Full Length Article



Evolution and Distribution of Mosaic Disease Caused by *Cowpea aphid borne mosaic virus* (CABMV) on Bambara Groundnut (*Vigna subterranea*) in Burkina Faso

Zongo Aboubié Elisabeth^{1*}, Ouattara Alassane², Neya Bawomon Fidèle³, Koala Moustapha^{1,4}, Néya Bouma James¹ and Traore Oumar^{1,4}

¹Laboratoire de Virologie et de Biotechnologie Végétales, Institut de l'Environnement et de Recherches Agricoles (INERA), 01 BP 476 Ouagadougou, Burkina Faso

²Laboratoire Biosciences, Université Joseph KI-Zerbo, Ouagadougou, 03BP 7021, Ouagadougou 03, Burkina Faso

³Centre Universitaire de Gaoua, Université Nazi BONI, Bobo Dioulasso, 01BP1091, Bobo-Dioulasso 01, Burkina Faso

⁴Laboratoire Nationale de Biosécurité (LNB), 06BP 10798 Ouagadougou, Burkina Faso

*For Correspondence: zongoelisabeth29@gmail.com

Received 15 November 2023; Accepted 13 January 2024; Published 14 February 2024

Abstract

Bambara groundnut is an important legume with great nutritional and economic potential. However, its production is limited by several constraints, including viral diseases. Among these diseases, mosaic disease caused by *Cowpea aphid borne mosaic virus* (CABMV) is the most dominant and frequent on Bambara groundnut. The aim of this study was to investigate the evolution of the disease caused by CABMV in a real environment to develop effective and sustainable control strategies. Sampling was carried out in farmers' fields in the localities of Dapélogo and Kamboinsé from central region of Burkina Faso. Symptomatic and asymptomatic samples were collected from Bambara groundnut and neighbouring crops, followed by periodic observations on the development of the disease in the fields of the two localities. Serological and molecular diagnostic tests revealed the presence of CABMV in the samples collected. The spread of the disease varied according to locality and evaluation period. The results showed a variation in the average number of diseased seedlings from 0.5 to 11.94 respectively at 15 days after sowing (DAS) and 55DAS. Disease incidence was severe in the Dapélogo locality with an average infection rate of 8.97% versus 5.25% in Kamboinsé. Plants aged between 15 and 45 days were the most favourable for viral infection. Cowpeas, peanuts and sesame crops were identified as CABMV reservoir hosts. The study provides for the first-time data on the epidemiology of CABMV infecting Bambara groundnut. This is important for the development of virus management strategies in the event of an epidemic. © 2024 Friends Science Publishers

Keywords: Alternative hosts; Bambara groundnut; Cowpea aphid borne mosaic virus; Incidence; Spread

Introduction

Bambara groundnut (*Vigna subterranean*) is one of the main legumes cultivated in West Africa, with 162,361.34 tons harvested from 222,296 ha in 2021. This production represented around 67.76% of the world production (FAOSTAT 2023). For the same year, Burkina Faso was the largest Bambara groundnut producer with 65965.95 tons harvested from 60673 ha followed by Zambia and Niger with 6316.89 and 52211.04 tons harvested from 25632 and 89104 ha, respectively (FAOSTAT 2023). Bambara groundnut is mainly grown for its seeds, which are sources of nutrients such as proteins, carbohydrates and starch (Sirivongpaisal 2008).

Despite this economic and nutritional importance, the crop is subject to numerous biotic stresses, in particular viral

diseases, which cause up to 85% of the losses (Zongo *et al.* 2018). Six viruses were mainly identified as causes of some diseases on Bambara groundnut in Burkina Faso. These viruses are *Cowpea aphid-borne mosaic virus* (CABMV), *Blackeye common mosaic virus* (BICMV), *Peanut mottle virus* (PeMV), *Cowpea mottle virus* (CPMoV) and two new species, *Bambara groundnut potyvirus* 1 (BGPV1) and *Bambara groundnut potyvirus* 2 (BGPV2) (Konate *et al.* 2017; Zongo *et al.* 2019).

Mosaic disease caused by *Cowpea aphid-borne mosaic* (CABMV) is a major threat to this crop among other diseases (Zongo *et al.* 2019). CABMV is identified naturally on Bambara groundnut and is the most prevalent in Burkina Faso. The disease is characterized by a mosaic with deformation of the leaves and stunting of the plants. CABMV is transmitted by the aphids, *Aphis craccivora* and *Aphis gossypii*, with transmission rates varying between 64 and 71% (Bashir and Hampton 1994). This virus could have major economic consequences if preventive measures are not adopted by farmers. Few data on the epidemiological aspects of CABMV are available on Bambara groundnut. Knowledge of these parameters is essential for developing a control strategy to prevent epidemics. The study will therefore assess the spread and distribution of CABMV in farmers' fields.

Materials and Methods

Sites, survey and sampling

Surveys of Bambara groundnut fields were carried out in two localities of Burkina Faso: Kamboinsé (Central region near Ouagadougou; latitude: $12^{\circ} 27'44''$ N, longitude: 1° 33'17'' W) and Dapélogo (Central plateau region; latitude: $12^{\circ}40'18$ N, longitude: $1^{\circ}32'22$ W). Four farmers' fields were selected, including two per locality, on the basis of the prevalence of CABMV in that area to assess the number of emerged and diseased plants. Diseased plants were counted every ten days from 15 DAS to 55 DAS. Each field was divided into four blocks to facilitate counting the total number of emerged and diseased plants. Disease severity was assessed on Bambara groundnut leaves showing the disease symptoms based on the rating scale of Gumedzoe *et al.* (1990) ranging from 1 (no symptom) to 5 (severe symptom).

To study the factors of propagation and the evolution of CABMV on Bambara groundnut, symptomatic and asymptomatic cultivated plants belonging to the legume family were also collected (Table 1). All the collected samples were packed in plastic bags, kept on ice and sent to the laboratory for identification tests described below.

Double antibody sandwich-enzyme-linked immunosorbent assay

Leaf samples collected from Bambara groundnut fields and bordering crops were subjected to double antibody sandwich-enzyme-linked immunosorbent assay (DAS-ELISA). For this test, polyclonal antibodies were used to detect the presence of CABMV in the samples. A DAS-ELISA kit (AC diagnostic) was used, following the supplier's recommendations. The positive threshold was twice the average of the absorbance of non-infected samples.

RNA extraction and RT-PCR diagnosis

Total RNA from all samples was extracted using trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. Detection of CABMV was carried out in an RT-PCR assay using the specific primer: Poty-GP1-5'CP-F/CGARAAGGARTTRCAAAGG/ Poty-GP1-5'CP-R

CAGCTGCGTCAGAGAAGTG according to the conditions described by Zongo *et al.* (2019). Amplified fragments were checked by electrophoresis on 1% agarose gels containing ethidium bromide and visualized under UV light to check the size of the amplified fragment. Samples with fragment sizes of approximately 600 bp were considered positive for CABMV.

Fields data analyses

All statistical analyses were performed using the statistical software R v. 4.3.1 (R Core Team 2023). Firstly, the prevalence of disease and that of virus infection were calculated as the percentage of Bambara groundnut plants positive DAS-ELISA and RT-PCR results, respectively, among all surveyed plants from each field from Dapélogo and Kamboinsé. The differences between fields were then examined using the Chi-square test based on a contingency table containing the collected data. Secondly, incidence of disease was calculated as the percentage of Bambara groundnut plants with symptoms of mosaic each 10 days during the 40 days of monitoring among all of plants from each field in both localities Kamboinsé and Dapélogo. Incidences were examined using the Chi-square test based on the contingency table containing the collected data. Thirdly, average disease severities were calculated for each of the four fields based on recorded disease notes of Bambara groundnut plants. Obtained data were compared pairwise among the four fields based on analysis of variance (ANOVA) and mean values were separated by Student-Newman–Keuls (SNK) multiple-range test (P = 0.05). Fourthly, the disease prevalence and virus infection rate were calculated as the percentage of plants with symptoms of mosaic and with positive DAS-ELISA and RT-PCR results, respectively, among all cultivated plants belonging to the legume family collected around Bambara groundnut fields. The differences between plants species were then examined using the Chi-square test based on a contingency table containing the collected data.

Results

Description of symptoms observed from Dapélogo and Kamboinsé

In the fields surveyed, mosaic leaf and deformation symptoms were observed from Bambara groundnut in both the localities with different levels of severity (Fig. 1).

Average severity scores of 3.5 and 3.9 were recorded in Dapélogo in fields 1 and 2, while they were 3.44 and 3.38 in kamboinsé fields 1 and 2, respectively. The highest severity indices were recorded in Dapélogo. Very severe symptoms were observed at 55 days after showing (DAS). However, no variation in symptom severity indices was observed in the other plots at 55 DAS (Fig. 2).

Locality	Especes	Varieties	number	
	Vigna unguiculata	KVX 61	06	
Dapélogo	Vigna unguiculata	Komcalé	05	
1 0	Sesamum indicum	-	13	
	Arachis hypogaea	-	07	
	Vigna unguiculata	Komcalé	08	
Kamboinsé	Vigna unguiculata	Tiligré	04	
	Arachis hypogaea	-	05	
			0.5	

 Table 1: List of cultivated plant species belonged to Fabaceae family collected around the Bambara groundnut fields in Kamboinse an Dapélogo in Burkina Faso



Incidence of Bambara groundnut mosaic leaf disease

Incidence of Bambara groundnut mosaic leaf disease was assessed in both localities based on the number of diseased plants. The Bambara groundnut mosaic leaf disease epidemic curves for the four fields show phases that vary in importance from field to field. There is an exponential phase in the infection rate of new plants from 25 to 45 days after infection, followed by a declining phase of variable duration (Fig. 3). The speed at which the disease spread through the fields varied considerably. The number of infected plants increased rapidly from 25 DAS onwards, peaking at 45 DAS. Between 35 and 45 days, the Dalpélogo fields recorded higher rates of new infection (3.9 and 3.82% respectively for Field 1 and 2) than the Kamboinsé fields (2.48% for field 1 and 2.2% for field 2). After 45 days, new infection rates dropped to an average of 1.55%. Comparison analysis of disease incidences according to date not showed significant difference between all fields ($P \ge 0.09$) except the field 1 and the Field 2 from Dapélogo and Kamboinsé which showed significant difference at 55 DAS (P = 0.03).

Frequency of Bambara groundnut disease and CABMV identified by DAS-ELISA and RT-PCR.

Both fields at Dapélogo showed the highest frequency of plant diseases with 10% (59/589 from the field 1) and 7.93% (56/706 in the field 2) at 55 DAS (Table 2, 3). However, at the same time, 5.83% (40/686) and 4.65% (36/774) diseased plants were recorded in fields 1 and 2 at Kamboinsé, respectively.

The DAS-ELISA diagnosis confirmed the presence of CABMV in some diseased plants from different fields (Table 2). In fact, 105 leaf samples including 50 and 55 samples from the Dapélogo and Kamboinsé sites respectively, were tested by DAS-ELISA and RT-PCR, confirming CABMV infection in 63 and 77 samples respectively, with an average detection rate of 60% and 73.33%. The Dapélogo site, specifically field 1, recorded the highest detection rate of 71.43%, compared with 59% in field 1 at Kamboinsé. The tests revealed that, in addition to the CABMV-positive samples, there were other potential potyvirus species or strains that were not detected by the polyclonal antibodies and CABMV-specific primer used,

 Table 2: Frequency of Bambara groundnut disease and CABMV detected based on DAS-ELISA and RT-PCR from Bambara groundnut collected from Dapélogo and Kamboinsé in Burkina Faso

			CABMV freque	ncy (Infected/tested samples)
		Disease frequency (Diseased plants/observed plants)	DAS-ELISA	RT-PCR
Dapélogo	Field 1	10.02% (59/589)	71.43% (15/21)	71.43% (15/21)
	Field 2	7.93% (56/706)	58.62% (17/29)	72.41% (21/29)
Kamboinsé	Field 1	5.83% (40/686)	59.46% (22/37)	75.68% (28/37)
	Field 2	4.65% (36/774)	50% (9/18)	72.22% (13/18)
Total		6.93% (191/2755)	60% (63/105)	73.33% (77/105)



Fig. 2: Infection levels of Bambara groundnut plants according to the symptom severity scale of Gumedzoe *et al.* (1990). Each point represents the average for fields. The symptom severity scale (left scale bar) ranges from 1 (no symptoms) to 5 (severe symptom). For each dot, vertical bars represent 95% confidence intervals. Identical letters on top of the bars indicate groups with non-significant differences in disease severity.

despite the serological relationships that exist between the viruses.

CABMV host range

Serological tests revealed that all samples from neighbouring crops were positive for CABMV. Variable infection rates were recorded according to plant species and varieties. The virus was identified in all variety of cowpea with infection rate ranging from 69.23 to 100%. Infection rates of 61.54% (8/13) and 50% (6/12) were recorded for sesame and peanut samples, respectively. Nevertheless, all neighbouring crops were identified as sources of CABMV propagation.

Discussion

The detection of CABMV in this study confirms the presence of the virus in Soudan Sahel area in Burkina Faso. In fact, early studies have reported this virus in Burkina

Table 3: Infection rate of CABMV

Species	Varieties	Infection rate (infected/tested)
Vigna unguiculata	Komcalé	69.23% (9/13)
Vigna unguiculata	KVX 61 1	100% (6/6)
Vigna unguiculata	Tiligré	100 (4/4)
Sesamum indicum	S42	61,54% (8/13)
Arachis hypogaea	-	50% (6/12)



Fig. 3: Bambara groundnut mosaic leaf disease incidence in farmers' fields form two localities in Burkina Faso (Dapélogo and Kamboinsé)

Faso (Néya *et al.* 2015; Palanga *et al.* 2016; Zongo *et al.* 2019). The four fields sampled showed variable incidences and symptoms. This observation could be linked to the presence of CABMV viral strains inducing severe symptoms that varied from one field to another. To this end, two strains of CABMV have been identified infecting Bambara groundnut in Burkina Faso by Konate *et al.* (2017) and Zongo *et al.* (2019).

Serological (DAS-ELISA) and molecular (RT-PCR) detection confirmed the close relationship between the symptoms observed and the virus. However, the ELISA test was less effective in detecting viruses (60%) than the RT-PCR test (73.33%). In fact, the specific primer of Zongo et al. (2019) used in RT-PCR amplifies a highly conserved area of the CABMV capsid protein gene, giving it a high detection efficiency of CABMV in samples even at low concentration. These results may be supported by the work of Sipahioğlu (2005) and Liebenberg et al. (2009), which showed that molecular RT-PCR tests were more effective than ELISA tests. Symptomatic samples that were negative to DAS-ELISA and RT-PCR diagnosis are either infected by other virus, whose antibodies and primers were not used in this study or to the fact that the symptoms observed were not of viral origin.

The progression of the disease caused by CABMV differed between the study fields. Although both sites were in the same agro-ecological zone and with the same plant material, they did not have the same level of infection. On the other hand, samples showing symptoms of viral infections tested negative. These results show that diagnosing viral diseases solely on the basis of symptoms is not always reliable. Indeed, symptoms caused by one particular virus may be confused with another.

The appearance and spread of the disease in the fields could also be linked to a number of factors. Symptoms observed at 15 days after sowing may be seed-borne, as the seeds used by growers are generally from previous harvests. It was reported on cowpea by Néya (2011) that CABMV was also seed-transmitted at a rate of 5%. Although a similar study has not been carried out on Bambara groundnut, the possibility that CABMV was transmitted by seed in certain fields cannot be ruled out. Furthermore, the increase in infection rates up to 45 days after planting (3.9 and 3.38% in Dapélogo and Kamboinsé fields) may be the result of mechanical contamination due to the shared use of farming tools and the abundance of insect vectors. It may also be associated with the phenological stage of seedlings (young seedlings) favourable to contamination (Aka *et al.* 2009).

The spread of the disease can cause severe damage to Bambara groundnut fields and considerably reduce yields. During the study, the spread of the disease was illustrated by multi-phase progression curves. As the disease is polycyclic, during different infection cycles, plants infected during a previous cycle serve as sources of inoculum for the following cycle (Zadoks and Schein 1979).

Phases of decline, characterized by new infections

after 45 days, may be explained by a strengthening of resistance mechanisms or factors linked to the age of the plant. Nevertheless, the progression of the disease in all fields seems to be linked to the lack of treatment against insects. During the course of the study, the presence of aphids was noted on both sites. Néya (2011) has shown that high populations of aphid vectors of CABMV are a factor of dissemination and are often responsible for high incidences of the disease, for which the absence of insecticide treatments results in high parasite pressure.

Observations in the fields based on symptoms revealed a low incidence of CABMV in the Kamboinsé fields. These differences also seem to be linked to several factors, including inoculum sources and human activities in the fields. Variations in environmental factors such as wind and rain could also be responsible for the spread of CABMV in the fields (Colhoun 1979). Crops adjacent to Bambara groundnut fields have been identified as alternative hosts for CABMV and may serve as a source of inoculum. In fact, peanut, cowpea, soybean and sesame fields in the Dapélogo locality were identified as being very close to Bambara groundnut fields. However, Bashir et al. (2002); Néya (2011) and González et al. (2013) reported that CABMV infects several species from different families, including Chenopodiaceae, Cucurbitaceae and Amaranthaceae, Solanaceae. This could also explain the strong spread of the disease in this locality. These hosts can harbour the virus, enabling it to be spread by insect vectors and other agroclimatic factors. It would therefore be necessary to keep the fields of these crops at a distance from the Bambara groundnut fields in order to avoid major epidemics. All these host species could play an important role in the maintenance and epidemiology of the virus. During the growing season, Arachis hypogaea and Vigna unguiculata could act as relay or vehicle plants for the disease. This is of particular concern, given that Bambara groundnut and these species are generally grown in association or in rotation, which could cause severe yield losses. Zongo et al. (2022) recorded yield losses of 80% on Bambara groundnut in Burkina Faso. Nevertheless, the same authors identified CABMV-tolerant Bambara groundnut genotypes. It would therefore be judicious to integrate them into varietal improvement programs in order to help reduce the spread of the disease in farmers' fields. Although wild species were not tested in this study, they could harbour the virus during the dry season. As such, they may play an important role as a source of inoculum for the spread of the virus in Bambara groundnut crops during the wet season. Virus control measures should therefore take all these plant species into account.

Conclusion

This study provided an overview of the spread of mosaic disease caused by CABMV on Bambara groundnut in growers' fields in Burkina Faso. The results showed a variation in the spread of the disease over time and space.

Young plants (15 to 45 days old) are favourable to infection by the CABMV virus. It would therefore be important to take into account the age of the plant when developing methods to combat the virus. The high frequency (10– 7.93%) of the disease recorded at the Dapélogo site is thought to be due to the presence of insect vectors and alternative hosts. The results showed that neighbouring crops, notably cowpeas, peanuts and sesame, are sources of CABMV spread and can cause high incidences of the disease or epidemics in Bambara groundnut fields. It would therefore be necessary to keep the fields of these crops at a distance from the Bambara groundnut fields in order to avoid major epidemics. The adoption of integrated management will help to combat the viruses more effectively.

Acknowledgements

The authors thank the Laboratoire Mixte International Patho Bios, Burkina Faso, for providing the technical platform. The author would like to thank the International Foundation for Science (IFS) for the financial support through the grant n° C/5884-1 awarded to A.E. Zongo.

Author Contributions

ZAE carried out tests, collected data; ZAE, AO analysed the data; ZAE, AO, NBF, KM and NBJ wrote and corrected the manuscript; OT supervised all the work.

Conflicts of Interest

All authors declare no conflict of interest

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Ethics Approval

Not applicable to this paper

References

- Aka AR, NK Koussi, TA Agnéroh, NA Amancho, A Sangaré (2009). Distribution et incidence de la mosaïque du concombre (CMV) dans des bananeraies industrielles au Sud-Est de la Côte d'Ivoire. Sci Nat 6:171–183
- Bashir M, RO Hampton (1994). Seed and aphid transmission of some isolates of blackeye cowpea mosaic and cowpea aphid-borne mosaic potyviruses. *Pak J Phytopaphol* 6:140–146

- Bashir M, Z Ahmad, A Ghafoor (2002). Cowpea aphid-borne mosaic potyvirus: A review. Intl J Pest Manage 48:155–168
- Colhoun J (1979). Predisposition by the environment. In: Plant Disease: An Advanced Treatise, Vol. IV, pp:75–96. JG Horsfall, EB Cowling (Eds). How Pathogens Induce Disease: Academic Press, New York, USA
- FAOSTAT (2023). Crop production. Food and Agriculture Organization, Rome, Italy. Available at: http://www.fao.org/faostat/en/#data/QC
- González SLR, AE Fariña, DD González, APOA Mello, JAM Rezende, EW Kitajima (2013). Alternative hosts of *Cowpea aphid-borne mosaic* virus (CABMV) in sesame (Sesamum indicum) crops grown in Paraguay. Trop Plant Pathol 38:539–542
- Gumedzoe MY, DY Sunu, G Thottappilly, A Asselin (1990). Importance du virus de la marbrure de niébé et du virus de la mosaïque jaune du niébé au Togo. *Phytoprotection* 71:85–91
- Konate MN, M Ouedraogo, BJ Neya, M Bangratz, E Palanga, H Nandkangre, A Ouoba, R Nanema, N Sawadogo, M Sawadogo (2017). Molecular characterization of virus isolates from genus Potyvirus infecting Vigna subterranea in Burkina Faso. Afr J Biotechnol 16:1953–1961
- Liebenberg A, MJ Freeborough, CJ Visser, DU Bellstedt, JT Burger (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 Immuno Strips as virus diagnostic assays. *Virus Res* 142:28–35
- Néya BJ (2011). Sérologie, Pathogénie, Epidémiologie et contrôle de la mosaïque, Cowpea Aphid-Borne Mosaic Virus (CABM) du niébé (Vigna unguiculata (L.) WALP.) Transmise par les pucerons (Aphis craccivora, A. gossypii) au Burkina Faso, p:218. Thèse unique. Université de Ouagadougou, Burkina Faso
- Néya BJ, PE Zida, D Sérémé, OS Lund, O Traoré (2015). Evaluation of yield losses caused by *cowpea aphid-borne mosaic virus* (CABMV) in 21 cowpeas (*Vigna unguiculata* (L.) Walp.) Varieties in Burkina Faso. *Pak J Biol Sci* 18:304–313
- Palanga E, D Filloux , DP Martin, E Fernandez, D Gargani, R Ferdinand, J Zabré, Z Bouda, JB Neya, M Sawadogo, O Traore, M Peterschmitt, P Roumagnac (2016). Metagenomic-based screening and molecular characterization of cowpea-infecting viruses in Burkina Faso. *PLoS One* 11:e0165188
- Sipahioğlu HM (2005). Comparison of DAS-ELISA and RT-PCR methods for the detection of Prunus Necrotic Ringspot Virus (PNRSV). Sci J Agric 15:153–158
- Sirivongpaisal P (2008). Structure and functional properties of starch and flour from Bambara groundnut. *Songkl J Sci Technol* 30:51–56
- Zadoks JC, RD Schein (1979). *Epidemiology and Plant Disease* Management, p:427. Oxford University Press, New York, USA
- Zongo AE, M Koala, BJ Neya, VSE Traore, PE Zida, A Ouoba, N Barro, O Traore (2022). Evaluation des accessions de voandzou pour la résistance au virus de la mosaïque du niébé (CABMV) au Burkina Faso. Evaluation of Bambara groundnut accessions for resistance to cowpea mosaic virus (CABMV) in Burkina Faso. Sci Nat Appl 41:33–47
- Zongo AE, BJ Néya, E Palanga, D Sérémé, M Koala, R Dimitri, S Longué, J Zabré, Z Bouda, N Barro, O Traoré (2019). Molecular characterization of a large diversity of bambara groundnut potyviruses (Vigna subterranea (L.) Verdc.) in Burkina Faso. Adv Biosci Biotechnol 10:270–281
- Zongo E, BJ Néya, VSE Traoré, E Palanga, J Zabré, N Barro, O Traoré (2018). Impact of cowpea mottle virus on the growth and yield of bambara groundnut (*Vigna subterranean* (L.) Verdc.). Amer J Plant Sci 9:2053–2062