

Full Length Research Paper

Surveys of virus diseases on pepper (*Capsicum* spp.) in South-west Nigeria

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Surveys to determine the incidence, diversity and distribution of viruses infecting pepper (*Capsicum* spp.) were conducted in six states (Oyo, Ondo, Osun, Ogun, Ekiti and Lagos) of South-west Nigeria in 2010 and 2011. Leaf samples from symptomatic and asymptomatic plants were collected at random from farmers' fields and were analyzed for viruses using antigen coated plate enzyme-linked immunosorbent assay (ACP-ELISA). Symptoms observed on infected plants included mosaic, mottling, puckering, reduction in leaf size, vein yellowing, leaf and fruit deformation and stunting. The average disease incidence was 79% in 2010 and 76% in 2011; the average disease severity score was 2.9 in both years. This study identified eight viruses in the leaf samples as follows: Potato virus Y (PVY), Potato virus X (PVX), Pepper veinal mottle virus (PVMV), Pepper mild mottle virus (PMMV), Tobacco mosaic virus (TMV), Cucumber mosaic virus (CMV), Tobacco etch virus (TEV) and Tomato mosaic virus (ToMV). Incidence of PVY was the highest (79%), followed by TEV (67%), CMV (61%), and PVMV (58%); lowest in ToMV (23%). Mixed infections were common in the farmers' fields and high incidence suggests the cultivars are highly susceptible to viral infections.

Key words: Pepper viruses, *Capsicum* spp., virus survey, enzyme-linked immunosorbent assay (ELISA), Nigeria.

INTRODUCTION

Chilli (*Capsicum* spp.) is one of the world's most popular vegetables; it is consumed, fresh or processed and used mainly as a spice and condiment (Sonago, 2003). Hot

peppers, fresh, dried or processed, are an important item in all local markets in Africa. Production of green chilli and peppers in Africa is estimated to be 2.88 million t on

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Abbreviations: DMRT, Duncan's multiple range test; ACP-ELISA, antigen coated plate enzyme-linked immunosorbent assay; PVY, potato virus Y; PVX, potato virus X; PVMV, pepper veinal mottle virus; PMMV, pepper mild mottle virus; TMV, tobacco mosaic virus; CMV, cucumber mosaic virus; TEV, tobacco etch virus; ToMV, tomato mosaic virus.

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363,937 ha of land (FAO, 2014); in West Africa the total production is 754,260 ton 108,452 ha with Nigeria contributing 500,000 ton 60,000 ha (FAO, 2014). Ogun State is the largest producer of *Capsicum* spp., in the south-west Nigeria with a production of 51.753t from 29,800 ha followed by Ekiti state with 48.85 t from 38,130 (APS, 2009). The two States are responsible for more than 50% of the total pepper production in the region (APS, 2009). The crop is cultivated mostly during the rainy season but also in the dry season in places where irrigation is available. It is mainly produced under subsistence conditions by smallholder farmers and is a significant source of household income. Peppers are used in stew and some local dishes all over the country although the types and quantities used vary in different areas. Four main varieties are grown in Nigeria: Bird peppers-atawera (*Capsicum frutescens*), Cayenne pepper or red pepper-Sombo (*C. frutescens*), Hot pepper-atarodo (*Capsicum chinense*), Sweet pepper-tatase (*Capsicum annum*). The fruits of these varieties vary in size, color, shape, and pungency but they are all hot. Both bird pepper and cayenne pepper are used either fresh or dried; atarodo and tatase are mostly used fresh (Bosland and Votata, 2000).

Pepper is susceptible to over 40 viruses (Kim et al., 2009). In Nigeria, most of the pepper fields exhibit complex symptoms of mosaic, mottle, leaf distortion, vein chlorosis and stunting that cause considerable losses in yield and plant vigor. Viral diseases caused in pepper are one of the most severe constraints in the production of the crop (Arogundade et al., 2012). Previous surveys showed high incidence of Pepper vein mottle virus (PVMV) and Cucumber mosaic virus (CMV) in Nigeria (Arogundade et al., 2012). However, most viral diseases said to be infecting pepper were identified based on the basis of symptoms expression. Identifying the specific common viral diseases of pepper would facilitate understanding and knowledge of how best to manage them to prevent production losses. Hence, this study aimed at determining the incidence and severity of viral diseases of pepper growing areas of south-west Nigeria.

MATERIALS AND METHODS

Survey and sample collection

Surveys were conducted in pepper farms for viral disease incidence and severity during the 2010 and 2011 planting seasons in six states: Oyo, Ogun, Osun, Ondo, Ekiti and Lagos. Thirty four farms were surveyed in 2010 and 25 in 2011. For each location within a state, farms not less than 0.5 acre (2000 m²) were selected for evaluation. Observation was taken on 30 plants per site by walking along a W-shaped path in a field with plants on each side spaced at equal distance from one another. Five plants were randomly sampled from the population of plant showing virus symptoms. In each local government area (LGA), 625 leaf samples from as many plants showing symptoms of mosaic, chlorosis, yellowing, stunting, mottle, necrosis, leaf deformation and leaf bunching (Figure 1) were collected and stored in zip-lock plastic sampling bags. Percentage

incidence of viral diseases was calculated and the severity score of the plants counted for disease incidence was calculated using the scale of 1-6: 1. No visible symptoms; 2. Mild mosaic/mottling/yellowing/mild necrosis on few leaves /branches of a plant (symptoms on less than 25% of the plant); symptom recovery; 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); and 6. Severe mosaic/puckering/mottling/yellowing/necrosis and severe stunting (entire plant), and death of the infected plants.

Mean of these scores ≥ 2 was expressed to determine the average severity of viral diseases in the field.

Antigen coated plate-enzyme-linked immunosorbent assay (ACP-ELISA)

Leaf samples were bagged separately and kept on dry ice while in the field until they were brought into the laboratory for further analysis. Leaf samples collected were stored at 4°C. These samples were tested using ACP-ELISA for the presence of Potato virus Y (PVY), Potato virus X (PVX), PVMV, Pepper mild mottle virus (PMMV), Tobacco mosaic virus (TMV), CMV, Tobacco Etch virus (TEV) and Tomato mosaic virus (ToMV) using homologous rabbit polyclonal antiserum available in the Virology Unit at IITA-Ibadan. About 0.1 g of the leaf sample was ground in 1 ml of carbonate coating buffer (0.015 M Na₂CO₃ and 0.0349 M NaHCO₃); 100 μ l was dispensed into each well of the ELISA plate. The plate was incubated at 37°C for 1 h and later washed three times with phosphate buffered saline (PBS) containing 0.05% (v/v) Tween-20 (PBS-T) with a 3 min interval between each wash. Polyclonal antiserum was cross-adsorbed in healthy pepper leaf sap extract (1:20 w/v) diluted in the conjugate buffer (PBS-T containing 0.02% (w/v) egg albumin and 0.2% (w/v) PVP-40,000). All the antisera used were diluted at 1:1000 (v/v) in conjugate buffer except CMV that was diluted at 1:3000 (v/v), and 100 μ l polyclonal antisera were used for virus detection. After incubating the ELISA plate for 1 h at 37°C, the plate was washed three times with PBS-T. One hundred (100) μ l of alkaline phosphatase conjugated anti-rabbit antibody diluted at 1:15000 (v/v) in conjugate buffer was used as secondary antibody and the plate was incubated at 37°C for 1 h. The plate was washed three times with PBS-T, and 100 μ l of 0.001 g/ml of *p*-nitrophenyl phosphate in 10% (v/v) diethanolamine buffer (pH 9.8) was added per well and incubated at room temperature for 1 h. Healthy pepper plants (*Capsicum* spp.) were used as the negative control. After 1 h the absorbance was measured at 405 nm in a BIO-RAD multiscan ELISA reader (ELx 800, Universal Microplate Reader). The samples were considered as virus positive when the ELISA reading was at least twice that of the healthy pepper leaf sap control.

Individual virus incidence was determined as a percentage of the total number of infected samples over the total leaf samples analyzed. Statistical analysis system (SAS) v2008 was used to analyze incidence and severity. Means were separated using Duncan's Multiple Range Test (DMRT) at 5% level of probability.

RESULTS

Disease incidence

The average disease incidence recorded was 79% in 2010 (Table 1) and 76% in 2011 (Table 2). The incidence



Figure 1. Viral disease symptoms observed on pepper in the farmers' fields. A= Sweet pepper plant infected with PVY, PVX, CMV and TEV; B= Hot pepper plant with CMV, PVY, PVX, TEV, PVMV, ToMV, TMV and PMMV; C= Long cayenne plant infected with PVY, PVX, PMMV, CMV and TEV; D= Hot pepper plant infected with PVY, PMMV, CMV and TEV.

and severity of infection varied significantly depending on the location. In general, the disease incidence was highest in Ekiti state (96.67%) in 2010, while Oyo had the least (66.90%) (Table 1). Ondo had the highest incidence in 2011 with 91.67%; followed by Oyo state with 80.17%, Osun had the least with 65.17% (Table 2). The south-west agro-ecological zone of Nigeria had an average disease severity score of 2.9 during the 2010 surveys and 2.8 in 2011. The disease severity was highest in Osun (average severity score of 3.1), closely followed by Ekiti (3.0). The lowest level of disease severity was recorded in Lagos state (2.6) during the 2010 surveys (Table 1). As for incidence, disease severity was highest in Ondo (3.3), followed by Oyo (2.9) during the 2011 surveys (Table 2).

Prevalence, distribution and relative occurrence of pepper viruses

Data generated from the survey revealed the relative

occurrence of viruses as follows: PVY 78.67%, PVX 57.17%, PVMV 57.5%, PMMV 43.0%, TMV 38.17%, CMV 61.17%, TEV 66.5% and ToMV 22.5% in the zone during the periods under survey (Table 3). The relative occurrence of PVY was highest in across all states compared to the other seven viruses. ToMV was not detected in Ekiti and Lagos (Table 3). The viruses detected were found to be distributed throughout the states surveyed. However, single infection were not detected in any of the states surveyed as all area had more than one viral infection in the infected plants (Table 4) and the viruses were distributed throughout south-west Nigeria (Figure 2).

DISCUSSION

The surveys showed that the incidence and severity of viral diseases of *Capsicum* spp., were generally high across the zone. A relatively high average of 78% incidence and an average severity index of 2.8 were

Table 1. Incidence and severity of virus diseases of pepper in south-west Nigeria in 2010.

State/local government area (LGA)	Locations	Incidence (%)	Severity
Oyo state			
Iseyin	Iseyin	96.6 ^{ab}	3.3 ^{abc}
Oyo East	Ogunroko	36.7 ^{fg}	2.0 ⁱ
Atiba	Araoyo	31.7 ^{fg}	2.8 ^{cdefg}
Oyo West	Abaoja	60.0 ^{de}	2.9 ^{cdefg}
Surulere	Onipanu	100 ^a	3.1 ^{bcd}
Oriire	Areago	73.3 ^{bcd}	2.5 ^{gh}
Ogbomosho South	Ogbomosho	70.0 ^{cde}	3.0 ^{bcdef}
Ekiti state			
Ikere	Ikere Ekiti	93.3 ^{abc}	3.0 ^{bcdef}
Ekiti South West	Ogotun Ekiti	100 ^a	3.0 ^{bcde}
Ido Osi	Aaye Ekiti	100 ^a	3.1 ^{bcd}
Ikole	Oko Isaba	93.3 ^{abc}	2.9 ^{bcdefg}
Osun state			
Oshogbo	Oshogbo	83.3 ^{abcd}	2.6 ^{efgh}
Atakunmosa West	Osunjela	93.3 ^{abc}	2.6 ^{efgh}
Orolu	Oko atan	70.0 ^{cde}	2.7 ^{defgh}
Oriade	Erinmo Ijesha	100 ^a	3.7 ^a
Ede South	Akoda and Alesin	100 ^a	3.7 ^a
Ogun state			
Abeokuta North	Ijale papa	70.0 ^{cde}	2.5 ^{fgh}
Odeda	Onidundu	93.3 ^{abc}	3.4 ^{ab}
Ewekoro	Wasini	73.3 ^{bcd}	2.8 ^{cdefg}
Yewa North	Aiyetoro	60.0 ^{de}	2.7 ^{defgh}
Ato	Odoputu	46.7 ^{ef}	3.0 ^{bcdef}
Lagos state			
Eredo LCDA	Erinmope	96.7 ^{ab}	2.8 ^{defgh}
Epe	Araga	20.0 ^g	2.0 ¹
Ijede	Igbodo	95.0 ^{ab}	2.9 ^{cdefg}
Ondo state			
Akure South	Akure	91.7 ^{abc}	3.3 ^{abc}
Akoko SouthWest	Ose	90.0 ^{abc}	2.9 ^{cdefg}
Akure North	Oba ile	93.3 ^{abc}	2.6 ^{efgh}
Ose	Elegbeka	73.3 ^{bcd}	2.3 ^{gi}
Average		78.74	2.86

Mean values followed by the same letter in different segment are not significant at P≥0.05 using the New Duncan Multiple Range Test (NDMRT).

observed in the two years surveys that were conducted. The serological method used in this study revealed the occurrence of eight viruses in pepper fields across the states studied. The PVY is the most commonly occurring plant virus in pepper followed by TEV, CMV, PVMV, PVX,

PMMV, TMV and ToMV. Previous studies also identified PVY and PVMV as the most common viruses infecting pepper (Green and Kim, 1991; Atiri, 1992; Fajinmi, 2010) in the same zone. The high occurrence of the viruses especially PVY, TEV, CMV, PVMV and PVX in the zone

Table 2. Incidence and severity of virus diseases of pepper in south-west Nigeria in 2011.

State/local government	Locations	Incidence (%)	Severity
Oyo state			
Ido	Gbekuba	100 ^a	3.4 ^{ab}
Iseyin	Iseyin	95.0 ^{ab}	2.8 ^{cdefg}
Surulere	Onipanu	64.2 ^{cd}	3.1 ^{abcde}
Egbeda	Osegere	76.7 ^{bc}	3.2 ^{abcd}
Atiba	Aba Olokun	65.0 ^{cd}	2.4 ^{gh}
Ekiti state			
Ikole	Oko-Isaba	68.3 ^{cd}	2.4 ^{gh}
Ekiti Southwest	Ogotun Ekiti	100 ^a	2.9 ^{b^{cdef}}
Ado	Iworoko	88.3 ^{ab}	2.8 ^{cdefg}
Ido-Osi	Aaye Ekiti	55.0 ^d	2.1 ^h
Osun state			
Ijesha East	Ilesha	63.3 ^{cd}	2.5 ^{fgh}
Ayedade	Odeomu	89.0 ^a	3.1 ^{abcde}
Atakunmosa East	Kajola	50.0 ^d	2.3 ^{gh}
Boluwaduro	Abalota	58.3 ^{cd}	2.7 ^{defg}
Oriade	Erinmo Ijesha	83.3 ^{ab}	3.3 ^{ab}
Ogun state			
Odeda	Alagbata	50.0 ^d	2.3 ^{gh}
Ewekoro	Baaye	68.3 ^{cd}	2.5 ^{fgh}
Yewa North	Joga Orile	95.0 ^{ab}	2.9 ^{b^{cdef}}
Abeokuta North	Denro	55.0 ^d	2.7 ^{defg}
Lagos state			
Eredo LCDA	Erinmope	96.6 ^{ab}	2.8 ^{defgh}
Epe	Araga	20.0 ^g	2.0 ¹
Ijede	Igbodo	95.0 ^{ab}	2.9 ^{cdefg}
Ondo state			
Akure South	Akure	100 ^a	3.5 ^a
Akure North	Oba-Ile	95.0 ^{ab}	3.9 ^a
Akoko Southwest	Ago Eleye	78.3 ^{bc}	2.6 ^{efg}
Owo	Igbo Oke	93.3 ^{ab}	3.3 ^{abc}
Average		76.12	2.82

Mean values followed by the same letter in different segment are not significant at $P \geq 0.05$ using the New Duncan Multiple Range Test (NDMRT).

Table 3. Frequency of pepper viruses detected in leaf samples collected from farmers' fields in south-west Nigeria.

State	Incidence of viruses detected (%)							
	PVY	PVX	PVMV	PMMV	TMV	CMV	TEV	ToMV
Oyo	69	50	65	31	15	58	56	13
Ekiti	76	52	38	29	38	29	62	0
Osun	97	78	97	83	52	91	95	57

Table 3. Contd

Ogun	76	24	24	29	29	35	49	6
Lagos	72	63	45	27	36	72	55	0
Ondo	82	76	76	59	59	82	82	59
Average	78.7	57.2	57.5	43.0	38.2	61.2	66.5	22.5

PVY, Potato virus Y; PVX, potato virus X; PVMV, PJEPPER veinal mottle virus; PMMV, pepper mild mottle virus; TMV, tobacco mosaic virus; CMV, cucumber mosaic virus; TEV, tobacco etch virus; ToMV, tomato mosaic virus.

Table 4. Distribution of viruses detected in the infected pepper plants in surveyed areas.

Location	LGA	State	Syptoms	PVY	PVX	PVMV	PMMV	TMV	CMV	TEV	ToMV
Oko Isaba	Ikole	Ekiti	Puc, Mo, M	1	1	1	1	1	0	1	0
Ogotun Ekiti	Ekiti Southwest	Ekiti	M, Mo, Puc	1	1	1	1	1	1	1	0
Iworoko	Ado	Ekiti	Puc, M, St, Y,Fd	1	0	1	0	0	1	1	0
Aaye	Ido-Osi	Ekiti	M, Puc, Mo, Y	1	1	1	1	1	1	0	0
Erimope	Eredo LCDA	Lagos	M,Mo	1	1	1	1	1	1	0	0
Araga	Epe	Lagos	Puc	1	1	1	1	1	1	1	0
Igbodo	Ijede LCDA	Lagos	M	1	1	1	0	0	1	0	0
Alagbata	Odeda	Ogun	Mo, M	1	0	0	0	0	1	0	0
Baaye	Ewekoro	Ogun	M, Vy, Puc, Lc	1	0	0	0	1	1	1	0
Joga Orile	Yewa North	Ogun	Vy,Lc,M,Puc, Y	1	0	0	1	0	0	0	0
Denro	Abeokuta North	Ogun	Puc,Vy,M	1	1	1	1	1	1	1	1
Akure	Akure South	Ondo	M,Puc,Mo,Y	1	1	1	0	0	1	1	0
Oba Ile	Akure North	Ondo	M,Mo,Puc	1	1	1	0	0	1	1	0
Ago Eleye	Akoko Southwest	Ondo	Puc,M,Y	1	1	1	1	1	1	1	1
Igbooke	Owo	Ondo	Y,M,Vy,Puc,Fd	1	1	1	1	1	1	1	1
Ilesha	Ilesha East	Osun	Mo,M	1	0	1	0	0	1	1	0
Erinmo Ilesha	Oriade	Osun	Mo,Puc,M,Lc	1	1	1	1	1	1	1	1
Odeomu	Ayedade	Osun	M,Y,Lc,Vy	1	1	1	1	1	1	1	1
Kajola	Atakunmosa East	Osun	M,Puc	1	1	1	1	1	1	1	1
Abalota	Boluwaduro	Osun	M,Y,Vy,Ld	1	1	1	1	1	1	1	1
Gbekuba	Ido	Oyo	M,Mo,Y	1	1	0	0	0	1	1	0
Iseyin	Iseyin	Oyo	M,Mo,Puc	1	1	1	0	0	0	1	0
onipanu	Surulere	Oyo	M,Mo,V	1	1	1	1	1	1	1	1
Osegere	Egbeda	Oyo	M,Lr	1	1	1	0	0	1	0	0
Aba Olokun	Atiba	Oyo	Y,Mo,Puc,M	1	1	1	1	1	1	1	0

Virus detected by antigen coated-plate enzyme-linked immunosorbent assay (ACP-ELISA); 1,Virus positive; 0, Virus negative. Lc, Leaf curl; Mo, Mottle; M, Mosaic; Puc, Puckering; Y, Yellowing; Ld, Leaf distortion; Vy, Vien yellowing; St, stunting; Fd, fruit deformation; PVY, potato virus Y; PVX, potato virus X; PVMV, pepper veinal mottle virus; PMMV, pepper mild mottle virus; TMV, tobacco mosaic virus; CMV, cucumber mosaic virus; TEV, tobacco etch virus; ToMV, tomato mosaic virus.

might be connected with their ability to remain infective for many months in alternative weed hosts together with a good breeding environment for the vectors of the virus that aids effective transmission (Agrios, 2005). Moreover, the major means for PMMV, TMV and ToMV to spread is through the use of infected pepper seeds as planting material as only a few seedlings need to be infected for the viruses to spread rapidly by vectors (AVRDC, 2014).

The rapid spread within crops could occur due to mechanical transmission by workers (from contaminated hands, clothing, and tools) during routine farm operations such as transplanting, pruning, grafting, and other farm activities.

Management necessitates good agronomic practices such as the control of weeds within and around pepper farms, which can reduce virus inoculum and breeding of

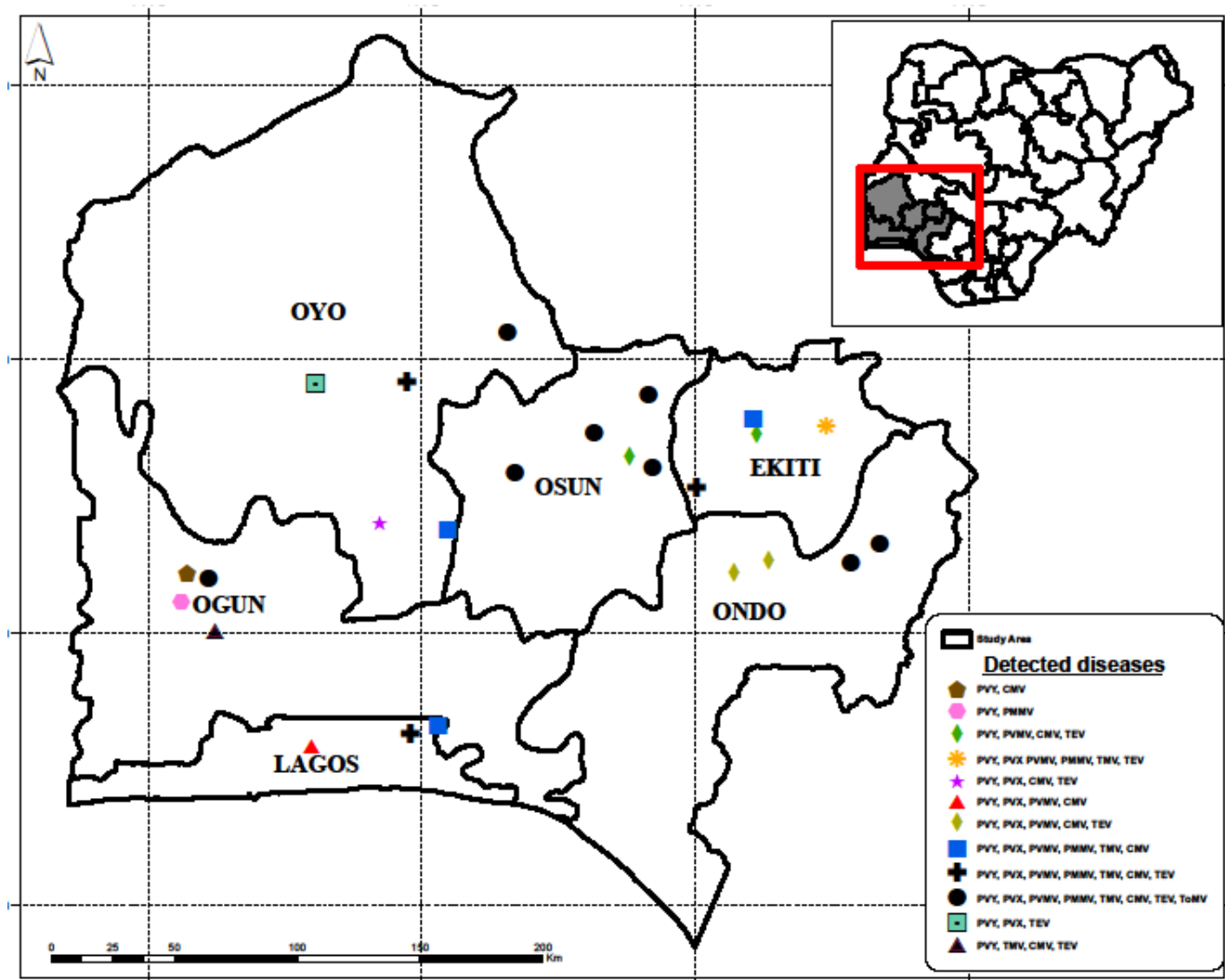


Figure 2. Distribution of eight viruses infecting pepper in south-west Nigeria.

vectors of plant viruses. Production and use of virus-free seeds by pepper farmers in Nigeria, breeding for virus resistance, and the adoption of efficient seed certification systems can reduce virus incidence and production losses due to plant virus infection.

Conflict of interests

The author(s) did not declare any conflict of interest.

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