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SEROTYPES AND PREVALENCE OF LEGUME VIRUSES IN NIGER STATE, SOUTHERN GUINEA SAVANNA OF NIGERIA

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SUMMARY

Field survey was conducted between September and October, 2015 to determine the serotypes and prevalence of legume viruses in Niger State, Southern Guinea Savanna of Nigeria. Niger State has 25 Local Government Areas (LGAs), grouped into three Agricultural zones. A multi-stage sampling procedure was employed to select three LGAs from each of the three zones, giving a total of 9 LGAs. Three villages/locations from the nine LGAs were randomly selected for the survey to give a total of 27 locations. Leaves were collected from cultivated cowpeas, groundnuts and soybean farms. Weeds showing virus and virus-like symptoms were also collected. The samples were preserved over silica gels in vial bottles and tested against major legume viruses [*Blackeye cowpea mosaic virus* (BICMV), *Cowpea aphid-borne mosaic virus* (CABMV), *Cowpea mild mottle virus* (CPMMV), *Cowpea yellow mosaic virus* (CPMV), *Cucumber mosaic virus* (CMV), *Cowpea mottle virus* (CPMoV) and *Southern bean mosaic virus* (SBMV)], using Antigen Coated Plate - Enzyme linked immunosorbent assay (ACP –ELISA). In all, 14.8 % of the total samples tested positive for BICMV, which was detected in Dabiri, Farin Shinge, Gidan Kwano and Tatiko. CPMMV was detected in Farin Shinge and Manigi, which accounted for 7.4 % of the total locations. In addition, CPMoV was found in Awuru, Gidan Kwano, Lemu, Manigi and Tatiko, which accounted for 18.5 % of the locations, whereas samples from other locations showed negative reaction. ELISA positive samples were detected in cowpea, groundnut, soybean and weeds (*Aeschynomene indica*, *Hyptis suaveolus*, and *Euphorbia hirta*). The occurrence of CPMoV and CPMMV in naturally infected legumes is believed to be the first report from Niger State. Inocula of the identified viruses could be utilized for breeding resistant cowpea, groundnut and soybean cultivars.

Keywords: ACP-ELISA; legume; survey; symptoms; virus; weed hosts

The major legumes in sub-Saharan Africa are cowpea (*Vigna unguiculata* L. Walp), groundnut (*Arachis hypogaea* L.) and soybean (*Glycine max* [L.] Merril) (11; 20). These crops contribute bulk of the protein in the diets of millions of people (17) and feed for livestock (24). Legumes play an important role in providing soil nitrogen to cereal crops such as maize, millet, and sorghum when grown in rotation, especially in areas where poor soil fertility is a problem. They are also an important

companion crop in most cereal-legume cropping systems due to the advantage of residual nitrogen, originating from the decay of roots and root nodules (12; 24). They also increase soil organic matter content and improve soil structure after soil incorporation. They do not require a high rate of nitrogen fertilization because their roots have nodules in which soil bacteria called *Rhizobia* help to fix nitrogen from the air.

It is estimated that the annual world cowpea crop is grown on 12.5 million hectares, and the total grain production is 3.9 million tonnes. More than 8 million hectares of cowpea are grown in West and Central Africa. Also, it is known that Nigeria is the largest producer with 4 million hectares (12). Nigeria, being the largest producer accounts for 45 % of the total on 1.15 million hectares annually (12). The major cowpea producing areas in Nigeria include Niger, Kwara, Kaduna, Borno, Taraba and Yobe States in the northern part while Oyo, Ogun and Ondo also produce appreciable quantities in the southern part of the country.

Cowpea farmers, particularly in sub-Saharan Africa are faced with two factors that constraint legume production. They are abiotic and the biotic factors (13). Diseases of these crop types which constitute biotic constraints are usually induced by fungi, bacteria, nematodes, viruses and the parasitic flowering plants such as witchweed *Striga gesnerioides* (Willd.) Vatke and *Alectra vogelii* Benth (15). Legumes are also attacked by a wide range of insect pests which can cause damage at all stages of plant growth (6). Additionally, viral diseases are considered to be a major limiting factor for the productivity of legumes in the tropical and sub-tropical countries (10).

Diseases caused by *Blackeye cowpea mosaic virus* (BICMV), *Cowpea aphid-borne mosaic virus*, (CABMV), *Cowpea yellow mosaic comovirus* (CPMV), *Southern bean mosaic virus* (SBMV) (9) *Cowpea mottle virus*, (CPMoV), *Cowpea golden mosaic virus*, (CPGMV), *Cucumber mosaic virus* (CMV), *Cowpea mosaic virus* (CMV), *Cowpea mild mottle virus*, (CPMMV) are the most economically important and are responsible for serious yield losses in legumes globally (4). They are also known to naturally infect several other crops such as fruits and vegetables and inflict significant losses (up to 100 %) (3).

Although a lot of cowpea, groundnut and soybean are cultivated in Niger State, there is scanty information on the occurrence and distribution of legume viruses. However, viral disease outbreak could induce significant reductions in quality and quantity of the produce. Plant breeding is an effective tool for adapting to new challenges of biotic and abiotic stresses in agriculture. Breeding for disease resistance/tolerance is a priority in the national breeding programme for controlling emerging diseases. Therefore, the information from virus types and distribution would encourage plant breeders to continually develop new cultivars that are resistant and could reduce losses in cowpea, groundnut and soybean production. Cultivation of resistant cultivars would increase the level of income to farmers and millions of consumers of these staple food sources would also take advantage of increased supply at relatively affordable prices. The objective of this study therefore, was to determine the serotypes and prevalence of legume viruses in Niger State, Southern Guinea Savanna of Nigeria.

MATERIALS AND METHODS

Description of the Study Area

Niger State is located in the Southern Guinea Savannah agro-ecological zone of

Nigeria (Latitude 6° 8′ E; Longitude 8° 44′ N). The State experiences distinct dry and wet seasons with an annual rainfall ranging from 1100 mm in the northern part to 1600 mm in the south with a mean of 1350 mm. The rainfall which peaks in September normally begins in April and ends in October. The temperature ranges between 35 and 37.5 °C with relative humidity between 60 and 80 %. Niger State occupies land area measuring 76,470 km^2 about 10 % of Nigeria's total land area, out of which about 85 % is arable.

Collection of Samples

Survey was conducted in major legume (cowpea, groundnut and soybean) producing areas of Niger State between September and October, 2015. Niger State has 25 Local Government Areas (LGAs), grouped into three Agricultural zones. A multi-stage sampling procedure was employed to select three LGAs from each of the three zones, giving a total of 9 LGAs. Three villages/locations were randomly selected for the survey to give a total of 27 locations. Ten farms were visited in each LGA and five leaves were randomly collected from each farm. They were collected from cowpea, soybean and groundnut plants with symptoms such as leaf mottling, mosaic, leaf curling, distortion, chlorotic spots and stunting during the vegetative stage (5 – 6 weeks after sowing), from the edges and within the fields. Samples were also collected from weed species growing within the legume fields. Weed species were identified in the laboratory of Department of Crop Production, Federal University of Technology (FUT), Minna, based on their morphological characteristics as described by Akobundu and Agyakwa (1). The leaf samples were preserved over silica gels in air-tight vial tubes. In each location, samples were collected at approximately 10 to 20 km away from the previous sampled farm (18). Geographical location of each field was recorded using Global Positioning System (GPS- 4300) equipment (Ethrex Garmin GPS, Taiwan).

Serological Detection of Legume Viruses

The sampled leaves were subjected to Antigen – Coated Plate Enzyme-Linked Immunosorbent Assay (ACP - ELISA) (16). Polyclonal antibodies raised against economically important legume viruses were used for virus detection. These were *Blackeye cowpea mosaic virus*, *Cowpea aphid-borne mosaic virus*, *Cowpea mild mottle virus*, *Cowpea yellow mosaic virus*, *Cucumber mosaic virus*, *Cowpea mottle virus* and *Southern bean mosaic virus*. Each sample was tested in duplicate wells of polystyrene microtitre plates. Healthy (negative) cowpea leaf and positive control (cv. TVU 76 infected leaf sample) were included.

Samples were ground in coating buffer at the rate of 100 mg/mL (1:10 w/v). Wells of microtitre plates were coated with 100 µL of each sample. The plates were incubated at 37 °C for 1 hour, washed thrice at 3 min intervals with Phosphate Buffered Saline-Tween (PBS-T) and tap-dried. One gram of healthy cowpea leaf was ground with 20 mL of conjugate buffer. The crude extract was filtered and rabbit antibody for various legume viruses was added at 1:10,000. One hundred µL of this was loaded into each well. The plates were incubated at 37 °C for 1 hour, washed thrice with PBS-T and tap-dried. This was followed by addition of 100 µL of anti-rabbit, goat antimouse diluted with conjugate buffer at the rate of 1:15,000 dilutions. Also, another round of incubation at 37 °C for 1 hour was performed and plates were tap-dried after washing with PBS-T. Substrate was prepared using *p*-nitrophenyl phosphate and diluted in substrate buffer at the rate of 1 mg/ml and 100 µL of the substrate solution was added to each well. The plates were then incubated in the dark

at room temperature (37 °C). Absorbance values were quantified at 405 nm using a microplate reader (MRX, Dynex Technologies, Inc., USA) after overnight. Values were scored positive when the mean optical density reading was at least twice that of the mean for the negative control.

RESULTS AND DISCUSSION

Prevalence of characteristic virus symptoms

The percentage incidence of characteristic virus symptoms observed on legumes (cowpea, groundnut and soybean) during the 2015 cropping season in Niger State are presented in Fig. 1. Mosaic was the most common symptom which accounted for 38.4 % of the symptoms across the locations. Leaf mottling was the next most prevalent foliar symptom which accounted for 20 % of the total. Leaf curl was another frequently encountered symptom during the survey with 15.8 % incidence. Chlorotic spots of about 12.5 % were observed during the survey. Some of the plants exhibited marked stunting and these accounted for about 7.3 % while puckering, leaf deformation, dead of plants and others represented 6 % of the symptomatic plants.

Identification of Legume Viruses

Results obtained from the identification of the viruses infecting legume crops in Niger State, using ACP - ELISA are shown in Table 1. *Blackeye cowpea mosaic virus* (BICMV), *Cowpea mild mottle virus* (CPMMV) and *Cowpea mottle virus* (CPMoV) were the only viruses detected in the legumes and weeds. These viruses occurred in single and mixtures of two or more on groundnut, cowpea and soybeans at the different locations surveyed. Specifically, 14.8 % of the total samples tested positive for BICMV, which was detected in Dabiri, Farin Shinge, Gidan Kwano and Tatiko. BICMV was detected in cowpea samples, and the following weeds: *Aeschynomene indica* (Linn.), *Amaranthus caudatum* L., *Centrosema pubescens* Benth, *Chenopodium amaranticolor* Coste & Reyn., *Desmodium scorpiurus* (Sw.) Desv. and *Euphorbia hirta* Linn., CPMMV was detected in Farin Shinge and Manigi, which accounted for 7.4 % of the total samples. CPMMV was found in groundnut and the following weeds: *Amaranthus caudatum* L., *Desmodium scorpiurus* (Sw.) Desv., *Hyptis suaveolus* Poit. and *Vicia faba* L. In addition, CPMoV was found in Awuru, Gidan Kwano, Lemu, Manigi, Mokwa and Tatiko, which accounted for 22.2 % of the locations, whereas samples from other locations showed negative reaction. CPMoV was found in soybean leaves and the following weeds: *Aspilia Africana* (Pers.) C.D Adams, *Cleome viscera* L., *Euphorbia hirta* Linn., *Heterotis rotundifollia* SM.

The virus symptoms observed on the sampled plants were in agreement with the earlier findings on viral infected legumes reported by Alegbejo and Kashina (5). The variation in symptoms was due to the presence of different viruses in the study area. Occurrence, distribution and spread of these pathogens might probably be influenced by environmental factors, presence of suitable and susceptible hosts as well as activities of vectors. Incidence and distribution of the viruses were natural and might have stemmed primarily from seed infection and weed hosts as observed from the cropping pattern (2). The differences observed in the symptoms from the different individual crops might be due to mixed infections of viruses on the crop, viral strains infecting the plant, crop cultivar and time of infection of the virus pathogen. Aliyu *et al.* (6) had earlier mentioned these factors to be responsible for symptom variation in cowpea plants infected with virus in Southern part of Nigeria. However, leaf samples that looked

like virus infected plants which tested negative might be suffering from nutrient deficiency.

Variation also noticed on the viral symptoms from different locations on the different legume crops surveyed was probably due to pathogenicity and virulence of the virus invading the various crops. The viruses identified in these locations are amongst the legume viruses reported to be present in Nigeria (22). Also, Taiwo *et al.* (23) maintained that BICMV was among the economically important viruses of legumes in Nigeria. The presence of two or more viruses within same location indicated the possibility of the locations and the immediate surroundings becoming hot spot of these viruses in the nearest future.

The detection of BICMV and CPMoV at Lemu and Dabiri, respectively, which are less than 5 km collaborated the findings of Aliyu *et al.* (6) who discovered two different viruses co-existing in the nearby fields in Kwara State, Nigeria. The implication of this is that subsequent mutation and replication of the viruses could simply result in several serotypes with varying degree of pathogenicity on the one hand and multiple infections of legume crops on the other hand. The occurrence of CPMoV and CPMMV in naturally infected cowpea is believed to be the first report from Niger State. BICMV was detected at Awuru and Gidan Kwano in mixture with CMoV. Multiple virus infections in field-grown plants might modify symptoms and essentially preclude field diagnosis (3). Aliyu *et al.* (6, 7) earlier reported CPMoV and CPMMV in Kwara State which is a neighboring State to Niger State.

From the locations where CPMoV was prevalent (Awuru, Gidan Kwano, Lemu, Manigi, Mokwa and Tatiko), Awuru is located at the riverine area which partially agreed with that of Alegbejo (4) who reported a high incidence of the pathogen in riverine areas of the middle belt of Nigeria, which has a Southern savanna climate and where a lot bambara groundnut (*Vigna subterranean* L. Verdc.) is grown. The seed borne nature of CPMoV (21), and recent detections suggested that the virus could be spreading through seeds to other parts of the Southern guinea agro-ecological zone of Nigeria. CPMMV has been reported to occur naturally in the middle belt of Nigeria which Niger State is inclusive (4). Odedara (19) reported CPMoV as a seed-borne virus and a major constraint to yield in legume fields because emerging plants are quickly exposed to viral inocula to produce greater damage at early stages of crop plant development. This showed how important the virus could be in the ecological zone.

The non detection of viruses in the other locations could be due to low virus concentration in the tested samples (16). It could also be argued that the negative reactions of some of the legume leaf samples to ELISA implied that they belonged to entirely different virus types (non-legume viruses). Moreover, the negative reactions of those samples in ELISA probably indicated that they belonged to other groups of the pathogens (8) or the symptom might be as a result of nutrient deficiency (12).

Different incidence levels of these viruses were recorded for all the locations studied, the distribution and spread of the pathogens could be attributed to the presence of suitable hosts (wild and cultivated crops), and even the presence and activities of vectors. The biotic and /or environmental factors that can influence the development, distribution and activity of the vectors can equally affect the distribution and spread of these viruses. Infection by the pathogens in the various areas surveyed was natural and might have primarily stemmed from seed infection and weed hosts (25). Since the

rate of seed transmission of viruses varied with legume types, cultivars and their interaction with individual virus, variation in incidence levels of the viruses was expected (14).

At survey time, the legume crops were at various stages of growth at the different locations. Virus concentration was influenced by the stages of growth which probably determined the incidence levels (9). Balogun (9) reported that epidemics occur at early stage in the growth of plants when they are most susceptible. The author reported that soybeans, groundnut and cowpea crops at pre-flowering stage exhibited higher incidence levels of the viruses than those at the flowering and pod-initiation stages.

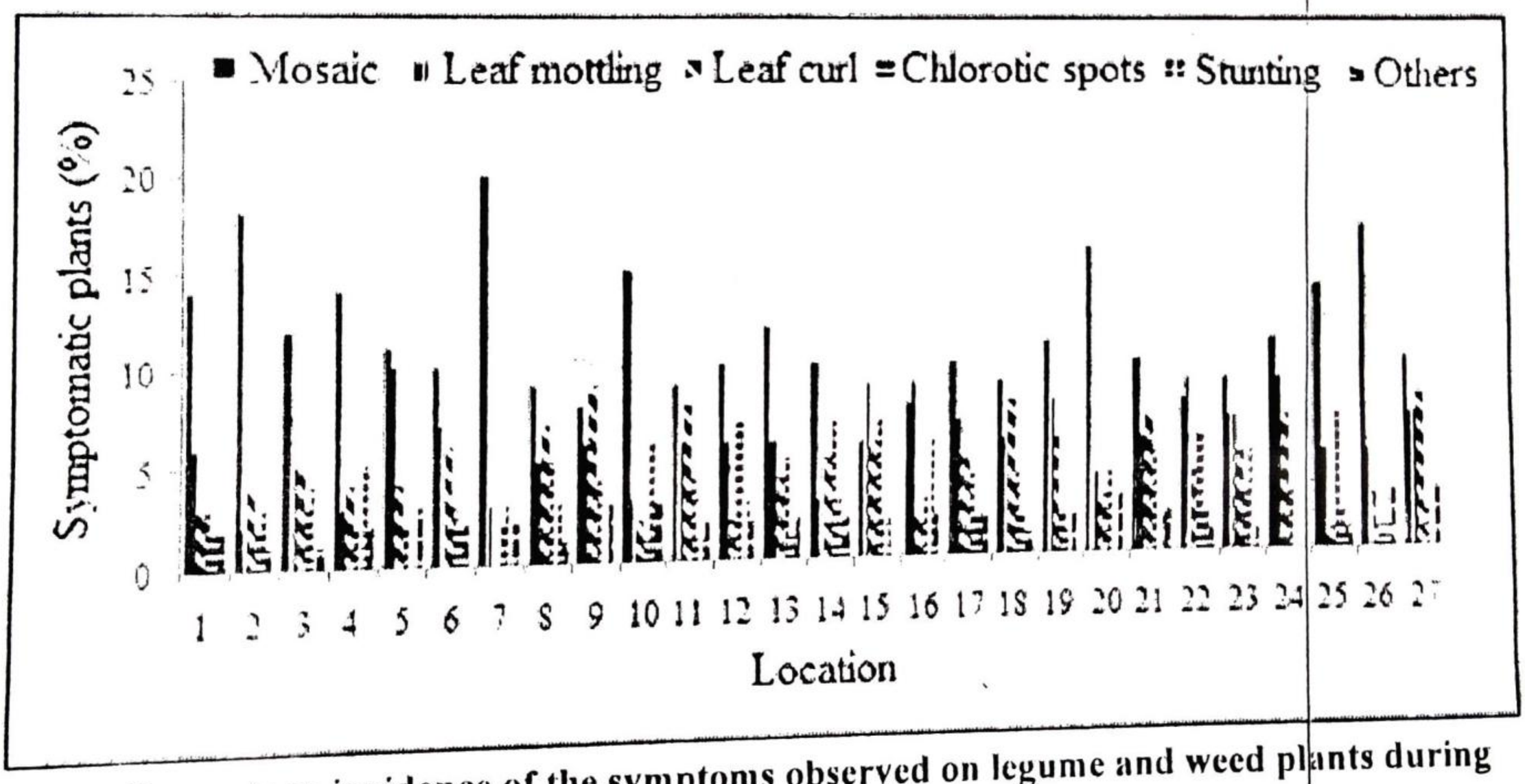

Fig. 1: Percentage incidence of the symptoms observed on legume and weed plants during 2015 wet session in Niger State, Southern Guinea Savanna of Nigeria

1 = Awuru; 2 = Ba'aburasa; 3 = Bokani; 4 = Dabiri; 5 = Farin Shinge; 6 = Gidan Kwano; 7 = Goje; 8 = Gonagi; 9 = Gwada; 10 = Lemu; 11 = Lumma; 12 = Kotseni; 13 = Kundu; 14 = Kuta; 15 = Makera; 16 = Manigi; 17 = Mokwa; 18 = Muvo; 19 = Paiko Jazu; 20 = Rafin Gora; 21 = Rokota; 22 = Shiroro; 23 = Tatiko; 24 = Tungan Makun; 25 = Zanchita; 26 = Zugurma; 27 = Zungeru

Table 1: ELISA of 7 virus pathogens from legume leaf samples across Niger State locations, 2015 season

Location	Polyclonal antibody						
	BICMV	CABMV	CMV	CPMMV	CPMoV	CPMV	SBMV
Awuru	0.273	0.132	0.142	0.314	0.354*	0.269	0.149
Ba'aburasa	0.112	0.121	0.102	0.211	0.122	0.117	0.100
Bokani	0.192	0.119	0.112	0.210	0.132	0.201	0.231
Dabiri	0.991*	0.173	0.238	0.235	0.157	0.217	0.217
Farin Shinge	0.854*	0.129	0.184	0.359*	0.149	0.142	0.146
Gidan Kwano	0.882*	0.171	0.225	0.176	0.473*	0.187	0.199
Goje	0.219	0.201	0.221	0.102	0.210	0.221	0.192
Gonagi	0.231	0.110	0.102	0.210	0.110	0.241	0.301
Gwada	0.121	0.090	0.211	0.214	0.211	0.219	0.21
Lemu	0.126	0.137	0.136	0.134	0.388*	0.162	0.146
Lumma	0.208	0.119	0.203	0.201	0.196	0.114	0.106
Kotseni	0.132	0.201	0.112	0.119	0.210	0.201	0.201
Kundu	0.209	0.221	0.301	0.104	0.203	0.119	0.116
Kuta	0.093	0.110	0.208	0.239	0.190	0.116	0.119
Makera	0.291	0.217	0.039	0.219	0.220	0.191	0.201
Manigi	0.135	0.137	0.144	0.523*	0.323*	0.169	0.151
Mokwa	0.171	0.167	0.197	0.276	0.543*	0.186	0.211
Muwo	0.130	0.156	0.201	0.200	0.172	0.110	0.192
Paiko Jazu	0.104	0.111	0.108	0.211	0.202	0.192	0.119
Rafin Gora	0.191	0.209	0.200	0.218	0.106	0.081	0.112
Rokota	0.201	0.110	0.118	0.196	0.100	0.196	0.091
Shiroro	0.110	0.210	0.099	0.117	0.124	0.115	0.107
Tatiko	0.458*	0.138	0.149	0.175	0.429*	0.135	0.141
Tungan Makun	0.100	0.112	0.109	0.103	0.201	0.119	0.099
Zanchita	0.108	0.110	0.201	0.194	0.109	0.201	0.114
Zugurma	0.129	0.22	0.201	0.113	0.221	0.192	0.201
Zungeru	0.202	0.168	0.11	0.232	0.109	0.201	0.211
Diseased control	2.562	2.138	2.915	2.424	2.876	2.454	2.899
Healthy control	0.294	0.138	0.245	0.161	0.141	0.242	0.246
Buffer	0.186	0.128	0.175	0.128	0.178	0.182	0.205

CABMV= *Cowpea aphid borne mosaic virus*, BICMV = *Blackeye cowpea mosaic virus*, CMV = *Cucumber mosaic virus* SBMV = *Southern bean mosaic virus*, CPMMV = *Cowpea mild mottle virus*, CPMoV = *Cowpea mottle virus*, CPMV = *Cowpea yellow mosaic virus*. * Virus positive.

CONCLUSION AND RECOMMENDATION

The survey results provided baseline information on the occurrence and distribution of legume viruses in Niger State. Virus diagnosis showed that three important legume viruses (BICMV, CPMoV and CPMMV) were prevalent in some specific locations surveyed. Specifically, 14.8 % of the total samples tested positive for BICMV, which was detected in Dabiri, Farin Shinge, Gidan Kwano and Tatiko. CPMMV was detected in Farin Shinge and Manigi, which accounted for 7.4 % of the total samples. In addition, CPMoV was found in Awuru, Gidan Kwano, Lemu, Manigi and Tatiko, which accounted for 18.5 % of the locations, whereas samples from other locations showed negative reaction. ELISA positive samples were detected in cowpea, groundnut, soybean and weed (*Aeschynomene indica*, *Hyptis suaveolus*, and *Euphorbia hirta*) leaf samples. The

occurrence of CPMoV and CPMMV in naturally infected cowpea is believed to be the first report from Niger State. Inocula of the identified viruses could be utilized for breeding resistant cowpea, groundnut and soybean cultivars.

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