

Responses of African yam bean (*Sphenostylis stenocarpa* [Hochst. Ex A. Rich]) accessions to viral diseases and serological identification of the associated viruses

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Abstract

African yam bean (*Sphenostylis stenocarpa*) is an orphan crop with lofty nutraceutical benefits grown in Africa. However, pests and diseases often hinder its productivity. Twenty accessions of African yam bean (AYB) were screened for resistance to viral diseases and symptomatic leaves were indexed for the presence of viruses. The Field experiments were conducted during the 2015/2016 cropping seasons (wet and dry seasons). The twenty accessions were grown in triplicates in a randomized complete block design (RCBD) on the field under natural infection and the resistance or susceptibility responses were recorded using visual symptom expression. The characteristic symptoms that were highly prevalent were leaf mosaic and leaf distortion. In both seasons, there were significant ($P < 0.05$) differences in the severity of these viral symptoms. Also, based on the severity of the symptoms observed in this study, 2 accessions were classified as resistant, 16 as susceptible and 2 accessions as highly susceptible. Two viruses were identified serologically namely Cowpea Mild Mottle Virus (CPMMV) and Blackeye Cowpea Mosaic Virus (BICMV). The sources of resistance identified in this study would be useful for breeders in developing virus resistant varieties for African yam bean improvement.

Keywords: African yam bean, Virus, Symptoms, ACP-Elisa, Screening.

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Introduction

African yam bean (AYB; *Sphenostylis stenocarpa*) is a tuberous legume which belongs to the family Fabaceae. It is also known as Diegemtenguere (Mali), Norouko and Roya (Sudan), Okpududu and Azanma (Igbo, Nigeria), Girigiri (Hausa, Nigeria) and Sese (Yoruba, Nigeria). It is mostly grown in Central (Gabon and Congo) and Western Africa (Nigeria, Cameroon,

Togo, Ghana, and Ivory Coast). Nigeria is more prominent for AYB production, as it is extensively cultivated in the western, eastern, southern and northern states of the country (Abbey and Berezi, 1998; Saka and Ajibade, 2004). AYB is mostly cultivated as a mixed crop with cassava, vegetables, yam and rice. The tubers of this crop possess higher protein content than sweet potato, Irish potato, yam, and cassava (Amoatey *et al.*, 2000). AYB records a higher amino acid

content compared to pigeon pea, cowpea, and bambara groundnut (Uguru and Madukaife, 2001). Despite its importance, as a crop with enormous potentials to provide food, feed, medicines, and insecticides for humans and livestock (Adewale *et al.*, 2008), it remains a neglected crop (Adewale *et al.*, 2012).

Its long maturation period, long cookability, need for stakes and high incidence of pests and diseases inspired its stigmatization as "neglected crop." These long jams subjected it to less research and farming attention thereby depriving it of a good breeding program and commercialization of research outputs (Adewale *et al.*, 2012), despite its qualification as a crop with scientific and economic benefits.

Pest and disease complex results in poor yield of AYB, which is one of the significant factors that affect productivity (Okigbo, 1973; Kay, 1987; Ameh and Okezie, 2005; Agu, 2008; Ogah, 2011). In Nigeria, unidentified viral diseases were reported to cause leaf malformation, leaf discoloration, leaf curl and plant stunting in AYB. Viral infected plants produced little or no flowers and pods (Ameh and Okezie, 2005). In order to appropriately and effectively manage the poor yield resulting from this infection, it is important to accurately diagnose the disease. Furthermore, the most efficient, feasible and cost-effective way of controlling legume viruses is through the development of resistant varieties (Taiwo, 2003). However, in past studies of viral diseases associated with AYB, there was no report of resistant sources or possible resistance to these diseases, which is a very important baseline information in any disease resistance breeding program.

Thus, this is a preliminary study, conducted to evaluate 20 accessions of AYB for possible sources of resistance to viral infection under natural field conditions and also to index associated viruses.

Materials and methods

Research location and planting materials

The field experiment was carried out at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta in Ogun State, Nigeria (Lat 7 °N, Long 3°231E). Twenty

accessions of AYB were planted in the wet season (2015) while sixteen AYB accessions were planted in the dry season (2015/2016) due to unavailability of the remaining four accessions in the gene bank. African yam bean seeds used in this study were sourced from the Genetic Resources Centre, International Institute of Tropical Agriculture, Ibadan.

Land preparation and experimental design

Prior to crop cultivation the land was cleared, ploughed twice and harrowed using a tractor. Field trials were conducted during the wet and dry seasons of 2015/2016 cropping periods. The wet season commenced in June and terminated in November 2015 and the dry season commenced in December 2015 and terminated in May 2016. The experiment was laid out in a Randomised Complete Block Design (RCBD) which was replicated three times. The total size of the field was 2649 m² while the size of the plot was 14 m by 20 m with a 1m space between plants and 0.7 m within plants. Line planting method was adopted to sow the African yam bean seeds; the planting density was 20 plants per plot .

The AYB seeds were sown on a loamy soil without fertilizer and herbicide application during both seasons. Staking of vines was carried out 3 weeks after planting and manual weeding was done when necessary, within the duration of the experiments. During the dry season, plants were planted in a Fadama area (a land that floods in the rainy season and its irrigable) and irrigated twice daily when necessary with watering can (early in the morning and in the cool evening).

Assessment of disease severity

Data were collected on the severity of the viral diseases. Diseases were rated on a scale of 0-4; 0= no symptom, 1= Mild mosaic with yellow mosaic, 2= Moderate mosaic without significant distortion, 3= Moderate mosaic with significant distortion, 4= Severe mosaic symptoms, distortion with stunting.

Disease rating scale was determined with disease scores of 0 – 0.99 (Highly resistant); 1.0 – 1.99 (Resistant); 2.0 – 3.99 (Susceptible); 4.0 and above (Highly susceptible).

Indexing Africa yam Bean for Viruses using Antigen Coated Plate (ACP) Enzyme linked immunosorbent assay (ELISA) Method

The serological protocol employed to detect the viruses infecting African yam bean was the Antigen-coated plate (ACP) ELISA method described by Kumar (2009). The leaf samples collected from the field were tested using antibodies specific for *Cowpea aphid borne mosaic virus* (CABMV), *Blackeye cowpea mosaic virus* (BICMV), *Cucumber mosaic virus* (CMV), *Southern bean mosaic virus* (SBMV), *Cowpea mottle virus* (CPMoV), *Cowpea yellow mosaic virus* (CYMV), *Cowpea mild mottle virus* (CPMMV), since AYB belongs to the same family as cowpea.

Data analysis

Data collected on disease severity were subjected to analysis of variance, and significant means were compared using Duncan multiple range test (DMRT) at 5 % probability level.

Results

Severity of characteristic viral symptoms on African yam bean during wet (2015) and dry (2015/2016) seasons

AYB manifested various characteristic viral symptoms (Fig.1; Plate 1) which includes, Mosaic (90%), distortion (70%), yellow chlorosis (5%) and stunting (55%).

Across the 20 accessions used in this trial, there was a significant ($P < 0.05$) difference in the severity of viral symptoms in both wet and dry seasons. In the wet season (2015) disease severity ranged from 0 to 4.75 while in the dry season (2015/2016), the severity score ranged from 2.60 to 3.40 (Table 1).

The highest severity values (4.75 and 3.40) were recorded in TSs 48 and TSs 23 in both seasons, respectively, while the least severity score (0.00) was recorded in TSs 10 and TSs 49. Similarly, TSs 7A recorded the least severity value (2.60) in the dry seasons.

The responses of the 20 accessions of AYB to viral infections are presented in Table 2. Based on disease reaction, 2 accessions were classified as resistant: 16 accessions as susceptible and 2 as highly susceptible to virus infection.

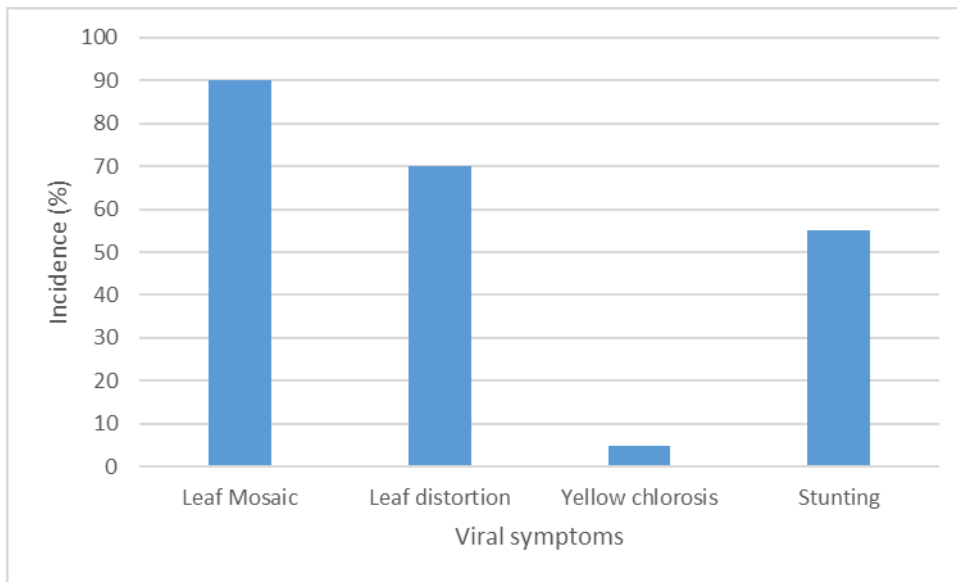


Fig 1: Incidence of characteristic viral symptoms observed in 20 accessions of African yam bean in two planting seasons (wet and dry seasons)



Plate 1: Characteristic viral symptoms observed on African yam bean leaves

Table 1: Severity of characteristic viral symptoms on African yam bean during wet (2015) and dry (2015/2016) seasons.

Accessions	Severity	
	Wet	Dry
TSs 1	4.15 ^a	2.83 ^{bc}
TSs 10	0.00 ^b	3.23 ^{ab}
TSs 10A	3.25 ^a	NA
TSs 23	4.40 ^a	3.40 ^a
TSs 24	4.03 ^a	3.00 ^{abc}
TSs 33	3.45 ^a	2.80 ^{bc}
TSs 40A	3.90 ^a	NA
TSs 48	4.75 ^a	NA
TSs 49	0.00 ^b	3.00 ^{abc}
TSs 57	3.00 ^a	2.90 ^{abc}
TSs 58	4.15 ^a	2.80 ^{bc}
TSs 61	4.07 ^a	2.90 ^{abc}
TSs 67	4.00 ^a	2.90 ^{abc}
TSs 69	4.20 ^a	2.97 ^{abc}
TSs 7A	2.40 ^{ab}	2.60 ^c
TSs 82	4.35 ^a	2.83 ^{bc}
TSs 84	4.35 ^a	2.80 ^{bc}
TSs 86	2.50 ^{ab}	3.17 ^{ab}
TSs 89	4.35 ^a	3.00 ^{abc}
TSs 9	3.83 ^a	NA

Means within the same column followed by the same letter are not significantly different at P<0.05 (DMRT)

TSs= Tropical *Sphenostylis stenocarpa*

NA= Not available

Table 2: Disease reaction of AYB accessions to viral diseases during wet (2015) and dry (2015/2016) seasons

Accessions	Pooled Severity means	Disease reaction
TSs 1	4.91	HS
TSs 10	1.62	R
TSs 10A	3.25	S
TSs 23	3.9	S
TSs 24	3.52	S
TSs 33	3.13	S
TSs 40A	3.9	S
TSs 48	4.75	HS
TSs 49	1.5	R
TSs 57	2.95	S
TSs 58	3.48	S
TSs 61	3.49	S
TSs 67	3.45	S
TSs 69	3.59	S
TSs 7A	2.5	S
TSs 82	3.59	S
TSs 84	3.58	S
TSs 86	2.84	S
TSs 89	3.68	S
TSs 9	3.83	S

TSs= Tropical *Sphenostylis sternocarpa*;

NA= Not available

R= Resistant

S= Susceptible

HS= Highly susceptible

Serological identification of Viruses associated with AYB using Enzyme-linked immunosorbent assay

During the first planting (wet) season using the bulk sampling method, none of the 20 symptomatic AYB leaf samples tested positive (0%) to the seven antibodies used in this study. However, during the second planting (dry) season, it was observed that 15 of the 16

symptomatic samples tested positive to CPMMV and BICMV.

CPMMV was recorded as the most prevalent virus with an incidence of 93.8% while BICMV was the least prevalent (6.3%). However, one sample (6.3%) tested positive for the combination of two viruses (CPMMV and BICMV).

Table 3: Occurrence of single and mixed virus infections on AYB leaf samples in first the (2015) and second (2015/2016) planting seasons

Planting season	No of samples	No of positive Samples								
		CPMMV (%)	CMV (%)	CMoV (%)	SBMV (%)	CYMV (%)	CYMV (%)	CABMV (%)	BICMV (%)	MIXED INFECTION (CPMMV +BICMV) (%)
Wet season	20	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dry season	16	15	ND	ND	ND	ND	ND	ND	1	1

ND= Not detected.

CABMV= *Cowpea Aphid borne mosaic virus*

CYMV= *Cowpea yellow mosaic virus*

CPMMV= *Cowpea mild mottle virus*

CMoV= *Cowpea mottle virus*

SBMV= *Southern bean mosaic virus*

CMV= *Cucumber mosaic virus*

BICMV= *Blackeye cowpea mosaic virus*

Discussion

In this study, evaluation of AYB accessions to viral infection was based on visual symptom expression only. The symptoms (leaf mosaic and leaf distortion) observed to be highly prevalent in AYB in this study corroborate the viral symptoms observed on most legumes especially cowpea (Vanderborght and Baudoin, 2001 and Akinjogunla, 2005). This evaluation showed that two accessions exhibited resistance to the viral infection, other accessions were either susceptible or highly susceptible. This means that most of the accessions used in this study cannot be incorporated in any disease resistant breeding program. Although, field screening for resistance under natural viral infection cannot rely only on

visual symptoms, as it has been postulated that different viruses exhibit similar symptoms. More so, some plants show virus-like symptoms due to nutritional deficiency, unfavorable weather condition, pest infestation, genetic abnormality, herbicide toxicity, insect or mite infestation (Naidu and Hughes, 2003).

ELISA detected no virus in the AYB samples collected in the first planting season (rainy season). However, 2 viruses which corroborate the reports of Hughes *et al.* (2003) and Ghorbani *et al.* (2007) were identified in the AYB leaf samples collected in the second planting season (dry season) namely CPMMV and BICMV. CPMMV is a member of the genus Carlavirus, which has been reported to cause leaf distortion

and it is usually transmitted by whitefly (*Bemisia tabaci*) in a non-persistent manner (Laguna *et al.*, 2006; Jeyanandarajah and Brunt, 1993). In this study, CPMMV recorded a high rate of occurrence, probably because of the large population of white flies in the environment due to the presence of alternate hosts near the crop. This observation verifies the report of El-Hammady *et al.* (2004) who reported that *Solanum incanum* L. acted as a perennial reservoir of CPMMV.

Blackeye cowpea mosaic virus (BICMV) is a seed-borne potyvirus which was first described to cause mosaic disease of cowpea by Anderson (1955), it was also detected in bambara groundnut by Thottappilly (1997). The low rate of occurrence of BICMV agrees with the study of Alegbejo and Kashina's (2001), on viruses of cowpea in Nigeria. They also reported a low occurrence of BICMV in cowpea. Furthermore, this virus was reported by Alegbejo and Kashina (2001), to be transmitted by Aphids (*Aphis craccivora*), an insect that infests AYB at the vegetative stage (Ameh and Okezie, 2005). The incidence of these viruses in the dry season, might have been as a result of the low relative humidity, which probably could have inhibited quick recovery in AYB genotypes (Edema *et al.*, 1997).

Furthermore, the ELISA results showed that some of the viruses occurred in mixtures such as CPMMV and BICMV in one sample. This result partially complies with the findings of Aliyu (2012) in which mixed infections such as Blackeye cowpea mosaic virus (BICMV) and Cowpea aphid borne mosaic virus (BICMV + CABMV) were detected in the cowpea leaf samples surveyed. Mixed viral infection is not a rare occurrence in field trials especially experiments conducted under natural infection. This kind of infection often alter and complicate symptom expression, thereby impeding a successful symptom-based field diagnosis (Aliyu *et al.*, 2012)

The inability to detect viruses in the AYB leaf samples collected during the wet season from all the 20 accessions could probably have resulted from low virus concentration in the AYB leaves, time of planting, AYB genetic abnormality, unfavorable weather conditions which curtail insect transmission or non-availability of the

precise antibodies to detect the virus in symptomatic AYB leaves, just as Aliyu *et al.* (2012) reported . The absence of CPMMV and BICMV in the wet season might be due to the weather condition as the low temperature and high relative humidity might have also inhibited the quick development of the vectors transmitting these viruses thereby impeding the transmission of these viruses in African yam bean genotypes. More so, due to the favorable weather conditions for the plant, it is possible that the AYB genotypes got to recover from the infection speedily (Tettey *et al.*, 2018).

Conclusion and Recommendation

African yam bean is a crop with great potential , considering its economic and research benefits. However, its susceptibility to viral diseases as shown in this study, can be devastating to its large-scale production for human and animal use. Due to the negative effects of chemicals to the environment and the fact that insecticides have been reported to be partially effective on the responsible vectors, efforts should be directed towards producing virus-free seeds and developing disease resistant varieties. However, the development of good disease resistant varieties is reliant on accurate virus identification and appropriate germplasm evaluation. Therefore, large-scale screening should be conducted in order to identify more possible sources of resistance. More so, the presumed resistance sources identified in this study should be further confirmed under screen house and field conditions using mechanical inoculation, serological and molecular methods such as real time polymerase chain reaction (RT-PCR) to detect the presence of the virus and quantity of the virus load after inoculation. This will ensure its suitability for use in any disease resistance breeding program for AYB improvement.

Declaration of Competing Interest

The authors have no conflict of interest.

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