

Impact of *Cowpea mottle virus* on the Growth and Yield of Bambara Groundnut (*Vigna subterranea* (L.) Verdc.)

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Abstract

Bambara groundnut [*Vigna subterranea* (L.) Verdc] is a food legume with high potential for food security in Sub-Saharan Africa. However, in addition to being a neglected crop, its production is limited by several constraints among which viral diseases are most cited. In order to contribute to the improvement of Bambara groundnut in Burkina Faso, local accessions of the crop were screened for resistance to *Cowpea mottle virus* (CPMoV), one of the most damaging viruses in grain legumes. Seven local accessions (C1 to C7) from two agro-ecological zones were evaluated by mechanical inoculation in field conditions in 2016 and 2017. The infected plants exhibited various symptoms of chlorosis, leaf deformation, growth retardation and plant stunting. CPMoV caused a significant reduction in the number of flowers and pods. As a result, grain yield was reduced by 49.5% to 83.9% depending on the accessions. The impact of the virus in yield loss was lowest in accessions C6 and C7 which indicated their possible use in the management of *Cowpea mottle virus* disease in bambara groundnut.

Keywords

Bambara Groundnut, *Cowpea mottle virus*, Impact, Yield Loss

1. Introduction

Bambara groundnut [*Vigna subterranea* (L.) Verdc.] is one of most important grain legumes particularly for rural communities in Burkina Faso. The crop is

adapted to various climatic and ecological conditions and fits well with the semi-arid climatic condition of the country. As a leguminous plant, it contributes to soil fertilization through the symbiotic fixation of atmospheric nitrogen [1] [2] [3]. Moreover, Burkina Faso is one of the main producers and exporters of bambara groundnut in West Africa. Besides the local market, it also supplies markets in countries such as Benin, Ghana, Nigeria and Togo [4].

Bambara groundnut is a high-energy plant which is rich in carbohydrates, proteins and minerals [4] [5]. Therefore, it is an excellent supplement to cereals and tubers that are the main food sources in sub-Saharan Africa. Moreover, this plant offers great potential for improvement due to its genetic diversity found in thousands of accessions harvested around the world [6] [7].

The production of bambara groundnut is constrained by a range of biotic factors including bacteria, fungi, insects, nematodes and viruses [8] [9]. *Cowpea mottle virus* (CPMoV, genus *Carmovirus*) is one of the common legume viruses which have been naturally found in bambara groundnut. CPMoV was described for the first time in Nigeria more than five decades ago (Robertson, 1963) but it was reported in Burkina Faso only recently [10]. It is transmissible mechanically and by the beetle vectors *Ootheca mutabilis* and *Paraluperodesquaternus* [11]. Significant yield losses caused by CPMoV in cowpea were reported to reach 75% in single infections and 100% in co-infection with other viruses [11] [12]. The recent report of the virus in Burkina Faso may undermine the country's effort in promoting the production grain legumes as part of the struggle for food security and poverty alleviation.

The aim of this study was to determine the possible impact of CPMoV on local accessions of Bambara groundnut in order to contribute to the management of cowpea mottle disease.

2. Material and methods

2.1. Plant Material and Virus Inoculation

Seven accessions (named C1 to C7) of Bambara groundnut landraces were used. Five of them were collected from farmers' saved seeds in different locations and the two remaining were from the seed bank of the Institute of Environment and agricultural Research (INERA). Seed coats of accessions C1, C2, C5 and C7 were entirely creamy while the remaining accessions had two-colored seed coats. Most their seed coats were creamy but they also included black or brown stripes.

CPMoV isolate BE273 collected from cowpea [10] was used as source of inoculum. The virus was first propagated by mechanical inoculation of cowpea plants kept in insect-proof cages. Two weeks after inoculation, symptom-bearing leaves from infected plants were harvested. Leaf samples were ground at the ratio of 1/10 (w/v) in 0.01 M sodium phosphate buffer, pH 7.4. The resulting slurry was squeezed through cheese cloth and carborundum 600 mesh was added to the extract. This extract was subsequently applied to two-week-old Bambara groundnut plants by carefully rubbing the upper surface of the leaves.

2.2. Experimental Design and Field Conditions

The experiment took place during the 2016 and 2017 cropping seasons (June to October) at INERA, Kamboinsé Research Station (12°28' N latitude, 1°32' W longitude). The field layout was randomized completely block design with four blocks and seven plots per block. The field was deeply ploughed and NPK fertilizer (14-23-14) was applied at 100 kg/ha. Seeds were planted in 80 cm spaced rows with 20 cm spacing in a single row. Two weeks after sowing, emerged plants were mechanically inoculated in two blocks and plants in the two remaining blocks were kept uninoculated. Sprays of insecticide consisting of a mixture deltamethrine and dimethoate were done fortnightly to minimize insect colonization of the field. For weed control, manual weeding was performed as necessary.

Data were collected from the five inner plants of the seven plants on each line. They included the date of symptoms appearance, type of symptoms, size of the plants, number of flowers at 50% flowering stage, weight of pods and seeds yield.

2.3. Serological Testing

CPMoV presence was diagnosed in plants using the double antibody sandwich format of the enzyme-linked immunosorbent assay method as described by [13]. Briefly, young trifoliolate leaves collected using cloves and placed in plastic sampling bags. Collected samples were processed by grinding the leaves with mortar and pestle in sample extraction buffer. The extract was centrifuged for 10 min at 8000 ×g and the supernatants were used for virus detection. A positive control sample and two negative controls consisting of healthy leaf sample extracts and extraction buffer only, respectively, were included in the tests. All samples were tested in triplicates and the reaction results were recorded as optical densities (OD) measured at 405 nm using a METERTECH Σ960 automatic microplate reader. A sample was considered positive for virus detection if the corresponding OD_{405nm} values were greater than the detection limit (mean OD_{405nm} from healthy sample negative control plus three times the standard deviation).

2.4. Data Analysis

Statistical analyzes of the data were carried out using the XLSTAT software, version 7.1, 2004 and the R software [14]. Means of the measured parameters were compared by analysis of variance (ANOVA) and post-hoc separation of the means was done according to the Newman Keuls test at 5% significance level.

3. Results

3.1. Dates of Onset and Description of Symptoms

The dates of onset of symptoms obtained after inoculation of CPMoV ranged from 4 to 8 days. At 8 days post-inoculation (dpi), all the inoculated plants were symptomatic. The symptoms varied according to Bambara groundnut accessions. They consisted mainly of mild or severe mosaic, leaf deformation and

stunting of the plants (**Figure 1**). No symptoms developed in non-inoculated plants throughout the whole study.

3.2. Serological Detection of CPMoV

All samples collected from inoculated plants at 14 dpi responded positively to the DAS ELISA test, confirming the presence of CPMoV in inoculated plants. OD_{405nm} values from inoculated plant samples ranged between 1.373 and 2.293 which were more than 10 to 18 times the detection threshold of 0.126. Therefore, no ambiguous reaction was recorded despite the discrepancies in the variation of OD_{405nm} values. The lowest values were recorded in C6 ($OD_{405nm} = 1.373$) and C7 ($OD_{405nm} = 1.548$), whereas more than 2 OD units were obtained from all other accessions. Samples from non-inoculated plants were also tested by ELISA at 15 days after flowering and were found negative.

3.3. Effect of *Cowpea mottle virus* on the Flowering

The time to flower varied from 33 to 42 days with an average of 37 days after sowing. Non-inoculated plants tended to flower earlier than diseased plants but

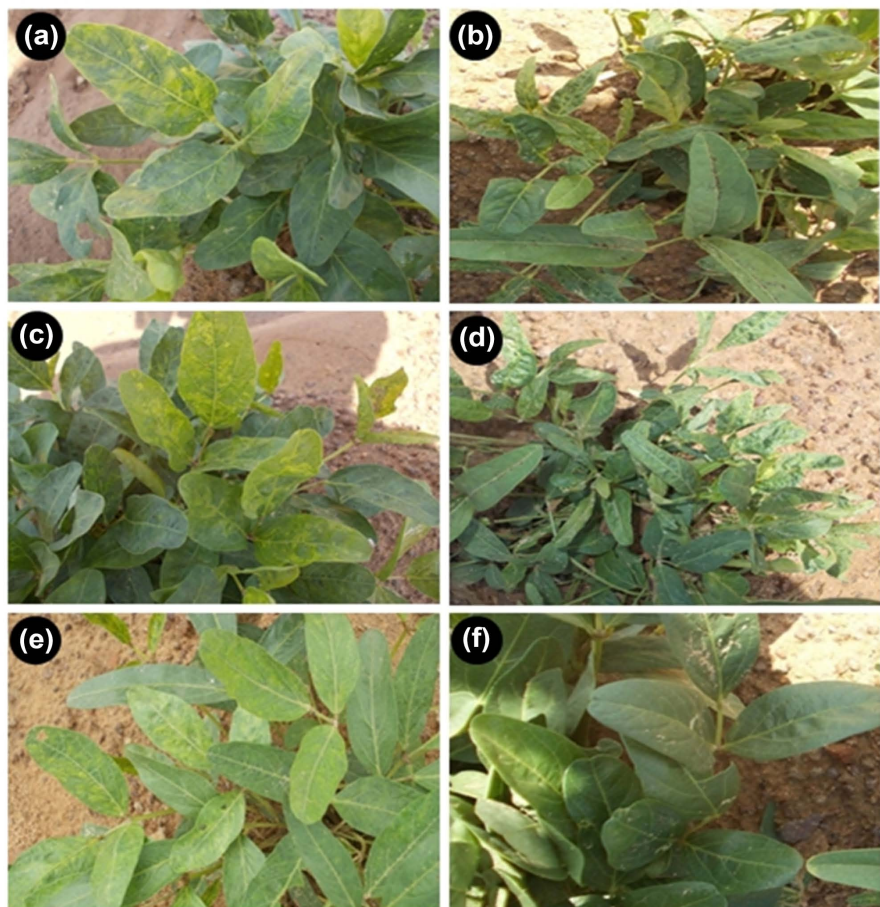


Figure 1. Diversity of symptoms caused by *Cowpea mottle virus* in Bambara groundnut. (a): mottling, (b): leaf deformation, (c): severe mosaic, (d): plant stunting, (e): mild mosaic, (f): healthy plant.

statistical analyses did not reveal any significant difference. Data on the number of flowers produced by both control and diseased plants are summarized in **Table 1**. On average, control and inoculated plants produced 11.5 flowers and 6.9 flowers, respectively in 2016. In 2017, the average per plant number of flowers produced by control plants was 8.8 whereas diseased plants produced 5.3 flowers on average. Statistical analyses indicated significant effect of the disease on the number of flowers produced in 2011 ($F = 17.7$, $P = 0.001$) as well as in 2017 ($F = 26.3$, $P = 0.0003$).

The effect of the disease on the number of flowers produced varied according to bambara groundnut accessions. In both years, control plants carried higher numbers of flowers for accessions C1, C5 and C7. No significant difference was observed for accessions C3 and C4 in any of the two years. For accessions C2 and C6, differences in flower production were significant in only one year.

3.4. Effect of Virus Infection on the Production of Pods

As summarized in **Table 1**, the average number of pods produced per plant was clearly affected by the diseased. Significant differences were found between inoculated and non-inoculated plants in both 2016 and 2017 ($P < 0.001$). Higher

Table 1. Effect of the disease on the per plant number of flower, number of pods and pod weight.

Accession	Number of flowers		Number of pods		Pod weight	
	control	diseased	control	diseased	control	diseased
2016						
C1	12.0 b	6.0 c	10.3 d	3.7 ef	7.7 bc	2.7 d
C2	9.7 bc	7.0 c	19.3 a	8.0 def	14.0 a	6.7 c
C3	12.0 b	7.7 bc	18.3 ab	3.3 f	14.3 a	2.7 d
C4	6.7 c	7.0 c	10.0 de	6.0 def	8.7 bc	4.7 cd
C5	12.0 b	6.0 c	17.0 abc	12.7 bcd	12.7 ab	9.3 bc
C6	12.3 b	6.3 c	11.7 cd	9.3 def	9.0 bc	7.7 bc
C7	16.0 a	8.0 bc	17.0 abc	8.0 def	9.7 bc	8.0 bc
2017						
C1	9.0 b	5.0 cd	13.7 bc	8.0 de	8.3 ab	4.7 bc
C2	9.0 b	5.0 cd	19.0 a	9.0 d	9.0 a	6.0 ab
C3	7.0 bcd	5.0 cd	8.3 de	2.0 f	9.0 a	1.3 d
C4	7.7 bc	6.0 bcd	17.0 ab	5.0 e	8.0 ab	2.7 cd
C5	7.7 bc	4.0 d	17.0 ab	12.0 cd	10.0 a	7.0 ab
C6	9.0 b	6.0 bcd	16.0 ab	12.0 cd	7.7 ab	7.0 ab
C7	12.0 a	6.0 bcd	15.7 ab	10.0 cd	8.0 ab	7.0 ab

C1-C7, bambara groundnut accessions; data with the same letter in the same parameter and the same line are not significantly different by the Student-Newman-Keuls test ($P < 0.05$).

production of pods was observed in healthy plants in 2017, regardless of the accessions. In 2016, differences in pod production were not significant in accessions C4, C5 and C6. Altogether, the number of pods in diseased plant was reduced by 20.5% - 82.0% and 25.0% - 75.9% in 2016 and 2017, respectively. Over the two years, reduction in the number of pods over the two years varied between 22.8% and 78.9%. Virus infection also resulted in a significant reduction of the average pod weight. Reduction in pod weight reached 52.1% and 64.9% in C2 and C1, respectively, but was highest (81.1%) in C3.

3.5. Effect of Virus Infection Grain Yield

In 2016, the average per plant seed yield differed significantly between inoculated and non-inoculated plants ($F = 341.7$, $P < 0.001$). There was also a significant accession effect ($P = 0.0003$) as well as disease x accession interaction ($P = 0.0002$). In average, healthy plants produced between 7.7 g/plant in accession C6 and 15.7 g/plant in accession C2 (**Figure 2(a)**). Altogether, healthy plants yielded 10.8 g of seeds per plant. Seed production in diseased plants was very low and varied from 1.3 to 4.4 g/plant in average (mean = 3.2 g). The lowest and highest yields were recorded in accessions C1 and C7, respectively.

Seed production in 2017 is illustrated in **Figure 2(b)**. Similarly to 2016, seed production in 2017 was significantly affected by virus infection ($P < 0.001$). Accession effect was also significant ($P = 0.0268$) no effect of the interaction between disease presence and bambara groundnut accession was observed ($P = 0.3445$). In average, healthy plants produced 8.7 g of seeds per plant which clearly contrasted with the level of seed production in diseased plants (2.7 g/plant).

Altogether, bambara groundnut accessions incurred severe yield loss caused by virus infection (**Table 2**). The highest losses were recorded in accessions C3 (83.9%) and C1 (81.0%). Accessions C6 and C7 were the least affected with yield losses of around 50%.

4. Discussion

All of Bambara groundnut accessions tested in this study developed characteristic symptoms of *Cowpea mottle virus* after inoculation with the virus. Symptoms were similar to those reported earlier [15] even if difference were noticed among accessions. The development of clearly visible symptoms indicated that all accessions were susceptible to the virus. Because virus inoculum was prepared from infected cowpea, our data showed that cowpea may be considered as a reservoir host for bambara groundnut infection in the field. This is particularly worrying, given that both cowpea and bambara groundnut are grown in overlapping areas [15]. Moreover, bambara groundnut is often grown after peanut fields are already in place.

Serological detection of CPMoV in inoculated plants was useful for confirming the etiology of symptoms developed in inoculated plants. Furthermore, non-inoculated control plants needed to remain uninfected by the virus for

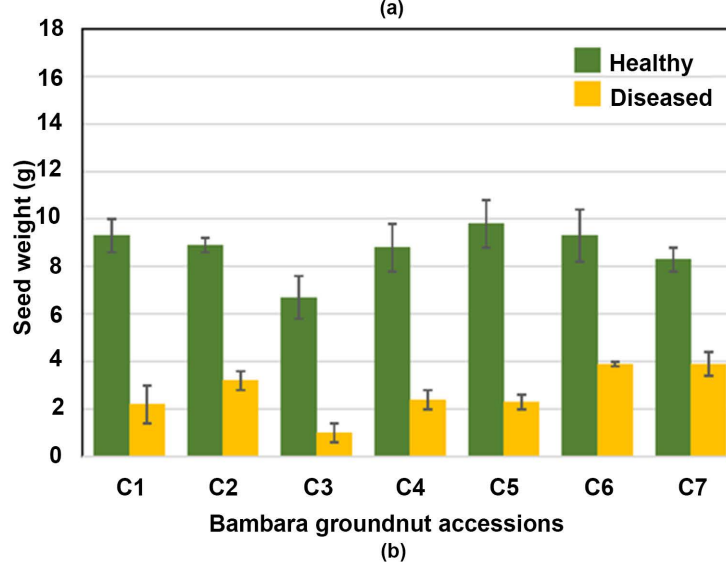
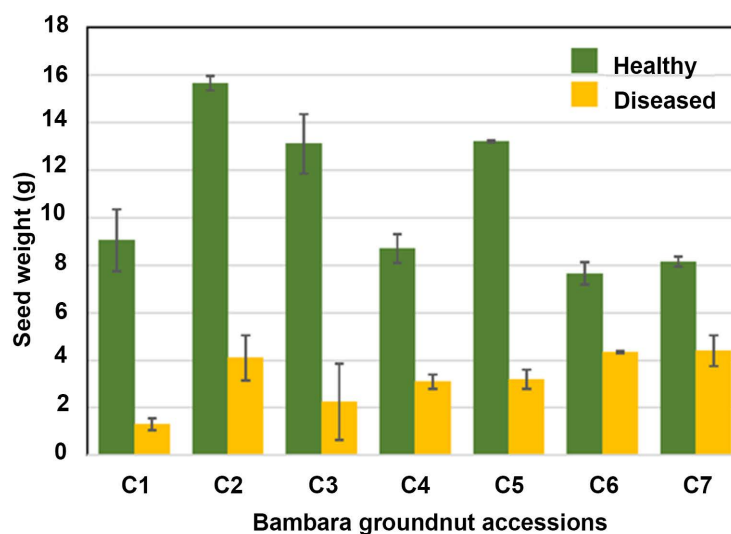


Figure 2. Average per plant grain weight in bambara groundnut accessions (C1-C7) for trials conducted in 2016 (a) and 2017 (b).

Table 2. Percentages of yield loss caused by virus infection bambara groundnut accessions.

Accession	Year		Mean \pm SD
	2016	2017	
C1	85.6	76.3	81.0 \pm 6.6
C2	73.8	64	68.9 \pm 6.9
C3	82.8	85.1	83.9 \pm 1.6
C4	64.4	72.7	68.6 \pm 5.9
C5	75.8	76.5	76.1 \pm 0.5
C6	43.4	58.1	50.7 \pm 10.4
C7	46.0	53	49.5 \pm 5.0

C1-C7, bambara groundnut accessions; SD, Standard deviation.

disease impact assessment. ELISA tests were also useful to confirm the absence of virus detection in non-inoculated plants after the flowering stage. Therefore, insecticide sprays were efficient in controlling insect vectors in order to prevent secondary spread of the cowpea mottle from inoculated plants. Possibly, insecticide sprays also protected plants in the trials from infection by other viruses which could occur from outside inoculum sources.

Overall, virus infection had negative impact on yield parameters despite differences among bambara groundnut accessions. The reduction in the average number of pod per plant which varied between 22.8% and 78.9% is in agreement with results (70.42%) found in CPMoV-infected cowpea [16]. The effect of virus infection on the average number of flowers was also similar to the impact of Cowpea aphid-borne mosaic virus (CABMV) on cowpea [17]. In both virus/host interactions, some plant genotypes were more affected than others and no significant effect of the virus infection was found in some cases.

In line with the reduction of the number of flowers and pods, grain yield was also affected by virus infection at levels as high as 83.9%. However, when most bambara groundnut accessions incurred more than 68% grain loss, accessions C6 and C7 were less affected with ca. 50% losses. Altogether, no resistance was apparent in the tested accessions. Although a very limited number of accessions were tested, resistance to CPMoV in bambara groundnut may be uncommon as reported in cowpea [18]. This hypothesis needs to be confirmed by testing as much genotypes as possible in order to identify adequate resistance sources. Such resistance sources are of great importance given the significant impact of the virus on grain yield and the recent identification of the CPMoV in farmers' fields. Resistance to the virus was found in the wild relative *Vigna vexillata* [18] and should be considered in breeding bambara groundnut for resistance to CPMoV. Indeed, in this study, yield losses were assessed in artificially inoculated plants but this should not differ significantly from field infected plants as losses of 70.42% were reported in cowpea [16]. Moreover, in field conditions, CPMoV usually occurs in mixed infections with other viruses such as CABMV, *Cucumber mosaic virus* or *Southern bean mosaic virus* [11] [15] [16]. Interestingly, some multiple infections do not result in significantly increased yield loss [12] [16].

5. Conclusion

This study is a contribution to the management of virus diseases in bambara groundnut. Seven accessions of this grain legume were tested for resistance to *Cowpea mottle virus* (genus *Carmovirus*). None of the accession showed resistance to the virus, which calls for testing a large number of accessions. However, two accessions (C6 and C7) showed some tolerance and were less subject to yield loss. Therefore, they can be used by farmers as part of cowpea mottle disease management. Additionally, testing seed lots is also recommended, as the virus was readily detected using Enzyme-linked immunosorbent assay.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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