GENETIC VARIATION FOR RESISTANCE TO YELLOW VEIN MOSAIC VIRUS AND NUCLEIC ACID HYBRIDIZATION TEST IN KENAF (*HIBISCUS CANNABINUS* L.)

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ABSTRACT

Kenaf is an important economic crop cultivated for the production of bast fibers which are used for industrial purposes. In spite of the uses of kenaf, its production is hindered by biotic stresses including viruses of which yellow vein mosaic virus (YVMV) is the most devastating. The effect of genotype and location on the incidence and severity of yellow vein mosaic disease (YVMD) in kenaf was determined and the presence of the virus in kenaf genotypes was detected with nucleic acid spot hybridization (NASH) test. Fifteen genotypes of kenaf were screened for tolerance to YVMV in three agro-ecological zones (Ibadan, Ikenne and Ilora) in Nigeria under rain-fed conditions in 2015 and 2016. Plot size of 2 m × 2 m with a spacing of 0.2 m x 0.5 m was used and the experiment was laid down in a randomized complete block design (RCBD) with three replicates. The NASH test revealed the presence of YVMV in kenaf plants. Significant ($p < 0.01$) variation among the genotype for low incidence and severity to YVMD was indicated. The environments affected the genotypes and influenced differential response of kenaf to YVMD across the locations. Ibadan (forest-savanna) was indicated as the most discriminating and representative environment to develop YVMD-resistant genotype for the ecology. Low incidence of YVMV was observed for HIB14, HIB23, Ex-shika 242, Tianung1, Tianung2, V1-100, V1-400 and IfekenD1-400 at Ibadan and Ikenne; AU-71 at Ilora2016 and HIB43, HIB31, HIB24 at Ilora2015. Genetic similarity was indicated for Ex-shika242, AU-71 and V1-400. Tianung1, Ex-Shika242 and V1-400 were recommended for release as resistant genotypes for the ecologies.

Keyword: Begomoviruses, diversity, fiber crop, incidence, Malvaceae

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a fiber crop belonging to the family Malvaceae; and it is one of the three largest fiber crops of economic importance (Keshk *et al*., 2006) along with cotton and jute (Singh, 2017). It is grown commercially in over 20 countries including India, China, Thailand and Vietnam (FAO, 2003). In Nigeria, kenaf is cultivated in more than twenty states including the Federal Capital Territory (Akubueze *et al*., 2014). It is used in making rope, cordage, canvas, sacks, carpet backing, fish nets and as a non-woody source of paper pulp (Paul *et al*., 2009). The bast and core fibers are used in building materials, adsorbents, textiles and automobile (Webber *et al*., 2002).

Despite its importance as an agricultural and industrial crop, its production continues to be limited by biotic stresses. Among the many diseases affecting kenaf, *Yellow vein mosaic virus* (YVMV) is the main biotic limiting factor (Chatterjee *et al*., 2005, 2008; Kareem *et al*., 2017). The virus belongs to the genus *Begomovirus*, family Geminiviridae. Begomoviruses are non-enveloped viruses, with nucleocapsid that is 38 nm long and 15 – 22 nm in diameter (Prajapat*et al*., 2010). The virus is transmitted by whitefly (*Bemisia tabaci*) (Chatterjee *et al*., 2008; Das *et al*., 2008) and symptoms induced include veinal chlorosis and leaf yellowing (Chatterjee *et al*., 2005; Ghosh *et al*., 2007). *Begomoviruses* are economically important plant viruses that cause significant yield losses to crops in tropical and subtropical regions (Brown and Bird, 1992; Ghosh *et al*., 2007). Therefore, control of this virus is important in order to increase kenaf production in Nigeria.

Identification of disease-resistant genetic materials is the most effective and environment-friendly disease control measure (Egesi *et al.,* 2009; Oloyede-Kamiyo *et al.,* 2018). Success in developing disease tolerant and resistant crop species depends on the amount of genetic variation for this trait in the germplasm. The higher the genetic diversity for the desirable traits the better chance to select potential parents with disease resistance for crop improvement. Therefore, diverse germplasm sources are needed in order to identify disease resistant genotypes in crop species. Field evaluation and identification of promising parental lines do not involve expensive technology, though the evaluation is usually influenced by environmental factor. Hence, complementing phenotypic diagnosis of viral diseases with other techniques will be more informative than phenotype diagnosis alone. Several molecular techniques have been employed to detect YVMV in kenaf, which include southern blot analysis (Chatterjee *et al*., 2005), nucleic acid hybridization test (Kareem *et al*., 2017) and polymerase chain reaction (Chatterjee and Ghosh, 2007). Nucleic acid hybridization test is sensitive and specific to detect YVMV (Harper and Creamer, 1995; Venkataravanappa *et al*., 2013). The technique allows rapid preparation of plant samples for hybridization-based detection and is commonly used for the detection of whitefly-transmitted geminiviruses (Brown and Bird, 1992). Geminviruses pose a threat to fiber crop in tropical and subtropical countries (Khan, 2000; Boulton, 2003). Due to dearth of information on kenaf viruses in Nigeria, this study was conducted to determine the influence of genotype and location on the incidence of YVMV disease, identify virus-resistant genotypes for improvement of kenaf and detect YVMV in kenaf using nucleic acid spot hybridization test.

MATERIALS AND METHODS

Genetic materials

Fifteen genotypes of kenaf collected from the Germplasm Units of Institute of Agricultural Research and Training (I.A.R.&T), Obafemi Awolowo University, Ibadan and National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, Nigeria were used in the study (Table 1).

Experimental layout

The genetic materials were evaluated for yellow vein mosaic disease at Ibadan

(Latitude 7˚38′N; Longitude 3˚84′E), Ikenne (Latitude 6˚54′N; Longitude 3˚42′E) and Ilora (Latitude 7˚81′N; Longitude 3˚82′E) in southwest Nigeria during the early growing seasons of 2015 and 2016. The average minimum and maximum temperatures, average precipitation and average humidity for the period of the experiment are presented in Table 2. The experiment was conducted using a randomized complete block design with three replicates. Plot size was 2 m × 2 m with four rows. Intra- and inter- row spacing was 0.2 m and 0.5 m, respectively. Three seeds were sown per hill and thinned to two seedlings per hill two weeks after planting to give a total of 80 plants per plot. Cultural practices carried out include application of NPK 15:15:15 fertilizer at 4 weeks after planting and application of insecticide (Laraforce) 8 and 10 weeks after planting. Plots were weeded twice as at when due.

Table 1. Description of kenaf genotypes used for the study

Code	Genotype	Type of genotype	Source†
G1	60-282	Local	I.A.R.&T
G2	AC-313	Local	I.A.R.&T
G3	AU-71	Local	I.A.R.&T
G4	Ex-shika242	Local	I.A.R.&T
G5	Ifeken100	Hybrid	I.A.R.&T
G6	IfekenDI-400	Hybrid	I.A.R.&T
G7	Tianung1	Local	I.A.R.&T
G8	Tianung2	Local	I.A.R.&T
G9	V1-100	Local	I.A.R.&T
G10	V1-400	Local	I.A.R.&T
G11	HIB14	Exotic	NACGRAB
G12	HIB23	Exotic	NACGRAB
G13	HIB24	Exotic	NACGRAB
G14	HIB31	Exotic	NACGRAB
G15	HIB43	Exotic	NACGRAB

†IAR&T = Institute of Agriculture, Research and Training, Ibadan, Nigeria; and NACGRAB = National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria.

Table 2. Location and climatic condition during the experiment

	Ibadan	Ikenne	Ilora
Ecology	Forest-savanna	Rain forest	Derived savanna
Latitude of experimental site	7˚38′N	6˚54′N	7˚81′N
Longitude of experimental site	3˚84′E	3˚42′E	3˚82′E
Altitude (m.a.s.l) †	182	73	278
2015			
Max.temp (ºC)	29.25	31.50	28.75
Min. temp (ºC)	22.25	20.50	22.50
Precipitation (mm)	155.00	159.50	198.25
Humidity (%)	82.00	75.75	79.28
2016			
Max.temp (ºC)	29.50	31.00	31.00
Min. temp (ºC)	22.75	21.00	22.25
Precipitation (mm)	159.50	163.75	196.75
Humidity (%)	81.00	77.25	83.25

†m.a.s.l = meters above sea level.

Incidence and severity of *Yellow vein mosaic virus*

Incidence of YVMV disease was assessed by counting the number of symptomatic plants and expressed as percentage of the total number of plants per plot. Virus severity was scored on a scale of 1 to 5, where 1 = symptom absent on leaves; 2 = 1 to 25% of leaves showing mild symptoms of chlorosis and mosaic; 3 = 26 to 50% of leaves showing moderate symptoms including leaf deformation and leaf wrinkling; 4 = 51 to 75% of leaves showing severe symptoms; and 5 = > 75% of leaves showing very severe symptoms (Qamar *et al*., 2015, with modifications).

Nucleic acid spot hybridization test

Leaf samples were collected from the kenaf genotypes and labeled appropriately. Genomic DNA was extracted from the samples using AG1Lysis Buffer (Agdia Inc., Elkhart, Indiana, USA) following the manufacturer's instructions but with some modifications (Kareem *et al*., 2017). *Yellow vein mosaic virus* infection was detected in the extracted DNA using nucleic acid spot hybridization (NASH) assay (Podleckis *et al*., 1993). A nylon membrane was loaded with 3.0 µl of the extracted DNA and was left to dry at room temp (25 ± 1°C). The DNA in the membrane was probed with labeled complementary sequences of Begomovirus in Agdia's laboratory (Elkhart, Indiana, USA). Samples were considered positive if visual spots were obtained on the membrane; otherwise, negative.

Data analysis

Analyses of variance were performed on plot basis for YVMV incidence and severity across three locations and two years using Statistical Analysis System version 9.1.1 (SAS, 2002). Means were separated via Duncan's multiple range test at $p < 0.05$. Genotype × environment interaction was partitioned with Genotype + G × E (GGE) biplot analysis using Plant Breeding Tools (PBTool, 2013) to determine the most discriminating and representative of the locations for the evaluation of tolerance among the kenaf genotypes, and assessed the incidence of YVMD across the locations. Single linkage cluster (SLCA) analysis was used to determine genetic diversity among the kenaf for tolerance to YVMV disease.

RESULTS

The result of ANOVA on the individual and interactive effects of year, location and genotype on virus incidence and severity revealed that mean squares for genotype (G) were significant ($p < 0.01$) for YVMV incidence and severity, location (L) was only significant for virus incidence while year was significant for virus severity. In addition, mean squares attributable to genotype × year (G × Y) interaction and G × Y × L were significant for both virus incidence and severity (Table 3).

Six environments were considered for the G × E analysis - Ibadan 2015 (E1), Ibadan 2016 (E2), Ikenne 2015 (E3), Ikenne 2016 (E4), Ilora 2015 (E5) and Ilora 2016 (E6). Biplot of the principal component axis 1 (PC1) and PC2 accounted for 81.0% of the total variation observed among the kenaf genotypes for YVMD incidence (Fig. 1).Projecting lines from the origin divided the biplot into sectors and revealed which genotypes had low incidence of YVMV in a specific environment. The environments

were separated into three mega environments. Low incidence of YVMD was revealed for HIB14 (G11), Ex-shika242 (G4), Tianung-1 (G7), Tianung-2 (G8), AC-313 (G2), V1-100 (G9), V1-400 (G10), IfekenD1-400 (G6), HIB23 (G6) at Ibadan 2015 (E1), Ibadan 2016(E2) and Ikenne 2015 (E3) and Ikenne 2016(E4); AU-71 (G3) at Ilora 2016 (E6); and HIB43 (G15), HIB31 (G14), HIB24 (G13) at Ilora 2015(E5).

Table 3. Mean squares of YVMV incidence and severity evaluated in kenaf across three locations in 2015 and 2016

Source of variation	df	Incidence	Severity
Block (location/year)	12	28.54^{ns}	0.23*
Year (Y)	1	15.79 ^{ns}	1.20**
Location (L)	2	1342.82**	0.23
Y x L	2	235.62*	0.90**
Genotype (G)	14	987.45**	3.44**
G×Y	14	948.06**	1.77**
G×L	28	651.20**	2.51**
G × Y × L	28	447.24**	2.41**
Error	168	58.00	0.11

** significant at p < 0.01, * significant at p < 0.05, ns = not significant

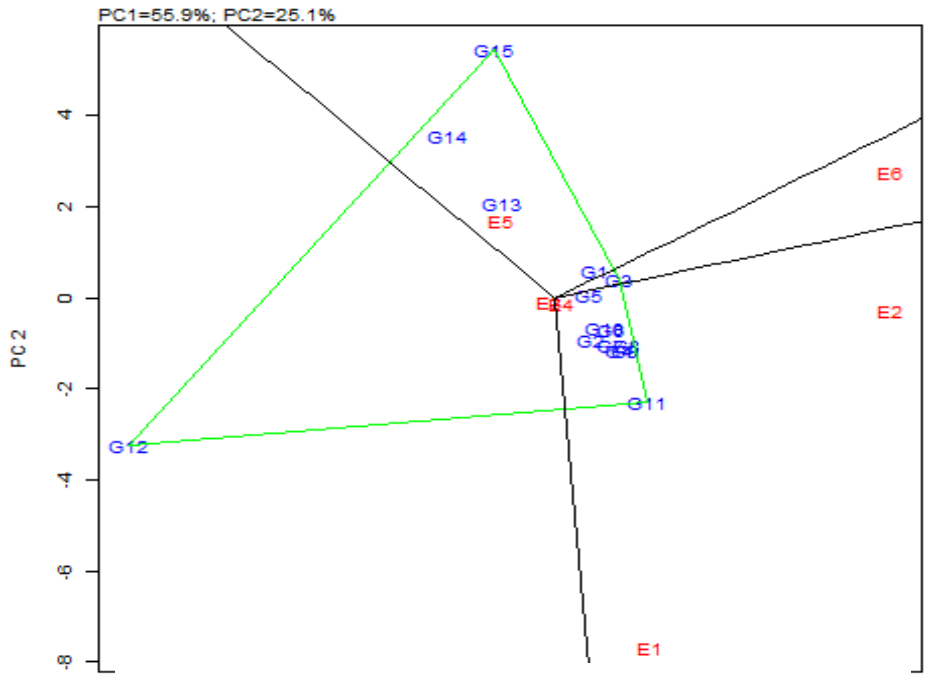

Figure 1. Specific response of kenaf genotypes to incidence of yellow vein mosaic virus across six environments

HIB23(G12) was not among the genotypes with lowest incidence for the disease in any of the environments. Discriminating ability of the environment was based on the length of the environment vector (Fig. 2) and representativeness on the size of the vector angle with average-environment axis (AEA). Ibadan 2015 (E1) then Ibadan 2016 (E2) and Ilora 2016 (E6) had long vectors of which Ibadan 2016 (E2) had the smallest angle with the AEA.

Mean YVMD incidence in six environments was approximated by the projections of their markers on the AEA(Fig. 3a). The double-arrow-headed lines separated the genotypes to below-average (left) and above-average YVMD incidence (right). Stability for high or low incidence of YVMD was measured by the genotype projection on the double-arrow headed line. Except HIB23 (G12), HIB24 (G14), HIB43 (G15) and HIB24 (G13), other genotypes had above average for low incidence to YVMD across the environments. Response of the genotypes over the environment was presented in Fig. 3bfor clarity on the stability of the genotypes for low incidence to YVMD. Consistently, Tianung1 (G7), Ex-Shika242 (G4), V1-400 (G10), Tianung2 (G8) and AU-71 (G3) had low incidence of YVMD in the six environments.

Severity of YVMD at Ibadan in 2016 and average over the six environments (Table 4) revealed high severity score for HIB23 and it was rated *susceptible* to YVMV. AU-71, Ex-Shika242,Tianung2, V1-100, HIB14 and HIB24 had low severity of YVMV and were rated *highly resistant* genotypes. *Yellow vein mosaic virus* was confirmed with the NASH test. Black spots on the membrane indicate the presence of *Begomovirus* DNAon the leaves (Fig. 4). Occurrence of YVMD was observed in the 15 kenaf genotypes in all the locations (Table 5) except for Ex-shika 242, Tianung2, V1-100 and HIB14 in Ibadan, AU-71 in Ilora, and HIB24 in Ikenne.

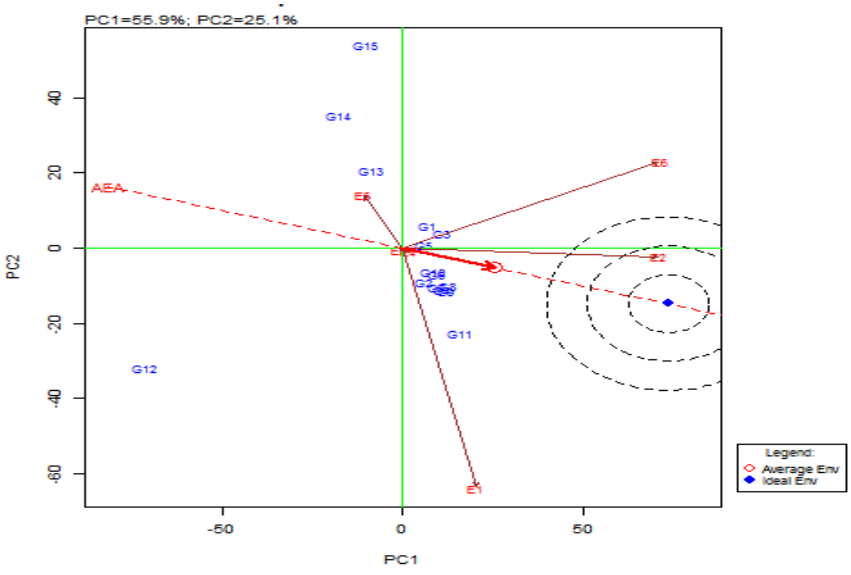

Figure 2. Representativeness and discriminating ability of six environments to evaluate incidence of yellow vein mosaic

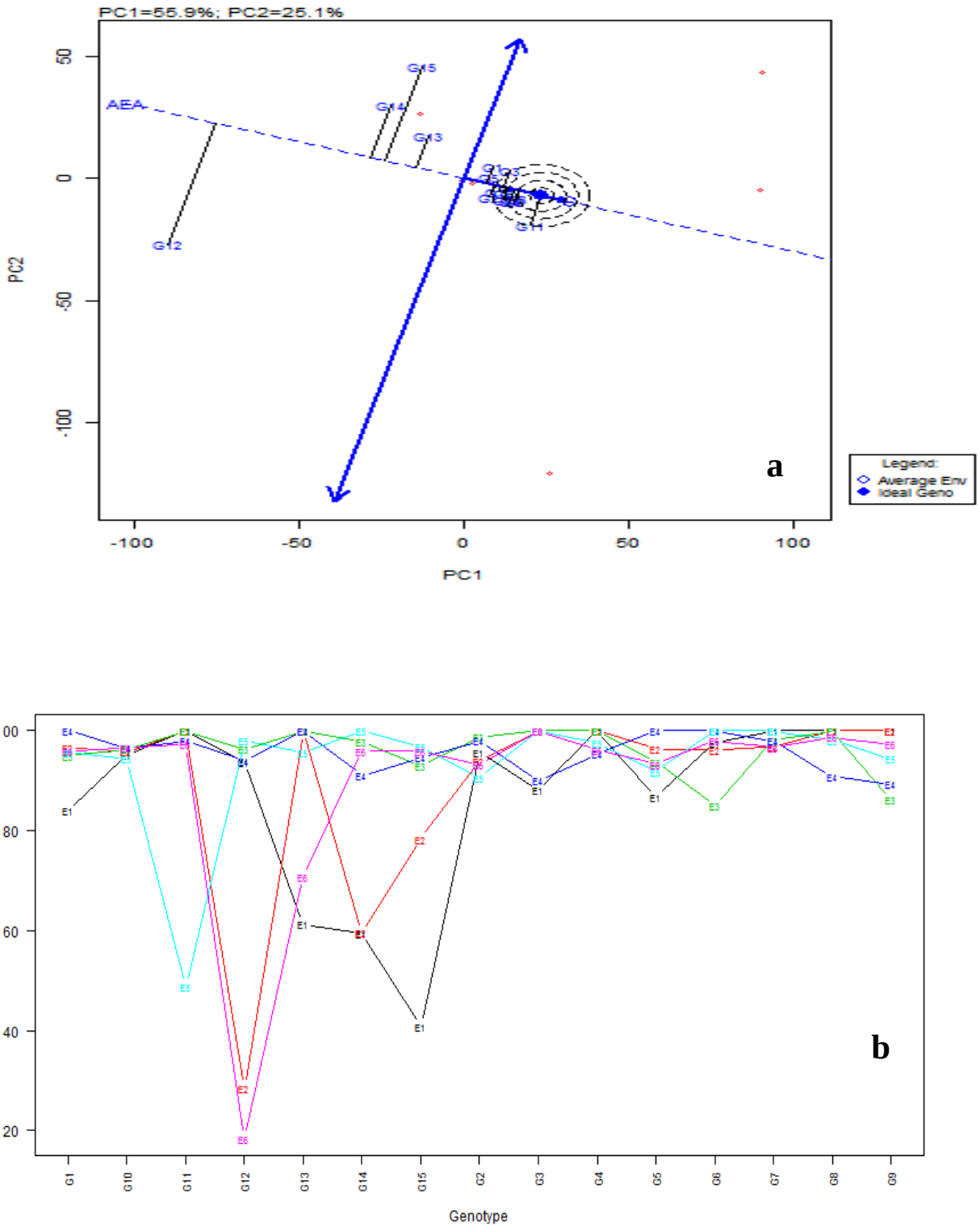

Figure 3. Stability (a) and response (b) of 15 kenaf genotypes to incidence of yellow vein mosaic disease in six

Table 4. Severity of *Yellow vein mosaic virus* (YVMV) in 15 varieties of kenaf

Genotype	y^a†	y^b†	Rating‡
60-282 (G1)	2.00c	2.25cde	MR
AC-313 (G2)	3.00ab	2.50abc	MS
AU-71 (G3)	1.00d	1.67gh	HR
Ex-shika 242 (G4)	1.00d	1.50h	HR
HIB14 (G5)	1.00d	1.75fgh	HR
HIB23 (G6)	3.50a	2.92a	S
HIB24 (G7)	1.00d	1.75fgh	HR
HIB31 (G8)	3.00ab	2.17cdef	MS
HIB43 (G9)	2.00c	2.58abc	MR
Ifeken100 (G10)	2.50bc	2.75ab	MS
IfekenDI 400 (G11)	2.00c	2.00defg	MR
Tianung1 (G12)	2.00c	1.83efgh	MR
Tianung2 (G13)	1.00d	1.75fgh	HR
V1-100 (G14)	1.00d	1.83efgh	HR
V1-400 (G15)	2.50bc	2.42bcd	MS

†Severity of YVMV at Ibadan in 2016 (y^a) and across the locations (y^b).
‡Highly resistant (HR), moderately resistant (MR), moderately susceptible (MS), susceptible (S).
§Means with the same letter along the column are not significantly ($p < 0.05$) different with Duncan's multiple range test.

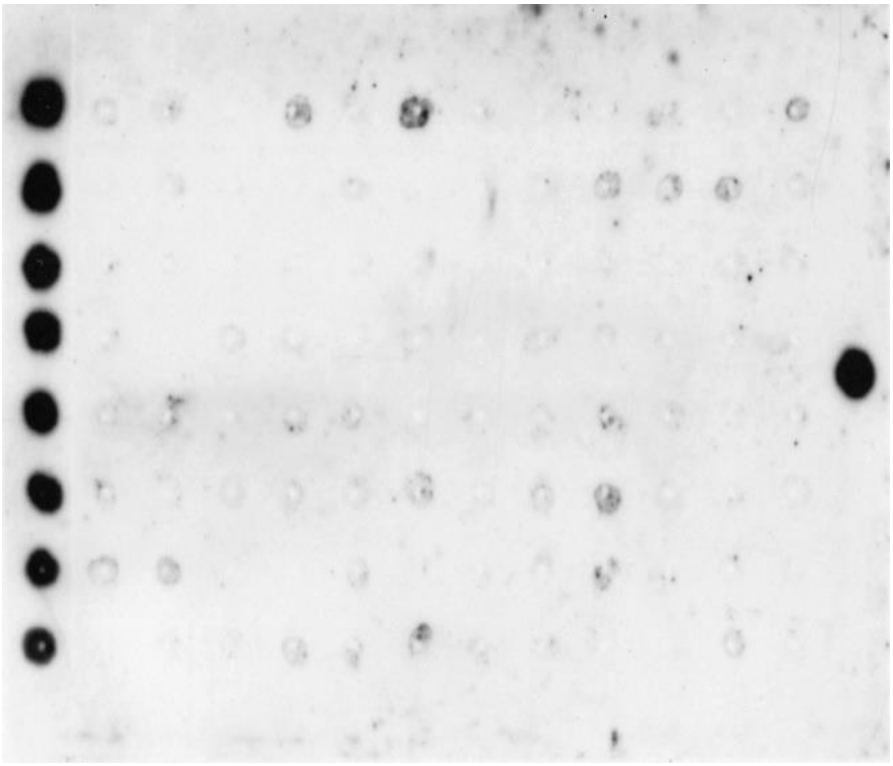

Figure 4. Spots of the presence of YVMV on hybridized nylon membrane

Table 5. Presence of yellow vein mosaic disease in kenaf genotypes at the locations during 2015 and 2016

Genotype	Ilora		Ikenne		Ibadan	
	2015	2016	2015	2016	2015	2016
Ex-shika 242	+	+	-	+	-	-
V1 100	+†	+	+	+	-	-
AU-71	-‡	-	-	+	+	-
Tianung-2	+	+	-	+	-	-
60-282	+	+	+	-	+	+
AC-313	+	+	+	+	+	+
V1 400	+	+	+	+	+	+
Tianung- 1	-	+	+	+	-	+
Ifeken DI 400	-	+	+	-	+	+
Ifeken 100	+	+	+	-	+	+
HIB 23	+	+	+	+	+	+
HIB 24	+	+	-	-	+	-
HIB 14	+	+	-	+	-	-
HIB 43	+	+	+	+	+	+
HIB 31	-	+	+	+	+	+

†+ = Presence of yellow vein mosaic virus (YVMV)
‡- = Absence of yellow vein mosaic virus (YVMV).

Data on incidence and severity of YVMV across the six environments were used to construct a dendrogram and reveal genetic diversity among the kenaf genotypes (Fig. 5). Similarity among the kenaf genotypes with low incidence and severity to YVMV were revealed. Tianung1 was grouped with Tianung2. Ex-shika242 was closely related to AU-71 and V1-400.

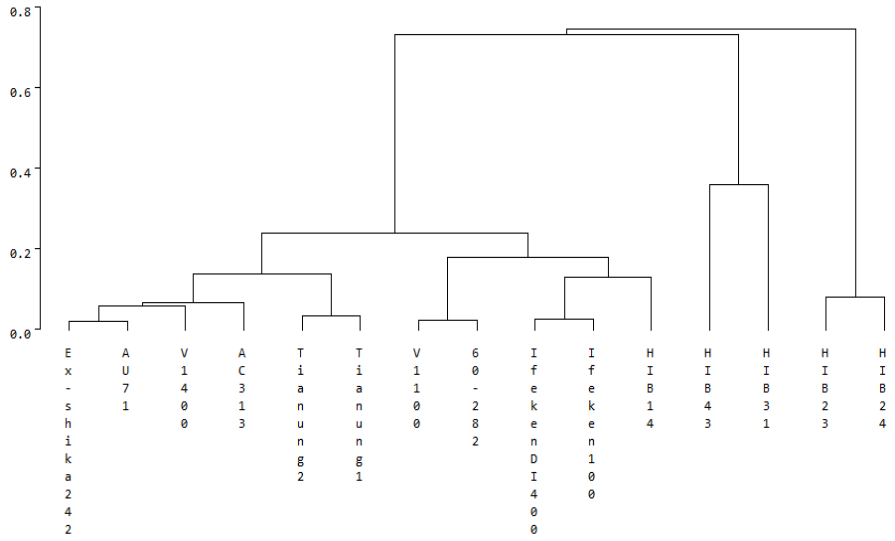

Figure. 5. Genetic diversity among 15 kenaf genotypes based on the mean incidence and severity of *Yellow vein mosaic virus* in six environments

DISCUSSION

Several studies have revealed that Geminviruses are significant plant pathogens of crop species in Africa, Australia, Southeast Asia, Latin America and the Caribbean, Southern Europe and southwestern United States, and that their infection results in reduced yield annually (Rojas *et al*., 2000; Morales and Anderson, 2001; Varma and Malathi, 2003).The presence of Begomovirus on kenaf in Southwest, Nigeria informs the need to develop strategies to control the resultant effect of the virus on the crop yield. Research efforts to identify and develop kenaf genotypes that are resistant to the disease are important.

Genetic variation observed among the kenaf genotypes in the study indicated the potential to select for superior genotypes with low incidence and severity to YVMD. Significant variation of the environment also affected the occurrence of the disease in the genotypes. Gerling *et al*. (1986) reported that disease incidence induced by geminivirus could depend on environment and other predisposing factors. The influence of the environment was revealed by differential symptoms of YVMD in the kenaf across the location. This created background to identify and develop resistant genotypes which are adapted to specific location and ecotype. Egesi *et al.* (2009) reported that genotypes do not respond in a similar manner to virus diseases.

The environmental effect may be attributed to different ecological zones of the locations of the experiment. Furthermore, environment with optimum effect on genetic diversity for desirable characters is important. This will help plant breeders to make better use of available and limited resources for cultivar improvement programme (Isik and Kleinschmit, 2005; Mohammadi *et al.*, 2008). Although, there was slight difference in the average temperature, humidity and precipitation among the locations, forest savanna (Ibadan) was the most discriminating and representative of the test environment. The agro-climatic condition of the zone should be considered to develop resistant kenaf for the ecology.

The positive result obtained from NASH test corroborates the work of other researchers which confirmed the presence of *Yellow vein mosaic virus* in kenaf (Chatterjee and Ghosh, 2007; Venkataravanappa *et al*., 2013; Kareem *et al*., 2017).

In conclusion, genotype and location as well as their interactions had significant effect on the response of kenaf to YVMD. Ex-shika 242, Tianung2, V1-100 and HIB14 with low incidence and severity to YVMD at Ibadan and AU-71 and HIB24 at Ilora can be exploited for specific adaptation. However, Tianung1, Ex-Shika242 and V1-400can be recommended for release as resistant genotypes for Southwest ecologies. These genotypes can further be recombined for selection of superior recombinants against strains of the virus.

Competing interests

We hereby declare that there was no competing interest among authors.

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