



## STATUS OF COMMON BEAN MOSAIC VIRUS IN COMMON BEANS IN MALAWI

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### ABSTRACT

Studies were conducted aimed at identifying and characterising bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) strains found in Malawi. To accomplish this, 400 bean seed samples collected from different markets around the country were used. The studies were conducted at Bunda College and Chitedze Research Station virology laboratory and glasshouse. Differential host plant analysis as well as an indirect Enzyme-Linked Immunosorbent Assay (ELISA) were conducted to determine BCMV/BCMNV strains present in the country.

Out of 400 samples, 53 were tested for bean common mosaic disease (BCMD) biological characteristics using indicator host plants Dubbele Witte, Redland Greenleaf C, Redland Greenleaf B, Michelite, Pinto 114-8, Black Turtle Soup, Jubila, Amanda, US92-1006, and CIAT MCR 2210 while 184 isolates (including the 53 tested on indicator plants) were tested for BCMV serological characteristics. Based on biological and serological results, BCMV and BCMNV isolates were assigned to five pathotypes, which included PG1, IV, VIa, VIb and VI. The isolates corresponding to these pathotypes were similar to NL1, NL6, NL3, NL5, and RU1, respectively. NL1 dominated and was found in all the Agricultural Development Divisions (ADD's) sampled except two. BCMNV isolates (NL3, NL5) were found in Lilongwe ADD only, however, isolates of both serotypes A and B were widely distributed in almost all the ADD's except Kasungu where seed was seemingly free from seedborne BCMV and BCMNV.

### INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is the most important grain legume crop in Eastern and Southern Africa. Since common beans provide an inexpensive source of proteins compared to animal sources, they are particularly important to low-income families and educational institutions in Malawi. Ninety percent of the beans in Malawi are grown by smallholder farmers with few inputs. The source of seed for these farmers is either saved from their previous harvests or bought from local markets (Msuku *et al.*, 1998). A study conducted to assess the status of seed-borne diseases in beans found that bean common mosaic virus is among the most important factors leading to low bean production in the country. The disease has been reported to affect production and hence yields depending on the virus strain, bean genotype and time of infection (Hart & Saettler, 1981). There is a need to develop resistant varieties to overcome this disease threat. A sound knowledge of the pathogen and its variants or strains are vital prerequisites for reliable breeding for resistance. Therefore the main objective of this study was to identify, characterise and determine the distribution of bean common mosaic virus (BCMV) and bean common mosaic necrosis virus (BCMNV) strains in Malawi using common bean seed collected from local markets.

### MATERIALS AND METHODS

**Bean seed collection.** A survey was conducted to study the status of seed-borne diseases in Malawi. During this survey a total of 400 bean samples were collected from 41 markets around the country from August to October 1995 (Table 1). This period was chosen because most farmers were preparing their fields, purchasing, and selecting seeds for the on-coming

growing season. All seeds were brought to Bunda College for laboratory and screenhouse studies.

**Isolation and identification of BCMV plant samples.** A total of 9 seeds from each lot were planted in pots with 3 seeds per pot. Nine seeds were considered an adequate sample size as previous findings indicated that levels of seed-borne infection in a given seed lot was very high (98%). The soil used was collected from virgin fields and was sterilised. Pots were placed in the screenhouse, watered twice or three times a day as needed, and were grown to maturity. Observations for presence/absence of BCMV symptoms were made from germination to blossom stage. Pots with one or all plants showing BCMV symptoms were removed as soon as symptoms were observed and were placed in another part of the screenhouse. Dimethoate was applied every two weeks to prevent aphids from vectoring disease. Leaves from plants that showed BCMV symptoms were collected before plants flowered and were used as a source of inoculum for characterisation on differential hosts (Table 2) and serological assay.

**TABLE 1.** Areas sampled within each Agricultural Development Divisions (ADD's) in Malawi from August to October 1995.

Agricultural Development Divisions (ADD's)					
Karonga	Mzuzu	Lilongwe	Machinga	Blantyre	Kasungu
Chitipa	Mzimba	Chimbiya	Katuli	Mwanankhu	Ntchisi
Misuku	Mzuzu	Mayani	Namwera	Chitakale	Chinthembere
Lufita	Rumphi	Lobi	Mayaka	Muloza border	
Karonga		Kwadaubwandire	Dzaone	Ruo	
		Mitundu	Thondwe	Thyolo	
		Nkhoma		Chifunga	
		Ntcheu		Bvumbwe	
		Kampepuza		Chisawani	
		Lizulu		Chizunga	
				Mbulumbuzi	
				Mwanamulanje	
				Mwanza	
				Nansadi	
				Neno	

**TABLE 2.** BCMV differential hosts used in this study to characterize pathogen isolates.

Cultivar Group	Differential Host	Genotype
1	Dubbele Witte	i Bc-u Bc-1 Bc-2 Bc-3 <sup>x</sup>
2	Redland greenleaf C	i bc-u bc-1 Bc-2 Bc-3
3	Redland greenleaf B	i bc-u bc-1 <sup>2</sup> Bc-2 Bc-3
4	Michelite	I bc-u Bc-1 bc-2 Bc-3
5	Pinto UI-114-8	I bc-u bc-1, bc-2 Bc-3
6	Monroe	I bc-u bc-1 <sup>2</sup> bc-2 <sup>2</sup> Bc-3
7	Black turtle soup	I Bc-u Bc-1 Bc-2 Bc-3
8	Jubila	I Bc-u bc-1 Bc-2 Bc-3
9	Amanda	I Bc-u bc-1 <sup>2</sup> Bc-2 Bc-3
10	92US - 1006	I ? ? bc-2 <sup>2</sup> Bc-3
11	CIAT MCR 2210	I ? ? ? bc-3

<sup>x</sup>Upper case gene symbol designates dominant allele and lower case designates recessive allele.

**Inoculum preparation and inoculation.** Sap was extracted from bean leaves that showed disease symptoms. Leaves were ground using a mortar in a 0.02 M phosphate buffer solution (pH 7.5) at the ratio of 1:10 (w/v). Extracts were filtered through a layer of cheesecloth to remove leaf debris. Using cotton wool, the sap was inoculated onto carborundum-dusted primary leaves of differential hosts at the primary leaf stage. Three plants of each differential host were inoculated and one was left as a control. The differential hosts were grown in the screenhouse at Chitedze Research Station. Dimethoate was applied every two weeks to control insects that might facilitate virus transmission. Plants were observed for BCMNV and BCMV symptoms 5-7 days after inoculation. Where symptoms were questionable, back inoculation was done using Dubbele Witte as a known susceptible host. The pathogenicity phenotypes of the differential reactions were compared with reactions produced by the standard strains. The unknown strains were identified as being identical or closely related to standard strains (Spence and Walkey, 1993, Saiz *et al.*, 1995).

**Serological assay.** Direct antigen coating (DAC) indirect enzyme-linked immunosorbancy assay (ELISA) was used for our serological assay. The antisera used included 197A to detect potyvirus, II463 to detect both serotypes A and B of BCMV, and I-2 (a monoclonal antibody) to detect BCMV serotype A. Leaves showing BCMV symptoms were homogenised in carbonate buffer pH 9.6 [3.8 g Na<sub>2</sub>CO<sub>3</sub>, 5.86 g NaHCO<sub>3</sub>, 40.0 g polyvinyl pyrrolidone (PVP), 4.0 g egg albumin, and 2000 ml distilled water] and centrifuged at 3200 rpm for five minutes. The homogenate was then loaded into micro-titer plates at 100 µl per well; four wells were used for each isolate. The border wells were not used to avoid border effects. The plates were left at room temperature for one hour then washed with phosphate buffered saline-tween (PBS-T pH 7.4) [32.0 g NaCl, 0.80 g KH<sub>2</sub>PO<sub>4</sub>, 11.6 g Na<sub>2</sub>HPO<sub>4</sub>, 2.0 ml polyethylene sorbitan monolaurate (Tween-20) and 4000 ml distilled water] four times to remove the unbound antigens. Blocking was done using milk/BSA to prevent non-specific reactions and this was also incubated for one hour at 37°C. The trapping antibodies were loaded at 100 µl per well at a dilution of 1:1000 in phosphate buffer pH 7.4 (PEP) (1200 ml PBS-T, 24.0 g PVP, and 2.4 g egg albumin). Incubation was done for one hour at 37°C and washing was done as previously described with PBS-T. Detecting antibodies in the form of goat anti-mouse alkaline phosphatase was added at the same rate and incubation was done for two hours at 37°C. The plates were washed three times with PBS-T and then with Tris Buffered Saline (TBS). The substrate P-nitrophenyl phosphate (sigma) dissolved in substrate buffer (97 ml Diethanolamin, 800 ml distilled water, filling up to 1000 ml with distilled water) was added to the well at the rate of 100 µl per well. The plates were placed in the dark for the colour to develop. After 30 minutes to one hour, absorbances at 405 nm were measured in a Titerk Multiscan Plus MK II ELISA reader. Data was analysed using MSTAT and separation of means was done using least significant differences (LSD). Reactions were considered positive when the average absorbance for the test samples were significantly higher than the average absorbance of the negative controls (buffer and healthy tissue) at P = 0.05. This threshold is almost similar to that used by Jafarpour *et al.*, (1979), Spence and Walkey (1993) and Saiz *et al.* (1995). In their studies, reactions were considered positive when the mean absorbances for the test samples were more than twice the mean absorbance of the negative control. Antiserum II197A failed to react positively with the positive controls NL8 and US5 in preliminary tests and therefore we deleted this antiserum from this study. If an isolate reacted with both antibodies, it was designated an A serotype, a necrotic strain of BCMV. If the sample only reacted with II463, it was designated a B serotype, a non-necrotic strain of BCMV. Negative and inconclusive tests were repeated and if the results were still not conclusive, this was indicated in the tables of results.

Microcomputer Statistical Program (MSTAT) was used for statistical analysis. The following model was used for the ELISA experiment.

$$Y_{ij} = \mu + T_i + \epsilon_{ij}$$

where  $i = 1, 2, \dots, 188$

$j = 1, \dots, 4$

$\mu$  = Overall mean

$T_i$  = effect due to the  $i^{\text{th}}$  isolate

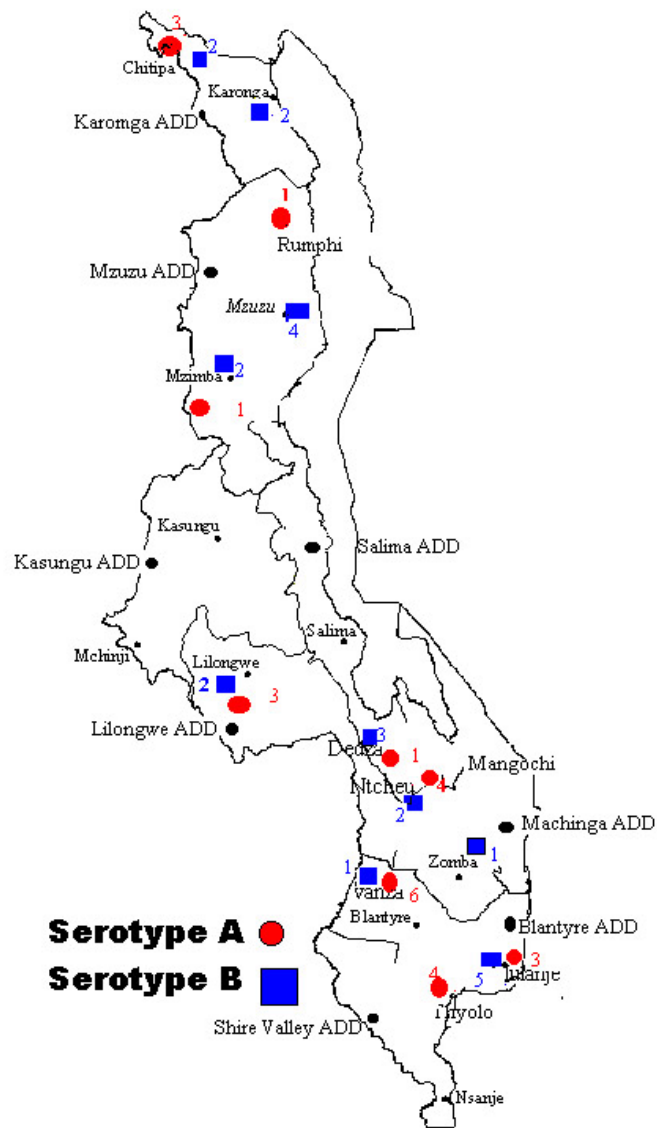
$\epsilon_{ij}$  = random error component

## RESULTS AND DISCUSSION

Both BCMV and BCMNV were isolated from the bean seedlots collected from local markets throughout Malawi (Figure 1). The isolates identified in this study could be classified into three pathogroups according to their pathogenicity phenotype on differential host cultivars, and could easily be classified as belonging to either the A or B serotype by ELISA. The pathogroups identified in this study included PG IV, PG VI, and PG I (Table 3).

Pathotype and serotype were strongly correlated in some cases. Where there was no correlation (data not shown) it could have been due to low virus titre in the inoculum resulting in failure to induce symptoms in some cultivars, or due to differences in temperature at which the experiments had been carried out. Femi Lana *et al.* (1988) reported reactions of some differential cultivars to standard strains NL1, NL3 and NY15 that differed from those described by Drijfhout (1978). These discrepancies were thought to be due to mutation in BCMV strains, temperature effects, and virus concentration in the inoculum. We found that PG IV isolates were infectious to the differential bean cultivars in host resistance groups 1, 2 and 3, in which they induced systemic mosaic symptoms. Isolates 50 and 176 from Lilongwe and Mzuzu ADDs respectively, differed from the standard strain NL6 in that they did not cause symptoms in host differential groups 8, 9, and 10. Failure to induce symptoms in these cultivars could be attributed to the fact that greenhouse temperatures under which these experiments were conducted were not favourable.  $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$  NL6-type strain is known to induce systemic necrosis in group 8 and 9 cultivars only at  $32^{\circ}\text{C}$  and not at  $26^{\circ}\text{C}$  (Spence & Walkey, 1993; Drijfhout, 1978). Four isolates, two from Lilongwe ADD (54, 154) and two from Blantyre ADD (266, 317) whose differential host reaction pattern conformed to those of NL6 were detected by both antisera I463 and monoclonal I-2 (data not shown). They may be mixtures of both serotype A and B isolates. Failure to induce systemic necrosis in a cultivar with unprotected *I* gene could be due to low virus titre in the inoculum, making diagnosis based on symptoms unreliable as reported by Abdallah (1995), and points out the need for strain purification before typing. Presence of an isolate similar to NL6 in Malawi confirms the findings of Spence and Walkey who reported this strain type in the country (Spence & Walkey, 1993).

The PG VIa isolates, 69 and 40 both from Lilongwe ADD, were infectious to bean differential hosts in host resistance groups 1-5, induced temperature insensitive systemic necrosis in cultivars in groups 8 (BT1) and 9 (Jubila), and produced localised necrotic lesions (92US-1006) in group 11 (Table 4). The reaction patterns in the differential bean cultivars produced by these isolates were identical to the standard strain NL3 as described by Drijfhout (unpublished data). Isolates similar to NL3 were previously reported in Malawi, however, this strain was only found in researchers' fields near Bunda College and at Matapwata Research Station (Spence & Walkey, 1993 and 1995). The presence of isolates similar to NL3 in seedlots collected from markets is therefore an indication that the strain has now been established in farmers' fields. Researchers can in future prevent the spread of seed-borne diseases by conducting seed inspection and quarantine before testing any material originating from outside the country.



**Figure 1.** BCMV (serotype A) and BCMNV (serotype B) isolated from seed lots collected at local markets in Malawi, from August to October 1995.

One isolate from Lilongwe ADD (isolate 51) was classified as a PG VIb. The isolate induced mosaic symptoms in cultivars in resistance groups 1-5, induced temperature insensitive systemic necrosis in BT1 and Jubila, and induced apical necrosis that was not followed by systemic necrosis in Amanda. Failure to complete systemic necrosis in Amanda may have been due to temperature effects or low virus titre in the inoculum. Silbernagel *et al.* (1986) reported that when infected plants are exposed to temperatures higher than 30°C for prolonged periods, certain *I* gene cultivars develop lethal systemic vascular necrosis. Local necrotic lesions were also induced by isolate 51 in cultivar 92US-1006. Isolate 51 was thus regarded as closely related to NL5 strain, and its presence in the same ADD and district as NL3 is of great interest. Since these isolates were collected from areas near to where bean researchers have plots (e.g., Dedza where Bean/Cowpea CRSP plots are located), it is possible that NL3 and NL5-like isolates have spread from these plots to farmers' fields.

**Table 3.** Foliage reaction of various bean genotypes to different BCMV and BCMNV isolates in Malawi with respect to the standard strains and their patho and sero groups.

Isolate	Cultivars <sup>x</sup>											Serotype & Pathogroup	
	DW	RGC	RGB	MICHE	P114	MON	BT1	JUB	AMA	92US	CIAT		
NL3	+ <sup>y</sup>	+	+	+	+	-	N+	N+	-	LL	-	A	PG VIa
69	+	+	+	+	+	-	N+	N+	-	LL	-	A	PG VIa
40	+	+	+	+	+	-	N+	N+	-	LL	-	A	PG VIa
NL5	+	+	+	+	+	-	N+	N+	N+	LL	-	A	PG VIb
51	+	+	+	+	+	-	N+	N+	AN	LL	-	A	PG VIb
NL6	+	+	+	-	-	-	+/-n	+/-n	+/-n	-	-	B	PG IVa
50	+	+	+	-	-	-	-	-	-	-	-	B	PG IVa
176	+	+	+	-	-	-	-	-	-	-	-	B	PG IVa
NL1	+	-	-	-	-	-	-	-	-	-	-	B	PG I
167	+	-	-	-	-	-	-	-	-	-	-	B	PG I
211	+	-	-	-	-	-	-	-	-	-	-	B	PG I
152	+	-	-	-	-	-	-	-	-	-	-	B	PG I
231	+	-	-	-	-	-	-	-	-	-	-	B	PG I
163	+	-	-	-	-	-	-	-	-	-	-	B	PG I
340	+	-	-	-	-	-	-	-	-	-	-	B	PG I
1	T+	-	-	-	-	-	-	-	-	-	-	B	PG I
RUI	+	+	+	+	+	-	+/-n	-	-	-	-	B	PG IVb
178	+	+	+	+	+	+	-	-	-	-	-	B	PG IVb
155	+	+	+	+	+	+	-	-	-	-	-	B	PG IVb
240	+	+	+	+	+	+	-	-	-	-	-	B	PG IVb
70	+	+	+	+	+	+	-	-	-	-	-	B	PG IVb

<sup>x</sup> DW=Dubbele Witte, RGC= Redla Greenleaf C, RGB= Redland Greenleaf B, MICHE= Michelite, P114= Pinto UI-114-8, MON= Monroe, BTI= Black turtle Soup, JUB= Jubila, AMA= Amanda, 92US= 92US- 1006, CIAT=CIAT MCR2210.

<sup>y</sup> + = systemic mosaic, - = absence of symptoms, T+= symptoms less pronounced but reacted positively with ELISA, N+= systemic necrosis, +/-n= systemic or no systemic necrosis (temperature dependent necrosis), LL= localised necrosis, AN= apical necrosis.

**Table 4.** Summary of BCMV and BCMNV isolates as identified by differential host cultivars, and their distribution in the six Agricultural Development Divisions (ADD's) in Malawi.

ADD	NL1	NL3	NL5	NL6	RUI-like	NOVEL	TOTAL
Karonga	4	0	0	0	0	0	4
Kasungu	0	0	0	0	0	0	0
Lilongwe	7	2	1	3	4	1	18
Machinga	0	0	0	0	0	0	0
Blantyre	6	0	0	2	5	0	13
Mzuzu	2	0	0	1	2	0	5
<b>Total</b>	19	2	1	6	11	1	40

There were 19 isolates whose reaction pattern in differential bean cultivars were closely related to standard strain NL1, and these dominated in our study (Table 4). These 19 isolates were infectious in host group 1 cultivar Dubbele Witte only, and only six were serotype B (NL6)(Table 4). One isolate (isolate 1, from Lilongwe ADD) failed to induce symptoms in any of the differential cultivars tested but was detected by antisera II463 which

detects both serotypes of BCMV. This isolate resembles some BCMV isolates adapted to non-bean hosts such as cowpea and adzuki (Myers, personal communication). The NL1 isolates were found in all ADD's sampled except Machinga and Kasungu. NL1 type strain has never been reported in Malawi before but has been reported in Tanzania. The presence of this strain in the country could be due to cross-border trade of agricultural product such as seed between the two countries. As BCMV is seedborne, it could have easily been introduced through this means. It could also be possible that this strain type has been present in the country but as there have been no intensive studies to identify BCMV and BCMNV strains in Malawi it was not detected. Work by Spence and Walkey (1993) to characterize BCMV and BCMNV strains for instance, only covered three of the 26 districts in Malawi.

Eleven isolates induced reaction patterns in differential bean cultivars that were similar to the Russian isolate (RU1) (Table 4). However only 4 of these isolates were confirmed serologically to belong to this strain type (Tables 3 & 4). Thirteen isolates remained symptomless after inoculation on differential hosts. The absence of mosaic or necrotic symptoms in BCMV- and BCMNV-inoculated bean genotypes may suggest different plant-virus interactions. Morales (1989) reported that such situations occur when the plant is truly immune: the plant escapes infection and though it could be infected, it remains symptomless.

The isolate induced hardly any symptoms in all the differentials tested but leaves from symptomless Dubbele Witte reacted positively with antisera II463. These results also support previous records by Jafarpour *et al.*, (1979) who noted that use of visual characterisation/differential host reaction besides being laborious and time consuming fail to detect masked infection. Serological assays are the most reliable and sensitive method for detecting BCMV and confirming diagnoses (Forster *et al.*, 1991). However Abdallah (1995) reported that polymerase chain reactions (PCR) is a more sensitive technique than ELISA. In her studies, Abdallah reported that about 39% of ELISA-negative samples tested positive for BCMV by PCR. The antibodies used in the ELISA test could not differentiate BCMV and BCMNV individual type-strains and therefore the effectiveness of the two methods could not be compared.

## **CONCLUSIONS**

Results from this study indicated that both BCMV and BCMNV are present in bean seed grown by farmers in Malawi. Both serotype A and B are widely distributed in the three regions of the country. There is a great need therefore that bean breeders in the country continue breeding for multiple resistance to the BCMV and BCMNV strains that have been found in the country. Breeders must also be prepared to breed for disease strains that have not yet been identified.

## **RECOMMENDATIONS**

- An in-depth characterisation of strain mixtures obtained in this study and a follow-up study to confirm the study results is strongly recommended; additionally, effective techniques such as Polymerase Chain Reaction (PCR) should be used to identify pathogen strains.
- Bean breeders in the country should consider the use of the gene *bc-2<sup>2</sup>* in addition to *bc-3* to ensure stability of BCMV and BCMNV resistance over time.
- CIAT MCR 2210 has shown to be resistant to all BCMV and BCMNV strains identified in this study and should be utilised in the bean breeding programme either directly as a resistant cultivar or indirectly as a source of resistance when making crosses. However, there is no data on adaptation and production potential of this cultivar in the country, therefore research is needed to determine if this cultivar is adapted and productive in Malawi.

## ACKNOWLEDGEMENTS

We would like to thank Dr. G. Mink for the antisera used in this study. Thanks to Dr. J. Myers and Dr. P. Guzman for the BCMV differential cultivars, and the B/C CRSP for the funding that made this work possible. Thanks to all bean team members, too many to mention, for the assistance rendered during this study.

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