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MOLECULAR DETECTION AND PHYLOGENY OF VIRUSES ASSOCIATED WITH PEPPER (*Capsicum* spp.) CULTIVATED IN SOME STATES IN NORTHERN GUINEA AND SUDAN SAVANNAH ZONES OF NIGERIA

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ABSTRACT

Surveys were conducted to assess the occurrence of viruses on pepper in 2020 and 2021 dry and wet seasons, respectively. Farms in Kaduna, Kano, Bauchi and Gombe States were visited for sampling and sample collection. Leaf samples from plants showing typical viral symptoms were collected for laboratory analysis. The genomic nucleic acid was isolated from the samples by phenol – chloroform separation and purification. The viral coat protein was amplified in Polymerase chain reaction using specific primers and sequenced. Sequences were aligned and used to search the GenBank database for viruses with high similarity. Sequences with high similarity scores were retrieved and used for phylogenetic analysis. Field occurrence of virus-like symptomatic pepper plants ranged from 10 – 70 %. Sequence analysis and phylogeny revealed that the virus infecting Pepper in locations surveyed was highly homologous (94.52%) to *Pepper veinal mottle virus*. This represents the first confirmation of *Pepper veinal mottle virus* on pepper in the study areas using novel molecular techniques. It is recommended that more extensive studies be conducted to cover the other northern states being the major pepper growing region of Nigeria. It should be emphasized that caution be exercised in the movement of planting materials across regions, especially between neighbouring countries because of the known impact of viruses on vegetable production in general, and pepper in particular.

Keywords: Nigeria, Pepper, Phylogeny, Savanna, Sequencing, Viruses

INTRODUCTION

Pepper (*Capsicum* spp.) is a member of the Solanaceae family that comprises many crop species. Globally, it occupies the position of being the second most important vegetable crop after tomato (Yoon *et al*., 1989; Aliyu, 2014). The crop is widely used in many parts of the world for different purposes (Amusa *et al*., 2004; Pamplona-Roger, 2007; Drafor, 2014).

The major pepper cultivation ecologies in Nigeria are the rain forest, derived savanna, guinea savanna and sudan savanna (Erinle, 1988). Bosland and Vativa (2000) puts the cultivation of pepper in Nigeria at an average yield of 1,021kg/ha. Pepper like many other crops, is susceptible to damage by pests and pathogens with potential consequences of economic yield losses if not controlled.

Virus diseases are major causes of losses in the pepper industry with potential of becoming the most limiting factor of its production (Makkouk and Gumpf, 1974). Notable symptoms of virus infection on pepper include mosaic, yellowing, leaf curling and mottling, dwarfism and death of infected plants with consequential reductions in crop growth and fruit yield (Jin *et al*., 2009). An estimated 68 viruses are known to infect peppers worldwide (Pernezny *et al*., 2003). Eleven of these viruses had been reported in Africa (Nono-Womdim, 2003) with *Pepper veinal mottle virus* (PVMV) and *Tomato yellow leaf curl virus* (TYLCV) being most widespread in the West Africa sub-region (Dafalla, 2001). Many other viruses of varying significance and impact had been detected and reported on pepper in Nigeria (Arogundade *et al*., 2012; Aliyu, 2014) mainly, using visual assessment and serological tests.

The research work done so far on viruses of tomato and pepper in Northern Nigeria has primarily dwelled on the use of field observations and serological tests. These procedures usually, are devoid of precision required for accurate detection of viruses, and are therefore, prone to the possibility of non-detection of key viruses for management and breeding purposes. Serological tests also, have the challenge of cross-reactions within closely related virus species. Conversely, molecular detection procedures of viruses are more precise in identification and diversity studies of viruses (Kumar, 2009). The current research was focused on establishing the genetic identity and determining the diversity of pepper-infecting viruses in some States in North West and East of Nigeria where the crop is cultivated in larger quantities. It was envisaged that the results obtained would reveal the identity of the viruses, place them in context with existing species elsewhere and provide a basis for proactive development of management strategies. The ultimate goal is to reduce the damage induced by the

viruses and enhance the sustainable and profitable production of pepper.

MATERIALS AND METHODS

Field sampling and sample collection

Surveys were conducted to assess the occurrence of viral diseases and collect leaf samples from pepper fields in Kaduna, Kano, Gombe and Bauchi States of Nigeria. Sampling and sample collections were be made in three farms of each crop per Local Government Area (LGA) and five LGAs in each State. Sampling was done along two diagonals on each farm. Infected and the total number of plants along each diagonal were counted. Disease incidence was expressed as a percentage of the diseased plants in the total population of plants assessed. Depending on the size of the farm, 10 – 15 symptomatic and asymptomatic samples were collected per farm. The pepper leaf samples collected were rolled into labelled vials containing glycerol for processing at the Virology Laboratory, Department of Crop Protection, Ahmadu Bello University, Zaria. The co-ordinates of each farm will be taken using a General Positioning System (GPS) and used to generate a map of the surveyed locations (Fig. 1).

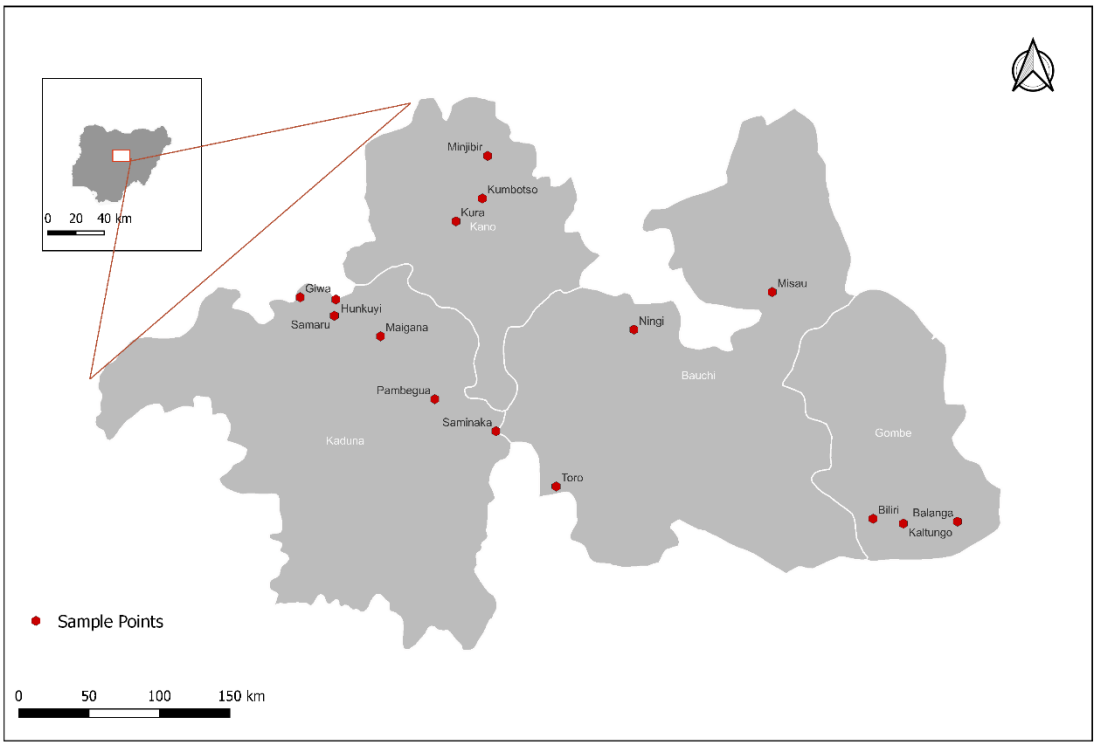

Figure 1: Map of States indicating locations where pepper farms were surveyed and samples collected.

Extraction and amplification of nucleic acid

The glycerol was washed off the samples using sterile distilled water and genomic DNA was purified from the leaf samples using the CTAB method described by Doyle and Doyle (1987). The concentration and quality of the DNA was determined using the NanoDrop (Nano Drop, USA) in 2 µl of DNA measured against a blank background and stored at -20 ºC until needed.

To check for viral coat protein, the extracted DNA was subjected to polymerase chain reaction (PCR) test using the universal core coat protein primers (core AV 5' GCC HAT RTA YAG RAA GCC MAG RAT 3' and core AC 5' GGR TTD GAR GCA TGH GTA CAN GCC 3') (Abdel-Salam *et al*., 2006) in 25 µl PCR mixture containing nuclease-free water (18.4 µl), buffer (2.5 µl), dNTPs (1.0 µl), primers (0.5 µl each), Taq polymerase (0.1 µl) and DNA (2.0 µl). The amplification cycles were one cycle of initial denaturation at 95 ºC for 1 min and 35 cycles of denaturation at 95ºC, annealing at 58 ºC and extension at 72 ºC for 1 min each, and a final cycle of extension for 10 min.

Sequence analysis and phylogeny

The amplicons were sequenced and used for sequence and phylogenetic relationship analyses. The coat protein (CP) gene sequences were edited and compared for similarity with sequences of other viral species in GenBank database using the BLASTn algorithm of the National Centre for Biotechnology Information (NCBI) for similarity. Highly homologous and non-homologous sequences were retrieved for pair wise comparison. The phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (Tamura *et al.*, 2013).

RESULTS AND DISCUSSION

Typical viral symptoms (Plate 1) were observed on the pepper plants during the field survey. Field incidence of symptomatic pepper plants ranged from 10 – 70 %. Presence of virus infection in the samples was confirmed by PCR yielding approximately 650 bp amplicon sizes (Fig. 2).

Plate 1: Pepper plants showing typical mottling (A), yellowing (B) and mosaic (C) symptoms of viral infections generally observed across locations in the study areas.

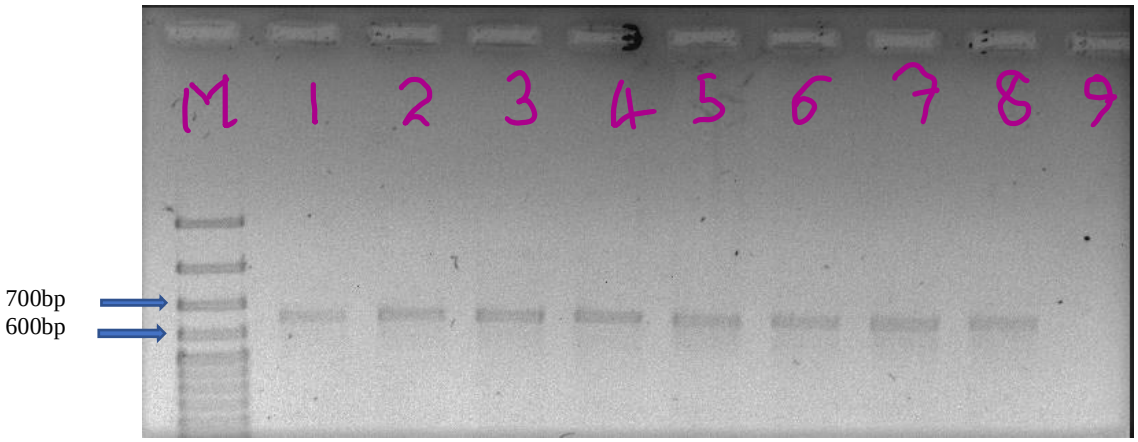

Figure 2: Polymerase chain reaction amplification of 650 bp viral coat protein gene from Pepper leaf samples. M = 1 Kb ladder; 1 – 8 = field leaf samples; 9 = healthy sample.

The sequences were used to check for sequence identity/diversity with other viruses are presented in Table 1. The highest sequence similarity was between isolate PepM10 (94.52%) and *Pepper veinal mottle virus* (LC438545.1), followed respectively, by isolates PepM7 (91.61%), PepG10 (87.19%), PepM9 (86.75%) and PepM4 (84.67%) (Table 1). The range of percentage sequence similarity with retrieved virus sequences was 70.30 to 94.52% (Table 1).

The phylogenetic relationship of sequenced pepper isolates was analyzed with selected viruses from the GenBank database. The sequences from pepper isolates clustered more with *Pepper veinal mottle virus*, while two isolates (PepM7 and PepM9) which are 100 % identical clustered distantly (54%) with the *Onion yellow dwarf virus* cluster (Fig. 3).

Table 1: Percent identity of sequenced pepper isolates with selected sequences of viral species from the GenBank database.

S/N	Virus species	Accession No	Percentage identity				
			PepM4	PepM7	PepM9	PepM10	PepG10
1	*African eggplant mosaic virus*	NC043537.1	78.38	77.78	77.81	77.30	77.96
2	*Bean yellow mosaic virus*	KX757143.1	-	-	-	74.55	-
3	*Chilli ringspot virus*	JQ234922.1	76.45	74.70	74.34	75.76	75.97
4	*Chilli veinal mottle virus*	MN508959.1	73.90	74.91	75.91	76.24	75.41
5	*Japanese yam mosaic virus*	KJ789139.1	-	-	-	-	-
6	*Maize dwarf mosaic virus*	MZ326124.1	-	71.83	-	-	-
7	*Pepper veinal mottle virus*	LC438545.1	84.67	91.61	86.75	94.52	87.19
8	*Soybean mosaic virus*	KF982784.1	-	-	-	75.09	-
9	*Sugarcane mosaic virus*	MG932076.1	-	-	72.62	-	-
10	*Sweet potato latent virus*	MK778600.1	-	70.30	-	-	-
11	*Tobacco vein banding virus*	L28616.1	76.77	-	74.60	74.15	76.51
12	*Wild tomato mosaic virus*	KF918754.1	76.92	75.87	-	75.68	-

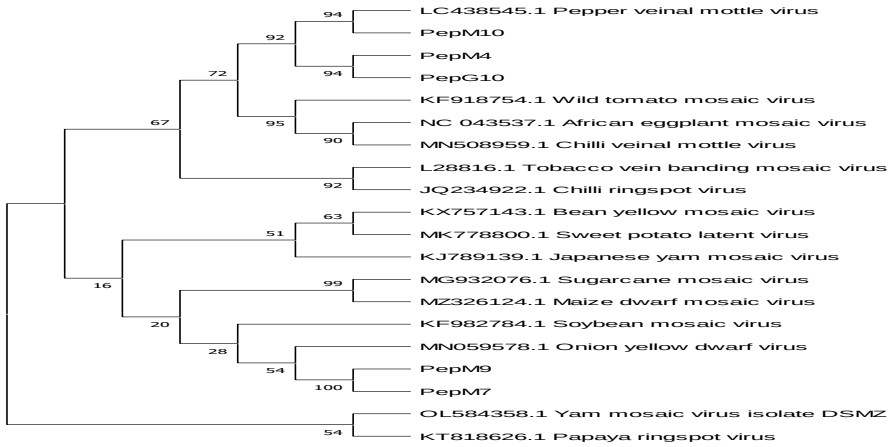

Figure 3: Phylogenetic relationship of sequenced pepper virus isolates with selected viruses based on coat protein gene sequences

In this study, field visits were conducted during which leaf samples from plants showing typical virus symptoms of mosaic, yellowing and leaf mottling. Molecular studies were conducted for diagnosis, genetic diversity and determination of phylogenetic relationships with associated viruses. In this study, based on the sequencing and sequence analysis, the virus sequenced from the pepper samples was identified to be *Pepper veinal mottle virus*. The identification of *Pepper veinal mottle virus* from sequenced isolates from pepper indicates that the virus is associated with the diseased symptoms observed on the field – grown pepper plants. The transmission and spread of the virus to pepper portends grave danger to pepper cultivation and vegetable growers in Nigeria (Arogundade *et al*., 2012) and globally (Hanssen *et al*., 2010; Thakur *et al*., 2018). Genetic variability is an adaptative mechanism that is common with plant viruses and favors the emergence of new virus strains/species (Ahmad *et al*., 2018). Researchers have at various times and in various locations reported the genetic

diversity and variability of begomoviruses from solanaceous crops (Idris *et al*., 2012; Khan *et al*., 2013; Kenyon *et al*., 2014). Factors often linked to this include changes in genomic sequences, presence of vectors, climatic conditions, changing cropping systems, frequent recombination and mutation of viral genomes (Seal *et al*., 2006).

To develop sustainable and durable disease management strategies against viruses require information on their genetic variability, evolution and host plant interaction. There is an urgent need for detailed studies on plant viruses and known or unknown host plants symptomatic or asymptomatic as well as weed species as likely alternate/alternative hosts in Nigeria. This information will prove valuable for resistance breeding and management purposes.

CONCLUSION AND RECOMMENDATIONS

The study provided information on the occurrence of viruses on pepper grown in some selected States in northern Nigeria. *Pepper veinal mottle virus* is identified as the major virus infecting pepper in Nigeria from sequence identity of isolates in the study area. The movement of planting materials must be done responsibly and legally to avoid inadvertent introduction and spread of viruses to hitherto, healthy environments and crops. This is critical to the protection of pepper and other economically important crops. Regular virus disease survey and monitoring is necessary for their effective and sustainable management.

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