Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Host plant resistance to bean common mosaic necrosis virus among snap bean cultivars in Kenya

Grace Wambui Watare, Bernard Mukiri Gichimu, Edith Esther Arunga*

Water and Agricultural Resource Management, University of Embu, Embu, Kenya

Vol. 63, No. 4: 418–427, 2023

DOI: 10.24425/jppr.2023.146876

Received: April 15, 2023
Accepted: July 20, 2023
Online publication: October 10, 2023

*Corresponding address:
arunga.esther@embuni.ac.ke

Responsible Editor:
Julia Minicka

Abstract

Snap bean production in Kenya is constrained by many pests and diseases, including the bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV). The occurrence of the dominant *I* gene in many snap bean cultivars has provided a measure of control over BCMV but the BCMNV overcomes this resistance. The current study aimed to screen a collection of locally grown snap bean commercial cultivars, landraces, breeding lines, and dry bean cultivars for the expression of resistance against BCMNV under both field and greenhouse conditions. The results showed that the evaluated snap bean cultivars were susceptible to BCMNV. The reactions of the genotypes to BCMNV varied from top, vein and local necrosis, mosaics, mottling, deformed leaves to stunted growth. Positive infection was confirmed through enzyme linked immunosorbent assays. The dry bean cultivars, which were used as resistant checks can be explored as sources of resistance to BCMNV in future breeding programs. Molecular analysis showed that the SW13 and eIF4E markers were reliable in confirming the presence or absence of the dominant *I* gene and the recessive *bc-3* gene, respectively. These molecular markers are useful in marker-assisted breeding programs.

Keywords: bean common mosaic necrosis virus (BCMNV), bean common mosaic virus (BCMV), molecular markers, pathotypes, snap bean

Introduction

Snap beans (*Phaseolus vulgaris* L.), also known as green or French beans, are common beans that are consumed as immature pods (Hagerty *et al.* 2016). As the leading export vegetable in Kenya, it contributes substantially to the Kenyan economy (HCDA 2020). However, its production is constrained by many pests and diseases, including the bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) (Kamiri *et al.* 2021).

These two related seed-borne and aphid-transmitted potyviruses (classified as BCMV serotypes A and B, respectively) are in some cases responsible for total yield loss in areas where they are prevalent (Worrall *et al.* 2015). In severe cases, most farmers are compelled to employ chemical control of the aphid vector. Unfortunately, this strategy is less effective because the aphids spread the virus in a non-persistent manner and chemical control is economically unsustainable for resource-poor farmers, especially in East Africa (Mangeni *et al.* 2014). Therefore, the deployment of host resistance is the best alternative strategy to control these diseases, and efforts have been made to develop cultivars that are resistant to these viruses.

Resistance to BCMV/BCMNV is controlled by the *I* gene, and five strain-specific recessive resistance genes, namely *bc-1*, *bc-2*, *bc-3*, *bc-4*, and *bc-u*^{d} (Soler-Garzon *et al.* 2021). Previously, highly effective host resistance to BCMV was conferred by the dominant *I* gene and was widely deployed in common bean breeding programs. However, the necrosis-inducing virus

(BCMNV) was able to overcome this resistance in genotypes carrying an unprotected *I* gene because of a hypersensitive response against BCMNV (Tang and Feng 2023). In addition, at high temperatures, BCMV can induce temperature-dependent systemic necrosis symptoms in genotypes carrying the *I* gene which can lead to plant death and total loss of the bean crop (Silbernagel *et al*. 2001).

Durable resistance to BCMV/BCMNV can be achieved by combining the dominant *I* gene with recessive genes, as they possess different mechanisms of resistance. For instance, cultivars possessing the *I* and *bc-3* or *bc-u* and *bc-3* are immune to all known strains of both BCMNV and BCMV (Tang and Feng 2023). Due to epistasis, *bc-3* masks the effects of the *I* gene, and as a result, the presence of *I* is undetectable phenotypically in a host carrying *bc-3*. These interactions prompted the development of DNA assays linked to each of the resistance genes that can be utilized in breeding programs for cultivar selection and for marker-assisted selection (Melotto *et al*. 1996; Johnson *et al*. 1997; Naderpour *et al*. 2010). The objective of this study was therefore, to characterize host plant resistance to BCMNV in commercial snap bean cultivars in Kenya in order to identify cultivars that confer resistance to BCMNV to be incorporated into breeding programs for long-term virus control.

Materials and Methods

Bean germplasm

Forty bean genotypes were used in this study, including 22 commercial snap bean cultivars, two local landraces, three breeding lines, three resistant checks (carrying both *I* and *bc-3*), two susceptible checks (Mitchelite and Cornell 49-2420) as reported by Kamiri *et al.* (2021), and eight differential cultivars (Table 1). The resistant checks were provided by the International Center for Tropical Agriculture (CIAT), Uganda while the differential cultivars were obtained from the Rwanda Agricultural Board. The differential cultivars possess a specific set of resistance genes known to confer resistance against different pathotypes of the virus (Silbernagel *et al*. 2001).

Field experiment

The field sites were located in Kirinyaga (0°34'S, 37°20´E) and Embu (0°34'S, 37°29´E) Counties, representing snap bean production in areas in Kenya, thus providing the most conducive environment for field evaluation. The experiments were conducted using randomized complete block design (RCBD) with four replications. The differential cultivars were not included in the field experiment. The test plants were sown

Table 1. Bean cultivars used in the screening for host resistance to BCMNV

S/No.	Variety	Status	S/No.	Variety	Status
1	Amy	commercial cultivar	21	Boston	commercial cultivar
2	Serengeti	commercial cultivar	22	Source	commercial cultivar
3	Monel	commercial cultivar	23	Cornel 49-2420	susceptible check
4	Morgan	commercial cultivar	24	Mitchelite	susceptible check
5	Teresa	commercial cultivar	25	MCM 1015	resistant check
6	Tausi	commercial cultivar	26	MCM 2001	resistant check
7	Fanaka	commercial cultivar	27	MCM 5001	resistant check
8	Samantha	commercial cultivar	28	MU#13	breeding line
9	Goldplay	commercial cultivar	29	MU#02	breeding line
10	Hawaii	commercial cultivar	30	MU#03	breeding line
11	Lomami	commercial cultivar	31	GBK 032 921	local landrace
12	Manakelly	commercial cultivar	32	GBK 032 952	local landrace
13	Mara	commercial cultivar	33	Pinto	differential cultivar
14	Vanilla	commercial cultivar	34	Red Mexican 34	differential cultivar
15	Widusa	commercial cultivar	35	Pure gold	differential cultivar
16	Seagull	commercial cultivar	36	Red Mexican 35	differential cultivar
17	Edge	commercial cultivar	37	Sanilac	differential cultivar
18	Moonstone	commercial cultivar	38	Sutter pink	differential cultivar
19	Enclave	commercial cultivar	39	Great northern 123	differential cultivar
20	Blazer	commercial cultivar	40	Great northern 31	differential cultivar

in single rows spaced 30 cm apart, and 10 cm between plants. All necessary agronomic practices for optimum growth were implemented in all experimental plots. The incidence of BCMNV was scored according to the presence/absence of symptoms and a cultivar was considered susceptible as long as symptoms were present.

Greenhouse experiment

Five samples of BCMNV-infected bean plants were collected from farmers' fields in Bungoma (0°34´N, 34°32´E), Embu (0°34'S, 37°29´E) and Kakamega (0°16´N, 34°46´E). The samples were pooled per site making a total of three isolates that were used for screening. These samples were transferred to a research laboratory in the Kenya Agricultural and Livestock Research Organization (KALRO) in Kakamega for inoculum isolation following the method described by Chilagane *et al.* (2013). The inoculum was then used to inoculate all 40 genotypes. The experiment was arranged in a completely randomized design (CRD) and replicated four times. The seeds of the test genotypes 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 at a ratio of 3 : 2 : 1. Plants were inoculated at 3-true leaf stage using BCMNV inoculum that was prepared by grinding the infected leaf samples with a mortar and pestle in 0.1% hydrogen phosphate buffer. The supernatant obtained was sieved through cheesecloth. The extracted sap was diluted in 0.02 M potassium phosphate buffer at a pH of 7.5 and gently rubbed on the entire leaf surface using carborundum powder as an abrasive. The plants were observed weekly for the development of symptoms and the final phenotypic evaluation was conducted in the fourth week post-inoculation. Data was recorded according to the presence/absence of BCMNV symptoms. Different BCMNV isolates were grouped into different pathogenicity groups based on the reaction of the host differential cultivar (Silbernagel *et al.* 2001).

Confirmation of BCMNV infection through DAS-ELISA test

The confirmation of BCMNV infection was conducted 3 weeks after inoculation using double antibody sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) following the manufacturer's instructions (BIOREBA, Switzerland). 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 used 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 to a final concentration of 1 mg/ml in substrate buffer was added and incubated at room temperature in the dark. Color development was assessed after 1 hour through quantitative measurements of the p-nitrophenol substrate conversion to yellow at 405 nm absorbance (A405). The presence of a yellow color indicated a positive infection, while the absence of a color change indicated negative infection (Fig. 1).

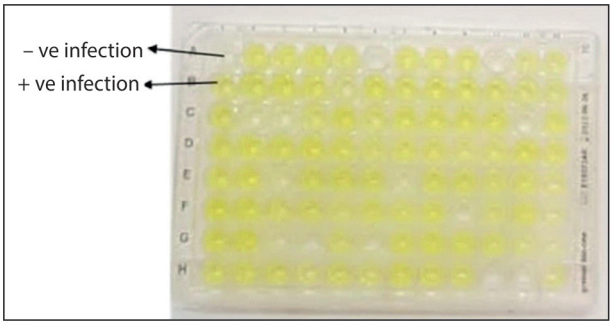

Fig. 1. Microtiter plate showing DAS-ELISA test for the presence of bean common mosaic necrosis virus (BCMNV) in snap bean cultivars

Identification of BCMV/BCMNV resistance genes by molecular markers

For genotypic evaluation, DNA was extracted from leaves of 15-day-old plants using the Mahuku (2004) protocol. PCR-based markers employed to detect the presence of *bc-1*[2], *bc-3* and *I*, and their respective PCR conditions are listed in Table 2. Each 10 µl PCR tube contained 1.5 µl of 50 ng · µl^{-1} DNA template, 0.5 µl of each reverse and forward primer (10 mM), 1 µl of 10x Dream Taq buffer (containing 20 mM $MgCl_2$), 1 µl of 2 mM dNTP and 0.05 µl of 500 U · µl^{-1} DNA *Taq* polymerase. The PCR products were separated by electrophoresis on a 1.2% agarose gel containing 0.5 µg · ml^{-1} ethidium bromide. For the cleaved amplified polymorphic sequence (CAPS) assay used for the elF4E marker, a 5 µl aliquot of the PCR product was *Rsa*I-digested in a 15 µL reaction before electrophoresis. A gel picture was obtained after visualization using an ultra-violet trans-illuminator and each individual was scored as (1) for the presence of a marker or (0) for the absence.

Table 2. Molecular markers linked to BCMV and BCMNV resistance genes

Marker	Locus	Annealing temperature	Size [bp]	Sequences of the primers (5′…3′)
SBD5	*bc-1²*	65°C	1250 cis	F: GTG CGG AGA GGC CAT CCA TTG GTG R: GTG CGG AGA GTT TCA GTG TTG ACA
SW13	*I*	60°C	690 cis	F: CAC AGC GAC ATT AAT TTT CCT TTC R: CAC AGC GAC AGG AGG AGC TTA TTA
ROC11	*bc-3*	55°C	420 trans	F: CCA ATT CTC TT T CAC TTG TAA CC R: GCA TGT TCC AGC AAA CC
eIF4E	*bc-3*	58°C	381/541 codominant	F: ACC GAT GAG CAA AAC CCT A R: CAA CCA ACT GGT ATC GGATT

Source: Melotto *et al.* (1996); Johnson *et al.* (1997); Nadepour *et al.* (2010)

Table 3. Response of snap bean cultivars to bean common mosaic necrosis virus (BCMNV) under field conditions

S/No.	Variety	Embu*	Kirinyaga	S/No.	Variety	Embu*	Kirinyaga
1.	Amy	1	1	17.	Blazer	1	0
2.	Monel	1	1	18.	Enclave	1	0
3.	Boston	1	1	19.	Manakelly	1	0
4.	Moonstone	1	1	20.	Mara	1	0
5.	Morgan	1	1	21.	Serengeti	1	0
6.	Edge	1	1	22.	Samantha	1	0
7.	Fanaka	1	1	23.	MU#02	1	0
8.	Seagull	1	1	24.	MU#03	0	1
9.	Goldplay	1	1	25.	MU#13	0	1
10.	Hawaii	1	1	26.	GBK 032 921	1	1
11.	Lomami	1	1	27.	GBK 032 952	1	1
12.	Tausi	1	1	28.	Cornell 49-2420 (SC)	1	1
13.	Teresa	1	1	29.	Mitchelite (SC)	1	1
14.	Vanilla	1	1	30.	MCM 1015 (RC)	0	0
15.	Source	1	1	31.	MCM 2001 (RC)	0	0
16.	Widusa	1	1	32.	MCM 5001 (RC)	0	0

1 – presence of disease; 0 – absence of disease; SC – susceptible check; RC – resistant check

Results

Response of snap bean genotypes to BCMNV under field conditions

The most common symptom of BCMNV in the field was top necrosis, starting from the shoot tip and progressing downwards to older plant parts (Fig. 2). Other frequently observed symptoms included mosaics, mottling, downward curling and stunting. Twenty-two genotypes were infected in Kirinyaga, while in Embu, 27 out of the 32 were infected (Table 3). None of the three dry bean resistant checks showed symptoms at either site.

Pathogen characterization

Based on the reaction of the differential cultivars to the three isolates, as expressed by the symptoms and confirmed by ELISA test, Bungoma and Kakamega isolates were classified as pathotype VI, whereas the Embu isolate was classified as pathotype III (Table 4). The symptoms expressed include mosaic, mottling, deformed leaves and stunted growth.

Response of snap bean genotypes to BCMNV under greenhouse conditions

The 32 bean genotypes reacted differently to infection by the three BCMNV isolates. The three isolates

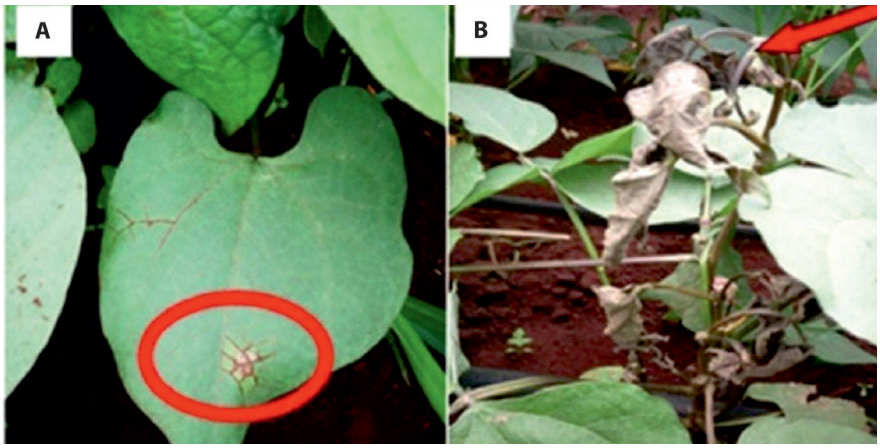

Fig. 2. Mosaic (A) and top necrosis (B) symptoms of bean common mosaic necrosis virus (BCMNV) expressed by susceptible snap beans under field conditions

Table 4. Reaction of bean common mosaic necrosis virus (BCMNV) common bean differentials cultivars to BCMNV isolates

S/No.	Differential cultivar	Host genes	Bungoma		Kakamega		Embu	
			symptom*	ELISA	symptom	ELISA	symptom	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	

*symptom: M – mosaics; MT – mottling; D – deformed leaves; ST – stunted growth; NR – no reaction. The positive and negative signs indicate presence and absence of infection respectively

successfully infected all of the 29 snap bean genotypes but were not able to infect the three resistant checks (Table 5). Positive BCMNV infection was confirmed in all the entries (including the asymptomatic ones) using the ELISA test.

Molecular marker analysis

ROC 11marker, which is in repulsion linkage with the *bc-3* gene, was detected in all snap bean genotypes except for the three dry bean cultivars that were used as resistant checks (Fig. 3A). This might indicate that only the three dry bean cultivars (resistant checks) carried the *bc-3* gene. Additionally, when the DNA was amplified using the elF4E primer pair, which also detects the presence of *bc-3*, each entry generated a 541 bp amplicon. Following *Rsa*I digestion, the amplicon produced by carriers of *bc-3* (the resistant checks) was cleaved into 381 bp and 160 bp products, whereas the amplicon that was produced by non-carriers was not cleaved (Fig. 3B). Furthermore, the findings revealed that all entries exhibited amplification of the anticipated 690 bp fragment associated with the SW13 marker, which is linked to the dominant *I* gene (Fig. 3C). Additionally, the SBD5 marker amplified a 1,250 bp amplicon (Fig. 3D), indicating the presence of the *bc-1²* gene in nine snap bean entries (Table 6).

Discussion

In recent years, BCMNV has become the dominant potyvirus affecting beans, which can be attributed to breeders' efforts to breed against BCMV by using the *I* gene (Worrall *et al.* 2015). The incidence of BCMNV in common bean growing regions of central and western Kenya concurs with previous studies (Mangeni *et al.* 2014; Mutuku *et al.* 2018). The differences observed in disease pressure at the two sites

Table 5. Reaction of snap bean cultivars to three bean common mosaic necrosis virus (BCMNV) isolates under greenhouse conditions

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

*reaction: M – mosaic; MT – mottling; D – deformed leaves; ST – stunted growth; TN – top necrosis; VN – vein necrosis; LN – local necrosis; NR – no reaction. **the positive and negative signs indicate the presence and absence of infection respectively

could be attributed to factors such as genotype susceptibility in terms of symptom expression, strains of the virus, and environmental factors such as humidity and temperature (Muute *et al.* 2021). The symptoms of BCMNV observed in this study are similar to previous studies (Mwaipopo *et al*. 2018; Mangeni *et al*. 2020; Deligöz *et al*. 2021, 2022). However, some cultivars were asymptomatic under field conditions, highlighting the need for artificial inoculation under controlled conditions and subsequent confirmation using ELISA tests on all genotypes.

Characterization of BCMNV isolates has previously been used to help understand the available strains in a specific region. The two pathotypes (III and VI) identified in this study have been reported in Kenya (Mangeni *et al*. 2014; Mutuku *et al*. 2018). The DAS-ELISA was useful in detecting BCMNV in asymptomatic plants, similar to previous studies (Kilic *et al.* 2020; Mangeni *et al.* 2020; Deligöz *et al*. 2022). The three dry bean cultivars (MCM 1015, MCM 2001 and MCM 5001) used as resistant checks consistently showed resistance, confirming their resistance to BCMNV. According to Kelly *et al*. (2003), the three cultivars possess the *I* and *bc-3* genes. These cultivars (initially CIAT breeding lines) are commercially grown in Uganda as K 131 (Okii *et al*. 2018), NABE

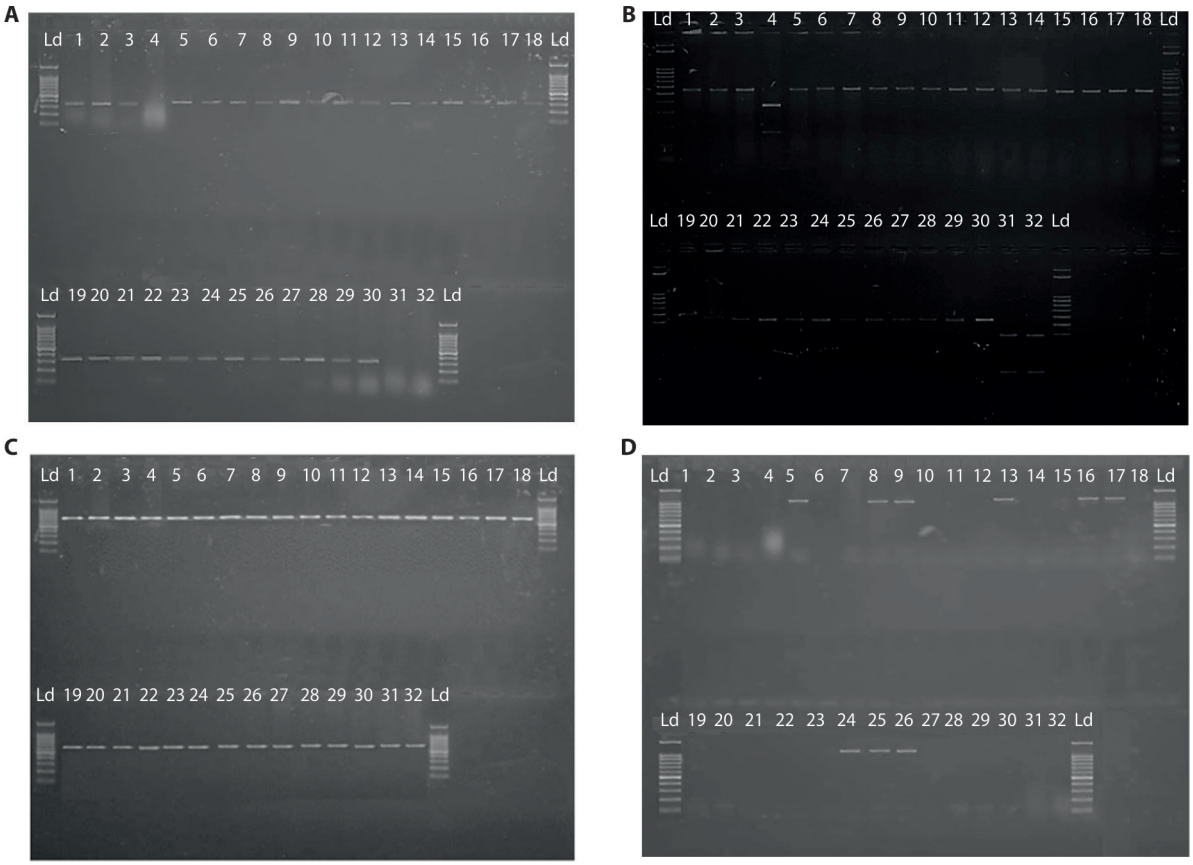

Fig. 3. Amplification of molecular markers linked to bean common mosaic necrosis virus (BCMNV) resistance genes *I*, *bc-3*, *bc-12*. A – SCAR marker ROC11 300 bp for *bc-3*; B – CAPS marker elf4e 541(381/160) for *bc-3*; C – SCAR marker SW13 690 bp for *I*; and D – SCAR marker SBD5 1250 bp for *bc-12*. Ld – 100 bp ladder. Entries 1–3, 5–30 are French bean cultivars; 4, 31–32 are resistant dry bean cultivars

Table 6. Molecular analysis of the snap bean cultivars to determine the presence of markers linked to bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) resistance genes

S/No.	Genotypes	SW13	ROC11*	elF4e**	SBD5
		(*I* gene)	(*bc-3*)	(*bc-3* gene)	(*bc-I²* gene)
1.	Amy	1	1	1	0
2.	Blazer	1	1	1	0
3.	Boston	1	1	1	0
4.	MCM 1015	1	0	1/1	0
5.	Goldplay	1	1	1	1
6.	Fanaka	1	1	1	0
7.	GBK 032921	1	1	1	0
8.	Hawaii	1	1	1	1
9.	Manakelly	1	1	1	1
10.	GBK 032952	1	1	1	0
11.	Lomami	1	1	1	0
12.	Enclave	1	1	1	0
13.	Mara	1	1	1	1
14.	Monel	1	1	1	0
15.	Morgan	1	1	1	0
16.	Moonstone	1	1	1	1

Table 6. Molecular analysis of the snap bean cultivars to determine the presence of markers linked to bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) resistance genes – *continuation*

S/No.	Genotypes	SW13	ROC11*	elF4e**	SBD5
		(*I* gene)	(*bc-3*)	(*bc-3* gene)	(*bc-1^2* gene)
17.	MU#02	1	1	1	1
18.	MU#03	1	1	1	0
19.	MU#13	1	1	1	0
20.	Samantha	1	1	1	0
21.	Vanilla	1	1	1	0
22.	Serengeti	1	1	1	0
23.	Source	1	1	1	0
24.	Widusa	1	1	1	0
25.	Seagull	1	1	1	1
26.	Teresa	1	1	1	1
27.	Tausi	1	1	1	1
28.	Mitchellite	1	1	1	0
29.	Cornell 49- 242	1	1	1	0
30.	Edge	1	1	1	0
31.	MCM 2001	1	0	1/1	0
32.	MCM 5001	1	0	1/1	0

*1 and 0 represent presence and absence of the marker respectively; **ROC 11 – presence of the marker indicates absence of the gene 1/1-381/160bps band after digestion with *RsaI* enzyme

3 and NABE 2, respectively. MCM 5001 and MCM 1015 were derived from a cross between IVT831629 and BAT 1554 cross, while MCM 2001 was derived from IVT831607 x RAB 71 cross. Given that BCMNV is prevalent in East Africa, these lines are particularly suitable for utilization in African breeding programs.

The deployment of molecular markers in this study was used together with phenotypic studies to determine their effectiveness in marker-assisted breeding (MAS). The marker SW13 is closely linked (1.3 ± 0.8 cM) in a coupling phase to the dominant *I* gene (Melotto *et al.* 1996). In the current study, the SW13 marker results corresponded to the phenotypic reactions of the genotypes which showed symptoms of top necrosis under field conditions. This emphasizes the usefulness of this marker for rapid identification of the dominant *I* gene for resistance breeding to BCMV. The top necrosis recorded in the field was a positive confirmation of the presence of the dominant *I* gene. This has made it possible to introgress the *I* gene into other cultivars to confer resistance to all known strains of BCMV (Chilagane *et al.* 2013)

The SCAR marker ROC 11 is linked to the *bc-3* gene in the 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 cultivars, whereas all 29 snap bean cultivars amplified the marker. However, the ROC 11 marker has been reported to have false positives and therefore its usage should be proceeded after validation (Chilagane *et al.* 2013). Based on the fact that the *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.* 2010). In this study, the CAPS marker elF4E was used to confirm the presence of the *bc-3* gene in the resistant checks and the absence of the gene in snap beans. Therefore, this marker is reliable in the identification of the *bc-3* gene. The absence of the *bc-3* gene in contemporary snap bean genotypes was also reported in Turkey by Deligöz *et al.* (2022). Nevertheless, the frequent use of the *bc-3* gene poses a risk for resistance breakdown and hence there is a need to use other alternative *bc* genes to protect the *I* gene (Feng *et al.* 2017).

Identification of *bc-1^2 in* genotypes possessing the dominant *I* gene is of utmost importance due to the epistatic interaction between *bc-2* and *bc-3*. In addition, MAS for *bc-1^2* is upfront since it does not require *bc-u* for its expression unlike the genotypes with the recessive *i* gene (Larsen *et al.* 2011). In this study, the SBD5 marker tightly linked to *bc-1^2*, was detected in nine snap 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 strains. Based on these results, the usefulness of the SBD5 marker for the selection of the *bc-1^2* gene in this snap bean panel cannot

be ascertained. This finding corroborates previous reports made by Pasev *et al.* (2014) and Deligöz *et al.* (2022) that the SBD5 marker was not reliable and should be supported with phenotypic data. A study by Mangeni *et al.* (2014) identified sources of *bc-1²* in dry beans, based on the SBD5 marker. However, because of the inconsistent results obtained from this study, further investigations are warranted to validate the applicability of the SBD marker in MAS. Moreover, it is imperative to develop molecular markers for the other recessive *bc* genes.

Conclusions

This study revealed that the majority of the snap beans grown in Kenya are susceptible to BCMNV, hence restricting their production in areas where the virus is prevalent. Therefore, the dry bean cultivars MCM1015, MCM 2001 and MCM 5001 can be utilized for gene pyramiding of the *I/bc-3* gene. The study established that the molecular markers SW13 and elF4e were reliable in the identification of genes that confers resistance to BCMNV and BCMV and therefore applicable in MAS involving the current germplasm. Further studies should focus on identifying additional molecular markers that can be used for a number of common bean market classes.

Acknowledgements

The authors are grateful to Kirkhouse Trust for financial support, Dr. Alice Kabeja of the Rwanda Agricultural Board and CIAT, for providing seeds of the checks/differential cultivars and KALRO Kakamega for providing greenhouse and laboratory facilities. While the research project was funded by Kirkhouse Trust, the design, execution and interpretation of the research remains wholly the responsibility of the authors.

References

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): 4343–4350. DOI: https://doi.org/10.5897/AJB2012.9651

Deligöz I., 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):7–18. DOI: https://doi.org/10.24326/asphc.2021.6.2

Deligöz İ., Sokmen M., Kutluk Yılmaz N., Özçelik 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): 5–13. DOI: https://doi.org/10.16955/bitkorb.1130635

Feng X., Guzmán P.J., Myers R., Karasev A.V. 2017. Resistance to Bean common mosaic necrosis virus conferred by the *bc-1* gene affects the systemic spread of the virus in common bean. Phytopathology 107 (7): 93–900. DOI: https://doi.org/10.1094/PHYTO-01-17-0013-R

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): 131–138. DOI: https://doi.org/10.21273/JASHS.141.2.131

HCDA. Horticultural Crops Development Authority. 2020 Horticulture Validation Report Nairobi, Kenya. [Available on: http://horticulture.agricultureauthority.go.ke/index.php/statistics/reports]. [Accessed on: 21st March 2023]

Johnson W.C., Guzmán P., Mandala D., Mkandawire A.B., Temple S., Gilbertson R.L., Gepts P. 1997. Molecular tagging of the *bc-3* gene for introgression into the Andean common bean. Crop Science 37 (1): 248–254. DOI: https://doi.org/10.2135/cropsci1997.0011183X003700010044x

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): 51–65. DOI: http://doi: 10.5897/AJB2020.17279

Kelly J.D., Gepts P. Miklas P.N., Coyne D. 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): 135–154. DOI: https://doi.org/10.1016/S0378-4290(03)00034-0

Kilic H.C., Hesna K.O., 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: 386–392. DOI: https://doi.org/10.31590/ejosat.705686

Larsen R.C., Druffel K.L., Wyatt S.D. 2011. The complete nucleotide sequences of bean common mosaic necrosis virus strain NL-5, NL-8 and TN-1. Archives of Virology 156 (4): 729–732. DOI: https://doi.org/10.1007/s00705-011-0945-8

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): 71–81. DOI: https://doi.org/10.1007/BF02773351

Mangeni B.C., Abang M., Omuse C.N., Leitich R., Arinaitwe W., Mukoye B., Kelly J., Were H. 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): 308–316.

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): 501–515. DOI: https://doi.org/10.1111/jph.12928

Melotto M., Afanador L., Kelly. J.D. 1996. Development of a SCAR marker linked to the *I* gene in common bean. Genome 39 (6): 1216–1219. DOI: https://doi.org/10.1139/g96-155

Mutuku J.M, Wamonje F.O., Mukeshimana G., Njuguna J., Wamalwa M., Choi S., Tungadi T., Djikeng A., Carr J.P., Harvey J. 2018. Metagenomic analysis of plant virus occurrence in common bean (*Phaseolus vulgaris*) in Central Kenya. Frontiers in Microbiology 9: 2939. DOI: https://doi.org/10.3389/fmicb.2018.02939

Muute N., Muli A.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):1–18. DOI: http:// doi: 10.9734/IJPR/2021/v8i330203

Mwaipopo B., Nchimbi-Msolla S., Njau P.J., Mark D., Mbanzibwa D.R. 2018. Comprehensive surveys of bean common mosaic virus and bean common mosaic necrosis virus and molecular evidence for occurrence of other *Phaseolus vulgaris* L. viruses in Tanzania. Plant Disease 102 (11): 2361–2370. DOI: https://doi.org/10.1094/PDIS-01-18-0198-RE

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): 255–263. DOI: https://doi.org/10.1111/j.1364-3703.2009.00602.x

Okii D., Mukankusi C., Sebuliba S., Tukamuhabwa P., Tusiime G., Talwana H., Ogong T., Namayanja A. Paparu P., Gepts P. 2018. Genetic variation, Heritability estimates and G × E effects on yield traits of Mesoamerican common bean (*Phaseolus vulgaris* L) germplasm in Uganda. Plant Genetic Resources 16 (3): 237–248. DOI: 10.1017/S1479262117000259

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): 19–25. DOI: https://doi: 10.1111/jph.12149

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: 1007–1020. DOI: https://doi.org/10.1007/s007050170132

Soler-Garzon A, McClean P. E., Miklas P.N. 2021. Coding mutations in vacuolar protein-sorting 4 AAA+ ATPase endosomal sorting complexes required for transport protein homologs underlie *bc-2* and new *bc-4* gene conferring resistance to Bean common mosaic virus in common bean. Frontiers in Plant Science 12: 769247. DOI: https://doi.org/10.3389/fpls.2021.769247

Tang M., Feng X. 2023. Bean common mosaic disease: etiology, resistance resource, and future prospects. Agronomy 13 (1): 58. DOI: https://doi.org/10.3390/agronomy13010058

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. p. 1–46. In: "Advances in Virus Research" (M. Kielian, K. Maramorosch, T. Mettenleiter, eds.). Academic Press, Burlington. DOI: https://doi.org/10.1016/bs.aivir.2015.04.002