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Original Article

RESISTANCE OF SOME COMMERCIAL GROUNDNUT CULTIVARS TO *COWPEA APHID-BORNE MOSAIC VIRUS* IN MINNA, SOUTHERN GUINEA SAVANNA OF NIGERIA

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ABSTRACT

Cowpea aphid-borne mosaic virus (CABMV) is one of the major viruses infecting many crop species in the family Fabaceae including groundnut. CABMV constitutes a serious limitation against legume productivity in Nigeria. Therefore, twenty commercial cultivars of groundnut were evaluated for resistance to the virus in Minna, Southern Guinea Savanna of Nigeria during the 2015 cropping season. The trial was conducted using Randomised Complete Block Design (RCBD) with three replications. Groundnut seedlings were infected with the virus through mechanical inoculation at 10 days after sowing and virus identity was confirmed using Enzyme-Linked Immunosorbent Assay (ELISA). The plants were assessed for disease incidence, disease severity (1=no symptom; 5=severe mosaic) as well as morphological and yield attributes. Data were analysed using general linear model and cultivar grouping was performed with cluster analysis. One hundred seed weight of infected SAMNUT 23 plants was the highest (54.9 g), followed by SAMNUT 25 and SAMNUT 26 which gave 50.2 and 49.4 g, respectively. The cultivars ICG-01276 and ICG-02189 exhibited the lowest reduction in dry haulm weight (31.1 %). Therefore, ICG-01276, ICG-02189, SAMNUT 23, 25 and 26 probably contained genes which could be utilized in breeding groundnut with durable resistance to CABMV in order to enhance high yield and food security.

Keywords: *Cowpea aphid-borne mosaic virus*; disease incidence and severity; pathogenicity; growth and yield; groundnut

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INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a leguminous crop in the family Fabaceae (syn. Leguminosae) (Hillocks *et al*., 2012). It originated in South America but is now cultivated in diverse environments throughout the world (Sreenuvasulu *et al*.,

2008). Groundnut is a popular crop in developing countries, Nigeria inclusive (Girei *et al*., 2013). It is an excellent source of oil, protein, minerals and vitamins that enhance growth and development in humans (Mukhtar, 2009). It is an important cash crop to both subsistence and commercial farmers in the tropics. Groundnut is widely used for manufacturing pharmaceuticals, butter, soaps, cosmetics, hair creams and paints. Additionally, its flour is utilized as ingredient in soup and confectionaries. In many parts of Africa, groundnut haulm is used as animal feed particularly during the off season and its oilcake is used as raw material in fertilizer industries (Ayele, 2010; Martey *et al*., 2015). The smallholder farmers commonly grow groundnut in mixture with cereal crops (Tsigbey *et al*., 2004) such as maize and guinea corn because of its ability to enhance soil fertility. Studies have shown that groundnut has the ability to fix atmospheric nitrogen in symbiotic association with rhizobia bacteria (Nyabyenda, 2005). These bacteria are able to penetrate the plants through root hairs, multiply there, form nodules and fix organic nitrogen in the nodules.

Presently, groundnut is produced in over100 countries around the world on a total estimated area of 21.8 million hectares. China, India, Nigeria, the United States, Myanmar, Sudan, Argentina, Tanzania, and Indonesia are the most prominent groundnut-producing countries (Food and Agriculture Organization, [FAO, 2013). In Nigeria, the greatest quantities of groundnut come from Niger, Kano, Jigawa, Zamfara, Kebbi, Sokoto, Katsina, Kaduna, Adamawa, Yobe, Borno, Taraba, Plateau, Nasarawa, Bauchi, and Gombe (NAERLS, 2011). Although there has been an annual increase in land area cultivated with groundnut in sub-Saharan Africa (SSA), productivity is usually low, being less than 1.0 tonne, contrary to the potential yield of 2 to 3 tonnes per hectare (Asibuo *et al*., 2008). The poor yield that is being experienced in many parts of Africa is also the case in Nigeria. Low groundnut yield could be attributed to several factors such as insect pests and disease attack.

Cowpea aphid-borne mosaic virus (CABMV) is a major limiting factor of leguminous crops in the tropics and subtropics (Sastry and Zitter, 2014). Though first observed in Italy (Lovisolo and Conti, 1966), the virus has been reported in many countries in different continents including Asia, Africa, Europe, North and South America, and Australia (Pio-Ribeiro *et al*., 2000). It has been confirmed in different parts of Nigeria since 1976 and is still regarded as a major threat to legume productivity (Shoyinka *et al*., 1997). The virus can reduce yield up to 87 % and complete crop loss may occur in highly susceptible cultivars (Alegbejo, 2015). To date, the most effective and sustainable management strategy against viral diseases is the cultivation of resistant cultivars. In Nigeria, several groundnut cultivars are being sold to farmers by commercial seed companies for planting. However, there is dearth of information on their level of resistance to CABMV. This information is critical for developing control strategies. One of such is the possibility of identifying resistant cultivar (s) that could be used for genetic improvement of the high yielding susceptible genotypes. Therefore, this study was conducted to screen some commercial groundnut cultivars for resistance to CABMV disease.

MATERIALS AND METHODS

Description of the Study Location

Two field trials were conducted at the Teaching and Research Farm of the Federal University of Technology, Minna during the 2015 cropping season. The bearing and elevation of the site (9^0 51'N, 6^0 44'E and 212 m above sea level) were captured using the Geographical Positioning System (GPS) equipment (GPS- 4300; Ethrex Garmin GPS, Taiwan). Minna belongs to the Southern Guinea Savanna ecological zone of Nigeria with average annual rainfall of about 1200 mm. Rainfall distribution is between April and early October with peak around September. Temperature ranges from 35 ^oC to 37.5 ^oC; the relative humidity is between 40 % and 60 % around January which later increases to between 60 and 80 % towards July. Generally, soils in Minna originated from basement complex rocks and are regarded as Alfisols (Adeboye *et al.*, 2011). The trial site has been under maize and yam cultivation in the last five years.

Treatments, Experimental Design and Field Establishment

Twenty groundnut cultivars constituted the treatments. They were FDRF7-61, FDRF7-67, ICG-01276, ICG-02189, ICG-5159, ICG-6654, ICG-92267, ICG-94169, ICG-IS-13003, ICG-IS-13986, ICGV-91317, ICGV-IS-76855, SAMNUT 10, SAMNUT 14, SAMNUT 21, SAMNUT 22, SAMNUT 23, SAMNUT 24, SAMNUT 25, and SAMNUT 26. These were laid out in Randomized Complete Block Design (RCBD) with three replications. The inoculated plots had a total size of 15 m by 2 m (30 m^2) containing 20 rows of 2 m long each with an alley of 3 m between the replicates. Uninoculated (control) plots of each cultivar also had a total size of 15 m by 2 m (30 m^2) containing 20 rows of 2 m long each with an alley of 3 m between the replicates as in the case of inoculated treatment. The inoculated and control plots were established side by side with isolation distance of 30 m in order to prevent virus contaminations (Sanders *et al.*, 1992).

Source of Seed

Seeds of the groundnut cultivars used for the study were acquired from Institute for Agricultural Research (IAR), Zaria, Nigeria. These are commercial cultivars already available to farmers across the country especially in Minna, Southern Guinea Savanna (SGS) agro-ecological zone of Nigeria where the study was conducted.

CABMV Inoculum Preparation and Inoculation Procedure

The Cowpea aphid-borne mosaic virus inoculum used for this experiment was obtained from the stock in the Department of Crop Production, Federal University of Technology, Minna, Nigeria. Its general characteristics have been reported by Taiwo (2001). The inoculum was multiplied in the screenhouse to obtain enough quantity required for the field work. This was carried out by planting susceptible cowpea (*Vigna unguiculata*, cv. Ife brown) cultivar into 20 pots at 5 plants per pot to arrive at 100 plants population. The plants were mechanically inoculated with CABMV inoculum at 10 days after sowing (Taiwo and Akinjogunla, 2006). This was accomplished by homogenizing one gram of CABMV infected leaf in one millilitre of 0.05 M phosphate buffer pH 7.2, using pre-cooled sterilized mortar and pestle. The buffer was prepared by dissolving 0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M L-cysteine in a litre of distilled water. Two microlitres of β- mercapto ethanol (which aided virus penetration through the cell wall

of the plant) was mixed with the extract just before used.

Leaves of the cowpea seedlings were dusted with 600-mesh carborundum (Fisher Scientific, Fair Lawn, NJ) powder. The inoculated leaves were rinsed with distilled water in order to prevent shading by the carborundum powder. Plants were observed daily until disease symptoms became fully evident at 2 to 3 weeks after inoculation. Symptomatic leaves were harvested and tested for CABMV in Enzyme-Linked Immunosorbent Assay (ELISA) as described by Kumar (2009). Extract of healthy cowpea leaves was used as negative control for validation of the test results. A discriminant CABMV polyclonal antibody was used for virus detection. Virus titres were quantified at 405 nm after one hour incubation of the ELISA plate, using a microplate reader (MRX, Dynex Technologies, Inc., USA). Readings were scored positive when the ELISA values exceeded two times the negative controls. Leaves of the positive samples were preserved on silica gels and these were used as source of inoculum during the field inoculations.

Field Establishment and Inoculations

Groundnut seeds were manually sown in the second week of August, 2015 on 5-m long ridges at the rate of two seeds per hole using 0.75 m × 0.20 m inter- and intra-row spacing, respectively. After emergence, groundnut seedlings were reduced to one plant per stand and manual weeding was carried out at 3, 5 and 8 weeks after sowing. The earlier preserved CABMV inoculum was used to infect groundnut seedlings on the field. Seedlings were infected with inoculum of CABMV at 10 days after sowing using the procedure described above.

Data Analysis

Disease incidence was recorded at 1 and 2 weeks after inoculation (WAI), as percentage of total plants exhibiting symptoms of CABMV infection. Disease severity was taken at weekly intervals for five weeks, commencing from 2 WAI. Disease severity assessment was based on percentage of the topmost leaf surface covered with symptom and general appearance of the plants. A visual 1 to 5 scoring scale was used (Arif and Hassan, 2002). On the scale, 1 = leaves without visible symptom; 2 = leaves exhibiting slight mosaic; 3 = distorted leaves with mosaic; 4 = stunted plants with distorted leaves and severe mosaic; and 5 = dead or stunted plants with severe mosaic. Disease severity scores were used to estimate Area Under Disease Progress Curve (AUDPC) (Shaner and Finney, 1977):

$$\text{AUDPC} = \sum_{i=1}^{n} [(Y_{i+1} + Y_i)/2] [X_{i+1} - X_i],$$

where:

Y_i = disease severity at the *ith* observation,

X_i = time (weeks) at the *ith* observation, and

n = total number of observations.

The AUDPC estimates were used to determine resistance classes according to Goktepe *et al.* (2007). Leaf diameter, plant height, number of branches per plant, number of days to 50 % flowering, fresh haulm weight per plant, dry haulm weight per plant, number of pods per plant, number of seeds per pod, and 100-seed weight were also recorded. The growth and yield data of infected and control plants of each cultivar were compared using analysis of variance (ANOVA). Statistical Analysis System (SAS, 2008) was used for ANOVA. Significance of *F* test was determined at 5 % probability level and cluster analysis was computed using Unweighted Pair Group Method with Arithmetic (UPGMA) mean.

RESULTS

Incidence and Severity of CABMV Infection

There were no symptoms of infection on the leaves of uninoculated plants throughout the period of evaluation. In contrast, CABMV induced characteristic symptoms in all (disease incidence = 100 %) the inoculated plants. Symptom was first sighted at 7 days after inoculation (DAI). This was mainly leaf mottling but at second week after inoculation (WAI), mosaic symptom was evident on some leaves. The intensity of symptoms varied among the cultivars from mild to moderate (Plate I) level throughout the period of evaluation. The resistant classes of the groundnut cultivars are presented in Fig. 1. The AUDPC analysis indicated significant ($p<0.05$) differences for resistance to CABMV infection. Only two cultivars (ICG-94169 and SAMNUT 14) were resistant to the virus while two others (ICG-01276 and ICG-5195) were moderately resistant. Conversely, a total of six cultivars (ICG-92267, SAMNUT 10, SAMNUT 21, SAMNUT 22, SAMNUT 23, and SAMNUT 26) were susceptible while ten cultivars (FDR7-61, FDR7-67, ICG-02189, ICG-6654, ICG-IS-13003, ICG-IS-13986, ICGV-91317, ICGV-IS-76855, SAMNUT 24, and SAMNUT 25) were highly susceptible to the virus.

Plate I: Mild (left) and moderate (right) level of infections in groundnut plants infected with *Cowpea aphid-borne mosaic virus*

Effects of CABMV Infection on Plant Growth and Yield Attributes

Healthy plants exhibited rapid growth, broad leaves with normal shape (Plate II). Infection by CABMV caused significant reduction in leaf diameter of infected plants as indicated in Fig. 2A. Reduction in leaf diameter was generally high and greater than 50 % in most of the cultivars. ICGV-IS-76855 suffered the highest (66.7 %) reduction in leaf diameter. However, SAMNUT 23, ICG-5195, and SAMNUT 26 also exhibited over 60 % reduction in leaf diameter. Among the cultivars, ICG-IS-13003 had the lowest (39.4 %) reduction in leaf diameter.

Plate II: Uninoculated groundnut plant showing broad leaves with normal shape

Uninoculated plants were generally tall while the virus infected plants exhibited shorter internodes. Some of the severely infected plants were markedly stunted. Although height reduction was highest (62.9 %) in ICG-6654, the value observed was similar to that (62.3 %) in infected plants of SAMNUT 21. In diseased plants of FDR7-61, ICG-IS-13003, ICG-02189, SAMNUT 14 and SAMNUT 26, height reduction was less than 40 %, whereas FDR7-67 showed the lowest (28 %) height reduction. In the remaining cultivars, height reductions varied between 41.7 and 57.9 % (Fig. 2B).

All the healthy plants produced significantly higher number of branches than their CABMV-infected counterparts (Fig. 3A). The infected plants of ICG-6654 showed the highest (81.9 %) reduction in number of branches per plant, followed by ICG-IS-13003 in which branches were reduced by 77.9 %. However, reductions in number of branches were also higher than 70 % in infected plants of ICG-5195, FDR7-61, SAMNUT 21, ICGV-IS-76855, ICG-92267 and SAMNUT 23. The lowest (52.9 %) reduction in number of branches was found in inoculated plants of ICG-94169 but the value obtained was statistically comparable to those observed in SAMNUT 10 (53.6 %) and SAMNUT 26 (53.5 %). Although flowering commenced in the healthy plants before the infected ones, the difference in number of days to 50 % flowering was not significant (Fig. 3B). In most cultivars, number of days to 50 % flowering was delayed for 1 day while in infected plants of SAMNUT 10 it was delayed for 5 days.

Fresh haulm weight of the CABMV-infected plants was significantly lower than the healthy plants in all the cultivars (Fig. 4A). With the exception of FDR7-61, SAMNUT 25 and SAMNUT 26, reduction in fresh haulm weight was greater than 60 %. ICG-02189 suffered the highest (80.2 %) reduction in fresh haulm weight while the lowest (52.1 %) was found in FDR7-61. Similarly, dry haulm weight of healthy plants was significantly higher than the CABMV-infected plants irrespective of the cultivar (Fig. 4B). However, unlike in fresh haulm weight, reduction in dry haulm weight which was the same in ICG-01276 and ICG-02189 was also the lowest (31.1 %) among all the cultivars studied. Reduction in dry haulm weight was highest (76.1 %) in FDR7-67, followed by SAMNUT 10. In ICG-5195, ICG-6654, ICG-IS-13003, ICG-94169, SAMNUT 14, SAMNUT 21, SAMNUT 23, SAMNUT 24 and SAMNUT 25, reduction in dry haulm weight varied between 60.2 and 76.1 %. In the remaining cultivars, it ranged from 50.2 to 59.6 % (Fig. 4B).

The effect of CABMV on number of pods per plant was significant in FDR7-61, ICG-01276, SAMNUT 10, SAMNUT 14 and SAMNUT 24 (Fig. 5A). In other cultivars, healthy plants produced more pods than the infected plants but the difference was not significant (p>0.05). In ICGV-91317 and ICGV-IS-76855 both inoculated and uninoculated plants produced the same number of pods per plant. Pod reduction was highest (46.2 %) in ICG-01276. Additionally, the result showed that pod reduction was less than 10 % in ICG-94169. For ICG-5195, ICG-92267, ICG-IS-13003, ICG-IS-13986 and SAMNUT 23, pod reduction was higher than 10 % but less than 20 %.

Both the infected and uninoculated plants produced two seeds per pod (Fig. 5B). Healthy plants produced large seeds with normal shape while most of the infected plants produced small and deformed seeds. One hundred-seed weight of uninoculated plants was higher than the CABMV-infected plants in ICG-02189, ICG-92267, ICG-94169, ICG-IS-13986, ICGV-IS-76855, SAMNUT 21, SAMNUT 22, SAMNUT 23 and SAMNUT 26 (Fig. 6). In the other cultivars, however, although healthy plants had higher seed weight than the infected plants, the difference between the two treatment groups was not statistically significant. Apart from FDR7-67 in which reduction in 100-seed weight was higher than 50 %, the remaining cultivars showed low level of reduction in seed weight. Reduction in 100-seed weight was lowest in ICG-5195 and the value obtained was less than 10 %. In ICG-6654 and ICG-01276, ICG-6654, ICG-92267, ICG-IS-13003, ICG-IS-13986, ICGV-91317, ICGV-IS-76855, SAMNUT 10, SAMNUT 14, SAMNUT 21, SAMNUT 22, SAMNUT 24, SAMNUT 25 and SAMNUT 26, 100-seed weight reduction in diseased plants was less than 20 %. In the remaining cultivars, reductions in 100-seed weight varied between 21 and 26 %.

Cluster analysis of the reductions in growth and yield parameters revealed that a total of 17 (FDRF7-61, ICG-5159, ICG-6654, ICG-92267, ICG-94169, ICG-IS-13003, ICG-IS-13986, ICGV-91317, ICGV-IS-76855, SAMNUT 10, SAMNUT 14, SAMNUT 21, SAMNUT 22, SAMNUT 23, SAMNUT 24, SAMNUT 25, and SAMNUT 26) cultivars belonged to the same group (Cluster 1), there were two (ICG-02189 and ICG-01276) cultivars in cluster 2 while only one (FDRF7-67) cultivar formed cluster 3 (Fig. 7).

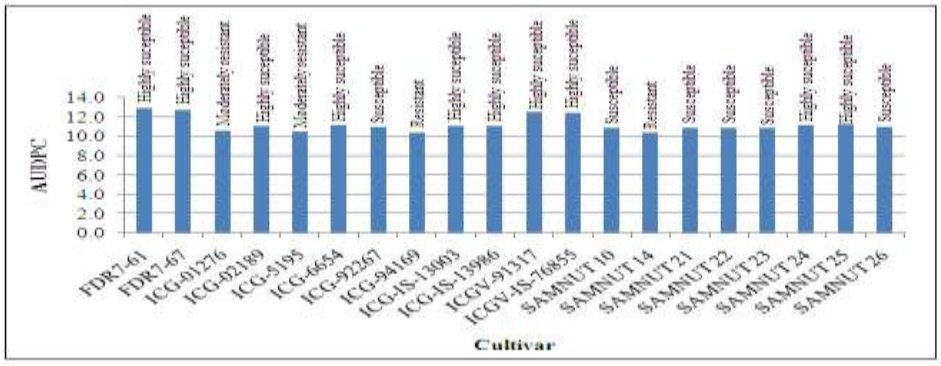

Fig. 1: Area Under the Disease Progress Curve (AUDPC) estimates and resistant classes of the groundnut cultivars infected with *Cowpea aphid-borne mosaic virus* during the 2015 cropping season in Minna, Southern Guinea Savanna of Nigeria

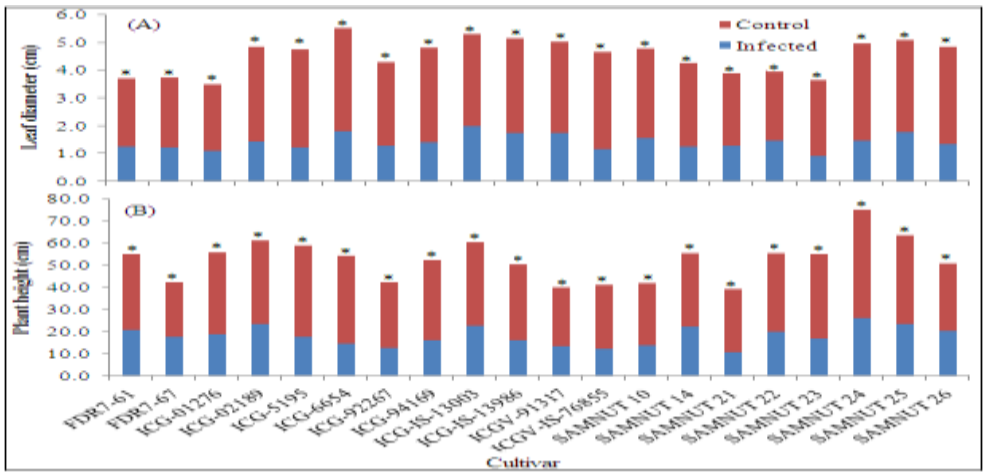

Fig. 2: Leaf diameter (A) and plant height (B) from healthy (control) and *Cowpea aphid-borne mosaic virus* infected groundnut cultivars during the 2015 cropping season in Minna, Southern Guinea Savanna of Nigeria. Asterisks on the bars indicate significant difference at 5 % probability level

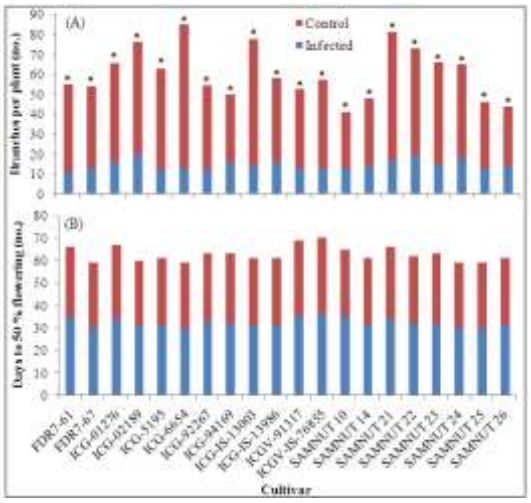

Fig. 3: Number of branches per plant (A) and days to 50% flowering (B) from healthy (control) and *Cowpea aphid-borne mosaic virus* infected groundnut cultivars during the 2015 cropping season in Minna, Southern Guinea Savanna of Nigeria. Asterisks on the bars indicate significant difference at 5 % probability level

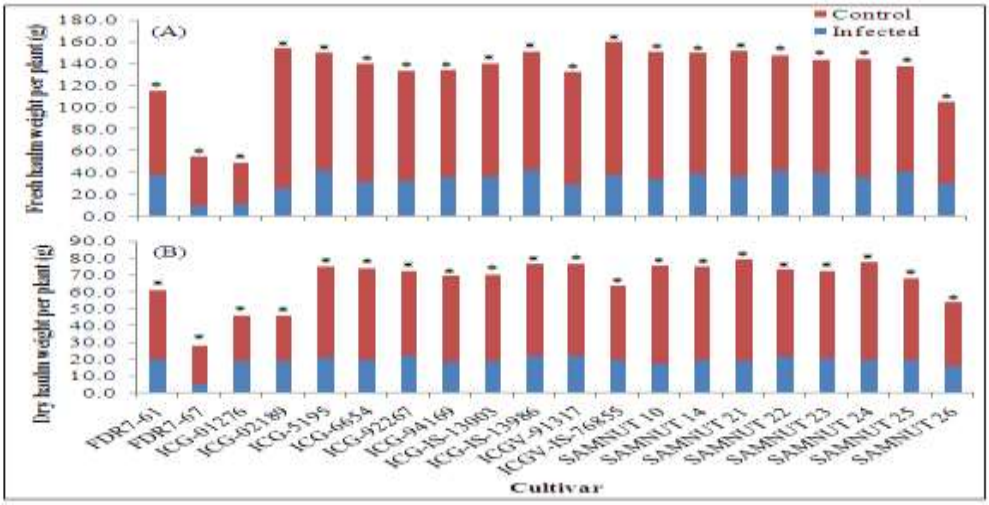

Fig. 4: Fresh haulm weight per plant (A) and dry haulm weight per plant (B) from healthy (control) and *Cowpea aphid-borne mosaic virus* infected groundnut cultivars during the 2015 cropping season in Minna, Southern Guinea Savanna of Nigeria. Asterisks on the bars indicate significant difference at 5 % probability level

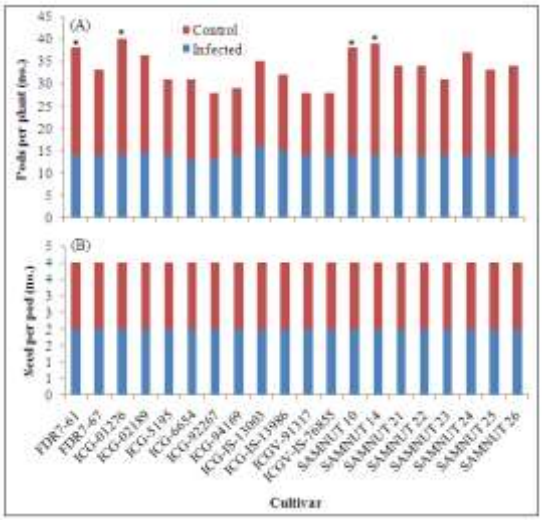

Fig. 5: Number of pods per plant (A) and number of seeds per pod (B) from healthy (control) and *Cowpea aphid-borne mosaic virus* infected groundnut cultivars during the 2015 cropping season in Minna, Southern Guinea Savanna of Nigeria
Asterisks on the bars indicate significant difference at 5 % probability level

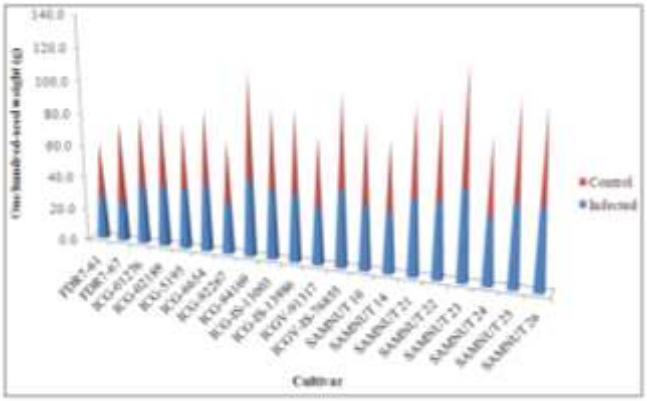

Figure 6: One hundred-seed weight from healthy (control) and *Cowpea aphid-borne mosaic virus* infected groundnut cultivars during the 2015 cropping season in Minna, Southern Guinea Savanna of Nigeria

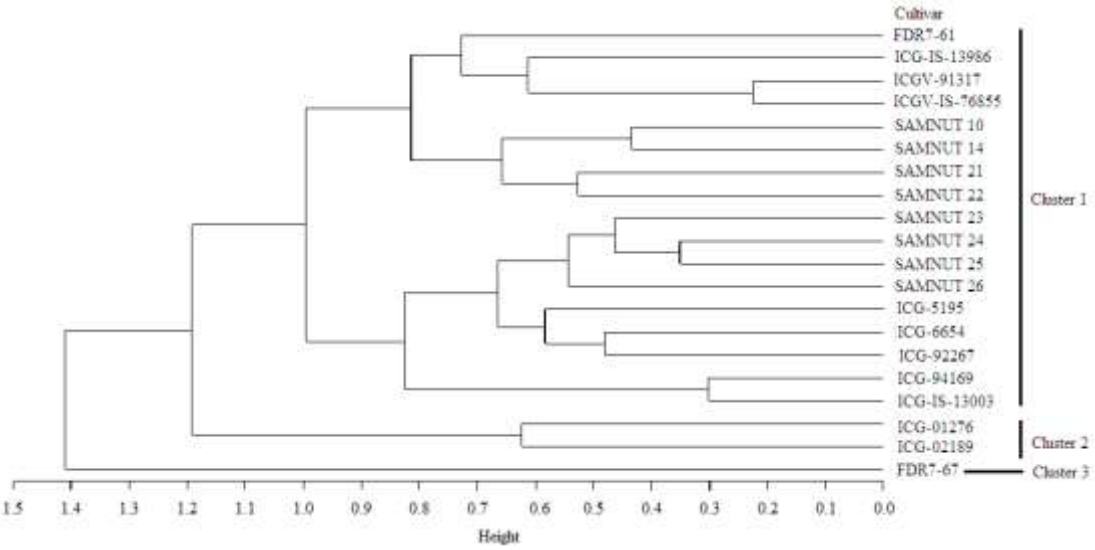

Figure 7: Dendogram of the percentage reductions in growth and yield parameters of groundnut cultivars infected with *Cowpea aphid-borne mosaic virus*, inferred from Unweighted Pair Group Method with Arithmetic (UPGMA) mean

DISCUSSION

Cowpea aphid-borne mosaic virus is one of the major viruses limiting groundnut productivity and adoption of resistant cultivars has been recommended as the best option (Thottappilly and Rossel, 1992). Because of the economic importance of groundnut, research has been intensified with the aim of ensuring adequate plant protection and maximum yield. The inability of all CABMV-infected groundnut plants to prevent symptom expression indicated that none of them was immune against the virus.

Immunity is the highest level of resistance which is recognized as absence of symptom after inoculation. However, the fact that the intensity of symptoms differed significantly among the infected plants revealed that pathogenicity of the virus was cultivar dependent (Fraser, 1990). The two cultivars (ICG-94169 and SAMNUT 14) in which symptom expression was mildest probably contained CABMV resistant gene (s). The same reason may hold for those cultivars (ICG-01276 and ICG-5195) which exhibited moderate level of resistance.

Moreover, the results obtained revealed that even though the inoculated plants permitted systemic spread of CABMV virus multiplication and symptom severity was suppressed (Fraser, 1987). These observations probably suggest that resistance to CABMV was under the influence of two or more genes. This argument is supported by the findings of workers such as Kim *et al*. (1989) who also reported that resistance to *Maize streak virus* (MSV) in inbred maize IB32 was quantitatively inherited through additive action of several genes. Similarly, Rodier *et al*. (1995) reported that resistance in CVR_3 – C_3 involved loci with major genes controlling high to complete resistance, and a locus with minor genes conferring partial resistance. The susceptible and highly susceptible cultivars were the most vulnerable possibly due to absence of CABMV resistant genes.

Growth and yield characters decreased drastically in infected plants of most groundnut cultivars owing to their poor genetic background. The magnitude of reductions in such plants revealed the level of CABMV pathogenicity on them. This corroborates the findings of Taiwo and Akinjogunla (2006) when some cowpea varieties were infected with CABMV. Some of the severely infected plants were stunted as a result of short internodes induced by the virus. This is consistent with the findings of Pio-Ribeiro *et al*. (1978). Although, none of the cultivars exhibited consistent low reduction in the evaluated parameters, the groundnut cultivar ICG-5195 which showed the lowest reduction in 100-seed weight could be described as the most promising. Seed weight is an important yield component because of its direct relationship with the total output. Reduction in seed weight was highest in diseased plants of FDR7-67 probably due to the carryover effects of high reductions in leaf diameter and number of branches per plant.The results of cluster analysis gave an insight into understanding average performance of the groundnut cultivars under CABMV attack. The cultivars in the same cluster with those that had the lowest reduction in leaf diameter, number of branches per plant, fresh haulm weight, number of pods per plant and 100-seed weight could be described as possible alternatives where CABMV poses serious challenge to groundnut production.

CONCLUSION AND RECOMMENDATIONS

This study revealed the susceptibility of some Nigerias commercial groundnut cultivars to CABMV. The cultivar SAMNUT 23 was the overall best for seed weight. Cultivation of this cultivar is recommended in CABMV prone environment in the Southern Guinea Savanna of Nigeria. Moreover, the cultivar probably contained CABMV resistant genes which could be exploited in breeding programmes. Although, groundnut is not normally produced as forage for livestock feed, if that is the intent of the grower, FDR7-61 which exhibited the lowest reduction in fresh haulm weight is recommended. On the other hand, ICG-01276 and ICG-02189 which had

the lowest dry haulm weight reduction appeared to be good sources of fodder in case of CABMV outbreak.

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