SURVEY OF THE SYMPTOMS AND VIRUSES ASSOCIATED WITH COWPEA (*VIGNA UNGUICULATA* (L).) IN THE AGROECOLOGICAL ZONES OF KWARA STATE, NIGERIA

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http://dx.doi.org/10.4314/ejesm.v5i4.S22

Received 24th September 2012; accepted 29th October 2012

Abstract

A comprehensive field survey was carried out in 2011 spanning thirty locations within the forest and savannah agroecologies of Kwara State, Nigeria. This was with the objective of having an overview of the prevalence of virus disease symptoms and to specifically identify the viruses infecting cowpea (Vigna unguiculata .L.) in Kwara State. Data were collected on the prevalence of some peculiar virus symptoms on cowpea plants on growing on farms in each location. Symptomatic cowpea leaves were also collected from each location for virus identification in the laboratory. The serological protocol employed for virus detection in the leaf samples was the antigen – coated plate Enzyme linked immunosorbent assay (ACP–ELISA) method. The results of the field survey indicated that the symptoms observed on the cowpea plants were peculiar to those associated with virus disease infection. The symptoms observed were leaf mottling (36.8%), mosaic (24.9%), leaf curl (15.6%), necrotic spots (8.2%) and other symptoms (14.5%). The result of the laboratory assay to detect the viruses present in the leaf samples revealed the presence of four (4) different viruses. The viruses identified were, Cowpea Aphid Borne Mosaic Virus (CABMV), Cowpea Yellow Mosaic Virus (CYMV), Blackeye Cowpea Mosaic Virus (BICMV) and Cowpea Mottle Virus (CPM$_o$V). These viruses were detected infecting the cowpea plants in mixtures of two or three. These results are indicative of the presence of viruses in all the ecological zones of Kwara State where cowpea is cultivated.

Key words: *Survey, Agroecology, Cowpea, Virus, Symptoms, ACP-ELISA.*

Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is an herbaceous short term, annual leguminous plant which is grown in many tropical and subtropical countries (Singh and Sharma, 1996). The crop is well adapted to stress and has excellent nutritional qualities (El- Ameen, 2008). It is a very important food source in developing countries where animal protein is limited (Tenebe *et al.,* 1995) thereby, supplementing the low protein menus due to high cost of animal source of protein (Ojeinelukwe, 2002; Fawole *et al*., 2006., Miko and Mohammed, 2007).

The African continent produces an estimated 8 million tonnes of grain legume seed (70% of total world production) from 17.7 million hectares of land (IITA, 2007). Nigeria is the world largest cowpea producer where about 2.1 million tonnes are produced per annum (IITA, 2001). Cowpea diseases induced by species of pathogens belonging to various pathogenic groups (fungi, bacteria, viruses, nematodes, and parasitic flowering plants) constitute one of the most important constraints to profitable cowpea production in all agro ecological zones where the crop is cultivated (Hampton *et al.* 1997).

Cowpea is infected by about 140 viruses worldwide (Hughes and Shoyinka, 2003), of which only nine had been reported to occur in Africa (Taiwo, 2003). Losses due to viral infections are estimated to be between 10 and 100% (Rachie 1985) and the complete loss of irrigated cowpeas in northern Nigeria had been attributed to virus infection (Rossel, 1977).

Viruses constitute major constraints in all agroecologies where cowpea is grown and for an effective diagnosis of virus diseases, it is expedient to carry out surveys and serological studies to identify the particular viruses prevalent in the area. The objective of this study therefore, was to assess the prevalence of characteristic virus symptoms on cowpea and serologically identify the viruses infecting cowpea in Kwara State, Nigeria.

Materials and Methods

A survey of some thirty (30) different farms within the agroecologies of Kwara State where cowpea was extensively grown, was carried out when the crops were at the vegetative growth stage. Table 1 shows the location, elevation and agroecology of the survey area.

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Table 1 Location and elevation of survey site

S/No	Town	Longitude/Latitude	Elevation (m)	Agroecology
1	Iloffa	$8^0\,05^I32.12^{II}$ N $5^0\,09^I55.44^{II}$ E	551.4	Rainforest
2	Ekenmeje	$8^0\,25^I46.87^{II}$ N $4^0\,50^I29.68^{II}$ E	443.2	Rainforest
3	Osi	$8^0\,04^I18.25^{II}$ N $5^0\,15^I08.47^{II}$ E	488.6	Rainforest
4	Odo-Owa	$8^0\,36^I56.19^{II}$ N $4^0\,66^I45.55^{II}$ E	373.9	Rainforest
5	Idera	$8^0\,04^I18.25^{II}$ N $5^0\,15^I08.47^{II}$ E	451.4	Rainforest
6	Idofian	$8^0\,16^I24.60^{II}$ N $4^0\,48^I17.76^{II}$ E	378.9	Rainforest
7	Igbaja	$8^0\,23^I22.55^{II}$ N $4^0\,53^I11.21^{II}$ E	429.2	Rainforest
8	Ilala	$8^0\,17^I14.68^{II}$ N $4^0\,44^I48.26^{II}$ E	396.9	Rainforest
9	Omu-Aran	$8^0\,08^I24.89^{II}$ N $5^0\,07^I25.98^{II}$ E	545.9	Rainforest
10	Ajasse-Ipo	$8^0\,14^I45.15^{II}$ N $4^0\,48^I42.80^{II}$ E	382.8	Rainforest
11	Oke-Ode	$8^0\,23^I32.14^{II}$ N $4^0\,58^I09.76^{II}$ E	444.4	Rainforest
12	Erin-Ile	$8^0\,05^I22.63^{II}$ N $4^0\,43^I22.65^{II}$ E	398.1	Rainforest
13	Ilemona	$8^0\,06^I59.55^{II}$ N $4^0\,39^I57.50^{II}$ E	411.2	Rainforest
14	Jimba-Oja	$8^0\,22^I41.67^{II}$ N $4^0\,42^I30.06^{II}$ E	406.6	Rainforest
15	Elerinjare	$8^0\,15^I33.43^{II}$ N $4^0\,44^I67.72^{II}$ E	386.2	Rainforest
16	Patigi	$8^0\,44^I22.56^{II}$ N $5^0\,45^I12.43^{II}$ E	118.3	Guinea savannah
17	Shonga	$9^0\,08^I23.16^{II}$ N $5^0\,04^I56.10^{II}$ E	76.8	Guinea savannah
18	Molete	$8^0\,39^I00.03^{II}$ N $4^0\,34^I43.01^{II}$ E	258.8	Guinea savannah
19	Alapa	$8^0\,36^I52.25^{II}$ N $4^0\,45^I16.22^{II}$ E	325.8	Guinea savannah
20	Shao	$8^0\,35^I31.21^{II}$ N $4^0\,33^I35.58^{II}$ E	305.1	Guinea savannah
21	Share	$8^0\,59^I44.61^{II}$ N $4^0\,97^I33.11^{II}$ E	504.1	Guinea savannah
22	Bubu	$8^0\,47^I07.75^{II}$ N $5^0\,17^I50.02^{II}$ E	240.5	Guinea savannah
23	Afon	$8^0\,33^I76.41^{II}$ N $4^0\,42^I62.22^{II}$ E	341.4	Guinea savannah
24	Awonga	$8^0\,45^I57.88^{II}$ N $5^0\,37^I07.86^{II}$ E	121.9	Guinea savannah
25	Kanbi	$8^0\,39^I18.64^{II}$ N $4^0\,33^I44.52^{II}$ E	304.9	Guinea savannah
26	Olooru	$8^0\,39^I41.17^{II}$ N $4^0\,35^I40.89^{II}$ E	315.2	Guinea savannah
27	Badi	$8^0\,58^I32.25^{II}$ N $4^0\,86^I11.65^{II}$ E	368.2	Guinea savannah
28	Alade	$8^0\,35^I18.25^{II}$ N $4^0\,55^I54.88^{II}$ E	439.5	Guinea savannah
29	Gwanara	$8^0\,53^I30.30^{II}$ N $3^0\,08^I02.82^{II}$ E	359.4	Guinea savannah
30	Kosubosu	$8^0\,54^I38.58^{II}$ N $3^0\,27^I02.28^{II}$ E	401.1	Guinea savannah

Thirty (30) plants per location were randomly sampled for virus disease symptoms by walking across a "W" shaped path in a field, with 5 plants per side spaced at an equal distance from each other. Furthermore, fresh cowpea leaf samples showing symptoms of virus infection were collected from each of the 30 locations. The leaves were put in air-tight polythene sachets and stored over ice at -20^0 C prior virus identification in the laboratory.

The Antigen - coated plate (ACP) – ELISA method described by Kumar (2009) was the serological protocol employed to detect the viruses infecting cowpea. The leaf samples were subjected to tests using antisera specific for Cowpea Aphid Borne Mosaic Virus (CABMV), Blackeye Cowpea Mosaic Virus (BlCMV), Cowpea Mosaic Virus (CMV), Cowpea Yellow Mosaic Virus (CYMV), Cowpea Mottle Virus (CPM_OV), Southern Bean Mosaic Virus (SBMV) and Cowpea Mild Mottle Virus

(CPMMV). The antisera used for serological testing were produced by International Institute of Tropical Agriculture (IITA), Virology and Molecular Diagnostic Unit, Ibadan-Nigeria.

Results

Prevalence of characteristic virus symptoms

Table 2 shows the Percentage incidence of characteristic virus symptoms observed on cowpea during the 2011 growing season in Kwara State. The results showed that leaf mottling was the most prevalent (36.8%) virus symptom in all of the locations surveyed. Further scrutiny of each location within agroecology indicated that Alapa and Afon (guinea savannah agroecology) had the highest percentage of mottling symptoms (61%), followed by Badi (58%), Idofian (53%) and Ilala (52%). The locations of Shonga (14%), Elerinjare (17%), Ekanmeje (18%) and Share (20%) had low percentage mottling symptoms. There was no mottling symptoms observed at Olooru. Leaf yellow mosaic was the next most rampant virus symptom observed during the survey with a mean percentage value of 24.9%. Gwanara in the guinea savannah agroecology, recorded the highest mosaic symptom incidence (51%), followed by Elerinjare 48%, Alade 43%, Share 40% and Idera 39%. The lowest values were reported at Olooru (9%), Kosubosu (10%), Shonga (11%), while the locations at Kanbi and Idofian had 14% incidence of mosaic symptoms.

Leaf curl was the third most recorded virus symptom observed and it had a mean value of 15.6% incidence. The locations at Oke-ode (forest agroecology), recorded the highest leaf curl symptom of 33%. The locations at Erin-ile, Ajasse - Ipo, Kanbi and Kosubosu had leaf curl incidence of 28%, 27%, 26% and 20%, respectively. Leaf curl viral symptoms was not reported in the location of Patigi in the savanna agroecology. The other symptoms, which were not easy to describe because of overlap of different symptoms and therefore referred to as "others" accounted for 14.5% of the total symptoms observed. Shonga (52%) had the highest number of samples with these symptoms followed by Ekenmeje (42%) and Olooru (40%). Ajasse - Ipo recorded 2%, while Oke-Ode, Afon, Alapa and Gwanara had 3% of samples manifesting "others" symptoms.

Necrotic spots was the least observed of the viral symptoms on cowpea during the survey, only 8.2% of leaf sampled for viruses exhibited necrotic spots. Slightly high occurrences were reported at Kanbi (30%), Jimba (21%), Olooru and Omu-Aran (20%). Necrotic spots were not observed in locations at Badi, Alade, Gwanara, Elerinjare and Oke-Ode.

Identification of the viruses using enzyme linked immunosorbent assay

The results of the identification of the viruses infecting cowpea in Kwara State using ELISA are shown in Table 3. The results indicated the presence of viruses in all agroecologies of Kwara State, although the distribution of the viruses varied across the different locations. The identities of the viruses were confirmed using specific antiserum to each virus using seven antibodies (CABMV, BICMV, CMV, CYMV, CMEV, SBMV and CPMMV). Four different viruses namely: Cowpea aphid borne mosaic virus (CABMV), Cowpea yellow mosaic virus (CYMV), Blackeye cowpea mosaic virus (BICMV), and Cowpea mottle virus (CPM_oV) were detected infecting cowpea in Kwara State. The viruses occurred in mixtures of two or three different viruses at the different locations and there was no infection by a single virus in all the locations.

The frequent mixtures of two viruses (CABMV + BICMV) were detected in the 14 locations of Ajasse, Idofian, Illala, Omu-Aran, Idera, Oke-ode, Erin-Ile, Jimba, Alapa, Shao, Bubu, Badi, and Kosubosu. The mixtures of three (3) viruses of CABMV, CPM_oV and BICMV were detected at Odo-Owa, and the combination of four (4) viruses (CABMV, CPM_oV and BICMV) were detected at Osi.

The locations that had negative virus results from the samples were at Iloffa, Ekenmeje, Igbaja, Ilemona, Elerinjare, Patigi, Shonga, Molete, Afon, Awoga, and Kanbi. The results showed that out of the 16 locations where viruses were detected, 10 were from the forest agroecology.

Table 2 Percentage incidence of characteristic virus symptoms observed on cowpea during the 2011 growing season in Kwara State

Symptoms	Location																													Percentage Mean
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
Mosaic	15	22	33	23	24	14	27	16	18	39	20	18	28	41	48	19	11	19	33	23	40	30	21	21	14	9	21	43	51	10	24.9
Mottling	39	18	27	44	36	53	43	52	38	44	44	31	29	21	17	46	14	38	61	51	20	31	61	41	28	0	58	41	36	43	36.8
Leaf Curl	13	7	20	17	27	20	18	20	14	7	33	28	18	11	6	0	15	21	3	6	15	21	10	8	26	31	11	11	10	20	15.6
Necrotic	8	11	15	11	11	5	4	3	20	5	0	10	5	21	0	4	8	11	0	10	10	3	5	9	30	20	0	0	0	6	8.2
Others	25	42	5	5	2	8	7	9	10	5	3	13	20	6	15	31	52	11	3	10	15	15	3	21	10	40	20	5	3	21	14.5

Key: 1= Iloffa 2 = Ekanmeje 3 = Osi 4 = Odo-owa 5 = Ajasse 6 = Idofian 7 = Igbaja 8 = Ilala 9 = Omu-Aran 10 = Idera 11 = Oke-Ode, 12 = Erin-Ile, 13 = Ilemona, 14 = Jimba, 15 = Elerinjare, 16 = Patigi, 17 = Shonga, 18 = Molete, 19 = Alapa, 20 = Shao, 21 = Share, 22 = Bubu, 23 = Afon, 24 = Awoga, 25 = Kanbi, 26 = Olooru, 27 = Badi, 28 = Alade, 29 = Gwanara, 30 = Kosubosu
Source: Field survey 2011

Table 3 Detection of viruses in cowpea leaf samples collected from selected locations in Kwara State during the 2011 growing season

S/N	Location	CABMV	BICMV	CMV	CYMV	CPM_oV	SBMV	CPMMV
		Absorbance Value (A405 nm)						
1	Illoffa	*	*	*	*	*	*	*
2	Ekanmeje	*	*	*	*	*	*	*
3	Osi	3.0	3.0	*	0.67	0.59	*	*
4	Odo-Owa	3.0	3.0	*	*	0.69	*	*
5	Ajasse-Ipo	3.0	3.0	*	*	*	*	*
6	Idofian	3.0	3.0	*	*	*	*	*
7	Igbaja	*	*	*	*	*	*	*
8	Ilalla	0.85	0.8	*	*	*	*	*
9	Omu-Aran	3.0	3.0	*	*	*	*	*
10	Idera	3.0	3.0	*	*	*	*	*
11	Oke-Ode	0.73	0.75	*	*	*	*	*
12	Ein-Ile	0.62	0.71	*	*	*	*	*
13	Ilemona	*	*	*	*	*	*	*
14	Jimba	3.0	3.0	*	*	*	*	*
15	Elerinjare	*	*	*	*	*	*	*
16	Patigi	*	*	*	*	*	*	*
17	Shonga	*	*	*	*	*	*	*
18	Molete	*	*	*	*	*	*	*
19	Alapa	3.0	3.0	*	*	*	*	*
20	Shao	1.17	3.0	*	*	*	*	*
21	Share	3.0	3.0	*	*	*	*	*
22	Bubu	3.0	3.0	*	*	*	*	*
23	Afon	*	*	*	*	*	*	*
24	Awoga	*	*	*	*	*	*	*
25	Kanbi	*	*	*	*	*	*	*
26	Olooru	*	*	*	*	*	*	*
27	Badi	3.0	3.0	*	*	*	*	*
28	Alade	*	*	*	*	*	*	*
29	Gwanara	*	*	*	*	*	*	*
30	Kosubosu	3.0	3.0	*	*	*	*	*
	Diseased	3.0	3.0	3.0	0.74	3.0	3.0	2.73
	Healthy	0.41	0.41	0.51	0.25	0.34	0.35	0.52
	Buffer	0.27	0.27	0.30	0.38	0.32	0.30	0.29

Discussion

The virus symptoms observed on cowpea in the surveyed fields

During the survey, preliminary diagnosis was based only on visual symptom expression and any symptomless plant or latent infections were not included for the evaluation. The results show that in all the agroecologies of Kwara State, leaf mottling (36.8%) was the most prevalent virus symptom followed by leaf mosaic (24.9%), leaf curl (15.6%), other symptoms (14.5%) and necrotic lesion (8.2%). Similar symptoms have been reported elsewhere on legumes infected by viral diseases (Vanderborght and Baudoin, 2001 and Akinjogunla, 2005).

The symptoms recorded are indicative of different viruses which infect cowpea in Kwara State. It has been postulated that symptoms produced are dependent on the particular viruses, the strain involved, the hybrid, species and age of plant, the time of the year and environmental conditions. The symptoms observed are also suggestive of the viruses likely to be serologically detected by ELISA. Some of the major symptoms

observed during the survey (mottling, mosaic, vein banding, chlorosis, leaf distortion, necrotic spots, stunting and plant death), are consistent with symptoms associated with infection by BICMV, CABMV, CM_EV and CYMV (Sekar and Sulochana 1988; Bashir, 1992).

The variations in the symptoms observed may be due to the type of viral strains infecting the plant, cowpea cultivar, the time of infection of the virus pathogen (time of the year and stage of plant growth), light intensity, environmental temperature, mixed infections and/or presence of yet unidentified pathogens. Jones *et al* (1991) had postulated these factors to be responsible for symptom variations in cowpea infected with virus.

Identification of Viruses by ELISA

The results of the virus identification showed the distribution of some viruses at different locations in the agroecology of Kwara State. Four (4) viruses namely, Cowpea aphid borne mosaic virus (CABMV), Cowpea yellow mosaic virus (CYMV), Blackeye cowpea mosaic virus (BICMV), and Cowpea mottle virus (CPMoV)], were positively identified as the viruses infecting cowpea in 16 locations. The four viruses identified in the locations are amongst the nine listed by Hughes *et al.* (2003), to be occurring in Nigeria. Also this is in partial agreement with Alegbejo and Kashina (2001) reported that the economically important viruses of cowpea in Nigeria include cowpea aphid-borne mosaic virus (CABMV), cowpea mosaic virus (CPMV) genus Comovirus and occasionally Southern bean mosaic virus (SBMV) genus Sobemovirus and Blackeye cowpea mosaic virus (BICMV) have a low rate of occurrence but may be widespread in some northern states.

The ELISA results also indicated that the viruses occurred in mixtures of two (CABMV + BICMV) in 14 locations and in mixtures of 3 viruses (CABMV + CYMV + BICMV or CABMV + CMEV + BICMV) in one location each. Multiple-virus infections are common among samples from field grown cowpeas and are known to modify and complicate symptoms, thus precluding field diagnosis based on symptoms. This result is in agreement with findings by Azzam and Makkouk (1985), in which mixed infections such as Blackeye cowpea mosaic virus (BICMV), Blackeye cowpea mosaic virus and Cucumber mosaic virus (BICMV+CMV), were detected in 65% of cowpea leaf samples assayed for viruses. It also compares well with the results of Shoyinka *et al*. (1997), in which mixtures of three viruses [Cowpea severe mosaic virus (CPSMV), Cowpea mild mottle virus (CMMV), and Cowpea aphid-borne mosaic virus (CABMV)] were observed in only two of the 108 cowpea samples.

The non detection of viruses in the other (14) locations could be as a result of low virus concentration in the cowpea leaf samples. Alternatively, it may be due to the presence of serologically variable strains of the viruses and the non availability of antibodies specific to the viruses. Mesfin *et al.* (1992) had shown that 19 of 24 isolates from grasses and cereal crops reacted with a polyclonal antiserum to a severe maize streak virus (MSV) isolate from maize, thus suggesting serological differences in the virus strain.

Conclusion and Recommendation

The survey result provided for the first time at first hand, a baseline information on the distribution of cowpea viruses in the agroecologies of Kwara State, virus diagnosis showed that four viruses were prevalent in all the locations surveyed. The viruses existed in combinations of two to three and were characterized by symptoms which were not specific to a particular virus. These facts present a good starting point for cowpea virus diseases diagnosis in Kwara state, Nigeria.

The viruses that were confirmed existing in the State have a wide crop range and are potentially very damaging to cowpea and other crops. There is the need, therefore, for constant monitoring of cowpea fields through regular disease surveys to identify new and emerging viruses. This will enable the deployment of effective environmental and management strategies necessary to prevent incidence and ameliorate virus disease problems on cowpea fields. This could ultimately be of agricultural importance for

sustainable food security and poverty eradication.

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