Evaluation of Cowpea Genotypes for Infection by Two Aphid-borne Viruses

*[1]Kehinde T. Kareem, [1]Ayodele A. Adegbite, [1]Olukemi T. Ayoola, [1]Racheal B. Olayinka, and [2]Qudrah O. Oloyede-Kamiyo

[1]Grain Legumes Improvement Programme, Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Oyo State, Nigeria, [2]Maize Improvement Programme for Southern Ecology, Institute of Agricultural Research and Training, Obafemi Awolowo University, PMB 5029, Moor Plantation, Ibadan, Oyo State, Nigeria
*E-mail address of corresponding author: kt_kareem@yahoo.com

Abstract: Cowpea is an important food legume utilized by millions of people in the sub-Saharan Africa and other countries. However, its production is hindered by biotic and abiotic factors of which virus is one of them. The study therefore, investigated the response of cowpea genotypes to two aphid-borne viruses during 2013 and 2014 growing seasons. Genotype IFE82-12 had the highest aphid score in 2014 while UAM1046-6-15 and UAM1051-1 recorded the highest virus severity in 2013. The virus incidence in 2013 was higher than 2014 with UAM1046-6-15 and LDPO-OBRI having the highest of 62.54% and 33.21% respectively. Serological reactions revealed that all genotypes were positive to both Cowpea aphid-borne mosaic virus (CABMV) and Cucumber mosaic virus (CMV) in 2013 but not all were positive to CMV in 2014. The highest grain yield in 2013 was 474.08 $kgha^{-1}$ produced by IT07K-318-33. In 2014, the highest significant grain yields were 888.89 and 870.37 $kgha^{-1}$ produced by IT07K-318-33 and IFE82-12 respectively. Despite the fact that genotypes IFE82-12, IT07K-293-2-1, IT07K-304-9 and IT08K-149-3 were susceptible to double infections in both years, their yields were appreciable and they can be regarded as potential candidates for breeding programmes.
[Kehinde.T. Kareem, Ayodele.A. Adegbite, Olukemi.T. Ayoola, Racheal.B. Olayinka, and Qudrah.O. Oloyede-Kamiyo. **Evaluation of Cowpea Genotypes for Infection by Two Aphid-borne Viruses.** *World Rural Observ* 2016;8(4):80-88]. ISSN: 1944-6543 (Print); ISSN: 1944-6551 (Online). http://www.sciencepub.net/rural. 13. doi:10.7537/marswro080416.13.

Key words: Genotype, incidence, resistance, severity, yield

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is an important leguminous crop in tropical and subtropical areas of Asia, Africa and Latin America, as well as parts of Southern Europe and the USA (Boukar et al. 2004). Cowpea is the most economically important indigenous African legume crop (Langyintuo et al. 2003) and Nigeria is reputed to be the highest producer of cowpea in the world (Taiwo and Akinjogunla 2006). Cowpea is an early maturing crop and therefore helps reduce the "hunger period" that often occurs prior to harvest in farming communities (Singh et al. 2002, Timko et al. 2007). However, cowpea production is constrained by a range of abiotic and biotic factors, including viral diseases and yields in sub-Saharan Africa are typically only 10–20% of the known cowpea yield potential. Viral diseases are a limiting factor in cowpea production and hence, identifying sources of resistance is an important objective of cowpea breeding programs (Singh et al. 2003). The majority of the viral diseases of cowpea lead to overall stunting, reduction in leaf size, mottling, mosaic, leaf chlorosis, leaf distortion, leaf curling, vein clearing, necrotic local lesion and death (Akinjogunla 2005).

Apart from infections caused by isolated viruses, mixed infections with more than one virus have been observed with relative frequency in cowpea under field conditions (Lima et al. 2005a). Simultaneous infections of Cowpea aphid-borne mosaic virus (CABMV) and Cucumber mosaic virus (CMV) have been reported to frequently occur, with high degree of incidence causing serious damages to crop productivity (Lima et al. 2005a). These viruses are transmitted by several insect species in a non-persistent manner and therefore, use of insecticides is not an effective method of control (Umaharan et al. 1997). Cowpea aphid-borne mosaic virus is an ssRNA virus belonging to the genus *Potyvirus.* It is one of the economically significant and cosmopolitan viruses known to inflict severe yield losses in cowpea. This seed-borne distinctive virus with flexuous filamentous particles, 750×12 nm (Damiri et al. 2013), is transmitted in a stylet-borne, non-persistent manner by several common species of aphids such as *Aphis craccivora, A. fabae, A. gossypii, A. medicaginis, Macrosiphum euphorbiae* and *Myzus persicae* (Damiri et al. 2013). The CABMV with wide geographical distribution has been reported from almost all the continents, where, cowpea is grown (Damiri et al. 2013). Cucumber mosaic virus (CMV) is the type

member of the genus *Cucumovirus*, family *Bromoviridae*. This virus has icosahedra particle of diameter of 28 nm and has a segmented genome of three single stranded RNAs (Palukaitis et al. 1992). It was first found in Cucumber in the USA (Price 1934) and first reported in cowpea by Robertson (1966). Cucumber mosaic virus has been confirmed to be very ubiquitous plant virus and is the most commonly found in the riverine area of the middle belt of Nigeria (Shoyinka et al. 1997).

The selection of cowpea cultivars with multiple resistances is fundamental to the control of mixed infections (Anderson et al. 1996). Genetic resistance is the best alternative in reducing crop losses due to these diseases. To identify host resistance, it is important to evaluate different genotypes under field conditions (Oloka et al. 2008, Goenaga et al. 2011, Maphosa et al. 2013). The development of resistant cultivars has been universally considered the most effective method to control diseases caused by viruses in cowpea, indicating that an increase in the number of virus resistant genotypes will generate more alternatives for breeders to produce resistant cultivars (Lima et al. 2005b, Assunção et al. 2005). This research therefore aims at (i) identifying new sources of single and double resistances in cowpea genotypes to CABMV and CMV, (ii) determining the relationship between aphid-borne viral infection and yield of cowpea.

Materials and methods

Sources of cowpea genotypes

The cowpea genotypes used in this study were obtained from International Institute of Tropical Agriculture (IITA), Ibadan, Institute of Agricultural Research, Zaria, University of Agriculture, Umudike and Institute of Agricultural Research and Training (IAR&T), Ibadan, Nigeria (Table 1). Fifteen genotypes were evaluated under field condition in two growing seasons.

Experimental sites

The study was conducted during 2013 and 2014 growing seasons at two locations within the Institute of Agricultural Research and Training, Ibadan, Nigeria. The 2013 experiment was located on Latitude 7°38'N; Longitude 3°84'E and 174.3 m above sea level while that of the 2014 was located on Latitude 7°37'N; Longitude 3°84'E and 177.1 m above sea level. Cowpea was previously grown on 2013 experimental site while kenaf was previously grown on 2014 experimental site.

Experimental design

The experiment was laid down in a randomized complete block design (RCBD) with three replications. Each cowpea genotype was planted at a spacing of 60 x 30 cm in a 3 x 3 m plot. Three seeds were planted per hole and later thinned to one after germination.

Table 1. Sources of cowpea genotypes used in the study

Cowpea genotype	Source
IAR-06-1006	IAR, Zaria
IFE82-12	IAR&T, Ibadan
IT04K-227-4	IITA
IT06K-111	IITA
IT06K-128	IITA
IT06K-270	IITA
IT07K-292-10	IITA
IT07K-293-2-1	IITA
IT07K-304-9	IITA
IT07K-318-33	IITA
IT08K-105-24	IITA
IT08K-149-3	IITA
LDPO-OBRI	University of Agriculture, Umudike
UAM1046-6-15	University of Agriculture, Umudike
UAM1051-1	University of Agriculture, Umudike

Data collection

Data were collected on aphid infestation, virus severity and incidence, virus serology and grain yield as explained below.

Scoring for aphids infestation and virus severity in cowpea genotypes

Data on the insect pest responsible for the transmission of CABMV and CMV in cowpea plants were taken between 20 to 30 days after planting. Four inner rows excluding I m row from both ends of each row were selected from each sub-plot for scoring. The target insect pest was *Aphis craccivora* Koch. All plants in the selected four middle rows were visually examined to score for severity or degree of infestation on a scale of 0-5 (where 0 = no aphids, 1= a few individual aphids, 2 = few small individual colonies, 3 = several small colonies, 4= large individual colonies, 5 = large continuous colonies) (Souleymane et al. 2013).

Viral infection severity was scored on a scale of 1-5, based on the extent of symptoms observed on leaves. 1 = no symptom on leaves or plant; 2 = 1 - 25% of leaves showing mild symptoms (chlorosis, mosaic, necrosis); 3 = 26-50% of leaves showing moderate symptoms (leaf deformation, leaf wrinkling, leaf reduction); 4 = 51-75% of leaves or plant showing severe symptoms (apical necrosis, stunting); 5 > 75% of leaves or plants showing very severe symptoms (Gumedzoe et al. 1997).

Assessment of virus incidence in cowpea genotypes

Virus incidence was estimated by counting the number of symptomatic plants and expressing it as a percentage of the total plants sampled (Kareem et al., 2012).

$$\text{Incidence (\%)} = \frac{\text{Number of symptomatic plants/plot x 100}}{\text{Total number of plants/plot}}$$

Reaction of Cowpea aphid-borne mosaic virus and Cucumber mosaic virus in cowpea genotypes using enzyme-linked immunosorbent assay (ELISA)

Leaves samples were collected from the cowpea genotypes before flowering during 2013 and 2014 growing seasons. Sap extracted from the leaves of each of the cowpea genotype was tested for the presence of CABMV and CMV. The presence of CABMV (Potyvirus) was determined by Antigen coated plate enzyme-linked immunosorbent assay (ACP-ELISA) using the manufacturer's instruction (Agdia-Bioford Inc, Elkhart, Indiana, USA). The presence of CMV was determined by double antibody sandwich (DAS) ELISA with antisera for CMV obtained from the same company above. The microtitre wells were inoculated with 100 µl of capture antibody and the plates incubated for 4 hrs at room temperature. Plates were washed three times with 1X phosphate buffer saline-tween 20 (PBST). One gram of leaf sample was weighed and ground in 10 ml of Agdia's general extract buffer (GEB). Extracted samples were coated into the duplicate wells of the microtitre plates at 100 µl per well. The procedure continued as for ACP-ELISA after the loading of sample extract. All samples were placed in duplicate wells. Absorbance values were read at 405 nm using a Microtitre Plate Reader (Biotek, ELx800). Samples were considered positive when the values of the test sample were greater than twice the mean of the sap of the healthy plant or negative controls.

Yield parameters of cowpea genotypes

Matured pods from each plot were harvested from the cowpea genotypes. The pods were dried for few days and then weighed on a weighing balance to determine the weights in g. The pods were shelled and the seeds were weighed on a weighing balance in g. The pod and seed weights were extrapolated to give their weights in kg^{-1}ha.

Data analysis

Combined analysis of variance was performed using PROC GLM of SAS (Version 9.2). Percentage data were transformed using arcsine transformation before analysis of variance. Means and standard error were estimated. Means were separated using Duncan Multiple Range Test at P = 0.05 and 0.01. Correlation analysis was also carried out.

Results

Mean squares estimates for the cowpea genotypes

Combined analysis of variance for the viral infection and yield traits in both seasons showed that mean square of genotypes was highly significant for all the traits studied except for aphid infestation. Mean square of season was significant for all the traits studied. There was significant genotype by season interaction for viral incidence and seed yield (Table 2).

Table 2: Mean squares from combined analysis of variance for the 15 cowpea genotypes under natural infections by two aphid-borne viruses in 2013 and 2014

source	df	Virus incidence	Virus severity	Pod	Seed	Aphids
Season	1	448.59*	0.07*	4795331.67**	274658.71**	17.78**
Rep	2	147.42	0.75	120641.67	34898.49	2.28
Genotype	14	632.83**	1.72**	282363.11**	118458.24**	1.58
Season x Genotype	14	278.83**	0.59	108710.3	66477.16**	1.37
Error	56	116.96	0.37	92117.71	38269.32	1.38

*, **: significant at P= 0.05 and 0.01 respectively, df: degree of freedom.

Aphid and virus severity

Apart from *Aphis craccivora* Koch which was the insect of interest in this study, other insects that were found attacking the crop on the field were; *Megalurothrip sjostedti*, *Maruca virtrata* and pod bug complex which include *Nezara viridula* and *Clavigralla tomintosicolliss*. The scores of aphids in 2013 were not significantly different ($P \leq 0.05$) and values ranged from 1.33 to 3.33. IFE82-12 had the highest aphid score of 4 in 2014 followed by IT04K-227-4 and IT07K-318-33 both with score of 2 (Table 3).

The highest significant virus severity score in 2013 was obtained from genotypes UAM1046-6-15 (4), UAM1051-1(4) and IT06K-111(3.67) while in 2014, there was no significant difference in the response of the genotypes to viruses and severity scores ranged between 2 and 3.17 (Table 3).

Table 3. Assessment of aphid and virus severity in 2013 and 2014

Cowpea genotypes	Aphid		Virus	
	2013	2014	2013	2014
IAR-06-1006	1.67^{a}±0.88	1.67^{b}±0.33	2.00^{b}±0.00	2.17^{a}±0.17
IFE82-12	2.00^{a}±1.00	4.00^{a}±0.00	2.00^{b}±0.00	2.50^{a}±0.5
IT04K-227-4	3.00^{a}±0.58	2.00^{ab}±0.00	2.00^{b}±0.00	3.17^{a}±0.73
IT06K-111	2.67^{a}±0.33	1.67^{b}±0.33	3.67^{a}±0.67	3.00^{a}±0.50
IT06K-128	2.33^{a}±0.33	1.67^{b}±0.33	2.67^{b}±0.33	2.8 ^{a}±0.60
IT06K-270	2.67^{a}±0.33	1.00^{b}±1.00	2.00^{b}±0.00	2.00^{a}±0.00
IT07K-292-10	2.67^{a}±0.33	1.33^{b}±0.67	2.00^{b}±0.00	2.00^{a}±0.00
IT07K-293-2-1	2.00^{a}±1.00	0.67^{b}±0.33	2.67^{b}±0.33	2.00^{a}±0.00
IT07K-304-9	3.00^{a}±0.00	1.00^{b}±0.58	2.33^{b}±0.33	2.67^{a}±.044
IT07K-318-33	3.33^{a}±0.88	2.00^{ab}±1.15	2.00^{b}±0.00	2.00^{a}±0.00
IT08K-105-24	2.33^{a}±0.67	1.00^{b}±0.58	2.33^{b}±0.33	2.00^{a}±0.00
IT08K-149-3	3.00^{a}±0.00	1.33^{b}±1.33	2.00^{b}±0.00	2.67^{a}±0.67
LDPO-OBRI	2.0^{a}±1.00	1.67^{b}±1.20	2.00^{b}±0.00	2.00^{a}±0.00
UAM1046-6-15	2.00^{a}±1.00	1.00^{b}±0.00	4.00^{a}±0.58	3.00^{a}±0.58
UAM1051-1	1.33^{a}±0.67	0.67^{b}±0.33	4.00^{a}±0.58	2.83^{a}±0.17

Values are means of three replicates ± standard error. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at P=0.05.

Viral incidence in cowpea genotypes

The highest viral incidence in 2013 was 62.54% followed by 36.62% and then 34.54% obtained from cowpea genotypes UAM1046-6-15, IT06K-111 and IT06K-128 respectively. Significantly low viral incidences were obtained from the following cowpea genotypes; IT04K-227-4, IT06K-270, IT07K-292-10, IT07K-318-33, IT07K-304-9, LDPO-OBRI and IT08K-105-24 in 2013 (Table 4). In 2014, LDPO-OBRI had the highest viral incidence of 33.21% while genotypes IT06K-270, IT07K-292-10, IT07K-318-33, IAR-06-1006 and IT08K-149-3 had the least incidence values (Table 4).

Table 4. Incidence of viruses in cowpea genotypes

Cowpea genotype	Virus incidence (%)	
	2013	2014
IAR-06-1006	18.71^{cd}±3.77	6.58^{b}±3.27
IFE82-12	23.4^{bcd}±6.12	22.74^{ab}±11.32
IT04K-227-4	6.96^{d}±0.73	22.5^{ab}±8.43
IT06K-111	36.62^{b}±7.79	23.99^{ab}±9.26
IT06K-128	34.54^{bc}±9.28	21.67^{ab}±9.69
IT06K-270	12.61^{d}±1.99	6.82^{b}±0.01
IT07K-292-10	7.77^{d}±1.15	4.06^{b}±0.11
IT07K-293-2-1	18.11^{cd}±4.75	11.39^{ab}±0.19
IT07K-304-9	12.76^{d}±2.52	14.6^{ab}±0.91
IT07K-318-33	9.11^{d}±1.53	6.43^{b}±0.04
IT08K-105-24	12.88^{d}±5.53	5.56^{b}±2.57
IT08K-149-3	19.67^{bcd}±3.25	12.25^{ab}±10.03
LDPO-OBRI	7.64^{d}±0.85	33.21^{a}±0.11
UAM1046-6-15	62.54^{a}±10.28	26.23^{ab}±16.18
UAM1051-1	24.07^{bcd}±6.25	22.37^{ab}±2.89

Values are means of three replicates ± standard error. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at P=0.05.

Reaction of cowpea genotypes to single and double infections of CABMV and CMV

All the cowpea genotypes showed positive reaction to CABMV and CMV in 2013. However, in

2014, all the genotypes were infected with CABMV while nine of the cowpea genotypes showed negative reaction to CMV. The genotypes that were negative to CMV were IAR-06-1006, IT04K-227-4, IT06K-128, IT06K-270, IT07K-318-33, IT08K-105-24, LDPO-OBRI, UAM1051-1 and UAM1046-6-15 (Table 5). Double infections of CABMV and CMV were present in all the genotypes in 2013. During the 2014 season, out of the 15 genotypes, nine were not doubly infected with CABMV and CMV while the remaining six genotypes showed positive reaction to the two viruses (Table 5).

Table 5. Reactions of cowpea genotypes to single and double infections of CABMV and CMV using enzyme-linked immunosorbent assay

Cowpea genotypes	CABMV		CMV		Double infection of CABMV+CMV	
	2013	2014	2013	2014	2013	2014
IAR-06-1006	+++	+	+++	-	+	-
IFE82-12	+++	+++	+++	+++	+	+
IT04K-227-4	+++	+++	+++	-	+	-
IT06K-111	+++	++	+++	++	+	+
IT06K-128	+++	+++	+++	-	+	-
IT06K-270	+++	++	+++	-	+	-
IT07K-292-10	+++	++	+++	+++	+	+
IT07K-273-2-1	+++	++	+++	++	+	+
IT07K-304-9	+++	+++	+++	++	+	+
IT07K-318-33	+++	+++	+++	-	+	-
IT08K-105-24	+++	++	+++	-	+	-
IT08K-149-3	+++	+++	+++	+	+	+
LDPO-OBRI	+++	+	+++	-	+	-
UAM1046-6-15	+++	+++	+++	-	+	-
UAM1051-1	+++	++	+++	-	+	-

+ = Mean ELISA value was twice the value of healthy control plants (positive reaction to virus); ++ = Mean ELISA value was three times the value of healthy control plants (high positive reaction to virus); +++ = Mean ELISA value was more than 3 times the value of healthy control plants (very high positive reaction to virus); - = negative reaction to virus

Yield of cowpea genotypes as influenced by aphid-borne viruses

Yield parameters were higher in 2014 than 2013. However, genotypes IT06K-270 and IT07K-292-10 did not have much difference in their individual yield in 2013 and 2014. Cowpea genotypes IT07K-318-33 and IT07K292-10 had the highest pod weights of 802.97 kg^{-1}ha and 811.11 kg^{-1}ha respectively in the year 2013. However, in the year 2014, the highest pod weights of 1,194.44 kg^{-1}ha and 1,177.78 kg^{-1}ha were obtained from cowpea genotypes IT07K-318-33 and IFE82-12 respectively (Table 6). The highest seed weight in 2013 was 474.08 kg^{-1}ha obtained from genotype IT07K-318-33 followed by 425.92 kg^{-1}ha produced by IT07K-292-10. Genotypes IFE82-12 and IT07K-318-33 had average seed weights of 870.37 kg^{-1}ha and 888.89 kg^{-1}ha respectively in 2014. Although, IT07K-318-33 was the highest in that year but it was not statistically different from IFE82-12 ($P = 0.05$) (Table 6).

Table 6. Pod and seed weights of cowpea genotypes

Cowpea genotypes	Pod weight (kg^{-1}ha)		Seed weight (kg^{-1}ha)	
	2013	2014	2013	2014
IAR-06-1006	18.52^{b}±16.67	124.99^{d}±7.22	7.41^{c}±6.67	55.56^{c}±0.00

IFE82-12	125.92^{ab}±85.11	1,177.78^{a}±0.01	57.33^{bc}±39.83	870.37^{a}±72.65
IT04K-227-4	455.56^{ab}±0.02	870.37^{ab}±44.09	188.89^{abc}±0.01	637.03^{ab}±14.53
IT06K-111	48.14^{b}±21.86	540.74^{bcd}±0.02	18.52^{c}±8.82	205.56^{bc}±1.27
IT06K-128	299.99^{ab}±0.02	718.52^{abc}±0.03	149.26^{abc}±0.01	444.44^{abc}±2.57
IT06K-270	496.29^{ab}±0.01	555.56^{bcd}±0.00	333.33^{abc}±0.01	355.56^{bc}±0.00
IT07K-292-10	811.11^{a}±0.02	944.44^{ab}±86.6	425.92^{ab}±99.55	472.22^{abc}±14.43
IT07K-293-2-1	411.11^{ab}±0.02	944.44^{ab}±86.6	207.41^{abc}±96.15	638.89^{ab}±0.01
IT07K-304-9	144.44^{ab}±68.1	870.37^{ab}±0.02	59.26^{bc}±27.28	611.11^{ab}±0.02
IT07K-318-33	802.97^{a}±0.06	1,194.44^{a}±0.01	474.08^{a}±0.03	888.89^{a}±86.60
IT08K-105-24	37.03^{b}±33.30	777.78^{abc}±2.65	18.52^{c}±16.67	583.33^{ab}±2.45
IT08K-149-3	140.74^{ab}±81.92	916.67^{ab}±43.3	62.97^{bc}±31.79	666.67^{ab}±28.87
LDPO-OBRI	48.14^{b}±29.63	287.03^{cd}±0.01	18.52^{c}±12.02	205.56^{bc}±95.26
UAM1046-6-15	555.55^{b}±50.00	870.37^{ab}±72.65	22.22^{c}±20.00	555.56^{ab}±76.38
UAM1051-1	166.67^{ab}±0.02	962.97^{ab}±88.19	103.69^{bc}±93.33	666.67^{ab}±86.60

Values are means of three replicates ± standard error. Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at P=0.05.

Correlation among infection and yield traits

Viral incidence has a very strong correlation with severity. Seed weight was also positively correlated with pod weight. There was a negative significant correlation between viral incidence and pod weight. However, aphid infestation was not significantly correlated with most of the traits studied (Table 7).

Table 7: Phenotypic correlation coefficients for the viral infection and yield traits in 2013 and 2014

Traits	Viral incidence	Viral severity	Pod	Seed	Aphid
Viral incidence		0.81**	-0.32*	-0.31ns	-0.16ns
Viral severity			-0.15ns	-0.15ns	-0.35ns
Pod				0.97**	0.35ns
Seed					0.39*
Aphid					1

*, **: significant at P= 0.05 and 0.01

Discussion

The significant genotypic effect from the combined analysis of variance suggests that there is a wide variation among the genotypes for resistance to infection by the two viruses and hence improvement could be made through breeding. Significant genotypic variation suggests possible improvement for a particular stress (Hallauer and Miranda 1988). The significant season by genotype interaction in some traits is an indication of environmental effect on expression of genes governing these traits. The highly significant effect of season corroborates with result of the aphids assessment which implies that aphid infestation varies with environmental conditions. Mohammed and Miko (2007) reported that variability of environmental factors has great influence on incidence and severity of crop diseases. The study revealed that all the fifteen cowpea genotypes had varying level of susceptibility to aphid infestation. This is an indication that none of the cowpea genotype was immune to *A. craccivora* infestation. The feeding habit of aphids can deprive plants of some of the nutrients required for pod and seed formation. Baidoo et al. (2012) reported that the activities of this pest rob the plant of essential food nutrients. Viral severity ranged from mild to severe symptoms among the genotypes. The severity could be attributed to the ability of aphids transmitting viruses in cowpea in a non-persistent manner. It has been reported that the viral infection of cowpea is transmissible through sap, seeds and insects like, *Mycus persicea*, *Meoythia quartena*, *Ootheca mutabilis*, *Paraluperodes quaternius*, *Aphis craccivora*, *Aphis gossypii*, and are readily transmissible in a non persistently manner (Taiwo 2003). Umaharan et al. (1997) also reported that viruses are transmitted by several insect species in a non persistent manner and therefore use of insecticides is not an effective method of control.

Incidence of aphid-borne viruses was higher in some genotypes especially in 2013. This could be due to the fact that the field used in 2013 was previously cropped with cowpea. Shoyinka et al. (1997) reported that CABMV had the highest incidence and was the most prevalent of the entire virus detected in a three year survey for the incidence and distribution of cowpea viruses. Amayo et al. (2012) and Orawu et al. (2005) also reported that CABMV and CMV were among the main viruses infecting cowpea in Uganda.

The reactions of cowpea genotypes to CABMV were very high in 2013 and 2014 irrespective of the experimental sites and the previous crops grown on the sites. This is because cowpea is the primary host of CABMV and its fast replication in this plant is not impossible. This confirms the report of Cisse et al. (2000) which stated that on cowpea field, the most frequent virus disease encountered is aphid-borne mosaic virus (CABMV). The reactions of genotypes to CMV were equally high in 2013 among the genotypes. This can be explained by the fact that CMV is the commonest virus which infects almost all plants and cowpea is not excluded. Edwardson and Christie (1991) have reported that CMV has the broadest host range of any known virus, infecting more than 1,000 species of plants, including monocots and dicots, herbaceous plants, shrubs, and trees. Six of the genotypes were susceptible to double infection by CABMV and CMV. This corroborates the work of Lima et al. (2005a) in which simultaneous infections of CABMV and CMV have been reported to frequently occur with high degree of incidence in Brazil, causing serious damages to crop productivity.

The yield in 2013 was low compared to the yield in 2014. This could be attributed to the fact that double virus infection was evident in all the genotypes during 2013. The research of Byoung-Cheorl et al. (2005) showed that viral diseases are among the most agriculturally important and biologically intriguing groups of plant pathogens, and they cause serious economic losses by reducing yields and quality of the crop. The reports of Pio‐Ribeiro et al. (2005) and Ghorbani et al. (2008) stated that the diseases caused by viruses have been responsible for great damage, causing serious losses in crop yield in several countries, including Brazil. Furthermore, it is obvious from the response of the genotypes that the experimental site used in 2013 had more inocula and volunteers than the site used in 2014. Maphosa et al. (2013) and Oloka et al. (2008) have reported that in order to identify host resistance, it is important to evaluate different genotypes under field conditions in different environments.

IT07K-318-33 and IT04K-227-4 are IITA lines used in this study with very good yield. Several improved cowpea varieties with resistance to Cowpea yellow mosaic virus and Cowpea aphid borne mosaic virus have been released for African growers by the International Institute of Tropical Agriculture (Asafo-Adjei et al. 2005, Mligo and Singh 2007, Toure´ and Singh 2005). IFE82-12, IT07K-293-2-1, IT07K-304-9, IT08K-149-3 are promising genotypes that can be used in cowpea virus resistance breeding programme. The development of resistant cultivars has been universally considered the most effective method to control diseases caused by viruses in cowpea, indicating that an increase in the number of virus‐resistant genotypes will generate more alternatives for breeders to produce resistant cultivars (Lima et al. 2005a and Assunção et al. 2005).

It was expected that some of the genotypes such as IAR06-1006 and LDPO-OBRI that were negative to CMV and with low CABMV concentration in 2014 would also have high yield but the reverse was the case. The report of Taiwo et al. (2007) revealed that in single virus infections, CABMV which induced the most severe symptoms had low absorbance values while Southern bean mosaic virus (SBMV) and Cowpea mottle virus (CMeV) which induced moderate symptoms had higher virus titres. This implies that CABMV infection in cowpea is a serious problem. The result of this study also showed that IFE82-12 that was susceptible to dual infection had a better yield in 2014. It could be that apart from the two viruses that were considered in this research, other viruses of importance in cowpea may be present which have serious effect on the two genotypes mentioned above. Amayo et al. (2012) and Orawu et al. (2012) have stated in their reports that breeding for disease resistance in cowpea is a complex problem because of the occurrence of multiple virus infections in a single field/plant. However, most of these viruses are transmitted by the same vector which offers an opportunity to utilize horizontal resistance to vector transmission in breeding programmes (Shoyinka et al. 1997).

The significant positive correlation between pod and grain yield is expected since seed yield is a function of number of pods. The negative correlation between viral incidence and pod yield implies that the higher the viral incidence, the lower the pod yield. This corroborates with previous researchers which reported negative correlation between disease traits and yield on various crops (Fokunang et al. 2000, AVRDC 2007). The non-significant correlation between aphid infestation and other parameters implies that the level of infestation of aphids on cowpea leaves *per se* does not translate to yield reduction but the extent of transmission of virus by the insect and the level of resistance by the cowpea genotypes. Sharma and Franzmann (2000) observed that variations in the susceptibilities and resistance among genotypes could be due to differences in their genetic make up.

In conclusion, of all the 15 cowpea genotypes evaluated for responses to single and double infections to aphid-borne viruses during 2013 and 2014 growing seasons at IAR&T, Ibadan, IT07K-318-33 was the best for grain yield. Although, IFE82-12 which is an IAR&T line recently released for its high yielding ability competed favourably with IT07K-318-33 in 2014 but not in 2013. Further research on IFE82-12

will prove its use in breeding programme for controlling viruses.

Aknowledgement

The authors thank the management of IAR&T for the financial support rendered for the actualization of this work. We also appreciate Late Prof. (Mrs) S.R. Akande for the experimental design and collection of IITA cowpea lines.

References

1. Akinjogunla OJ. Effects of single and mixed inoculation with viruses on symptomatology, growth, yield and nutritive content of cowpea: (*Vigna unquiculata*) M.Sc. Thesis. University of Lagos, Nigeria. 2005.
2. Amayo R, Arinaitwe AB, Mukasa S B, Tusiime G, Kyamanywa S, Rubaihayo P, Edema R. Prevalence of viruses infecting cowpea in Uganda and their molecular detection. African Journal of Biotechnology 2012; 11: 14132–14139.
3. Anderson EJ, Kline AS, Morelock TE, McNew RW. Tolerance to Blackeye cowpea mosaic potyvirus not correlated with decreased virus accumulation or protection from cowpea stunt disease. Plant Disease 1996;80:847–852.
4. Asafo-Adjei B, Singh BB, Atuahen-Amankwash G. Registration of 'Asontem' cowpea. Crop Science 2005;45:2649.
5. Assunção IP, M‑Filho LR, Resende LV, Barros MCS, Lima GSA, Coelho RSB, Lima JAA. Genes diferentes podem conferir resistência ao Cowpea severe mosaic virus em caupi. Fitopatologia Brasileira 2005;30:274–278.
6. AVRDC. 2007. Evaluation of chilli rootstocks for grafted sweet pepper production during the hot-wet and hot-dry seasons. In: *AVRDC Progress report*. Asian Vegetable Research and Development Centre, Publication Number 07–691. Shanhua, Taiwan. *2004;* pp. 70–72.
7. Baidoo PK, Baidoe-Ansah D, Agbonu I. Effects of neem (*Azadirachta indica* A. Juss) products on *Aphis craccivora* and its predator, *Harmonia axyridis* on cowpea. American Journal of Experimental Agriculture 2012; 2:198–206.
8. Boukar O, Kong L, Singh BB, Murdock L, Ohm HW. AFLP and AFLP-derived SCAR markers associated with *Striga gesnerioides* resistance in cowpea (*Vigna unguiculata* (L.) Walp.). Crop Science 2004; 44: 1259–1264.
9. Byoung-Cheorl K, Inhwa Y, Molly MJ. Genetics of plant virus resistance. Annual Review of Phytopathology 2005; 43: 581–621.
10. Cissé N, Thiaw S, Baldé M, Ndiaye M, Ndiaye A, Wade M. Breeding cowpea for the sahelian zone and resistance to striga and disease. Bean/Cowpea CRSP, Midcourse 2000 Research Meeting, April 9-14, 2000. Senegal. p. 9–10.
11. Damiri BV, Al-Shahwan IM, Al-Saleh MA, Abdalla OA, Amer MA. Identification and characterization of Cowpea aphid-borne mosaic virus isolates in Saudi Arabia. Plant Pathology 2013; 95: 79–85.
12. Edwardson JR, Christie RG. Cucumoviruses. CRC Handbook of viruses infecting legumes. CRC Press, Boca Raton, Fla. 1991; p. 293–319.
13. Fokunang CN, Ikotun T, Dixon AGO, Akem CN. Field reaction of cassava genotypes to anthracnose, bacterial blight, cassava mosaic disease and their effects on yield. African Crop Science Journal 2000;8: 179–186.
14. Ghorbani SGM, Shahraein N, Elaginia SA. Serodiagnosis of cowpea (*Vigna unguiculata*) viruses in Guilan province, Iran. Iranian Journal of Virology 2008;1:28–31.
15. Goenaga R, Gillaspie AG, Quiles A. Field performance of cowpea genotypes grown under virus pressure in Puerto Rico. African Crop Science Journal 2011;19: 97–103.
16. Gumedzoe MYD, Thottapily G, Asselin A. Occurrence of southern bean mosaic virus (SVMV) in Togo and its interaction with some cowpea cultivars. African Crop Science Journal 1997;5: 215–222.
17. Hallauer AR, Miranda JB. Eds. Heredity variance: Mating Design. Quantitative genetics in maize breeding. 2nd edition. USA: Iowa State Univ. Press, Ames. 1988; 45–114.
18. Kareem KT, Alamu OO, Arogundade O. Indigenous knowledge: Application of mulches in the control of okra mosaic virus. Nigerian Journal of Plant Protection 2012;26:173–182.
19. Langyintuo AS, Lowenberg-DeBoer J, Faye M, Lambert D, Ibro G, Moussa B, Kergna A, Kushwaha S, Musa S, Ntoukam G. Cowpea supply and demand in West and Central Africa. Field Crops Research 2003;82:215–231.
20. Lima JAA, Sittolin IM, Lima RCA. Diagnose e estratégias de controle de doenças ocasionadas por vírus. In: Freire‑Filho, F.R., Lima, J.A. de A., and Ribeiro, V.Q. (Eds.). Feijão caupi: avanços tecnológicos. Brasília: Embrapa Informação Tecnológica, 2005a; p.404–459.
21. Lima JAA, Nascimento AKQ, Silva GS, Camarço RFEA, Gonçalves MFB. *Crotalaria paulinea*, novo hospedeiro natural do vírus do mosaico severo do caupi. Fitopatologia Brasileira 2005b;30:429–433.
22. Maphosa M, Talwana H, Tukamuhabwa P. Assessment of comparative virulence and resistance in soyabean using field isolates of

soyabean rust. Journal of Agricultural Science 2013;5: 249–257.
23. Mligo JK, Singh BB. Registration of 'Vuli-1' cowpea. Crop Science 2007;47:437–438.
24. Mohammed IB, Miko S. Climatic variables under rain-fed and irrigated crop production: A Basis for yield variation. Journal of Science, Management and Technology 2007;3: 26–31.
25. Oloka HK, Tukamuhabwa P, Sengooba T, Asundram S. Reaction of exotic soybean germplasm to *Phakopsora pachyrhizi* in Uganda. Plant Disease 2008;92: 1493–1496.
26. Orawu M, Melis R, Demilliano W, Laing M, Adipala E. Occurrence and prevalence of cowpea virus diseases in Uganda. African Crop Science Proceedings 2005;7: 1279–1283.
27. Orawu M, Melis R, Laing M, Derera J. Genetic inheritance of resistance to cowpea aphid-borne mosaic virus in cowpea. Euphytica 2012;189: 191–201.
28. Palukaitis P, Roossinck MJ, Dietzgen RG, Francki RIB. Cucumber mosaic virus. Advance Virus Research 1992;41**:**281–348.
29. Pio‑Ribeiro G, Assis Filho MF, Andrade GP. Doenças do caupi. In: Kimati, H., Amorim, L., Rezende, J.A.M., Bergamin Filho, A., Camargo, L.E.A. (Eds.). Manual de fitopatologia: doenças das plantas cultivadas. 4. ed. São Paulo: Agronômica Ceres. 2005; p.215–216.
30. Price WC. Isolation and study of some yellow strains of cucumber mosaic virus. Phytopathology 1934;68: 1260–1265.
31. Robertson DG. Seed-borne viruses in cowpea in Nigeria. B. Sc. Thesis University of Oxford, U.K. 1966.
32. Sharma HC, Franzanmann BA. Biology of the legume pod borer, *Maruca vitrata* (Fabricius) and its damage to pigeon pea and Adzuki bean. Insect Science and its Application 2000;20: 99–108.
33. Shoyinka SA, Thottappilly G, Adebayo GG, Anno-Nyako FO. Survey on cowpea virus incidence and distribution in Nigeria. International Journal of Pest Management 1997;43:127–132.
34. Singh BB, Ehlers JD, Sharma B, Freire Filho FR. Recent progress in cowpea breeding. In Fatokun, C. A., Tarawali, S. A., Singh, B. B., Kormawa and P. M. Tamò, M. (Eds.), Challenges and opportunities for enhancing sustainable cowpea production. Proceedings of the World Cowpea Conference III held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 2002; pp. 21–40.
35. Singh BB, Ajeigbe HA, Tarawali SA, Ferna´ndez-Rivera S, Abubakar M. Improving the production and utilization of cowpea as food and fodder. Field Crops Research 2003;84:169–177.
36. Souleymane A, Aken'Ova ME, Fatokun CA, Alabi OY. Screening for resistance to cowpea aphid (*Aphis craccivora* koch) in wild and cultivated cowpea (*Vigna unguiculata* L. Walp.) Accessions. International Journal of Science, Environment and Technology 2013;2: 611–621
37. Taiwo MA. Viruses infecting legumes in Nigeria: case history. In: Plant Virology in Sub-Saharan Africa Edited by: Hughes J d'A, Odu, B. Proceedings of a conference organized by IITA, Ibadan, Nigeria. 2003; p. 93–115.
38. Taiwo MA, Akinjogunla OJ. Cowpea Viruses: Quantitative and qualitative effect of single and mixed viral infection. African Journal of Biotechnology 2006;5: 1179–1187.
39. Timko MP, Ehlers JD, Roberts PA. Cowpea. In Kole, C. (Ed.), *Genome Mapping and Moleclular Breeding in Plants.* Volume 3, Chapter 3, Pulses, Sugar and Tuber Crops. Berlin: Springer-Verlag. 2007.
40. Toure´ MA, Singh BB. Registration of 'Sangaraka' cowpea. Crop Science 2005;45:2648.
41. Umaharan P, Ariyanayagam RP, Haque SQ. Resistance to cowpea severe mosaic virus determined by three dosage dependent genes in *Vigna unquiculata* L. Walp. Euphytica 1997;95: 49–55.

12/25/2016