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Screening of *Abelmoschus esculentus* and *Abelmoschus callei* cultivars for resistance against okra leaf curl and okra mosaic viral diseases, under field conditions in South Eastern Nigeria

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Of all the several factors that limit okra production in West Africa, okra mosaic virus disease (OMVD) and okra leaf curl disease (OLCD) are rank among the most severe. Twenty three (23) *Abelmoschus esculentus* and *Abelmoschus callei* cultivars retrieved from the local farmers were screened in the open field for the resistance against OMVD and OLCD over two cropping seasons in Nsukka, South Eastern Nigeria. The degree of susceptibility was determined by the calculation of yield decline (YD) due to changes in the fruit yield with exposure to the disease causing agents as well as virtual scoring of the exposed plants. The results show that all the *A. esculentus* cultivars were susceptible to the two diseases. The findings indicate that contrary to general belief, not all *A. callei* cultivars are resistant to these viral diseases. Any cultivar with a YD score of less than 10% was identified as a potential candidate for the disease resistant genes. Only Ebi Ogwu, Ojo ogwu, Tongolo, VLO, Oru ufie and Ogolo met this criterion. The potentials of transferring these resistant genes from *A. callei* cultivars to the vulnerable *A. esculentus* cultivars are discussed.

Key words: Okra, screening, resistance, viral diseases.

INTRODUCTION

In West Africa, two distinct species of okra, *Abelmoschus esculentus* (L.) Moench, which is the conventional or early okra and *Abelmoschus caillei* (A. Chev) Stevels, late okra (Siemonsma and Hamon, 2004) are grown. Late okra is also named West African okra, since its distribution is restricted to the West African region (Martin et al., 1981; Siemonsma, 1982; Hamon and Hamon 1991). These okra types respond differently to changes in natural photoperiod and this has been one of the major means of distinction between them. Early okra has a Critical Day Length (CDL) of 12.5 h whereas late okra has a CDL of 12.25 h (Njoku, 1958; Oyolu, 1977; Nwoke, 1980; Siemonsma, 1982; Udengwu, 1998). World production of okra (both species) as fresh fruit-vegetable

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is estimated at 6 million t/year. Okra production in West and Central Africa is estimated at 500,000 to 600,000 t annually based on available consumption data. West African okra makes up half of this amount, which is about 5% of total world production of okra (Siemonsma and Hamon, 2004). Of the several factors that limit okra production in West Africa, Okra Mosaic Virus Disease (OMVD) and Okra Leaf Curl Disease (OLCD) diseases rank among the most severe. They are caused by a complex of monopartite begomoviruses and a promiscuous betasatellite with an associated parasitic DNA1 satellite in Mali (Kon et al., 2009). They can occur singularly or in combination in plants and when they occur in combination in okra plants their effects are often devastating (Kon et al., 2009). Givord and Hirth (1973) identified the okra mosaic virus (OMV) and noted that it occurs naturally in the field. Its symptoms on okra include chlorosis, mosaic and vein banding. Okra mosaic virus (OMV), transmitted by flea beetles (*Podagrica*), is widespread but damage is much less important than that caused by leaf curl, transmitted by whitefly (*Bemisia tabaci)*. These viruses must be controlled through control of the vectors (Siemonsma and Hamon, 2004).

Venkataravanappa et al. (2011) isolated from okra exhibiting typical begomovirus symptoms (leaf curl and yellow vein) four groups of beta satellite sequences- Okra Leaf Curl Beta Satellite (OLCuB), Bhendi yellow vein beta satellite (BYVB); Bhendi yellow vein India beta satellite (BYVIB) and Croton yellow vein mosaic beta satellite (CroYVMB). Their detailed analysis of the sequences showed that OLCuB, BYVB and BYVIB share highest identity with respect to βC1 gene. βC1 being the only gene encoded by beta satellites, the product of which is the major pathogenicity determinant of begomovirus-beta satellite complexes and is involved in overcoming host defenses based on RNAi.

In West Africa, climate influences (i) virus disease outbreaks, (ii) the rate of development and activity of virus vectors, and also of their migration, and (iii) the phenology of crops, weeds and wild hosts that habour plant viruses (Atiri et al., 2000). These viral diseases have been implicated in declined okra production in many okra growing regions of the world. The disease YVMV transmitted by the whitefly (*Bemisia tabaci*) is reported to be one of the most destructive plant diseases in India causing great loss by affecting quality and yield of fruits, as high as 93.80% depending on age of plant at the time of infection (Sastry and Singh, 1974). Additionally it has been stated that the unavailability of quality seed and heavy incidence of biotic stresses particularly yellow vein mosaic virus (YVMV) are the most important reasons for low yield of okra in India (Chattopadhyay et al., 2011). Prabu et al. (2007) reported that the incidence of Yellow Vein Mosiac Virus (YVMV) in wild and cultivated lines of okra was observed more in summer as compared to kharif season at hot spots in western Maharashtra. Rainfall, temperature and wind are identified as key weather components in virus pathosystems involving maize (cereal), okra (vegetable) and cassava (tuber crop), and are therefore important in determining the most suitable period in which to undertake crop protection measures (Atiri et al., 2000). According to Siemonsma and Hamon (2004), West African okra which is known to be resistant to yellow vein mosaic virus (YVMV), a major cause of crop failure of common okra in Asia, whose vector is the whitefly (*B. tabaci*), has already been introduced into several American and Asian countries for research purposes through germplasm exchange.

In their screening studies for four seasons to identify the source of resistance to okra yellow vein mosaic virus in Maharashtra, Prabu et al. (2007) reported that the wild species *Abelmoschus angulosus* was found completely free from yellow vein mosaic virus symptoms. The wild *Abelmoschus tetraphyllus* lines, *Abelmoschus moschatus* lines (1, 2, 3, 4 and 5), *A. caillei*-2 and *Abelmoschus manihot* spp. tetraphyllus were found highly resistant while the wild lines *A. tetraphyllus*-5, *A. manihot* (L.) Medikus and *A. manihot* spp. *manihot* were found to be resistant whereas *Abelmoschus tuberculatus* lines (1, 2 and 3), *Abelmoschus ficulneus* lines (1 and 2) and *A. caillei* -1 were found moderately susceptible to YVMV. Among the cultivated *A. esculentus* types, none was found resistant to YVMV. Despite the fact that several workers had conducted serious research works on how to reduce the incidence of the disease; through the use of insecticides (Sastry and Singh, 1973; 1974; Chakraborty and Mukhopadhyay, 1977; Khan and Mukhopadhyay, 1985; Ramachandran and Summanwar, 1986; Bhagat et al., 1997; Raghuraman et al., 2007; Aktar et al., 2009; Gupta et al., 2009), screening for resistant cultivars (Arora et al., 1992; Sharma et al., 1993; Singh and Gupta, 1991; Bora et al., 1992; Nath and Saikia, 1992; Rashid et al., 2002); inducing mutation for resistance to yellow vein mosaic virus disease of okra (Dalve et al., 2012); screening of plant extracts for antiviral properties against okra vein mosaic virus infection of okra (Pun et al., 1999) and management of yellow vein mosaic and leaf curl disease by manipulation of agronomic practices (Singh et al., 1989; Amoatey and Acquah, 2010; Kalita and Dhawan, 2010), the incidence of the disease is estimated to still be on the increase in important okra growing regions of the world. The threat posed by the diseases to global okra production calls for coordinated action to identify elite resistant cultivars that can facilitate production of durable resistant cultivars. There is urgent need to screen available okra land races for resistance to these two severe okra viral diseases with a view to identifying cultivars, which are highly immune to the two viral diseases. Such a move may not only minimize, but can even eliminate, the use of chemical insecticides for the control of the vectors; with the concomitant impact of boosting okra production in the region. This present study reports on field screening of 23 selected landraces of

Table 1. Details of the scale used.

Virtual infestation classification	Score	Virtual Infestation Classification	Score
Very highly immune	9	Mildly susceptible	5
Highly immune	8	Susceptible	3-4
Immune	7	Highly Susceptible	2
Slightly immune	6	Very Highly Susceptible	1
		Hyper-susceptible	0
Immune - very highly immune	6-9	Hyper-susceptible - Mildly Susceptible	0-5

$$\text{Yield decline (YD)} = \frac{\text{Mean weight of protected plants} - \text{mean weight of un-protected plants}}{\text{Mean weight of un-protected plants}} \times 100$$

both early and late okra types, for the identification of cultivars with the viral resistant genes. These will be used in the improvement of promising but susceptible cultivars, for the prevention of further erosion of okra germplasm and thus ensure maintenance of a broad genetic base for the crop.

MATERIALS AND METHODS

Fifteen (15) *A. esculentus* cultivars: Ogbu Oge, Nnobi fat, Obimo girdle, Osukwu, Mpi ele, Ogba mkpe, Iloka, Awgu early, Nwa obala, Lady finger, Kpum zua, Hawzua, Kano dwarf, Odiche Nnobi long and eight late okra cultivars: Ojo ogwu, Tongolo, AOAU, Oru ufie, Ebi ogwu, Ogolo, VLO and Ala nwagboho were used in screening studies. The preliminary field work was carried out in the Botanic garden, University of Nigeria, Nsukka in the late nineties while the substantive work was carried out in the Department of Crop Science experimental fields, University of Nigeria, Nsukka, in 2008 and 2009 following standard agricultural practices. The viral diseases were confirmed by the Plant Pathology and Virology unit of our department. The 23 cultivars were grown in four replications using Randomized Complete Block Design (RBCD). Two sets of plantings were done in different locations, 50 m apart, to ensure the absence of interactions between the two locations based on treatment. In one location, the plants were protected through weekly spraying with Vetox, foliar insecticide to control the *Bemisia* flies and other insects that might be involved in the transmission of the causative viral organisms. The second location apart from being unprotected (no spraying) had three replicated rows of Iloka, the *A. esculentus* cultivar that had chronically shown susceptibility to both okra viruses, over several generations, in each block. Stands of the cultivar were also as used as boarder crops, all aimed at ensuring adequate availability of both the vectors and the viral organisms in each of the blocks. All the plantings were done on flat beds measuring 6.5 x 6.0 m for the protected plots and 7.5 x 7.0 m for the un-protected plants. Well cured poultry manure was worked into the beds at the rate of 20.5 kg per plot, one week before planting. The 23 cultivars were planted in randomized rows for the four blocks using the table of random numbers. Plants were spaced 30 x 30 cm. Three pre-germinated seeds of each cultivar were planted inside holes 2.5 cm deep. The plants were later thinned down to one per stand, nine days after the emergence of the first two opposite juvenile leaves. There were 20 experimental plants per row giving rise to 460 plants per block. There were 1840 experimental plants per set of planting. Data were not collected from the additional replicated stands of the chronically infested cultivar as well as the border crops that served as rich sources of both the viruses and the *Bemisia* flies.

The plants were rain-fed throughout the period of the studies. Harvesting of fresh marketable fruits from each of the locations was carried out every five days. Upon termination of the experiment the data on yield per plant was subjected to ANOVA and the mean yield per cultivar for each of the two groups of planting were compiled and used to determine the yield decline (YD) as shown in the formula presented above.

Based on the calculated YD the cultivars were categorized using the following YD scale scores and symbols; i) immune scale (%): 000 = very highly immune: 0-5; 00 = highly immune: above 5-10; 0 = slightly immune above: 10-30; 2) susceptibility scale: + = Susceptible: above 30-50; ++ = highly susceptible: above 50-60; +++ = very highly susceptible: above 60-70; ++++ = hyper-susceptible: above 80.

Scoring based on virtual evaluation for resistance was carried out at the transitional stage between the vegetative and reproductive phases of growth, which was approximately 30 days after seed germination (ASG) for early okra and 90 days after seed germination (ASG) for late okra, since they have different critical day length and consequently different times for transition from vegetative to reproductive phase (Udengwu, 1998). Scores were recorded for both Okra Leaf Curl (OLC) and Okra Mosaic (OM) viruses using a scale of 0-9. The most susceptible scored 0 while the most immune scored 9. Details of the scale are shown in Table 1.

RESULTS

Table 2 shows that cultivars Nos. 1-8 (*A. callei* cultivars) had a yield decline (YD) range of 4.70% (Ebi Ogwu) to 28.57% (AOAU) for the first year of planting. The second planting YD ranged from 4.34% (Ebi Ogwu) to 29.40% (AOAU). On the basis of the YD assessment the categorization of the *A. callei* cutivars for both diseases are shown in Table 3. Ebi Ogwu whose YD is within the 0-5%, is designated as Very Highly Immune (VHI), 000. Ogolo, Oru Ufie, Ojo Ogwu, Tongolo and VLO with YD range of 5-10% are classified as Highly Immune (HI), 00; Alanwanghoho and AOAU with YD range of 10-30% are

Table 2. Mean yield (t/h) of 23 cultivars of early and late okra, exposed to *Bemisia* white flies (unprotected) and protected (*Bemisia* flies controlled), under field conditions for 2 years.

Cultivar	Yield (t h^{-1}) 1st year of planting			Yield(t h^{-1}) 2nd year of planting		
	Protected plants (PP)	Non protected plants (NPP)	Yield decline (%)	Protected plants (pp)	Non protected plants (npp)	Yield decline (%)
Ogolo (L)	3.60±0.21	3.40±0.12	5.88	0.72±0.08	0.66±0.11	6.06
Ebi Ogwu (L)	3.09±0.13	2.95±0.22	4.74	0.48±0.07	0.46±0.07	4.34
Oru Ufie (L)	2.20±.09	2.08±0.32	5.76	0.82±0.14	0.76±0.09	7.89
Ojo Ogwu (L)	2.48±0.17	2.30±0.42	7.82	0.79±0.19	0.73±0.12	8.21
Tongolo (L)	3.00±0.16	2.75±0.21	9.09	0.55±0.06	0.51±0.17	7.84
VLO(L)	3.10±0.19	2.87±0.31	8.01	0.92±0.05	0.85±0.02	8.23
Alanwa. (L)	2.00±0.11	1.60±0.13	25.00	0.52±0.01	0.42±0.14	23.81
AOAU (L)	3.60±0.13	2.80±0.35	28.57	1.10±0.24	0.85±0.14	29.40
Nnobi long (E)	5.87±0.08	3.20±0.18	83.40	2.00±0.07	1.13±0.21	76.99
Lady finger (E)	2.53±0.07	1.42±0.27	78.16	1.80±0.06	1.04±0.11	73.07
Ogba mkpe (E)	3.00±0.26	2.01±0.39	49.25	1.20±0.11	0.75±0.13	60.00
Awgu Early (E)	5.03±0.35	2.88±0.25	74.65	2.20±0.32	1.30±0.04	69.23
Iloka (E)	3.02±0.25	1.36±0.15	122.05	1.82±0.12	0.86±0.08	112.79
Ogbu oge (E)	2.26±0.08	1.50±0.07	50.66	1.10±0.08	0.72±0.21	52.77
Odiche (E)	2.20±0.08	1.30±0.21	69.23	0.90±0.14	0.56±0.09	60.71
Kano Dwarf (E)	3.00±0.18	1.92±0.18	56.25	1.30±0.14	0.85±0.07	52.94
Hawzua (E)	2.00±0.06	1.10±0.17	81.81	1.00±0.07	0.56±0.05	78.54
Obimo Girdle (E)	5.87±0.25	3.50±0.05	67.71	2.40±0.18	1.34±0.10	79.10
Nnobi fat (E)	2.53±0.09	1.50±0.11	68.66	1.92±0.21	1.20±0.17	60.00
Osukwu (E)	3.00±0.14	1.80±0.18	66.66	1.04±0.13	0.58±0.12	79.30
Mpi Ele (E)	5.03±0.28	3.10±0.24	62.25	2.10±0.24	1.25±0.08	68.00
Nwa Obala (E)	3.00±0.35	1.70±0.12	76.47	1.30±0.08	0.76±0.17	71.05
Kpum Zua (E)	2.26±0.41	1.40±0.07	61.42	1.20±0.14	0.76±0.12	57.89
$LSD_{(0.05)}$	0.74	0.28		0.20	0.01	

classified as slightly immune (SI), 0. For the *A. esculentus* cultivars, Nos. 9-23, the YD ranged from 49.25% (Ogba mkpe) to 122% (Iloka), for the first year of planting and second year of planting 52.77% (Ogbu Oge) to 112.79% (Iloka) for the second year of planting. Their categorization was as follows: Ogba mkpe and Awgu Early (first year for OLC disease) with a YD range of 30-50% was categorized as susceptible (SB), +; Lady finger, Awgu Early (first year OM disease and second year), Ogbu Oge, Odiche, Kano Dwarf, Nnobi fat, Osukwu, Mpi Ele and Kpum Zua are grouped as Highly susceptible (HS), ++. Nnobi long. Hawzua and Nwa obala with YD range of 60-70% were grouped as very highly susceptible (VHS), +++. Lastly, Iloka with YD above 80% was classified as Hyper Susceptible (HPS), +++. The responses of the cultivars for the two years were also scored based on virtual inspection of the incidence of both diseases, Table 4. This was carried out using a virtual scale range of 0-9. Scores between 0 to 4 were grouped as hyper susceptible - susceptible, those that scored from 5 - 9 were categorized as Immune - very highly immune. For cultivars nos. 1-8 representing the *A. callei* cultivars, Ebi Ogwu with a score of 9 is designated as Very Highly Immune (VHI). Alanwangboho, AOAU and Ojo Ogwu with score range of 5 - 6 are designated as Slightly Immune. The rest with score range of 7-8 are designated as Highly Immune (HI).

For the *A. esculentus* cultivars, Nos. 9-23, essentially for the two years and for the two diseases, Iloka with a virtual score of 0 is designated as Hyper-susceptible (HPS). Lady finger with a score of 1 is classified as Very Highly Susceptible (VHS). Nnobi long, Hawzua and Nwa obala, with a score of 3 are classified as Susceptible (SC). The other nine cultivars with a score of 2, are classified as highly susceptible (HSP). The yield of the 23 okra cultivars for the two years studies, for both the protected and the un-protected plants, were subjected to analysis of variance. The ANOVA table is shown in Table 5. The results indicate that the variance ratios were very highly significant for both the unprotected and the protected plants for the two years. Figure 1 shows Alanwangboho, an *A. callei* cultivar with curled leaves and mosaic pattern (CLMP) as well as aborted fruits (ABF) due to the OLC and OM diseases. The plants were still able to bear fruits (FF) despite the attack though there were evidences of abortion of fruits (ABF). An *A.*

Table 3. Summary of responses of 23 okra cultivars to exposure to okra leaf curl and okra mosaic viral diseases under field conditions, for two cropping seasons.

Cultivar		First cropping season		Second cropping season	
	Okra type	*Okra leaf curl*	*Okra mosaic*	*Okra leaf curl*	*Okra mosaic*
Ogolo	Late	00	00	00	00
Ebi ogwu	,,	000	000	000	000
Oru ufie	,,	00	00	00	00
Ojo ogwu	,,	00	00	00	00
Tongolo	,,	00	00	00	00
VLO	,,	00	00	00	00
Ala nwagboho	,,	0	0	0	0
AOAU	,,	0	0	0	0
Nnobi Long	Early	+++	+++	+++	+++
Lady finger	”	++	++	++	+++
Ogba mkpe	”	+	+	+	+
Awgu early	”	+	++	++	++
Iloka	”	++++	++++	++++	++++
Ogbu Oge	”	++	++	++	++
Odiche	”	++	++	++	++
Kano dwarf	”	++	++	++	++
Hawzua	”	+++	+++	+++	+++
Obimo girdle	”	++	++	++	++
Nnobi fat	”	++	++	++	++
Osukwu	”	++	++	++	++
Mpi ele	”	++	++	++	++
Nwa obala	”	+++	+++	+++	+++
Kpum zua	”	++	++	++	++

Score	Yield decline (%)	Score	Yield decline (%)
000 =	Very Highly Immune:	0-5	+ = Susceptible: Above 30-50
00 =	Highly Immune:	Above 5-10	++ = Highly Susceptible: Above 50-60
0 =	Slightly Immune:	Above 10-30	+++ = Very Highly Susceptible: Above 60-70
			++++ = Hyper-susceptible: Above 80

Key to Table 2 legend

Classification	Leaf curl disease	Leaf mosaic disease
Very highly immune	Very few veins affected, leaf shape still intact	Faint localized yellow and green dots on leaf surface
Highly immune	Many veins affected, leaf shape still intact	Faint yellow and green dots covering about one quarter of the leaf surface.
Slightly immune	Mid-rib affected with distortion of leaves	Yellow and green dots covering more than half of the leaf surface
Susceptible	Mid-rib and veins affected, leaves highly distorted, apical bud affected	Almost all the surface of the leaves is covered but no chlorosis.
Highly susceptible	Curling of leaves observed, apical bud severely affected. Intense curling and distortion of leaves, apical buds, severe abortion of flower buds, drastic reduction of leaf size, defoliation.	Entire leaf surface covered with yellow and green dots, slight chlorosis observed Entire leaf surface covered with yellowish green patches, leaf chlorosis, reduction of leaf size, defoliation
Very highly susceptible Hyper susceptible	Most intense curling and distortion of leaves, apical buds. Very severe abortion of flower buds. Distortion of shape of fruits	Very severe dis-colouration of leaves and fruits, fruits creamy in colour and shaped like e-plant fruits. Severe abortion of fruits

callei cultivar, AOAU, also showing CLMP with many healthy looking flower buds (HFB) but virtually no fruits (AFB) is shown in Figure 2.

A hyper susceptible *A. esculentus* cultivar, Iloka is shown in Figure 3. The curled and wrinkled leaves with mosaic pattern (LCMP); the destroyed apical bud (DAB), the discoloured and drastically reduced leaves (DDLR); the complete abortion of all flower buds (AFB) coupled with premature leaf abscission (PLA) are shown as devastating effects of the two diseases. The normal healthy *A. callei* cultivar is shown with the cultivar, VLO in Figure 4a while Kpum Zua (Figure 4b) showed a typical

Table 4. Summary of Virtual inspection of scores of 23 okra cultivars to exposure to okra leaf curl and okra mosaic viral diseases under field conditions, for 2 cropping seasons.

Cultivar	Okra type	1st Cropping season		2nd Cropping season	
		Okra leaf curl	*Okra mosaic*	*Okra leaf curl*	*Okra mosaic*
Ogolo	Late	7	7	7	7
Ebi ogwu	,,	9	9	9	9
Oru ufie	,,	8	7	7	8
Ojo ogwu	,,	6	6	7	6
Tongolo	,,	7	8	8	7
VLO	,,	7	7	6	7
Ala nwagboho	,,	5	5	5	5
AOAU	,,	5	5	5	5
Nnobi Long	Early	3	3	3	3
Lady finger	”	1	1	2	1
Ogba mkpe	”	4	4	4	4
Awgu early	”	4	2	2	2
Iloka	”	0	0	0	0
Ogbu Oge	”	2	2	2	2
Odiche	”	2	2	2	2
Kano dwarf	”	2	2	2	2
Hawzua	”	3	3	3	3
Obimo girdle	”	2	2	2	2
Nnobi fat	”	2	2	2	2
Osukwu	”	2	2	2	2
Mpi ele	”	2	2	2	2
Nwa obala	”	3	3	3	3
Kpum zua	”	2	2	2	2

Virtual inspection legend
0-5 Hyper-susceptible - mildly susceptible
6-9 Immune - very highly immune

Virtual infestation classification	Score
Very Highly Immune	9
Highly Immune	8
Immune	7
Slightly Immune	6
Mildly susceptible	5
Susceptible	3-4
Highly Susceptible	2
Very Highly Susceptible	1
Hyper-susceptible	0

example of a normal healthy *A. esculentus* cultivar.

DISCUSSION

In the field both viral diseases were observed to occur together on vulnerable cultivars. According to Kon et al. (2009), they can occur singly or in combination in plants and when they occur in combination in okra plants their effects are often devastating. This is the prevailing situation among vulnerable *A. esculentus* cultivars in the region.

From the results out of the eight late okra cultivars screened, only Ebi Ogwu with less than 5% YD was categorized as VHI. AOAU was more susceptible than Alanwangboho. The important point to note here is that not all the screened *A. callei* cultivars were immune to these viral diseases, contrary to the belief held by some scholars working outside the region. Consequently, screening of any *A. callei* germplasm for vulnerability to these diseases is imperative before declaring it as a resistant cultivar.

However, the degree of vulnerability of the vulnerable *A. callei* cultivars, Alanwangboho and AOAU, was nowhere near to the devastating situation found among the *A. esculentus* cultivars, especially Iloka. The acquisition of resistance to these viral diseases by *A. callei* whose distribution is restricted to the West African region (Singh and Bhatnagar, 1976) and which is believed to have evolved from the region (Harlan, 1971), might not be unconnected with their larger number of chromosomes (2n=194) as against the smaller chromosome number of (2n=130) for *A. esculentus* cultivars (Singh and Bhatnager, 1976). *A. callei* which is

Table 5. Analysis of variance of yield of 23 cultivars of early and late okra, exposed to *Bemisia* white flies (unprotected) and protected (*Bemisia* flies controlled), under field conditions for 2 years.

Item	Sum of squares	Degree of freedom	Mean square	Variance ratio
ANOVA of protected plants for first year of planting				
Total	96.95	68		
Block	0.50	2	0.25	1.25 NS
Variety	87.62	22	3.98	19.91 ***
Error	8.83	44	0.20	
ANOVA of un- protected plants for first year of planting				
Total	39.40	68		
Block	0.03	2	0.013	0.44 NS
Variety	38.08	22	1.73	59.65 ***
Error	1.29	44	0.03	
ANOVA of protected plants for second year of planting				
Total	22.53	68		
Block	0.06	2	0.03	2.0 NS
Variety	21.79	22	0.99	66.0 ***
Error	0.68	44	0.02	
ANOVA of un-protected plants for second year of planting				
Total	5.35	68		
Block	0.02	2	0.01	1.00 NS
Variety	4.88	22	0.22	22.0 ***
Error	0.44	44	0.01	

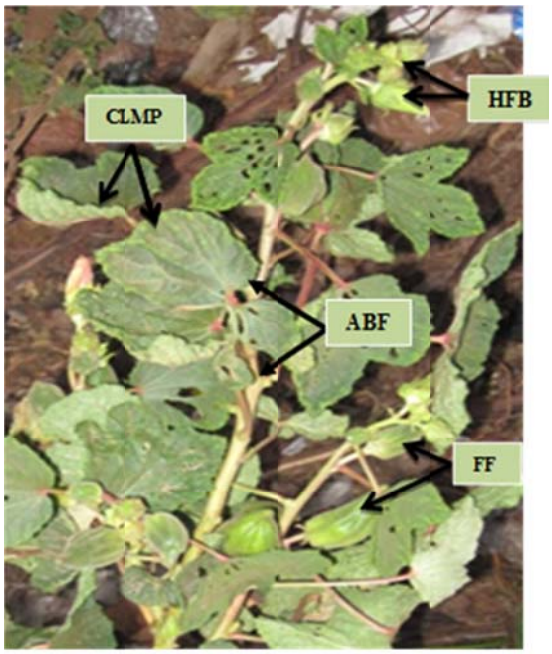

Figure 1. Late Okra Alanwagboho showing moderate attack of Leaf Curl and mosaic virus. HFB, Healthy looking flower buds; FF, Fresh fruits; CLMP, Curled leaves with mosaic pattern; ABF, Aborted fruits.

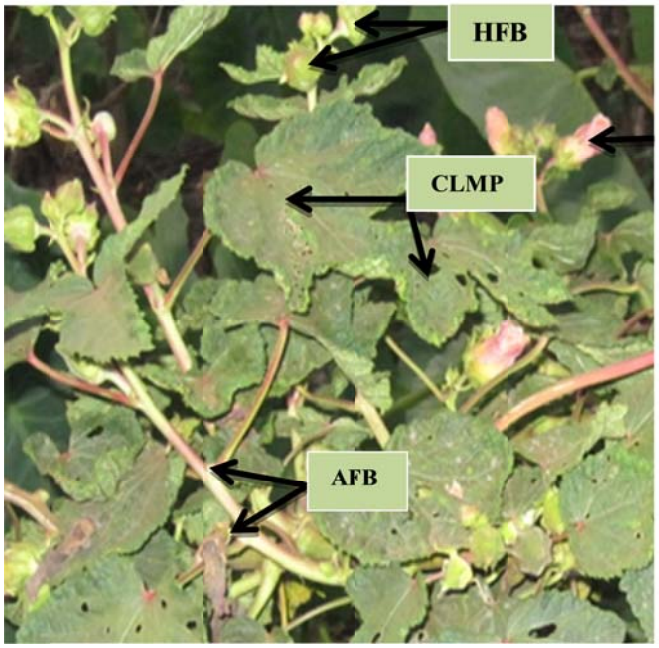

Figure 2. Late Okra AOAU showing more serious moderate attack of Leaf curl and mosaic virus with abortion of flower buds. CLMP, Curled leaves with mosaic pattern; Heal HFB, Healthy looking flower buds; ABF, Aborted fruits.

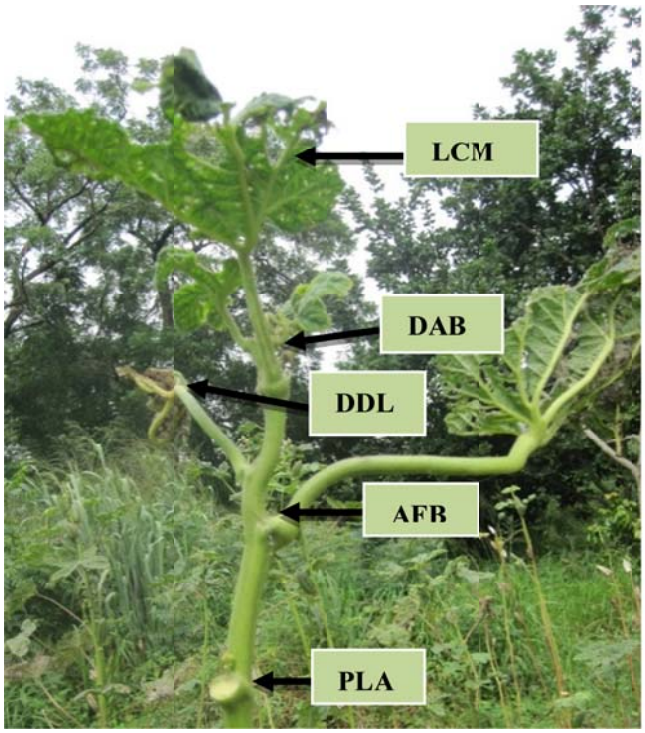

Figure 3. Chronic infestations of Iloka (Early Okra cultivar) by Okra Leaf curl and mosaic viral disease. LCMP, Leaf showing curling and mosaic pattern; DAB, destruction of apical bud; DDLR, discolouration and drastic leaf reduction; AFB, Abortion of flower buds; PLA, premature leaf abscission.

indigenous to West Africa is suspected to have conserved some gene constellations during the course of evolution which possibly conferred on them resistance to these diseases among many others. The acquisition of these disease resistant genes are thought to have occurred early in the evolution of *A. callei* in the equatorial jungles of West Africa, where this edible species can still be found growing wild (an ability which *A. esculentus* lacks) among weeds and other wild plants.

Martin and Ruberte (1978) stated that the exact origin of okra is not known but the presence of wild varieties in Ethiopia, in the region of the Upper Nile river, and the presence of primitive perennial varieties in West Africa, suest an African origin. The possession of this exceptional wild resistant ability by *A. callei* is similar to the report of Prabu et al. (2007), who noted that the five wild *Abelmoschus* species they screened showed strong resistance to Okra Yellow Vein Mosaic Virus in India. Further studies will make it possible for these resistant genes to be identified and sequenced for easy transfer to the more disease prone *A. esculentus* cultivars as well as other vulnerable plants. The existence of several wild species of *Abelmoschus* that possess genes resistant to these viruses as reported by Prabu et al. (2007) obviously broadens the source of resistant genes for the improvement of *A. esculentus*. On the other hand, the occurrence of some *A. callei* cultivars that showed varied degrees of susceptibility to these viral diseases is viewed as a recent event probably occasioned by over domestication which might have failed to provide the natural wild environment needed to stimulate the synthesis of the pertinent mRNA(s) from the genome of the plant, for the production of the necessary protein(s), needed for the conferment of resistance. This strongly argues in support of conserving the rainforests and their resources which are repositories of numerous wild genes that can have positive impact on the problem of food security in Sub-Saharan Africa. In the words of Atiri et al. (2000), the intricate interrelationships among cropping seasons, intercrop periods and virus incidence in West Africa must be thoroughly understood in order to develop ecologically based and sustainable management practices.

The vulnerability of the *A. esculentus* cultivars may not be unconnected with their lower chromosome number of 2n=130 (Singh and Bhatnager, 1976). It could be that *A. esculentus* genome lack these disease resistant genes that *A. callei* had evolved that provides protection against these viral diseases as well as other diseases. This view is supported by the observation that *A. esculentus* cultivars do not survive in the wild. They easily succumb to even weeds when not fully attended to under cultivation, talk less thriving in the wild like the robust *A. callei* cultivars. Worthy of mention is the chronic manifestation of these diseases by Iloka, an *A. esculentus* cultivar. The situation was so serious that planting of this cultivar was discontinued because of its hyper degree of manifestation of the two diseases in the field. The cultivar was seen as a repository of the viral causing organisms, since the disease always manifested on this cultivar for up to four generations. It is suspected that the viruses had become seed borne in this cultivar from where viral reserves are supplied from generation to generation. The cultivar however served the useful purpose of serving as a rich and ready source of the viruses throughout the studies. Among the other *A. esculentus* cultivars screened, the degree of susceptibility varied with Ogba mkpe showing the least vulnerability. This however does not qualify the cultivar to serve as a source of genes for conferring resistance to the diseases on other early okra cultivars. More extensive screenings involving more early okra cultivars is required for the possible identification of better resistant early okra cultivar. In their own report Prabu et al. (2007), noted that among the cultivated *A. esculentus* types they screened, none was found resistant to YVMV.

This present study was carried out during the traditional planting season of April to September which is known to be more humid than the other months of the year. The season of growth is known to be favourable to the multiplication of the *Bemisia* vector of the two diseases as well as to insects generally. By extension the season could therefore be said to favour the viruses. Reduction

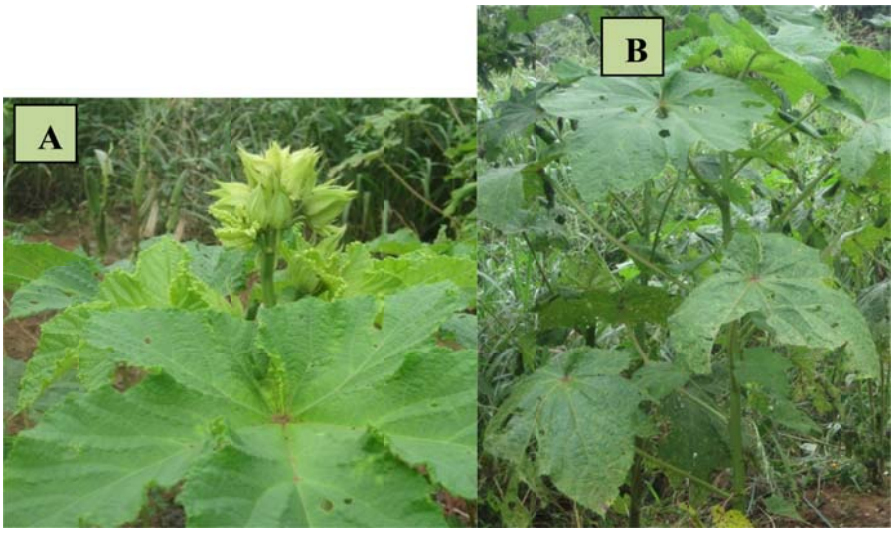

Figure 4. A. Normal healthy late okra (VLO) with a crown of inflorescence**. B.** Normal healthy early okra plants (Kpum Zua).

of the incidence of the two diseases could be based on the cultural practice of growing *A. esculentus* cultivars essentially during the hotter non-growing season, under irrigation. There is however the need to conduct a study to compare the response of the cultivars to the incidence of these diseases during the growing and non-growing seasons, to confirm this assertion. Martin and Ruberte (1978) reported that okra is a warm-season crop. It prospers in the hot, dry tropics and in the hot, humid tropics, but it is out of place and unproductive in cool highlands. High temperatures are necessary for seed germination and growth. Prabu et al. (2007) noted that the incidence of yellow vein mosiac virus (YVMV) in wild and cultivated lines of okra was observed more in summer as compared to kharif season at hot spots in western Maharashtra.

In their own report, Atiri et al. (2000) noted that rainfall, temperature and wind are key weather components in virus pathosystems involving maize (cereal), okra (vegetable) and cassava (tuber crop), and are therefore important in determining the most suitable period in which to undertake crop protection measures. Furthermore, they noted that in West Africa, climate influences (i) virus disease outbreaks, (ii) the rate of development and activity of virus vectors and also of their migration, and (iii) the phenology of crops, weeds and wild hosts that habour plant viruses. These factors need to be taken into consideration in efforts geared towards the control of the incidence of the diseases in the region.

Meanwhile, any cultivar with a YD score of less than 10% is a possible candidate for the disease resistant genes. Consequently, only the following late okra cultivars have been earmarked as late okra cultivars with promising genes that can resist these viral diseases; Ebi Ogwu, Ojo ogwu, Tongolo, VLO, Oru ufie, and Ogolo. More local land races when collected from the local farmers will still be screened to further identify more resistant cultivars. Identification of the resistant cultivars is just one part of the solution. Transferring the disease resistant genes from one *A. esculentus* resistant cultivar (if identified), to a vulnerable one may not provide a lasting solution. Nerkar (1999) reported that a Pusa Sawani cultivar tolerant to Okra Yellow Vein Mosaic disease was developed in India using a strain from I.C. 1542, which stabilized okra cultivation in the country in the 1970s.

However, later Pusa Sawani became severely affected by Okra Yellow Vein Mosaic virus. The reason for this is not quite clear. Transferring of these genes from the more rugged *A. callei* to *A. esculentus* invariably becomes a research imperative which incidentally is almost impossible, adopting the conventional breeding protocols, because of the well-known existence of barrier to gene flow between these two edible *Abelmoschus* species (Fatokun et al., 1979; Martin et al., 1981; Siemonsma, 1982; Hamon and Hamon, 1991; Siemonsma and Hamon, 2004).

This calls for utilization of modern biotechnological gene transfer techniques for rapid molecular breeding for cultivars resistant to these viral diseases. These viral diseases of okra constitute forms of abiotic stress. In the words of Nicot et al. (2005); plants exposed to virulent and avirulent pathogens respond with a range of defense and damage-limiting mechanisms. Many of the responses are also induced by other stress-causing agents, which reflect the common elements in different stress situations (Collinge and Boller, 2001). It is an established fact that numerous studies on the defense and stress mechanisms in plants have been based on gene expression (Kirch et al., 1997; Collinge and Boller, 2001; Bezier et al., 2002; Dean et al., 2002). Transcriptome studies could be helpful in providing a better under-

standing of plant stress responses due to these viral diseases and through these studies, numerous novel stress-responsive genes could be discovered (Nicot et al., 2005).

Through the use of cDNA, the genes responsible for resistance to these viral diseases can be isolated from *A. callei* and used for rapid transfer to vulnerable *A. esculentus* cultivars using *Agrobacterium* mediated, or other gene transfer protocols. This could provide fast and effective means of trouble-shooting the menace of the two diseases in the region.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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