-Garzón *et al*., 2021). These two genes were found to be linked. Bean breeders have effectively utilized SCAR markers linked to both *I* and *bc-3* gene to develop improved germplasm with resistance to BCMV and BCMNV (CIAT, 2001). The developed lines were evaluated in East Africa where the disease is prevalent for instance in Uganda (Okii *et al*., 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Evaluation of host resistance to BCMNV among selected French bean cultivars

3.1.1 Genotypes

A set of 32 entries, comprising 23 commercial varieties, two local landraces, two breeding lines of French beans together with five dry bean varieties was assembled for testing for resistance to BCMNV under field conditions (Table 3.1). Three of the dry bean breeding lines (MCM 2001, MCM 5001 and MCM 1015) carry both *I* and *bc-3* were used as the resistant checks. Mitchelite and Cornell 49-2420 are both highly susceptible to BCMNV. These two cultivars were reported to be highly susceptible to BCMNV by Kamiri (2021) while screening French bean cultivars for resistance to anthracnose under field conditions. For greenhouse screening, 8 differential cultivars (Table 3.2) were added for purposes of characterizing the BCMNV virus pathotypes. The seeds for the check varieties were provided by the International Centre for Tropical Agriculture (CIAT), Uganda while the French bean genotypes were obtained from various seed companies in Kenya, research organizations and the National Gene bank of Kenya. The seed for the differential cultivars were obtained from Rwanda Agricultural Board.

Table 3.1: Bean genotypes used in the screening for host resistance to BCMNV

S/No.	Variety	Status	S/No.	Variety	Status
1	Amy	Commercial Cultivar	17.	Edge	Commercial Cultivar
2.	Serengeti	Commercial Cultivar	18.	Moonstone	Commercial Cultivar
3.	Monel	Commercial Cultivar	19.	Enclave	Commercial Cultivar
4.	Morgan	Commercial Cultivar	20.	Blazer	Commercial Cultivar
5.	Teresa	Commercial Cultivar	21.	Boston	Commercial Cultivar
6.	Tausi	Commercial Cultivar	22.	Source	Commercial Cultivar
7.	Fanaka	Commercial Cultivar	23.	Cornel 49-2420	Susceptible Check
8.	Samantha	Commercial Cultivar	24.	Mitchelite	Susceptible Check
9.	Goldplay	Commercial Cultivar	25.	MCM 1015	Resistant Check
10.	Hawaii	Commercial Cultivar	26.	MCM 2001	Resistant Check
11.	Lomami	Commercial Cultivar	27.	MCM 5001	Resistant Check
12.	Manakelly	Commercial Cultivar	28.	MU#13	Breeding Line
13.	Mara	Commercial Cultivar	29.	MU#02	Breeding Line
14.	Vanilla	Commercial Cultivar	30.	MU#03	Breeding Line
15.	Widusa	Commercial Cultivar	31.	GBK 032 921	Local Landrace
16.	Seagull	Commercial Cultivar	32.	GBK 032 952	Local Landrace

Table 3.2: BCMV/BCMNV common bean differential cultivars used in this study

Host group	Host gene	Differential Cultivar
1	*Ii*	Sutter Pink
2	*bc-1bc-1*	Puregold Wax
3	*bc-1^{2}bc-1^2*	Great Northern 123 (Ul-123)
4	*bc-2bc-2*	Sanilac
5	*bc-2bc-2*	Red Mexican 34
6	*bc-2^{2}bc-2^2*	Red Mexican 35
7	*bc-2^{2}bc-2^2*	Great Northern 31
8	*I bc-3*	MCM 5001

Source: Djifhout (1978)

3.1.2 Field experiments

3.1.2.1 Experiments sites

Two field experiments were conducted in this study. The first experiment was a pilot experiment where 10 French bean cultivars and 3 dry bean breeding lines (MCM 1015, MCM 2001 and MCM 5001) were tested in two locations; University of Embu and Kenya Agricultural and Livestock Research Organization (KALRO) in Kakamega to confirm the incidence of BCMNV. The University of Embu site (0° 30' S, 37° 27' E) is located in Embu West Sub-County at an elevation of 1480 m above sea level. The field has been previ ously used for screening of common bean breeding materials for multiple disease resistance. The area has a mean temperature of 19 °C with a maximum of 25 °C and a minimum of 10 °C. The soils are mainly *Humic Nitisols* derived from basic volcanic rocks. The average annual rainfall is 1,252 mm and is received in two distinct rainy seasons; short rains (mid-October to December) and long rains (March to June). The KALRO Kakamega site (0° 16' N, 34°46' E) is located in Kakamega, Lurambi Sub-county at an elevation of 1523 m above sea level. The soils are *ferralo-Orthic Acrisols*. The annual rainfall is about 1950 mm (Jaetzold *et al.,* 2006). The site was selected as a disease hotspot because of the high severity of BCMNV and other common bean pathogens (Mangeni *et al.,* 2014). The experiment was conducted between October and December in 2019.

The second experiment evaluated 32 bean genotypes in Kirinyaga and Embu regions. These sites represent the major French bean production zones thus provided the most conducive environments for field evaluation. The Kirinyaga site (0° 34'S, 37°20'E) is located in Kirinyaga Central Sub-County between, at an elevation of 1,287 meters above sea level. The area receives an annual rainfall of 1095 mm and has an average annual temperature of 21 °C. The Embu site (0° 34'S, 37° 29'E) was located in Mbeere North sub-county at an elevation of 1598 m.a.s.l with an average temperature of 25°C and receives an average annual rainfall of 1,252 mm. The experiment was conducted between April to June 2021.

3.1.2.2 Experimental design

The field experiments were laid out in randomized complete block design (RCBD), replicated four times in both sites. Seeds were sown in single rows measuring 2 m spaced 50 cm apart, with a 20 cm spacing between adjacent plants. Each experimental unit comprised of 10 plants. Primary and secondary cultivations were carried out in order to achieve a fine tilth. An application of 200 Kg/ha di-ammonium phosphate (DAP) fertilizer was made, followed by two applications of 50kg/ha calcium ammonium nitrate (CAN); the first at 2-3 leaf stage and the second after flowering. BCMNV incidence was scored on the basis of the presence/absence of symptoms.

3.1.3 Greenhouse experiment

3.1.3.1 Pathogen collection and characterization

Samples of BCMNV-infected plants growing in farmers' fields in Bungoma (0° 34´N, 34°32´E), Embu (0° 34'S, 37°29´E) and Kakamega (0°16´E, 34°46´N) were collected and pooled on the basis of their sampling site. These samples were transferred to the KALRO research laboratory for inoculum isolation following the method described by Chilagane *et al*. (2013). The greenhouse experiment that was carried out at KALRO Kakamega involved the inoculation of each of the 3 isolates of the virus on a set of 8 differential host varieties along with the 32-entry panel. The experiment was replicated 4 times and arranged in a completely randomized design (CRD) in the greenhouse. Seeds were sown in individual plastic pots measuring 20 cm in diameter and 16 cm in height and were filled with sterilized soil, farmyard manure and sand, in a ratio of 3:2:1. Each pot received 5 g triple superphosphate at planting, followed by 2 applications of 1.25 g calcium ammonium nitrate; first at trifoliate stage and after flowering.

Plants were inoculated at trifoliate stage using BCMNV inoculum that was prepared by grinding 5 g of severely infected leaf samples with a mortar and pestle in 0.1% hydrogen phosphate buffer. The supernatant obtained was sieved using a cheesecloth. The extracted sap was diluted in 0.02M KPO_4 buffer at pH of 7.5. Inoculation was carried out on primary leaves (7 days after planting) using the carborundum powder as an abrasive. The carborundum powder and the inoculum were gently rubbed on the entire leaf surface. The

plants were observed weekly for the development of symptoms for up to 4 weeks. Data was taken based on the type of symptom expressed. An ELISA test was done to ascertain that the symptoms were caused by BCMNV-positive infection and not any other pathogen. Different BCMNV isolates exist with different virulence hence they are categorized into different pathogenicity groups based on the host differential cultivar reaction as described by Djifhout (1978). Each pathotype corresponds to a known set of resistance genes within the pathogenicity group. The mode of resistance is categorized into 2 groups; specific isolate-recessive genes which include *bc-1*^{2}, *bc-2*^{2} and *bc-3* and the dominant *I* gene (Feng *et al*., 2017).

3.1.3.2 Confirmation of BCMNV infection through DAS-ELISA test

The confirmation of BCMNV infection was done using a double antibody sandwich enzyme linked immunosorbent assay (DAS- ELISA) to BCMNV antiserum conducted 3 weeks' post-inoculation as described by Were *et al.* (2004). Microtiter plates were coated with BCMNV IgG diluted 1:1,000 (v/v) in coating buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$, 0.20 g NaN_3, dissolved in 900 ml H_2O, and pH adjusted to 9.6 by adding HCl up to 1 L) and incubated for 4 hours at 30 °C. The leaf sap extracts prepared from ground infected leaf tissues 1:10 (w/v) in sample extraction buffer (PBST + 2% PVP) were added and incubated overnight at 4 °C. Positive and negative controls were preliminary intended to verify the performance of the assay. The IgG alkaline phosphatase conjugate, diluted 1: 1,000 (v/v) in conjugate buffer (PBST + 2% PVP + 0.2% egg albumin [Sigma A-S253]), was added and incubated for 5 hours at 30°C. The substrate, p-Nitrophenyl phosphate dissolved into a final concentration of 1 mg/ml in substrate buffer was added and incubated at room temperature in the dark. The colour development was assessed after 1 hour through quantitative measurements of the p-nitrophenol substrate conversion into yellow colour at 405 nm absorbance (A405). A yellow colour indicated positive infection while lack of colour change indicated negative infection.

3.2 Identification of host resistance against BCMNV among French bean lines

Genomic DNA was extracted from the young leaves (15 days old) of each of the French bean cultivars, 5 dry bean varieties and 3 breeding lines following Mahuku (2004)

protocol (Appendix 1). Molecular markers linked to *bc-1*^{2}, *bc-3* and *I* were used (Table 3.3).

Table 3.3: Molecular markers linked to BCMV and BCMNV resistance genes used in the study

SCAR marker	Size (bp)	Primers sequences	Tagged Locus	References
SBD5	1250 Cis	F: 5'- GTG CGG AGA GGC CAT CCA TTG GTG-3' R: 3'- GTG CGG AGA GTT TCA GTG TTG ACA-5'	*bc-1*^{2}	Miklas *et al.*, 2000
SW13	690 Cis	F: 5'-CAC AGC GAC ATT AAT TTT CCT TTC-3' R: 3'-CAC AGC GAC AGG AGG AGC TTA TTA-5'	*I* *Pse-3*	Haley *et al.*, 1994 Melotto and Kelly, 1998; Fourie *et al.*, 2004
ROC11	420 Trans	F: 5'-CCA ATT CTC TT T CAC TTG TAA CC-3' R: 3'- GCA TGT TCC AGC AAA CC-5'	*bc-3*	Johnson *et al.*, 1997
eIF4E	381/541 codominant	F: 5′-ACC GAT GAG CAA AAC CCT A-3′ R: 3'-CAA CCA ACT GGT ATC GGATT-5'	*bc-3*	Naderpour *et al.*, 2010

The polymerase chain reaction (PCR) for each marker was made up of a total volume of 10 µl comprising of 5 ηg/ul DNA, 0.5 uM of each specific reverse and forward primer, 5x Bioline MyTaq Reaction buffer (5 mM dNTPs, 15 mM, MgCl2, stabilizers and enhancers), 1.2 units of *Taq* DNA Polymerase (Bioline) made up to the volume using molecular grade water. The PCR regime comprised of an initial denaturation (94°C/5 minute), followed by 35 cycles of denaturation (94°C/10 seconds), primer pair-specific annealing step and an extension step (72°C/2 minute), and was completed by a final extension step (72°C/5 minute). The reaction products were separated by electrophoresis in 1.2% agarose gel, pre-strained with EtBr (0.5µg/ml). For the cleaved amplified polymorphic sequence (CAPS) assay used for the elF4 marker, a 5 µL aliquot of the PCR product was *Rsa*I-digested in a 15 µL reaction, according to the manufacturer's procedures before electrophoresis. After amplification, a volume of 3 µl of each amplicon was resolved on 1.2% agarose gel containing ethidium bromide, run in 1x Sodium borate

buffer at 100 volts for 1 hour. The gel was visualized using a UV trans-illuminator and photographed using a Canon® camera. The gel picture obtained for each individual was scored as (1) for the presence of a marker or (0) for the absence. The band size was estimated using a 50 base pair ladder. However, the ROC11 marker is linked in repulsion to the *bc-3* gene that conditions resistance against BCMNV (Johnson *et al*., 1977). Therefore, the presence and absence of the amplification was an indication of a susceptible and resistant cultivar respectively.

3.3 Introgression of the *bc-3* gene in French bean cultivars

3.3.1 Genotypes

Three dry bean accessions (MCM 1015, MCM 2001 and MCM 5001) known to carry both *I* and *bc-3* (CIAT, 2001) were used as donor parents in a marker-assisted backcross breeding scheme. These lines were provided by CIAT Uganda (https://alliancebioversityciat.org/regions/africa/uganda). The recipient French bean varieties were Amy, Serengeti and Vanilla, selected on the basis of their popularity with local producers and their excellent market acceptance. The seed was sourced from a registered stockist and their authenticity was confirmed with Kenya Plant Health Inspectorate Service (KEPHIS) by scratching a sticker label on the packet and sending the unique codes as a short message service (SMS) to 1393. All 3 varieties were genotyped with SW13 the marker and confirmed to carry the *I* gene. Ten plants of each donor and recipient parent were grown to make the necessary crosses, using the French bean varieties as the female parents. Starting from the BC_1F_1 generation, marker-assisted crossing was applied to select the progenies that retained the *bc-3* gene across generations.

3.3.2 Hybridization of parental lines

Artificial hybridization was carried out by emasculating the female flower followed by transfer of the pollen from the opened male flowers (Bliss, 1980) (Plate 3.1). Marker assisted backcrossing (MAB) was used in order to incorporate *bc-3* gene from the donor parent to the recurrent parents which resulted in a total of 9 F_1 hybrid lines (3 donors and 3 recurrent plants) (Figure 3.1). Prior to the initiation of the breeding program, ten plants of each donor and recipient parent were grown to facilitate the necessary crosses.

Plate 3.1: Hook emasculation method (A) and successfully cross-pollinated French bean flower (B).

After the first crossing of the recurrent and the donor parents, morphological (seed and flower color, growth habit and hypocotyl pigmentation) was utilized to identify the true F_1 hybrids. The selected F_1 plants were then backcrossed to the recurrent parents for three more generations and one round of selfing. At each first filial generation of each backcross, morphological marker (seed and flower color, growth habit and were utilized for selection.

The SCAR marker ROC11 was utilized at BC_1F_2 and BC_3F_2 to ensure the retention of the *bc-3* gene. The BC_3F_2 progenies generated after selfing were genotyped using both the SCAR marker ROC11 and CAPS marker elF4E to confirm the successful introgression of the *bc-3* gene. The selections were then screened using the SCAR marker SW13 to confirm the presence of the *I* gene. The presence of the marker was scored as 1 and the absence as 0.

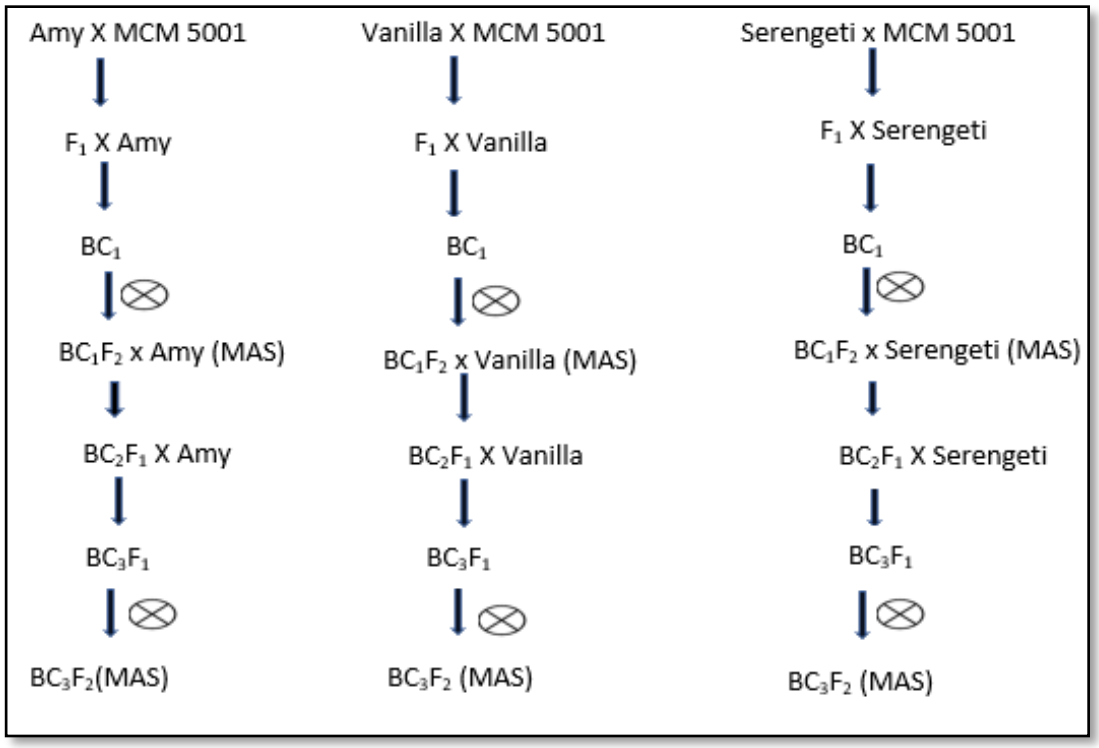


Figure 3.1: Marker-assisted backcrossing scheme to introgress *bc-3* gene into French beans

NB: The same scheme was applied to the other two donor parents (MCM 1015 and MCM 2001).

3.3.3 Marker genotyping

In order to track the *bc-3* gene in the backcross segregating population, DNA analysis was done as described in section 3.2. Two molecular markers that are linked to the *bc-3* gene were used in this study in order to track the successful introgression of the *bc-3* gene in the backcross segregating population. ROC 11 is a SCAR marker that is tightly linked to the *bc-3* gene in repulsion (Johnson *et al*., 1997) while elF4E is a CAPS marker that is obtained after *Rsal* restriction enzyme digestion. The breeding lines were first screened using ROC11 marker and the results were validated using elF4E CAPS marker. This was because ROC11 is a dominant marker. Further screening was carried out in order to confirm the presence of the *I* gene using SCAR marker SW13.

3.3.4 Evaluation of the BC_3F_2 French bean breeding lines

Selected BC_3F_1 plants were selfed to obtain the BC_3F_2 populations that were evaluated under field conditions alongside the recipient and donor parents for disease screening and for early generation testing. The field experiment was conducted in Mbeere North Sub-county in Embu County. The site is located between 0° 34´S and 37° 29´E at an elevation of 1,598 m above sea level. The mean annual rainfall is 1,252 mm and the mean air temperature is 25 °C. These conditions are conducive for the development of BCMNV. A nearby BCMNV screening nursery acted as the source of inoculum while a susceptible variety Rojo was used as a spreader to intensify the disease pressure. The field experiment was laid out in a randomized complete block design with four replications. The experimental plots comprised of 2 m rows with 50 cm inter-row spacing and 20 cm intra-row spacing. A pre-sowing application of 200 kg/ha di-ammonium phosphate fertilizer was done, followed later by two splits each of 50 kg/ha calcium ammonium nitrate fertilizer top dressing applications at 2-3 leaf stage and after flowering. BCMNV incidence was scored on the basis of the presence/absence of disease symptoms (Morales, 2003). The susceptibility percentage was calculated and reported as the proportion of infected plants over the total number of plants per genotype.

The genotypes were also characterized for agro-morphological traits including growth habit, pod length, pod diameter, pod suture string, pod wall fiber and the number of pods per plant. Based on the pod diameter, pods were graded into three standard categories: extra fine (6 mm), fine (6-8 mm) and bobby (8-9 mm). The number of pods per plant was determined by counting the total number of pods in a plot and dividing by the total number of plants in the plot. The pod fiber wall was quantified by snapping ten pods at the midpoint, and rating fiber strands protruding from the snapped pods on a scale of 1-5 (1 = no visible fiber strands, 3 = less than or equal to 3 visible fiber strands, 5 = more than 3 visible fiber strands). This scale was a modification of a scale of 0-2 used by Hagerty *et al*. (2016). The pod's strings were gently pulled from the calyx along the adaxial suture of the pod after boiling the pods in a water bath for 30 minutes at 100 °C. The average pod suture string values among the ten pods were used for analysis. Harvesting was carried out over a period of four weeks at two-day intervals.

3.3. 5 Data Analysis

Genstat software v.15 (https://vsni.co.uk/software/genstat) was used to subject the agro-morphological data to an analysis of variance and multiple-mean separation, applying Tukey's honest significant difference at a 5% probability level. Significance of correlation coefficients (r) was also determined and statistical table was used to establish relationships between the agronomic traits. The statistical model was:

$$Y_{ij} = \mu + \pi_i + \beta_j + \varepsilon_{ij}$$

where Y_{ij} = individual observation; μ=overall mean; πi= effect due to the i^{th} genotype; β_j= effect due to j^{th} replicate; ε_{ij} = estimate of experimental error.

CHAPTER FOUR

RESULTS

4.1 Evaluation of host resistance in French beans

4.1.1 Season 1

A total of ten French bean genotypes were evaluated for BCMNV host resistance against the resistant checks (MCM 5001, MCM 1015, MCM 2001). The screened French bean genotypes exhibited typical BCMNV symptoms such as mosaic, mottling, stunting, vein necrosis and leaf curling. In Embu, more genotypes were relatively higher than in Kakamega and all the screened genotypes were susceptible except the resistant checks MCM 5001, MCM 1015 and MCM 2001 (Table 4.1). In Kakamega, 80% of the French bean genotypes were susceptible to BCMNV but Boston, Moonstone and Vanilla did not show any BCMNV symptoms. The resistant checks did not show any signs of infection to BCMNV in both sites (Table 4.1).

Table 4.1: Response of French beans to BCMNV under field conditions in Embu and Kakamega during the long rainy season 2019.

		BCMNV symptoms	
S/no.	**Variety**	**Embu**	**Kakamega**
1.	Amy	1	1
2.	Boston	1	0
3.	Fanaka	1	1
4.	Gold Play	1	1
5.	Hawaii	1	1
6.	Lomami	1	1
7.	MCM 1015	0	0
8.	MCM 2001	0	0
9.	MCM 5001	0	0
10	Moonstone	1	0
11	Seagull	1	1
12	Serengeti	1	1
13	Source	1	1
14	Vanilla	1	0

[a]1=present, 0=absent

4.1.2 Season 2

A total of 32 genotypes were evaluated under field conditions in Embu and Kirinyaga during the long rain season of 2020. The most common symptom was top necrosis (Plate 4.1), beginning at the plant shoot and progressing downwards to older plant parts. Other common viral symptoms such as mosaics, mottling, downward curling and stunted growth were also observed. The tested genotypes showed signs and symptoms of BCMNV and none of BCMV.

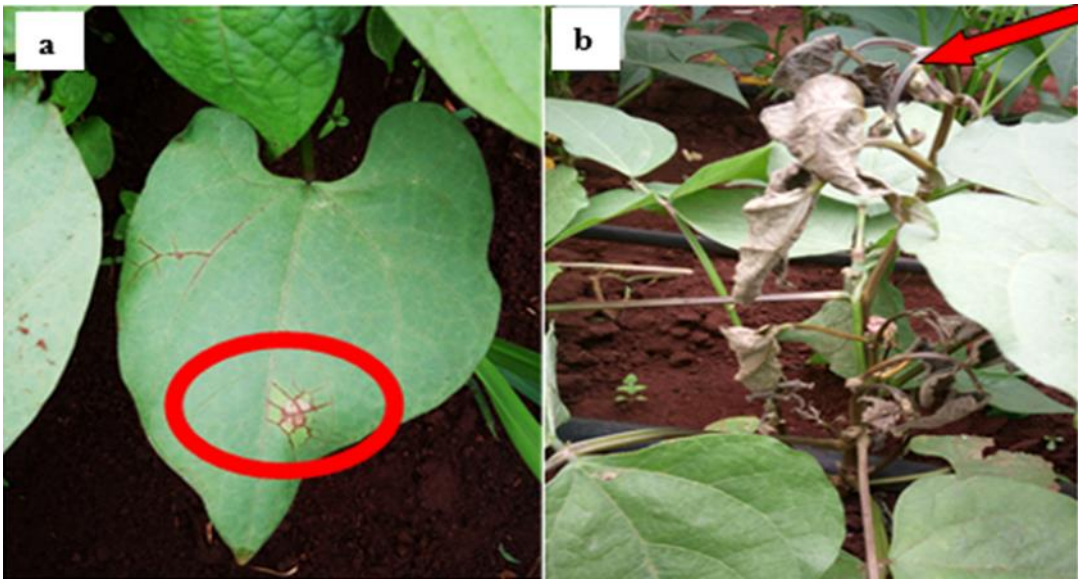


Plate 4.1: Mosaic (a) and top necrosis (b) symptoms of BCMNV disease expressed by susceptible plants in the field

At the Kirinyaga site, 69% were infected, while 84% of the genotypes were infected in Embu (Table 4.2). None of the three virus-resistant dry bean checks showed any symptoms at either site.

Table 4.2: Response of French bean genotypes to BCMNV under field conditions in Embu and Kirinyaga Counties during the long rain season 2020.

		BCMNV symptoms[a]				BCMNV symptoms	
S/No.	Variety	Embu	Kirinyaga	S/No.	Variety	Embu	Kirinyaga
1	Amy	1	1	17	MCM 5001	0	0
2	GBK 032 921	1	1	18	Mitchelite	1	1
3	Blazer	1	0	19	Monel	1	1
4	Boston	1	1	20	Moonstone	1	1
5	Cornell49-2420	1	1	21	Morgan	1	1
6	Edge	1	1	22	M13	0	1
7	Enclave	1	0	23	MU#02	1	0
8	Fanaka	1	1	24	MU#03	0	1
9	GBK 032 952	1	1	25	Samantha	1	0
10	Goldplay	1	1	26	Seagull	1	1
11	Hawaii	1	1	27	Serengeti	1	0
12	Lomami	1	1	28	Source	1	1
13	Manakelly	1	0	29	Tausi	1	1
14	Mara	1	0	30	Teresa	1	1
15	MCM 1015	0	0	31	Vanilla	1	1
16	MCM 2001	0	0	32	Widusa	1	1

[a]1=present, 0=absent

4.2 Pathogen characterization

The isolates collected from Bungoma and Kakamega induced similar symptoms to those of strain NL-3 and were therefore classified as pathotype VI while those from Embu induced symptoms similar to NL-8 hence classified as pathotype III (Table 4.3). The symptoms expressed include mosaic, mottling, deformed leaves and stunted growth. The ELISA test confirmed that the plants were infected with BCMNV (Plate 4.2).

Table 4.3: Reaction of differential cultivars to different BCMNV isolates

	Differential		Bungoma		Kakamega		Embu	
S/No.	cultivar	Host genes	Reaction[a]	ELISA[b]	Reaction	ELISA	Reaction	ELISA
1	Pinto	*bc-1 bc-2²*	M, ST	+	M, ST	+	NR	-
2	Red Mexican 34	*bc-2*	MT, D	+	M	+	MT, D	+
3	Pure gold	*bc-1*	M	+	M	+	NR	-
4	Red Mexican 35	*bc-2²*	NR	-	NR	-	NR	-
5	Sanilac	*bc-2*	M, ST	+	ST	+	ST, D	+
6	Sutter pink	*Ii*	M	+	MT, ST	+	ST	+
7	Great northern 123	*bc-1²*	ST, D	+	ST, D	+	NR	-
8	Great northern 31	*bc-2*	NR	-	NR	-	NR	-
	Pathotype		VI		VI		III	

[a]M - mosaic, MT - mottling, D - deformed leaves, ST - stunted growth, NR - no reaction;
[b]+ = positive, - = negative

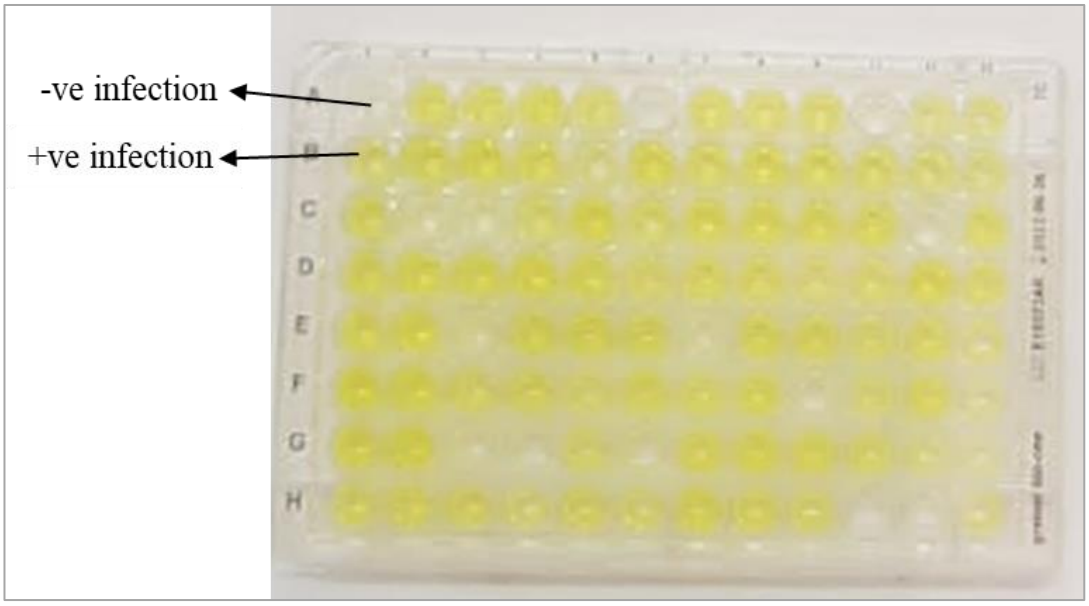


Plate 4.2: Microtitre plate showing DAS- ELISA results for BCMNV

4.3 Response of French bean genotypes to BCMNV under greenhouse conditions

The 32 entries reacted differently to infection with the three BCMNV isolates: symptoms observed included top necrosis, mosaics, mottling, deformed leaves, stunted growth and no symptoms (Plate 4.3) (Table 4.4). The presence of BCMNV in these plants (including the symptomless ones) was confirmed using the ELISA test. The three resistant dry bean check varieties MCM 1015, MCM 2001 and MCM 5001 were all resistant to each of the three isolates (Table 4.4).

Plate 4.3: Mottling (a), mosaic (b), stunted growth (c) and top necrosis (d) symptoms on beans

Table 4.4: Disease symptoms and ELISA test reactions on the French bean genotypes

		Bungoma isolate		Kakamega isolate		Embu isolate	
S/No.	**Genotypes**	**Reaction[a]**	**ELISA[b]**	**Reaction**	**ELISA**	**Reaction**	**ELISA**
1.	Amy	M	+	M, MT	+	NR	+
2.	Blazer	MT, ST	+	M	+	MT	+
3.	Boston	M	+	MT	+	MT, D	+
4.	Goldplay	TN	+	ST	+	ST	+
5.	Fanaka	MT, D	+	M	+	M	+
6.	GBK 032921	D	+	M, ST	+	D, MT	+
7.	Hawaii	M, LN	+	VN	+	MT	+
8.	Manakelly	M	+	M	+	D	+
9.	GBK 032952	D	+	M, ST	+	ST	+
10.	Lomami	M	+	M	+	MT, D	+
11.	Enclave	M	+	M	+	M, D	+
12.	Mara	M	+	M	+	M	+
13.	Monel	MT, D	+	M	+	MT	+
14.	Morgan	MT	+	M	+	M	+
15.	Moonstone	D, M	+	M	+	M	+
16.	MU#02	M	+	M, ST	+	MT	+
17.	MU#03	M	+	M	+	NR	+
18.	MU#13	MT	+	M	+	M	+
19.	Samantha	M	+	M, ST	+	M	+
20.	Vanilla	M, D	+	MT	+	M	+
21.	Serengeti	M, D	+	M	+	M, D	+
22.	Source	M	+	M, ST	+	M	+
23.	Widusa	M	+	M	+	M	+
24.	Seagull	M	+	MT	+	M	+
25.	Teresa	M	+	MT	+	D	+
26.	Tausi	M	+	MT	+	M	+
27.	Mitchellite	M, D	+	M, D	+	M, D	+
28.	Cornell 49- 242	TN	+	TN	+	TN	+
29.	Edge	VN	+	M	+	ST	+
30.	MCM 1015	NR	-	NR	-	NR	-
31.	MCM 2001	NR	-	NR	-	NR	-
32.	MCM 5001	NR	-	NR	-	NR	-
	Pathotype	VI		VI		III	

[a]M = mosaics; MT = mottling; D = deformed leaves; ST = stunted growth; TN = top necrosis; VN = vein necrosis; LN = Local necrosis; NR = no reaction. Entries 1-27 = French bean genotypes; 28-32 = dry bean genotypes used as cheks. [b]+ = positive, - = negative

4.4 Molecular characterization of BCMNV resistance

4.4.1 SCAR marker ROC11 and CAPS marker elF4E

All cultivars except the resistant checks were amplified by the ROC11 marker visualized as a 300 bp band (Plate 4.4 A), indicating that only the MCM series possess the *bc-3* gene. However, the size of the band that was amplified differed from the expected. When the

DNAs were amplified using the elF4E primer pair (a marker that also assays for the presence of *bc-3*), each sample generated a 541 bp amplicon. Following *Rsal* digestion, the amplicons produced by carriers of *bc-3* (MCM 2001, 5001 MCM 1015) were cleaved into a 381 bp and a 160 bp product, whereas those produced by non-carriers were not cleaved (Plate 4.4 B).

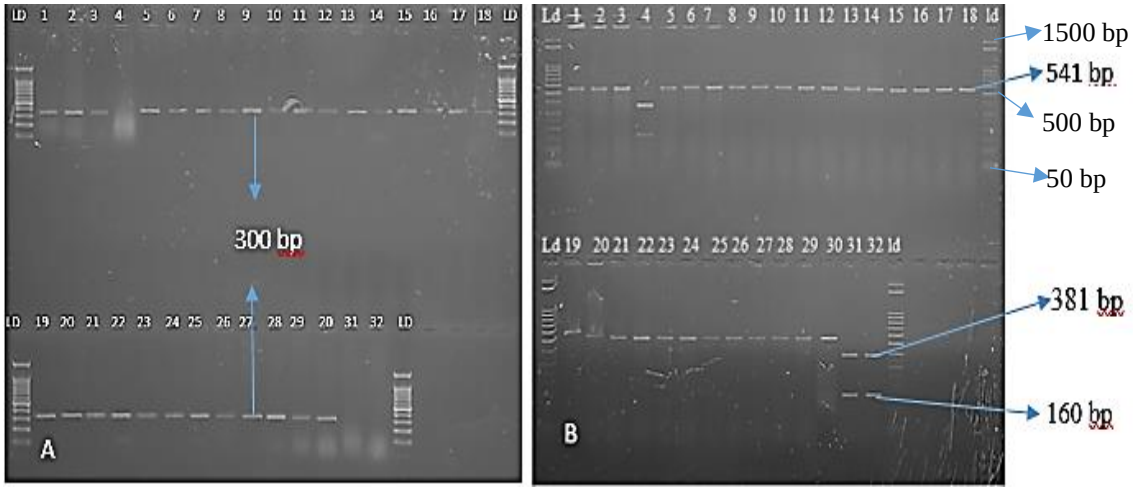


Plate 4.4: Amplification of molecular markers linked to BCMNV resistance genes *bc-3*; SCAR marker ROC11 420 bp (A) and CAPS marker elF4E 541(381/160) (B). Entries 1-3, 5-30 are French bean genotypes; 4, 31-32 are resistant dry bean genotypes.

4.4.2 SCAR markers SW13 and SBD5

The SW13 marker is a dominant marker that is tightly linked to the dominant *I* gene that confers resistance to BCMV. All the tested samples amplified the *I* gene fragment of the expected size (690 bp) and appeared as a single polymorphic band in agarose gel (Plate 4.5 A**)**. This implied that all the tested materials (both French bean varieties and dry bean genotypes) possessed the *I* gene. On the other hand, the presence of the target gene *bc-1*^{2} was identified by the dominant SCAR marker SBD5. It amplified the *bc-1*^{2} gene as a single DNA fragment of the expected size of 1250 bp in 28% tested genotypes. All the nine genotypes were French bean varieties namely Mara, MU#02, Manakelly, Teresa, Tausi, Goldplay, Moonstone, Seagull and Hawaii (Plate 4.5B).

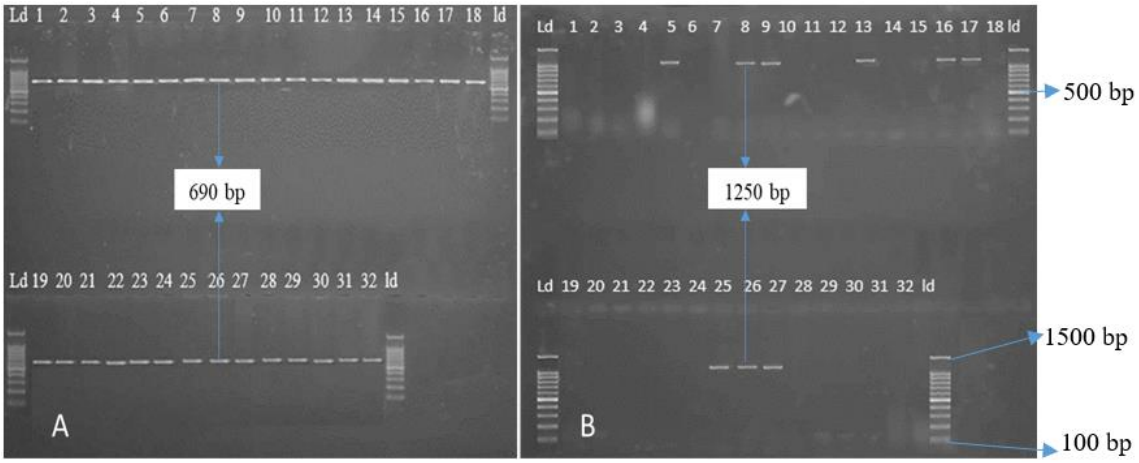


Plate 4.5: Amplification products of SCAR marker SW13 associated with dominant *I* gene (A) and SCAR marker SBD5 1250 bp for *bc-1*^{2} (B). Entries 1-3, 5-30 are French bean genotypes; 4, 31-32 are resistant dry bean genotypes.

From the above molecular analysis results, the 32 genotypes were grouped into three: Group 1 had the three resistant dry bean genotypes carrying markers for both the *I* and *bc-3* genes; Group 2 had the 9 French bean genotypes carrying markers for both the *I* and *bc-I*^{2} genes; while group 3 had 20 French bean genotypes carrying the marker for the *I* gene (Table 4.5). Therefore, none of the 28 French bean genotypes that were tested in this study combined the *I* gene and the *bc-3* gene.

Table 4. 5: Molecular analysis and ELISA test of the French bean genotypes

Group	Genotypes	ELISA Reaction	SW13 (*I gene*)	ROC11 (*bc-3*)	elF4E (*bc-3*)	SBD5 (*bc-1*^{2})
1	MCM 1015; 2001; 5001	Resistant	Present	Present	Present	Absent
2	Goldplay; Manakelly; Mara; Moonstone; MU#03; Seagull; Tausi; Teresa	Susceptible	Present	Absent	Absent	Present
3	Amy; Blazer; Boston; Edge; Enclave; Fanaka; GBK 032921; GBK 032952; Hawaii; Lomami; Monel; Morgan; MU#02; MU#13; Samantha; Serengeti; Source; Vanilla; Widusa; Mitchellite; Cornell 49-242	Susceptible	Present	Absent	Absent	Absent

4.5 Selection of breeding lines using molecular and morphological markers

The presence of the SW13 marker, which tags the dominant *I* gene, was confirmed in both the donor and the recipient parents. However, the *bc-3* gene was found exclusively in the donor accessions. Out of the 50 F_1 seeds generated from the French bean × dry bean crosses, 29 were selected as true hybrids using morphological markers (seed and flower color, growth habit and hypocotyl pigmentation). The materials were backcrossed to form a population of BC_1F_1 progenies which were then selfed to obtain 416 BC_1F_2 progenies, out of which 225 lacked the ROC11 amplicon and so were considered to be likely carriers of *bc-3* (Table 4.6). Some of these were used as parents for the second backcross which resulted in a total of 85 BC_2F_1 progenies, out of which 67 were selected morphologically and advanced to BC_3F_1. Some of the resultant BC_3F_1 progenies were then selfed to produce a total of 716 BC_3F_2 seeds (Table 4.6). To confirm the retention of the *bc-3* gene, the BC_3F_2 progenies were first screened with the ROC11 marker resulting in 123 selections (Plate 4.6) which were further screened with the elF4E marker resulting in 19 *bc-3* homozygotes and 13 heterozygotes (Plate 4.7; Table 4.7). The selected progenies were then monitored for the retention of *I* using the SW13 marker (Plate 4.8) resulting in 22 genotypes carrying the *I* gene but only 19 lines carried both *bc-3* and *I* genes (Table 4.7) and formed the final selections. However, these selections showed some variable levels of disease susceptibility in the field ranging from 7 – 21% (Table 4.7) which may require further screening and selfing to fix the genes.

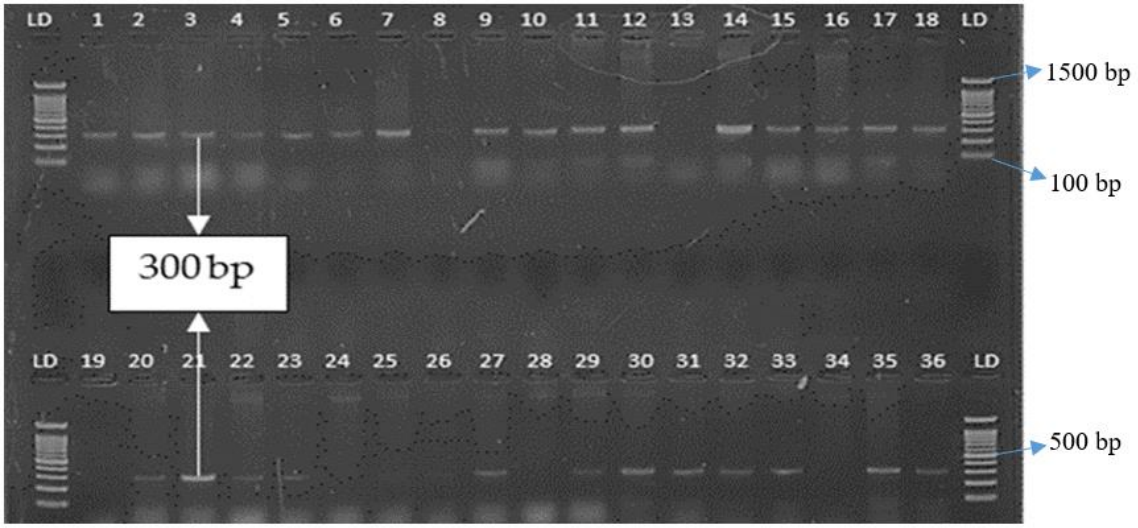


Plate 4.6: BC_3F_2 amplification products for marker ROC 11 (420 base pair), linked to *bc -3* gene in repulsion (absence of the band indicates the presence of *bc-3* gene and vice versa); Ladder 100bp

Table 4.6: Number of seeds obtained from a French × dry bean cross and selected breeding lines at different backcross and selfing generations. `

Crosses	F_1		BC_1F_2		BC_2F_1		BC_3F_1	BC_3F_2
	Number of seeds obtained	Selected	Number of seeds obtained	Selected	Number of seeds obtained	Selected	Number of seeds obtained	Number of seeds obtained
Amy / MCM 2001	5	3	40	20	10	7	67	176
Amy / MCM 5001	3	2	60	16	10	8	48	87
Amy /MCM 1015	4	3	52	25	8	7	27	42
Vanilla /MCM 2001	6	4	50	36	10	8	29	43
Vanilla / MCM 5001	4	3	38	27	9	7	32	76
Vanilla / MCM 1015	7	3	57	30	10	9	75	49
Serengeti / MCM 2001	6	4	42	21	10	9	89	32
Serengeti / MCM 5001	7	3	49	32	9	6	53	80
Serengeti /MCM 1015	8	4	28	18	9	6	36	131
Total Progenies	**50**	**29**	**416**	**225**	**85**	**67**	**456**	**716**

Table 4.7: Phenotypic and genotypic selection data for the *bc-3* and *I* gene selection at BC_3F_2

Cross combination	SCAR ROC 11	CAPs elF4E	SCAR SW13	Both SW13 and elF4E	Susceptible Plants %
Amy / MCM 2001	16	4	2	2	17
Amy / MCM 5001	10	6	4	4	11
Amy /MCM 1015	8	4	4	4	20
Vanilla /MCM 2001	15	5	5	3	20
Vanilla / MCM 5001	16	2	1	1	19
Vanilla / MCM 1015	22	2	1	1	7
Serengeti/MCM 2001	13	3	2	1	11
Serengeti / MCM 5001	12	3	1	1	21
Serengeti /MCM 1015	11	3	2	2	11
Total Progenies Selected	**123**	**32**	**22**	**19**	

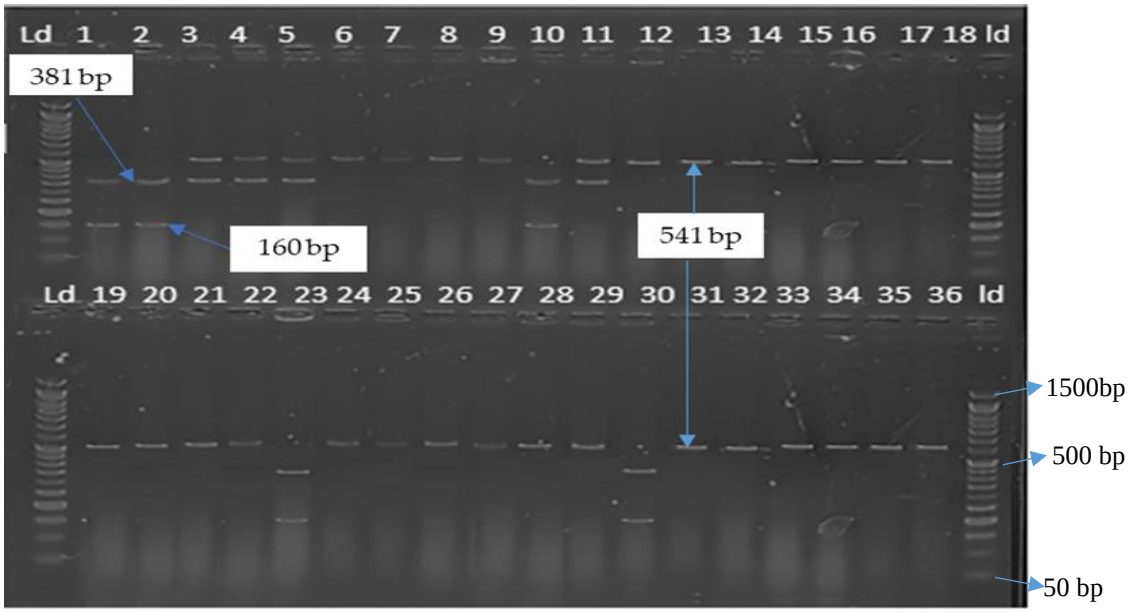


Plate 4.7: Gel plates of BC_3F_2 breeding population (1-36) showing the CAPS marker elF4E linked to *bc-3* after digestion of 541-bp fragment with *Rsal* enzyme into 381bp and 160bp (1, 2, 10, 23 and 30); ld is 50bp ladder

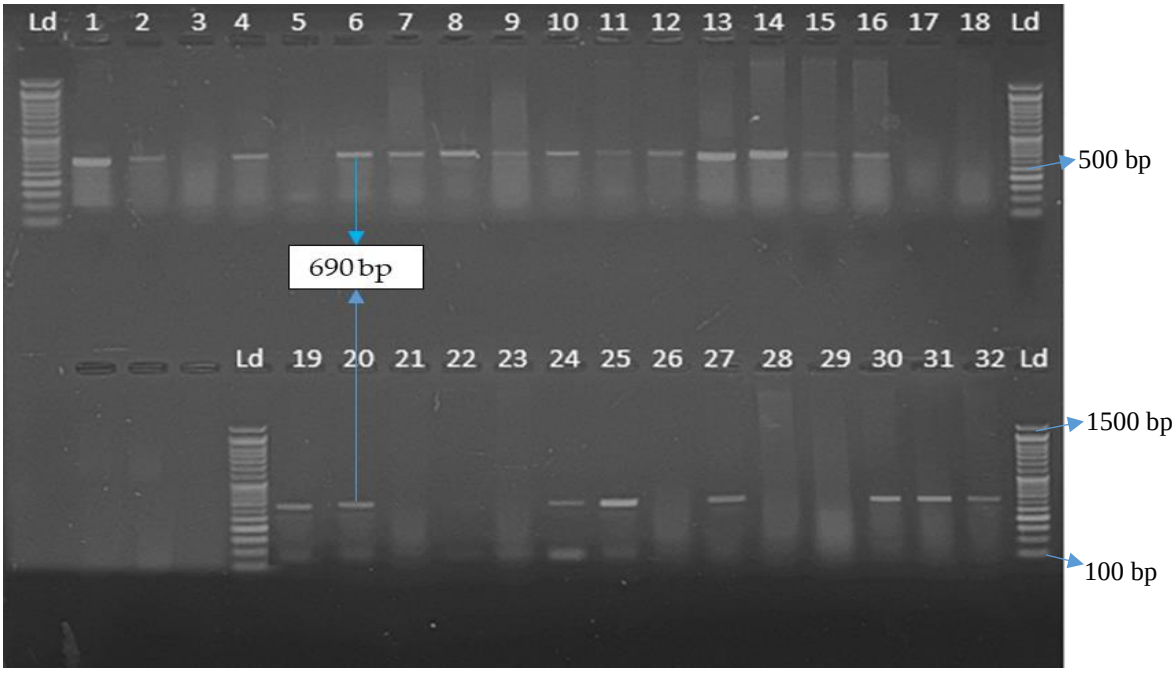


Plate 4.8: Gel plates showing the SW13 (690bp) linked to *I* gene fragment in BC_3F_2 breeding population that had previously been selected by elF4E CAPs marker; ld is 100bp ladder.

4.6 Phenotypic characterization of selected backcrosses for agro-morphological traits

The analysis of variance that was carried out on the agro-morphological traits showed that the selected progenies differed significantly ($p < 0.05$) with respect to pod length, pod suture string, pod diameter, pod fibre, the number of pods per plant and pod yield, but were not significantly different in the number of days to flowering (Table 4.8). Pod length varied from 9.4 to 14.2 cm, with hybrids developed from Vanilla/MCM 5001 hybrids producing the longest pods.

Pod length also positively correlated to number of pods per plant (r= 0.307*) (Table 4.9) and pod weight (r= 0.40**). In terms of productivity, the selections formed between 22 and 38 pods per plant, with Vanilla hybrids being the most productive. Pod weight was highly correlated to pod weight (r= 0.928***). The total pod weight per plant varied from 51.4 g to 96.4 g and the Vanilla/MCM 5001 hybrid produced the highest total pod weight. The days to 50% flowering varied from 40 to 42 days with the majority of the breeding

line flowering at 41 days. The cross between Amy/MCM 5001 flowered early with 40 days. Pod diameter varied from 6.98 mm to 8.88 mm. All the breeding lines attained fine market class pod diameter 6.9 -8 mm. This parameter was negatively correlated to pod length (*r*= -0.60***). Pod suture string length varied from 5.13-5.75 cm. The selected Amy/MCM5001 hybrids produced the longest pod suture string of 5.75 cm. All of the selections produced pods that had some visible pod fibre. Overall, most of the selections outperformed their recipient parent with respect to pod length, the number of pods produced per plant and total pod weight.

Table 4.8: Agro-morphological diversity of BC_3F_2 French bean breeding lines evaluated under field conditions

Genotype	D50%F[a]	Plant height (cm)	Pod diameter (mm)	Pod suture string (cm)	Pod length (cm)	Pods per plant	Pod weight per plant (g)	Pod fiber	Growth habit
Amy	41.25	37.00 a	6.98 a	2.18 a	9.42 a	21.57a	51.43 a	1.00 a	Determinate
Amy x MCM 1015	41.00	32.17 a	7.07 ab	5.18 bc	10.19 ab	25.28ab	61.65 a-c	3.21 b	Determinate
Amy x MCM 2001	42.00	101.38 fg	7.56 c-e	5.30 c	10.57 a-c	25.59ab	86.28 b-d	3.15 b	Indeterminate
Amy x MCM5001	40.50	97.50 e-g	7.87 d-f	5.75 c	10.68 a-d	27.30ab	67.94 a-d	3.22 b	Indeterminate
MCM 1015	41.00	108.54 g	8.03 f	10.48 e	10.68 a-d	27.83ab	78.01 a-d	5.00 c	Indeterminate
MCM 2001	41.50	94.46 d-g	8.74 g	9.10 e	10.87 b-d	30.34ab	88.63 cd	5.00 c	Indeterminate
MCM 5001	40.25	69.17 b-c	8.88 g	7.46 d	11.23 b-e	30.46ab	51.98 ab	5.00 c	Indeterminate
Serengeti	41.00	39.77 ab	7.19 a-c	3.20 a	11.43 b-e	31.22ab	94.04 cd	1.00 b	Determinate
Serengeti x MCM 1015	42.00	34.75 a	6.97 a	5.73 c	11.84 c-f	32.97ab	61.62 a-c	3.22 b	Determinate
Serengeti x MCM 2001	41.50	78.44 c-e	7.48 b-d	5.53 c	11.93 c-f	32.98ab	92.54 cd	3.12 b	Indeterminate
Serengeti x MCM 5001	41.50	82.81 c-f	7.69 d-f	5.15 bc	11.96 d-f	32.99ab	79.54 a-d	3.20 b	Indeterminate
Vanilla	42.00	36.77 a	7.00 a	3.60 ab	14.23 h	34.29b	99.95 d	1.00 a	Determinate
Vanilla x MCM 1015	41.50	47.33 ab	7.14 a-c	5.26 c	12.84 fg	36.40b	72.38 a-d	3.17 b	Determinate
Vanilla x MCM 2001	42.00	51.46 ab	7.22 a-c	5.13 bc	13.70 gh	37.24b	78.00 a-d	3.12b	Determinate
Vanilla x MCM 5001	42.00	74.00 cd	7.91 e-f	5.70 c	14.23 h	37.55b	96.35 cd	3.28b	Indeterminate
Grand mean	41.40	66.40	7.58	5.65	11.60	30.94	77.40	3.24	
P Value	0.895	<.001	<.001	<.001	<.001	0.43	0.30	<.0001	
CV%	3.7	21.5	3.5	11.4	7.1	23.7	26.7	4.9	

[a]D50%F 50% days to flowering; Means sharing the same letter are not significantly different at $P \leq 0.05$ according to Tukey's test

Table 4.9: Correlation coefficients of agronomic traits of the BC_3F_2 French bean breeding lines

Variables	D50%F					
Pod number	0.07	**Pod number**				
Pod weight	0.09	0.93***	**Pod weight**			
Pod length	0.20	0.31*	0.40**	**Pod length**		
Pod fiber	-0.07	-0.31	-0.01	0.62***	**Pod fiber**	
Plant height	-0.02	0.18	0.22	-0.41**	0.64***	**Plant height**
Pod diameter	-0.18	-0.03	-0.01	0.49***	0.62***	0.19

*, **, *** Correlation coefficient significant at significant at p< 0.05, 0.01 and 0.001.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The tested genotypes showed different reactions to BCMNV with the majority being infected with the virus across the sites. This implied that most of the genotypes possess the resistant genes to BCMV but lacks the important resistance genes to BCMNV. In recent years, BCMNV has become the dominant potyvirus affecting beans (Tang & Feng, 2022) which can be attributed to the breeder's effort to breed against the strains of BCMV by the use of the *I* gene. The identification of BCMNV in common bean and French bean growing regions in Kenya concurs with earlier studies (Omunyi *et al*., 1995; Mutuku *et al*., 2018). BCMNV has also been documented in other African countries such as Rwanda (Kabeja, 2020), Tanzania (Mwaipopo *et al*., 2017) and Zambia (Mulenga *et al*., 2022). The extensive prevalence of BCMNV across these regions emphasizes the importance of implementing common bean breeding programs to develop resistance against this virus throughout the affected areas. The difference in disease pressure and spread across the field that was observed this study is similar to what was reported earlier by Muute *et al*. (2021).

These differences could be attributed to factors such as susceptibility of the genotypes, strains of the virus, and environmental factors such as solar radiation, humidity and temperature (Muengula *et al*., 2012). There was high disease pressure in the Embu site as compared to the Kirinyaga site. This was attributed to the difference in temperature and altitude as the Embu site was located at a slightly higher altitude and was also slightly warmer than the Kirinyaga site. Therefore, not all high-altitude zones receive lower temperatures than the low altitude zones. According to Muute *et al*. (2021), low temperature favours the feeding activities of the aphids thus resulting in increased incidence of BCMNV. Environmental temperature variation during the growth period also leads to symptoms deviation due to viral infection (Kapil *et al*., 2011). Therefore, successful infection under field conditions depended on the host factors (resistance or susceptibility levels), environmental factors, presence of disease inoculum, and the

feeding activities of the vector. This implies that a susceptible cultivar may escape infection in the field hence the need for artificial inoculation under controlled conditions.

This study identified pathotypes III and VI this is in line with previous studies carried out in Kenya (Mutuku *et al.,* 2018; Mangeni *et al.,* 2020). The BCMNV isolates are grouped into different pathogenicity groups based on the reaction to the standard differential which are associated with a known set of resistance genes (Djifhout, 1978). In addition, a previous survey carried out by Kabeja, (2020) in Rwanda, identified these two pathotypes to be widespread with NL-3 being the dominant strain (pathotype VI). BCMNV pathotype VI isolates have been found in Africa while those of pathotype III have been found to be dominant in Rwanda, Kenya, South Uganda, Tanzania and Burundi (Coyne *et al*., 2003). Characterization of BCMNV isolates has previously been used to help understand the available strains in a specific region. Screening of the genotypes using specific BCMNV pathotypes was of almost importance in this study in order to confirm the field evaluation data and determine host resistance genes. BCMNV isolate characterization has previously been used to help understand the available strains in a specific region. The BCMNV isolates were obtained from farmer's fields expressing BCMNV symptoms this clearly shows that majority of the common beans produced lack the resistance genes against this potyvirus. Furthermore, it was apparent that the *bc-3* gene is useful in the management of BCMNV in Kenya. However, the frequent use of the *bc-3* gene poses a risk for resistance breakdown (Feng *et al*., 2014) and hence there is a need to use other alternative *bc* genes to protect the *I* gene.

The ELISA test confirmed that the symptoms expressed during the greenhouse screening were due to BCMNV infection. ELISA test has been previously utilized in different studies to identify common bean viruses either from leaf or seed samples (Mwaipopo *et al*., 2017; Deligoz *et al*., 2021). The test is a cost-effective method that simply uses a polystyrene plate that binds with antibody linked to the enzyme substrate reaction (Boonham *et al*., 2014). BCMV and BCMNV has been categorized into two distinct viruses using ELISA. The DAS-ELISA is a useful serological tool for the identification of BCMNV as observed in previous studies (Peyambari *et al.,* 2006; Mangeni *et al.,* 2020; Kilic *et al.,* 2020). It has also been used to detect symptomless plants that have a high

virus titer during mechanical inoculation. Most common bean plant viruses exhibit similar symptoms therefore, it is impossible to distinguish them based on symptoms alone. These results are similar to previous finding by Mangeni *et al*. (2020) that utilized DAS-ELISA in order to confirm the BCMNV presence in diseased plants samples collected from the fields. Kabeja, (2020) also used DAS- ELISA during a survey carried out in Rwanda aimed at characterization of BCMNV. In this study, greenhouse results identified all the French beans evaluated to be susceptible to bean common mosaic necrosis virus while the MCM 1015, MCM 2001 and MCM 5001 were resistant. This clearly shows that the French bean cultivars screened in this study are resistant to BCMV however, this leaves them vulnerable to BCMNV. The results further corroborates with previous studies carried out by Deligoz *et al*. (2021) who screened a panel of French beans that were reported to be resistant to BCMV but susceptible to BCMNV. This implies that French bean breeders should explore more sources of resistance among the French bean germplasm or rely on dry bean germplasm which could slow the breeding process because of linkage drag associated to poor pod quality. The resistance levels that were expressed by MCM 1015, MCM 2001 and MCM 5001 are attributed to the fact that the varieties possess the *I/bc-3* gene combination (CIAT, 2001). These genotypes can be utilized as donor parents for both *I* and *bc-3* gene.

The phenotypic variations observed in the field necessitated the screening of the current French bean germplasm in Kenya under controlled conditions as well as using molecular markers to confirm the results. This was particularly important in order to remove the background noise and inconsistencies that were observed in the field. The BCMNV is controlled by race-specific recessive *bc* genes. The three recessive genes *bc-1*^{2}, *bc-2*^{2,} and *bc-3* have been proven to act constitutively by limiting the virus movement in the plant (Kelly *et al.,* 2003). Studies have also revealed that it's only in the presence of the recessive *bc-3* gene that the dominant *I* gene confers resistance to all known strains of both BCMV and BCMNV (Larsen *et al.,* 2008). Therefore, combining the dominant *I* and *bc-3* gene is the only effective way to curb this virus.

The SCAR marker ROC11 is linked to *bc-3* gene in repulsion phase and therefore, the absence of the band is an indication of the presence of the gene and vice versa (Johnson

*et al.,*1997). In this study, the absence of the marker was reported in the three dry bean varieties whereas all 27 French bean varieties amplified the marker. However, the ROC11 marker have false positives and therefore its usage should be proceeded after validation (Chilagane *et al*., 2013). The marker amplified a 300 bp band which differed from the expected 420 bp. Similar observations were reported by Pasev *et al.* (2014) who identified a similar band size in their breeding lines and concluded that the difference in band size could be due to deletions in the sequences of the gene. The immune reaction observed in the resistant checks MCM 1015, MCM 2001 and MCM 5001 is attributed to the fact that they possess both the *I* and the *bc-3* gene.

The resistance of potyviruses are highly influenced by the translation initiation factors (TlFs) (Robaglia & Caranta, 2006). These translation initiation factors act by restricting the replication of potyviruses. Based on the fact that *bc-3* gene locus in beans has been found to be associated with a mutation in a sequence encoding elF4E protein, a stable CAPS marker was developed (Naderpour *et al*., 2015). The CAPS marker elF4E identified the *bc-3* gene present in the resistant checks (MCM 1015, MCM 2001 and MCM 5001). This study did not identify any French bean that possesses the marker for *bc-3* gene hence pointing towards the contribution of the dry beans as the sources of the *bc-3* gene. These findings also confirmed the field data as the genotypes that were selected to possess the *bc-3* gene were immune in the field. Similar results were reported by Deligoz *et al*. (2022).

The marker SW13 was developed from a RAPD marker $OW13_{690}$ and closely linked (1.3± 0.8 cM) in a coupling phase to the dominant *I* gene (Haley *et al.,*1994; Melloto *et al.,* 1998; Fourie *et al.,* 2004). The dominant *I* gene was first mapped at the terminal position of linkage group 02 (LG02) by Perez *et al.* (2010). The marker facilitates the selection of genotypes that possess the *I* gene and breeders utilize marker-assisted selection (MAS) in absence of the pathogen (Miklas *et al.,* 2006; Pastor- Corrales *et al.,* 2007). In the current study, the SW13 marker results corresponded to the phenotypic reactions of the genotypes. The presence of the hypersensitive symptom in the field was the positive confirmation of the genotypes possessing the dominant *I* gene. This emphasizes the usefulness of this marker for rapid identification of the dominant *I* gene for resistance breeding to BCMV. This has made it possible to introgress the *I* gene into other cultivars

to confer resistance to all known strains of BCMV. In addition, breeders are combining the *I* gene with other recessive genes into a single a cultivar in order to confer resistance to BCMNV a more destructive virus. The usefulness of the SW13 marker has been explored in other bean breeding programs in selecting for halo blight resistance (HBB) as well as selecting against colour intensifying gene (B locus) (Morales & Castano, 1992).

The recessive genes *bc-1*^{2}, *bc-3*, and *bc*-2^2 act by restricting the virus movement within the plant (Kelly *et al.,* 2003). Marker-assisted selection for *bc-1*^{2} in genotypes possessing the dominant *I* gene is upfront since it does not require *bc-u* for its expression unlike the genotypes with the recessive *I* gene (Singh & Singh, 2015). Furthermore, the MAS of *bc-1*^{2} is of paramount importance due to the epistatic interaction between *bc-2* and *bc-3*. A SCAR marker SBD5 tightly linked to *bc-1*^{2} was suggested by Miklas *et al*. (2000) and has been found to be useful in common beans of Mesoamerican origin. However, the marker SBD5 has been shown to be unreliable and less reproducible in cranberry and kidney beans (Milkas *et al.,* 2008). In this study, the SBD5 marker tightly linked to *bc-1,*^{2} was detected in nine French bean cultivars which were susceptible to BCMNV pathotype III, whereas the Great Northern 123 differential cultivar known to possess the *bc-1*^{2} gene, was resistant to the virus strain. Based on these results, the usefulness of the SBD5 marker for the selection of *bc-1*^{2} gene in this French bean panel cannot be ascertained. This finding corroborates the previous report by Pasev *et al.* (2014) and Deligoz *et al*. (2022) that the SBD5 marker was not reliable and should be supported with phenotypic data. Further investigations are warranted to validate the applicability of the SBD marker in MAS involving the French bean genotypes. Moreover, it is imperative to develop molecular markers for the other recessive *bc* genes.

This study was successful in introgressing the recessive *bc-3* gene into elite local commercial French bean varieties using marker-assisted selection. Marker-assisted selection has been exploited in common bean breeding programs (Kelly *et al.,* 2003; Miklas *et al.,* 2006). It allows the elimination of genotypes with inferior traits in the early generation selection. When the marker is located near the gene of interest, breeders are able to track the gene through the segregating materials which is a major advantage (Chilagane *et al.,* 2013). The genetic markers are not identified as the target genes

themselves but act as chromosome landmarks to help in the introgression of economic important genes (Collard *et al*., 2005). Backcross breeding together with molecular markers has aided the incorporation of disease resistance while maintaining the recurrent genetic background (Chukwu *et al.*, 2020). Marker-assisted selection has been exploited for the target locus selection and also recovery of the recurrent parent background. The *bc-3* gene which is inherited as a single gene been identified through morphological and molecular screening (Mukeshimana *et al*., 2005; Chilagane *et al*., 2013). Recessive genes have been discovered to control the resistance of the most known plant viruses (Coyne *et al*., 2003). They play a major role in the resistance to the diseases caused by the different pathogenic viruses (Truniger & Aranda, 2009). Introgression of resistance genes using conventional methods is time-consuming as it involves progeny tests in order to identify cultivars possessing the genes. The study therefore confirmed that marker-assisted backcross breeding is an efficient method of introgressing genes conferring disease resistance as earlier demonstrated by Kelly *et al*. (2003).

Although field randomization takes care of the disease spread in the field during the phenotypic selection, the use of molecular markers is of paramount importance because it gives a clear confirmation of the presence of the resistance genes. Chilagane *et al*. (2013) recommended the use of marker-assisted backcross breeding as it allows the breeders to track a gene of interest through a segregating population. In addition, marker assisted selection (MAS) has been combined with convectional breeding in order to intensify the process of selection (Njuguna, 2014). This study ultimately resulted in the selection of 19 French bean lines combining field resistance against BCMNV. The release of these selections to farmers as improved French bean varieties is expected to expand the production of common bean even to the hotspots of this lethal virus.

This study utilized the ROC11 marker which has been available as a tag for *bc-3* for over two decades (Johnson *et al*., 1997). The major disadvantage of this *trans-dominant* marker is that it is linked to *bc-3* gene in repulsion meaning that the presence of an amplicon is associated with the allele responsible for susceptibility rather than for resistance. The back-crossing program used in this study was designed to include a progeny test between each backcross generation as recommended by Chilagane *et al*. (2013). This was

important because the profiling of the ROC11 marker cannot distinguish between a *Bc-3bc-3* heterozygote and a *bc-3bc-3* homozygote. In addition, ROC 11 selections at BC_3F_2 generation were confirmed by the elF4E CAPs. The marker is a dominant marker that is converted to a co-dominant state upon digestion with the *Rsal* restriction enzyme. It has the additional advantage of lying within a gene encoding a protein known to play an important role in the translation of viral RNA (Naderpour *et al*., 2010). The elF4E marker has been exploited elsewhere to identify materials carrying *bc-3* (Pasev *et al*., 2014; Ruhimbana *&* Mutlu, 2019). The combined use of the two markers negated the shortcomings of the ROC 11 marker to enable the successful selection of the desired lines. Further, the use of the elF4E CAPs marker to tag the *bc-3* gene offered an opportunity to confirm the original results of Naderpour *et al.* (2010). Consequently, study recommends the use of elF4E CAPs marker in the selection of *bc-3* gene.

The comparison between the genotypic and phenotypic evaluation of BCMNV resistant genes concluded that none was superior to the other. This agrees with previous studies carried out by Mukeshimana *et al*. (2005) and Namayanja *et al*. (2006) which found that the molecular and phenotypic data analyses did not have any significant difference. This indicated that phenotypic and genotypic selection compliments each other. Therefore, there is a need to incorporate molecular markers to accelerate selection, especially in traits with low heritability and in a case where one gene mask the other (Chilagane *et al*., 2013).

Development of resistant French bean lines with desired export quality, more productive and have a longer harvesting period could increase productivity of French bean farming. The development of French bean varieties that are disease resistant, high-quality for export, more productive, and have a longer harvesting period could potentially increase the efficiency of French bean farming (Mondo *et al*., 2022). This could also benefit farmers who want to access the European Union market, which has stringent safety and quality standards that are becoming increasingly difficult to meet. The main challenge affecting French beans breeding is the introgression of novel traits without disturbing pod quality (Singh *et al*., 2015) since the marketability of the crop is dependent on its production of tender, seedless pods. While market requirements for French beans can vary, there are certain pod aspects that are commonly considered. The dry beans market

majorly focuses on the seed characteristics while French beans classes are based on pod characteristics (Kimutai, 2018). This becomes complicated where the donor parent is the common bean whose economic value is based on the quantity of the dry beans (Robaglia & Caranta, 2006). This notwithstanding, the 19 French bean hybrid selections obtained in this study combined the field resistance against BCMNV with some desirable agro-morphological traits including the desirable pod quality and yields. This was enabled by the inclusion of a progeny test between each backcross generation as recommended by Chilagane *et al*. (2013). Consequently, the 19 hybrid lines that were ultimately selected were either comparable to or better than their recipient parents with respect to days to 50% flowering, pod diameter, pod length, number of pods per plant and pod weight. This achievement confirmed the earlier report by Chukwu *et al*. (2020) that the use of marker-assisted selection in backcross breeding enables the incorporation of disease-resistance genes while maintaining the recurrent genetic background. However, some traits particularly the plant height (growth habit), pod suture string and fiber tended to significantly increase in the hybrids as compared to the recipient parents. Plant height may not be a major market concern as the pod quality. Pods exhibiting a short suture string and little or no fiber are preferred in the French bean market. This reduction in pod quality is attributed to the linkage drag associated to the use of dry bean as *bc- 3* donor and can be improved by further rounds of marker-assisted backcrossing. Fortunately, the pod suture string is largely controlled by a single dominant gene which can be an easier target by the breeders (Hagerty *et al*., 2006).

Early maturing French bean varieties are preferred by producers since they allow the crop to be harvested before the price responds to a glut in the market. For this trait, the selections did not differ significantly from the parental genotypes. All the test genotypes attained the 50% flowering after approximately 40 days. This was in line with the observation by Ndegwa *et al*. (2011) that the majority of French bean varieties reach flowering at 39-43 days after sowing.

In terms of pod length, all the 19 lines were within the recommended length of 10-14 cm for the fine (at least 10 cm) and extra fine (12-14 cm) pods (Wahome *et al*., 2013; HCDA, 2020). This agrees with Ndegwa *et al.* (2011), who reported pod lengths of 11 to 18 cm

among climbing French bean lines. Wahome *et al.* (2013) recorded the pod length of KSB French bean lines ranging from 9.7 to 11.7 cm while climbing French bean lines ranging from 10.5 to 11.4 cm. A close association between this parameter and number of pods per plant and pod weight per plant was established. Dhillon *et al*. (2017) found similar results where there was a significant positive correlation between pod length, number of pods per plant and pod weight per plant. French beans produced for processing recommended length is 10 cm to 16 cm longer than this are incompatible with the processing machines (HCD, 2016). They also have an oval or round shape for fresh market purposes due to their durability and attractive appearance (Kimutai, 2018).

For the pod diameter, none of the test genotypes (both parents and hybrids) produced extra-fine pods (less than 6mm); they all fell under the class of fine pods whose diameter is between 6 – 9 mm (Arunga *et al*., 2015). However, since all of them had a pod diameter of less than 8 mm, it was an indication that they have a potential of producing extra-fine pods with additional backcrosses. Pod diameter was found to have a negative significant correlation to pod length. This is in contrast to Prakash *et al.* (2015) who recorded a positive correlation between pod diameter and pod length. The larger pod size can be attributed to the use of dry bean cultivars as the source of *bc-3* gene. Previous studies by Arunga *et al*. (2010) reported on the use of dry beans to improve French bean varieties. However, the use of dry beans as a source of resistance has been previously linked to linkage drag of poor pod quality traits and should be used when there are no suitable germplasm among French beans. Therefore, more backcrosses should be carried out in order to improve the pod aspects of these breeding lines.

In terms of yield, all the selected lines produced a higher number of pods than their parents, indicating their comparatively higher productivity potential since the number of pods per plant is a major component of yield (Checa & Blair, 2012; Cabral *et al*., 2018). The number of pods per plant has been previously used to indirectly select for pod yield in French beans. In this study variation in the number of pods obtained agrees with Njau (2016) who observed pod variations between varieties. This was attributed to the environment, production systems, varieties and crop management. A highly significant positive correlation was found between number of plants per and pod weight. These

results were similar to those found by Araujo *et al*. (2012) where number of pods per plant was found to be highly correlated to pod weight per plant. Additionally, Checa & Blair (2012) found that the number of pods per plant in French beans was closely related to the yield. Similar findings have been observed in common bean where number of pods per plant had the highest correlation to grain yield (Cabral *et al*., 2011). This highlights the potential of using the number of pods per plant for selection and identification of high yielding cultivars in French beans.

5.2 Conclusion

This study revealed that the majority of the French beans grown in Kenya are susceptible to BCMNV hence restricting their production in areas where the virus is prevalent. This necessitates the need for pyramiding resistance genes to aid in the management of the disease. It was apparent that the *bc-3* gene is useful in management of BCMNV in Kenya. However, genotypic screening revealed that the French bean genotypes possessed the *I* gene; although none carried the *bc-3* gene implying susceptibility to BCMNV.

Genotypic screening utilized the SW13 and elF4E markers, which were reliable in the identification of the *bc-3* and *I* genes, that confer resistance to BCMNV and BCMV, respectively. This emphasizes their efficiency and reliability in MAS involving the current germplasm. However, this study could not ascertain the usefulness of the SBD5 marker for the selection of $bc\text{-}1^2$ gene in French beans. Classical common bean breeding can be integrated with marker-assisted backcrossing in the introgression of disease-resistant genes. Marker-assisted backcrossing for *I* and *bc-3* gene can effectively utilize SW13 and elF4E molecular markers.

The MCM 1015, MCM 2001 and MCM 5001 dry bean genotypes were used as donor parents in the introgression of both *I* and *bc-3* genes, that successfully resulted in 19 lines that can be further improved for release or for use as sources of resistance for future breeding programs. Therefore, these dry bean genotypes can be utilized in pyramiding of the *I/bc-3* gene. Although resistance to BCMNV has been confirmed in the dry bean gene pool, sources within the French bean gene pool would simplify the development of BCMNV-resistant varieties.

5.3 Recommendations

5.3.1 Recommendations derived from this study

1. Adoption of resistant common bean genotypes by the farmers would contribute in the reduction and spread of BCMNV to the French beans.

2. Utilization of molecular markers SW13 and elF4E in the introgression and selection of the *I* and the *bc-3* gene by plant breeder.

3. Marker-assisted pyramiding of BCMV and BCMNV resistance genes into elite French bean cultivars.

5.3.2 Recommendations for further research

1. Collection and characterization of BCMNV isolates from diverse geographical areas to identify the variability and prevalence of BCMNV strains in Kenya.
2. Development of high throughput and cost-effective molecular markers tagging *bc-1*^{2} and *bc-3* genes.
3. Utilization of other recessive genes to confer resistance to BCMNV to avoid the risk of *bc-3* gene breakdown due to selection pressure.
4. Further improvement and testing of the 19 breeding lines with resistance to both BCMV and BCMNV for release as a locally adapted French bean varieties.
5. Sequencing of BCMNV isolates in Kenya to identify specific strains.

REFERENCES

Adams, I. P., Miano, D. W., Kinyua, Z. M., Wangai, A., Kimani, E., Phiri, N., & Souza-Richards, R. (2013). Use of next-generation sequencing for the identification and characterization of Maize chlorotic mottle virus and Sugarcane mosaic virus causing maize lethal necrosis in Kenya. *Plant Pathology*, *62*(4), pp.741-749.

Agbeci, M., Grangeon, R., Nelson, R. S., Zheng, H., & Laliberté, J. F. (2013). Contribution of host intracellular transport machineries to intercellular movement of turnip mosaic virus. *PLoS pathogens*, *9*(10), pp.100-683.

Araujo, L. C. D., Gravina, G. D. A., Marinho, C. D., Almeida, S. N. C. D., Daher, R. F., & Amaral Júnior, A. T. D. (2012). Contribution of components of production on snap bean yield. *Crop Breeding and Applied Biotechnology*, *12*, pp. 206-210.

Arunga, E. E., Van Rheenen, H. A., & Owuoche, J. O. (2010). Diallel analysis of Snap bean (*Phaseolus vulgaris* L.) varieties for important traits. *African Journal of Agricultural Research, 5*(15), pp.1951-1957.

Arunga, E. E., Ochuodho, J. O., Kinyua, M. G., & Owuoche, J. O. (2012). Characterization of *Uromyces appendiculatus* isolates collected from snap bean growing areas in Kenya. *African Journal of Agricultural Research, 7*(42), pp. 5685-5691.

Arunga, E. E., Kinyua, M., Ochuodho, J., Owuoche, J., & Chepkoech, E. (2015). Genetic diversity of determinate French beans grown in Kenya based on morpho-agronomic and simple sequence repeat variation. *Journal of Plant Breeding and Crop Science*, *7*(8), pp. 240-250.

Beaver, J. S., Rosas, J. C., Myers, J., Acosta, J., Kelly, J. D., Nchimbi-Msolla, S., Misangu, R., Bokosi, J., Temple, S., Arnaud-Santana, E. & Coyne, D.P. (2003). Contributions of the Bean/Cowpea CRSP to cultivar and germplasm development in common bean. *Field crops research*, *82*(2-3), pp.87-102.

Beshir, H. M., Bueckert, R., & Tar'an, B. (2016). Effect of temporary drought at different growth stages on snap bean pod quality and yield. *African Crop Science Journal*, *24*(3), pp.317-330.

Bliss, F. A. (1980). Common bean. In: W. R. Fehr, H. H. Hadley, (eds) *Hybridization of crop plants*, (pp. 273-284). John Wiley and Sons, Ltd.

Bragard, C., Caciagli, P., Lemaire, O., Lopez-Moya, J. J., MacFarlane, S., Peters, D., & Torrance, L. (2013). Status and prospects of plant virus control through interference with vector transmission. *Annual review of phytopathology*, *51*, pp.177-201.

Bock, K. R., Guthrie, E. J., Meredith. G. E. &. Njuguna, J. M. (1976). Virus of food legumes. *In East Africa Agriculture and Forest Research Organization*. Annual Report, Nairobi, Kenya.

Boonham, N., Kreuze, J., Winter, S., van der Vlugt, R., Bergervoet, J., Tomlinson, J., & Mumford, R. (2014). Methods in virus diagnostics: from ELISA to next generation sequencing. *Virus Research, 186,* pp. 20-31.

Buruchara, R. A., & Gathuru, E. M. (1979). Preliminary information on a severe strain of common bean mosaic virus isolated from beans (*Phaseolus vulgaris L.*) in Kenya. In *Contribution to a Symposium on Grain Legume Improvement in Eastern Africa.* Kabete, Nairobi.

Buruchara, R., Chirwa, R., Sperling, L., Mukankusi, C., Rubyogo, J.C., Mutonhi, R., & Abang, M.M. (2011). Development and delivery of bean varieties in Africa: The Pan-Africa Bean Research Alliance (PABRA) model. *African Crop Science Journal*, *19*(4), pp.227-245.

Cabral, P. D. S., de Souza, L. C., da Costa, G. F., Silva, F. H. L., & Soares, T. C. B. (2018). Investigation of the genetic diversity of common bean (*Phaseolus vulgaris* L) cultivars using molecular markers. *Genetics and Molecular Research*, *17*(4), pp. 1-11.

CIAT (2001). Bean Improvement. Developing Lines with Multiple Disease Resistance. Available on http://www.ciat.Cgiar.org/beans/disease-resist.htm. Accessed on 29th May 2022.

Checa, O. E., & Blair, M. W. (2012). Inheritance of yield-related traits in climbing beans (*Phaseolus vulgaris* L.). *Crop Science*, *52*(5), pp.1998-2013.

Chilagane, L. A., Tryphone, G. M., Protas, D., Kweka, E., Kusolwa, P. M., & Nchimbi-Msolla, S. (2013). Incorporation of resistance to angular leaf spot and Bean Common Mosaic Necrosis Virus diseases into adapted common bean (*Phaseolus vulgaris L.*) genotype in Tanzania. *African Journal of Biotechnology*, *12*(27).

Chiumia, L., & Msuku, W. A. (2001). Status of common bean mosaic virus in common beans in Malawi. In *Bean Seed Workshop, Arusha, January 12*(14).

Chukwu, S. C., Rafii, M. Y., Ramlee, S. I., Ismail, S. I., Oladosu, Y., Muhammad, I. I., ... & Yusuf, B. R. (2020). Recovery of recurrent parent genome in a marker-assisted backcrossing against rice blast and blight infections using functional markers and SSRs. *Plants*, *9(11)*, pp.1411.

Collard, B. C., Jahufer, M. Z., Brouwer, J. B., & Pang, E. C. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*(1-2), pp.169-196.

Coyne, D. P., Steadman, J. R., Godoy-Lutz, G., Gilbertson, R., Arnaud-Santana, E., Beaver, J. S., & Myers, J. R. (2003). Contributions of the Bean/Cowpea CRSP to management of bean diseases. *Field crops research*, *82*(2-3), pp.155-168.

Deligoz, I., Arlı-Sökmen, M., & Tekeoglu, M. (2021). Phenotypic and molecular screening of dry bean (*Phaseolus vulgaris* L.) breeding lines for resistance to bean common mosaic virus and bean common mosaic necrosis virus. *Acta Scientiarum Polonorum Hortorum Cultus*, *20*(6), pp.17-18.

Deligöz, I., Sokmen, M., Yilmaz, N. K., Ozçelik, H., & Tekeoğlu, M. (2022). Screening of snap and dry bean (*Phaseolus vulgaris* L.) genotypes for resistance to Bean common mosaic virus and Bean common mosaic necrosis virus. *Plant Protection Bulletin*, *62*(4), pp.5-13.

Dhillon, T. S., Dhall, R. K., & Gill, B. S. (2017). Correlation and path analysis studies in French bean (*Phaseolus vulgaris* L.) for yield and yield attributing traits. *Vegetable Science*, *44*(1), pp. 131-133.

Drijfhout, E. (1978). *Genetic interaction between Phaseolus vulgaris and bean common mosaic virus with implications for strain identification and breeding for resistance*. PhD, Wageningen University and Research.

FAOSTAT. (2020). Food and agriculture data. Available on http://www.fao.org/home. Accessed on 4th June 2022.

Farm Link Kenya (2018). French Beans farming Kenya. Available on http://www.farmlinkkenya.com/. Accessed on 20th April, 2019.

Feng, X., Myers, J. R., & Karasev, A. V. (2015). Bean Common Mosaic Virus isolate exhibits a novel pathogenicity profile in common bean, overcoming the *bc-3* resistance allele coding for the mutated eIF4E translation initiation factor. *Phytopathology*, *105*(11), pp.1487-1495.

Feng, X., Poplawsky, A. R., Nikolaeva, O. V., Myers, J. R., & Karasev, A. V. (2014). Recombinants of Bean common mosaic virus (BCMV) and genetic determinants of BCMV involved in overcoming resistance in common bean. *Phytopathology*, *104*(7), pp.786-793.

Ferreira, J. J., Campa, A., Kelly, J. D., Varshney, R. K., & Tuberosa, R. (2013). Organization of genes conferring resistance to anthracnose in common bean. *Translational Genomics for Crop Breeding*, *1*, pp.151-181.

Flores-Estévez, N., Acosta-Gallegos, J. A. & Silva-Rosales, L. (2003). Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus in Mexico. *Plant disease*, *87*(1), pp.21-25.

Fulano, A. M., Lengai, G. M., & Muthomi, J. W. (2021). Phytosanitary and Technical Quality Challenges in Export Fresh Vegetables and Strategies to Compliance with Market Requirements: Case of Smallholder Snap Beans in Kenya. *Sustainability*, *13*(3), pp.1546.

Fourie, D., Miklas, P., & Ariyaranthe, H. (2004). Genes conditioning halo blight resistance to races 1, 7, and 9 occur in a tight cluster. *Annual Report-Bean Improvement Cooperative*, *47*, pp. 103-104.

Freyre, R., Skroch, P. W., Geffroy, V., Adam-Blondon, A. F., Shirmohamadali, A., Johnson, W. C., ... & Gepts, P. (1998). Towards an integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. *Theoretical and Applied Genetics*, *97*, pp. 847-856.

Gepts, P. (1998). Origin and evolution of common bean: past events and recent trends. *Horticultural Science*, *33*(7), pp.1124-1130.

Grangeon, R., Agbeci, M., Chen, J., Grondin, G., Zheng, H., & Laliberté, J. F. (2012). Impact on the endoplasmic reticulum and Golgi apparatus of turnip mosaic virus infection. *Journal of Virology*, *86*(17), pp.9255-9265.

Grubben, G. J., & Denton, O. A. (2004). Plant resources of tropical Africa 2. Vegetables. *Plant resources of tropical Africa 2. Vegetables.*

Hagerty, C. H., Cuesta-Marcos, A., Cregan, P., Song, Q., McClean, P., & Myers, J. R. (2016). Mapping snap bean pod and color traits, in a dry bean× snap bean recombinant inbred population. *Journal of the American Society for Horticultural Science*, *141*(2), pp.131-138.

Haley, S. D., Afanador, L., & Kelly, J. D. (1994). Identification and application of a random amplified polymorphic DNA marker for the *I* gene (potyvirus resistance) in common bean. *Phytopathology*, *84*(2), pp.157-160.

Horticultural Crops Directorate Authority (HCDA) validated report (2016). Available on https://www.agricultureauthority.go.ke/wp-content/uploads/2016/09/Horticulture-2015_2016-Validated-Report3.pdf. Accessed on 30th May 2019.

Horticultural Crops Development (HCD). 2020 Horticulture Validated. Available on http://horticulture.agricultureauthority.go.ke/index.php/statistics/reports. Accessed on 21st March 2022

Hull, R. (Ed.). (2013). *Plant virology*. (5th ed.). Academic press: *Elsevier*

Ivanov, K. I., Eskelin, K., Lohmus, A., & Mäkinen, K. (2014). Molecular and cellular mechanisms underlying potyvirus infection. *Journal of General Virology*, *95*(7), pp.1415-1429.

Jaetzold, R., Schmidt, H., Hornetz, B., & Shisanya, C.A. (2006). Farm Management Handbook of Kenya. Natural Conditions and Farm Information, 2nd edition, vol. 11/C. Ministry of Agriculture/GTZ, Nairobi, Kenya.

Jiang, G. L. (2013). Molecular markers and marker-assisted breeding in plants. *Plant Breeding from Laboratories to Fields*, 3, pp.45-83.

Johnson, W. C., Guzman P., Mandala D., Mkandawire A. B., Temple S., Gilbertson R. L., & Gepts P. (1997). Molecular tagging of the *bc-3* gene for introgression into Andean common bean. *Crop Science*. (37), pp.248-254.

Jordan, R., & Hammond, J. (2008). Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus (Genus Potyvirus; Potyviridae). *Encyclopedia of Virology*, (*1)*, pp.288-295.

Juma, R. U. (2012). *Evaluation of bean varieties (Phaseolus vulgaris L.) for resistance to bean common mosaic virus (BCMV) and bean common necrosis virus across a soil fertility gradient in Western Kenya*. MSc Thesis, University of Eldoret.

Kabeja, A. (2020). *Gene ecology of the climbing common bean (Phaseolus vulgaris)-Bean Common Mosaic Virus/Bean Common Mosaic Necrosis Virus (BCMV/BCMNV) relationship in Rwanda: a key for the development of virus-resistant bean*s. PhD Thesis, University of California, Davis.

Kamiri, A. K. (2021). *French bean resistance to bean anthracnose (colletotrichum lindemuthianum) in Kenya*. MSc Thesis, University of Embu, Kenya.

Kamiri, A. K., Arunga, E. E., Rotich, F., & Otsyula, R. (2021). Response of French bean genotypes to *Colletotrichum lindemuthianum* and evaluation of their resistance using SCAR markers. *African Journal of Biotechnology*, *20*(2), pp. 51-65.

Kapil, R., Sharma, P., Sharma, S. K., Sharma, O. P., Sharma, O. P., Dhar, J. B., & Sharma, P. N. (2011). Pathogenic and molecular variability in Bean common mosaic virus infecting common bean in India. *Archives of Phytopathology and Plant Protection*, *44*(11), pp. 1081-1092.

Kelly, J. D., Gepts, P., Miklas, P. N. & Coyne, D. P. (2003). Tagging and mapping of genes and QTL and molecular marker-assisted selection for traits of economic importance in bean and cowpea. *Field Crops Research, 82*(2-3), pp.135-154.

Kelly, J. D., & Vallejo, V. A. (2004). A comprehensive review of the major genes conditioning resistance to anthracnose in common bean. *Horticultural Science*, *39*(6), pp. 1196-1207.

Kiliç, H. Ç., Hesna, K. Ö. K., & Yardimci, N. (2020). Bean common mosaic virus and bean common mosaic necrosis virus infections in bean production areas in The Lakes Region of Turkey. *Avrupa Bilim ve Teknoloji Dergisi*, (19), pp. 386-392.

Kimani, P. M., Narla, R. D., Ugen, M., Onyango, C., Kibet, S., & Musoni, A. (2016). Development and validation of new French bean varieties for Eastern Africa. *In Bean Improvement Cooperative (BIC)* (59), pp. 227-228.

Kimutai, J. J. (2018). *Inheritance and selection for yield components and multiple disease resistance in snap bean*. MSc Thesis, University of Nairobi. Kenya.

Kulkarni, H. Y. (1973). Notes on East African Plant Virus Diseases: 5. Identification and Economic Importance of Sugar-Cane Mosaic Virus in Maize in East Africa. *East African Agricultural and Forestry Journal*, *39*(2), pp.158-164.

Larsen, R. C., & Miklas, P. N. (2004). Generation and molecular mapping of a sequence characterized amplified region marker linked with the Bct gene for resistance to Beet curly top virus in common bean. *Phytopathology*, *94*(4), pp.320-325.

Larsen, R. C., Miklas, P. N., Eastwell, K. C. & Grau, C. R. (2008). A strain of Clover yellow vein virus that causes severe pod necrosis disease in snap bean. *Plant Disease, 92*(7), pp.1026-1032.

Larsen, R. C., Druffel, K. L., & Wyatt, S. D. (2011). The complete nucleotide sequences of bean common mosaic necrosis virus strains NL-5, NL-8 and TN-1. *Archives of Virology*, *156*(4), pp.729-732.

Mahuku, G. S. (2004). A simple extraction method suitable for PCR-based analysis of plant, fungal, and bacterial DNA. *Plant Molecular Biology Reporter,* 22(1), pp. 71-81.

Mangeni, B. C., Were, H. K., Ndong'a, M., & Mukoye, B. (2020). Incidence and severity of bean common mosaic disease and resistance of popular bean cultivars to the disease in western Kenya. *Journal of Phytopathology*, *168*(9), pp.501-515.

Mangeni, B. C., Abang, M. M., Awale, H., Omuse, C. N., Leitch, R., Arinaitwe, W., Mukoye, B., Kelly, J. D., & Were, H. K. (2014). Distribution and pathogenic characterization of bean common mosaic Virus (BCMV) and bean common mosaic necrosis virus (BCMNV) in western Kenya. *Journal of Agri-Food and Applied Sciences, 2*(10), pp.308-316.

Mavric, I., & Vozlic, S. J. (2004). Virus diseases and resistance to Bean common mosaic and Bean common mosaic necrosis potyvirus in common bean (*Phaseolus vulgaris* L.). *Acta Agriculturae Slovenica, 83*(1), pp.181-190.

Mbeke, A. M., Kirui, S. C., Kibet, N. C., Welinga, A. M., Musyoki, S. K. & Nguta, C. M. (2014). Effects of Nitrogen Application on Snap beans Production in Koibatek District Kenya. *International Development and Sustainability, 3*(*5*), pp.1013-1025.

Melgarejo, T. A., Lehtonen, M. T., Fribourg, C. E., Rännäli, M. & Valkonen, J. P. (2007). Strains of BCMV and BCMNV characterized from lima bean plants affected by deforming mosaic disease in Peru. *Archives of Virology, 152*(10), pp.1941-1949.

Melotto, M. & Kelly J. D. (1998). SCAR markers linked to major disease resistance genes in common bean. *Annual Report Bean Improvement Cooperative. (41*), pp. 64-65.

Messiaen, C. M., Seif, A. A., Jarso, M. & Keneni, G. (2004). *Pisum sativum* L. In: Grubben, G.J., Denton, O.A. (Eds.), *Plant Resources of Tropical Africa*. Vegetables. PROTA Foundation, Wageningen, Netherlands.

Miklas, P. N., Delorme, R., Stone, V., Daly, M. J., Stavely, J. R., Steadman, J. R., & Beaver, J. S. (2000). Bacterial, fungal, and viral disease resistance loci mapped in a recombinant inbred common bean population (Dorado'/XAN 176). *Journal of the American Society for Horticultural Science, 125*(4), pp.476-481.

Miklas, P. N., Smith, J. R., & Singh, S. P. (2006). Registration of common bacterial blight resistant white kidney bean germplasm line USWK-CBB-17. *Crop science*, *46*(5), pp.2338-2339.

Miklas, P. N., Fourie, D., Wagner, J., Larsen, R. C., & Mienie, C. (2009). Tagging and mapping Pse-1 gene for resistance to halo blight in common bean differential cultivar UI-3. *Crop science*, *49*(1), pp.41-48.

Morales, F.J. and Castaño, M. (1992). Increased disease severity induced by some comovirus in bean genotypes possessing monogenic dominant resistance to bean common mosaic potyvirus. *Plant Disiease, (76)*, pp.570-573.

Morales, F. J. (2006). History and current distribution of begomoviruses in Latin America. *Advances in Virus Research*, (*67)*, pp.127-162.

Mondo, M. J., Kimani, P. M., & Narla, R. D. (2019). Validation of effectiveness marker-assisted gamete selection for multiple disease resistance in common bean. *African Crop Science Journal, 27*(4), pp.585-612.

Mulenga, R. M. (2022). *Molecular Characterization of Viruses Infecting Common Bean (Phaseolus Vulgaris L.) and Reaction of Bean Genotypes to Virus Infection*. PhD Thesis, University of Nairobi, Kenya.

Muengula, M., Ngombo, A., Kalonji, A., Tshiendesha-Musokandu, P., Dekwize-Diakabi, S., Kayembe, R., ... & Kalonji-Mbuyi, A (2012). Occurrence of Cassava Mosaic Disease Related to Agro-ecosystem in Farmer's Fields located in Kongo Central Province, Democratic Republic of Congo. *Asian Journal of Biology*, 3(1), pp. 1-7.

Mukeshimana, G., & Kelly, J. D. (2003). Evaluation of Rwandan varieties for disease resistance. *Annual Report-Bean Improvement Cooperative*, (46*)*, pp. 145-146.

Mukeshimana, G., Paneda, A., Rodríguez-Suárez, C., Ferreira, J. J., Giraldez, R. & Kelly, J. D. (2005). Markers linked to the *bc-3* gene conditioning resistance to bean common mosaic potyviruses in common bean. *Euphytica*, *144*(3), pp.291-299.

Mukeshimana, G., Butare, L., Cregan, P. B., Blair, M. W., & Kelly, J. D. (2014). Quantitative trait loci associated with drought tolerance in common bean. *Crop Science*, *54*(3), pp. 923-938.

A Muute, N., Muli, B., & Charles, O. (2021). Evaluation of bean common mosaic disease and associated aphid vector, *aphis fabae* L., on common bean (*Phaseolus vulgaris* L.) production in lower eastern Kenya. *International Journal of Pathogen Research*, *8*(3), pp.1-18.

Mutuku, J. M., Wamonje, F. O., Mukeshimana, G., Njuguna, J., Wamalwa, M., Choi, S. K., ... & Harvey, J. J. (2018). Metagenomic analysis of plant virus occurrence in common bean (*Phaseolus vulgaris* L) in Central Kenya. *Frontiers in Microbiology*, pp 2939.

Mwaipopo, B., Nchimbi-Msolla, S., Njau, P., Tairo, F., William, M., Binagwa, P., Kweka, E., Kilango, M. & Mbanzibwa, D. (2017). Viruses infecting common bean (*Phaseolus vulgaris L.*) in Tanzania: A review on molecular characterization, detection and disease management options. *African Journal of Agricultural Research*, *12*(18), pp.1486-1500.

Myers, J. R., & Baggett, J. R. (1999). Improvement of snap beans: In: Singh S. P.(ed) Common bean improvement for the 21st century. *Spinger*, Dordrecht.

Myers, J.R., Mink, G.A., Mabagala, R., (2000). Surveys for bean common mosaic necrosis virus in East Africa. *Annual Report Bean Improvement Cooperative*. (43), pp.13–14.

Naderpour, M., Lund, O. S., Larsen, R., & Johansen, E. (2010). Potyviral resistance derived from cultivars of Phaseolus vulgaris carrying bc-3 is associated with the homozygotic presence of a mutated eIF4E allele. *Molecular plant Pathology*, *11*(2), pp.255-263.

National Farmers Information Service (NAFIS). (2017). French Beans Value Chain Development Murang'a County. Available online: http://www.nafis.go.ke/vegetables/ (accessed on 20th June 2021).

Namayanja, A., Buruchara, R., Mahuku, G., Rubaihayo, P., Kimani, P., Mayanja, S., & Eyedu, H. (2006). Inheritance of resistance to angular leaf spot in common bean and validation of the utility of resistance linked markers for marker assisted selection outside the mapping population. *Euphytica*, *151*(3), pp.361-369.

Nellist, C. F., Ohshima, K., Ponz, F., & Walsh, J. A. (2022). Turnip mosaic virus, a virus for all seasons. *Annals of Applied Biology*, 180(3), 312-327.

Njau, S. N. (2016). *Selection for yield potential, disease resistance and canning quality in runner and snap bean lines and populations*. *MSc* University of Nairobi, Kenya.

Njau, P. J. R., & 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 seedlots in Tanzania. *Seed science and technology*, *28*(1), pp. 85-92.

Ndegwa, A. M., Chege, B. K., Wepukhulu, S. B., Wachiuri, S. M. & Kimanira, J. N. (2011). Evaluation of advanced French bean (*Phaesolus vulgaris* L.) breeding lines for resistance to bean rust, yield potential and pod quality. *KARI-CIAT* Report, pp.9.

Ndunguru, J., & Kapinga, R. (2007). Viruses and virus-like diseases affecting sweet potato subsistence farming in southern Tanzania. *African Journal of Agricultural Research*, *2*(5), pp.232-239.

Njuguna, S. M. (2014). *Marker assisted gamete selection for multiple disease resistance in Mesoamerican bean genotypes and race typing of angular leaf spot pathogen in Kenya.* PhD Thesis, University of Nairobi, Kenya.

Ogala, B. O. (2013). *Management of thrips in French bean by use of integrated pesticide application regimes in Embu East and Mwea East Districts*. PhD Thesis, University of Nairobi, Kenya.

Okii, D., Mukankusi, C., Sebuliba, S., Tukamuhabwa, P., Tusiime, G., Talwana, H., ... & Gepts, P. (2018). Genetic variation, heritability estimates and GXE effects on yield traits of Mesoamerican common bean (*Phaseolus vulgaris* L) germplasm in Uganda. *Plant Genetic Resources*, *16*(3), pp.237-248.

Omunyin, M. E. (1995). *Molecular basis of resistance to soybean mosaic virus: reverse transcription (RT)-PCR and strain complementation analysis in soybeans*. PhD Thesis, Lewa state University.

Opole, R. A., Mathenge, P. W., Auma, E. O., Van Rheenen, H. A. & Almekinders, C. J. (2003). On-farm seed production practices of common bean (*Phaseolus vulgaris* L.). In *African Crop Science Conference Proceedings*. (6), pp.722-725.

Otim, M., Obia, P. O., Mugagga, I., & Ugen, M. (2011). Farmer's perception and management of pests and diseases on snap beans in Uganda. In: M. Katafire, M. Ugen and M. Mcharo (eds.), proceedings of the Regional Stakeholder's Workshop. ASARECA, Entebbe, Uganda, pp.83-95.

Pasev, G., Kostova, D., & Sofkova, S. (2014). Identification of genes for resistance to Bean common mosaic virus and Bean common mosaic necrosis virus in snap bean (*Phaseolus vulgaris* L.) breeding lines using conventional and molecular methods. *Journal of Phytopathology*, *162*(1), pp.19-25.

Pastor-Corrales, M. A., Kelly, J. D., Steadman, J. R., Lindgren, D. T., Stavely, J. R. & Coyne, D. P. (2007). Registration of six great northern bean germplasm lines with

enhanced resistance to rust and bean common mosaic and necrosis potyviruses. *Journal of Plant Registrations*, *1*(1), pp.77-79.

Pérez-Vega, E., Pañeda, A., Rodríguez-Suárez, C., Campa, A., Giraldez, R., & Ferreira, J. J. (2010). Mapping of QTLs for morpho-agronomic and seed quality traits in a RIL population of common bean (*Phaseolus vulgaris* L.). *Theoretical and Applied Genetics*, *120*(7), pp.1367-1380.

Peyambari, M., Habibi, M. K., Mosahebi, G. & Izadpanah, K. (2006). Determination of seed-born percentages of bean common mosaic necrosis virus (BCMNV) in three genotypes of *Phaseolus vulgaris. Communications in Agricultural and Applied Biological Sciences*, *71*(3), pp.1221-1227.

Pierce W. H., & Hungerford C. W. (1929). A note on longevity of bean Mosaic Virus. *Phytopathology*; (19), pp.605.

Powell, G. (2005). Intracellular salivation is the aphid activity associated with inoculation of non-persistently transmitted viruses. *Journal of General Virology*, *86*(2), pp. 469-472.

Prakash, J., Ram, R. B., & Meena, M. L. (2015). Genetic variation and characters interrelationship studies for quantitative and qualitative traits in french bean (*Phaseolus vulgaris L*.) under Lucknow conditions. *Legume Research*, *38*(4), pp. 425-433.

Robaglia, C., & Caranta, C. (2006). Translation initiation factors: a weak link in plant RNA virus infection. *Trends in Plant Science*, *11*(1), pp.40-45.

Ruhimbana, C., & Mutlu, N. (2019). Marker-assisted pyramiding potyvirus resistance genes into Rwandan common bean (*Phaseolus vulgaris* L.) genotypes. *Mediterranean Agricultural Sciences*, *32*(3), pp.381-385.

Robaglia, C., & Caranta, C. (2006). Translation initiation factors: a weak link in plant RNA virus infection. *Trends in Plant Science*, *11*(1), pp.40-45.

Santosa, M., Maghfoer, M. D., & Tarno, H. (2017). The Influence of Organic and Inorganic Fertilizers on the Growth and Yield of Green Bean, (*Phaseolus vulgaris* L.) Grown in Dry and Rainy Season. *Agrivita*, *39*(3), pp.296.

Sastry, K. S., & Sastry, K. S. (2013). Plant virus transmission through vegetative propagules (asexual reproduction). In: Seed-borne plant virus diseases. Springer, India.

Silbernagel, M. J., Mills, L. J., & Wang, W. Y. (1986). Tanzanian strain of bean common mosaic virus. *Plant disease*, *70*(9), pp.839-841.

Silbernagel, M. J., Mink, G. I., Zhao, R. L., & Zheng, G. Y. (2001). Phenotypic recombination between bean common mosaic and bean common mosaic necrosis potyviruses in vivo. *Archives of Virology*, *146*(5), pp.1007-1020.

Singh, B. K., & Singh, B. (2015). Breeding perspectives of snap bean (*Phaseolus vulgaris* L.). *Vegetable Science, 42(1)*, pp.1-17.

Singh, R. J. (2005). Landmark research in grain legumes. *Genetic Resources, Chromosome Engineering, and Crop Improvement Series: Grain legumes*, (1), pp.1-9.

Strausbaugh, C. A., Miklas, P. N., Singh, S. P., Myers, J. R., & Forster, R. L. (2003). Genetic characterization of differential reactions among host group 3 common bean cultivars to NL-3 K strain of Bean common mosaic necrosis virus. *Phytopathology*, *93*(6), pp.683-690.

Sofkova, S., Poryazov I., & Kiryakov I. (2010). Breeding green beans (*Phaseolus vulgaris* L.) for complex disease resistance. *Genetics and Breeding, 38(3)*, pp.77-88.

Soler-Garzón, A., McClean, P. E., & Miklas, P. N. (2021). Genome-wide association mapping of *bc-1* and *bc-u* reveals candidate genes and new adjustments to the host-pathogen interaction for resistance to Bean common mosaic necrosis virus in common bean. *Frontiers in Plant Science*, *12*, pp.699569.

Tang, M., & Feng, X. (2022). Bean Common Mosaic Disease: Etiology, Resistance Resource, and Future Prospects. *Agronomy*, *13*(1), pp.58.

Truniger, V., & Aranda, M. A. (2009). Recessive resistance to plant viruses. In *Advances in virus research (75)*, pp. 119-231.

Tryphone, G. M., Chilagane, L. A., Protas, D., Kusolwa, P. M., & Nchimbi-Msolla, S. (2013). Marker assisted selection for common bean diseases improvements in Tanzania: Prospects and future needs. In Sven, B. A. (Ed), *Plant breeding from laboratories to fields*. InTech.

USAID-KAVES. (2015). USAID-KAVES French bean value chain analysis. Retrieved from https://pdf.usaid.gov/pdf_docs/pa00m2s4.pdf.

Vetten, H. J., Lesemann, D. E., & Maiss, E. (1992). Serotype A and B strains of bean common mosaic virus are two distinct potyviruses. In *Potyvirus taxonomy* pp. 415-431.

Vidyakar, V., Lal, G. M., Singh, M. K., & Kumar, A. (2017). Study on genetic diversity in French bean (*Phaseolus vulgaris* L.). *Journal of Pharmacognosy and Phtochemistry*. pp.184-187.

Wahome, S. W., Kimani, P. M., Muthomi, J. W., Narla, R. D., & Buruchara, R. (2011). Multiple disease resistance in snap bean genotypes in Kenya. *African Crop Science Journal*, *19*(4), pp. 289-302.

Wahome, S. W., Kimani, P. M., Muthomi, J. W., & Narla, R. D. (2013). Quality and yield of snap bean lines locally developed in Kenya. *International Journal of Agronomy and Agricultural Research*, *3*(7), pp.1-10.

Wasonga, J., Marcial, C., Pastor-Corrales, A., Timothy, G., Porch, P., & Griffiths, D. (2010). Targeting gene combinations for broad spectrum rust resistance in heat-

tolerant French beans developed for tropical environments. *Journal of American Society of Horticulture Science* 135(6), pp.521-532.

Were, H. K., Winter, S., & Maiss, E. (2004). Viruses infecting cassava in Kenya. *Plant Disease, 88*(1), pp.17-22.

Westwood, J. H., Yoder, J. I., & Timko, M. P. (2010). The evolution of parasitism in plants. *Trends in Plant Science, 15*(4), pp.227-235.

Westwood, J. H., & Stevens, M. (2010). Resistance to aphid vectors of virus disease. In *Advances in Virus Research.* (76), pp. 179-210.

Worrall, E.A., Wamonje, F.O., Mukeshimana, G., Harvey, J. J., Carr, J. P., & Mitter, N. (2015). Bean Common Mosaic Virus and Bean common mosaic necrosis virus: relationships, biology, and prospects for control. In *Advances in virus research.* Academic Press.

Xu, L., & Hampton, R. O. (1996). Molecular detection of Bean common mosaic and Bean common mosaic necrosis potyviruses and pathogroups. *Archives of virology*, *141*(10), pp.1961-1977.

Yadav, B. V., Srinivasulu, B., Reddy, P. S., & Balakrishna, M. (2015). Influence of sowing dates on growth and yield of French bean (*Phaseolus vulgaris* L.) varieties under Rayalaseema Region of Andhra Pradesh. *J. of Agroecology and Natural Resource Manage*, *2*(2), pp.145-149.

APPENDICES

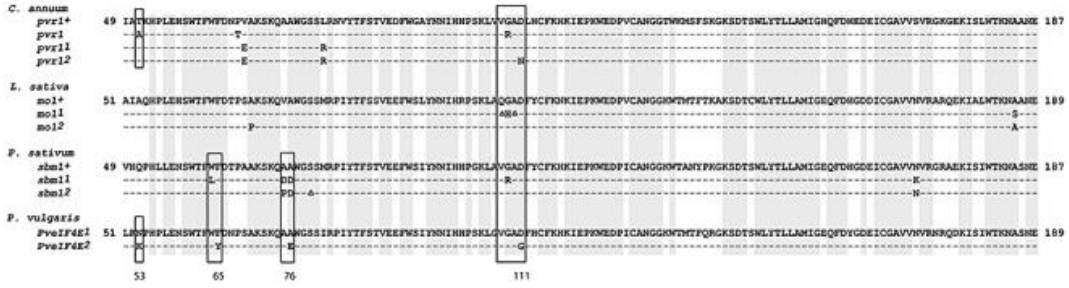
Appendix 1: DNA extraction protocol

1. Transfer DNA pulled from five young randomly selected leaves per cultivar (150 mg) to a sterilized 1.5-mL Eppendorf (micro-centrifuge) tube containing 300µL of TES extraction buffer (0.2 M Tris-HC1 pH 8], 10 mM EDTA [pH 8], 0.5M NaC1, 1% SDS) and acid-washed, sterilized sea sand or 0.5-mm glass beads.
2. Macerate the leaves for 2 min with a hand-held disposable homogenizer that fits the 1.5-mL microcentrifuge tube.
3. Vortex samples for 30 s and add an additional 200 µL of TES extraction buffer containing proteinase K (final concentration of 50 µg/mL).
4. Vortex to thoroughly mix and place tubes in a water bath at 65°C for 30 min.
5. Add one-half volume (250 µL) of 7.5 M Ammonium acetate.
6. Mix and incubate the samples on ice or at -5°C in the refrigerator for 10 min.
7. Centrifuge for 15 min at 15,000rpm.
8. Transfer the supernatant to a new tube and add an equal volume (500µL) of ice-cold isopropanol.
9. Incubate tubes at -20°C for 1-2 h.
10. Centrifuge for 10 min at 15,000 rpm to pellet the DNA. Decant the supernatant and wash DNA pellet with 800µL of cold 70% ethanol.
11. Turn tubes upside-down on clean sterile paper towels for 10-15 min to air-dry DNA.
12. Elute DNA from the pellet with twice-repeated extractions with 250µL of 1XTE buffer (10 mM Tris-HC1 [pH 8], 1 mM EDTA), each time centrifuging to avoid collecting pelleted polysaccharides.
13. Transfer DNA solution to a 1.5-mL microcentrifuge tube, add 5µl of RNaseA (20 mg/mL), and incubate at 37~ for 60 min.
14. Recover DNA and air-dry as described above.

Appendix 2: ANOVA for agro- morphological traits of BC_3F_2 French bean breeding lines

Traits	Source of variation	d.f.	s.s.	m.s.	F pr
50 % Days to Flowering	Genotype	14	17.900	1.279	0.895
Plant height	Genotype	14	39921.2	2851.5	<.001
Pod diameter	Genotype	14	21.27415	1.51958	<.001
Pod fiber	Genotype	14	12.9443	0.92460	<.001
Pod length	Genotype	14	96.2810	6.8772	<.001
Pod weight per plant	Genotype	14	13319.0	951.4	0.296
Pod per plant	Genotype	14	1589.9	113.6	0.425
Pod suture string	Genotype	14	247.8043	17.6489	<.001

Appendix 3:Amino acid polymorphism in eukaryotic translation factor 4E (elF4E)



Source: Naperpour *et al*., 2010