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The Susceptibility of Two Bambara Groundnut Varieties to Blackeye Cowpea Mosaic Virus and Cowpea Yellow Mosaic Virus Infection

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Abstract
Bambara groundnut is a high-energy plant which is rich in carbohydrates, proteins and minerals. The crop attracts low patronage by farmers, consumers and researchers and is still commonly being grown by poor farmers at the subsistence level in Nigeria. A greenhouse experiment was conducted to determine the susceptibility of Nsuka Red and Ayaba Cream Bambara groundnut varieties to *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea yellow mosaic virus* (CPYMV) inoculation. The viruses were mechanically applied on the Bambara groundnut varieties at 14 days after planting. The results indicated that the highest virus disease incidence (11.83 - 18.51%) was in Nsuka Red Bambara groundnut variety inoculated with BICMV. The effects of the viruses on plant height showed that Ayaba Cream Bambara groundnut variety inoculated with CPYMV had taller plants (8.92 – 29.54cm) compared to the other virus inoculation treatments. The yield parameters showed that the significantly lowest yields such as; pod length (16.10mm), number of pods (7.94), number of seeds per pod (0.22) and weight of seeds per plant (2.36g) were gotten on Nsuka Red Bambara groundnut variety that was inoculated with BICMV. The results obtained from the study suggest that Nsuka Red and Ayaba Cream Bambara groundnut varieties were susceptible to BICMV and CPYMV with consequent reductions in growth and yield of plants. There is need therefore to control virus spread on Bambara groundnuts for higher productivity, increased income and food security. The Ayaba Cream Bambara groundnut variety which was more tolerant to the viruses could be recommended to farmers and crop breeders in virus disease management.

Keywords: Virus incidence, food security, inoculation, buffer, legume, susceptibility

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Introduction

Bambara groundnut (*Vigna subterranea* L.) belongs to the family Leguminosae and originated in the sahelian region (Begemann, 1988). It is an indigenous African crop grown across the continent from Senegal to Kenya and from the Sahara to South Africa and has the third highest production of the grain legumes, coming after cowpea and groundnut (Diabate *et al*., 2005). It is widely grown in Nigeria and in other African countries like Ghana, Cameroon, Ivory Coast and Togo (Klu *et al.*, 2001).

Bambara groundnut seed contains sufficient quantities of protein, carbohydrate and fat. On average, the seeds contain 63% carbohydrate, 19% protein and 6.5% oil (Akanni *et al.*, 2000). The seeds can be eaten fresh, or grilled while still immature. In many West African countries, the fresh seeds are boiled with salt and pepper, and eaten as a snack (Atiku *et al.*, 2004; Bamshaiye *et al.*, 2011). In East Africa, the seeds are roasted, then pulverized and used to make a soup, with or without condiments. Bread made from Bambara groundnut flour has been reported in Zambia (Linnemann, 1990).

In Nigeria, a common use of Bambara groundnut is to make steamed products, such as 'akara' and 'moin-moin' (Obizoba, 1983). A trial of Bambara groundnut milk was carried out which compared in flavor and composition with milk prepared from cowpea, pigeon pea and soybean (Brough *et al.*, 1993). The gross energy value of Bambara groundnut seed is greater than that of other common pulses such as cowpea, lentil and pigeon pea (FAO, 1982). According to Padulosi *et al.* (2002) the potential of neglected and under-utilized crops such as Bambara groundnut could be exploited for overcoming food deficits in the continent.

Bambara groundnut has a reputation for resisting pests, and compares favorably with other legumes such as groundnut or cowpea in this regard. In humid environments, however, fungal diseases such as Cercospora leaf spot, Fusarium wilt and Sclerotium rot are common (Begemann, 1988). Thottappilly and Rossel (1997) reported eight viruses infecting Bambara groundnut in farmer's fields in Nigeria: *Cowpea aphid-borne mosaic potyvirus*, *Blackeye cowpea mosaic potyvirus*, *Peanut mottle potyvirus*, *Cowpea mottle carmovirus*, *Cowpea yellow mosaic virus*, *Cowpea mild mottle carlavirus*, *Cucumber mosaic cucumovirus* and *Southern bean mosaic sobemovirus*.

Bambara groundnut attracts low patronage by farmers and researchers and is still commonly being grown by poor farmers at the subsistence level in Nigeria, hence neglected and underutilized. In addition, the effect of virus infection on the crop will limit its production and productivity. The objective of the study therefore was to determine the susceptibility of two widely cultivated Bambara groundnut varieties in Kwara State (Nsuka Red and Ayaba Cream) to *Blackeye cowpea mosaic virus* and *Cowpea yellow mosaic virus* infection.

Materials and Methods

Experimental site, source of seeds and virus inoculum

The potted experiment was carried out in the Greenhouse of the Crop Protection Department, Faculty of Agriculture, University of Ilorin – Nigeria. The Bambara groundnut seed varieties Nsuka Red and Ayaba Cream were obtained from the Ministry of Agriculture, Ilorin-Kwara State. The virus inoculum used for the study were *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea yellow mosaic virus* (CPYMV) both acquired from the International Institute for Tropical Agriculture (IITA), Ibadan-Nigeria.

Soil sterilization, experimental design and virus inoculation procedure

The soil used was steam sterilized to a temperature of 80°C for 30 minutes, followed by an 8-minute resting period which would result in 100% kill of all weeds and soil pathogens (Van Loenen *et al.*, 2003). The soil was then potted into plastic perforated buckets of 10 litre capacity prior to the sowing of the Bambara groundnut seeds. The experiment was set up in the Greenhouse as a Complete Randomized Design (CRD). Four seeds of the groundnut varieties were sown per pot and thinned to 2 stands at 7 days after sowing. The virus inoculations were applied singly on each groundnut variety at 2 weeks after planting (WAP) at the rate of 30 μL of inoculum per leaf. The control plants were buffer inoculated alone. These constituted the treatments that had 5 replications each and a total of 48 pots with two plants each.

The viral isolates were extracted from the infected leaves by homogenization, using mortar and pestle in 0.05M Phosphate buffer at pH 7.2 at the rate of 1g leaf sample to 5 ml of buffer. In all cases, the two plants per pot were mechanically inoculated. The inoculation was done by mechanical transmission of virus through sap. The sap was applied on the surface of the oldest leaves previously sprinkled with carborundum. The sap was applied by gently rubbing the leaves with a cotton wool dipped in the sap after which all the plants were rinsed with water to reduce inoculation stress on them (Aliyu, 2018). The experimental site is located on latitude 8^0 26 N, longitude 4^0 29 E and about 344.7m above sea level with an average maximum temperature of 38°C, average relative humidity of 77.5% and 7.1 hours of sunshine daily.

Data collection and Statistical analysis

Data were collected from the 2^{nd} to 8^{th} week after inoculation (WAI) for the following parameters: plant height (cm), number of leaves per plant, number of leaves showing virus disease symptoms and number of branches per plant. The percentage virus incidence was measured as percentage of inoculated plants eliciting virus disease symptoms relative to the total number of leaves on any given plant and expressed as percentage (Ibrahim *et al.,* 2017).

The pods were harvested at 120-130 days after planting and yield parameters measured were: pod length (mm), number of pods per plant, number of seeds per pod and the weights of seeds per plant. All collected data were subjected to analysis of variance (ANOVA) using the Statistical Package for the Social Sciences SPSS version 15.0. Significant differences among treatment means were separated using New Duncan Multiple Range Test at 5% level of probability.

Results and Discussion

Effect of treatments on percentage disease incidence

The percentage virus incidence as shown in Table 1 indicated that the inoculated viruses had significant effect on percentage disease incidence on the two Bambara groundnut varieties. The result showed that from the 3^{rd} to 8^{th} week after inoculation (WAI), the highest disease incidence was in Nsuka Red variety inoculated with BICMV (11.83 to 18.51%). The treatment with the second highest virus incidence was Ayaba Cream variety inoculated with BICMV and the values ranged from 7.64% in the 3^{rd} WAI to 15.51% at 8^{th} WAI. The inoculation with CPYMV had a mild incidence value of 4.11 - 10.44% in Nsuka Red and 3.26 - 9.92% in Ayaba Cream variety. In all cases however, virus incidence were significantly lowest in the control treatments with between 1.86 - 6.65% in Nsuka Red variety and 1.66 - 5.97% in Ayaba Red variety.

The finding is an indication of the susceptibility of the Bambara groundnut varieties to virus infection. The result signified higher infection with BICMV in comparison with CPYMV, however varietal assessment showed Ayaba Cream to be the more tolerant variety. This phenomenon is not uncommon as similar observations were also detected by Zongo *et al.* (2018). It was then concluded that this development is as a result of battle for survival between viruses and their host plants. It is envisaged that host plants as a means of defense use mechanisms such as antiviral RNA silencing but virus pathogens fight back using silencing-repressors. The strain of the virus, immune competence of the host, and innate resistance of the host therefore would have significant effect on the incidence of the disease induced. This proposition is also supported by Boualem *et al.* (2016).

Effect of treatments on plant height

The effect of the treatments on plant height (Table 2) showed that the un-inoculated plants were the tallest. The virus infected plants irrespective of the virus inoculated and Bambara groundnut variety exhibited markedly stunted growth. The effect was more pronounced on Nsuka Red variety inoculated with BICMV (4.3 - 19.9cm), followed by Ayaba Cream variety inoculated with BICMV (6.4 - 22.4cm). The significantly taller plants were in the varieties inoculated with CPYMV and the heights ranged from 7.8 - 26.1cm in Nsuka Red variety to 8.9 - 29.5cm in Ayaba Cream variety. Plant height estimate is one of the essential components used in the evaluation of the effect of virus disease on plant physiological processes and therefore is considered to be of economic importance especially when there is reduction in plant height. In the present study, plant height was significantly affected by virus infection at various growth stages and the most significant effect was with the BICMV infection. This finding is in agreement with Taiwo and Akinjogunla (2006); Ehinmore and Kareem (2010) who reported severe stunting in other arable crops such as *Vigna unguiculata* and *Amaranthus spinosus* infected with viruses. It can thus be concluded that stunted growth was induced by plant reaction to virus presence in the inoculated plants.

Table 1: The effect of treatments on percentage virus incidence at different weeks after inoculation

	Weeks after mechanical inoculation						
Treatment	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
NR X BICMV	0.83^{a}	11.83^{a}	14.4^{a}	15.4^{a}	16.5^{a}	17.8^{a}	18.51^{a}
AYC X BICMV	0.82^{a}	7.64^{b}	10.4^{b}	12.1^{b}	13.1^{b}	13.93^{b}	15.51^{b}
NR X CPYMV	0.73^{a}	4.11^{c}	5.31^{cd}	6.03^{cd}	8.33^{c}	9.66^{c}	10.44^{cd}
AYC X CPYMV	0.64^{a}	3.26^{c}	4.64^{d}	4.92^{d}	7.52^{d}	8.32^{d}	9.92^{d}
NR X Buffer	0.05^{a}	1.86^{d}	1.82^{e}	2.11^{e}	4.41^{e}	5.91^{e}	6.65^{e}
AYC X Buffer	0.06^{a}	1.66^{d}	1.72^{e}	2.04^{e}	3.16^{e}	5.42^{e}	5.97^{e}
S.E.M	0.96	0.84	0.94	0.99	1.15	0.79	0.99

Key:

NR X BICMV = Nsuka Red variety inoculated with *Blackeye cowpea mosaic virus*
NR X CPYMV = Nsuka Red variety inoculated with *Cowpea yellow mosaic virus*
AYC X BICMV = Ayaba Cream Variety inoculated with *Blackeye cowpea mosaic virus*
AYC X CPYMV = Ayaba Cream Variety inoculated with *Cowpea yellow mosaic virus*
NR X Buffer = Nsuka Red Variety buffer inoculated
AYC X Buffer = Ayaba Cream Variety buffer inoculated
Means within a column followed by the same letter (s) are not significantly different using the New Duncan Multiple Range Test at $P \geq 0.05$.

Table 2: The effect of treatments on plant height (cm) at different weeks after inoculation

	Weeks after mechanical inoculation						
Treatment	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
NR X BICMV	2.24^{a}	4.33^{e}	5.51^{f}	7.22^{f}	10.86^{f}	14.25^{e}	19.94^{e}
AYC X BICMV	2.63^{a}	6.42^{d}	8.12^{e}	12.16^{e}	15.24^{e}	18.63^{d}	22.41^{d}
NR X CPYMV	2.81^{a}	7.82^{c}	10.22^{d}	15.37^{d}	21.13^{d}	23.32^{c}	26.13^{c}
AYC X CPYMV	2.72^{a}	8.92^{bc}	13.44^{cd}	18.24^{c}	25.52^{cd}	27.24^{b}	29.54^{b}
NR X Buffer	2.81^{a}	10.54^{a}	16.67^{ab}	20.72^{ab}	31.16^{b}	32.81^{a}	33.44^{a}
AYC X Buffer	2.93^{a}	10.73^{a}	17.73^{a}	21.03^{a}	32.24^{a}	33.33^{a}	33.56^{a}
S.E.M	0.93	0.89	0.68	1.01	0.76	1.43	1.62

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at $P \geq 0.05$.

Effect of treatments on average number of leaves

The effect of the treatments on average number of leaves per plant (Table3) indicated that there were significant differences from 3rd to 8th WAI. The effect was more pronounced in virus inoculated plants which on the average produced lower number of leaves per plant compared to the control. The effect was however more significant in BICMV than in CPYMV inoculated plants. The average number of leaves in Nsuka Red inoculated with BICMV (25.41- 64.83) and Ayaba Cream inoculated with BICMV (28.14 - 65.32) was the significantly lowest. However, the significantly highest number of leaves (48.91 - 80.33) was obtained in the control treatment. The result obtained is in agreement with Pazarlar *et al.* (2013) and Hull (2002) who observed that reduction in the average number of leaves per plant is a consequence of less effective functioning of chloroplasts commonly associated with virus infection.

Effect of treatments on average number of branches per plant

The infection of the Bambara groundnut varieties with either BICMV or CPYMV significantly decreased the average number of branches per plant (Table 4). The values for Nsuka Red inoculated with BICMV (1.81 – 5.83), Ayaba Cream inoculated with BICMV (2.14 - 6.34), Nsuka Red inoculated with CPYMV (3.12 - 7.85) and Ayaba Cream inoculated with CPYMV (3.74 - 7.71) were significantly lower than the control treatments. The reduction in number of branches in the virus infected plants could be due to alterations in the physiology and morphology of the Bambara plants associated with BICMV and CPYMV infection. This view is supported by Wintermantel (2005); Taiwo and Akinjogunla (2006).

Table 3: The effect of treatments on average number of leaves / plant at different weeks after inoculation

	Weeks after mechanical inoculation						
Treatment	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
NR X BICMV	13.24[a]	25.41[d]	35.64[f]	40.33[e]	50.67[c]	58.63[d]	64.83[c]
AYC X BICMV	13.63[a]	28.14[c]	36.13[ef]	41.35[d]	51.54[c]	59.45[c]	65.32[c]
NR X CPYMV	13.81[a]	32.45[b]	38.24[de]	44.43[c]	54.35[b]	63.16[b]	70.86[b]
AYC X CPYMV	13.76[a]	33.92[b]	39.83[cd]	45.16[c]	55.16[b]	64.07[b]	71.74[b]
NR X Buffer	13.83[a]	48.91[a]	60.62[ab]	65.72[ab]	69.66[a]	73.89[a]	80.33[a]
AYC X Buffer	13.84[a]	49.74[a]	61.14[a]	66.02[a]	68.47[a]	72.93[a]	79.53[a]
S.E.M	0.64	0.62	0.78	0.86	2.38	0.64	0.78

Means within a column followed by the same letter(s) are not significantly different using the New Duncan multiple Range Test at $P \geq 0.05$

Table 4: The effect of treatments on average number of branches per plant at different weeks after inoculation

	Weeks after mechanical inoculation						
Treatment	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**
NR X BICMV	0.22[a]	1.81[d]	2.82[f]	3.06[d]	3.44[c]	4.54[d]	5.83[d]
AYC X BICMV	0.34[a]	2.14[cd]	3.01[ef]	3.93[cd]	4.76[c]	5.33[c]	6.34[c]
NR X CPYMV	0.35[a]	3.12[b]	3.94[d]	4.81[b]	5.26[b]	6.16[b]	7.85[b]
AYC X CPYMV	0.34[a]	3.74[b]	4.13[cd]	4.94[b]	5.51[b]	6.04[b]	7.71[b]
NR X Buffer	0.56[a]	4.96[a]	5.74[ab]	6.72[a]	7.84[a]	8.81[a]	9.52[a]
AYC X Buffer	0.42[a]	5.31[a]	6.03[a]	6.93[a]	7.92[a]	8.91[a]	9.94[a]
S.E.M	0.01	0.92	0.22	0.11	0.08	0.04	0.06

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at $P \geq 0.05$

Table 5: The effect of treatments on yield parameters

Treatment	Pod length/plant (mm)	Average number of pods/plant	Average number of seeds/plant	Weight of seeds/plant (g)
NR X BICMV	16.10[d]	7.94[e]	0.22[f]	2.36[e]
AYC X BICMV	17.31[c]	8.62[d]	0.46[e]	2.73[d]
NR X CPYMV	19.46[b]	10.41[c]	0.74[d]	3.48[c]
AYC X CPYMV	19.83[b]	11.35[b]	0.89[cd]	4.74[b]
NR X Buffer	25.13[a]	16.01[a]	1.52[ab]	7.76[a]
AYC X Buffer	25.86[a]	16.73[a]	1.61[a]	8.04[a]
S.E.M	0.77	0.04	0.01	0.05

Means within a column followed by the same letter(s) are not significantly different using the New Duncan Multiple Range Test at $P \geq 0.05$

Effect of treatments on yield parameters

The Bambara groundnut varieties incurred significant yield reductions caused by BICMV and CPYMV infections (Table 5). However, the lowest pod length (16.10mm), average number of pods per plant (7.94), average number of seeds per plant (0.22) and weight of seeds per plant (2.36g) were recorded in Nsuka Red variety inoculated with BICMV. Infection with CPYMV on Nsuka Red variety was milder with significantly ($P \geq 0.05$) higher yield parameters. The values were 19.46mm (pod length), 10.41 (average number of pods per plant), 0.74 (average number of seeds per plant) and 3.48g (weight of seeds per plant). In comparison, the control treatment produced the significantly ($P \geq 0.05$) highest yield parameters. It can be deduced from the study that the reductions observed is a consequence of BICMV and CPYMV inoculations. Previous studies by Amayo *et al*, (2012) and Aliyu *et al*, (2012) had also posited that viruses influenced host physiological and photosynthetic abilities with attendant reduction on yield parameters of infected plants.

Conclusion and Recommendation

The study indicated that Nsuka Cream and Ayaba Red Bambara groundnut varieties were susceptible to BICMV and CPYMV infection. It is therefore appropriate to control the spread of the viruses in Bambara groundnut and to other arable crops. Ayaba Red Bambara groundnut variety which was found to be the more tolerant variety is hereby recommended to farmers and crop breeders for enhanced productivity and food security.

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