

# Incidence and severity of bean common mosaic disease and resistance of popular bean cultivars to the disease in western Kenya

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## Abstract

The common bean (*Phaseolus vulgaris*) is a high protein crop and the main legume in the cropping system of western Kenya. Despite its importance, common bean yields are low (<1.0 t/ha) and declining. Bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) are the most common and most destructive viruses and can cause a yield loss as high as 100%. In Kenya, a limited number of cultivars and exotic genotypes with resistance to BCMV and BCMNV strains have been reported. This study sought to determine the distribution and screen popular cultivars for resistance to the viruses. In October 2016 and May 2017, two diagnostic surveys for bean common mosaic disease (BCMD) were conducted in seven counties of western Kenya namely Bungoma, Busia, Homa Bay, Nandi, Vihiga, Kakamega and Siaya. Leaf samples showing virus-like symptoms were collected and analysed by ELISA. Sixteen popularly grown bean cultivars together with cowpea (*Vigna unguiculata*), soybean (*Glycine max*), green grams (*Vigna radiata*) and groundnut (*Arachis hypogaea*) were planted in a greenhouse in a completely randomized block design with three replicates. The plants were inoculated with BCMNV isolate at 3-leaf stage. Data were taken weekly for 3 weeks on type of symptoms expressed and number of plants infected. In total, 270 bean farms were visited. Symptoms of mosaic, downward curling, local lesions, stunting or a combination of these were observed during both surveys. Mean virus incidence was higher in the short rain season (50.2%) than in the long rain season (35.6%). The mean BCMD severity on a scale of 0–3 was highest (2.3) in Kakamega County and lowest (0.5) in Siaya. On variety resistance tests to BCMNV isolate, 10 bean cultivars were susceptible, four tolerant and two resistant. BCMNV is widely distributed across counties probably because of use of uncertified seeds by farmers and inoculum pressure from seed and aphid vector. For improved yields of common bean, farmers should be advised to plant certified seeds for all legumes in the cropping system.

## KEYWORDS

BCMNV, BCMV, disease incidence, severity

## 1 | INTRODUCTION

Western Kenya is one of the food baskets of the country and a region with approximately 1/3 of the country's population. The common bean (*Phaseolus vulgaris* L) is a high protein (22 g/100 g) crop and the main grain legume in the cropping system of the region. Small-scale farmers mainly grow the crop. Besides providing food for humans, feed for animals and improving soil fertility by fixing nitrogen, it also improves the incomes of the farmers. Despite its importance, common bean productivity is declining and yields obtained of <1.0 t/ha (Kayondo et al., 2014) are low compared to a production potential of 1,400–2,000 kg/ha (Katungi, Farrow, Chianu, Sperling, & Beebe, 2009). Decreasing yield is attributed to poor access to improved seeds, declining soil fertility, drought, high incidence of pests (e.g., aphids (*Aphis* spp), bean stem maggot (*Ophiomyia* spp), borers (*Dectes* spp)), diseases (e.g., root rots [*Rhizoctonia* spp, *Fusarium* spp and *Pythium* spp], viruses, anthracnose [*Colletotricum lindemuthianum*], angular leaf spot [*Phaeoisariopsis griseola*] and rust [*Uromyces appendiculatus*]) and unpredictable weather. Variations in weather conditions such as temperature, rainfall, humidity, wind patterns and length of daylight hours due to general climate change impact on arthropod vector reproduction and development, their distribution, and feeding behaviour, and in turn all can influence virus replication and transmission (Tabachnick, 2010). Increase in temperature due to global warming is a critical determinant of increased virus transmission efficiency, symptom expression and severity (Caminade, McIntyre, & Jones, 2019).

One key biotic stress factor that reduces yield is plant viruses. The common bean is one of the most susceptible legume crops to virus infection. The bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) cause significant yield losses (Chiquito-Almanza et al., 2017) and are both seed-borne and aphid-transmitted (Flores-Estévez, Acosta-Gallegos, & Silva-Rosales, 2003; Gamez, 1973; Melgarejo, Lehtonen, Fribourg, Rannali, & Valkonen, 2007). The two viruses are closely related and belong to the Family *Potyviridae*, genus *Potyvirus*, which is the largest of the eight genera currently assigned to the family by the International Committee on Taxonomy of Viruses (ICTV, 2013).

The serological relationships of the two viruses (formally serotype A and serotype B and treated as strains of BCMV) were determined and from sequence analysis, it was agreed that they were treated as distinct viruses (Vetten, Lesemann, & Maiss, 1992).

Bean common mosaic virus and BCMNV are the most common and most destructive viruses that infect common beans in Kenya (Mangeni, Abang, & Kelly, 2014) as well as a range of other cultivated and wild legumes (Morales, 2006). Yield losses due to BCMV and BCMNV can be as high as 100% (Damayanti et al., 2008; Li et al., 2014; Mutuku et al., 2018; Mwaipopo, Nchimbi-Msolla, Njau, Mark, & Mbanzibwa, 2018; Saqib, Nouri, Cayford, Jones, & Jones, 2010; Singh & Schwartz, 2010; Verma & Gupta, 2010).

Mottling and malformation of the primary leaves is an indication that the primary infection occurred through seed (Bos, 1971). Systemically infected plants may have smaller and fewer pods, and

infected pods may sometimes be covered with small, dark green spots and mature later than uninfected pods.

Breeding for genetic resistance to BCMV and BCMNV is the most durable form of managing the viruses. Bean cultivars possessing the dominant *I* gene are resistant to BCMV however susceptibility to BCMNV-induced black root disease (Worrall et al., 2015). Available recessive resistant genes are virus strain-specific and therefore difficult to breed bean cultivars with a broad resistance to the existing strains of BCMV and BCMNV based on one of these genes alone. Marker-assisted selection can be utilized to pyramid the recessive genes (*bc-u*, *bc-1*, *bc-1<sup>2</sup>*, *bc-2*, *bc-2<sup>2</sup>* and *bc-3*), with the dominant *I* gene in order to provide broad-spectrum possible resistance (Pasev, Kostova, & Sofkova, 2014).

This study therefore sought to determine the incidence and severity of bean common mosaic disease (BCMD) and resistance of popular bean cultivars to the disease.

## 2 | MATERIALS AND METHODS

### 2.1 | Survey and sample collection

In October 2016 and May 2017, two diagnostic surveys for BCMD were conducted in seven counties of western Kenya namely Bungoma, Busia, Homa Bay, Nandi, Vihiga, Kakamega and Siaya. A stratified random sampling procedure was adopted to determine BCMD symptom incidence and severity. The disease incidence was assessed according to Were, Winter, and Maiss (2004), Were et al. (2014) as the proportion of diseased plants in an area. Incidence was scored as the presence or absence of virus disease symptoms using a rating scale where low incidence = 1%–20%; moderate incidence = 21%–49%; and high incidence = 50%–100%. Disease symptom severity was scored on a scale of 0–3 according to Odu, Asiedu, Hughes, Shoyinka, and Oladiran (2004) where 0 = no disease symptoms on plants, 1 = mild foliar disease symptoms, 2 = moderate foliar disease symptoms and 3 = severe distortion, malformation of leaves or stem and stunting. At least 5–15 samples (depending on the size of the field) were taken along a diagonal. One trifoliate symptomatic leaf sample was collected from the sampled plant and stored in polythene bags in a cool box prior to transfer to the laboratory for serological analysis. Sample location, cultivar and symptoms were recorded.

### 2.2 | Enzyme-Linked Immunosorbent Assay (ELISA)

For all ELISA tests, microtitre plates (Greiner Microlon medium binding) were used and generally volumes for each reactant were kept at 100 µl/well.

Between incubations, three intensive washing steps each lasting 3 min were carried out by repeated soaking of the plates in washing buffer for 4 min. Antibodies were provided by Dr. Stephan Winter of DSMZ, Germany.

## 2.2.1 | Sample preparation

Leaf tissue from plants with virus-like symptoms was ground 1:10 (w/v) in sample extraction buffer (PBST + 2% PVP (Serva PVP-1S polyvinylpyrrolidone)). To detect BCMNV, Double Antibody Sandwich (DAS)-ELISA was conducted essentially as described (Were et al., 2004) following manufacturer's instructions. Microtitre plates were coated with BCMNV IgG diluted 1:1,000 (v/v) in coating buffer (1.59 g sodium carbonate [Na<sub>2</sub>CO<sub>3</sub>], 2.93 g sodium bicarbonate [NaHCO<sub>3</sub>], 0.20 g sodium azide [NaN<sub>3</sub>], dissolved in 900 ml H<sub>2</sub>O, adjusted pH to 9.6 with HCl and made up to 1 L) and incubated for 2 hr at 37°C. To block, 2% skimmed milk in PBST (200 µl/well) was added and incubated for 30 min at 37°C. The extracts of sap prepared from ground leaf tissues of virus-infected plants 1:10 (w/v) in sample extraction buffer (PBST + 2% PVP) were added and incubated overnight at 4°C. Extracts from healthy and of BCMNV infected plants were used as negative and positive controls, respectively. 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 2 hr at 37°C. The substrate, *p*-Nitrophenyl phosphate diluted 1 mg/ml in substrate buffer (DEA + H<sub>2</sub>O + NaN<sub>3</sub>) was added and incubated for 1 hr at 37°C or until there was colour change before 1 hr. Quantitative measurements of the *p*-nitrophenol substrate conversion resulting in yellow colour were made by determining the absorbance at 405 nm (A405) in a BioTek® model spectrophotometer (Labsystems Co.). Twice the mean absorbance readings of healthy controls were used as the positive thresholds.

To detect BCMV, Triple Antibody Sandwich (TAS) ELISA was conducted as described (Were et al., 2004) and following the manufacturer's instructions. Microtitre plates (96 wells) were coated with BCMV IgG diluted 1:1,000 (v/v) in a coating buffer and incubated for 2 hr at 37°C. Blocking was done as above. Sap extracts prepared as described above were added and incubated overnight at 4°C. 100 µl/well of MAbs raised against BCMV and diluted 1:100 (v/v) in conjugate buffer added and incubated for 2 hr at 37°C were used for detection. As explained above, extracts from healthy and of BCMV infected plants were used as negative and positive controls, respectively. Alkaline phosphatase-labelled rabbit anti-mouse RaM-AP, (DSMZ) diluted 1:1,000 v/v in conjugate buffer was added and the plates incubated for 45 min at 37°C. Substrate addition, incubation and absorbance readings were done as described above.

## 2.3 | Variety resistance screening to BCMV and BCMNV

### 2.3.1 | Seed germination and mechanical inoculation

Five seeds from each of 16 popularly grown bean varieties; and five cowpea, one soybean, one green gram and one ground nut were sowed in plastic pots with three replicates in a greenhouse. Inoculation was done following the protocol developed by Mandal, Csinos, Martinez-Ochoa, and Pappu (2008) with minor modifications

to address the limitations in plant virus mechanical or manual transmission that may not be efficient in certain host species, resulting in many "escapes". The BCMNV isolate used in inoculation had been maintained in infected bean in a greenhouse at Kenya Agricultural and Livestock Research Institute (KALRO)-Kakamega, Kenya.

The inoculum was prepared by grinding BCMNV-infected leaves at the rate of 1.0 g tissue and 10 ml of 0.1 M phosphate buffer, pH7.0 containing 0.2% sodium sulphite using a chilled pestle and mortar. The test plants were inoculated at the 3-leaf stage with BCMNV isolate from western Kenya beside a healthy control. Data were taken on type of symptoms expressed by plants and the number of plants showing symptoms weekly for 3 weeks. Systemic infection was determined at the end of third week postinoculation by DAS ELISA. Susceptible plants expressed typical symptoms of BCMNV. Symptomatic plants positive for BCMNV by DAS ELISA were graded susceptible, asymptomatic plants positive for BCMNV graded tolerant while asymptomatic plants negative for BCMNV graded resistant.

## 3 | RESULTS

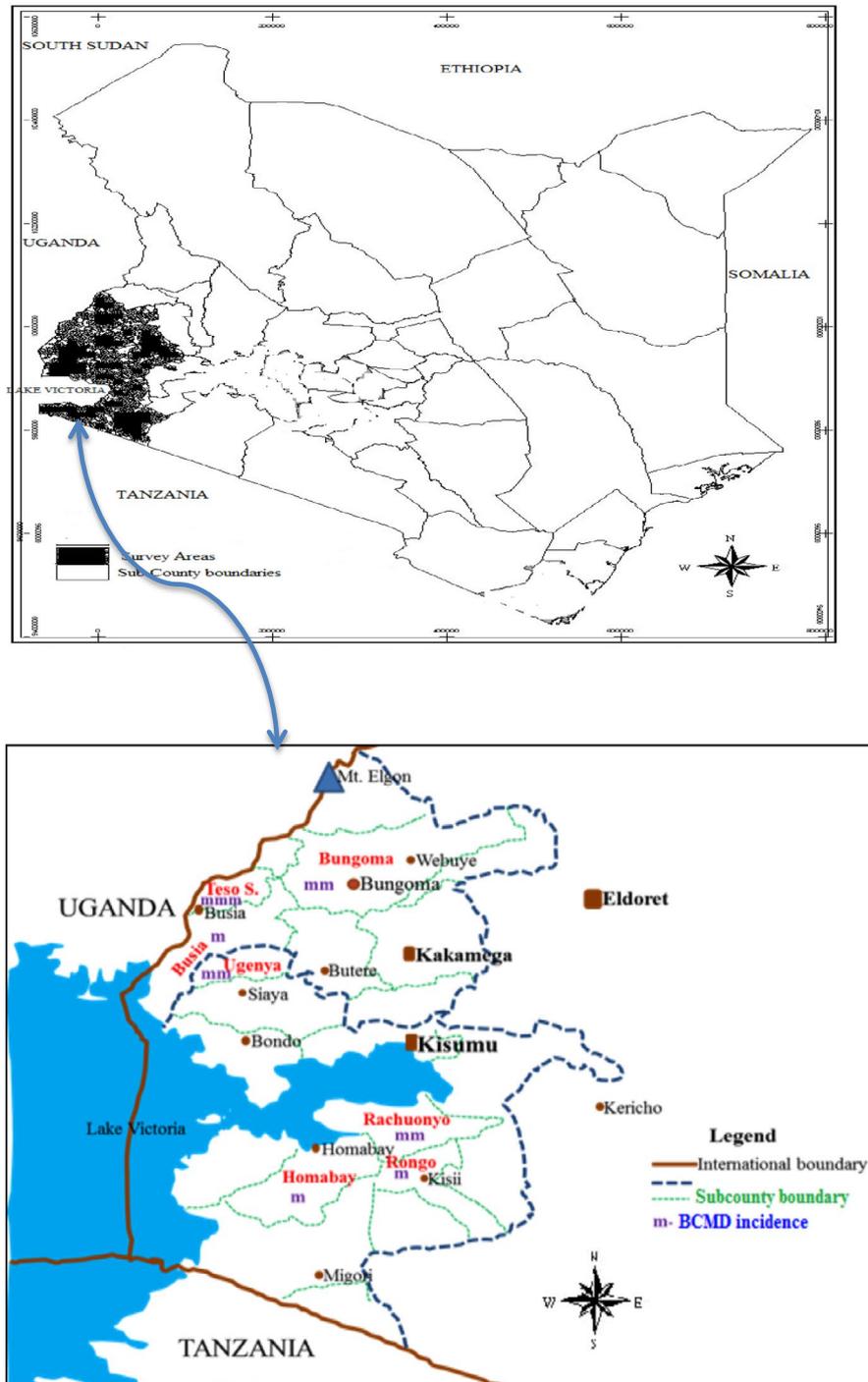
### 3.1 | Incidence and severity of BCMNV

Two hundred and seventy farms were surveyed, 150 in the long rain season and 120 in the short rain season. The survey covered areas as low as 1,164 m above sea level, a farm in Busia County, to areas as high as 1,600 m, a farm in Kakamega County. The southernmost farm (S00.70061) surveyed was in Homa Bay County, while the northernmost (N00.69718) was in Bungoma County. The westernmost farm surveyed (E034.19242) was in Busia County, while the easternmost (E034.81551) was in Kakamega County (Figure 1).

Typical BCMNV and other virus-like symptoms of mosaic, leaf distortion, downward curling, mottling, vein necrosis, local lesions, stunting or a combination of these were observed during both surveys (Figure 2).

The average temperature and rainfall during June were 22.5°C and 525 mm in the western areas and 16.5°C and 225 mm in eastern areas, respectively (Min Env. and Mineral Res. 2018). The results of visual symptom scoring in the field had mean virus disease symptom incidence higher in the short rain season (41.8%) than in the long rain season (35.6%).

Across counties (Table 1), Kakamega County had the highest mean virus incidence (47.6%), while Siaya had the lowest (31.6%) in the short rain season. In the long rain season, it was highest in Bungoma (44.3%) and lowest in Siaya (29.4%). Most popular common bean varieties found on the farms were Rosecoco (152 farms), Wairimu (64 farms), Yellow (29 farms), KK8 (18 farms), Punda (five farms) and Tulu (two farms). Punda had the highest mean viral incidence observed (56.3%) followed by KK8 (48.2%), Wairimu (42.7%), Rosecoco (40.5%) and Tulu (40.0%), while Yellow had the lowest (39.3%) in the short rain season. In the long rain season, Rosecoco had highest mean incidence (44.1%) while yellow the lowest (35.0%).



**FIGURE 1** Map of western Kenya showing counties surveyed [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

From Table 2, the sum of samples positive for BCMV was from beans (15), groundnut (6) and cowpea (2), while for BCMNV the sum of positive samples was from beans (21), groundnut (2) and cowpea (1) collected during the short rain season (Figure 3) (Table 3). During the long rain season, the sum of samples positive for BCMV was from beans (23), groundnut (2) and cowpea (4), while for BCMNV the sum of positive samples was from beans (31), groundnut (4) and cowpea (0) (Figure 4) (Table 4). Most samples from across the counties were found having mixed infections of both BCMV and BCMNV as detected by antibodies for the two viruses. Mixed infection with the two viruses causing BCMD was found in samples from the all the counties surveyed.

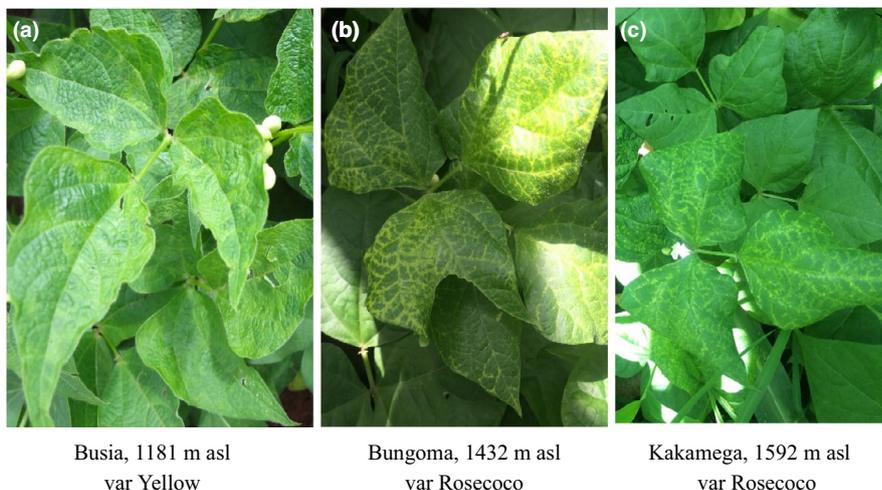
Symptomatic samples negative for the two viruses may have been due to other viruses inducing similar symptoms or mineral deficiency.

Viral disease severity varied within and between fields and in counties. The mean BCMD severity in the long rain season was highest (1.5) in Bungoma County and lowest (0.5) in Busia, Siaya and Vihiga.

### 3.2 | Variety resistance screening

Sixteen popularly grown common bean cultivars in western Kenya inoculated with BCMNV BG12 isolate from western Kenya

**FIGURE 2** Some virus-like symptoms observed in the field during survey that were found positive for BCMNV. Above: (a) Shrivelled leaves with mosaic on variety Yellow in Busia County at 1,181 meters above sea level (m asl); (b): leaves of Rosecoco variety showing yellow-net vein banding in Bungoma County and 1,432 m asl; and (c): leaves of Rosecoco variety in Kakamega County showing vein banding and curling downwards at 1,592 m asl [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



**TABLE 1** Mean bean common mosaic disease incidence and severity observed during the short and long rain seasons, respectively, in western Kenya

County	Season	Number of fields	Mean incidence	Mean Severity
Busia	LR	25	33.6 <sup>a</sup>	0.5
	SR	20	44.1 <sup>b</sup>	0.2
Bungoma	LR	25	44.3 <sup>b</sup>	1.5
	SR	20	47.4 <sup>d</sup>	0.2
Homa Bay	LR	20	35.4 <sup>a</sup>	1.2
	SR	15	44.3 <sup>b</sup>	1.7
Kakamega	LR	20	38.4 <sup>c</sup>	1.0
	SR	20	47.6 <sup>d</sup>	0.3
Siaya	LR	20	29.4 <sup>e</sup>	0.5
	SR	15	31.6 <sup>a</sup>	1.0
Vihiga	LR	20	32.0 <sup>a</sup>	0.5
	SR	15	42.8 <sup>b</sup>	1.2
Nandi	LR	20	33.0 <sup>a</sup>	1.3
	SR	15	40.5 <sup>b</sup>	0.6
Total	LR	150	35.6 <sup>a</sup>	1.0
	SR	120	41.8 <sup>b</sup>	1.5

Note: Means with the same letter(s) within a column are not significantly different at 0.05 level.

Disease incidence-proportion of diseased plants per field.

Disease severity-amount of disease on individual plants.

ANOVA was used to compare means, and least significant difference (L.S.D.) values were used to separate the significant different means at  $p \leq .05$ . Disease incidence among the counties varied significantly ( $p = .05$ ). There was a strong positive correlation between viral disease incidence and severity ( $r = 0.843$ ;  $p < .001$ ), and therefore, severity increased with increase in disease incidence.

Abbreviations: LR, long rain season; SR, short rain season.

(Kakamega) in a greenhouse exhibited typical virus symptoms such as leaf mosaic, down ward leaf curl and yellowing as shown on popular variety GLP 2 (Figure 5).

From Table 5, four bean varieties (Imbeko, KK/RIL5/Red 13, Okwoto, RIL05/CAL 194) were symptomless with BCMNV BG 12 isolate from western Kenya however tested positive for BCMNV by DAS ELISA. Two bean varieties (KK RIL05 and KK 072) were symptomless and negative for BCMNV by DAS ELISA.

Successful infection was determined 3 weeks postinoculation by both symptomatology and DAS ELISA.

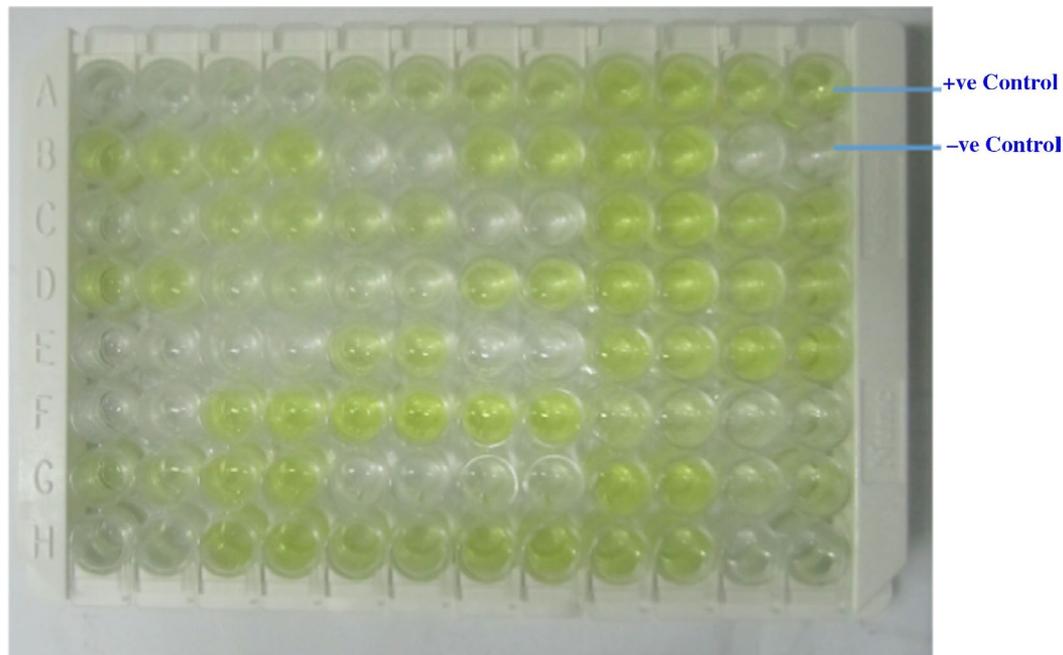
Popularly grown grain legumes, groundnut cv "Red Valencia", soybean cv "Nyala", green grams cv "Local", cowpea cv "Local cream", "Local black", "K-80", "KVU 270-1" and "M66" screened for host range expressed distinct symptoms of stunted growth, shortened internodes, thickened stems, necrosis, dwarfism with bushy appearance, yellowing with chlorosis lesions, mixed mosaic, reduced leaf area with twisted and distorted leaves curling downwards and upwards (Figure 6).

## 4 | DISCUSSION

Bean common mosaic virus and BCMNV presence in western Kenya as detected by serology concurs with earlier studies by Mutuku et al., (2018). The viruses increase the risks of farming as a livelihood strategy or a commercial enterprise by decreasing agricultural yields, raising production costs and limiting marketability of food and feed legumes (Akinyemi, Wang, Zhou, Qi, & Wu, 2016; Nicaise, 2014). Despite the importance of beans, virus effects are largely unrecognized by most farmers from western Kenya. In this study, higher disease incidence was observed in the short rain season than in the long rain season, a finding that concurs with studies previously by (Mangeni et al., 2014) who found high virus incidence in common bean fields. This may be attributed to the following: firstly, there is more rain in the long rain season than in the short rain season, which negatively interferes with insect vector populations and hence their ability to transmit viruses; secondly, it has been said that most farmers buy certified seed in the long rain season and use home-saved seed for the short rain season. This action coupled by the fact that there are more aphids transmitting and spreading the virus faster in

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

**TABLE 2** BCMD ELISA results of samples from short and long rain seasons



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.380	0.337	0.178	0.226	0.751	0.581	0.949	1.460	0.797	0.523	0.872	0.492
B	0.519	0.470	1.205	0.521	0.165	0.190	0.599	0.461	0.647	0.621	0.181	0.181
C	0.248	0.206	0.573	0.426	0.454	0.599	0.162	0.261	0.453	0.437	0.927	0.832
D	0.708	0.861	0.195	0.180	0.243	0.323	0.459	0.998	0.488	0.577	0.452	0.428
E	0.194	0.159	0.137	0.279	0.626	0.531	0.266	0.146	0.531	0.642	0.619	0.545
F	0.177	0.198	0.649	0.422	0.450	0.416	0.410	0.625	0.944	1.124	0.131	0.125
G	1.394	0.523	2.127	0.625	0.163	0.134	0.126	0.138	0.437	0.530	0.491	0.530
H	0.140	0.134	0.492	0.492	0.492	0.492	0.492	0.492	0.492	0.492	0.181	0.181

**FIGURE 3** ELISA microtitre plate and plate map showing DAS-ELISA results for some samples positive for BCMNV [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

fields; and thirdly, poor agronomic (untimely control of weeds and other volunteer plants that could be potential hosts for the viruses) and cultural practices such as mixed cropping with other legume crops that are hosts favour the spread of the virus. This is supported by the fact that most farmers do not recognize this virus problem

and so they unknowingly spread the virus by planting seed with high virus load, very minimal crop rotation, and inadequate weed and pest control measures as was observed in some fields.

ELISA detected more BCMNV in bean samples collected from different parts of western Kenya. It appears that BCMNV is the most

**TABLE 3** DAS ELISA spectrophotometric absorbance values conducted for BCMNV

ELISA microtitre plate well	Sample ID	Season	Virus	Spectrophotometric absorbance at 405 nm	Result
A1	141	Short rain	BCMNV	0.380	-
A2	145	Short rain	BCMNV	0.337	-
A3	44	Short rain	BCMNV	0.178	-
A4	46	Short rain	BCMNV	0.226	-
A5	140	Short rain	BCMNV	0.751	+
A6	45	Short rain	BCMNV	0.581	+
A7	47	Short rain	BCMNV	0.949	+
A8	49	Short rain	BCMNV	1.460	+
A9	79	Short rain	BCMNV	0.797	+
A110	225	Short rain	BCMNV	0.523	+
A11	56	Short rain	BCMNV	0.872	+
A12	BCMNV (+) control		DSMZ	0.492	+
B1	432	Short rain	BCMNV	0.519	+
B2	429	Short rain	BCMNV	0.470	+
B3	61–398 g <sup>*</sup>	Short rain	BCMNV	1.205	+
B4	399	Short rain	BCMNV	0.521	+
B5	48	Short rain	BCMNV	0.165	-
B6	220	Short rain	BCMNV	0.190	-
B7	237	Short rain	BCMNV	0.599	+
B8	182	Short rain	BCMNV	0.461	+
B9	180	Short rain	BCMNV	0.647	+
B10	173	Short rain	BCMNV	0.621	+
B11	222	Short rain	BCMNV	0.181	-
B12	Negative control		BUFFER	0.181	-
C1	224	Short rain	BCMNV	0.248	-
C2	226	Short rain	BCMNV	0.206	-
C3	177	Short rain	BCMNV	0.573	+
C4	175	Short rain	BCMNV	0.426	+
C5	181	Short rain	BCMNV	0.454	+
C6	231	Short rain	BCMNV	0.599	+
C7	57	Short rain	BCMNV	0.162	-
C8	404	Short rain	BCMNV	0.216	-
C9	77–231 <sup>*</sup>	Short rain	BCMNV	0.453	+
C10	80–157 <sup>*</sup>	Short rain	BCMNV	0.437	+
C11	133–99 <sup>*</sup>	Long rain	BCMNV	0.927	+
C12	98	Long rain	BCMNV	0.832	+
D1	135	Long rain	BCMNV	0.708	+
D2	91	Long rain	BCMNV	0.861	+
D3	185	Short Rain	BCMNV	0.195	-
D4	177	Short Rain	BCMNV	0.180	-
D5	178	Short Rain	BCMNV	0.243	-
D6	17–	Short Rain	BCMNV	0.323	-
D7	293	Long rain	BCMNV	0.459	+

(Continues)

TABLE 3 (Continued)

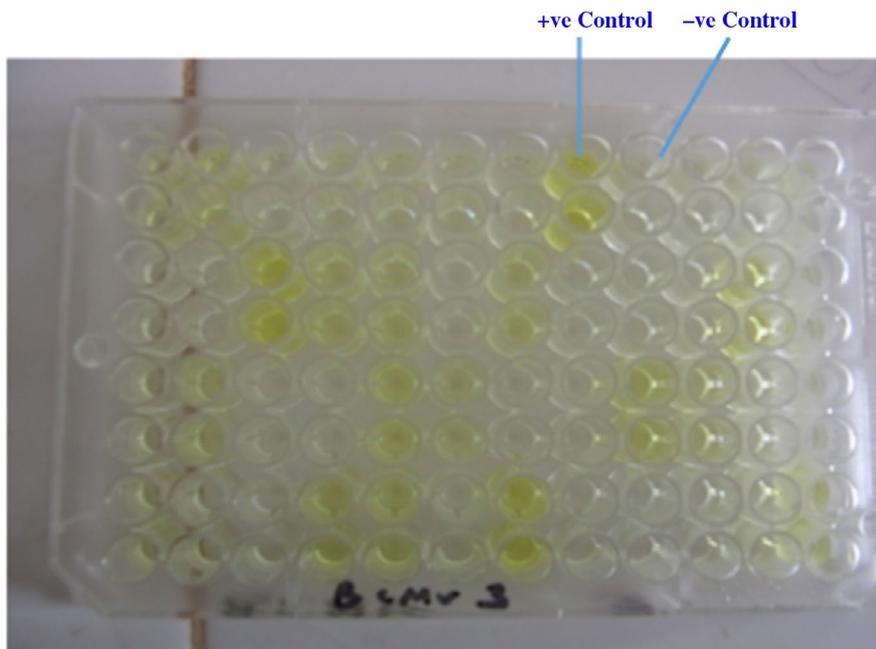
ELISA microtitre plate well	Sample ID	Season	Virus	Spectrophotometric absorbance at 405 nm	Result
D8	289	Long rain	BCMNV	0.998	+
D9	264	Long rain	BCMNV	0.488	+
D10	294	Long rain	BCMNV	0.577	+
D11	291	Long rain	BCMNV	0.452	+
D12	252	Long rain	BCMNV	0.428	+
E1	235	Short rain	BCMNV	0.194	-
E2	232	Short rain	BCMNV	0.159	-
E3	78	Short rain	BCMNV	0.137	-
E4	157	Short rain	BCMNV	0.279	-
E5	146-252 <sup>†</sup>	Long rain	BCMNV	0.626	+
E6	253	Long rain	BCMNV	0.531	+
E7	99	Long rain	BCMNV	0.266	-
E8	92	Long rain	BCMNV	0.146	-
E9	257	Long rain	BCMNV	0.531	+
E10	123	Long rain	BCMNV	0.642	+
E11	129	Long rain	BCMNV	0.619	+
E12	40	Long rain	BCMNV	0.545	+
F1	287	Long rain	BCMNV	0.177	-
F2	265	Long rain	BCMNV	0.198	-
F3	36	Long rain	BCMNV	0.649	+
F4	31	Long rain	BCMNV	0.422	+
F5	54	Long rain	BCMNV	0.450	+
F6	33	Long rain	BCMNV	0.416	+
F7	51	Long rain	BCMNV	0.410	+
F8	167-51 <sup>†</sup>	Long rain	BCMNV	0.625	+
F9	29	Long rain	BCMNV	0.944	+
F10	31	Long rain	BCMNV	1.124	+
F11	250	Long rain	BCMNV	0.131	-
F12	11	Long rain	BCMNV	0.125	-
G1	15	Long rain	BCMNV	1.394	+
G2	63	Long rain	BCMNV	0.523	+
G3	58	Long rain	BCMNV	2.127	+
G4	65	Long rain	BCMNV	0.625	+
G5	255	Long rain	BCMNV	0.163	-
G6	256	Long rain	BCMNV	0.134	-
G7	258	Long rain	BCMNV	0.126	-
G8	259	Long rain	BCMNV	0.138	-
G9	67	Long rain	BCMNV	0.437	+
G10	70	Long rain	BCMNV	0.530	+
G11	68	Long rain	BCMNV	0.491	+
G12	384	Long rain	BCMNV	0.530	+
H1	55	Long rain	BCMNV	0.140	-
H2	45	Long rain	BCMNV	0.134	-
H3	BCMNV (+) control		DSMZ	0.492	+

(Continues)

TABLE 3 (Continued)

ELISA microtitre plate well	Sample ID	Season	Virus	Spectrophotometric absorbance at 405 nm	Result
H4	BCMNV (+) control		DSMZ	0.492	+
H5	BCMNV (+) control		DSMZ	0.492	+
H6	BCMNV (+) control		DSMZ	0.492	+
H7	BCMNV (+) control		DSMZ	0.492	+
H8	BCMNV (+) control		DSMZ	0.492	+
H9	BCMNV (+) control		DSMZ	0.492	+
H10	BCMNV (+) control		DSMZ	0.492	+
H11	(-) control		BUFFER	0.181	-
H12	(-) control		BUFFER	0.181	-

Note: <sup>1</sup>ID's labeled in two numbers were used to differentiate samples picked from different plants showing virus like symptoms on the same farm.



	1	2	3	4	5	6	7	8	9	10	11	12
A	-	+	-	a	-	-	-	+	-	-	-	-
B	+	+	-	-	-	-	-	+	-	-	-	-
C	-	-	+	+	+	-	+	-	-	-	+	-
D	-	-	+	+	+	-	+	-	-	-	+	-
E	+	+	-	-	+	+	-	-	+	+	-	-
F	+	+	-	-	+	+	-	+	+	+	-	-
G	-	-	-	+	+	-	-	-	-	-	+	-
H	+	+	-	+	+	-	+	-	-	-	+	-

FIGURE 4 ELISA microtitre plate and plate map showing TAS-ELISA results for some samples positive for BCMV [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 4 TAS ELISA spectrophotometric absorbance values conducted for BCMV

ELISA microtitre plate well	Sample ID	Season	Virus	Spectrophotometric absorbance value at wavelength of 405nm	Result
A1	157	Short rain	BCMV	0.207	-
A2	152	Short rain	BCMV	0.659	+
A3	153	Short rain	BCMV	0.137	-
A4	154	Short rain	BCMV	0.209	-
A5	94	Short rain	BCMV	0.175	-
A6	95	Short rain	BCMV	0.209	-
A7	96	Short rain	BCMV	0.157	-
A8	BCMV (+) control		DSMZ positive	0.531	+
A9	(-) control		BUFFER	0.181	-
A10	99	Short rain	BCMV	0.179	-
A11	100	Short rain	BCMV	0.130	-
A12	155	Short rain	BCMV	0.173	-
B1	101	Short rain	BCMV	0.652	+
B2	88	Short rain	BCMV	0.689	+
B3	89	Short rain	BCMV	0.151	-
B4	87	Short rain	BCMV	0.523	+
B5	159	Short rain	BCMV	0.170	-
B6	162	Short rain	BCMV	0.527	+
B7	59	Short rain	BCMV	0.187	-
B8	163	Short rain	BCMV	0.657	+
B9	165	Short rain	BCMV	0.148	-
B10	227	Short rain	BCMV	0.209	-
B11	126	Short rain	BCMV	0.246	-
B12	231	Short rain	BCMV	0.156	-
C1	122	Short rain	BCMV	0.202	-
C2	123	Short rain	BCMV	0.154	-
C3	218	Short rain	BCMV	0.622	+
C4	228	Short rain	BCMV	0.594	+
C5	127	Short rain	BCMV	0.711	+
C6	128	Short rain	BCMV	0.209	-
C7	219	Short rain	BCMV	0.568	+
C8	32	Short rain	BCMV	0.180	-
C9	33	Short rain	BCMV	0.203	-
C10	35	Short rain	BCMV	0.256	-
C11	34	Short rain	BCMV	0.547	+
C12	156	Short rain	BCMV	0.136	-
D1	37	Short rain	BCMV	0.164	-
D2	38	Short rain	BCMV	0.184	-
D3	143	Short rain	BCMV	0.587	+
D4	142	Short rain	BCMV	0.518	+
D5	36	Long rain	BCMV	0.777	+
D6	163	Long rain	BCMV	0.171	-
D7	155	Long rain	BCMV	0.968	+

(Continues)

TABLE 4 (Continued)

ELISA microtitre plate well	Sample ID	Season	Virus	Spectrophotometric absorbance value at wavelength of 405nm	Result
D8	329	Long rain	BCMV	0.321	-
D9	163	Long rain	BCMV	0.188	-
D10	319	Long rain	BCMV	0.136	-
D11	164	Long rain	BCMV	0.497	+
D12	314	Long rain	BCMV	0.343	-
E1	160	Long rain	BCMV	0.617	+
E2	165	Long rain	BCMV	0.487	+
E3	93-166*	Long rain	BCMV	0.253	-
E4	91	Long rain	BCMV	0.586	+
E5	166	Long rain	BCMV	0.626	+
E6	95-161*	Long rain	BCMV	0.867	+
E7	161	Long rain	BCMV	0.157	-
E8	96	Long rain	BCMV	0.196	-
E9	318	Long rain	BCMV	0.544	+
E10	320	Long rain	BCMV	0.545	+
E11	101	Long rain	BCMV	0.143	-
E12	151	Long rain	BCMV	0.294	-
F1	321	Long rain	BCMV	0.661	+
F2	102	Long rain	BCMV	2.290	+
F3		Long rain	BCMV		-
F4	323	Long rain	BCMV	0.123	-
F5	228	Long rain	BCMV	1.124	+
F6	191	Long rain	BCMV	2.172	+
F7	190	Long rain	BCMV	0.303	-
F8	351	Long rain	BCMV	0.405	+
F9	187	Long rain	BCMV	0.453	+
F10	375	Long rain	BCMV	0.973	+
F11	189	Long rain	BCMV	0.123	-
F12	372	Long rain	BCMV	0.413	-
G1	374	Long rain	BCMV	0.379	-
G2	109	Long rain	BCMV	0.176	-
G3	377	Long rain	BCMV	0.128	-
G4	385	Long rain	BCMV	0.335	+
G5	379	Long rain	BCMV	0.625	+
G6	380	Long rain	BCMV	0.138	-
G7	378	Long rain	BCMV	0.236	-
G8	191	Long rain	BCMV	0.128	-
G9	186	Long rain	BCMV	0.137	-
G10	121	Long rain	BCMV	1.587	+
G11	94	Long rain	BCMV	0.422	+
G12	198	Long rain	BCMV	0.117	-
H1	187	Long rain	BCMV	0.526	+
H2	120	Long rain	BCMV	1.391	+

(Continues)

TABLE 4 (Continued)

ELISA microtitre plate well	Sample ID	Season	Virus	Spectrophotometric absorbance value at wavelength of 405nm	Result
H3	194	Long rain	BCMV	0.122	-
H4	185	Long rain	BCMV	0.528	+
H5	184	Long rain	BCMV	0.731	+
H6	97	Long rain	BCMV	0.130	-
H7	195	Long rain	BCMV	0.538	+
H8	166	Long rain	BCMV	0.226	-
H9	93-166*	Long rain	BCMV	0.253	-
H10	161	Long rain	BCMV	0.157	-
H11	95-161*	Long rain	BCMV	0.867	+
H12	96	Long rain	BCMV	0.196	-

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



Leaf mosaic, downward leaf curl and yellowing on GLP2 Control

**FIGURE 5** Symptoms expressed on varietal screening for resistance to BCMNV BG12 isolate. ELISA spectrophotometric absorbance value at wavelength of 405 nm for bean variety GLP2 was 0.777, while the negative control had 0.180 [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

predominant virus in common bean in this region. Bean losses are exacerbated by simultaneous infection with two or more viruses, as co-infected plants exhibit severe stunting and have little to no usable yield (Hobbs et al. 2003). Moreover, Taiwo, Kareem, Nsa, and Hughes (2007) suggested that multiple viral infections of cowpeas (a common legume and a host of BCMNV) might result in complete yield loss; therefore, seeds of cultivars with multiple virus resistance are recommended as a means of control. However, co-infection can, in at least some cases, attenuate the effects of individual viruses on plant-vector interactions to the extent that such effects are adaptive for the virus and hence have adverse effects on disease transmission (Peñaflor, Mauck, Alves, De Moraes, & Mescher, 2016). Some viruses infecting beans were not expected to be found by ELISA because the antisera was limited to detection of BCMNV and BCMV the causative agents of BCMV. Therefore, under agricultural intensification system of farming, mostly used in the region, there is a mixture of two or

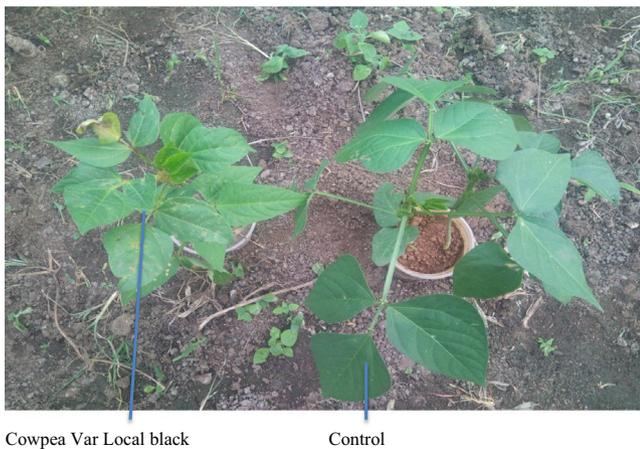
more crops per season per plot or in adjacent plots. Since the vectors of these viruses are polyphagous, they may probe on the popular legumes in the western Kenya indiscriminately thereby picking and spreading the viruses on compatible host plants. With evolution and the effects of climate change, BCMNV may find refuge other host plants. High incidence of BCMV is an indication that not much care is taken to control them, probably because most farmers do not recognize virus diseases and link symptoms to other causes such as mineral deficiency of poor soils. This observation is supported by the fact that most farmers plant their own seed (Opole, Mathenge, Auma, Van Rheenen, & Almekinders, 2003), which have been selected not based on viral disease considerations. Therefore, these farmers need awareness education on virus diseases and how they can be controlled.

Seed-borne viruses have great potential to reduce bean growth and yield because the plant germinates already infected (Marcenaro & Valkonen, 2016; Maule & Wang, 1996). It has been reported (Johansen, Edwards, & Hampton, 1994) that even low seed-borne transmission rates of viruses may be sufficient to cause severe disease epidemics when combined with efficient spread by vectors to susceptible crops. Several virus control measures have been examined and are in use but host plant resistance seems the most economical, practical and environmentally friendly option (Bashir & Hampton, 1996a, 1996b; Wagara & Kimani, 2007). Because BCMNV and BCMV detected by serology were in mixed infections, breeding for single virus resistance may not be of much help. It is therefore worth the effort to breed for multiple virus resistance as suggested by Orawu, Melis, Laing, and Derera (2013) to counter this problem in cowpea. Resistant bean varieties (KK 072 and KK RIL 05) observed in this study present a potential source of resistance in the management of BCMNV. The cultivars could possess the right combinations of resistance genes against BCMNV. Previous studies (Mangeni et al., 2014) have shown these two varieties contain SCAR DNA markers SW13 tightly linked to BCMV dominant resistance *I* gene and have not been probed for recessive resistance genes required to protect the *I* gene that induces hypersensitive black rot symptom of BCMV. The dominant *I* gene is known to inhibit all known

**TABLE 5** Reaction of test plants to BCMNV isolate

Test plant	Variety	BCMNV symptoms	Number of plants inoculated with BCMNV	Number of symptomatic plants at 3 weeks after inoculation	Number of ELISA-positive plants
Bean	GLP 2	ST, D	5	5	5
Bean	RIL 05	D, M	5	4	4
Bean	KK20	ST, M	5	5	5
Bean	KK RIL05	Symptomless	5	0	0
Bean	Imbeko	Symptomless	5	0	5
Bean	Yellow	M, D	5	5	5
Bean	Rosecoco	Y, M	5	5	5
Bean	Wairimu	M	5	5	5
Bean	KK 8	M, Y	5	4	3
Bean	Punda	M	5	5	5
Bean	GLPX92	Y, M	5	5	5
Bean	KK15	Y	5	4	4
Bean	KK/RIL5/Red 13	Symptomless	5	0	5
Bean	KK072	Symptomless	5	0	0
Bean	Okwoto	Symptomless	5	0	4
Bean	KK RIL05/CAL 194	Symptomless	5	0	3
Groundnut	Red Valencia	M	5	2	2
Soybean	Nyala	Y	5	5	3
Green grams	Local	M	5	4	4
Cowpea	Local cream	D	5	3	3
Cowpea	Local red	D	5	4	3
Cowpea	Local black	D, Y	5	4	3
Cowpea	K-80	D	5	3	3
Cowpea	KVU 270-1	D	5	4	4
Cowpea	M66	D	5	3	3

Note: Key: D-deformed leaves, M-mosaic, Y-yellowing, ST-stunting and C-chlorosis (severity scale: 1-mild, 2- moderate and 3-severe).



**FIGURE 6** Veinal yellowing and stunting on cowpea var Local black inoculated to BCMNV BG12 isolate. ELISA spectrophotometric absorbance value at wavelength of 405 nm for cowpea var Local black was 0.530, while the negative control had 0.130 [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

strains of BCMV but can be overcome by necrosis-inducing strains, the BCMNV (Miklas et al., 2000). The dominant *I* gene, however, can be combined with appropriate recessive resistance genes in order to protect it. These combinations can restrict, prevent or delay extreme hypersensitive response in plants infected with BCMNV (Bello et al., 2014). High levels of BCMNV causing viruses have been revealed by our study in bean crops in all growing counties of western Kenya. This is because the viruses are seed-borne and the climate favours virus vector insects (aphids) coupled by the fact that farmers plant their own seed not certified as virus free. The results indicate that seeds are the major source of virus infection, a finding supported by earlier report (Demski, 1975; Johansen et al., 1994; Sastry, 2013) that observed infected seed increased virus incidence by 25% in groundnuts compared to certified seed. Disease-free seed is laudable because insects spread the virus from some source, which if absent there is a likelihood of aphids infected with persistently transmitted plant viruses, becoming a source of inoculum. Despite

the absence of infected plants in the vicinity, if aphids travel far (or carried on clothing/farm equipment) or aphids residing on other plants—they can be a valid source for virus inoculum.

## 5 | CONCLUSION

This study has shown clearly that BCMD is widespread across all the counties in western Kenya and still a major disease in common bean. Viral symptom incidence was found to be high and widely distributed in counties. Higher incidence was recorded during the short rain season due to abundance of aphid vectors than in the long rain season. Symptoms of BCMV and BCMNV are indistinguishable in the field especially in western Kenya, and hence, the two viruses can be distinguished through serology and molecular means. Severity of viral symptoms could be due to mixed infection by two viruses, different strains and abiotic factors in counties or a combination of these.

Majority of popular bean varieties grown in western Kenya are susceptible to BCMNV when inoculated mechanically. Other popular legume varieties such as those of cowpea, groundnut and green grams grown in western Kenya are susceptible to BCMNV infection and therefore potential hosts for the virus. Use of virus resistant variety is the best alternative and durable method to alleviate occurrence of BCMD. Identification of BCMD-resistant legume genotypes is very much essential and screening to identify stable resistance source. However, the nature of disease resistance being complex makes the identification of resistant and susceptible lines cumbersome through conventional screening techniques, and therefore, DNA-based molecular markers such as RAPD, RFLP, AFLP and SSR will be useful in rapid identification of resistance genes linked to certain virus resistance in diverse bean genotypes for efficient breeding and production of suitable varieties (Manjunatha, Rangaswamy, Sah, Nagaraju, & Rudraswamy, 2017).

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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