

RESEARCH ARTICLE

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The Occurrence and Distribution of Viruses Infecting Groundnut (*Arachis hypogaea* L.) in Kwara State of Nigeria

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Abstract

A field survey was conducted on farms in twenty-two (22) locations in the guinea savannah agroecology of Kwara state, Nigeria. Virus disease incidence was estimated on each field by assessing fifty (50) plants for virus symptoms. Virus severity was scored on a scale of 1- 6 on the extent of leaf damage and percentage number of leaves showing symptoms of the fifty plants counted for disease incidence. The second stage of the study involved the use of Antigen-Coated Plate Enzyme-Linked Immunosorbent assay (ACP-ELISA) for presence of Groundnut Rosette Virus (GRV), Cowpea aphid borne mosaic virus (CABMV), and Cucumber mosaic virus (CMV) on collected field samples. The field survey indicated that virus incidence ranged from 5% - 65% with the highest value in Oke-Ose and Batakipan respectively. The severity fluctuated between mild mosaic mottling and necrosis on few leaves and branches of the plants to severe mosaic/ puckering/mottling/yellowing/necrosis symptoms on entire plants. The serology trial revealed presence of the 3 tested viruses in 100% of locations in the study area. The viruses were found occurring either singly or in mixtures of two or three on the groundnut samples; although CMV was the most prominent with detection in all samples. This pioneer study, revealed the intense occurrence and sporadic distribution of plant viruses on groundnut in Kwara State of Nigeria. The information obtained is germane in the design of effective management to mitigate the undesirable effect of virus infection on the crop.

Keywords: food security, incidence, legume, plant pathology, serology, severity

1. Introduction

Groundnut (*Arachis hypogaea* L.) is a leguminous oil seed crop cultivated in the semi-arid and subtropical regions of the world. It is an important seed and staple food crop grown in Nigeria and more than 100 countries of the world [16]. In contrast to some other crops, which strip the soil of its nutrients, groundnuts naturally improve the soil that they grow in by adding much-needed Nitrogen. It is called a self-fertilizing crop, nevertheless, it is very exhaustive crop compared to other legumes because a very little portion of the plant residue is left in the soil after harvest [24]. Worldwide, groundnut is grown on nearly 24.6 million hectares with a total production of 41.3 million tones and with an average yields of 1676 kg/ha [9]. Groundnut is usually grown as a

smallholder crop in the semi-arid tropics under rain fed conditions and therefore serves as one of the prime source of big income for the resource-poor subsistence farmers. It is an important crop in many countries, especially in sub-Sahara Africa, where it is a good source of easily digestible protein (25%-34%), cooking oil (48%-50%) and vitamins E, K and B. The haulms are a good source of feed for livestock, especially during the dry season when fresh green grasses are not available. This serves as an additional source of income for farmers in the dry season when the fodder is in high demand [2].

In sub Saharan Africa, 32 viruses induce yield losses in groundnut and the major ones are Cowpea aphid-borne mosaic virus (CABMV), Cucumber mosaic virus (CMV), Indian peanut clump virus (IPCV), Peanut clump virus (PCV), Peanut mottle virus (PeMoV),

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Peanut streak virus (PStV) and Cowpea mild mottle virus (CPMMV) [20]. All these viruses are transmitted through groundnut seed and are regarded as important quarantine pests. Cowpea aphid-borne mosaic virus has been known in Nigeria since 1976 as a devastating virus disease of legume in all the agro-ecological zones [21].

Kwara state, Nigeria lies within a region described as tropical climate and characterized by double rainfall maxima and has tropical wet and dry climate [17]. The rainy season begins at about the end of March and lasts until early September with an annual range of 1000 mm to 1500 mm. Temperature is uniformly high and ranges between 25°C and 30°C in the wet season throughout the season except in July – August when the clouding of the sky prevents direct insolation while in the dry season it ranges between 33°C to 34°C [1]. The climate of Kwara state supports tall grass interspersed with short scattered trees. This attribute predisposes the people of Kwara State to make farming their major occupation. Food crops produced in the state include groundnut, yam, cassava, water yam and sweet potato and these constitute the main staple food aside cereals [14].

The pressing need to increase food production in the tropics to feed the burgeoning population requires that crop yields must be increased without prejudicing the resource base for future generations. Farmers risk high losses due largely to lack of information on the status of viruses infecting groundnut. Such information is needed towards effective management to mitigate the undesirable effect of virus infection on the crop in the State.

The objectives of the study therefore were to:

- (i) determine by field surveys, the incidence and severity of viruses infecting groundnut in Kwara State-Nigeria.
- (ii) detect using Enzyme-linked Immunosorbent assay (ELISA), the presence of three viruses namely: *Cucumber mosaic virus* (CMV), *Cowpea aphids borne mosaic virus* (CABMV) and *Groundnut rosette virus* (GRV) on groundnut in Kwara State -Nigeria.

2. Material and Methods

2.1 Location, sampling technique and estimation of virus incidence

A virus disease survey of 22 groundnut fields with farm size not less than 2000m² was carried out in the 2017/2018 cropping season across the agroecology in

Kwara State of Nigeria (Table 1). Virus disease incidence was estimated in each field by assessing fifty plants observations per field for virus disease symptoms. This was done by walking in “M” shaped path counting five plants per side spaced at an equal distance from each other. Incidence was estimated using the formula given below:

$$\frac{\text{number of infected plant} \times 100}{50 (\text{plant population})}$$

2.2 Virus severity index

Virus severity index was scored on a scale of 1-6 on extent of leaf damage and percentage number of leaves showing symptoms of the fifty plants counted for disease incidence. Mean of these scores (approximated to the nearest whole number) was expressed to determine the average severity of virus disease in the field [5]. A modified version of scale was adopted for the study whereby:

- 1= No visible symptoms
- 2 = Mild mosaic/mottling/yellowing/mild necrosis on few leaves /branches of a plant
- 3 = Moderate mosaic/puckering/mottling/yellowing/necrosis on many leaves/plants and vein clearing (symptoms cover 50% of the plant)
- 4 = Severe mosaic/puckering/mottling/yellowing/necrosis (symptoms on entire plant)
- 5 = Severe mosaic/puckering/mottling/yellowing/necrosis and severe stunting (entire plant)
- 6 = Severe mosaic/puckering/mottling/yellowing/necrosis and severe stunting (entire plant), deformation and death of the infected plants.

Symptomatic and asymptomatic groundnut leaf samples (20) were then randomly collected from each field surveyed and separately sealed in dispenser sachets labeled and preserved over ice prior serological analysis using Enzyme Linked-Immunosorbent Assay (ELISA).

2.3 Enzyme - Linked Immunosorbent Assay (ELISA) protocol

The moisture on groundnut leaves were removed by blotting with absorbent paper and the leaves were cut into pieces and placed in a specimen bottle with Calcium chloride (CaCl₂) prior to ELISA testing. The samples were then tested for the presence of three

viruses. Antigen-coated plate enzyme-linked immunosorbent assay (ACP-ELISA) was used to test for Groundnut rosette virus (GRV), Cowpea aphid borne mosaic virus (CABMV), and Cucumber mosaic virus (CMV). The antibodies were obtained from the stock of the international institute for tropical Agriculture (IITA), Ibadan- Nigeria. The leaves were ground in coating buffer pH 9.6 (Na_2CO_3 1.59g, NaHCO_3 2.93g dissolved in one liter of distilled water) at a ratio of 1:10 (w/v), 100 μl of extract was dispensed per well. The positive and negative controls were the diseased and buffer respectively. The enzyme conjugate, alkaline phosphate was diluted by adding 1 μl anti-rabbit alkaline in 1500ml conjugate buffer and

mixed thoroughly. Then 100 μl of this was dispensed into each well of the ELISA plate and then incubated at 37°C for one hour. The p-nitrophenylphosphate (PNPP) substrate solution was prepared at a concentration of 1mg/ml with substrate buffer. One hundred μl (microliter) of PNPP solution was added to each well and the plate incubated in the dark for one hour at room temperature to allow for colour development. The optical density (OD) values were measured at absorbance of 405nm (A_{405}), using a Biotek (ELx800, Universal Micro plate Reader). An optical density value two times greater than the mean of the negative controls was considered as positive [6].

Table 1: Location of survey site

S/n	Location	Latitude (N)	Longitude (E)	Altitude (m)
1	Oke-Oyi	08 ⁰ 33'35.0	004 ⁰ 40'09.4	365
2	Oke-Ose	08 ⁰ 33'34.9	004 ⁰ 40'07.4	363
3	Oke-Oloka	08 ⁰ 39'09.6	004 ⁰ 51'23.7	365
4	Oke-Olokoo	08 ⁰ 39'09.8	004 ⁰ 51'19.5	368
5	Isamu	08 ⁰ 38'56.4	004 ⁰ 51'26.6	349
6	Tepatan	08 ⁰ 33'32.9	004 ⁰ 36'12.3	313
7	Gidaje	08 ⁰ 34'20.9	004 ⁰ 36'02.3	333
8	Agiya	08 ⁰ 34'14.7	004 ⁰ 36'04.6	330
9	Abati	08 ⁰ 32'57.9	004 ⁰ 35'52.9	284
10	Yana-Shonga	08 ⁰ 51'08.7	005 ⁰ 01'05.9	272
11	Patidzuru	08 ⁰ 51'16.6	005 ⁰ 00'40.1	283
12	Fedudangi	08 ⁰ 51'13.4	005 ⁰ 00'50.3	286
13	Batakpan	08 ⁰ 51'19.2	005 ⁰ 00'10.7	274
14	Fenjewe	08 ⁰ 49'02.2	004 ⁰ 59'27.7	274
15	Gaa-Filani	08 ⁰ 49'04.8	004 ⁰ 59'39.9	284
16	Gurelade	08 ⁰ 48'50.9	004 ⁰ 59'38.0	282
17	Mukadam	08 ⁰ 17'26.0	004 ⁰ 47'15.2	397
18	Obaloyan	08 ⁰ 17'09.5	004 ⁰ 48'07.7	392
19	Sanmara	08 ⁰ 14'29.2	004 ⁰ 50'26.4	412
20	Araromi-Ipo	08 ⁰ 15'05.4	004 ⁰ 49'22.1	399
21	Oke-Odo	08 ⁰ 16'04.9	004 ⁰ 49'04.3	387
22	Gbagede	08 ⁰ 16'11.6	004 ⁰ 48'51.0	385

Sources: field survey, 2017/2018

3. Results

3.1 Incidence of virus disease on groundnut in Kwara State of Nigeria

Table 2 below shows the result of the incidence of the virus disease on groundnut in Kwara state of Nigeria. The result indicated that virus incidence ranged from 5% - 65% in the guinea savannah agroecology of Kwara State, Nigeria. A further analysis confirms the highest incidence of 65% in Oke-Ose and Batakpan

and this was followed by Gaa-Fulani with 60%. The locations of Fenjewe, Araromi-Ipo, Patidzuru, Oke Oloka and Isamu recorded 55%; whereas Oke-Olokoo had a value of 50%. The 3 locations of Oke-Oyi, Tepatan, Agiya and Sanmara had incidence value of 40%, followed by Yana-Shonga with 35% and Obaloyan, Gurelade, Fedudagi and Abati with 30%. Lower virus incidence was obtained at Mukadam (25%), Oke-Odo, and Araromi-Ipo (20%), Gidaje

(15%). However, the lowest incidence level was at Gbagede (5%).

3.2 Severity of virus disease on groundnut in Kwara State of Nigeria

The severity of virus disease on groundnut in Kwara State (Table 3) indicated a severity index of between 2 to 4 in all of the 22 locations of study. This suggests that virus severity on groundnut in the study area fluctuated between mild mosaic mottling and necrosis on few leaves and branches of the plants to severe mosaic/puckering/ mottling/ yellowing/necrosis symptoms on entire plants. A severity index of 4 reported at Isamu and Agiya were however the highest. The locations at Oke-Olokoo, Tepatan, Fenjewe, Gaa-filani, Gurelade and Obaloyan had severity index of 3. While the other 14 remaining locations of Oke-oyi, Oke-Ose, Oke-Oloka, Gidaje, Abati, Yana-Shonga, Patidzuru, Fedundagi, Batakpan, Mukadam, Sanmara, Araromi-Opi, Oke-Odo and Gbagede had the lowest severity index (2) in the study area, indicating mild

mosaic/ mottling/yellowing/mild necrosis on few leaves and branches.of the plant.

3.3 Identification of viruses using Enzyme - Linked Immunosorbent Assay (ELISA)

The identification by ELISA result is on Table 4. The result showed that the three (3) viruses tested; *Cucumber mosaic virus* (CMV), *Cowpea aphids borne mosaic virus* (CABMV) and *Groundnut rosette virus* (GRV) were detected on all of the groundnut samples. These viruses were established to be occurring on the samples either singly or in mixtures of two or three as the case may be.

Further assessment of the result (Table 4), revealed that *Cucumber mosaic virus* (CMV) was detected in all of the locations. *Groundnut rosette virus* (GRV) was found in samples from 5 locations namely: Oke-Ose, Isamu, Patidzuru, Fedudangi and Batakpan. Likewise, *Cowpea aphids borne mosaic virus* (CABMV) was detected in samples from 5 locations of Oke-Ose, Isamu, Tepatan, Agiya and Batakpan.

Table 2: Incidence of virus diseases on Groundnut in Kwara State of Nigeria

S/N	Location	Incidence (%)
1	Oke-Oyi	40
2	Oke-Ose	65
3	Oke-Oloka	55
4	Oke-Olokoo	50
5	Isamu	55
6	Tepatan	40
7	Gidaje	15
8	Agiya	40
9	Abati	30
10	Yana-Shonga	35
11	Patidzuru	55
12	Fedudangi	30
13	Batakpan	65
14	Fenjewe	55
15	Gaa-Filani	60
16	Gurelade	30
17	Mukadam	25
18	Obaloyan	30
19	Sanmara	40
20	Araromi-Ipo	55
21	Oke-Odo	20
22	Gbagede	5

Table 3: Severity of virus diseases on Groundnut in Kwara State of Nigeria

S/N	Location	Severity
1	Oke-Oyi	2
2	Oke-Ose	2
3	Oke-Oloka	2
4	Oke-Olokoo	3
5	Isamu	4
6	Tepatan	3
7	Gidaje	2
8	Agiya	4
9	Abati	2
10	Yana-Shonga	2
11	Patidzuru	2
12	Fedudangi	2
13	Batakpan	2
14	Fenjewe	3
15	Gaa-Filani	3
16	Gurelade	3
17	Mukadam	2
18	Obaloyan	3
19	Sanmara	2
20	Araromi-Ipo	2
21	Oke-Odo	2
22	Gbagede	2

Table 4: Viruses identified using Enzymes Linked Immunosorbent Assay (ELISA)

S/n	Location	VIRUSES		
		GRV	CMV	CABMV
1	Oke-Oyi	0.641 (-)	4.371 (+)	0.271 (-)
2	Oke-Ose	3.532 (+)	3.437 (+)	1.191 (+)
3	Oke-Oloka	0.511 (-)	3.748 (+)	0.253 (-)
4	Oke-Olokoo	0.645 (-)	3.812 (+)	0.189 (-)
5	Isamu	0.817 (+)	3.272 (+)	1.251 (+)
6	Tepatan	0.912 (-)	3.319 (+)	1.342 (+)
7	Gidaje	0.565 (-)	3.355 (+)	0.181 (-)
8	Agiya	0.481 (-)	3.412 (+)	1.175 (+)
9	Abati	0.361 (-)	3.352 (+)	0.999 (-)
10	Yana-Shonga	0.364 (-)	4.312 (+)	0.114 (-)
11	Patidzuru	2.541 (+)	4.106 (+)	0.196 (-)
12	Fedudangi	3.732 (+)	4.610 (+)	0.353 (-)
13	Batakpan	3.842 (+)	4.116 (+)	1.943 (+)
14	Fenjewe	0.721 (-)	3.514 (+)	0.126 (-)
15	Gaa-Filani	0.817 (-)	3.014 (+)	0.220 (-)
16	Gurelade	0.437 (-)	2.612 (+)	0.564 (-)
17	Mukadam	0.471 (-)	3.146 (+)	0.210 (-)
18	Obaloyan	0.291 (-)	2.664 (+)	0.284 (-)
19	Sanmara	0.149 (-)	4.601 (+)	0.245 (-)
20	Araromi-Ipo	0.389 (-)	3.791 (+)	0.300 (-)
21	Oke-Odo	0.814 (-)	3.632 (+)	0.310 (-)
22	Gbagede	0.342 (-)	0.147 (+)	0.345 (-)

Diseased	2.916	1.510	1.889
Healthy	0.400	0.276	0.383
Healthy	0.764	0.314	0.380
Buffer	0.691	0.284	0.263

Key: CMV = *Cucumber mosaic virus*, CABMV = *Cowpea aphid borne mosaic virus*, GRV = *Groundnut rosette virus*

(-) = denotes absence of the virus

(+) = denotes presence of the virus

OD = Optical density values (nm) of ELISA are in parenthesis

The detection of two or more viruses infecting groundnut were from samples at 6 locations of: Oke-Ose (GRV+CMV+CABMV), Tepatan (CMV + CABMV), Agiya (CMV+CABMV), Patizuru (GRV+CMV), Fedundagi (GRV+CMV) and Batakpan (GRV+CMV+CABMV). An overall assessment of the result confirmed GRV, CMV and CABMV existence either as single or mixed virus infections in all of the tested samples.

4. Discussion

This is the first report which determined the occurrence, distribution and prevalence of *Cucumber mosaic virus* (CMV), *Cowpea aphids borne mosaic virus* (CABMV) and *Groundnut rosette virus* (GRV) infecting groundnut in the main growing areas in Kwara State of Nigeria. The study provides important information which could enable informed research and management decisions to be made on plant-viral epidemiology [3]. Even though there were variations in virus incidence among the locations, the study revealed generally high incidence on groundnut in the study area. Previous reports by [13] [18] had indicated disparity in the incidence of virus infection on groundnut from location to location over a period of time. It was posited that virus infection was governed by environmental factors such as rainfall and temperature which affected aphid multiplication, movement, and consequently their ability to cause secondary spread of the virus. The variations observed in virus incidence in the present study could also be attributed to changing environmental conditions within the agroecology. This position is also in agreement with those of [11].

The present study described variation in virus severity index in the study area. The symptoms on the groundnut plants were majorly discernable by mosaic patterns, mottling and necrotic symptoms. The severity of virus symptoms could be a limiting factor for its use

in breeding and selection programs. This is because infected seed stocks may carry the viruses to succeeding planting seasons, which could be dispersed regionally through germplasm exchange [10]. The growth stage of the crops, cultural practices deployed and time of sowing may differ from one location to another and could be responsible for the disparity in severity index. Furthermore, there may be genetic inheritability traits among the varieties grown which mediated some degree of resistance to virus infection [4] [3].

The three viruses confirmed by ELISA to be prevalent on groundnut in the study are known to be widespread on legumes and other arable crops with consequent yield losses [21]. The fact that CMV was the most prevalent virus reported in the study could be as a result of the wide host range and very active vectors of the virus. This finding is supported by [12], which similarly reported CMV as a major biotic constraint to groundnut productivity in sub Saharan Africa. CMV has the widest host range for any plant virus, including more than 1200 species in over 100 families of dicotyledonous and monocotyledonous angiosperms [7] [8]. The detection of GRV and CABMV by ELISA showed the viruses occurred frequently in the samples tested irrespective of whether the varieties are resistance or susceptible and is in agreement with [23]. The detection of multiple virus infection on groundnut in the study area is a common phenomenon in nature, and a number of important virus diseases of plants are the outcomes of interactions between causative agents. Multiple infections lead to a variety of intrahost virus-virus interactions, many of which result in the generation of variants showing novel genetic features, and thus change the genetic structure of the viral population [22]. The observation of multiple viral infections in the study is an indication of the probability of more severe diseases than a single infection [19] [15].

5. Conclusions

The visual field assessments revealed considerable virus incidence and severity in the surveyed areas. Laboratory tests using ELISA also elucidated the prevalence of viruses which infect groundnut in Kwara State of Nigeria. The factors put together could result in considerable yield losses and food insecurity not only on groundnut but other arable crops in the study area.

An effective and applicable groundnut virus-disease management requires accurate diagnosis and understanding of the life and disease cycle of new and emerging viruses. It is therefore important and expedient to conceive timely virus surveys supplemented with serology to avert losses.

6. References

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