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Research Article

Characterization and Distribution of Potyvirus Species Infecting Sweet Potato (*Ipomoea batatas*) in Burkina Faso

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Abstract

Virus species belonging to the genus *Potyvirus* are the most common viruses infecting sweet potato crop. Among these viruses, Sweet potato Feathery Mottle Virus (SPFMV) is the most damaging and widespread in the world. To assess the potyvirus disease on sweet potato in Burkina Faso, a total of 300 samples were collected from the nine largest sweet potato producing regions. Samples were analyzed using RT-PCR and products were Sequenced. Bioinformatic analyzes were performed to know the strains of the viruses. The results revealed that SPFMV is the main Potyvirus infecting sweet potato in Burkina Faso with a prevalence of about 28.33%. A total of seven isolates of SPFMV were successfully sequenced and used for phylogenetic analyzes. These isolates have shown 99% nucleotide identity with the phylogroup A-II (SPFMV-O), and some of them had 95% nucleotide identity with phylogroup B (SPFMV-RC). This study showed that SPFMV is the main Potyvirus-infecting sweet potato in Burkina Faso.

Keywords: Sweet potato; Potyvirus; Sweet Potato Feathery Mottle Virus; Burkina Faso

Introduction

Viral diseases are known as common problem in sweet potato production worldwide with crop losses [1]. These diseases can cause losses of up 56 - 98% of the crop [2]. More than 30 virus species belonging to the 9 following families are known to affect negatively sweet potato production: *Bromoviridae*, *Bunyaviridae*, *Caulimoviridae*, *Closteroviridae*, *Comoviridae*, *Flexiviridae*, *Geminiviridae*, *Luteoviridae* and *Potyviridae* [3].

The *Potyviridae* family is the largest and most economically damageable group of plant viruses with 176 member species and 31 tentative species [4,5]. All members of this family are single-stranded, positive-sense RNA (ssRNA+) viruses. *Potyviridae* family is subdivided into 12 genera: Arepavirus, *Bevemovirus, Brambyvirus, Bymovirus, Celavirus, Ipomovirus*, *Macluravirus*, *Poacevirus*, *Potyvirus, Roymovirus, Rymovirus*, *Tritimovirus* (ICTV, 2020). They are transmitted to plants via a range of vectors such as aphids, whiteflies, mites, fungi, through different transmission modes [4,6].

Most of viruses belonging to the genus *Potyvirus* are transmitted by aphids in a non-persistent and non-circulative manner [4,6]. In this group, Sweet potato Feathery Mottle Virus (SPFMV) is one of

the most constraining virus in sweet potato production worldwide, and in synergy with the Sweet potato Chlorotic Stunt Virus (SPCSV) (*Closteroviridae, Crinivirus*), cause losses of 70 to 100% [1,7-11].

This study was then initiated to assess viral disease caused by Potyviruses on sweet potato and to contribute to the improvement of its production in Burkina Faso. Specifically, this study aimed to characterize potyviruses infecting sweet potato at the country level using molecular tools.

Materials and Methods

Samples collection

During March 2015 to October 2016, 300 sweet potato young leaves with typical symptoms as well as symptomless ones were collected from Hauts-Bassins, Cascades, Boucle du Mouhoun, Centre-Ouest, Centre-Est, Centre-Sud, Sud-Ouest, and Centre regions of Burkina Faso (Figure 1). The leaf samples were immediately putted into paper envelopes, dried at 37 °C, and stored at the INERA plant virology laboratory located at Kamboinsé Research Station. Cuttings were also collected and grown in an insect proof greenhouse.

Figure 1: Sampling locations in different regions of Burkina Faso.

RNA extraction and RT-PCR for potyviruses detection

Total RNA was extracted from dry leaf samples using RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. Reverse transcription (RT) was performed on extracted RNA using MMLV reverse transcriptase (Promega) and random hexamers (Promega) as primers at 42°C for 1h (Prasanth and Hegde, 2008) to produce cDNA. PCR was first carried out for potyviruses amplification using degenerated primers such as Oligo1n forward (ATGGTHTGGTGYATHGARAAYGG) and Oligo2n reverse (TGCTGCK-GCYTTCATYTG), with H=A/C/T; Y=C/T; R=A/G and K=G/T [12]. Then, primers specific to the SPFMV CP gene CP1A (5′-GCAGAG-GATGTCCTATTGCACACC-3′) and CP1S (5′ AGTGGGAAGGCACCATA-CATAGC-3′), were used in second time, with Maximo Taq DNA polymerase (GeneON). The PCR were performed in 50 µl reaction volumes using 2.5 µl cDNA and 0.2 µM each of primers CP1A/ CP1S. The conditions were 94°C for 3 minutes; then 30 cycles of 94°C for 30s, 56.3°C for 30s and 72°C for 1 minute and a final cycle of 72°C for 10 minutes previously described by Prasanth and Hegde (2008).

Sequencing and bioinformatic analysis

PCR products from RT-PCR were sequenced by the Sanger method by Genewiz Company (UK). Contigs were cleaned and assembled *de novo* using Geneious v. 8.1.7 (Biomatters Ltd). All the sequences were subjected to the BLAST search tools in NCBI using Geneious and subsequently to pairwise sequence comparison [13]. Using ClustalW in MEGA v. 7.0.14, the sequences were aligned with homologous sequences retrieved from GenBank [14]. Evolutionary history was inferred using maximum likelihood with the Tamura-Nei model [14]. Phylogenetic reconstruction was performed with bootstrap support values of 1,000. The tree was visualized and edited using FigTree v. 1.4.3.

Results

Symptoms observed in sweet potato field

Most observed viral symptoms on field plants were vein-clearing, mosaic and stunting. Chlorotic spots and purpling were also observed on older leaves. Symptoms among the collected samples were sometimes mild regardless of geographical location and sweet potato variety (Figure 2).

Figure 2: Leaf symptoms on sweetpotato plants affected by viruses. (a) vein-clearing, (b, c) Mild mosaic.

Potyvirus detection using universal primers and its distribution

Molecular detection by RT-PCR of a portion of coat protein gene yielded a product of approximately 350 bp. This product indicates

the presence of potyvirus in the analyzed sample. A total of 85, i.e. 28.33% of the samples tested were positive for Potyvirus. These positive samples are scattered at different percentages over all the regions surveyed on the national territory. Sud-Ouest and Haut-bassins are the regions most affected by Potyvirus with prevalence of 38.7% and 36.6% respectively. The Centre-Ouest, Cascades and Centre-Sud regions follow with prevalence's of 28.6%, 25.6%, 22% and 14% respectively. The Boucle du Mouhoun and Centre-Sud regions share the same prevalence of 13%. The Centre region seems to be unaffected by Potyviruses with absence of Potyvirus in all the samples analyzed from this region.

Detection of Sweet potato feathery mottle virus (SPFMV) by RT-PCR

Using the CP1S-CP1A primers to characterize SPFMV, only 6% of the samples were found positive. These positives samples showed RT-PCR product of approximately 1,000 bp (Figure 3). Our data showed that the Est region is slightly more affected by the SPFMV with a total prevalence of 11%. The Hauts Bassins, Mouhoun, Centre-Ouest, and Cascades regions follow with prevalence rates of 9.8%, 8.7%, 5.7%, and 2.6%, respectively. For the Sud-Ouest, Centre-Est, Centre and Centre-Sud regions, no sample was tested positive for the primers used.

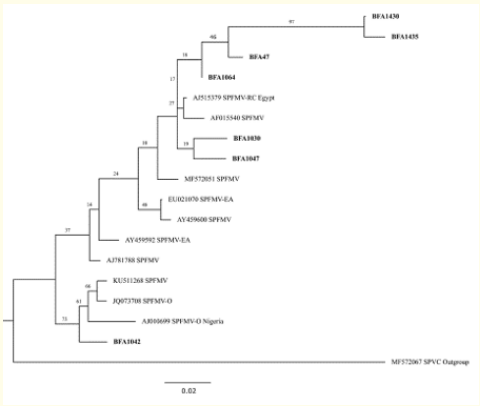

Figure 3: Maximum likelihood phylogenetic trees obtained from alignment of partial sequence of CP genes from sweet potato feathery mottle virus (SPFMV). The comparisons made were between phylogroup A-II (O) and B (RC) from this study and partial CP gene sequences obtained from GenBank. The tree was created in MEGA X using ClustalW with 1000 replicates. Bootstrap values are percentages with values shown at the branch. The tree was rooted with sweet potato virus C (MF572067). Sequence symbols: from this study (blod), from GenBank (black).

Potyvirus species characterized

Using the Oligo1n-Oligo2n and CP1S-CP1A primers 7 sequences were successfully obtained by sanger sequencing. Isolates sequenced are all SPFMV species regardless the primers used. BLASTn search showed that sequences from isolates BFA1430, BFA1435, BFA47, BFA1064, BFA1030 and BFA1047 share highest nucleotide identity (99%) with SPFMV isolates EF990653, MF572048 and AB509458. Isolate BFA1042 shares 95% nucleotide identity with isolate AJ010699.

Partial CP gene from the 7 SPFMV isolates from Burkina Faso were compared with 31 SPFMV isolates retrieved from GenBank (Table 1). Then phylogenetic analyses showed that our isolates clustered with phylogroup A-II (SPFMV-O) and B (SPFMV-RC). Thus, BFA1430, BFA1435, BFA47, BFA1064, BFA1030 and BFA1047 clustered with isolates belonging to phylogroup B and BF1042 with isolates belonging to phylogroup A-II (Figure 3).

Isolate	Phylogroup	Geographical origin	Accession no.
BFA47	B (RC)	Burkina Faso	This study
BFA1030	B (RC)	Burkina Faso	This study
BFA1042	B (RC)	Burkina Faso	This study
BFA1047	B (RC)	Burkina Faso	This study
BFA1064	B (RC)	Burkina Faso	This study
BFA1430	B (RC)	Burkina Faso	This study
BFA1435	B (RC)	Burkina Faso	This study
Egypt9	B (RC)	Egypt	AJ515379
Aus3D	B (RC)	Australia	MF572052
Canar3	B (RC)	Canary Island	AY459600
K1	B (RC)	South Korea	AF015540
TM68A-Out-group	SPVC	East Timor	MF572067
AM-MB2	A-II (O)	Spain	KU511268
BAU	A-II (O)		AJ010699
Umfume-o	A-II (O)	South Africa	JQ073708
Mbl2	A-I (EA)	Uganda	AJ781788
Fe	A-I (EA)	Peru	EU021070
54/9S	A-I (EA)	Kenya	AY459592

Table 1: Isolates and phylogroup of sweet potato feathery mottle virus (SPFMV) detected or used for phylogenetic analysis in this study. In bold are virus isolates described in this study.

Discussion and Conclusion

This study highlights the presence sweet potato's Potyvirus in Burkina Faso. These viruses are unevenly distributed over all the survey areas with an overall prevalence of 28.33%. This seems consistent with study of Tibiri., *et al.* [15] which reported a prevalence rate of 34%. Potyviruses are known to be the most widespread viruses on sweet potato around the world, especially in tropical and subtropical regions [3,16,17]. Tropical and subtropical regions are favorable for sweet potato cultivation and therefore certainly to the proliferation of aphids [3]. Furthermore, the SPFMV presence in Hauts-Bassins, Cascades, Sud-Ouest, Boucle du Mouhoun, Centre-Ouest, Centre-Est, Est and Centre-Sud regions could be explain by sweet potato quite important cultivation in these regions [15].

Both CP1S/CP1A SPFMV specific primers and poty-universal primers (Oligo1n/Oligo2n) [18] used in this study allowed the characterization of only SPFMV. However, using poty-universal primers, prevalence was 28% while the prevalence of specific primers was 6%. Using specific primers, rate of prevalence was low compared to what was expected. This suggests CP1S/CP1A primers used would not be specific to all SPFMV isolates.

BLASTn (NCBI) search showed all our Potyvirus isolates were found to be close to SPFMV with nucleotide identity from 95 to 99%. This supports hypothesis that the CP1S and CP1A primers may not be specific enough for some of the SPFMV isolates in our samples. Thus, sequencing and bioinformatics analyses performed lead to the conclusion that the prevalence of SPFMV in Burkina Faso is 28.33%.

According to our findings, SPFMV alone was present in Burkina Faso as a potyvirus infecting sweetpotato. So, efforts to improve diagnosis must be carried out through the design of other more efficient diagnostic tools.

This work will also make it possible to strengthen the tools for monitoring sweet potato viral diseases in Burkina Faso and also to provide healthy cuttings to farmers.

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