Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 6(3): 1090-1101, June 2012

ISSN 1991-8631

International Journal of Biological and Chemical Sciences

Original Paper

http://indexmedicus.afro.who.int

Genetic diversity among yam mosaic virus (YMV) isolates infecting yam of the complex *Dioscorea cayenensis rotundata* in Togo

Kwasi Dzola AYISAH * and Yawovi Mawuena Dieudonné GUMEDZOE

Université de Lomé, Ecole Supérieure d'Agronomie, Laboratoire de Virologie et de Biotechnologies Végétales, B.P. 1515 Lomé, Togo.

* *Corresponding author, E-mail: dayisah@yahoo.fr; Tél. : +228 225 41 97; Fax: +228 221 85 95*

ABSTRACT

Phylogenetic analyses were carried out using partial CP-encoding nucleotide sequences and 3´-UTR of 31 YMV isolates originated from the five major yam-growing areas of Togo (Maritime, Plateaux, Central, Kara and Savannas Regions). The aim of this study is to know the genetic structure of YMV populations in Togo in order to develop yam resistant genotypes against the virus. Three major clusters of YMV isolates were observed. Clustering of the 31 isolates did not correlate with the geographical origin but, isolates from Maritime, Plateaux and Central Regions, were genetically more variable than isolates from Kara and Savannas Regions. Comparison of the 31 isolates of Togo with 15 nucleotide sequences from Genbank and originated from other parts of Africa and Caribbean, showed that few isolates from Togo were close to the isolates belonging to phylogenetic groups I and III of Africa but the majority were rather, genetically different..These results revealed that YMV isolates infecting yams in Togo, are genetically variable and they are different a little from isolates of other African countries, suggesting a high pathogenic potential of YMV populations in the various yam–growing areas of Togo and consequently problems of improved yam varieties introduction in the country .

Keywords: Yam mosaic virus, *Dioscorea cayenensis rotundata*, molecular variability, genetic group, phylogenetic analysis, yam-growing area.

INTRODUCTION

Yam mosaic virus Genus *Potyvirus* (YMV) is the most widespread and economically important viral disease affecting yams of the complex *Dioscorea cayenesis rotundata* in West Africa. It is detected in all yam-growing regions on most of edible yams in West Africa, in the West India (Brunt et al., 1996), in Caribbean (Goudou-Urbino et al. 1996; Bousalem et al., 2000). YMV, as other species of *Potyvirus*, is transmitted mechanically and by several aphid species in a non-persistent manner (Odu et al., 2004). The virus causes significant losses (Thouvenel and Dumont, 1990; Amusa et al., 2003). In Togo, YMV is detected in all yam-growing areas on the complex *D. cayenesis rotundata*. Up to now, farmers continue to use infected planting material to renew their yam plantations since there are few methods to control the virus. The only efficient and the cheapest method to manage the viral disease is the use of resistant genotypes. But to be successful, it is very important to understand the genetic diversity of the pathogen since viruses, exceptionally the populations of YMV from West Africa as

DOI : http://dx.doi.org/10.4314/ijbcs.v6i3.15

reported by Bousalem et al. (2000), are subject to molecular variability and rapid evolution which may generate genetic variants susceptible to overcome the resistance of planting material introduced.

The diversity among YMV isolates was firstly studied immunologically with monoclonal antibodies by Goudou-Urbino et al. (1996). Molecular diversity of YMV isolates was conducted using different regions of RNA genome and it was established that the coat protein gene was the most variable region of YMV isolates (Duterme et al., 1996; Aleman-Verdaguer et al., 1997; Moury et al., 2002). Phylogenetic analyses of nucleotide sequences of coat protein region of YMV isolates originated from Africa and Caribbean, structured the virus populations into 9 distant molecular groups and correlations were found between groups and the yams host species infected and the geographical origins of the isolates. The highest genetic diversity was found among isolates originating from Africa and collected on the complex *D. cayenensis rotundata* (Bousalem et al., 2000). According to that study, the YMV isolates from Africa were structured in six groups. But in Togo, up to now, no study has been conducted on the genetic variability of YMV isolates infecting yams, particularly the complex *D. cayenensis rotundata*, the most cultivated species in the country. The aim of this study is to determine the genetic structure of the populations of YMV isolates infecting yams of the complex *D. cayenensis rotundata* in all yam-growing areas in Togo. This approach may help developing efficient and sustainable control strategies towards selecting resistant yam varieties. Therefore, we undertake the molecular variability analyses of 31 YMV isolates collected from five yam-growing regions using primers designed in CP and 3'end UTR regions of the viral genome.

MATERIALS AND METHODS

Virus isolates studied

Thirty-one YMV isolates, representing five major yam production areas of Togo (Maritime Region, Plateaux Region, Central Region, Kara Region, Savannas Region), were sampled among 157 isolates previously screened by RT-PCR on leaves of different cultivars of *D. cayenensis rotundata* (Table 1). The number of isolates chosen per region took into account the situation of yam cropping and the incidence of YMV in every region. Isolates were preserved at -20 °C in CIRAD-AMIS, Montpellier until the analyses. Additional sequences of the partial coat protein core region of 15 YMV isolates were retrieved from Genbank database (Table 2). The isolates were from Africa (Togo, Nigeria, Benin, Ivory Coast, Guinea, Ghana, Burkina Faso and Cameroon) and Caribbean (Guadeloupe).

Genome sequencing

Viral genomes of the 31 YMV isolates were previously amplified by RT-PCR with primer pair YMVCP1F& YMVUTR1R (Mumford and Seal, 1997) using one step RT-PCR QIAgen kit. The Nucleotides sequences were obtained by direct sequencing of PCR products (586 bp) at Génome Express Laboratory in France.

RT-PCR for sequencing

Microfuge tubes (0.5 ml, Starlab) were coated with sap (25 µl) obtained by grinding 0.25 g of leaves sample in carbonate buffer pH 9.6. The microfuge tubes were incubated overnight at +4 °C then, washed two times with PBST sterilised and one time with DEPC water. After this step, 25 µl of RT- PCR reaction mixture (QIAgen one step RT-PCR kit) containing a pair of primers, were added to each of microfuge tubes. The primers YMV-CP-1F: 5'ATC CGG GAT GTG GAC AAT GA 3' and YMV-UTR-1R: 5'TGG TCC TCC GCC ACA TCA AA 3' (Mumford and Seal, 1997) were used to amplify a partial Coat protein coding region and the 3'end of UTR region of viral genome.

The RT-PCR reactions were carried out in a Biometra thermocycler. The cycling conditions were: one cycle of RT (50 °C for 30 mn followed by 95 °C for 15 mn), 35 cycles of PCR (1mn of denaturation at 94 °C

Table 1: Lists of the 31 YMV isolates used for molecular variability studies of YMV populations in Togo.

YMV isolates	*Dioscorea* species	Geographic origin (Region)	Reference
M2-4	*Dioscorea cayenensis rotundata*	Maritime Region	This study
M9-30	*D. cayenensis rotundata*	«	This study
M1-4	*D. cayenensis rotundata*	«	This study
P16-28-T11	*D. cayenensis rotundata*	Plateaux Region	This study
P3-20	*D. cayenensis rotundata*	«	This study
P5-28	*D. cayenensis rotundata*	«	This study
P6-9	*D. cayenensis rotundata*	«	This study
P8-15	*D. cayenensis rotundata*	«	This study
P9-13	*D. cayenensis rotundata*	«	This study
P14-13	*D. cayenensis rotundata*	“	This study
P19-11-T1	*D. cayenensis rotundata*	“	This study
P18-1-T4	*D. cayenensis rotundata*	“	This study
P21-2-T14	*D. cayenensis rotundata*	“	This study
P15-11-T2	*D. cayenensis rotundata*	“	This study
P17-15-T20	*D. cayenensis rotundata*	“	This study
P20-20-T17	*D. cayenensis rotundata*	“	This study
C1-3	*D. cayenensis rotundata*	Central Region	This study
C3-22	*D. cayenensis rotundata*	“	This study
C5-22	*D. cayenensis rotundata*	“	This study
C6-7	*D. cayenensis rotundata*	“	This study
C9-25	*D. cayenensis rotundata*	“	This study
K11-6-T6	*D. cayenensis rotundata*	Kara Region	This study
K2-5	*D. cayenensis rotundata*	“	This study
K5-5	*D. cayenensis rotundata*	“	This study
K7-5	*D. cayenensis rotundata*	“	This study

K9-5	*D. cayenensis rotundata*	"	This study
K10-6	*D. cayenensis rotundata*	"	This study
S2-21	*D. cayenensis rotundata*	Savannas Region	This study
S1-21	*D. cayenensis rotundata*	"	This study
E1-21	*D. cayenensis rotundata*	"	This study
E7-21	*D. cayenensis rotundata*	"	This study

Table 2: Additional coat protein-encoding sequences of Yam mosaic virus (YMV) isolates retrieved from Genebank database.

YMV isolates	Geographic origin	Fragment size	GeneBank accession number.	Reference
BFC54.	Burkina Faso	1144	AJ244051	Bousalem and al.2000
CAM1/C1.	Cameroon	1180	AJ244054	Bousalem and al.2000
CAM2/C31.	"	1180	AJ244055	"
CGU2/C4.	Guadeloup	1180	AJ24404	Bousalem and al.2000
DrCDI1.	"	617	AJ305449	"
DrBen2.	Benin	617	AJ305451	Mumford and al.2001
DrBen3.	Benin	616	AJ305450	Mumford and al.2001
DmGui36.	Guinea	617	AJ305452	Mumford and al.2001
DrGui214.	"	616	AJ305438	Mumford and al.2001
DrCDI27.	Côte d'Ivoire	617	AJ305448	Mumford and al.2001
DrGha5.	Ghana	617	AJ305437	Mumford and al.2001
DrNig8.	Nigeria	617	AJ305439	Mumford and al.2001
DrNigN20.	Nigeria	618	AJ305440	Mumford and al.2001
DrGha4.	Ghana	617	AJ305436	Mumford and al.2001
DrTog3.	Togo	617	AJ305442	Mumford and al.2001

1mn of annealing at 55 °C, 1mn of extension at 72 °C) and 10mn of extension at 72 °C to end the cycles. At the end of the RT-PCR reactions, 12 µl of cDNA amplified of each of the microfuge tubes were mixed with 2 µl of 6X load buffer. The mixtures loaded on 12% agarose gel, were run in 0.5X TBE buffer for 26 mn at 100 v. The agarose gel, after the electrophoresis, was coloured in Ethidium Bromide for 15 mn and washed for 5 mn. The electrophoretic bands were visualised on a UV trans-illuminator.

Sequences analysis

The viral nucleotide complementary sequences obtained in this study were assembled using Vector NTI (advance suite 10.0) software package.

Multiple alignments of nucleotide sequences were made using BioEdit 7.0 (Hall, 1999) software package. Phylogenetic trees were constructed and displayed with DARWING 5.0.144 (Perrier et al., 2003) software package using neighbour-joining method with 1000 bootstrapped replicates. The pair-wise dissimilarity between sequences was calculated using Jukes–Cantor (Jukes and Cantor, 1969) multiple substitutions correction model.

The 31 nucleotide sequences were compared, then, with other 15 YMV isolates nucleotide sequences retrieved in GenBank (NBCI database). Nucleotide sequences, as described above, were multi-aligned using BioEdit 7.0 software package and phylogenetic trees were constructed and displayed with DARWING 5.0.144 software package.

RESULTS

Phylogenetic analyses

Phylogenetic analyses were carried out using partial CP-encoding nucleotide sequences and 3´-UTR of 31YMV isolates described in this study. The size of the amplified products ranged from 541 bp to 590 bp. Three major groups or clusters (A1, A2, A3), supported by low bootstrap values (<60%), were observed (Figure 1). In each cluster, isolates coming from various Regions of the country were found. None of the three clusters was identifiable with any specific Region of the country. However, isolates from certain Regions of the country were specifically identifiable to some genetic groups as the case of isolates from the Savannah Region which were all clustered in group A2. Indeed, as indicated by Figure 1, the Cluster A1 contained 10 isolates originated from Maritime (1 isolate), Plateaux (6 isolates) and Central (3 isolates) Regions. The Cluster A2 was formed by 11 isolates originated from the five prospected Regions: Maritime (1 isolate), Plateaux (1 isolate), Central (3 isolates), Kara (4 isolates) and Savannas Regions (4). However, the majority of the isolates grouped in Cluster A2 were originated from Kara (36.36% of the isolates) and Savannas (100% of the isolates from this area) Regions. The Cluster A3 was formed by 10 isolates originated from Maritime (1 isolate), Plateaux (6 isolates), Central (1 isolate) and Kara (2 isolates) Regions. Identity between the three Clusters varied between 91.8% and 98.1% (Table 3).

Geographic distribution of YMV isolates molecular variability in Togo

The phylogenetic tree analyses revealed that the molecular variability of YMV isolates was low in Kara and Savannas Regions. On the contrary, the variability of the isolates was high in Maritime, Plateaux and in the Central Regions (Table 4). Indeed, all the isolates of the Savannas Region were grouped only in Cluster A2 whereas isolates of Kara Region were grouped in Cluster A2 and in Cluster A3. Isolates from Maritime, Plateaux and Central Regions were grouped in the three Clusters (A1, A2, and A3). In addition, as indicated by Figure 1, the three genetic groups (clusters) were differently represented in the five Regions. For example, Cluster A2 was represented in all the five Regions of Togo while the cluster A3 was present in four of the five Regions. The cluster A1 was found only in three Regions.

Phylogenetic relationships between YMV isolates from Togo and from the rest of Africa and Caribbean

Nucleotide sequences of the 31 YMV isolates obtained in this study were compared with 15 CP sequences retrieved from Genbank. Phylogenetic analysis clustered the 46 YMV isolates in four main groups or clusters B1, B2, B3, B4 (Figure 2) with, however, low bootstrap values (<60%). The introduction of isolates from the rest of the world did not change greatly the typology of the phylogenetic tree; the YMV isolates from Togo (the 31 isolates) were mainly grouped in three clusters, B1, B2, B3, corresponding to the three main clusters (A1, A2, A3) obtained above in Figure 1 except isolate C3-2 originated from Central Region. Isolate C3-22, which initially clustered in cluster A1 (Figure 1), segregated from isolates from Togo to group with isolates from West Africa and Caribbean in cluster B3. However, the isolates from Togo were also clustered with some isolates from other parts of the world. Cluster B1 contained 10 isolates originated from Togo (with isolate drtog3 of Genbank), 1 isolate of Cameroon (cam1/c1) and 1 isolate of Guinea (dmgui214). Cluster B2 contained 10 isolates originated from Togo (all the isolates of clusters A2) and 1 isolate from Benin. Cluster B3 was formed by 11 isolates from Togo (all the isolates of cluster A3 in Figure 1), 1 isolate of Burkina Faso (bfc54), 1 isolate of Cameroon (cam2/c31) and 1 isolate of Ivory Coast (drcdi27). Cluster B4 was formed by 9 YMV isolates originated from very different geographical origins. It contained only 1 isolate of Togo (C3-22) and 8 isolates originated respectively from Ghana (drgha4, drgha5), Nigeria (drnign20, drnig8.), Ivory Coast (drcdi1.), Guinea (dmgui36) and Guadeloupe (cgu2/c45). Identity between the four clusters varied from 90.8% to 97.5% (Table 5).

Table3 : Identity (%) within and among the three clusters or the 31 YMV isolates from Togo.

	Cluster A1	**Cluster A2**	**Cluster A3**
Cluster A1	95.3-100		
Cluster A2	94.4-98.1	95.5-100	
Cluster A3	91.8-96.1	92.8-96.7	94.0-98.9

Table 4: YMV isolates genetic variability within the five yam-growing regions in Togo: percentage of isolates of each of the five Regions grouped in the three clusters.

YMV isolates geographical origins (Regions)		**Cluster A1**	**Cluster A2**	**Cluster A3**
Southern Regions	Maritime Region	33.33%	33.33%	33.33%
	Plateaux Region	46.15%	07.69%	46.15%
	Central Region	60.00%	20.00%	20.00%
Northern Regions	Kara Region	0.00%	66.67%	33.33%
	Savannas Region	0.00%	100.0%	0.00%

Table 5: Identity (%) within and among the four clusters or the 46 YMV isolates from Togo and from Genebank.

	Cluster B1	Cluster B2	Cluster B3	Cluster B4
Cluster B1	95.7-100			
Cluster B2	94.4-97.5	95.5-100		
Cluster B3	90.8-96.3	91.8-97.9	90.8-98.9	
Cluster B4	93.0-96.9	92.0-99.3	89.7-96.3	94.0-99.3

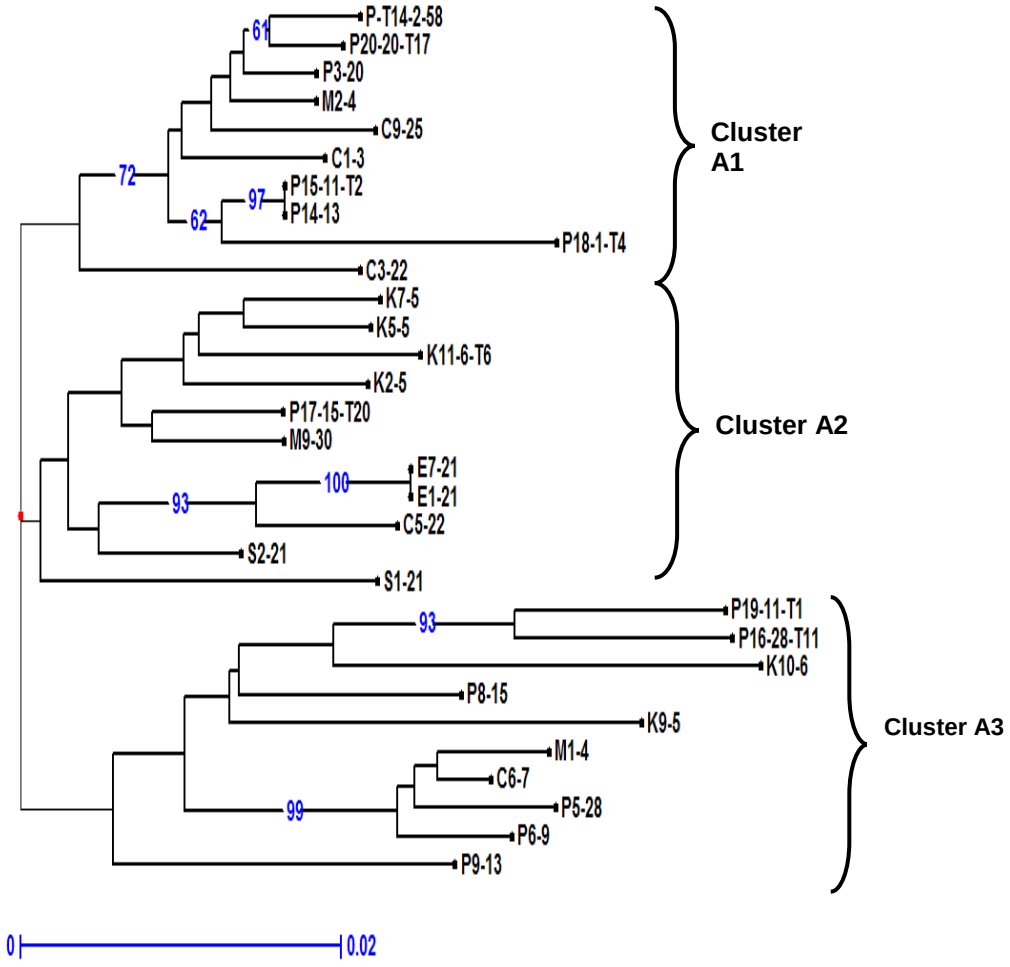

Figure 1: Phylogenetic tree based on the partial CP and 3'end UTR region sequences of 31 YMV isolates using neighbour-joining method. Bootstrap values exceeding or equal to 60 are presented. Origins of the isolates are the following: Maritime Region (isolates M); Plateaux Region (isolates P); Central Region (isolates C); Kara Region (Isolates K); Savannas Region (isolates S).

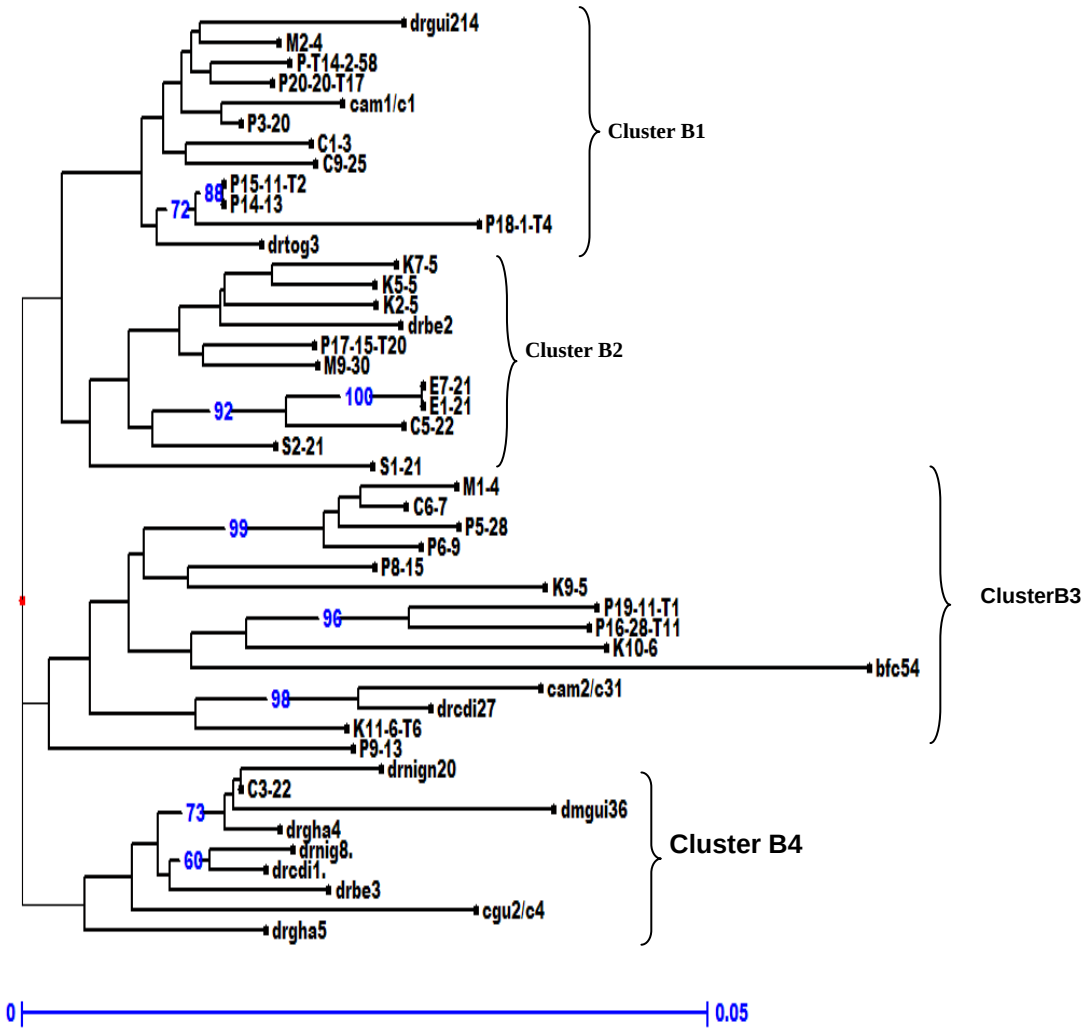

Figure 2: Phylogenetic analysis of 31 YMV isolates of our study and 15 YMV isolates from Genebank (described in Table 2) using neighbour-joining method. Branch lengths are proportional to genetic distance. Bootstrap values exceeding or equal 60 are presented on branches.

DISCUSSION

The intra-specific genetic variability of YMV isolates in Togo was investigated using a partial CP-encoding and 3'- UTR nucleotide sequences of 31 isolates collected in five major yam production Regions. The phylogenetic analysis indicated a high genetic variability within these YMV isolates. The high intra-specific genetic variability of YMV isolates infecting the complex *D. cayenensis rotundata* was already reported in yams-growing areas and exceptionally in West Africa (Aleman-Verdaguer et al., 1997; Bousalem et al., 2000) and it was representative to Potyviruses (Shukla et al., 1994).

Clustering of the 31 isolates did not correlate with their geographical origins; isolates from different regions were often clustered in the same group. But the level of the genetic variability of YMV isolates populations in each of agro-ecological area varied greatly. For instance, all the isolates from Savannas Region were grouped in only one Cluster (cluster A2) and the isolates from Kara Region were grouped in two clusters (A2, A3), whereas isolates in Maritime, Plateaux and in Central Regions were grouped in three Clusters (A1, A2 A3). Isolates from Savannas and Kara Regions seemed, therefore, genetically less variable than isolates from Plateaux, Maritime and Central Regions. These variations could be associated with different factors, such as the importance of exchanging planting materials between the different yam-growing areas (Urbino et al., 1997; Sedzro, 2004), to mutations due to virus isolates-yam host species interactions, to recombination events (Bousalem et al., 2000; Schneider and Roossinck, 2001,) or to virus transmission modalities in the agro-ecological areas. Some studies indicated a preferential selection of some genetic variants within a viral population for vector transmission (Perry and Francki, 1992; Suga et al., 1995). Other studies have also shown that, the transmission by aphids may cause variability by submitting constantly the viruses to adaptation stresses to the different host plants on which these vectors are trying to feed (Fereres et al., 1992; Hammond-Kosack et Jones, 2000). Indeed, at the northern parts of Togo (Kara, Savannas Regions), a great part of the vegetation is destroyed during dry seasons, actually with the Potyviruses as these viruses could not live in died cells (Lecoq, 1992). Therefore, the principal modality of viruses' transmission would be yam tubers. In this case, it is possible that only the same virus genotypes would be transmitted. On the contrary, at the southern areas (Maritime, Plateaux Regions) where the vegetation is present all the year, the transmission of the viruses may be realised both by vectors from weeds or infected yam plants on the fields and infected yam tubers. In this case, different virus genotypes could be transmitted. However, for a better comprehension of the present genetic structure of the viral population in relation with the agro-ecological areas, an epidemiological study would be necessary. In addition, some genetic groups are more represented in some areas than others. This is the case, for example, of cluster A1 which is present only in three Regions (Maritime, Plateaux and Central Regions) and cluster A2 which is found in the five areas. These variations in the distribution of the genetic groups of YMV in the five Regions of Togo, could be associated, as explained above, to the importance of exchanges of planting materials between different yam-growing areas or to virus transmission modalities in the agro-ecological areas.

Furthermore, the comparison of the 31 isolates of our study with 15 YMV isolates of Genbank, revealed that isolates of Togo have bonds with some of the six phylogenetic groups of YMV identified in Africa by Bousalem et al. (2000). For instance, isolate Cam1/C1 from Cameroon which was clustered with isolates from Maritime, Plateaux and Central Regions in cluster B1, belonged to African Phylogenetic Group III. Cluster B3, beside isolates from Togo, contained also one isolate from Burkina Faso (Bfc54) which belonged to African Phylogenetic Group I. Another interesting case was the separation of isolate C3-22 from isolates of Togo and its clustering with isolates of the rest of the World in cluster B4. This suggested that, although this isolate was originated from Togo, it was more closely related to isolates of Ghana, Nigeria, Guinea and Guadeloupe than to isolates of Togo. However, on the six phylogenetic groups of YMV identified in Africa (Bousalem et al. 2000), only two were found in Togo. In addition, it is noticed that only 42% of the nucleotidic sequences retrieved in Genbank, have phylogenetic relationships with the isolates of Togo. In fact, except isolate C3-22 from Central Region, all the isolates from Togo were grouped in three of the four

clusters obtained (Clusters B1, B2, B3) as described previously above. Cluster B2 which grouped isolates of Savannas and Kara Regions contained only one isolate of Genbank (isolate drbe2 from Benin, a neighbouring country of Togo). In the same way, the clusters B1 and B3 grouped respectively only 14.29% and 21.43% of the isolates of Genbank. The majority of the isolates of Genbank (57.14%) were grouped in the cluster B4. These results showed that YMV isolates of Togo were not sufficiently mixed with the YMV isolates of the rest of Africa and demonstrated a genetic specificity of these isolates, suggesting low level exchanges of planting material between farmers of Togo and others countries. In Togo, planting materials are produced by farmers themselves and the few quantity offered by yam seeds producers (15%) are sold on the local market because of transport problems (Sedzro, 2004). Genetic specificities of YMV populations due to low exchanges of yam planting materials with other regions have been reported in Guyana and Burkina Faso (Urbino et al., 1997).

The results obtained in this study revealed that yams of the complex *D. cayenensis rotundata* in Togo were infected by different strains of YMV isolates even in the same locality. That suggests a high pathogenic potential of YMV populations in the various yam production areas of the country. In addition, the majority of YMV populations from Togo seemed to be genetically specific to the country. This situation could create problems of longevity of the resistant planting materials which will be introduced on the territory; the durability of resistances to viruses being related to the virus populations evolution potential (McDonald and Linde, 2002a, 2002b). Indeed, in Togo, loss of resistance of improved yam cultivars introduced from IITA (International Institute of Tropical Agriculture) to control viral diseases and to increase yams yield, was already reported by ITRA (Institut Togolais de Recherche Agronomique) (2003). A case of loss of resistance related to the variability of the viruses in different agro-ecological zones was reported on sweet potato in Uganda (Mwanga et al., 1991). Sweet potato cultivars recognized very resistant to viral diseases in Nigeria, were found very strongly contaminated in Uganda because of the presence on this territory of other viral strains much more virulent than those of the zone of selections. It will be useful to take into account this genetic structure of the populations of YMV in Togo in the search for resistant varieties of yam for the control of the virus; and in accordance with results obtained during this study, the use of local genetic resources of yam would be the best approach.

The Maritime, Plateaux and Central Regions which individually lodge the three genetic groups of the YMV, could be useful for the research and the screening of yam resistant genotypes to the virus; the resistances identified in these zones could be exploited durably in other areas of the country. The distribution of the isolates of cluster A2 in all yam-growing areas of Togo should also be considered in yam selection programs by using these isolates for the screening of yam resistant genotypes to the YMV unless they are the most virulent.

The study of YMV-yam hosts' interactions and of the pathogenic variability of the three genetic groups of the virus, would be very important to complete this study and to choose appropriate isolates to screen YMV resistant clones of yams

ACKNOWLEDGEMENTS

The authors acknowledge the Service de Coopération et d'Action Culturelle of the French government in Lomé, Togo and the Agence Universitaire de la Francophonie for financially supporting this study. The authors thank M. Filloux (CIRAD-AMIS, France) and M. Agboka, (ESA-UL, Togo) for their precious assistance and advice.

REFERENCES

Aleman-Verdauger ME, Goudou-Urbino C, Dubern J, Beachy RN, Fauquet C. 1997. Analysis of the sequence diversity of the

PI, HC, P3, NIb, and CP genomic regions of several yam mosaic potyvirus isolates: Implications for the intraspecies molecular diversity of potyviruses. *J. Gen. Virol*., **78**: 1253-1264.

Amusa NA, Adegbite AA, Muhammed S, Baiyewu RA. 2003. Yam diseases and its management in Nigeria. *Afr. J. Biotechnol.,* **2**: 497-502.

Bousalem M, Douzery EJP, Fargette D. 2000. High genetic diversity, distant phylogenitic relationships and intraspecies recombination events among natural populations of *Yam mosaic virus*: a contribution to understanding potyvirus evolution. *J. Gen. virol.,* **81**: 243-255.

Brunt AA, Crabtree K, Dalwitz MJ, Gibbs A, Watson L. 1996. *Viruses of Plants. Description and Lists from the Database.* CAB International/Cambridge University Press: Wallingford/Cambridge, UK.

Duterme O, Colinet D, Kummert J, Lepoivre P. 1996. Determination of the taxonomic position and characterization of *Yam mosaic virus* isolates based on sequence data of the 5'-terminal part of the coat protein cistron. *Arch. Virol.,* **141**: 1067-1075.

Fereres A, Blua MJ, Perring TM. 1992. Retention and transmission characteristics of Zucchini yellow mosaic virus by *Aphis gossypii* and *Myzus persicae* (Homoptera : Aphididae). *J. Econom. Entom.,* **85**: 759-765.

Goudou-Urbino C, Givord L, Quiot JB, Boeglin M, Konate G Dubern J. 1996. Differentiation of yam mosaic virus isolates using symptomatology, Western blot assay and monoclonal antibody. *J. Phytopathol.,* **144**: 235–240.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.,* **41**: 95–98.

Hammond-Kosack K, Jones JDG. 2000. Responses to plant pathogens. In *Biochemistry and Molecular Biology of Plants,* Buchanan BB, Gruissem W, Jones RL (eds). Amer. Soc. Pl. Physiol.: Rockville, Maryland, USA; 1102-1156.

ITRA (Institut Togolais de Recherche Agronomique). 2003. Cultures vivrières. Rapport annuel 2003. Ministère de l'Agriculture, Ministere de l'Agriculture, de l'Élevage et de la Pêche, Lomé Togo, 64p.

Jukes TH, Cantor C. 1969. Mammalian protein metabolism. In *Evolution of Protein Molecules,* Munro HN (ed). Academic Press: New York; 21–132.

Lecoq H. 1992. Les virus des cultures de melon et de courgette de plein champ (I et II). *PHM-Rev. Hort.,* **323**: 23-28; **324**: 15-25.

McDonald BA, Linde C. 2002a. Pathogen population genetics. Evolutionary potential, and durability resistance. *Ann. Rev. Phytopathol.*, **40**: 349-379.

McDonald BA, Linde C. 2002b. Pathogen population genetics and durability resistance. *Euphytica.,* **124**: 163-180.

Moury B, Morel C, Johansen E, Jacquemond M. 2002. Evidence for diversifying selection in Potato virus Y and in the coat protein of other potyviruses. *Gen. Virol.,* **83**: 2563-2573.

Mumford RA, Seal SE. 1997. Rapid single-tube immunocapture RT-PCR for the detection of two yam potyviruses. *J. Virol. Meth.,* **69**: 73–79.

Mwanga ROM, p'Obwoya CNO, Otim-Nape GW, Odongo B. 1991. Sweetpotato improvement in Uganda. In *The Role of Root Crops in Regional Food Security and Sustainable Agriculture,* Alvarez N, Asiedu R (eds). Proc. East. South. Afr. Regional Root Crops Workshop 4th: IITA, Ibadan, Nigeria; 59-67.

Perrier X, Flori A, Bonnot F. 2003. Data analysis methods. In *Genetic Diversity of Cultivated Tropical Plants,* Hamon P, Seguin M, Perrier X, Glaszmann JC (eds). Enfield, Science Publishers: Montpellier; 43–76

Perry KL, Francki RIB. 1992. Insect-mediated transmission of mixed and reassorted

cucumovirus genoms RNAs. *J. Gen. Virol.,* **73**: 2105-2114.

Schneider WL, Roossinck MJ. 2001. Genetic diversity in RNA virus quasispecies is controlled by host-virus interactions. *J. Virol.,* **75**: 6566-6571.

Sedzro KM. 2004. Acteurs du secteur semencier et sources d'approvision-nement en matériel de plantation des plantes à racines et tubercules au Togo. Semences et matériel de plantation en Afrique Occidentale. *Bulletin du Réseau sur les Semences en Afrique Occidentale,* **12**: 7-11.

Shukla DD, Ward CW, Brunt AA. 1994. Genome structure, variation and function. In *The Potyviridae*. CAB International: Wallingford, UK; 74-110.

Suga H, Uyeda I, Yan J, Murao K, Kimura I, Tiongco ER, Cabautan P Koganezawa H. 1995. Heterogeneity of rice ragged stunt oryzavirus genomes segment-9 and its segregation by insect vector transmission. *Arch. Virol.,* **140**: 1503-1509.

Thouvenel J-C, Dumont R. 1990. Perte de rendement de l'igname infectée par le virus de la mosaïque de l'igname en Côte d'Ivoire. *Agron. Trop.*, **45**: 125-129.

Urbino C, Bousalem M, Pinel A, Fargette D, Dubern J. 1997. Les virus de l'igname : caractérisation immunologique et moléculaire du virus de la mosaïque de l'igname. In *L'Igname, Plante Séculaire et Culture d'Avenir*, Berthaud J, Bricas N, Marchand J-L (eds). Actes du Séminaire International: Montpellier, France; 205-211.