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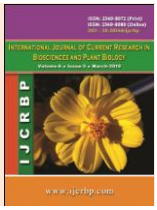

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Molecular genetic identification of viruses affecting pepper crop (*Capsicum* spp.) in Western North of Benin

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ABSTRACT

Chilli (*Capsicum* spp.) is a spice of economic importance and is widely cultivated for its fruits in Benin. Viral infestations are one of the major constraints that hamper agricultural production. The objective of the present work is to identify the viruses that affect off-season pepper cultivated in the Donga Department in north west Benin. All the communes of the Department were prospected and 16 fields were sampled. A total of 43 pepper accessions with leaves showing symptoms of virus diseases were collected. Six (6) phytovirus-specific primer pairs belonging to three botanical genera (Begomovirus, Tobamovirus and Potyvirus) were used for molecular identification. The results revealed the presence in the Department of Phytovirus of the genus Begomovirus present on 53.43% of the accessions, the genus Potyvirus present on 37.20% of the accessions and at last the genus Tobamovirus present on 6.97% of the accessions. The geographic distribution of the infestation remains globally high in all communes of the Department with 83.33% of the accessions in Copargo; 65.2% in Bassila, 53.83% in Djougou, and 75% in Ouaké. These results are significantly important for the definition of an improvement policy of off-season chilli production by setting up a program to control viral diseases.

Introduction

Agricultural productivity improving is one of the major concerns in sub-Saharan Africa to save food security (Assogba-Komlan et al., 2009). In Benin, agricultural production can cover more than 80% of national food needs. Nevertheless, this production still faces to many constraints with consequent food insecurity. Truck farming has become an activity that responds effectively to urban food demand (Assogba-Komlan et al., 2009). Truck farming products are common in people's eating habits. Based on the production, peppers are the second largest truck farming crop after tomatoes (FAO, 2014). It belongs to the genus of *Capsicum* and Solanaceae family. Its order is Solanales of Dicotyledonous class and Magnoliophyta phylum (Adetula and Olakojo, 2006; Dagnoko et al., 2013). It is a spice widely grown in the world. In Benin, it is grown in almost all Departments. Pepper is cultivated in normal season and counter season. It is produced in home

gardens, perimeters of agricultural development, in mono-culture or in association with other crops such as tomato, corn, peanut, cowpea, soy, cassava, yam, etc.

The importance of peppers in human nutrition is crucial (Adetula and Olakojo, 2006). Leaves, fruits and roots are all used in sauce preparation (Assogba-Komlan et al., 2009). Chili can be eaten fresh, fried, in sauce or canned. It is often combined with other vegetables. Also known for its medicinal properties, the fruits of *Capsicum* spp. are used in traditional medicine for their antimicrobial properties due to the secondary metabolites they contain (Jin et al., 2009). Despite all these potentialities, the pepper crop remains subject to many biotic constraints (insect pests, various infectious diseases) and abiotic constraints that cause significant yield losses. In Benin, it is currently noted, an emergence of viral diseases that cause yield losses estimated at nearly 90% (Orobiyi et al., 2013). The improvement of the production of this important species becomes an urgent action. In the first step, it is needed to identify the viruses that affect the crop to control their expansion. The general objective of this work is to identify some groups or species of viruses that affect the pepper crop in Western North of Benin.

Materials and methods

Area of study and survey

This study took place in four townships (communes) of Donga Department: Djougou, Bassila, Copargo and Ouaké (Fig. 1). This Department covers an area of 11,126 km². It has 177 villages in the four townships. It constituted of 26 districts and located in Western North Benin with Djougou as capital. It belongs to agro-ecological zone IV characterized by tropical climate. Fourteen villages were selected according to their areas of pepper production and high virus incidence in the zone. In total, 16 fields were surveyed. In each field, the severity was recorded on 15 randomly selected plants expressing pepper virus symptoms (Fanou et al., 2017).

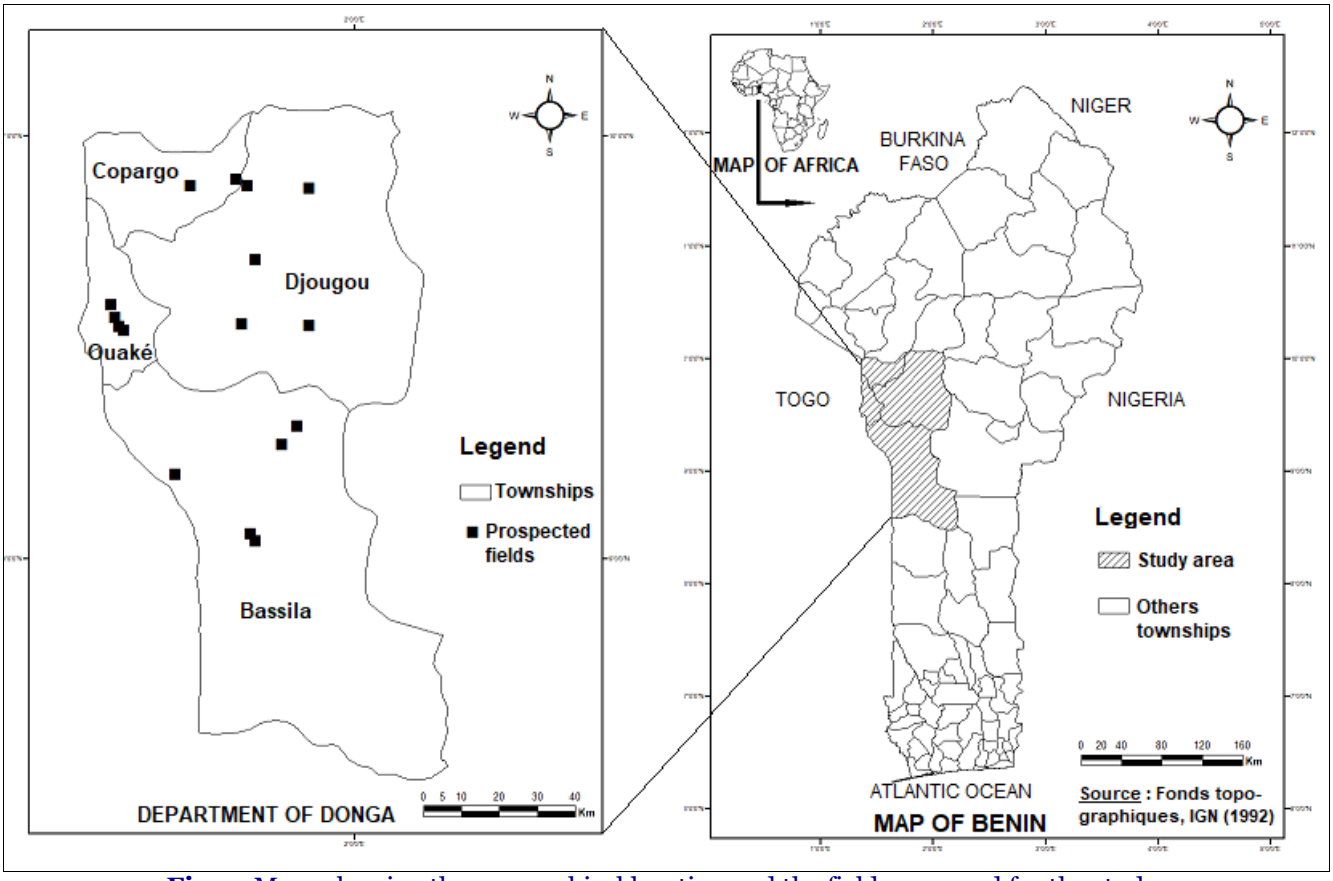

Fig. 1: Maps showing the geographical location and the fields surveyed for the study.

Plant material

The plant material used consists of forty-two (42) leaves samples of young pepper accessions showing virus symptoms collected in different fields surveyed. The collected leaves were kept in aluminum paper and chilled until they were back to the laboratory.

DNA extraction

Total genomic DNA is isolated from the collected infected pepper leaves using the protocol described by Dellaporta et al. (1983) with minor modifications. In summary, (i) About 0.2 g of leaf sample was crushed in 500 µl of extraction buffer (50 µl TrisHCl with Ph = 8.0, 8.5 µl of EDTA 0.5 M and 5 µl of βME 100%); (ii) Add 33 µl 20% SDS in each tube, (iii) Brief vortex the mixture and incubate at 65 °C in water bath for 10 min; (iv) Add 160 µl of potassium acetate to the cool mixture, vortex and centrifuge at 10,000 rpm for 10 min; (v) Transfer the supernatant to a new sterile eppendorf tube containing 200 µl of cold isopropanol and incubate at 4 °C for 20 min, centrifuge the solution at 10,000 rpm for 10 min to precipitate the DNA; (vi) Remove the supernatant, wash the DNA pellet with 500 µl of 70% ethanol and dry the DNA at room temperature; (vii) Dissolve the DNA in 50 µl of TE buffer and stored at -20 °C until further use.

Polymerase chain reaction (PCR) amplification and specific primers used

Polymerase chain reaction amplifications were carried out in 25 µl reaction consisting 3 µl of template DNA, 2.5 µl of 10X HCl buffer, 2.5 µl of each primer (F and R).), 1.25 µl of $MgCl_2$, 0.75 µl of dNTPs, 0.75 µl of Taq polymerase and ultra-pure water (Meck water). The reactions are carried out in Peltier-Effect Cycling thermocycler according to a specific amplification program for each primers (Eman et al., 2006; Mnari-Hattabe et al., 2006; El-Araby et al., 2009; Fajinmi et al., 2011).

Four genera and five species of plant virus that generally affect Solanaceae specifically pepper crop were targeted in the study. These are Begomoviruses, Potexvirus, Tobamoviruses and Potyviruses. Specifically, these are three Potyviruses (Pepper mottle virus, Pepper veinal mottle virus, Potato virus Y), a Tobamovirus (*Pepper Mild-Mottle Virus*) a Potexvirus (Potato virus X) and Begomoviruses in general (Eman et al., 2006; Mnari-Hattabe et al., 2006; El-Araby et al., 2009; Fajinmi et al., 2011). The specific primers used are summarized in Table 1. The revelation of PCR products was made by using Ethidium Bromide (BET) on a transilluminator (high performance) after electrophoresis on 2% agarose gel.

Table 1. List of pepper virus tested and primers used.

Virus (acronyme)	Name	Genera	Primer	Size (bp)
PepMoV	*Pepper mottle virus*	*Potyvirus*	P3 : (5'AATGCAAAGCCAACATTC-3') M4 : (5'CTAATACGAACACCAAGCAT-3')	345
PMMoV	*Pepper Mild-Mottle Virus*,	*Tobamo-virus*	P12/3 : (5'-ACAgCgTTTggATCTTAgTAT-3') P12/3A (5'gTgCggTCTTAATAACCTCA-3')	836
PVMV	*Pepper veinalmottle virus*	*Potyvirus*	5'(TC(G/A/T/C)A(T/C)CAT(G/A/T/C)ACCCACAT(G/A/T/C)CC3' 5' ATGGTiTGGTG(T/C)AT(A/T/C)GA(G/A)AA(T/C)GG)3'	650
PVX	*Potato virus X*	*Potexvirus*	v1 : S 5'GAYACNATGGCNCARGCNGCNTGG3' c2 : AS 5' YTGNGCNGCRTTCATYTCNGCYTC 3'·	300
PVY	*Potato virus Y*	*Potyvirus*	S: 5'TCAAGGATCCGCAAATGACACAATTGATGCAGG 3' AS: 5'AGAGAGAATTCATCACATG1TCITGACTCC3'	801
Begomo-virus		*Begomo-virus*	S : 5'AATGCAAAGCCAACATTCGTTAGTGCTGC-3') AS : (5'CTAATACATACGATCGTCGTAGTCGAACACCAAGCAT-3')	700 - 800

Data analysis

The presence and absence of specific band were respectively correlated to an infected plant or non virus-infected plant or the corresponding virus in order to calculate the percentage of infected sample per commune and to assess the infection of viruses and genera of viruses tested in the study area. Infection of the viruses in the collection was correlated with the different pepper genetic groups in the study area.

Results and discussion

Molecular identification of viruses reveals the presence of four targeted genera with specific size bands (Fig. 2). These are Begomoviruses detected on 53.43% of the samples, Potyviruses on 32.57% of the samples, Potexviruses detected on 4.65% and Tobamoviruses present on 6.97% of the collected samples (Table 2).

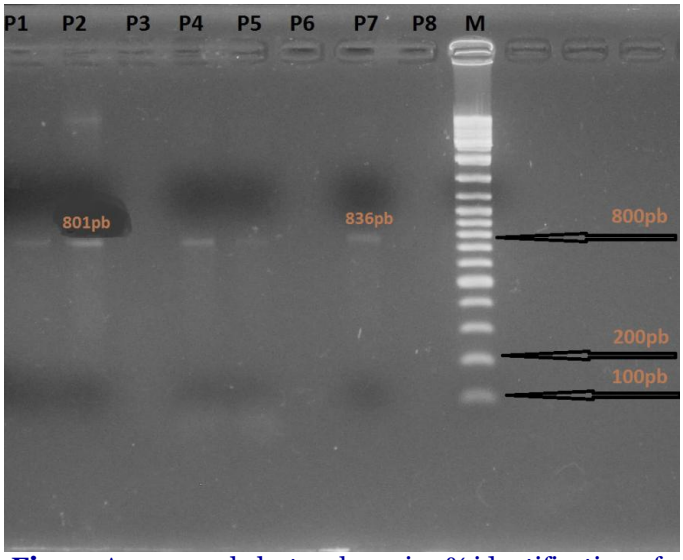

Fig. 2: Agarose gel electrophoresis 2% identification of PMMoV (836 bp) and PVY (801 bp) viruses.

Table 2. Percentage of infections by viruses and virus genus.

Virus	Genus	Infection by genus (%)
PVMV PepMoV PVY	*Potyvirus*	32.57
PVX	*Potexvirus*	4.65
PMMoV	*Tobamovirus*	6.97
Begomovirus	*Begomovirus*	53.43

This identification shows that pepper crop of counter-season in Donga is infected by viruses of four genera. Similar results have been obtained in Egypt (Eman et al., 2006); in Tunisia (Mnari-Hattab et al., 2006); in Nigeria (Fajinmi et al., 2011); in Egypt (El-Araby et al., 2009). Also, Tiendrebeogo in 2010 by working on the characterization and epidemiological aspect of Begomoviruses infesting the vegetables and the cassava in Burkina Faso has detected Potyviruses, Tobamoviruses and Begomoviruses on peppers (*Capsicum annum* and *Capsicum frutescens*). In addition, the first work concerning the identification of tomato and peppers viruses grown in north east of Benin using ELISA tests showed seven viruses grouped in the genus of Cucumovirus, Tobamovirus, Potyvirus and Begomovirus (Afouda et al., 2013). The surveys conducted by these authors both in dry season and rainy season did not show a significant difference for the kinds of viruses infecting the pepper. In our study, Potato Virus X (PVX) (genus: Potexvirus), was detected while Afouda et al. (2017) detected viruses of the genus Cucumovirus and the genus Polerovirus during the survey in southern and north east Benin. These results show that peppers grown in Benin whatever the season may be infected by several kinds of viruses. The large detection of Begomoviruses in this study may be explained by the fact that pepper crop in Donga Department are mostly associated with other crops such as cassava and tomato which are highly susceptible to Begomoviruses (Kabemba et al., 2017). Indeed, Begomoviruses infect many dicotyledonous plant species and cause enormous economic damage in many highly cultivated plants such as tomato, bean, cassava and cotton. The low presence of Potyvirus and Potexvirus Tobamovirus compared to Begomovirus is explained by the fact that in the present study, Begomovirus primers are not specific to a particular type of virus but detect all virus of the genus unlike the genus Tobamovirus, Potexvirus and Potyvirus where the primers detected a specific virus. Two of three virus species targeted of Potyvirus, Potexvirus and Tobamovirus targeted in this study were found to be present on the infected plants. This is for Potyvirus, Pepper Mottle Virus (PepMoV), and Potato Virus Y (PVY); for Potexvirus, Potato Virus X (PVX) and for Tobamovirus, Pepper Mild Mottle Virus (PMMoV). In total, of five targeted virus species, four were

found to affect the pepper crop in Donga Department. The degree of infestation varied from one species of virus to another. It is 20.93% for Pepper Mottle Virus, 11.62% for Potato Virus Y, 6.97% for Pepper Mild Mottle Virus and 4.65% for Potato Virus X.

Regarding the geographical distribution of these viruses, it is presented in all the townships of the Department or 83.33% of the sample collected in Copargo, 75% of the sample of Ouaké, out of 62.5 % of the Bassila sample and finally 53.83% of the Djougou samples (Table 3).

Table 3. Proportion of infected sample with viruses and virus genus identified by township.

Townships	Number of sample	Infected samples	Infection proportion (%)
Bassila	16	10	62.5
Djougou	13	7	53.84
Copargo	06	5	83.33
Ouake	08	6	75
Total	**43**	**28**	**65.11**

This important distribution could be explained to the horizontal transmission either by wind or by vectors such as insects as reported by Yao et al. (2013). Known symptoms of these viruses include mosaic, curling leaf curl, leaf curl and foliar shrinkage (Fanou et al., 2017), which at the score 0.05 show high severity in Pepper field of Donga. Of the twenty-eight sample revealed infected by the viruses tested, eighteen (18) or 64.28% belong to genetic group I (Group I) and the other ten (10) or 35.11% belong to the second genetic group (Group II) from the molecular genetic characterization of the sample (Fanou et al., 2017). This strong presence of viral diseases in group I could be explained by the high presence in this group of *C.chinense* species (65.55%) which is more susceptible to viruses than *C. annuum* and *C. frutescens* species present in group II (Mahbou, 2010).

Conclusion

This study revealed the presence of Pepper mottle virus (PepMoV), Potato virus Y (PVY), Potato virus X (PVX) among the viruses that affect pepper crop in Donga Department belonging to the group of Potyviruses and Pepper Mild-Mosaic Virus (PMMoV) belonging to the group of Tobamoviruses. In general, the study also revealed the presence of Begomovirus genus. The degree of infestation of these viruses varied by species and by genus. The infestation also varied according to the groups of pepper varieties. Thus, the genetic group I essentially characterized by accessions of the species *C. chinense* is more susceptible than the genetic group II characterized by *C. annuum* and *C. frutescens* species. For a better productivity of the pepper production in Donga department other research actions should be undertaken:

- ✓ the identification of specific strains of viruses of the Begomovirus group affecting pepper crops
- ✓ the identification of other virus genus and species that affect pepper crops;
- ✓ the extension of this study to the other departments in Benin where pepper is practiced in order to develop an integrated biological control strategy;
- ✓ the development of a policy to promote pepper cultivation by setting up a virus eradication program.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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