academicJournals

Vol. 16(39), pp. 1953-1961, 27 September, 2017
DOI: 10.5897/AJB2017.16168
Article Number: 956F0B266135
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Molecular characterization of virus isolates from genus *Potyvirus* infecting *Vigna subterranea* in Burkina Faso

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Received 26 July, 2017; Accepted 18 September, 2017

Bambara groundnut (*Vigna subterranea*) is an African legume with a great nutritional, economic and social potential. However, one of the main constraints to this crop is viral diseases which reduced yields. Indeed, approximately 12 viruses have been reported to infect bambara groundnut. Among these, only four were reported from Burkina Faso, namely, cowpea aphid-borne mosaic virus (CABMV), bean common mosaic virus strain-blackeye cowpea mosaic (BCMV-BlCM), peanut mottle virus (PeMoV), and cowpea mottle virus (CPMoV). This study was carried out in order to identify and characterize the main viruses occurring in bambara groundnut from Burkina Faso using serological and molecular tests. 140 plants were sampled in the three agro-climatic zones of Burkina Faso. Both *Potyvirus* CABMV and BCMV-BlCM were identified in 8.57% of the samples when double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) test was used and 14.29% of the samples when reverse transcription-polymerase chain reaction (RT-PCR) test was used. Phylogenetic tree based on 476 nt in coat protein showed that all Burkina Faso CABMV isolates clustered together with Uganda isolate (KT726938). However, two groups were distinguished within these isolates. Burkina Faso BCMV-BlCM isolates are strengthly clustered with BCMV and BlCMV group. This study reports the first molecular characterization of CABMV and BCMV-BlCM infecting Bambara groundnut in Burkina Faso.

Key words: Bambara groundnut, cowpea aphid-borne mosaic virus (CABMV), bean common mosaic virus strain-blackeye cowpea mosaic (BCMV-BlCM), double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), reverse transcription-polymerase chain reaction (RT-PCR).

INTRODUCTION

Bambara groundnut [*Vigna subterranea* (L.) *Verdc*.] is an indigenous legume that originated from Africa precisely in the northern part of Nigeria and Cameroun (Goli et al., 1997). It is mainly grown for human consumption and

plays an important socio-economic role in tropical Africa (Nadembega, 2016). Its seeds contain an average of 63% carbohydrate, 19% protein and 6.5% oil (Mkandawire, 2007). This well balanced composition makes it a complete food, thus Bambara groundnut could be used to alleviate nutritional problems especially for the rural population (Bamshaiye et al., 2011). In Burkina Faso, it is ranked second seed legume after cowpea [*Vigna unguiculata* (L.) Walp] in terms of production and consumption (Ouédraogo et al., 2008).

However, viral diseases are one of the major constraints to Bambara groundnut production. Indeed, viral disease can cause yield loss of about 60% (Brink et al., 2006). Approximately, 12 virus species were known to infect bambara groundnut in Africa (Thottappilly and Rossel, 1997). These include cowpea mottle virus (CPMoV) (Robertson, 1966; Rossel, 1977; Shoyinka et al., 1978), cowpea aphid borne mosaic virus (CABMV) (Gumedzoe, 1985) from Nigeria, peanut mottle virus (PeMoV) from Kenya (Bock et al., 1978), voandzeia necrotic mosaic virus, voandzeia mosaic virus, southern bean mosaic virus (SBMV) and voandzeia distortion mosaic virus from Cote d'Ivoire (Fauquet and Thouvenel, 1987), bean common mosaic virus strain black eye cowpea mosaic (BCMV-BlCM) formerly known as blackeye cowpea mosaic virus (BlCMV), cowpea mosaic virus (CPMV), cowpea mild mottle virus and cucumber mosaic virus (CMV) from Nigeria (Thottappilly and Rossel, 1997) and an unidentified *Potyvirus* from Togo (Bird and Corbett, 1988). From the listed virus, CABMV and BCMV-BlCM from the genus *Potyvirus*, are reported to be most damageable on cowpea (Udaya Shankar et al., 2009). They are transmitted by seed at the rates of about 40 and 30%, respectively (Frison et al., 1990), by sap inoculation and by aphids in a non-persistent manner (Zettler and Evans, 1972; Bock, 1973; Brunt et al., 1990).

Among these viruses found to occur on bambara groundnut, only four have been reported from Burkina Faso using serological and biological tests. They include CABMV, BCMV-BlCM, PeMoV and CPMoV (Sérémé, 1989; Drabo et al., 1997; Néya, 2011). However, none of the studies have molecular characterized bambara groundnut viruses from this country. Whereas, improving our knowledge in the virus characterization would help to establish some effective control strategies against bambara groundnut viral diseases (Frenkel et al., 1992).

In this study, serological and molecular detection tests were used to screen for the presence of viruses within bambara groundnut leaves samples collected in the Sudan (humid), Sudan-Sahel (sub-humid), and Sahel (dry) agro-climatic zones of Burkina Faso. Virus identified was further characterized for their phylogenetic relationship with GenBank viruses.

MATERIALS AND METHODS

Plant sampling

During the months of September to October of the year 2016, a sampling was carried out on bambara groundnut fields. Symptomatic leaves showing leaf curling, stunting and mosaic diseases were sampled in the three agro-climatic zones of Burkina Faso. Sampling concerned farmers' fields and experimental plots. A total of 140 samples were collected with 5 in the sahel zone, 125 in the sudan-sahel zone and 10 in the sudan zone. Leaves collected were placed on melting ice before storage at -80°C. Each collected sample was divided into three parts. The first was used for double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) serological tests, the second for reverse transcription-polymerase chain reaction (RT-PCR) molecular tests and finally the third part was retained as eventual inoculum.

Serological detection test

The presence of viruses CABMV, BCMV-BlCM, CPMoV, CPMV and PeMoV in the samples collected were detected by using their corresponding polyclonal antibodies from ELISA detection kits. ELISA detection kits were purchased from AC Diagnostics, Inc. (USA) and a DAS-ELISA (Clark and Adams, 1977) was performed following the manufacturer's protocol.

RNA extraction, RT-PCR and sequencing

For molecular characterization, RNA was extracted from collected samples leaves using Trizol reagent (Invitrogen, USA) extraction protocol as described in Longué et al. (2017).

The cDNAs were constructed in two steps. Firstly, 5 µl mixture of total RNA and oligo dT (10 µM) were incubated at 70°C for 5 min and immediately placed on ice for 5 min. Secondly, a mixture constituted of 1.25 µl of dNTPs (10 mM), 5 µl of M-MLV RT Buffer (5x) (Promega, Corp. USA), 0.5 µl of enzyme M-MLV RT RNase (200 U) (Promega, Corp. USA) and H_2O qsp was added to the previous 5 µl mixture. Then, the total volume of 25 µl was incubated at 40°C for 1 h followed by 70°C for 15 min to generate cDNA.

PCR was performed in a final volume of 25 µL containing: 4 µl of Go Taq 5x Buffer (Promega, Corp. USA), 0.5 µl of dNTPs (10 mM), 0.1 µl of Go Taq G2 polymerase (200 U) (Promega, Corp. USA), 2 µl of cDNA, 0.5 µl of each forward and reverse primers (Table 1) and H_2O qsp. The amplifications cycle was as follow: 94°C for 5 min, 35 cycles of 94°C for 30 s, Ta (°C) for 30 s and 72°C for Ext (s), and a final elongation of 72°C for 7 min.

The degenerated primers pair P077/P078 (Table 1) (Marie-Jeanne et al., 2000) was used for further identification of *Potyvirus* in the samples. To sequence the *Potyvirus* (CABMV and BCMV-BlCM), the whole coat protein gene, primers P105 and P106 were designed based on GenBank available sequences. Then, primers combination P105/P078 and P077/P106 (Table 1) were used in independent PCR. Amplification products were separated by electrophoresis on a 1% agarose gel containing ethidium bromide and then visualized under UV light. Expected amplicons for 12 samples were direct sequenced with forward and reverse primers using the Sanger method (GENEWIZ, UK).

Sequences analyses and phylogenetic construction

Sequences obtained were trimmed and assembled using DNAMA

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Table 1. List of primers used in this study.

Primers pair	Sequences (5'-3')	Ta (°C)	Ext (Sec)	Expected fragment length (bp)
P077 P078	ATGGTHTGGTGYATHGARAAYGG CARATGAARGCMGCAGCA	55	30	327
P105 P078	GCYCCDTAYATHGCRGARWCWGC TGCTGCKGCYTTCATYTG	52.5	60	≈800
P077 P106	ATGGTHTGGTGYATHGARAAYGG CACAGTTAKCRTYTCRYG	52.5	60	≈400

Single letter code: H = A/C/T; Y = C/T; R = A/G; K = G/T; Ta, annealing temperature; Ext, extension time.

Table 2. Potyvirus detection in DAS-ELISA and RT-PCR according to sampling area.

Samples code	Samples name	Detection test used		Agro-climatic zones
		DAS-ELISA	RT-PCR	
E1	L86-E111	+	+	Sudan-sahel
E2	203-KVS246-1	+	+	Sudan-sahel
E3	306-KVS235-100GY	+	+	Sudan-sahel
E4	310-E119	+	+	Sudan-sahel
E5	210-KVS235	+	+	Sudan-sahel
E6	209-E125	+	+	Sudan-sahel
E10	L9-E25	+	+	Sudan-sahel
E15	215-E56 A	+	+	Sudan-sahel
E18	308-KVS246-2	-	+	Sudan-sahel
E51	E56 A-KBS2	+	+	Sudan-sahel
E113	Ech-DED2	-	+	Sudan-sahel
E138	KVS246-KBS2	-	+	Sudan-sahel
E139	L85-E117	+	+	Sudan-sahel
E140	120-KVS235-100GY	+	+	Sudan-sahel
E141	220-KVS235-100GY	-	+	Sudan-sahel
E142	Ech-KBS	+	+	Sudan-sahel
E99	Ech-PO4	-	+	Sudan
E100	Ech-SAP5	-	+	Sudan
E101	Ech-LEO2	-	+	Sudan
E104	Ech-SAP3	-	+	Sudan

+ = Positive response to the detection tool used; - = negative response to the detection tool used.

software version 7.2.0. A consensus sequences of about 476 (partial coat protein) to 950 nt (covered the complete coat protein) were reconstituted with the tree primer pair P077/P078, P105/P078 and P077/P106. Sequences were then compared to Genbank available sequences using Basic Local Alignment Search Tool (BLAST; hhttp://www.ncbi.nlm.nih.gov/blast) (Altschul et al., 1997).

Phylogenetic tree was constructed based on 476 nt obtained from 12 samples. Sequences were aligned using MUSCLE (Edgar, 2004) with default setting. Maximum likelihood phylogenetic tree was performed in MEGA 6.0 (Tamura et al., 2013) using the Tamura Nei parameter (TN93+G+I) nucleotidic substitution model. Bootstrap method at 1000 replicates was adopted to support the branches.

The pairwise nucleotide identities were performed using SDT software version 1.2 (Muhire et al., 2014). Sequences obtained in this study have been submitted to Genbank (Table 3).

RESULTS

Virus detection in DAS-ELISA and RT-PCR tests

DAS-ELISA was efficient to identify only CABMV and BCMV-BlCM from the genus *Potyvirus,* respectively in 7.14 and 1.43% samples to make a total of 8.57% (12/140) positive samples (Table 2). None samples was detected positive to CPMoV, CPMV and PeMoV antibodies. However, RT-PCR tests were more efficient in *Potyvirus* detection (14.29%, 20/140) using primers pair P077/P078 (Marie-Jeanne et al., 2000). Beside positive samples in DAS-ELISA, eight new samples become positives (Table 2). Figure 1 shows the detection

Table 3. List of virus isolates from the genus *Potyvirus* from Burkina Faso and some GenBank accessions analyzed in this study.

Accession number	Plant host	Country	Virus names
MF277031	*V. subterranea*	Burkina Faso	CABMV
MF277032	*V. subterranea*	Burkina Faso	BCMV-BlCM
MF277033	*V. subterranea*	Burkina Faso	CABMV
MF277034	*V. subterranea*	Burkina Faso	CABMV
MF277035	*V. subterranea*	Burkina Faso	CABMV
MF277036	*V. subterranea*	Burkina Faso	CABMV
MF277037	*V. subterranea*	Burkina Faso	CABMV
MF277038	*V. subterranea*	Burkina Faso	CABMV
MF277039	*V. subterranea*	Burkina Faso	CABMV
MF277040	*V. subterranea*	Burkina Faso	BCMV-BlCM
MF277041	*V. subterranea*	Burkina Faso	CABMV
MF277042	*V. subterranea*	Burkina Faso	BCMV-BlCM
AB458596.1	*Cucurbita pepo*	Syria	Zucchini yellow mosaic virus
AF348210.1	-	Zimbabwe	CABMV
AJ132414.1	*V. unguiculata*	Nigeria (Ibadan)	CABMV
AJ312438.1	*V. unguiculata*	China	BCMV
D10053.1	*Passiflora* species	South Africa	South African passiflora virus
DQ666332	*Phaseolus vulgaris*	Columbia	BCMV
EU660586.1	*Cucumis melo*	France	Watermelon mosaic virus
KC777407.1	*Passiflora* species	Brazil	CABMV
KT726938.1	*V. unguiculata*	Uganda	CABMV
AY575773.1	-	Taiwan	BCMV-BlCMV
Y17822.1	*V. unguiculata*	Nigeria (Monguno)	CABMV
Y18634.1	*V. unguiculata*	Morocco	CABMV

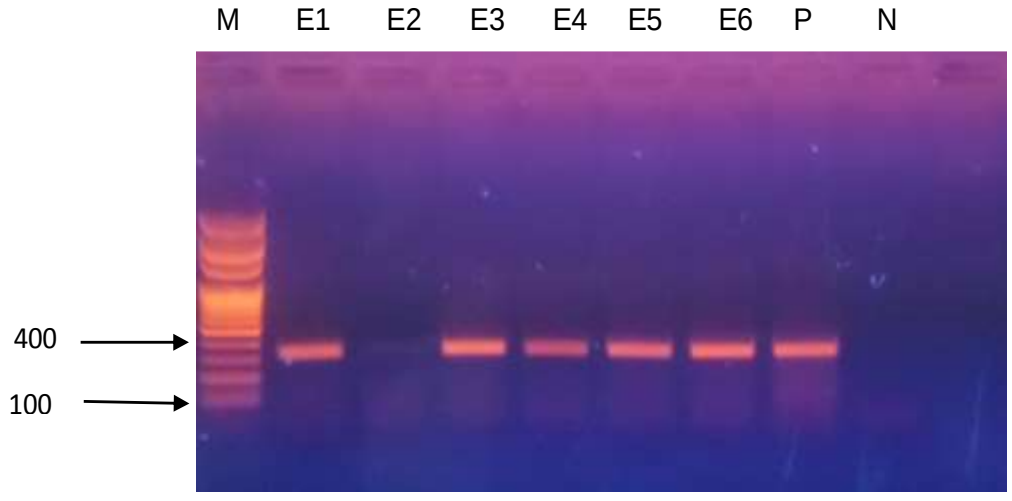

Figure 1. Reverse transcription-polymerase chain reaction (RT-PCR) bands in agarose gel with *Potyvirus* primer couple 1 (Marie-Jeanne et al., 2000). M represents marker (100 bp DNA ladder); N represents negative control; P represents positive control; E1-E6 represent RNA of samples tested.

of *Potyvirus* at 327 bp in some samples. Moreover, primers pair P105/P078 and P077/P106 succeeded in amplifying their corresponding fragments (Figure 2).

The BlastN analyses based on consensus sequences

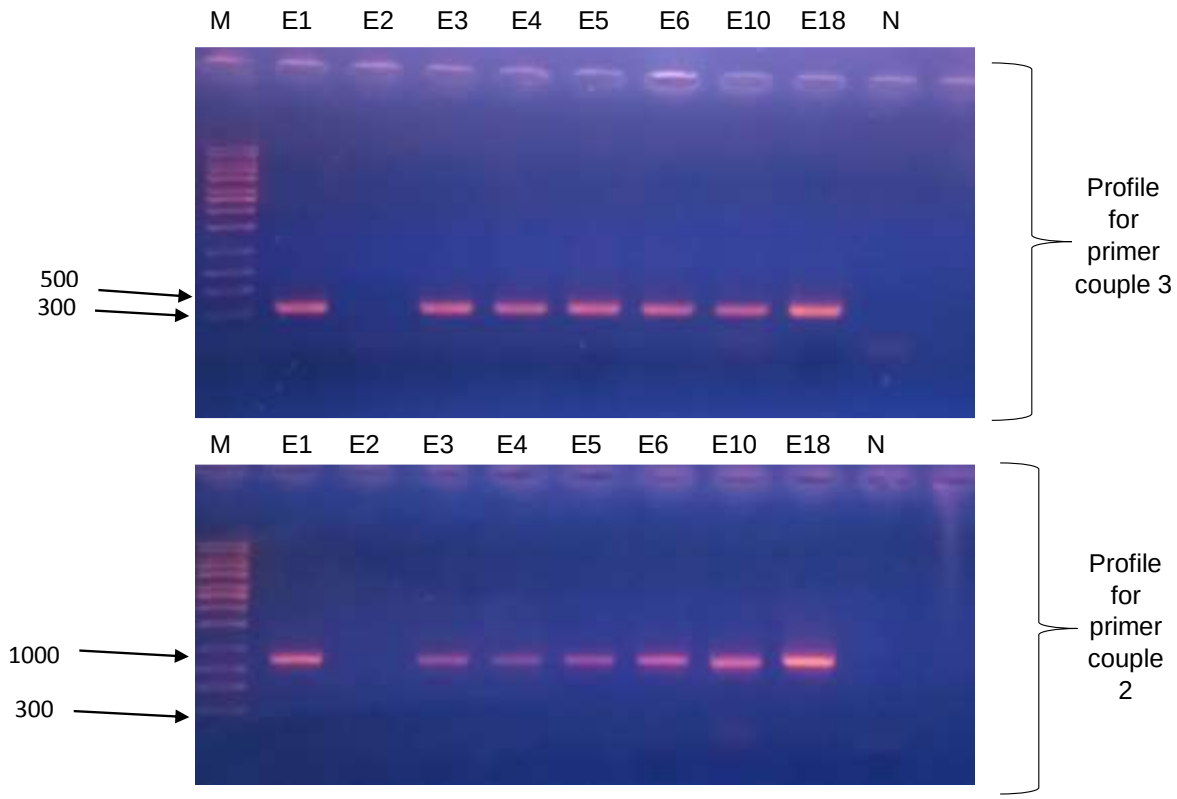

Figure 2. Reverse transcription-polymerase chain reaction (RT-PCR) bands in agarose gel with *Potyvirus* primer couple 2 and 3. M represents marker (1Kb DNA ladder); N represents negative control; E1-E18 represent RNA of samples tested.

(476-950 nt) revealed that nine samples were identified to CABMV Genbank accession number KT726938 at 88 to 98% identity (Table 3) whereas, three samples were identified at 97 to 98% identity to BCMV Genbank accession number AJ312438 (Table 3). These confirmed the identification of CABMV and BCMV-BlCM in DAS-ELISA test.

Among the three agro-climatic zones, sudan zone was the most infected by *Potyvirus* (40%, 4/10), followed by the Sudan-sahel zone (12.8%, 16/125) when none positive sample was detected in the sahel zone (Table 2).

Phylogenetic and nucleotide identity analyses

The maximum likelihood phylogenetic tree (Figure 3) showed that the 5' partial coat protein sequence (476 nt) was sufficient to separate the two *Potyvirus* CABMV and BCMV-BlCM.

All Burkina Faso CABMV isolates clustered together with Uganda isolate (KT726938). However, they might form two distinct groups. The first one is most closed to Uganda isolate (KT726938) at bootstrap 86%. Members of this group shared together 99.6 to 100% identity and 97.5 to 97.7% identity to Uganda strain (Table 4). Members of the second group showed in an isolate group and shared together 83.4 to 99.4% identity. However, pairwise identities between Uganda isolate (KT726938) and members of the second group (85.1 to 93.3%) (Table 4) were low. Furthermore, identities between the two groups of CABMV ranged from 84.9 to 92.4%.

In Burkina Faso, all CABMV isolates and Uganda isolate nested to the Nigeria isolate (Y17822) at bootstrap 97 (Figure 3).

Burkina Faso BCMV-BlCM isolates unambiguously nested with BCMV and BlCMV group and particularly clustered with BCMV (AJ312438) Chinese strain at bootstrap 97. These isolates shared high nucleotide identity together (97.3 to 100%), while they shared 97.3 to 97.5% identity with Chinese isolate (AJ312438).

DISCUSSION

Several viruses have been reported to infect bambara groundnut (Thottappilly and Rossel, 1997). This study identified only *Potyvirus* CABMV and BCMV-BlCM infecting bambara groundnut in Burkina Faso both in DAS-ELISA and RT-PCR tests. This may be associated to the high prevalence of these viruses (over 65%)

Table 4. Percentage of nucleotide identity between Burkina Faso isolates of cowpea aphid-borne mosaic virus (CABMV) and blackeye cowpea mosaic virus strain of bean common mosaic virus (BCMV-BlCM) and Genbank related sequences based on 476 nucleotides in the coat protein.

Accession No.	Country	E1	E2	E3	E4	E5	E6	E10	E18	E113	E138	E139	E142
BCMV (AJ312438)	China	67.8	97.3	66.9	68.1	66.9	66.9	66.9	68	66.9	97.3	64.3	97.5
BlCMV (AY575773)	Taiwan	66.7	94.1	64.5	68.3	64.5	64.5	64.5	68.1	64.5	94.1	65.5	94.3
BCMV (DQ666332)	Colombia	67	84	66.7	67	66.7	66.7	66.7	66.8	66.7	84	66.6	84
WMV (EU660586)	France	67.1	68.7	65.5	66.9	65.5	65.5	65.5	68.5	65.5	68.7	66.7	68.5
ZYMV (AB458595)	Syria	66.7	67.7	66	64.5	65.8	66	65.8	67.3	65.8	67.7	66.8	67.7
CABMV (KT726938)	Uganda	93.1	68.5	97.5	93.1	97.7	97.5	97.7	93.3	97.7	68.5	85.1	68.5
CABMV (Y17822)	Monguno	84.2	69.3	84.2	84.5	84.5	84.2	84.5	84	84.5	69.3	78.4	69.3
CABMV (AJ132414)	Nigeria	80.3	67.7	81.8	80.1	82	81.8	82	80.8	82	67.7	81.2	67.7
CABMV (Y18634)	Morocco	80.3	67	81.6	80.3	81.8	81.6	81.8	80.8	81.8	67	81.6	67
CABMV (KF725712)	Brazil	77	69.1	75.2	77.2	75	74.8	75	77.2	75	69.1	77.1	69.1
CABMV (AF348210)	Zimbabwe	77.5	67.4	76.1	77.7	76.3	76.1	76.3	77.9	76.3	67.4	76.1	67.4

E1, MF277031; E2, MF277032; E3, MF277033; E4, MF277034; E5, MF277035; E6, MF277036; E10, MF277037; E18, MF277038; E113, MF277039; E138, MF277040; E139, MF277041; E142, MF277042.

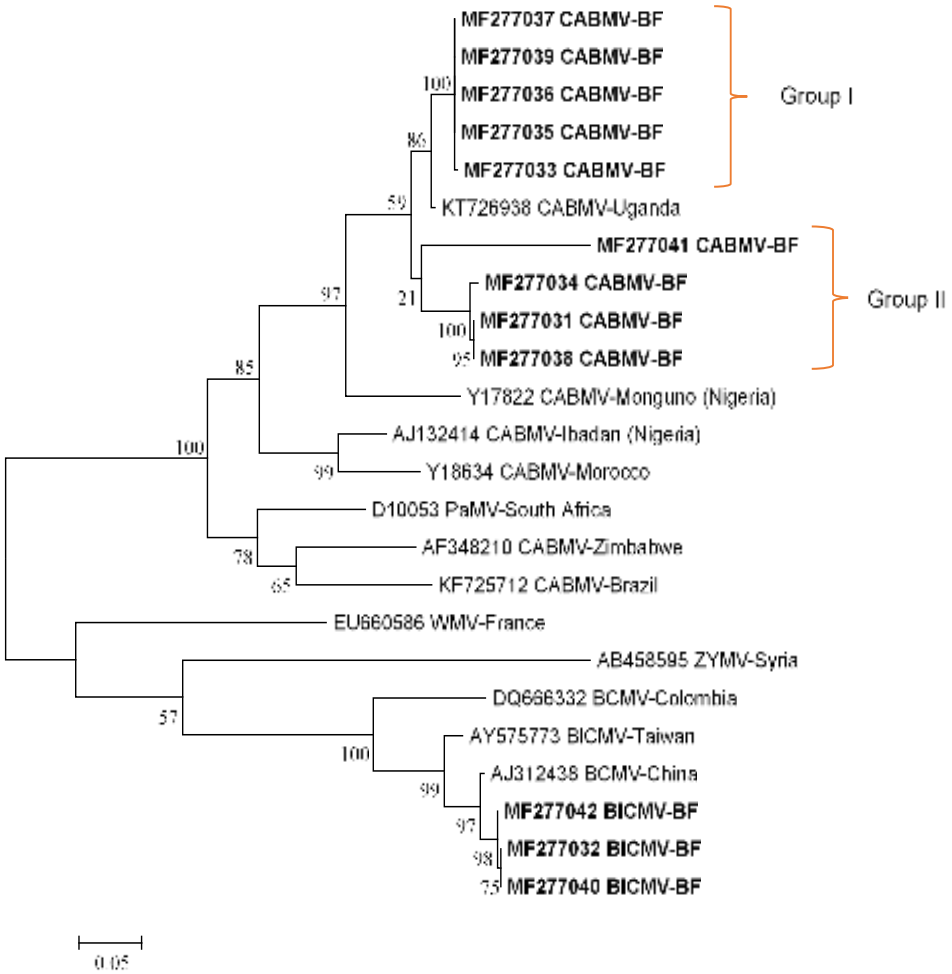

Figure 3. Maximum likelihood phylogenetic tree based on 476 nt in coat protein gene showing relationship between Burkina Faso isolates of CABMV and BCMV-BlCM (MF277031 to MF277042) and Genbank related species (Table 3). Bootstrap method was adopted at 1000 replicates.

reported in the country on cowpea crop (Néya, 2011; Palanga et al., 2016). Bambara groundnut and cowpea are two legume crops cultivated under the same climatic conditions and sometime in association. However, their prevalence in DAS-ELISA (8.57%) was less than in RT-PCR (14.29%). Indeed, some specificity in the coat protein structure of some virus strain may result in their false detection in serological test, whereas primers used in RT-PCR were specific and degenerated (Table 1) to amplified maximum of CABMV and BCMV-BlCM strains. On the other hand, some studies reported the efficiency of ELISA tests in plant virus detection (Konaté and Néya, 1996; Akinjogunla et al., 2008; Lima et al., 2012), but Gillaspie et al. (1999), Sipahioğlu (2005) and Liebenberg et al. (2009) showed that PCR and RT-PCR molecular tests were more sensitive.

Sudan zone (humid) was observed to be the most infected (40%) than sudan sahel (sub humid) (12.8%) and sahel (dry) (0%). Thus, climate might have an influence on *Potyvirus* infection and distribution in bambara groundnut crop. Indeed, Dabiré (1992) and Néya et al. (2008) also showed that the propagation of CABMV epidemic in cowpea crop and aphid population (vectors of these *Potyvirus*) decreased from the sudan zone to the sahel. Elsewhere, Estay et al. (2009) reported that aphids' population increase is a key factor to the spread of viruses and it depend on climate. In other parts, the prevalence of CABMV in cowpea was also found higher in the sudan than the sudan-sahel and the sahel (Néya, 2011; Palanga et al., 2016).

However, this study reports that CABMV was most prevalent than BCMV-BlCMV even in DAS-ELISA (7.14 against 1.43%, respectively) and RT-PCR (10.71 against 2.86%, respectively). This is in agreement with the study of Néya (2011) and Palanga et al. (2016) work on cowpea.

The none identification of CPMV, CPMoV and PeMoV might explain their absence in the 140 bambara groundnut samples tested. However, Sérémé (1989) had already reported CPMoV and PeMoV on bambara groundnut in the country.

The high identity obtained in BlastN identification of CABMV (88 to 98%) and BCMV-BlCM (97 to 98%) is in agreement with the ICTV *Potyvirus* species demarcation (<76% nucleotide identity in the coat protein) (Wylie et al., 2017).

The phylogenetic tree supports the occurrence of the two *Potyvirus* species CABMV and BCMV-BlCM on bambara groundnut in Burkina Faso. The specificity of nucleotide sequence to distinguish these two viruses was reported by Grisoni et al. (2006) and Palanga et al. (2016). Two groups within Burkina Faso CABMV isolates were observed on the phylogenetic tree (Figure 3). When the first group (MF277036, MF277033, MF277035, MF277037, MF277039) shared high nucleotide identity (97.5 to 97.7%) with Ugandan isolate (KT726938), the second group (MF277031, MF277034, MF277038, MF277041) was at 85.1 to 93.3% identity to the same Ugandan isolate. According to the *Potyvirus* strain criteria demarcation proposed by Shukla and Ward (1988) (with nucleotides sequence identity >95% in the coat protein), members of the first group and the Ugandan isolate (KT726938) might be the same strain. However, member of the second group might still be an isolate strain. These results might support the presence of two strains of CABMV infecting bambara groundnut in Burkina Faso. Indeed, previous study based on serological test revealed the presence of four serotypes I, II, III and IV within CABMV species infecting legume crops in Burkina Faso (Néya, 2011). Beside the Ugandan isolate (KT726938), all Burkina Faso CABMV isolates were closed to Nigerian isolate (Y17822) (78.4 to 84.5% identity).

The high seed-transmission of CABMV on cowpea (3 to 100%) was shown by Konaté and Néya (1996), Néya (2002) and Barro et al. (2016). It was considered to be the first source of infection to initiate CABMV epidemic (Néya et al., 2007). Bambara groundnut or other legume crops seeds trade between countries or seeds exchange between research centers could explain the strong relationships between Uganda and Burkina Faso isolates.

Burkina Faso BCMV-BlCM isolates (MF277032, MF277040 and MF277042) shared 97.3 to 97.5% high identity to China BCMV (AJ312438) and 94.1 to 94.3% to Taiwan BlCMV (AY575773). Since Khan et al. (1993) showed that BlCM and BCMV are strain of the same species, our study supports that Burkina Faso BCMV-BlCM and Chinese BCMV isolate (AJ312438) might be the same strain according to (Shukla and Ward, 1988) strain criteria demarcation.

Altogether, our study reports the first molecular characterization of virus from genus *Potyvirus* in bambara groundnut from Burkina Faso.

Conclusion

This study reveals the occurrence of CABMV and BCMV-BlCMV infecting bambara groundnut in Burkina Faso. However, CABMV was the most prevalent. Beside, RT-PCR was the most accurate tool for these virus detection in bambara groundnut; the phylogenetic analyses permit the understanding of evolutionary relationships between Burkina Faso isolates and others Genbank available species. However, further analysis based on the whole coat protein gene in amino acid or nucleotide and increase of samples number is required for better characterization of virus from genus *Potyvirus* occurring in bambara groundnut fields in Burkina Faso.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to the Mc Knight Foundation for their financial and technical support. They also thank LMI-PathoBios (Laboratoire Mixte International-PathoBios) for the technical assistance provided throughout this study.

REFERENCES

Akinjogunla OJ, Taiwo MA, Kareem KT (2008). Immunological and molecular diagnostic methods for detection of viruses infecting cowpea (*Vigna unquiculata).* Afr. J. Biotechnol. 7(13):2099-2103.

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25(17):3389-3402.

Barro A, Sawadogo M, Kiebre Z, Neya BJ (2016). Evaluation de la résistance de quelques lignées et écotypes de niébé (Vigna unguiculata (L.) WALP.) au cowpea aphid-borne mosaic virus au Burkina Faso. Int. J. Innov. Appl. Stud. 15(2):387-394.

Bamshaiye OM, Adegbola JA, Bamishaiye EI, (2011). Bambara groundnut: An Under Utilized Nut in Africa. Adv. Agric. Biotechnol. 1:60-72.

Bird AE, Corbett MK, (1988). Bambara groundnut mosaic caused by a seed-transmitted potyvirus. Phytopathology 78:860.

Bock KR (1973). East African strains of cowpea aphid-borne mosaic virus. Ann. Appl. Biol. 74(1):75-83.

Bock KR, Guthrie EJ, Meredith G (1978). Viruses occurring in East Africa that are related to peanut mottle virus. Ann. Appl. Biol. 89:423 428.

Brink M, Grubben GJH, Belay G, AGROOH (2006). Ressources végétales de l'Afrique tropicale 1 : Céréales et légumes secs. Edition M. Brink. Wageningen University, Wageningen, Netherlands, 328 p.

Brunt AA, Crabtree K, Gibbs AJ (1990). Viruses of tropical plants. Wallingford (CT): CAB International.

Clark MF, Adams AN (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.

Dabiré CLB (1992). Contribution à l'étude des problèmes phytosanitaires du niébé au Burkina. Lutte intégrée contre les ennemis des cultures vivrières dans le sahel. INSAH. Séminaires et Colloques. 1:295-304.

Drabo I, Sérémé P, Dabiré C, (1997). Country reports. In: Heller J, Begemann F, et Mushonga J, Eds. Conservation and improvement of Bambara groundnut (*Vigna subterranea* (L.) Verdc.). BP 550 Hararé, Zimbabwé. Pp. 19-26.

Edgar RC (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32:1792-1797.

Estay SA, Lima M, Harrington R (2009). Climate mediated exogenous forcing and synchrony in populations of the oak aphid in the UK. Oikos 118(2):175-182.

Fauquet C, Thouvenel JC (1987). Plant viral diseases in the Ivory Coast. ORSTOM, Paris, Documentation Techniques 46:243.

Frenkel MJ, Jilka JM, Shukla DD, Ward CW (1992). Differentiation of potyviruses and their strains by hybridization with the 3' non-coding region of the viral genome. J. Virol. Methods 36(1):51-62.

Frison EA, Bos L, Hamilton RI, Mathur SB, Taylor JD, (1990). FAO/IBPGR technological guidelines for the safe movement of legume germplasm. Rome, Italy: Food and Agriculture Organization of the United Nations, International Board for Plant Genetic Resources.

Gillaspie Jr. AG, Mitchell SE, Stuart GW, Bozarth RF (1999). RT-PCR method for detecting cowpea mottle carmovirus in *Vigna* germ plasm. Plant Dis. 83:639-643.

Goli AE, Begemann F, Ng NQ (1997). Characterization and evaluation of IITA's bambara groundnut collection. In. Heller J, Begemann F, et Mushonga J, Eds. Conservation and improvement of bambara groundnut (*Vigna subterranea* (L.) Verdc.), BP 550, Hararé, Zimbabwé, pp. 101-118.

Grisoni M, Moles M, Farreyrol K, Rassaby L, Davis R, Pearson M (2006). Identification of potyviruses infecting vanilla by direct sequencing of a short RT-PCR amplicon. Plant Pathol. 55(4):523-529.

Gumedzoe MY (1985). Studies of variability of the cowpea aphid-borne mosaic (CAbMV) complex in Nigeria. Doctorate thesis, University of Lawal, Quebec, Canada.

Khan JA, Lohuis D, Goldbach R, Dijkstra J (1993). Sequence data to settle the taxonomic position of bean common mosaic virus and blackeye cowpea mosaic virus isolates. J. Gen. Virol. 74(10):2243-2249.

Konaté G, Néya BJ (1996). Rapid detection of cowpea aphid-borne mosaic virus in cowpea seeds. Ann. Appl. Biol. 129(2):261-266.

Liebenberg A, Freeborough MJ, Visser CJ, Bellstedt DU, Burger JT, (2009). Genetic variability within the coat protein gene of Grapevine fanleaf virus isolates from South Africa and the evaluation of RT-PCR, DAS-ELISA and ImmunoStrips as virus diagnostic assays. Virus Res. 142(1-2):28-35.

Lima JAA, Nascimento AKQ, Radaelli P, Purcifull DE (2012). Serology applied to plant virology. In. Serological diagnosis of certain human, animal and plant diseases. InTech.

Longué RDS, Galzi-Pinel A, Zingua I, Semballa S, Fargette D, Barro N, Traoré O (2017). Genetic diversity and molecular epidemiology of *Rice Yellow Mottle Virus* in Central African Republic. Int. J. Virol. 13:80-89

Marie-Jeanne V, Loos R, Peyre J, Alliot B, Signoret P, (2000). Differentiation of Poaceae potyviruses by reverse transcription-polymerase chain reaction and restriction analysis. J. Phytopathol. 148(3):141-151.

Mkandawire CH (2007). Review of BG production in Sub Sahara Africa. Agric. J. 2(4):464-470.

Muhire BM, Varsani A, Martin DP (2014). SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9(9):e108277.

Nadembega S (2016). Food production and food security in Burkina Faso: Case of the bambara groundnut in three communes of the three agro-ecological zones. Diploma of Master 2, *Université Catholique de l'Afrique de l'Ouest, Bobo Dioulasso,* 90 p.

Néya BJ, (2002). Variabilité sérologique et aspects épidémiologiques du virus de la mosaïque du niébé (*Vigna unguiculata* [L.] Walp.) transmis par des pucerons au Burkina Faso. Mémoire de DEA, Université de Ouagadougou.

Néya BJ (2011). Sérologie, pathogénie, épidémiologie et contrôle de la mosaïque Cowpea aphid-borne mosaic virus (CABMV) du niébé (*Vigna unguiculata* (L.) Walp.) transmise par des pucerons (*Aphis craccivora*, *A. gossypii*) au Burkina Faso. Thèse de doctorat*,* Université de Ouagadougou.

Néya BJ, Zabré J, Millogo RJ, Guinko S, Konaté G (2007). Effect of cowpea seeds contamination rate by the Cowpea aphid borne mosaic virus on epidemics development. Pak. J. Biol. Sci. 10(24):4365-4374.

Néya BJ, Zabré J, Millogo RJ, Guinko S, Konaté G (2008). Propagation of the CABMV from infected seeds in three zones of Burkina. Plant Pathol. J. 7(1):75-84.

Ouédraogo M, Ouédraogo TJ, Tignéré JB, Balma D, Dabiré BC, Konaté G (2008). Characterization and evaluation of accessions of Bambara groundnut. Sci. Nat. 5(2):191-197.

Palanga E, Filloux D, Martin DP, Fernandez E, Gargani D, Ferdinand R, Zabré J, Bouda Z, Neya JB, Sawadogo M, Traore O, Peterschmitt M, Roumagnac P, (2016). Metagenomic-based screening and molecular characterization of cowpea-infecting viruses in Burkina Faso. PLoS One. 11(10):1-21.

Robertson DG, (1966). Seed-borne viruses of cowpea in Nigeria. BSc thesis, University of Oxford, 111 p.

Rossel HW (1977). Preliminary investigation on the identity and ecology of legume virus diseases in northern Nigeria. Trop. Grain Legume Bull. 8:41-46.

Sérémé P (1989). Seed-borne pathogens of bambara groundnut (*Vigna subterranea* (L.) Verdc.) in Burkina Faso and their significance. A report of research conducted from September-December, 1982 at the Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen, Denmark. 49 p.

Shoyinka SA, Bozarth RF, Reese J, Rossel HW (1978). Cowpea mottle virus: a seed-borne virus with distinctive properties infecting cowpea in Nigeria. Phytopathology 68:693-699.
Shukla DD and Ward CW (1988). Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. J. Gen. Virol. 69:2703-2710.
Sipahioğlu HM (2005). Comparison of DAS-ELISA and RT-PCR Methods for the Detection of *Prunus Necrotic Ringspot Virus* (PNRSV). J. Agric. Sci. 15(2):153-158.
Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30(12): 2725-2729.
Thottappilly G, Rossel HW (1997). Identification and characterization of viruses infecting bambara groundnut (*Vigna subterranea*) in Nigeria. Int. J. Pest Manage. 43(3):177-185.
Udaya Shankar AC, Nayaka CS, Kumar BH, Shetty SH, Prakash HS, (2009). Detection and identification of the blackeye cowpea mosaic strain of Bean common mosaic virus in seeds of cowpea from southern India. Phytoparasitica 37(3):283-293.
Wylie SJ, Adams M, Chalam C, Kreuze J, Lopez-Moya JJ, Ohshima K, Praveen S, Rabenstein F, Stenger D, Wang A, Zerbini FM, ICTV Report Consortium (2017). ICTV virus taxonomy profile: Potyviridae. J. Gen. Virol. 98(3):352-354.
Zettler FW, Evans IR (1972). Blackeye cowpea mosaic virus in Florida: host range and incidence in certified cowpea seed. Proceedings of the Florida State Horticultural Society 85(99):101.