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## DETECTION OF COWPEA APHID-BORNE MOSAIC VIRUS (CABMV) IN COWPEA BY REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

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### ABSTRACT

The occurrence of CABMV on cowpea (*Vigna unguiculata* L. Walp) in Uganda was described recently in several studies. This study developed and optimised a reverse transcription polymerase chain reaction (RT-PCR) based assay for the detection of CABMV in leaf samples, and compared it to previous RT-PCR and ELISA assays. Use of the forward primer (CABFF1, 5'-GGT AAC AAY AGT GGR CAA CC-3') and the reverse primer (CABRR1, 5'-CTG AGC ACT CMA ACC GGG-3') yielded a product of ~ 1,642 bp. Amplicon sequencing and subsequent BLASTN analysis showed that Ugandan isolates were 89.3-94.3% identical indicating they belong to the same strain of CABMV. Phylogenetic analysis also placed the Ugandan isolates in the same cluster different from other isolates but closer to those from Burkina Faso. However, the previously reported RT-PCR assay (GF/GR primer pair) did not give the expected PCR fragment (221 bp) and gave no virus hits upon amplicon sequencing and sequence analysis. The ELISA assay did not differentiate between positive and negative samples. The newly developed RT-PCR assay for detecting CABMV, described in this study, has important applications for plant quarantine, resistance breeding, host range studies as well as epidemiological studies for the control of CABMV in the country.

**Key Words:** NCM-ELISA, RT-PCR, sequencing, *Vigna unguiculata*

### RÉSUMÉ

L'apparition de CABMV sur le niébé (*Vigna unguiculata* (L.) en Ouganda a été décrite récemment dans plusieurs études. Cette étude a développé et optimisé un essai basé sur la transcription inverse- réaction en chaîne par polymérase (RT-PCR) pour la détection du CABMV dans les échantillons de feuilles, et la comparé aux essais précédents basés sur la RT-PCR et ELISA. L'usage de l'amorce direct (CABFF1, 5'-GGT AAC AAY AGT GGR CAA CC-3') et d'amorce réverse (CABRR1, 5'-CTG AGC ACT CMA ACC GGG-3') a donné ~ 1,642 bp. Le séquençage d'amplicon et l'analyse subséquente de BLASTN ont montré que les isolats d'Ouganda étaient à 89,3-94,3% identiques indiquant qu'ils appartiennent à la même souche de CABMV. Les analyses phylogénétiques ont aussi placé les isolats Ougandais dans la même classe qui est différente des autres isolats mais proche de ceux du Burkina-Faso. Néanmoins, les essais de RT-PCR (GF/GR paire d'amorces) précédents n'ont pas donné les fragments PCR espérés (221 bp) et n'ont donné aucune détection de virus à partir du séquençage d'amplicon et de l'analyse de la séquence. L'essai de l'ELISA n'a pas différencié entre les échantillons positifs et négatifs. L'essai RT-PCR nouvellement développé pour détecter le CABMV, décrit dans cette étude, a d'importantes

applications pour la mise en quarantaine de la plante, sélection pour la résistance, les études des gammes d'hôtes ainsi que les études épidémiologiques pour le contrôle du CABMV dans le pays.

*Mots Clés:* NCM-ELISA, RT-PCR, séquençage, *Vigna unguiculata*

## INTRODUCTION

Over 20 viruses have been reported on cowpea in several parts of the world (Hughes and Shoyinka, 2003; Lima *et al.*, 2005), of which over eight viruses infect cowpea in Africa (Hughes and Shoyinka, 2003). While some of these viruses occur occasionally with local or minor importance, some are widely spread with significant economic importance (Hughes and Shoyinka, 2003). *Cowpea aphid-borne mosaic virus* (CABMV), of the family *Potyviridae* and genus *Potyvirus*, is one of the viruses reported to cause the most important viral disease on cowpea in Africa (Taiwo, 2003). CABMV was first reported in Uganda in 1981 (Anon, 1981) and is now widely distributed in the main cowpea growing areas of the country (Orawu *et al.*, 2005, 2015).

Using next-generation sequencing (NGS), the first complete genome sequence for a Ugandan isolate (Serere 1) of CABMV was recently published by Mbeyagala *et al.* (2018). Transmission of CABMV through seed is variable, but can be as high as 80% (Ojuederie *et al.*, 2009), and is efficiently vectored by several aphid species such as *Aphis craccivora*, *A. fabae*, *A. gossypii*, *A. medicaginis*, *Macrosiphum euphorbiae* and *Myzus persicae* (Bashir *et al.*, 2002).

The worldwide nature of CABMV occurrence (Bashir *et al.*, 2002) poses a serious threat of further spread through seed shipments. CABMV alone can cause yield losses of up to 60% (Neya *et al.*, 2015), but can also interact with other viruses leading to severe mosaic diseases (Lima *et al.*, 2005; Taiwo *et al.*, 2007).

Previous molecular diagnostic work on the same virus was reported by Gillaspie *et al.* (2001) on groundnuts. Their study yielded a diagnostic amplicon of 221 bp. However,

validation of this assay on CABMV infected plants from Uganda did not yield the same amplicon (Amayo, 2008). This could have been due to difference in the strains and the non-specificity of the primers used in a different host. This study was designed to optimise molecular detection of CABMV in cowpea, through design of new primer sets and reverse transcriptase-polymerase chain reaction (RT-PCR) conditions.

## MATERIALS AND METHODS

**Plant samples.** Forty leaf samples from plants characteristic virus symptoms were collected from farmers' fields in Serere and Ngora districts in eastern Uganda during first season of 2017. The leaves were dried by placing them between paper towels; and inserted in ziploc bags, and silica gel added. The bags were placed horizontally in a plastic container and tightly closed. Complete drying of samples was achieved in 2-3 days at room temperature, with at least a single change of silica gel. The dried leaf samples were used for RNA extraction and virus detection by RT-PCR. Forty fresh leaf samples were also collected and kept in cool boxes (4-8 °C) containing ice for serological assay, using Nitrocellulose membrane based ELISA (NCM-ELISA).

**Nitrocellulose membrane based ELISA (NCM-ELISA).** NCM-ELISA procedure was carried out as described by Fuentes (2010). Positive and negative controls, as well as the 1<sup>st</sup> antibody and the 2<sup>nd</sup> antibody (conjugated with alkaline phosphatase), were obtained from DSMZ (Braunschweig, Germany). The substrate-chromogen solution, BCIP/NBT (5-Bromo-4-chloro-3-indolyl phosphate/nitro-blue tetrazolium chloride), was obtained from

Sigma (St. Louis, USA). Colour development was stopped by discarding the substrate solution and immersing the membrane in distilled water for 10 min. Positive reactions were identified by purple coloration of blots; while negative samples remained blue.

**RNA extraction and cDNA synthesis.** Dried leaf samples were ground into powder using sterile mortars and pestles; with the help of liquid nitrogen. RNA extraction was carried out using AccuZol reagent (Bioneer, Seoul, Republic of Korea), according to the manufacturer's instructions. Quality was checked using TAE/Formamide agarose gel electrophoresis, as described by (Masek *et al.*, 2005); and quantified using NanoDrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). RNA samples showing intact 18S and 28S subunits on agarose gel were used for cDNA synthesis. The oligo(dT)<sub>18</sub> primed synthesis of complementary DNA (cDNA) on 2 µg of total RNA was achieved using RevertAid Reverse Transcriptase (RT), a recombinant M-MuLV RT according to manufacturer's instructions (Thermo Fisher Scientific, Massachusetts, USA).

**Primer sequences used.** Primers for virus detection were designed based on available sequence data (from DDBJ/ENA/ GenBank) from full genome sequences for the forward primers, and partial sequences at the 3'-untranslated regions (3'-UTR) of CABMV, for reverse primers. The sequences were aligned using multiple alignment softwares; ClustalX Version 1.83 (Thompson *et al.*, 1997) and MEGA7 (Kumar *et al.*, 2016). Following the multiple alignment, five primer pairs (Table 1) were designed with the forward/left primer for the first pair starting at the conserved GNNSGQ motif located in the center of the nuclear inclusion protein b (NIb) (Zheng *et al.*, 2010). Subsequent forward primers were located downstream of the GNNSGQ motif. Reverse/right primer sets were designed in the 3'UTR regions. An additional primer pair (GF/GR) designed by Gillaspie *et al.* (2001) was also included.

**Polymerase chain reaction (PCR) conditions.** Following optimisation of PCR conditions, only CABFF1/CABRR1 out of the five primer pairs was effective; thus further sections on methodology, results and

TABLE 1. Primers used for detection of CABMV in Ugandan cowpea samples

Name	Primer sequence (5'-3')	Predicted size (bp)	Source
CABFF1 CABRR1	GGT AAC AAY AGT GGR CAA CC CTG AGC ACT CMA ACC GGG	1,642	This study
CABFF2 CABRR2	GAG AGG YTR GTK TTC TTT GC GGC CTC YCY GCT AAG TTC	1,514	This study
CABFF3 CABRR3	TGA ATT AYG AYT TCT CAG AAA G CTG RAT ATA TGC GTA CTA TTT AC	1,333	This study
CABFF4 CABRR4	AGA GAR GAR TTA TGG TTC ATG CTA AAA CCA ACC ATT AGC	1,281	This study
CABFF5 CABRR5	TAC ATA CCA AAR CTW GAG C CCA CAC TRR CAT ATA TAG	1,275	This study
GF GR	CGCTCAAACCCATTGTAGAA TATTGCTTCCCTTGCTCTTTC	221	Gillaspie <i>et al.</i> (2001)

discussions are limited to this primer set as well as GF/GR primer set designed by Gillaspie *et al.* (2001). The PCR mix consisted of 10  $\mu$ l of 2X PCR Master mix (Bioneer, Seoul, Republic of Korea), 0.25  $\mu$ M of each primer, 1  $\mu$ l of cDNA and 8  $\mu$ l of sterile nuclease free water giving a total reaction volume of 20  $\mu$ l. The PCR conditions for CABFF1/CABRR1 was 94 °C for 2 min, followed by 35 cycles of 94 °C (30 sec), 58 °C (90 sec), 72 °C (90 sec) for denaturation, annealing and extension, respectively; with a final extension at 72°C for 5min. The PCR conditions used for GF/GR were as described by Gillaspie *et al.* (2001).

PCR products were analysed by electrophoresis in 1% agarose gel in 1× TAE buffer, and stained with ethidium bromide. Gel electrophoresis was carried out at 100V for 60 min. After electrophoresis, images of the PCR products were captured/visualised using an inbuilt camera in an E-Gel® Imager System (Thermo Fisher Scientific, Massachusetts, USA) under UV light.

**Amplicon sequencing and sequence analysis.** The PCR products were purified from the gel using Wizard SV Gel and PCR Clean-Up System (Promega, Madison, USA), following the manufacturer's instructions, and directly sequenced. Sequencing was carried out at Macrogen Inc (Macrogen Europe Laboratory, Amsterdam, The Netherlands). Sequence data were compared to published DNA sequences using a Basic Local Alignment Search Tool (BLAST), as described by Johnson *et al.* (2008). Related sequences identified through BLASTN analysis were used in phylogenetic analysis. A total of 21 sequences, including an out group (*Barley yellow dwarf virus-BYDV*) were included in the analysis.

Multiple sequence alignment and computation of nucleotide sequence identities were done using the BioEdit software version 7.0.5 (Hall, 1999). Phylogenetic relationships were inferred using the Maximum Likelihood method, based on the Tamura-Nei model (Tamura and Nei, 1993) implemented in

MEGA7.0 (Kumar *et al.*, 2016). Initial tree(s) for the heuristic search were obtained automatically, by applying Neighbor-Joining and BioNJ algorithms, to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The trees were drawn to scale, with branch lengths measured in the number of substitutions per site.

Statistical significance of branching for phylogenetic trees was assessed using bootstrap analyses of 1,000 different alignments from the original dataset, and reported as percentage.

## RESULTS

**Virus detection by NCM-ELISA.** CABMV was detected in all plant samples by NCM-ELISA, including the positive and negative controls. NCM-ELISA, therefore, was not able to distinguish between positive and negative samples, since all samples gave positive signals.

**Virus detection by RT-PCR.** Among the six primer pairs that were designed, only one primer (CABFFI/CABRRI) was able to give CABMV amplification when tested on the positive controls. The primer pair was, therefore, used to differentiate all collected samples into negative and positives. CABFFI/CABRRI primer pair yielded the expected PCR product of approximately 1,642 bp (Fig. 1). However, the GF/GR primer designed by Gillaspie *et al.* (2001) produced a PCR product of about 400 bp instead of the expected 221 bp (Fig. 2).

**BLAST and pairwise sequence comparison of CABMV isolates.** BLAST analysis (BLASTN) of nucleotide sequences from CABFF1/CABRR1 amplicons from Ugandan CABMV isolates showed similarity to coat protein (CP) sequences of other CABMV isolates in the DDBJ/ENA/GenBank (Fig. 3). However, BLAST analysis of sequence data

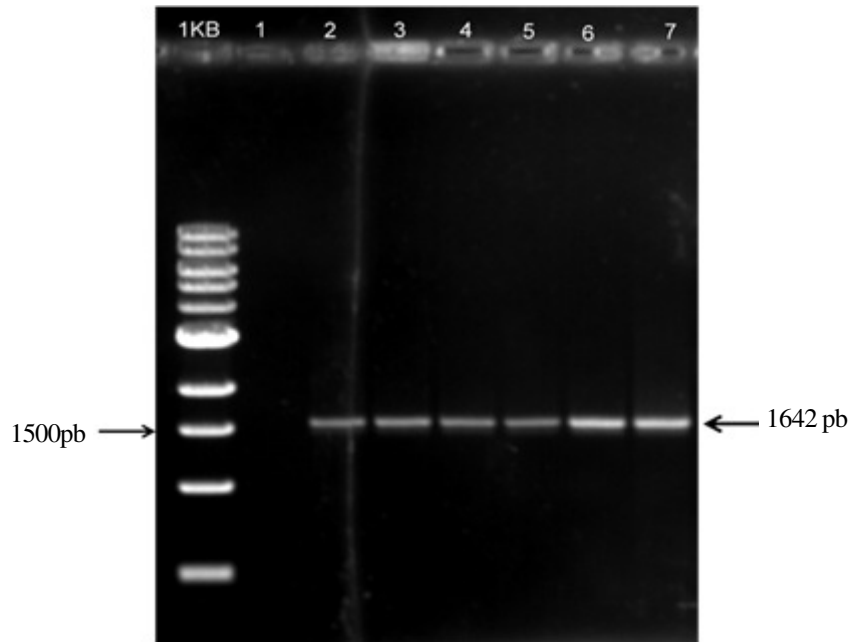


Figure 1. RT-PCR product for CABMV resulting from CABFFI/CABRR1 primer pair: Lane 1: Negative control, lanes 2-6: Samples, lane 7: Positive control.

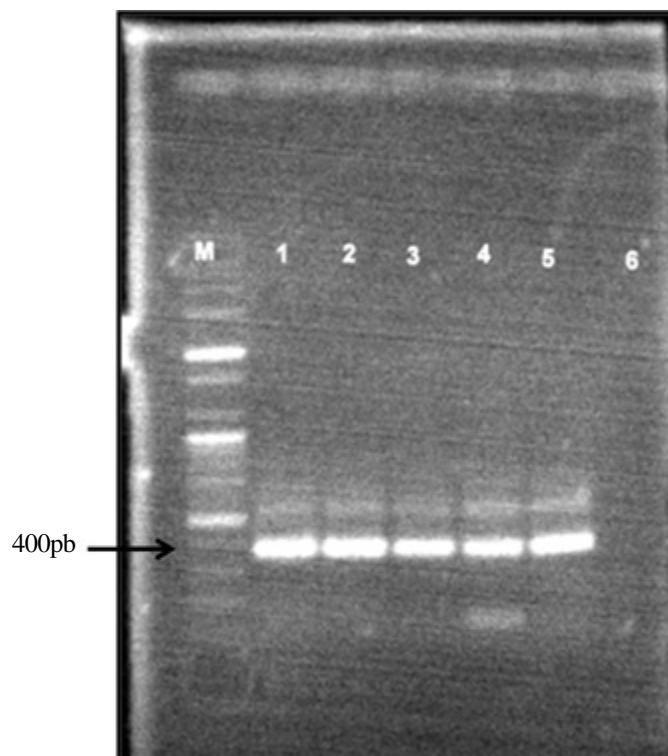


Figure 2. RT-PCR product for CABMV for GF/GR primer pair: M is 100 bp ladder, lane 1-5: Samples, lane 6: Negative control.



Cowpea aphid-borne mosaic virus isolate BF-E1 coat protein gene, partial cds  
 Sequence ID: [MF277031.1](#) Length: 828 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
1419 bits(768)	0.0	808/828(98%)	0/828(0%)	Plus/Minus
CDS: Putative 1	275	* Q G H R M G L L S H M N Q N V D T A T		
Query	147	TTACTGCCCATGCCTCATCCCAAGAAGGGAGTGCATGTTTGTATTGACATCAGTGGCAGT		206
Sbjct	828	.....C.....A.....G..		769
CDS:coat protein, pa	275	Q G H R M G L L S H M N Q N V D T A T		
CDS: Putative 1	256	H R E T N E S T T A V N G D L G F M R T		
Query	207	GTGCCTCTCAGTGTCTCCTACAGTGTGTCGCAACATCCCATCCAGGCCAACATCCTGGT		266
Sbjct	768	.....G.....		709
CDS:coat protein, pa	256	H R E T N E S T T A V N G D L G F M R T		
CDS: Putative 1	236	N V N S L A A A K M Q A I A E R A R D S		
Query	267	GTAAAGTTCGACAGGGCTGCTGCTTTCATCTGTGCTATTGCTTCTCGTGTCTATCAGA		326
Sbjct	708	.....		649
CDS:coat protein, pa	236	N V N S L A A A K M Q A I A E R A R D S		
CDS: Putative 1	216	T K S T V E Y F D F A Y R A L S K D R L		
Query	327	AGTTTATAGAGTCACTTCGTAGAAATCAAAGCATACCTTGCCTAAGCTCTATCCCTTAA		386
Sbjct	648	.....G.....G.....		589
CDS:coat protein, pa	216	T K S T V E Y F D F A Y R A L S K D R L		
CDS: Putative 1	196	N R L L G Y R P M Y F G E S N R M E I Y		
Query	387	ATTCCTGAGTAGTCCATACCTGGGCATGTAGAACCCTCGGAATTTCTCATCTATATA		446
Sbjct	588	.....A.....		529
CDS:coat protein, pa	196	N R L L G Y R P M Y F G E S N R M E I Y		
CDS: Putative 1	176	A E A A D S F H H M I Q R L T P K A N E		
Query	447	CGCTTCAGCTGCGTCAGAGAAGTGGTGCATTATTTGCTAAGTGTGGGTTTGCATTTTC		506
Sbjct	528	.....		469
CDS:coat protein, pa	176	A E A A D S F H H M I Q R L T P K A N E		

Figure 3. NCBI BLAST of sequence data for the RT-PCR fragments from CABFF1/CABRR1 primer.

from GF/GR primer fragments gave no virus hits (there were no significant alignments) (Fig. 4). Nucleotide sequences for three Ugandan CABMV isolates (CABMV-H1, CABMV-H2 and CABMV-H5) were deposited in the DDBJ/ENA/GenBank under accession numbers MH151199, MH151200 and MH151201, respectively. Sequences for these isolates contained a single open reading frame (ORF) of 278 aa encoding the entire coat protein (CP) followed by a non coding region of 147 nt, 150 nt and 144 nt, excluding the poly A tail for CABMV-H1, CABMV-H2 and CABMV-H5, respectively.

The Ugandan CABMV isolates exhibited 62-75% nucleotide identity with CABMV isolates from Brazil, 72-75% identity with and isolate from Nigeria and 68-75% identity with isolates from Burkina Faso (Table 2). The molecular diversity among the Ugandan CABMV isolates was very limited, ranging from 89-94% with the lowest identity observed between CABMV-H1 and CABMV-H5.

#### Phylogenetic analysis of CABMV isolates.

Phylogenetic analysis showed little divergence among CP sequences for the three Ugandan isolates as shown by the very short branch lengths. It is, therefore, possible that the three isolates belong to the same strain of CABMV. The Ugandan isolates were closely related to other CABMV isolates from Burkina Faso, but very different from Brazilian isolates (Fig. 5).

## DISCUSSION

#### Virus detection by NCM-ELISA.

NCM-ELISA test was unable to distinguish between positive and negative samples, since all samples gave positive signals. ELISA tests are often associated with giving false signals (false positives) and this could be associated with cross reaction of antibodies with plant proteins or other viruses (Dietzgen *et al.*, 2001; Gutierrez *et al.*, 2003; Kashif, 2009). Cross reaction makes it difficult to correctly distinguish/separate infected from non-

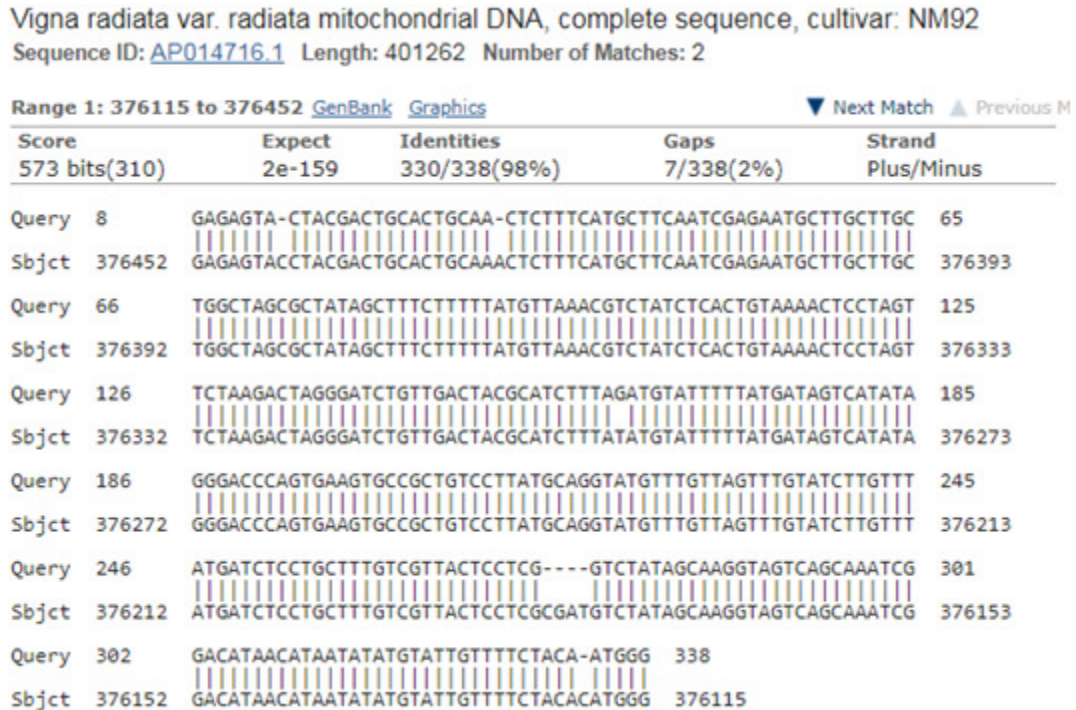


Figure 4. NCBI BLAST of sequence data for the RT-PCR fragments from GF/GR primer.

infected samples, rendering accurate virus detection impossible. Cross adsorption of membranes with extracts (plant sap) from healthy plants was reported as efficient in preventing reaction of antibodies with plant proteins in sweet potato (Gutierrez *et al.*, 2003). The potential of cross adsorption with cowpea plant extracts, therefore, needs to be evaluated.

**Virus detection by RT-PCR.** An RT-PCR assay optimised in this study using a newly designed CABFFI/CABRR1 primer was able to detect CABMV in the infected cowpea plant samples that were tested. This primer set yielded the expected amplification of 1,642 bp (Fig. 1). While reverse transcriptase PCR (RT-PCR) is a reliable method for diagnosis and allows for further characterisation of cowpea viruses (Akinjogunla *et al.*, 2008), not all RT-PCR assays are efficient. An earlier protocol described by Gillaspie *et al.* (2001) failed to detect CABMV in positive samples in this

study (Fig. 2), as was observed by Amayo (2008). However, other studies using the protocol by Gillaspie *et al.* (2001) reported its ability to give the expected fragment size (221 bp) (Byarugaba, 2008; Amayo *et al.*, 2012). Non-specificity of the RT-PCR assays could be attributed to nucleotide differences in sequences used to design diagnostic primers. Primers designed by Gillaspie *et al.* (2001) were based on a single Brazilian CABMV strain and, thus were strain specific. However, in the present study, alignment of multiple sequences from different strains improved the specificity of the diagnostic primer (CABFFI/CABRR1) and, therefore, improved its efficiency for detection of CABMV in Uganda.

**BLAST, pairwise sequence comparison and phylogenetic analysis of CABMV isolates.** Molecular analysis of the CP fragments amplified using the CABFFI/CABRR1 clearly identified them as CABMV belonging to the family *Potyviridae* (Fig. 3). In the present

TABLE 2. Pairwise sequence comparison matrix for complete coat protein nucleotide sequences of three Ugandan isolates and other isolates of CABMV<sup>a</sup>

Virus isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
CABMV-BF-E4																					
CABMV-BF-E1	99.1																				
CABMV-BF-E5	95.2	95.2																			
CABMV-BF-E10	95.2	95.2	100																		
CABMV-BF-E6	95.4	95.4	99.8	99.8																	
CABMV-M3	64.7	64.6	64.6	64.6	64.5																
CABMV-M2	64.8	64.7	64.9	64.9	64.9	98.6															
CABMV-SP	63.5	63.4	63.3	63.3	63.2	95.1	94.6														
CABMV-Lns10	64.0	63.7	63.3	63.3	63.2	84.8	84.9	83.4													
CABMV-Lns9	64.0	64.0	63.6	63.6	63.5	84.7	84.9	83.1	99.3												
CABMV-Lns4	63.9	63.8	63.2	63.2	63.1	84.7	84.9	83.3	99.2	98.9											
CABMV-Lns3	63.9	63.8	63.4	63.4	63.3	84.9	85.0	83.4	99.6	99.3	99.0										
CABMV-Lns2	64.0	63.9	63.5	63.5	63.4	84.9	85.1	83.4	99.6	99.7	99.0	99.6									
CABMV-DF-Brs	63.8	63.7	63.1	63.1	63.0	91.9	91.8	90.4	85.8	85.9	86.0	85.9	85.9								
CABMV-F-144	64.9	64.8	65.0	65.0	64.9	98.1	99.4	94.6	85.0	84.9	85.0	85.1	85.2	92.0							
CABMV-F-101	63.4	63.3	63.6	63.6	63.5	96.2	97.4	96.5	83.4	83.3	83.0	83.5	83.6	90.1	97.4						
CABMV-A132	56.1	55.9	55.3	55.3	55.2	77.3	77.6	76.0	77.3	77.1	77.0	77.3	77.3	77.8	77.6	76.0					
CABMV-H1	74.5	74.4	72.4	72.4	72.5	69.0	69.1	68.0	74.7	74.5	75.0	74.7	74.6	68.2	69.2	68.0	72.1				
CABMV-H2	71.6	71.5	69.7	69.7	69.7	66.7	66.8	65.7	72.1	71.9	72.0	72.1	72.1	65.8	66.9	66.0	74.8	94.3			
CABMV-H5	69.6	69.5	67.7	67.7	67.8	63.2	63.3	62.5	68.6	68.3	68.0	68.6	68.5	62.6	63.3	62.0	73.4	89.3	93.4		
BYDV-Montana	30.1	30.0	29.8	29.8	29.8	30.0	30.1	29.5	29.6	29.7	30.0	29.6	29.6	29.3	30.1	29.0	25.5	27.7	26.8	25.3	

<sup>a</sup>Pair wise comparisons made with BioEdit version 7.0.4.1, derived using ClustalW multiple alignments with the following parameters: BLOSUM matrix, Gap open = 10, Gap extension = 0.1



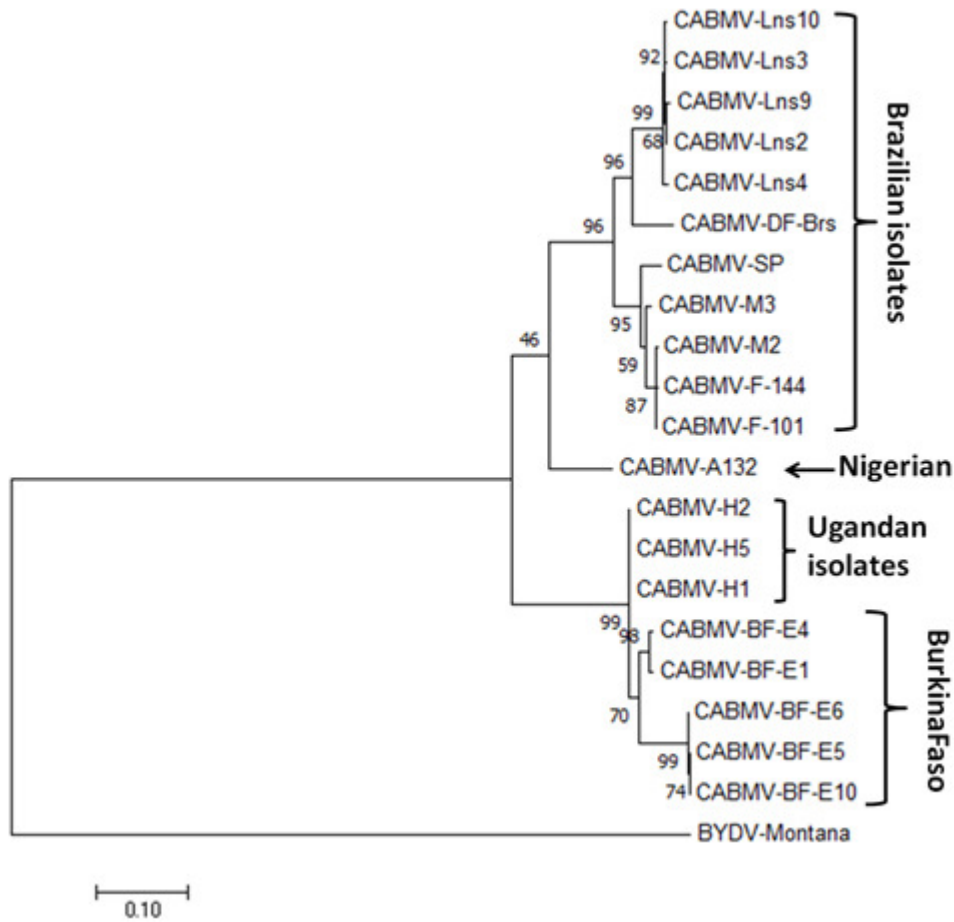


Figure 5. Phylogenetic tree based on alignments of complete coat protein nucleotide sequences of three CABMV isolates from Uganda and isolates from other countries. The tree was rooted on the sequence of Barley yellow dwarf virus.

study, sequence analysis of fragments from the assay by Gillaspie *et al.* (2001) did not give any hits related to CABMV (Fig. 4) and, therefore, was unable to detect the virus in Uganda. The criteria set by the International Committee for Taxonomy of Viruses (Adams *et al.*, 2011) and other taxonomic studies (Adams *et al.*, 2005) suggested a benchmark for species demarcation in the family *Potyviridae* as a nucleotide sequence identity of less than 76%, either in the CP or over the whole genome. Using this criteria, the three virus isolates from cowpea are all the same species (Table 2) belonging to the same strain of CABMV (Fig. 5).

### CONCLUSION

Using a newly designed primer pair (CABFFI/CABRR1), we have optimised a reliable and accurate RT-PCR assay for detection of CABMV in cowpea leaf samples. The assay produced a PCR fragment of approximately 1,642 bp, which upon sequencing yielded the complete CP region of CABMV. The new assay clearly distinguished between positive and negative samples that could not be distinguished using NCM-ELISA. Sequencing of amplicons for the Ugandan CABMV isolates tested using the assay and subsequent phylogenetic analysis showed that they belong

to the same strain of CABMV. Further studies utilising the new diagnostic assay are needed to determine the distribution of CABMV in the main cowpea growing areas of the Uganda. The assay will also be applicable in cowpea breeding to aid selection of virus resistant progenies. Additionally, this assay will contribute to efficient screening of samples before exist or entry (international exchange of germplasm and quarantine protection) into the country, thus limiting the spread of the virus. Since CABMV has a wide host range infecting both legumes and non legumes, the new assay can also be used to detect CABMV in alternative hosts in the country.

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#### REFERENCES

- Adams, M.J., Zerbini, F.M, French, R., Rabenstein, F., Stenger, D.C. and Valkonen, J.P.T. 2011. Family *Potyviridae*. In: King, A.M.Q., Adams, M.J., Carstens, E.B. and Lefkowitz, E.J. (Eds.), *Virus Taxonomy*, Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London, UK. pp. 1069-1089.
- Adams, M.J., Antoniw, J.F. and Fauquet, C.M. 2005. Molecular criteria for genus and species discrimination within the family *Potyviridae*. *Archives of Virology* 150(3):459-479. <https://doi.org/10.1007/s00705-004-0440-6>.
- Akinjogunla, O.J., Taiwo, M.A. and Kareem, K.T. 2008. Immunological and molecular diagnostic methods for detection of viruses infecting cowpea ( *Vigna unguiculata* ). *African Journal of Biotechnology* 7(13):2099–2103.
- Amayo, R. 2008. Occurrence and transmission of seed-borne cowpea viruses in Uganda. MSc. Thesis, Makerere University, Kampala, Uganda. 70pp.
- Amayo, R., Arinaitwe, A.B., Mukasa, S.B., Tusiime, G., Kyamanywa, S., Rubaiyaho, P.R. and Edema, R. 2012. Prevalence of viruses infecting cowpea in Uganda and their molecular detection. *African Journal of Biotechnology* 11(77):14132-14139. <https://doi.org/10.5897/AJB11.398>.
- Anonymous. 1981. Viruses, virus-like diseases and mycoplasma-like diseases. In: *Pest control in tropical grain legumes*. Centre for Overseas Pest Research, London, United Kingdom. pp. 95-110.
- Bashir, M., Ahmad, Z. and Ghafoor, A. 2002. Cowpea aphid-borne mosaic potyvirus: A review. *International Journal of Pest Management* 48(2):155–168. <https://doi.org/10.1080/09670870110118722>.
- Byarugaba, A.A. 2008. Occurrence and pathogenesis of cowpea viruses in Uganda. MSc. Thesis, Makerere University, Kampala, Uganda. 69pp.
- Dietzgen, R.G., Callaghan, B., Higgins, C.M., Birch, R.G., Chen, K. and Xu, Z. 2001. Differentiation of peanut seed-borne potyviruses and cucumoviruses by RT-PCR. *Plant Disease* 85(9):989-992.
- Fuentes, S. 2010. NCM-ELISA detection of sweetpotato viruses. OP21, Version 56. International Potato Center (CIP), Lima, Peru. 5pp.
- Gillaspie, A.G., Pio-Ribeiro, G., Andrade, G.P. and Pappu, H.R. 2001. RT-PCR detection of seedborne cowpea aphid-borne mosaic virus in peanut. *Plant Disease* 85:1181-1182.
- Gutierrez, D.L., Fuentes, S. and Salazar, L.F. 2003. Sweet potato virus disease (SPVD): Distribution, incidence, and effect on sweet potato yield in Peru. *Plant Disease* 87:297-302.

- Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41:95–98.
- Hughes, J. d’A. and Shoyinka, S.A. 2003. Overview of viruses of legumes other than groundnut in Africa. In: *Proceedings of a conference organized by IITA/ : 4-8 June 2001*. International Institute of Tropical Agriculture. Ibadan, Nigeria. pp. 553-568.
- Johnson, M., Zaretskaya, I., Raytselis, Y., Merezhuk, Y., McGinnis, S. and Madden, L. T. 2008. NCBI BLAST: A better web interface. *Nucleic Acids Research* 36(2):W5–W9. <https://doi.org/10.1093/nar/gkn201>.
- Kashif, M. 2009. Detection and characterization of viruses of sweet potatoes (*Ipomoea batatas* L.) from Guatemala and Honduras. MSc. Thesis, University of Helsinki, Finland. 72 pp.
- Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870-1874.
- Lima, J.A.A., Sittolin, I. M. and Lima, R.C.A. 2005. Diagnosis and strategies to control diseases caused by viruses. In: Freire Filho, F.R., Lima, J.A. de A. and Ribeiro, V.Q (Eds.), *Cowpea beans: technological advances*. DF: Embrapa Technological Information; Teresina: Embrapa Meio-Norte, Brasília, Brazil. Chapter 11. pp. 403-459.
- Masek, T., Vopalensky, V., Suchomelova, P. and Pospisek, M. 2005. Denaturing RNA electrophoresis in TAE agarose gels. *Analytical Biochemistry* 336(1):46-50. <https://doi.org/10.1016/j.ab.2004.09.010>.
- Mbeyagala, E.K., Tukamuhabwa, P., Bisikwa, J., Holton, T. and Mukasa, S.B. 2018. Next-generation sequencing reveals the first complete genome sequence of *Cowpea aphid-borne mosaic virus* from Uganda. *Genome Announcements* 6:e01491-17. <https://doi.org/10.1128/genome.A.01491-17>.
- Néya, B.J., Zida, P.E., Sérémé, D., Lund, O.S. and Traoré, O. 2015. Evaluation of yield losses caused by cowpea aphid-borne mosaic virus (CABMV) in 21 cowpea (*Vigna unguiculata* (L.) Walp.) varieties in Burkina Faso. *Pakistan Journal of Biological Sciences* 18 (7):304-313. <https://doi.org/10.3923/pjbs.2015.304.313>.
- Ojuederie, O.B., Odu, B.O. and Ilori, C.O. 2009. Serological detection of seed borne viruses in cowpea regenerated. *African Crop Science Journal* 17(3):125-132.
- Orawu, M., Melis, R., Milliano, W.D., Laing, M. and Adipala, E. 2005. Occurrence and prevalence of cowpea virus diseases in Uganda. *Africana Crop Science Proceedings* 7:1279-1283.
- Orawu, M., Obuo, J. and Omadi, R. 2015. Distribution and detection of cowpea viruses infecting cowpea in Uganda. *American Journal of Plant Sciences* 6(5):574-581. <https://doi.org/10.4236/ajps.2015.65062>.
- Salem, N., Ehlers, J. and Roberts, P. 2010. Biological and molecular diagnosis of seed-borne viruses in cowpea germplasm of geographically diverse sub-Saharan origins. *Plant Pathology* 59:773–784. <https://doi.org/10.1111/j.1365-3059.2010.02285.x>.
- Tamura, K. and Nei, M. 1993. Estimating the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and Evolution* 10(3):512-526. [10.1093/oxfordjournals.molbev.a040023](https://doi.org/10.1093/oxfordjournals.molbev.a040023).
- Taiwo, M.A. 2003. Viruses infecting legumes in Nigeria/ : case history. In: *Proceedings of a conference organized by IITA/ : 4-8 June 2001*. International Institute of Tropical Agriculture. Ibadan, Nigeria. pp. 365-380.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. 1997.

- The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25:4876-4882.
- Zheng, L., Rodoni, B.C., Gibbs, M.J. and Gibbs, A.J. 2010. A novel pair of universal primers for the detection of potyviruses. *Plant Pathology* 59(2):211-220. <https://doi.org/10.1111/j.1365-3059.2009.02201.x>.