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DISTRIBUTION AND PATHOGENIC CHARACTERIZATION OF BEAN COMMON MOSAIC VIRUS (BCMV) AND BEAN COMMON MOSAIC NECROSIS VIRUS (BCMNV) IN WESTERN KENYA

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ABSTRACT

Common bean (*Phaseolus vulgaris* L) is an important food legume crop in Kenya as a major source of protein in human diet. The yield is 530 kg ha^{-1} and the country production is estimated at 529,265 tons. Bean production is declining in Kenya due to various abiotic and biotic factors. Virus diseases are a major yield reduction factor in bean production. Among the viruses infecting beans, *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrosis virus* (BCMNV) are most wide spread. The current status of BCMV in western Kenya is not well documented. The main objective of this study was to carry out a diagnostic survey for Bean common mosaic disease (BCMD) in bean growing areas of western Kenya, characterize its causal agent and evaluate host resistance to BCMV and BCMNV. BCMD incidence varied across the AEZs being high in LM2 (17.90%) and lowest in LM3 (3.75%). Pathogenic characterization studies of isolates of BCMV and BCMNV on common bean collected from western Kenya revealed the presence of three pathogroups PG IV, PG VI and Pathogroup VII. Serological analysis of the samples revealed mixed infections of BCMV and BCMNV in common bean. Positive samples for BCMV and BCMNV detected by enzyme linked immunosobent assay, ELISA, were categorized into 3 isolates X, Y and Z. Two new pathogroups IV and VII belonging to BCMNV are shown to occur for the first time in the region. The presence of two SCAR DNA markers SW13 and SBD5 associated with the dominant *I* gene and recessive *bc-1²* resistance gene respectively to BCMV in twelve lines from the popular Andean Diversity Panel (ADP) from Kenya confirmed the presence of *bc-1²* gene in 75% of the lines whereas the *I* gene was present in only 3 (25%) of the lines

Keywords: *Common bean (Phaseolus vulgaris L), Bean common mosaic disease, Bean common mosaic virus, Bean common mosaic necrosis virus, Pathogroups, Screening.*

INTRODUCTION

The common bean is widely grown in the medium and high rainfall regions of the Kenya by small-scale subsistence farmers (Wortmann ., 1998; Mwaniki, 2002: Wagara, 2007). Although per capita consumption has increased to 66 kg per year in Kenya (Buruchara , 2011). The yields of 530 kg ha^{-1} and the country production estimated at 613,902 metric tons (FAO, 2012) are lower compared to a production potential of 1400 – 2000 kg ha^{-1} (Katungi ., 2009) but attainment of that potential is constrained by diseases. Among the diseases, Bean common mosaic disease caused by BCMV and BCMNV is a widespread disease of common bean in Kenya (KARI/CIAT, 1991; Gad, 2003; Odendo ., 2004). Disease incidence as high as 100% with yield losses of 35–98% in beans has been reported to be caused by BCMV and BCMNV (Hampton, 1975; Galves & Morales, 1989; Wortmann ., 1998; Albrechtsen 2006).

BCMV and BCMNV is both seed-borne and aphid-transmitted in a non-persistent manner (Hongying ., 2002). Depending on the common bean cultivar and stage of development, seed transmission rate varies from less than 1% to 50 % (Hong-Soo Choi ., 2006). In susceptible common bean genotypes at typical growing temperatures (26-28˚C), a severe mosaic, curling of the leaves, vein banding, mottled and malformed pods can appear (Bos, 1971). At elevated temperatures (above 30˚C) systemic necrosis appears. The type of symptom induced depends on type of infection (either seed borne or vector transmitted), cultivar type, strain of the virus, and age of the plant at infection. The BCMV may incite three types of symptoms: mosaic, systemic necrosis (black root), or local lesions or leaf malformation.

Based on symptomatology and serology, several strains of BCMV exist with different virulence and have been categorized into pathogenicity groups PGI (NL 1, US 1, PR 1), PGII (NL 7), PGIII (NL 8), PGIVa (US 5), PGIVb (US 4; US 3, NL 6), PGVa (US 2), PGVb (NL 2), PGVIa (NL 3), PGVIb (NL 5), and PGVII (US 6, NL 4) based on their virulence on 11 differential cultivars established by Drijfhout, (1978). These strains fall into two different serotypes: serotype A comprises NL 8, NL 3 and NL 5 pathotypes; and serotype B encompassing the other non necrosis inducing strains of BCMV (Vetten ., 1992). Serotype A has been renamed as *Bean common mosaic necrotic virus* (BCMNV) based on serological and symptomatic differences between the two groups (McKern ., 1992; Vetten ., 1992). The strains in PG I, PG II and PG III have one pathogenicity gene, the strains in PG IV and PG V have two pathogenicity genes, and the strains in PG VI and PG VII have three pathogenicity genes. According to this grouping, BCMV strains were classified into PG I, PG II, PG III, PG IV, PG V, PG VI and PG VII (Drijfhout ., 1978; Drijfhout, 1994; Silbernagel ., 2001).

Different strains of BCMV and BCMNV can be distinguished based on their reactions on differential hosts (Drijfhout ., 1978; Tu, 1986; Omunyin ., 1995; Saiz ., 1995)

PGs are determined by the ability of virus isolate to systemically infect a set of differential bean cultivars possessing defined combinations of recessive and dominant resistance genes.

The knowledge of the spectrum of strains of BCMV and BCMNV in Kenya is lacking and is an important pre-requisite to exploit disease management through host resistance, which is the only durable and economic method of managing BCMD.

The host resistance genes involved in BCMV and BCMNV resistance form two groups; The dominant strain unspecific gene is gene *I* and the recessive strain unspecific gene is *bc-u*. All strain specific genes, *bc-1*, *bc-1²*, *bc-2*, *bc-2²* and *bc-3*, are recessive and are independently inherited except for the allelic pairs *bc-1, bc-1²* and *bc-2, bc-2²*. The dominant *I* gene is known to inhibit all known strains of BCMV but can be overcome by necrosis-inducing strains, the BCMNV. They stimulate systemic hypersensitive response and infected plants develop systemic lethal necrosis (Irena and Jelka, 2004). However, dominant *I* gene can be protected by combining it with recessive resistance genes. These combinations can restrict, prevent or delay extreme hypersensitive response in plants infected with BCMV.

The objectives of this study were, therefore, to: (1) assess the distribution and severity of BCMD in bean growing areas of western Kenya; (2) characterize the causal agent (BCMV or BCMNV) using differential cultivars; (3) screen for resistance to BCMV and BCMNV in popularly grown bean varieties/lines from western Kenya using DNA SCAR markers.

MATERIALS AND METHODS

Two extensive surveys were conducted that covered 15 sub counties (Fig. 6) in the major bean production areas of western Kenya. The sub counties are in the following agro-ecological zones (AEZs): Lower Midland 1 (LM1), Lower Midland 2 (LM2), Lower Midland 3 (LM3), Lower Midland 4 (LM4), Upper Midland 1 (UM1), Upper Midland 3 (UM3), and Lower Highland1 (LH1). The survey covered an altitudinal range from 1137 masl in LM4 (S00.33663; E034.34580) to 1995 masl (N00.11519; E035.10233) in LH1 during the short and the long rain seasons. Farms were surveyed in June and December 2013 and where possible samples were taken from two fields per farm for serological and molecular analysis. A GPS device (Magellan Triton "Windows CE Core 5.0" X11-15302) was used to measure the coordinates and altitude of the location. Data obtained from the surveys (altitude, virus disease incidence and severity) were recorded. Visual symptoms were assessed just before flowering and classed as mosaic, leaf rolling, dwarfing, chlorosis, or a combination of these. Each field was divided into quarters, 100 randomly

selected plants were observed in each quarter, and the results were expressed as percentage of symptom-bearing plants in the field.

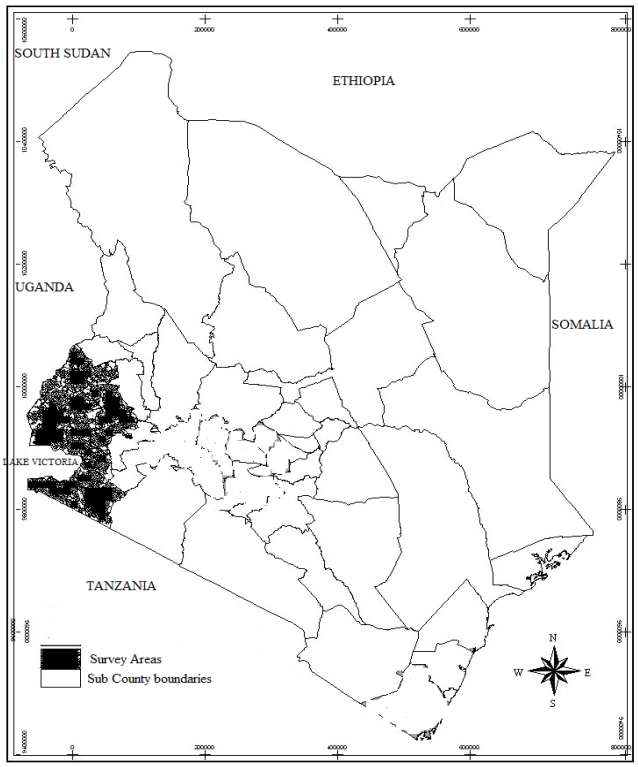

Figure 6. Map of Kenya showing locations of sub counties of western Kenya surveyed

Detection of BCMV and BCMNV

Enzyme linked immunosorbent assay (ELISA) was used in detection of BCMV and BCMNV. Detection of BCMV was performed using triple antibody sandwich, TAS ELISA while double antibody sandwich ELISA detected BCMNV.

TAS ELISA was done as described by Thomas ., (1986) but with minor modifications. Microtitre plates (96 wells) were coated with BCMV IgG diluted 1:1000 (v/v) in a coating buffer and incubated for 2h at 37°C. After washing, blocking was done by adding 2% skimmed milk in PBST (200µl/well) and incubated for 30 min at 37°C. Sap extracts prepared by grinding 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 plants and of plants infected with known BCMV were used as negative and positive controls, respectively. MAbs raised against BCMV were used in detecting antibodies at dilutions of 1:100 (v/v) in conjugate buffer. 100 µl of each supernatant dilution were loaded onto microtiter plates and incubated for 2 h at 37°C. After washing the plates, an alkaline phosphatase labeled Rabbit-anti-mouse RaM-AP, (DSMZ, Germany) diluted 1:1000 v/v in conjugate buffer was added (100 µl/well) and the plates incubated for 45 min at 37°C. The substrate, p-Nitrophenyl phosphate diluted 1 mg/ml in substrate buffer (DEA+H_2O +NaN_3) was added and incubated for 1h and 2h at 37°C. Quantitative measurements of the p-nitrophenol substrate conversion resulting in yellow color were made by determining the absorbance at 405 nm (A_{405}) in a Biotek® model spectrophotometer (Labsystems Co., Finland). The mean absorbance readings of non-infected controls were determined and twice the values were used as the positive thresholds.

DAS-ELISA was done essentially as described by Clark and Adams (1977). For detection of BCMNV by DAS-ELISA, Microtiter plates were coated with BCMNV IgG diluted 1:1000 (v/v) in coating buffer and incubated for 2h at 37°C. Sap extracts prepared as described above were added and incubated overnight at 4°C. Extracts from healthy plants and of plants infected with known BCMNV were used as negative and positive controls, respectively. IgG alkaline phosphatase conjugate, diluted 1:1000 (v/v) in conjugate buffer, was added and incubated for 2h at 37°C. Substrate addition, incubation and absorbance readings were as described above.

Characterization of BCMV and BCMNV isolates using differentials

A standard set of differential host cultivars at Michigan State University (MSU) USA, Bean breeding program were planted in pots in a greenhouse and inoculated with BCMV isolates prepared from systemically infected leaves macerated in a chilled sterilized 0.01M cold phosphate buffer (K_2HPO_4 +KH_2PO_4), pH 7.0, containing 0.2% sodium sulphite and 0.01M Mercapto-

ethanol (1:6 [w/v] tissue: buffer). Inoculation on plants at the three-leaf stage was done using the rub method on carborundum-dusted leaves. The plants were observed weekly for symptom development for 5 weeks and samples for each observation were taken for ELISA tests to confirm if symptoms were due to infection or other factors.

Evaluation of host resistance genes to BCMV using SCAR DNA markers.

Twelve genotypes selected from the Andean diversity panel (ADP) from Kenya and promising lines were sown in pots containing super mix humus rich soil and 1g of di-ammonium phosphate per plant in the greenhouse at MSU using a standard procedure used by MSU bean breeding program; Eight seeds per popular bean variety from Kenya and promising lines were sown per pot. After germination, the seedlings were thinned to six plants. The test plants were inoculated with BCMV isolates X, Y, Z, NL 3 and NL 4 inoculum as described above and examined daily for BCMD symptom development one week after inoculation on weekly basis for 5 weeks.

Data collected included the type of symptoms expressed as mosaic, mild mosaic, leaf rolling, local lesions and top necrosis on leaves and stems.

Total DNA was extracted from plant tissues using the CTAB method as described by Murray and Thompson (1980). Plant leaves were ground in liquid nitrogen using a mortar and pestle and homogenized in extraction buffer (Tris + NaCl + EDTA + β- Mercaptoethanol) (20 ml/g leaf tissue). To the slurry, 100 µl of 20 mg/ml proteinase K was added and incubated for 15 min on ice while stirring. The mixture was transferred to a new tube, adjusted to 2% sodium dodecylsulphate (SDS) and incubated at 65°C for 35 min. To pellet cell debris, the homogenate was centrifuged for 10 min at 10,000 rpm at room temperature. The supernatant was adjusted to 1.4 M NaCl before adding 0.1 volume of adjust buffer (CTAB + Tris + EDTA) and then adjusting it to 1% RNase A (20 mg/ml). This was then incubated at 65°C for 10 min and allowed to cool on ice for 15 min. To further denature plant cell constituents and release nucleic acid, supernatants were washed twice with an equal volume of chloroform and centrifuged at 10,000 rpm for 10 min.

Total nucleic acids were precipitated by addition of 0.8 vol. isopropanol followed by incubation on ice for 30 min and centrifugation at 10,000 rpm for 20 min at 4°C. The final pellets were washed with 70% ethanol, dried at 37°C for 5 min and resuspended in appropriate amount of sterile bi-distilled water.

DNA samples were then amplified using primer pair of Forward and Reverse primers for SW13 and SBD5 markers. The set of SW13 primers used to amplify genomic DNA were, SW1; Forward 5'CAC AGC GAC ATT AAT TTT CCT TTC 3'; Reverse 5' CAC AGC GAC AGG AGG AGC TTA TTA 3' (Haley ., 1994; Melotto ., 1996, Fourie ., 2004) and SBD5 Forward 5' GTG CGG AGA GGC CAT CCA TTG GTG 3'; Reverse 5' GTG CGG AGA GTT TCA GTG TTG ACA 3' (Miklas ., 2000). Three microliter of total DNA was subjected to PCR in a total volume of 24 µl. The reaction mix consisted of 2.4 µl of 10 × reaction buffer, 4.8 µl of 10 mM dNTPs, 3 µl of each primer, and 0.3 µl of *Taq* DNA polymerase (Invitrogen), and 13.5 µl of water. Reactions were run in an Eppendorf Mastercycler under the following thermal cycling conditions: For SW13, 34 cycles of 10s at 94ºC, 40s at 67ºC, and 2 min at 72ºC; followed by one cycle of 5 min at 72ºC while for SBD5, 34 cycles of 10s at 94ºC, 40s at 65ºC, and 120s at 72ºC; followed by one cycle of 5 min at 72ºC.

The predicted length of DNA amplified by primer combination SW 13 is 690 bp and that of SBD 5 is 1250 bp. To determine the presence and size of the PCR products, 10 µl of the cycled PCR reaction mix were analyzed by electrophoresis in 1% agarose gels containing 0.3 µg/ml Ethidium Bromide in TAE buffer. The gel was run at 120 volts and maximum current for 45 min before being viewed under UV light and photographed.

RESULTS AND DISCUSSION

RESULTS

The survey was from the lowest Midland 4 (LM4) agro eco zone at an elevation of 1137m above sea level (S00.33663; E034.34580) to the lower Highland 1 (LH1) agro eco zone at an elevation of 1995m above sea level (N00.11519; E035.10233). A total of 498 bean farms were visited, 329 during long rain and 169 during short rain season.

Symptoms observed in the field during short and long rain seasons were mosaic on leaves, leaf distortion, downward curling and mottling, vein necrosis and local lesions.

193 samples from the field subjected to ELISA revealed the presence of 49 BCMNV positive and 40 BCMV positive samples as shown on Table 1. The total number of samples from the long rain season positive was 16 for BCMV and 46 for BCMNV while during the short rain season, 24 samples were BCMV positive and 3 BCMNV positive. Three samples from lower highland 1 had mixed infection with both BCMV and BCMNV from the short rain season. BCMNV infection was very high during the short rain season as compared to BCMV. Mixed infection with the these two viruses causing BCMD was found in samples from the AEZs LH 1, LM1, LM 2 and UM 1 during short rain season as compared to only LH 1 during long rain season that had three samples.

Table 1. ELISA results of samples collected from short and long rainy seasons

		BCMV/BCMNV presence			
		BCMV (Short rain)	BCMNV (Short rain)	BCMV (Long rain)	BCMNV (Long rain)
AEZONE	N				
LH1	45	5	21	3	3
LM1	23	6	15	2	0
LM2	20	4	7	9	0
LM3	26	0	1	4	0
LM4	22	0	0	3	0
UM1	57	1	2	3	0
Total	193	16	46	24	3

Agro ecological zones (AEZ); LM1 – lower midland zone 1; LM2 – lower midland zone 2; LM3 – lower midland zone 3; LM4 – lower midland zone 4; UM1 – upper midland zone 1; LH1 – lower highland zone 1

Results of inoculation of known differential cultivars with two known and three unknown isolates in the greenhouse as shown is Fig. 1, Fig. 2 and Table 2, the isolates X, Y and Z appear to belong to 3 pathogenicity groups, IV, VI and VII. SCAR DNA markers (SW15, SBD5 tightly linked to I and bc-1^2 were used to identify the presence or absence of these important resistance genes to BCMV as shown in Table 3, Fig. 4 and Fig. 5).

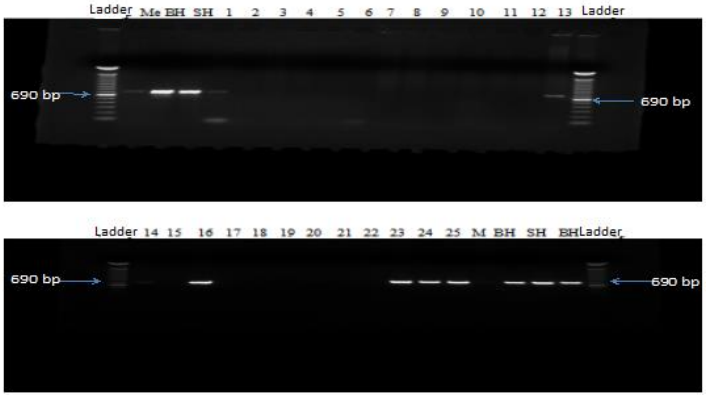

Figure 4. Gel electrophoresis of bean lines evaluated for presence of SW13 marker tightly linked to I gene; Me = Merlot, BH=Blackhawk, SH=Seahawk

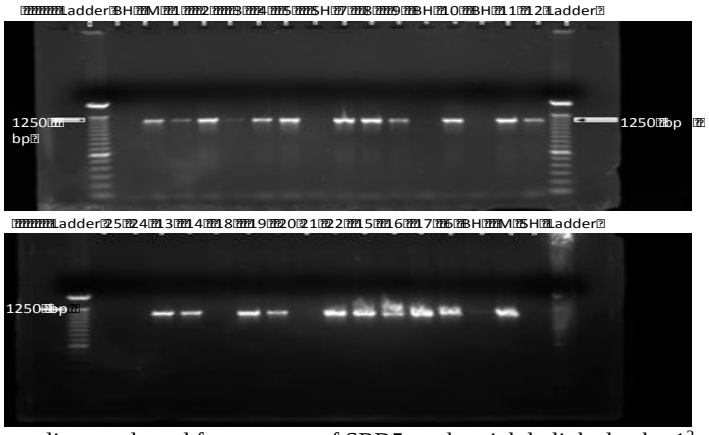

Figure 5. Gel electrophoresis of bean lines evaluated for presence of SBD5 marker tightly linked to bc-1^2 gene; BH=Blackhawk, M=Merlot

Othello US-5 Control 96 US 1006

Figure 1; Othello and 92 US 1006 differentials inoculated with Z isolate of BCMV showing BCMD symptoms in the greenhouse, 1-US-5 control.

Othello Tebo WA Olathe

Figure 2; Differentials Othello and Olathe inoculated with Z, Tebo WA(NL 4)- control.

Table 2. Reaction of Differential cultivars to the virus isolates

		Reaction of host genotype to virus strain				
Differential cultivar	Genotype	NL-3	US-5	Isolate X	Isolate Y	Isolate Z
Black hawk	II	Top necrosis	Top Necrosis	Top Necrosis	Top Necrosis	Top Necrosis
Olathe	ii, bc-1^2	Mild mosaic	Mild Mosaic	Mosaic	Mosaic	Mosaic + Mottling
Othello	ii, bc-2^2	No reaction	Mild mosaic	No reaction	Mosaic	Interveinal yellowing
Raven	II. bc-3	No reaction	No reaction	No Reaction	No Reaction	No reaction
92 US 1006	II, bc-2^2	Local lesions	Mild Mosaic	Necrotic lesions	Top Necrosis	Mosaic
Kodiak	II, bc-1^2	Vein necrosis	Mild Mosaic	Vein necrosis	Top Necrosis	Local Lesions
G99750	ii, bc-3	No reaction	No reaction	No Reaction	No reaction	Mild Mosaic
Y11405	ii	Mild mosaic	Mild mosaic	Mosaic	Mosaic+ leaf Mottling	Mosaic

Reactions in Table 4 included Classical leaf mosaic, yellowing was expressed by differential cultivars inoculated with isolate Y and Z whereas Differential cultivars inoculated with isolate X had Necrotic lesions localized initially in 92 US 1006 but Vein necrosis was expressed in Kodiak

NL 3 was used as a control for serotype A while US 5 as a control for serotype B.

I wish to propose since country code for Kenya is KE we use it to identify the isolates as KE X, KE Y and KE Z

Three local lines, Tulu, KK072 and RIL 05 were found to posses the I gene while none of the 12 ADP lines from western Kenya had the I gene (Table 3). However, all the 12 ADP lines were found to possess the recessive resistance gene bc-1^2 (Table 4) while only 7 of the popularly grown bean lines from western Kenya were found to carry this gene. The rest of the 12 popular lines screened for resistance were susceptible to the common strains of BCMV and BCMNV in pathogroups IV, VI and VII that were found infecting common bean across western Kenya. ADP lines from western Kenya were susceptible to strains US 5, NL 3 and NL 4 and the three isolates from Western Kenya belonging to Pathogroup IV, VI and VII.

Table 3. Results of SW13 and SBD5 marker on ADP and Kenyan lines

No.	Lines	SW13	SBD5	No.	Lines	SW13	SBD5
	Merlot	-	+	14	Teso	-	+
	Blackhawk	+	-	15	Rose coco	-	+
	Seahawk	+	-	16	Tulu	+	+
1	ADP 1	-	+	17	GLP2	-	+
2	ADP 39	-	+	18	KK/RIL5/Red13	-	-
3	ADP 186	-	+	19	Osogo	-	+
4	ADP 24	-	+	20	Rosebella	-	+
5	ADP 188	-	+	21	KK 20	-	-
6	ADP 466	-	+	22	GLP 585	-	+
7	ADP 467	-	+	23	KK 072	+	-
8	ADP 480	-	+	24	RIL 05	+	-
9	ADP 481	-	+	25	II-bc-3	+	-
10	ADP 482	-	+		Merlot	-	+
11	ADP 483	-	+		Blackhawk	+	-
12	ADP 528	-	+		Seahawk	+	-
13	Punda	-	+				

Positive controls for SW 13, Blackhawk and Seahawk; Negative control for SW 13, Merlot.
Positive controls for SBD 5, Merlot; Negative control for SBD 5, Blackhawk and Seahawk.
Punda, Rosecoco, GLP 2, Osogo, Rosebella, KK20, RIL 05, Tulu, Teso, GLP 585, KK/RIL 05/Red 13 are among the most the popular bean varieties in western Kenya

DISCUSSION

Strains that cause BCMD exist with different virulence and have been categorized into discrete pathogenicity groups. The results of serological tests and symptom expression showed that the isolates X and Y belong to serotype A in pathogroup VI, whereas isolate Z induced symptoms attributable to pathogroup IV and VII under serotype B.

The controls NL 3, NL 4 and US 5 were used to test for resistance since the symptoms observed in the field during survey resembled the documented symptoms of the strains on susceptible genotypes (Drijfhout, 1978; Vetten ., 1992).

Analysis of resistance genes revealed three lines from Western Kenya expressed top necrosis, a response to virus infection that results in plant death. This is characteristic of cultivars possessing the I gene. The I gene by itself conditions either an immune or temperature-dependent hypersensitive resistance to BCMV strains. However, when the unprotected I gene is challenged by BCMNV strains, a temperature-independent hypersensitive response called top necrosis or black root results from systemic necrosis and leads to plant death (Irena and Jelka, 2004).

Presence of bc-1^2 alone is ineffective against strains of BCMV and BCMNV present in western Kenya and therefore combination with the dominant I gene is key to confer resistance. Symptoms observed including vein necrosis, local lesions and mosaic expressed by hosts varied from mild to severe as shown in Fig. 3. This suggests differences in recognition events between products of host resistance genes and matching pathogen avirulence gene. A specific reaction by the host to virulence factors from the pathogen is genetically controlled in order for resistance to symptom formation to take place. The BCMV and BCMNV strains found in Kenya are mostly temperature independent necrotic strains that are more virulent on cultivars. Furthermore, it is noted to occur in mixed infection with other strains inducing mosaic, therefore causing severe infection on beans. Genetics of resistance to BCMV and BCMNV in various lines and land races from East.

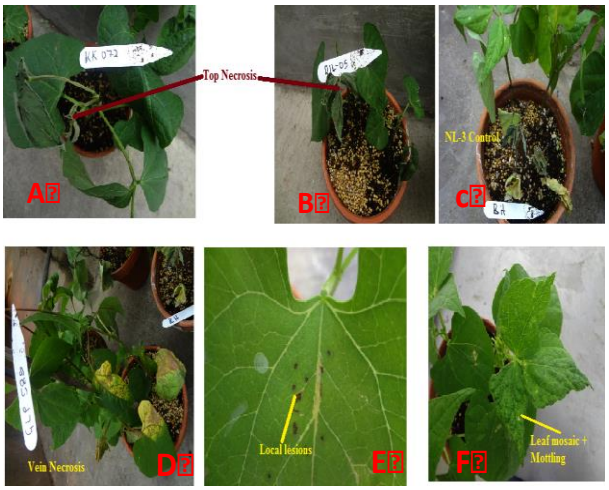

Figure 3. Symptoms induced by NL-3 strain of BCMNV in some Kenyan bean varieties screened

The reaction induced in Fig. 3 A and B above is top necrosis, beginning with the plant shoot and progresses downwards to older plant parts. C – top necrosis on a control for presence of I gene. D-GLP 585 with vein necrosis on leaves starting at point of inoculation and spreads from cell to cell. E- local lesions-necrotic spots localized at inter-veinal region. F- leaf mosaic and mottling

Africa has been explored on a limited scale. Evaluation of available bean lines and breeding for resistance against BCMV is the most durable method of managing BCMD.

The dominant I gene confers immunity or temperature-dependent hypersensitive response to most strains of BCMV, and temperature-independent systemic necrosis to strains of BCMNV (CIAT 2012). The I gene has been tagged with the dominant SCAR marker SW13, which is commonly employed in breeding programs (Drijfhout ., 1978).

The I gene in combination with other recessive genes (bc-1^2, bc-2^2, bc-3) provides resistance to all strains of BCMV and BCMNV, but due to their epistatic interaction among them and the dominance nature of current markers it is not possible to indirectly select plants with desirable allele combinations (Melotto ., 1996).

In conclusion, The seven popularly grown bean lines from western Kenya that were found to possess the I gene provide the right genetic background in which to introgress other resistance genes, including root rot resistance. Interestingly neither KK20 nor KK072, which have root rot resistance, carries the I gene. Analysis of bean lines and land races from Kenya and East Africa to identify available resistance genes for pyramiding would increase efficiency in breeding for resistance against BCMV/BCMNV and improve yield among local bean cultivars.

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REFERENCES

Albrechtsen SE. 2006. Testing methods for seed transmitted viruses: principles and protocols. Oxfordshire (UK): CABI Publishing.

Barnett OW. 1986. Surveying for plant viruses: Design and considerations. In: Plant Virus Epidemics, pp. 147-166. Academic Press. Australia.

Bello MH and Miklas PN. 2014. Development of potential breeder-friendly markers for the *I* gene using bulked segregant analysis and whole-genome sequencing. Annual Bean Report. BIC. 2014, Vol. 57 pg 17-18

Berger PH, Wyatt SD, Shiel PJ, Silbernagel MJ, Druffel K and Mink GI. 1997. Phylogenetic analysis of the Potyviridae with emphasis on legume-infecting potyviruses. Archives of Virology 142: 1979-1999.

Bos L. 1971. Bean common mosaic virus. Descriptions of Plant Viruses 73

Buruchara R, Chirwa R, Sperling L, MukankusI C, Rubyogo JC, Muthoni R and Abang MM. 2011. Development and delivery of bean varieties in africa: the pan- africa bean research alliance (PABRA) model. African Crop Science Journal, Vol. 19, No. 4, pp. 227 - 245

Clark MF and Adams AN. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34: 475-483.

Clark MF and Bar-Joseph M. 1984. Enzyme immunosorbent assays in plant virology. Pages 51–85 in Methods in virology, edited by K. Maramorosch and H. Koprowski. Academic Press, New York, USA.

Cooper JI and Edwards ML. 1986. Variations and limitations of enzyme-amplified immunoassays. Pages 139–154 in Developments and applications in virus testing, edited by R.A.C. Jones and L. Torrance. Association of Applied Biologists, Wellesbourne, UK.

Gad L and Thottappilly G. 2003. Virus and Virus-like Diseases of Major Crops in Developing Countries. Kluwer Academic Publishers, Dordrecht. 800.

Drijfhout E. 1991. Bean common mosaic virus. In: Compendium of bean diseases. Hall R. (Ed.). APS Press, The American Phytopathological Society, Minnesota: 37-39.

Drijfhout E, Silbernagel MJ and Burke DW. 1978. Differentiation of strains of Bean common mosaic virus. Neth. J. Plant Pathology, 84: 13-26.

Drijfhout E. 1978. Genetic interaction between Phaseolus vulgaris and bean common mosaic virus with implications for strain identification and breeding for resistance. Pudoc, Wageningen: Agricultural Research Reports. 872 p.

FAOSTAT. 2012. Food and Agriculture Organization at www.fao.org

Galvez GB and Morales FJ. 1989. Aphid transmitted viruses In: Schwartz HF and Pastor Corrales MA (edn): Bean production problems in the tropics, 2nd edn. CIAT, Cali, Colombia, pp 333-361

Haley SD, Afanador L and Kelly JD. 1994. Identification and application of a random amplified polymorphic DNA marker for the I gene (potyvirus resistance) in common bean. Phytopathology 84:157-160.

Hongying Z, Jiong C, Jianping C, Michael JA and Mingsheng LT. 2002. BCMV isolates causing different symptoms in Asparagus bean in China differ greatly in the 5 parts of their genomes. Archives of Virology 147, 1257-1262.

Hong-Soo cH, Mi- Kyeong K, Jin woo P, Jeong-Soo K, Were HK, Jang-Kyung CH and Takanami Y. 2006. Occurrence of Bean common mosaic virus infecting peanut in Korea. Plant Pathology J. 22(1):97-102.

Katungi E, Farrow A, Chianu J, Sperling L and Beebe S. 2009. Common bean in Eastern and Southern Africa: a situation and outlook analysis. CIAT.

Kelly JD. 1997. A review of varietal response to bean common mosaic potyvirus in Phaseolus vulgaris. . Plant Varieties and Seeds 10: 1-6.

Manyi MM, Kabwe KN, Claude B, Patrick TD, Winter S and Mbuyi AK. 2012, Incidence, Severity and Gravity of Cassava Mosaic Disease in Savannah Agro-Ecological Region of DR-Congo: Analysis of Agro-Environmental Factors. American Journal of Plant Sciences, 2012, 3, 512-519.

Mckern NM, Mink GL, Barnett OW, Mishra A, Whittaker LA, Silbernagel MJ, Ward CW and Shukla DD. 1992. Isolates of Bean common mosaic virus comprising two distinct potyvirus. Phytopathology 82, 923-929.

Melotto M, Afanador L and Kelly JD. 1996. Development of a SCAR marker linked to the I gene in common bean. Genome 39:1216-1219.

Ministry of Agriculture and Rural Development (MOARD). 2004. Annual Report. Nairobi, Kenya.

Ministry of Agriculture. 2013. Kenya is food secure, Annual Report. Nairobi, Kenya.

Mink GI, Vetten HJ, Ward CW, Berger PH, Morales FJ, Myers JM, Silbernagel MJ and Barnett OW. 1994. Taxonomy and classification of legume-infecting potyviruses: A proposal from the Potyviridae Study Group of the Plant Virus Subcommittee of ICTV. Archives of Virology 139: 231-235.

Mukeshimana G, Paneda A, Rodriguez-Suarez C, Ferreira JJ, Giraldez R and Kelly JD. 2005. Markers linked to the bc-3 gene conditioning resistance to bean common mosaic potyviruses in common bean. Euphytica 144(3): 291-299.

Murray HG and Thompson WF. 1980. Rapid isolation of high molecular weight DNA. Nucleic Acids Research 8, 4321-4325

Mwaniki AW. 2002. Assessment of bean production constraints and seed quality and health of improved common bean seed. MSc Thesis, University of Nairobi, 113 pp.

Odendo M, David S, Kalyebara R, Ostyula R and Buruchara R. 2004. The key role of beans in poverty alleviation: Lessons from the impact of Improved bean varieties in western Kenya. Occasional Publication Series, No.43.

Omunyin ME, Gathuru EM and Mukunya DM. 1995. Pathogenicity groups of Bean common mosaic virus isolates in Kenya. Plant Disease 79: 985-989.

Silbernagel MJ, Mink GI, Jhao RL and Zheng GY. 2001. Phenotypic recombination between bean common mosaic and bean common mosaic necrosis potyviruses in vivo. Archives of Virology.146:1007–1020.

Vetten HJ, Lesemann DE and Maiss E. 1992. Serotype A and B strains of Bean common mosaic virus are two distinct potyviruses. Archives of Virology. (Suppl. 5):415-431.

Wagara IN. 2005. Molecular and virulence characterization of Phaeoisariopsis griseola and reaction of bean germplasm to races of the angular leaf spot pathogen. Ph.D. Thesis, University of Nairobi. 166 pp.

Wagara IM and Kimani PM. 2007. Resistance of nutrient-rich bean varieties to major biotic constraints in Kenya. African Crop Science Conference Proceedings Vol. 8. pp. 2087-2090.

Wortmann CS, Kirkby RA, Elude CA & Allen DJ. 1998. Atlas of common bean (Phaseolus vulgaris L.) production in Africa. CIAT publication No.297. 133pp.

Xu L and Hampton RO. 1996. Molecular detection of Bean common mosaic and Bean common mosaic necrosis potyviruses and pathogroups. Archives of Virology 141: 1961-1977.