

Evaluation of Common Bean Cultivars Resistance to the Bean Common Mosaic Necrosis Virus 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 and can cause a yield loss as high as 100%. In Kenya, limited cultivars and exotic genotypes with resistance to BCMV and BCMNV strains have been reported. Reports indicate BCMNV being a widespread virus compared to BCMV in western Kenya. This study therefore, sought to screen popular cultivars for resistance to the virus. Sixteen popularly grown bean cultivars together with cowpea (*Vigna unguiculata*), soybean (*Glycine max*) and groundnut (*Arachis hypogaea*) were planted in a green house in a completely randomized block design with three replicates. The plants were inoculated with BCMNV Enzyme Linked Immunosorbent Assay (ELISA) positive isolate at 3-leaf stage. Data was taken weekly for 3 weeks on type of symptoms expressed and number of plants infected. Ten bean cultivars were susceptible, 4 tolerant and 2 resistant. For improved yields of common bean, farmers should be advised to plant certified seeds for all legumes in the cropping system.

Keywords: Beans, BCMNV, Resistance, Screening

1. Introduction

Western Kenya is one of the food baskets of the country, and a region with about 1/3 of the country's population. The common bean (*Phaseolus vulgaris* L.), is a high protein (22g/100g) crop and the main legume in the cropping system of the region. Small-scale farmers mainly grow the crop. Besides providing food, feed and improving soil fertility, it also improves the incomes of the farmers. Despite its importance, common bean productivity is declining and yields obtained of less than 1.0 t ha⁻¹ are low compared to a production potential of 1400 – 2000 kg ha⁻¹ [1, 2]. This has happened at a time when a greater variety of high protein food sources are needed to ensure food security. Decreasing yields are attributed to poor access to improved seeds, declining soil fertility, drought, and high incidence of pests, diseases and unpredictable weather. One key biotic stress

factor that reduces yield is plant viruses, and the common bean is one of the most susceptible legumes to virus infection. The bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) cause significant yield losses and are both seed borne [3]. 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 [4].

The serological relationships of the two viruses (formally serotype A and serotype B and treated as strains of BCMV) was determined and from sequence analysis, it was agreed that they be treated as distinct viruses [5].

BCMV and BCMNV are the most common and most destructive viruses that infect common beans (*Phaseolus vulgaris* L.) as well as a range of other cultivated and wild legumes [6]. Yield losses due to BCMV and BCMNV can be as high as 100% [7].

Mottling and malformation of the primary leaves is an indication that the primary infection occurred through seed [8]. 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-induce black root disease [9]. 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-1²*, *bc-2*, *bc-2²* and *bc-3*), with the dominant *I*-gene in order to provide broad spectrum possible resistance [10].

This study therefore sought to determine the resistance of popular bean cultivars to the disease.

2. Materials and Methods

2.1. Seed Germination and Mechanical Inoculation

Five seeds from each of the 16 popularly grown bean varieties; and cowpea, soybean, green grams and ground nut were sowed in plastic pots with 3 replicates in a greenhouse.

The inoculum was prepared by grinding BCMNV infected leaves at the rate of 1.0 g tissue and 10 ml of 0.1M phosphate buffer, pH7.0 containing 0.2% sodium sulfite using a chilled pestle and mortar. The test plants were separately inoculated at the 3 leaf stage with BCMNV isolate from western Kenya. Data was 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 second week post inoculation by Double Antibody Sandwich ELISA. Susceptible plants expressed typical symptoms of BCMV. Symptomatic plants positive for BCMNV by DAS ELISA were graded susceptible, symptomatic plants positive for BCMNV graded tolerant while asymptomatic plants negative for BCMNV graded resistant.

2.2. Enzyme-Linked Immunosorbent Assay (ELISA)

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

Between incubations, 3 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.

Leaf tissues of virus-inoculated plants were ground 1:10 (w/v) in sample extraction buffer (PBST + 2% PVP (Serva PVP-1S polyvinyl pyrrolidone). To detect BCMNV, Double Antibody Sandwich (DAS)-ELISA was conducted essentially as described following manufacturer's instructions [11]. Microtiter plates were coated with BCMV IgG diluted 1:1000 (v/v) in coating buffer ((1.59 g sodium carbonate

(Na₂CO₃), 2.93 g sodium bicarbonate (NaHCO₃), 0.20 g sodium azide (NaN₃), Dissolved in 900 ml H₂O, adjusted pH to 9.6 with HCL and made up to 1l) and incubated for 2h at 37°C. To block, 2% skimmed milk in PBST (200µl/well) were 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 BCMV infected plants were used as negative and positive controls, respectively. IgG *alkaline phosphatase* conjugate, diluted 1:1000 (v/v) in conjugate buffer (PBST + 2% PVP + 0.2% egg albumin (Sigma A-S253)), was added and incubated for 2h at 37°C. The substrate, *p*-Nitrophenyl phosphate diluted 1 mg/ml in substrate buffer (DEA+H₂O +NaN₃) was added and incubated for 1h at 37°C or until there was colour change. Quantitative measurements of the *p*-nitrophenol substrate conversion resulting in yellow color were made by determining the absorbance at 405 nm (A405) in a Biotek® model spectrophotometer (Labsystems Co., Finland). Twice the mean absorbance readings of healthy controls were used as the positive thresholds.

3. Results

Sixteen popularly grown common bean cultivars in western Kenya inoculated with BCMNV isolate from western Kenya (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 1).



Leaf mosaic and yellowing on GLP2 Control

Figure 1. Symptoms expressed on varietal screening for resistance to BCMNV isolate.



Control

Figure 2. Veinal yellowing and stunting on Cowpea var 'Local' inoculated with BCMNV.

Table 1. 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

Key: D-deformed leaves, M-mosaic, Y-yellowing, ST-stunting, C-chlorosis,, (Severity scale:1-Mild, 2- Moderate, 3- Severe)

From Table 1, Sixteen popularly grown common bean cultivars in western Kenya inoculated with BCMNV BG12 isolate from western Kenya (Kakamega) in a greenhouse exhibited typical virus symptoms such as leaf mosaic, downward leaf curl and yellowing as shown on popular variety GLP 2 (Figure 1). 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 three weeks post inoculation by both symptomatology and DAS ELISA. Popularly grown grain legumes, groundnut cv 'Red Valencia', soybean cv 'Nyala', greengrams 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 2).

4. Discussion

BCMNV presence in western Kenya as detected by serology concurs with earlier studies by Mutuku *et al.*, (2018)

[12]. BCMNV 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 [13]. Despite the importance of beans, virus effects are largely unrecognised by most farmers from western Kenya. Seed borne viruses, have great potential to reduce bean growth and yield because the plant germinates already infected [14]. It has been reported 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 [15]. Several virus control measures have been examined and are in use but host plant resistance seems the most economical, practical and environmentally friendly option [16]. 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 (2013) to counter this problem [17]. High levels of viral diseases have been revealed by our studies in bean crops in all growing counties of Western Kenya. This is because, most of the viruses are seedborne and the climate favours virus vector insects (aphids) coupled by the fact that farmers plant their own seed not certified for virus freedom. The results indicate that seeds are the major source of virus infection, a finding supported by earlier report that observed that infected seed increased virus incidence by 25% in

groundnuts compared to certified seed [18]. This is laudable because insects spread the virus from some source, which if absent there will be no virus.

5. Conclusion

This study shows most popular bean varieties grown in western Kenya are all susceptible to BCMNV when inoculated mechanically. Other popular legume varieties such as those of cowpea, groundnut and greengrams grown in western Kenya are also 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 to assess genetic diversity of genotypes.

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