Assessment of sweet potato (*Ipomoea batatas* [L.] Lam.) accessions for resistance to sweet potato feathery mottle virus.

ABSTRACT

Sweet potato~~es~~ play~~s~~ an important role in human and animal nutrition. In many parts of the world, it has been used as an emergency crop, enabling populations to survive difficult situations. Despite its adaptation to a variety of environments and growing conditions, sweet potato production is subject to a number of abiotic and biotic constraints, including ~~sweet potato~~ viruses. Sweet potato feathery mottle induced by sweet potato feathery mottle virus (SPFMV) is one of the most damaging virus diseases of sweet potato (*Ipomoea batatas)*. Genetic control is considered as very promising mean the ~~combat~~ control the disease. Microsatellite markers (SSR) were assessed for their possible use in marker assisted selection (MAS) of sweet potato for resistance to SPFMV. A collection of 86 sweet potato accessions and 131 F1 hybrids were evaluated for resistance to the virus by assessing the accumulation of viral particles by Enzyme-linked immunosorbent assay (ELISA). Up to 22.1% of the accessions and 29.8% of F1 hybrids showed resistance to the virus as they did not develop any symptom and ELISA failed to detect the virus in leaf samples. Assessment of SSR markers clustering structure and use of principal component analysis indicated that seven SSR markers were correlated to virus accumulation. Validation of these correlations should allow the use of these markers in MAS to improve sweet potato for resistance to SPFMV.

Keys words: sweet potato~~, *Ipomoea batatas*~~, SPFMV, viral accumulation, SSRs markers, Burkina Faso.

1. INTRODUCTION

Sweet potato (*Ipomoea batatas* [L.] Lam.) is one of the world's most important tuber crops. It is the seventh most important food crop after wheat, rice, maize, potato, barley and cassava, and the fourth most important in tropical regions [1]. It is the second most important tuber crop after potatoes [2]. ~~On the other hand~~Furthermore, in Africa, sweet potato ranks third among tuber crops after cassava (*Manihot esculenta* Crantz) and yam (*Dioscora* spp.). In Burkina Faso, its production was estimated at around 92.800 tonnes in 2023, with an area of 40.86 ha and an average yield of 20.3tonnes/ha [1].

Sweet potato production is limited by several abiotic (drought, irregular rainfall) and biotic (pests, weeds and ~~diseases~~phytopathogens) constraints. Because sweet potatoes are grown by cuttings, they are highly susceptible to disease, particularly viral diseases. Sweet potato virus disease (SPVD) is one of the most devastating viral diseases of sweet potato crops, with crop losses ranging from 56 to 98% [3,4,5]. SPVD is caused by synergistic co-infection between sweet potato feathery mottle virus (SPFMV) and sweet potato chlorotic stunt virus (SPCSV).

SPCSV ~~Sweet potato chlorotic stunt virus~~ has been reported in several regions of Burkina Faso, where it appears to be the main virus infecting sweet potato in the country [6[sz1]].

The first means of ~~combating~~ controlling SPFMV[sz2] were cultural methods (use of healthy cuttings) and the control of insect vectors [7]. Increasingly, genetic control is being considered with a view to integrated management. For example, resistant varieties have been popularized in Uganda. However, resistant varieties developed in a given country or geographical region may prove susceptible in other regions [8]. It is therefore essential to evaluate germplasm at local level in order to determine the sources of resistance present.

The aim of this work is to contribute to the improvement of sweet potato in Burkina Faso. The aim was to evaluate sweet potato varieties collected from growers or introduced into Burkina Faso for research purposes against SPFMV. This work is structured around the following main points:(i) Diversity of viral symptoms on sweet potato;(ii) Detection of SPFMV in genotypes and hybrids

from the sweet potato collection; (iii) a final section devoted to correlations between viral concentration and SSRs microsatellite markers.

2. **MATERIALS AND METHODS**

2.1. Plant material

The *Sweet potato* collection of Environmental and Agricultural Research Institute (INERA), Burkina Faso was used. This collection is held at the Kamboinsé Environmental, Agricultural Research and Training Center. It comprises 86 entries, 70 of which were collected in Burkina Faso, while the remainder were introduced. The Burkina Faso genotypes were collected between December 2008 and January 2010 in the main sweet potato production regions: Cascades regions, High bassins region, Center-West, South Central, East Central and East [9]. Entries are kept in triplicate in 20-liter buckets since their collection in a screened shelter with high inoculum pressure (Table 1).

Table 1: Entries for the sweet potato collection.

Entries		Entries	Entries		Entries
BF 2		BF 59	BF 7		BF 44
BF 12		BF 153	BF 10		BF52
BF 15		CIP	BF 17		BF 53
BF 19		Commensal	BF 21		BF54
BF 49		Ejumula	BF 25		BF55
BF 51		TIB	BF 45		BF 57
BF 63		BF 4	BF 46		BF 60
BF 65		BF 8	BF 56		BF61
BF 66		BF 11	BF 58		BF 62
BF 87		BF13	BF68		Simon
BF 89		BF20	BF 64		BF 77
BF 94		BF23	BF 67		BF 80
BF 98		BF 24	BF 74		BF 81
BF 112		BF 27	BF 88		BF83
BF 115		BF32	BF 92		BF 100
BF 155		BF 33	BF 93		BF 114
Diabo Local		BF 34	BF 95		BF 119
Ejumula 2		BF35	BF 99		BF 120
Jewel Léo		BF36	BF 141		Taînug1
Résisto		BF 41	BF C1		Tiébélé2
BF 1		BF 42	Caromex		Taînug440129
BF 3		BF 43	Kakamega		

Table 2: Accessions of F1 hybrids.[sz3]

Entries		Entries		Entries
BF59xTIB-6		BF92xTanning-4		BF92xTanning-5
BF77xTanning-3		BF77xRésisto-4		BF82xTanning-21
BF24xRésisto-20		BF77xTanning-10		BF92xCIP-6
BF82xCIP-6		BF80xRésisto-2		BF59xRésisto-4
BF59xRésisto-8		BF80xtanning-1		BF59xCIP
BF59xTanning-9		BF80xTanning-3		BF82xCIP-17
BF59xRésisto-9		BF2x4Résisto-16		BF80xCIP-1
BF82xCIP-5		BF59xCIP-1		BF92xRésisto-13
BF59xRésisto-24		BF24xRésisto-12		BF82xRésisto-5
BF24xRésisto-34		BF24xRésisto-38		BF92xTIB-1
BF92xCIP-5		BF77xRésisto-3		BF59xTanning-9
BF92xRésisto-7		BF80xRésisto-4		BF59xTanning-2
BF82xTanning-5		BF92xTanning-7		BF59xTanning-3
BF13xRésisto-3		BF59xTanning-3		BF59xCIP-4
BF24xRésisto-15		BF82xTIB-9		BF24xTIB-3
BF80xRésisto-1		BF82xTanning-8		BF13xTIB-1
BF24xRésito-16		BF82xTanning-18		BF59xRésisto-9
BF82xCIP-16		BF82xRésisto-35		BF80xCIP-7

Entries		Entries		Entries
BF24xCIP-1		BF92xTanning-4		BF59xTIB-3
BF13xRésisto-3		BF59xRésisto-4		BF59xTIB-5
BF24xTIB-1		BF77xTanning-1		BF24xCIP-2
BF92xTIB-2		BF82xTanning-42		BF24xCIP-4
BF59xTIB-4		BF24xTanning-9		BF59xRésisto-2
BF59xRésisto-7		BF92xCIP-2		BF12xTanning-7
BF82xTanning-10		BF59xTanning-7		BF59xCIP-3
BF77xRésisto-4		BF77xRésisto-5		BF77xRésisto-5
BF24xRésisto-20		BF77xTanning-2		BF82xTanning-14
BF59xRésisto-5		BF59xRésisto-10		BF13xTIB-3
BF24xRésisto-13		BF59xRésisto-13		BF59xTanning-1
BF24xRésisto-39		BF77xTanning-7		BF82xTIB-6
BF59xRésisto-3		BF82xTIB-8		BF59xCIP-6
BF24xRésisto-23		BF77xTanning-6		BF24xTanning-2
BF24xTanning-10		BF77xRésisto-7		BF82xCIP-14
BF24xTanning-5		BF59xTIB-2		BF82xRésisto36
BF24xTanning-1		BF92xRésisto-2		BF82xCIP-21
BF82xCIP-4		BF82xTanning-24		BF77xCIP-2
BF59xRésisto-12		BF82xTanning-18		BF13xTanning-1

Entries		Entries		Entries
BF59xRésisto-5		BF24xRésisto-24		BF92xCIP-4
BF13xTIB-2		BF77xRésisto-1		BF82xTIB-11
BF92xRésito-10		BF24xRésisto-3		BF82xTanning-16
BF77xTIB-1		BF82xRésisto-12		BF24xTanning-11
BF13xCIP-1		BF24xTIB-2		BF82xTanning-15
BF77xCIP-1		BF82xCIP-13		BF24 xRésist0-18
BF82xCIP-5		BF92xCIP-3		BF59xTanning-14

2.2. **Serological detection of SPFMV**

SPFMV were tested in plant extracts using single-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In brief, leaf sample were individually extracted by grinding 0.4 g of tissue in 5 ml[sz4] of extraction buffer and Immunoplates (Nunc) were coated for 2h. To minimize nonspecific binding, wash buffer contained 2% skimmed milk powder was added and incubated in duplicate overnight at 37°C for 30 min. Then, SPFMV antiserum was added, followed by incubation of anti-rabbit alkaline phosphatase conjugate at 37°C for 2 h. Para-nitrophenolphosphate substrate was added at 1 mg/ml in 9.7% diethanolamine, pH 9.8. Sap extracts from known Sweet potato feathery-infected and healthy plants were used as positive and negative controls, respectively. Plates were incubated for 1 h at 37˚C and absorbance values were measured at 405 nm using a Metertech Σ960 multiskan microplate reader. Sample with absorbance values more than twice that of the healthy controls were considered positive for the virus sweetpotato virus diseases.

2.2.2 Genotyping of sweet potato entries

Genotyping data for the various sweet potato entries were generated by [9]. They were obtained by analysis of sweet potato leaf samples by the DNAL[sz5] and marks laboratory (Quebec, Canada). A total of 30 SSR markers comprising 212 alleles were used. The corresponding primer sequences are shown in Table 3.

Table 3. Primer sequences for the 30 SSR markers used for genotyping sweet potato accessions.

Markers	Primer sequences	Markers	Primer sequences
IbL16_F	GTCTTGCTGGATACGTAGAACA	IBS12_F	CAGTTATCAATTCCCACCTACC
IbL16_R	GGGAGAAGTAAGAGAACCGATA	IBS12_R	TTGCTGTTTATAGGCTTTGTC
IbL32_F	GGGATGAAGGAGAGAATGAGTA	IBS84_F	CAAAGATGAAGCAAGTAAGCAG
IbL32_R	TTGAAAACCTAGAGAGAAAGGG	IBS84_R	ACTAATGTTGATCTACGGACCC

Markers	Primer sequences	Markers	Primer sequences
IbL46_F	CTGAAATTAGGGATTGAAGAGG	IBS85_F	AACTACTCATGGGGAGAACAAC
IbL46_R	TCCAATCACTCCTTGTTTTCTC	IBS85_R	CTAACGAAAGTTTGGACATCTG
IbO2_F	TGTGGATCTGTTCTTTGAACC	IBS86_F	AGAAACTGAAAACTAAGCTCGC
IbO2_R	TTCCATGTGGAGTGTGAAGTAT	IBS86_R	GCTATGCGTTTACAGAAACAAG
IBS100_F	TGCTATAGTTACGTGGACGAAG	IBS97_F	GTTACCAGGAATTACGAACGAT
IBS100_R	TTTAATGCTGATGTGGATGC	IBS97_R	CTCTCTACAAAAACTCACAGCG
IBS134_F	CTTCAATCACCTGAAACTCTGA	IbU13_F	GCAACCAATCTACAGCAAACTA
IBS134_R	AATATCGCTATGTTCTTGGGaC	IbU13_R	CAGATAAAGTCCCCATTTCTTC
IBS137_F	TcAACAGACGTCTTCACTTACC	IbU20_F	GGAGAGCAAGTGGAGAAAGTAT
IBS137_R	TCGATAGTATGATGTGAATCGC	IbU20_R	ACTCCTAGACCCACAATTGAAC
IBS139_F	CTATGACACTtCTGAGAGGCAA	IbU31_F	CCGCAGAAAAAGTTCAGATT
IBS139_R	AGCCTTCTTGTTAGTTTCAAGC	IbU31_R	GCAACTTTTCTTCTTCCGTAAC
IBS144_F	TCGAACGCTTTCTACACTCTT	IbU33_F	TTTGAAGAAGATGAGAGCGAC
IBS144_R	CTGTGTTTATAGTCTCTGGCGA	IbU33_R	TCAGAAAGACGATACACTAGAGAGA
IBS147_F	TGTGTACATGAGTTTGGTTGTG	IbU4_F	GGCTGGATTCTTCATATTTAGC
IBS147_R	GAAGTGCAACTAGGAAACATGA	IbU4_R	GCTTAATGGATCAGTAACACGA
IBS156_F	TTGATTCCACTATGACTTGAGC	IbU6_F	GGGGTAGAGAGAAGAGAGTGAC
IBS156_R	ACACCAACCCTTATATGCTTTC	IbU6_R	CCAGGTGAGAGTGTCTTTCAA
IBS166_F	TCCGTCTTTCTTCTTCTTCTTC	IBS24_F	AGTGCAACCATTGTAATAGCAG
IBS166_R	ATACACTAACTGCATCCAAACG	IBS24_R	TCCTTTCtTcATCATGCACtAc
IBS18_F	GCCAAGGATGAAGGATATAGAa	IBS33_F	ATCTCTtCATACcAATCGgAaC
IBS18_R	ACAAcCAAACTAGCTAAAAGCC	IBS33_R	CaATgaTAGCGGAGATTGAAG
IBS19_F	TCCTATGAGTGCCCTAAGAATC	IBS72_F	CTACTCTCTGCTGGTTTATCCC
IBS19_R	CTCCTTCGTCTTCTTCTTcTTC	IBS72_R	CTAGTGGTCTCTCTTCCTCCAC
IBS199_F	TAACTAGGTTGCAGTGGTTTGT	IBS82_F	GACATAATTTGTGGGTTTAGGG
IBS199_R	ATAGGTCCATATACAATGCCAG	IBS82_R	GAAATGGCAGAATGAGTAAGG

2.2.3. Statistical analysis of data

Relationships between phenotypic markers (scores from serological reactions) and SSR markers were determined by analyzing the similarity matrix between markers using the DendroUPGMA computer program available at http://genomes.urv.cat/UPGMA/ [11]. This program was also used to construct a UPGMA (Unweighted Pair Group Methodwith Arithmeticmean) dendrogram to visualize the results of the similarity analysis.

The data obtained using the various markers were also analyzed using the PCA (Principal Component Analysis) method. This method was carried out using STATISTICA statistical software version 6.0 (STATSOFT, FRANCE~~E, version 6.0~~).

3. Results

3.1. Symptom diversity

A wide range of symptoms was observed in the sweet potato collection (Figure 1). The majority of plants showed symptoms of feathery variegation, as illustrated in Figure 1A. Various other symptoms of variegation or mosaic associated or not with leaf deformations were also observed.

In some cases, very severe leaf deformation in the form of leaf blade curling was observed (Figure 1H).

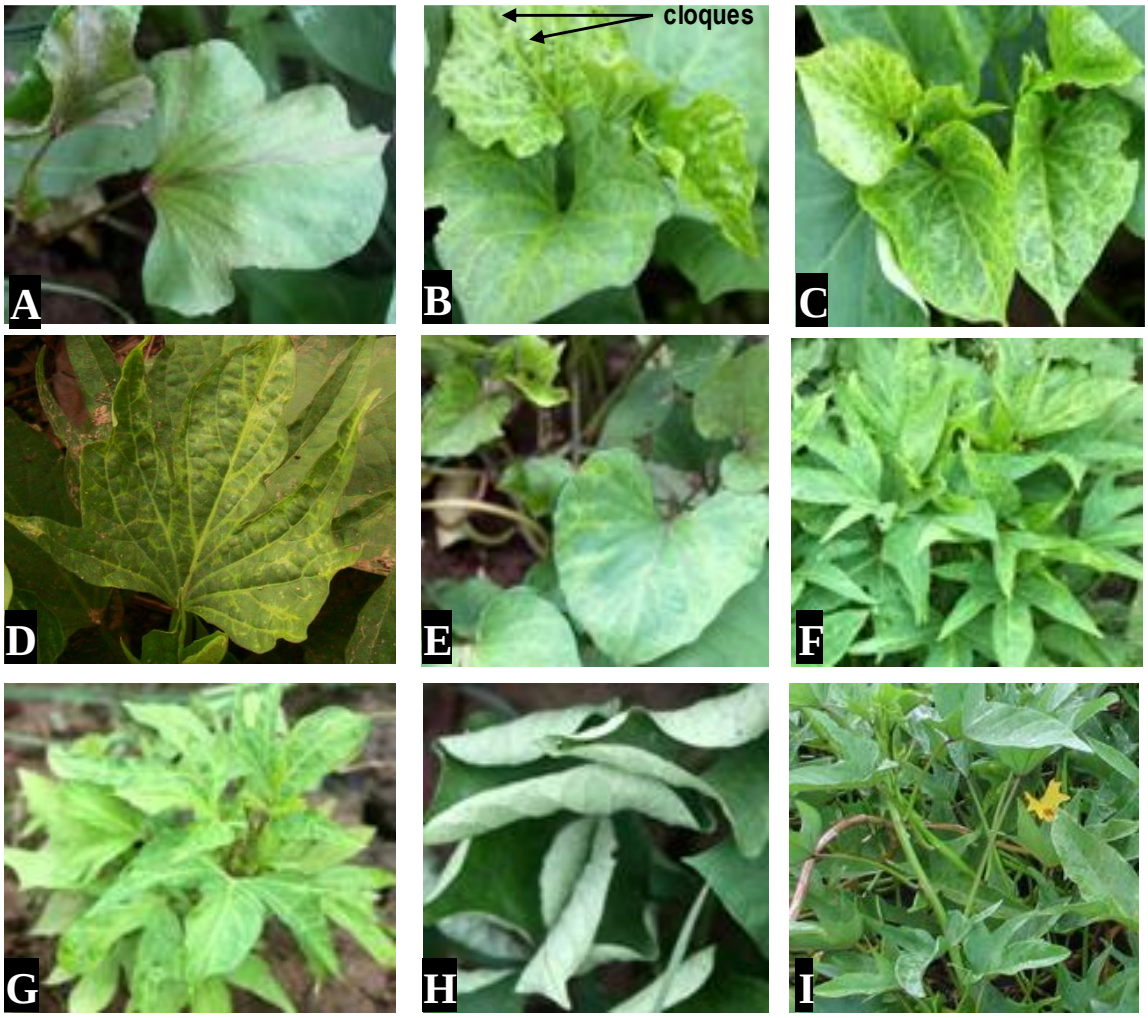

Figure 1. *Diversity of symptoms observed in plants in the sweet potato collection. A: symptoms of leaf deformation with mild mosaic; B: interveinal chlorosis with severe blistering of the leaf blade; C: interveinal chlorosis with variegation; D: interveinal chlorosis with leaf blade embossing; E: symptoms of variegation; F: yellow mosaic; G: severe symptoms of yellow mosaic with stunting of the plant; H: leaf curling with mild mosaic; I: symptomless plant.*

3.2. Detection of SPFMV in genotypes from the sweet potato collection

The results of serological detection of SPFMV in sweet potato leaf samples are reported in Table 4. The virus was detected in 77.9% (67/86) of samples, while no detection was possible in the remainder (19/86 or 22.1%). The majority of sweet potato genotypes in which the virus was not detected were collected in Burkina Faso. For the genotypes in which the virus was detected, OD405 nm values enabled us to distinguish three groups corresponding respectively to scores 1, 2 and 4. Genotypes in group 2 were the most numerous (61.2%). Those in group 1 accounted for 37.3%, while group 3 contained only the Taînug440129 genotype introduced from China. SPFMV was detected in leaf samples irrespective of the type of symptoms observed.

Table 4. Serological detection by ELISA of SPFMV in sweet potato leaves

Entries	D405 nm[a]	SPFMV[b]	Score	Entries	D405 nm	SPFMV	Score
BF 2	0,156 ± 0,025	-	0	BF 59	0,433 ± 0,05	+	1
BF 12	0,138 ± 0,015	-	0	BF 153	0,471 ± 0,015	+	1
BF 15	0,150 ± 0,029	-	0	CIP	0,467 ± 0,064	+	1
BF 19	0,309 ± 0,006	-	0	Commensal	0,421 ± 0,042	+	1
BF 49	0,229 ± 0,02	-	0	Ejumula	0,489 ± 0,077	+	1
BF 51	0,180 ± 0,05	-	0	TIB	0,528 ± 0,052	+	1
BF 63	0,294 ± 0,036	-	0	BF 4	0,695 ± 0,042	+	2
BF 65	0,134 ± 0,048	-	0	BF 8	0,924 ± 0,086	+	2
BF 66	0,187 ± 0,033	-	0	BF 11	0,832 ± 0,048	+	2
BF 87	0,285 ± 0,013	-	0	BF13	0,972 ± 0,09	+	2
BF 89	0,274 ± 0,01	-	0	BF20	1,039 ± 0,143	+	2
BF 94	0,185 ± 0,003	-	0	BF23	1,021 ± 0,059	+	2
BF 98	0,267 ± 0,012	-	0	BF 24	0,846 ± 0,056	+	2
BF 112	0,288 ± 0,015	-	0	BF 27	0,702 ± 0,06	+	2
BF 115	0,202 ± 0,007	-	0	BF32	1,087 ± 0,032	+	2
BF 155	0,121 ± 0,032	-	0	BF 33	0,924 ± 0,054	+	2
Diabo Local	0,295 ± 0,034	-	0	BF 34	0,961 ± 0,04	+	2
Ejumula 2	0,154 ± 0,016	-	0	BF35	1,046 ± 0,039	+	2
Jewel Léo	0,084 ± 0,008	-	0	BF36	1,160 ± 0,058	+	2
Résis to	0,290 ± 0,011	-	0	BF 41	0,603 ± 0,071	+	2
BF 1	0,439 ± 0,031	+	1	BF 42	0,610 ± 0,105	+	2
BF 3	0,524 ± 0,03	+	1	BF 43	0,665 ± 0,057	+	2
BF 7	0,562 ± 0,018	+	1	BF 44	0,948 ± 0,032	+	2
BF 10	0,340 ± 0,028	+	1	BF52	0,972 ± 0,024	+	2
BF 17	0,481 ± 0,067	+	1	BF 53	0,733 ± 0,035	+	2
BF 21	0,348 ± 0,008	+	1	BF54	1,049 ± 0,091	+	2
BF 25	0,349 ± 0,023	+	1	BF55	1,103 ± 0,081	+	2
BF 45	0,382 ± 0,056	+	1	BF 57	0,757 ± 0,048	+	2
BF 46	0,505 ± 0,034	+	1	BF 60	0,607 ± 0,091	+	2
BF 56	0,410 ± 0,022	+	1	BF61	1,028 ± 0,052	+	2
BF 58	0,586 ± 0,067	+	1	BF 62	0,750 ± 0,063	+	2
BF68	0,989 ± 0,086	+	2	Simon	1,028 ± 0,027	+	2
BF 64	0,456 ± 0,036	+	1	BF 77	0,800 ± 0,084	+	2
BF 67	0,465 ± 0,02	+	1	BF 80	0,799 ± 0,036	+	2
BF 74	0,315 ± 0,102	+	1	BF 81	0,670 ± 0,045	+	2
BF 88	0,351 ± 0,036	+	1	BF83	0,970 ± 0,165	+	2
BF 92	0,405 ± 0,057	+	1	BF 100	0,825 ± 0,042	+	2
BF 93	0,466 ± 0,031	+	1	BF 114	0,930 ± 0,039	+	2

Entries	D405 nm[a]	SPFMV[b]	Score	Entries	D405 nm	SPFMV	Score
BF 95	0,411 ± 0,043	+	1	BF 119	0,887 ± 0,049	+	2
BF 99	0,421 ± 0,042	+	1	BF 120	0,834 ± 0,054	+	2
BF 141	0,940 ± 0,049	+	2	Taînug1	0,841 ± 0,048	+	2
BF C1	0,908 ± 0,042	+	2	Tiébélé2	2,010 ± 0,508	+	2
Caromex	0,689 ± 0,06	+	2	Taînug440129	2,528 ± 0,109	+	4
Kakamega	1,137 ± 0,049	+	2	Témoin sain	0,143 ± 0,065	-	0[sz6]

[a]*Average of three replicates (six replicates for the healthy control) ±sample* [b]*Detection (+) or non-detection (-) of SPFMV in the samples analyzed. The detection threshold was 0.300.*

3.3. Detection of SPFMV in F1 hybrids

Out of a total of 131 F1 sweet potato hybrids, SPFMV could not be detected in 29.8% when leaf samples were analyzed using the ELISA test (Table 5). In the majority of hybrids (51.9%), the virus was detected but with a low score of 1. Scores of 2 and 3 were obtained in 12.2% and 6.1% of hybrids respectively. No hybrids showed reactions with a score of 4.

Table 5. Serological detection by ELISA of SPFMV in sweet potato leaves

Entries	D405 nm[a]		Score	Entries	D405 nm		Score
BF59xTIB-6	0,016 ± 0,000	(-)	0	BF59xResisto-4	0,232 ± 0,029	(+)	1
BF77xTanning-3	0,016 ± 0,011	(-)	0	BF82xCIP-17	0,247 ± 0,045	(+)	1
BF24xResisto-20	0,021 ± 0,002	(-)	0	BF80xCIP-1	0,249 ± 0,030	(+)	1
BF82xCIP-6	0,022 ± 0,003	(-)	0	BF92xResisto-13	0,259 ± 0,027	(+)	1
BF59xResisto-8	0,023 ± 0,002	(-)	0	BF82xResisto-5	0,259 ± 0,100	(+)	1
BF59xTanning-9	0,024 ± 0,011	(-)	0	BF92xTIB-1	0,260 ± 0,084	(+)	1
BF59xResisto-9	0,024 ± 0,004	(-)	0	BF59xTanning-9	0,260 ± 0,097	(+)	1
BF82xCIP-5	0,026 ± 0,009	(-)	0	BF59xTanning-2	0,274 ± 0,085	(+)	1
BF59xResisto-24	0,030 ± 0,005	(-)	0	BF59xTanning-3	0,276 ± 0,065	(+)	1
BF24xResisto-34	0,031 ± 0,021	(-)	0	BF59xCIP-4	0,282 ± 0,066	(+)	1
BF92xCIP-5	0,034 ± 0,027	(-)	0	BF24xTIB-3	0,302 ± 0,069	(+)	1
BF92xResisto-7	0,041 ± 0,023	(-)	0	BF13xTIB-1	0,309 ± 0,026	(+)	1
BF82xTanning-5	0,044 ± 0,050	(-)	0	BF59xResisto-9	0,312 ± 0,066	(+)	1
BF13xResisto-3	0,049 ± 0,038	(-)	0	BF80xCIP-7	0,314 ± 0,001	(+)	1
BF24xResisto-15	0,053 ± 0,015	(-)	0	BF59xTIB-3	0,319 ± 0,033	(+)	1
BF24xResisto-16	0,056 ± 0,037	(-)	0	BF59xTIB-5	0,319 ± 0,006	(+)	1
BF82xCIP-16	0,057 ± 0,004	(-)	0	BF82xTanning-10	0,326 ± 0,049	(+)	1
BF24xCIP-1	0,080 ± 0,043	(-)	0	BF24xCIP-2	0,329 ± 0,005	(+)	1
BF13xResisto-3	0,085 ± 0,042	(-)	0	BF24xCIP-4	0,329 ± 0,024	(+)	1
BF24xTIB-1	0,086 ± 0,034	(-)	0	BF59xResisto-2	0,330 ± 0,291	(+)	1
BF59xTIB-4	0,100 ± 0,008	(-)	0	BF12xTanning-7	0,331 ± 0,053	(+)	1
BF59xResisto-7	0,109 ± 0,007	(-)	0	BF59xCIP-3	0,337 ± 0,021	(+)	1

Entries	D405 nm[a]		Score	Entries	D405 nm		Score
BF80xResisto-2	0,111 ± 0,008	(-)	0	BF77xResisto-5	0,339 ± 0,067	(+)	1
BF77xResisto-4	0,117 ± 0,031	(-)	0	BF82xTanning-14	0,344 ± 0,032	(+)	1
BF24xResisto-20	0,121 ± 0,034	(-)	0	BF13xTIB-3	0,348 ± 0,119	(+)	1
BF59xResisto-5	0,124 ± 0,061	(-)	0	BF59xTanning-1	0,348 ± 0,099	(+)	1
BF24xResisto-13	0,130 ± 0,012	(-)	0	BF82xTIB-6	0,354 ± 0,007	(+)	1
BF24xResisto-39	0,130 ± 0,012	(-)	0	BF59xCIP-	0,358 ± 0,083	(+)	1
BF59xResisto-3	0,133 ± 0,031	(-)	0	BF24xTanning-2	0,368 ± 0,052	(+)	1
BF24xResisto-23	0,136 ± 0,052	(-)	0	BF82xCIP-14	0,375 ± 0,021	(+)	1
BF24xTanning-10	0,137 ± 0,013	(-)	0	BF82xResisto-36	0,395 ± 0,070	(+)	1
BF24xTanning-5	0,151 ± 0,039	(-)	0	BF82xCIP-21	0,395 ± 0,043	(+)	1
BF24xTanning-1	0,151 ± 0,000	(-)	0	BF77xCIP-2	0,403 ± 0,090	(+)	1
BF82xCIP-4	0,153 ± 0,065	(-)	0	BF13xTanning-1	0,415 ± 0,207	(+)	1
BF59xResisto-12	0,153 ± 0,040	(-)	0	BF92xCIP-4	0,423 ± 0,031	(+)	1
BF59xResisto-5	0,158 ± 0,015	(-)	0	BF82xTIB-11	0,425 ± 0,019	(+)	1
BF13xTIB-2	0,164 ± 0,089	(-)	0	BF82xTanning-16	0,428 ± 0,005	(+)	1
BF92xResisto-10	0,171 ± 0,006	(-)	0	BF92xTanning-4	0,436 ± 0,042	(+)	1
BF77xTIB-1	0,184 ± 0,095	(-)	0	BF77xResisto-4	0,440 ± 0,108	(+)	1
BF13xCIP-1	0,204 ± 0,058	(+)	1	BF77xTanning-10	0,441 ± 0,119	(+)	1
BF77xCIP-1	0,208 ± 0,008	(+)	1	BF92xTIB-2	0,447 ± 0,038	(+)	1
BF82xCIP-5	0,209 ± 0,080	(+)	1	BF80xResisto-2	0,449 ± 0,032	(+)	1
BF24xTanning-11	0,209 ± 0,098	(+)	1	BF80xTanning-1	0,470 ± 0,145	(+)	1
BF82xTanningx15	0,216 ± 0,013	(+)	1	BF80xTanning-3	0,471 ± 0,084	(+)	1
BF92xTanning-5	0,224 ± 0,035	(+)	1	BF24xResisto-16	0,477 ± 0,050	(+)	1
BF82xTanning-21	0,226 ± 0,028	(+)	1	BF59xCIP-1	0,478 ± 0,241	(+)	1
BF24xResisto-12	0,487 ± 0,109	(+)	1	BF82xTanning-42	0,582 ± 0,047	(+)	1
BF92xCIP-6	0,502 ± 0,434	(+)	1	BF24xTanning-9	0,605 ± 0,085	(+)	2
BF24xResistox38	0,517 ± 0,035	(+)	1	BF92xCIP-2	0,632 ± 0,100	(+)	2
BF77xResisto-3	0,526 ± 0,076	(+)	1	BF59xTanning-7	0,655 ± 0,108	(+)	2
BF80xResisto-4	0,530 ± 0,275	(+)	1	BF77xResisto-5	0,663 ± 0,149	(+)	2
BF92xTanning-7	0,534 ± 0,034	(+)	1	BF77xTanning-2	0,687 ± 0,188	(+)	2
BF59xTanning-3	0,536 ± 0,012	(+)	1	BF59xResisto-10	0,720 ± 0,052	(+)	2
BF82xTIB-9	0,537 ± 0,094	(+)	1	BF59xResisto-13	0,730 ± 0,028	(+)	2
BF82xTanning-8	0,544 ± 0,066	(+)	1	BF77xTanning-7	0,752 ± 0,015	(+)	2
BF82xTanning-18	0,544 ± 0,026	(+)	1	BF82xTIB-8	0,759 ± 0,011	(+)	2
BF82xResisto-35	0,545 ± 0,214	(+)	1	BF77xTanning-6	0,759 ± 0,110	(+)	2
BF92xTanning-4	0,557 ± 0,071	(+)	1	BF77xResisto-7	0,760 ± 0,071	(+)	2
BF59xResisto-4	0,569 ± 0,175	(+)	1	BF59xTIB-2	0,779 ± 0,076	(+)	2
BF77xTanning-1	0,575 ± 0,090	(+)	1	BF92xResisto-2	0,839 ± 0,093	(+)	2
BF28xTanning-24	0,882 ± 0,182	(+)	2	BF24xTIB-2	1,256 ± 0,084	(+)	3
BF82xTanning-18	0,917 ± 0,145	(+)	2	BF82xCIP-13	1,351 ± 0,189	(+)	3
BF24xResisto-24	1,009 ± 0,347	(+)	2	BF92xCIP-3	1,394 ± 0,194	(+)	3

Entries	D405 nm[a]		Score	Entries	D405 nm		Score
BF77xResisto-1	1,150 ± 0,111	(+)	3	BF24x Resisto-18	1,440 ± 0,184	(+)	3
BF24xResisto-3	1,205 ± 0,030	(+)	3	BF59xTanning-14	1,777 ± 0,162	(+)	3
BF82xResisto-12	1,221 ± 0,110	(+)	3	Témoins sain	0,140 ± 0,020	(-)	0

[a]Average of three replicates (six replicates for the healthy control) ±sample; (+) and (-) indicate detection and non-detection of SPFMV in the samples tested, respectively. The detection threshold was 0.200.

3.4. Correlations between viral concentration and genotypic markers

Using ELISA reaction scores and genotyping data with SSR markers, correlations between the different variables were determined. Significant correlations were observed between viral concentration (ELISA reaction scores) and 33 SSR alleles. Of these 33 alleles, six were identified as redundant, leading to results identical to those of other markers. These redundant markers were eliminated from further analysis, which was carried out with 27 SSR alleles.

Relationships between viral concentration and markers were determined by calculating the similarities (using Pearson's coefficient) shown in Table 6 [11]. The highest similarities were observed with SSRs IbU6-147, IBS82-140, IBS134-141, IbL32-152 and IbL32-155, with rates ranging from 61.8% to 71.8%. The lowest similarities between 0.8% and 3% were obtained with SSRs IBS137-136, IBS139-286, IbL16-158, IBS147-194 and IBS18-177.

Marker classification based on the similarity matrix is illustrated in Figure 2. The SSR markers closest to the phenotypic marker are IbL32-152, IBS139-328, IbL32-155, IBS33-176, IBS82-140, IBS134-141 and IbU6-147.

Tableau 6: Table 6 Similarity matrix between phenotypic marker (viral concentration) and SSR markers

	virus	IbL16158	IbL32152	IbL32155	IbL46163	IbL46169	IBS12255	IBS12259	IBS12273	IBS18172	IBS18177	IBS18179	IBS19138	IBS24142	IBS33176	IBS82122	IBS82140	IBS134141	IBS137136	IBS139286	IBS139328	IBS147194	IBS147196	IbU4165	IbU6140	IbU6147	IbU33156
	1	4	11	12	18	20	32	33	37	42	44	45	49	58	63	70	74	114	120	122	133	148	149	178	188	191	212
1	1																										
4	0,012	1																									
11	0,653	0,28	1																								
12	0,618	0,227	0,773	1																							
18	0,428	0,078	0,123	0,359	1																						
20	0,305	0,277	0,248	0,201	0,298	1																					
32	0,406	0,138	0,342	0,266	0,517	0,35	1																				
33	0,104	0,052	0,187	0,12	0,056	0,046	0,077	1																			
37	0,301	0,03	0,085	0,069	0,192	0,342	0,217	0,016	1																		
42	0,327	0,068	0,193	0,157	0,437	0,779	0,494	0,036	0,439	1																	
44	0,03	0,426	0,055	0,338	0,179	0,131	0,079	0,211	0,045	0,102	1																
45	0,267	0,329	0,632	0,652	0,34	0,057	0,127	0,174	0,091	0,208	0,162	1															
49	0,458	0,191	0,378	0,396	0,359	0,013	0,338	0,024	0,157	0,063	0,18	0,109	1														
58	0,104	0,052	0,187	0,12	0,056	0,046	0,077	0,315	0,016	0,036	0,211	0,174	0,024	1													
63	0,57	0,236	0,731	0,961	0,279	0,064	0,177	0,125	0,072	0,018	0,352	0,68	0,372	0,125	1												
70	0,449	0,275	0,579	0,788	0,069	0,243	0,088	0,145	0,083	0,19	0,362	0,762	0,122	0,145	0,746	1											
74	0,696	0,079	0,78	0,803	0,38	0,027	0,328	0,194	0,082	0,186	0,212	0,454	0,439	0,194	0,762	0,614	1										
114	0,653	0,037	0,708	0,773	0,396	0,248	0,391	0,076	0,085	0,193	0,008	0,414	0,459	0,076	0,731	0,579	0,744	1									
120	0,008	0,491	0,105	0,3	0,067	0,043	0,162	0,345	0,046	0,105	0,552	0,459	0,139	0,345	0,315	0,422	0,088	0,034	1								
122	0,01	0,001	0,069	0,239	0,062	0,024	0,099	0,503	0,032	0,072	0,394	0,287	0,002	0,503	0,249	0,289	0,143	0,009	0,456	1							
133	0,563	0,211	0,776	0,591	0,159	0,038	0,405	0,145	0,083	0,161	0,267	0,44	0,37	0,145	0,542	0,432	0,711	0,629	0,097	0,107	1						
148	0,028	0,478	0,025	0,307	0,228	0,268	0,017	0,392	0,224	0,028	0,517	0,194	0,033	0,083	0,319	0,371	0,061	0,177	0,433	0,278	0,321	1					
149	0,332	0,104	0,234	0,239	0,516	0,141	0,345	0,055	0,032	0,219	0,001	0,257	0,273	0,055	0,186	0,197	0,284	0,295	0,083	0,11	0,197	0,141	1				
178	0,297	0,052	0,148	0,003	0,056	0,168	0,228	0,028	0,572	0,232	0,078	0,063	0,149	0,028	0,009	0,145	0,03	0,148	0,061	0,055	0,079	0,083	0,055	1			
188	0,458	0,191	0,5	0,439	0,155	0,013	0,228	0,024	0,058	0,063	0,127	0,271	0,178	0,101	0,457	0,204	0,439	0,459	0,087	0,002	0,533	0,202	0,273	0,024	1		
191	0,718	0,182	0,688	0,819	0,327	0,234	0,32	0,027	0,08	0,183	0,061	0,475	0,43	0,027	0,778	0,632	0,686	0,799	0,033	0,218	0,697	0,053	0,279	0,14	0,512	1	
212	0,041	0,104	0,13	0,239	0,062	0,092	0,099	0,503	0,032	0,072	0,394	0,287	0,07	0,503	0,249	0,289	0,082	0,009	0,533	0,899	0,107	0,278	0,11	0,055	0,002	0,157	1

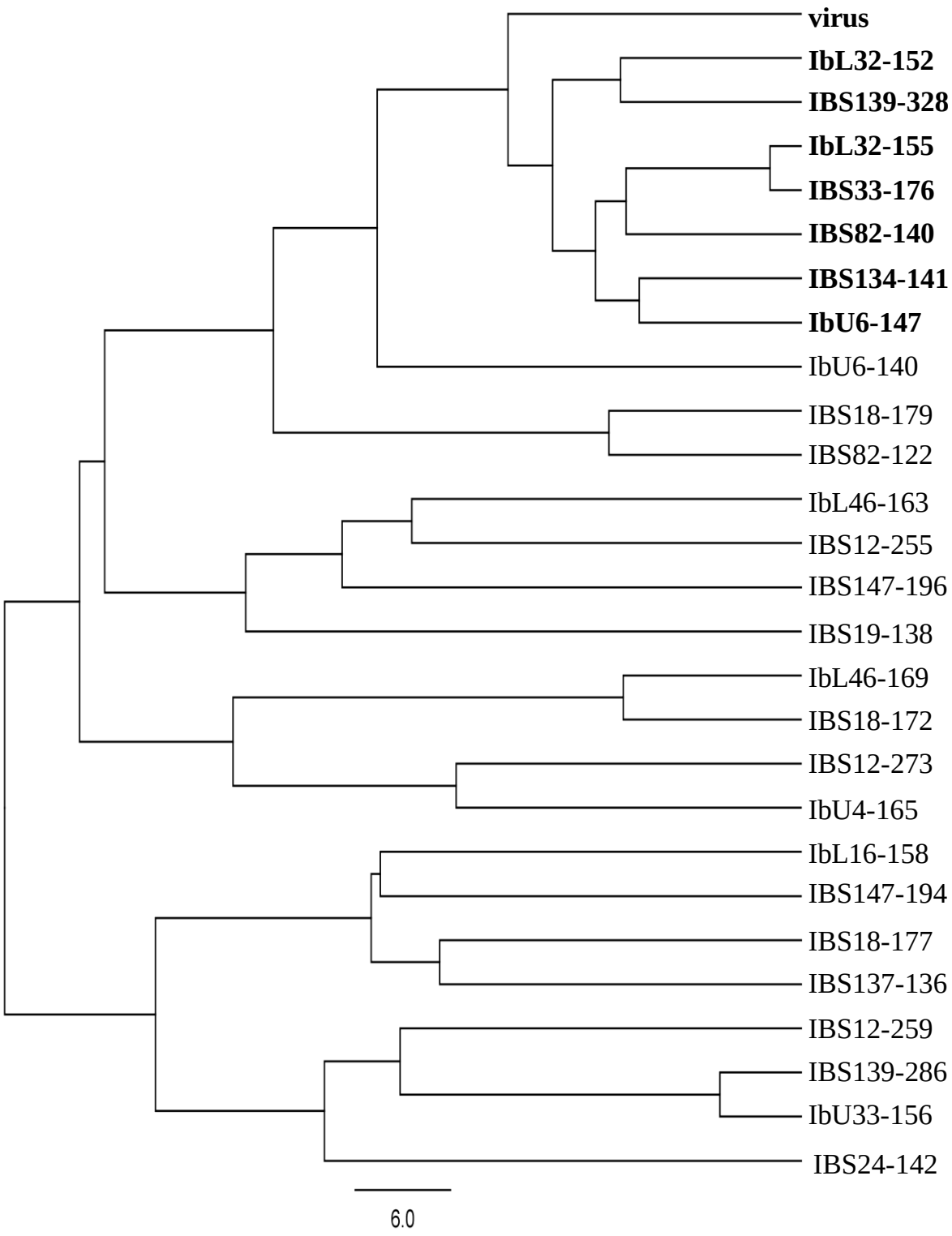

Figure 2. UPGMA-type dendrogram showing relationships between markers.

3.5. Grouping by principal component analysis

Principal component analysis (PCA) was performed to better visualize the relationships between markers. The results presented in three dimensions (Figure 3) show the clustering of the phenotypic marker with the following SSR markers: IbL32-152, IBS139-328, IbL32-155, IBS33-176, IBS82-140, IBS134-141, IbU6-147 and IbU6-140.

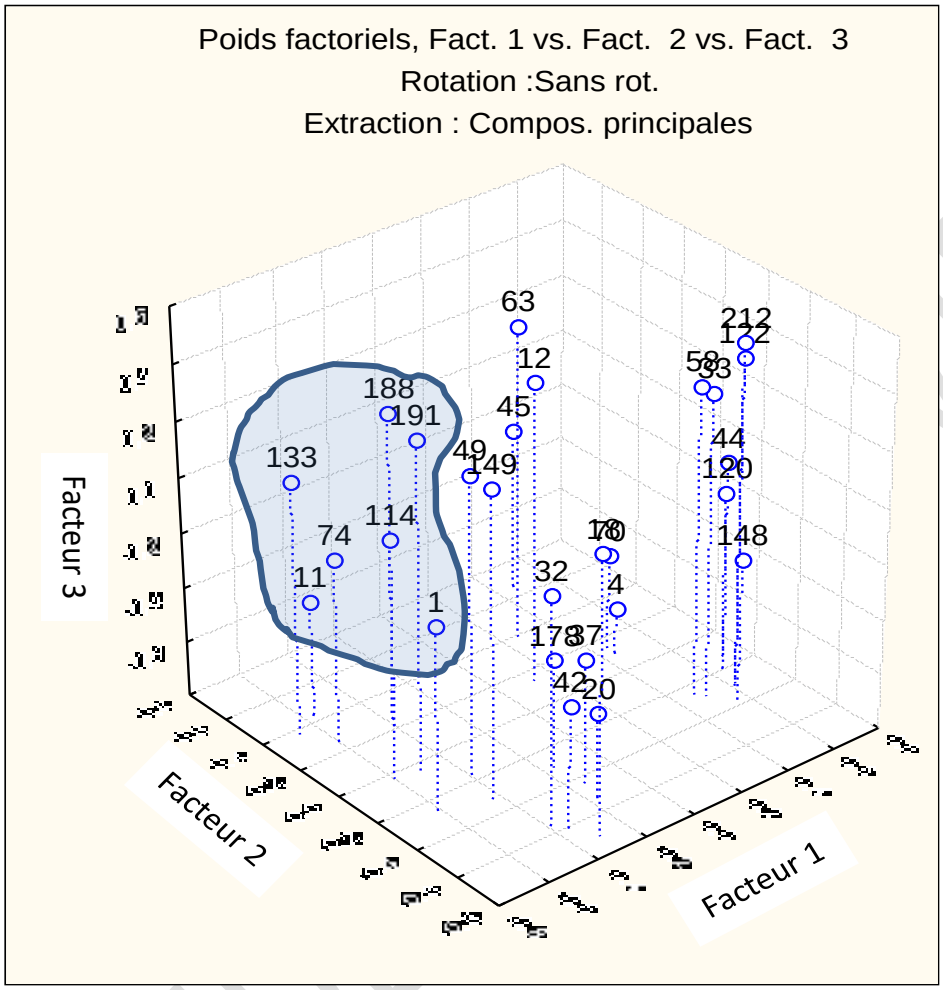

Figure 3: Three-dimensional representation of the results of the principal component analysis. SSR markers close to the phenotypic marker (1) are: IbL32-152 (11), IBS139-328 (133), IbL32-155 (12), IBS33-176 (63), IBS82-140 (74), IBS134-141(114), IbU6-147 (191) and IbU6-140 (188).

4. DISCUSSION

Although sometimes characteristic, the symptoms of viral plant diseases are often complex, originating from various pathogens or even the effect of abiotic factors [12]. This is all the more true[sz7] in the case of sweet potatoes, as several viruses transmitted by ubiquitous vectors such as aphids and whiteflies infect this plant. In the course of this work, which focused on SPFMV, a wide variety of symptoms were observed in sweet potatoes. Some of them were very close to the type

of symptoms reported when the virus was first described [13]. The detection of SPFMV in plants showing various types of symptoms may appear surprising on the leaves in the case of atypical symptoms such as that shown in figure 1H. This result reflects the occurrence of secondary transmission between plants in the sweet potato collection. It also suggests that the plants may have been subject to multiple infections by different viruses, despite the fact that only SPFMV is currently reported in Burkina Faso [6].

In most cases, SPFMV is detected using a serological test on nitrocellulose membrane; [14,15]. This is a qualitative detection method for determining the presence or absence of the virus in the sample analyzed. Virus detection by microtiter plate ELISA carried out in the course of this work offers the added advantage of being able to quantify viral accumulation in the samples analyzed. This also enabled the phenotypic evaluation of potato genotypes by assigning them ELISA reaction scores.

The strong reactions observed in the introduced genotypes confirm the earlier findings of several authors on the frequent existence of very severe local strains of the virus [16]. In agreement with these authors, several sweet potato genotypes from the local germplasm, as well as F1 hybrids, have been shown to be highly unfavorable to virus accumulation. National programs could take advantage of this availability of resistance sources to improve ~~sweetpotato~~sweet potato for SPFMV resistance. Furthermore, the introduction of sweet potato varieties should be carried out with great care to avoid highly susceptible varieties and, above all, the introduction of exotic virus strains into the local environment.

Analysis of ELISA reactions in relation to SSR markers revealed significant correlations between virus accumulation rates and seven of the SSR markers. This was confirmed by structuring the different markers in a dendrogram based on the similarity matrix and in a principal component analysis. The SSR markers identified provide additional knowledge in the search for molecular markers for virus resistance in sweet potato, in particular SPFMV. Only a few AFLP [17] and SSR [18, 19] markers have been reported for ~~sweetpotato~~sweet potato virus disease caused jointly by SPFMV and SPCSV. The availability of molecular markers for the improvement of ~~sweetpotato~~sweet potato against viral diseases is of the utmost importance. Indeed, although transmission is generally via infected cuttings, they make it possible to reduce the tedious recourse to transmission by grafting or insect vectors.

5. CONCLUSION

In the course of this work, SSR-type molecular markers were analyzed for their possible use in marker-assisted selection of sweet potato for resistance to SPFMV. A large collection of sweet potatoes was evaluated to determine the existence of resistant genotypes. Analysis of the results of the serological evaluation in relation to 30 SSRs markers revealed that seven of these markers. These could be used to compensate for the unavailability of molecular markers in marker-assisted selection for SPFMV resistance in sweet potato. However, it is essential to refine their ability to distinguish virus-resistant from susceptible genotypes. Genotyping F1 hybrids could help validate the results obtained. Indeed, their serological analysis has revealed highly contrasting phenotypes in terms of viral accumulation.

The identification of molecular markers for sweet potato resistance to SPFMV should be combined with those for SPCSV, given the synergistic effects of the two viruses. First, it is necessary to determine the presence of SPCSV and its geographical distribution in Burkina Faso.

In the particular context of sweet potato, the identification of genotypes with very low virus accumulation (resistant genotypes) is a major asset for the varietal improvement of the crop for resistance to SPFMV. In the short term, these hybrids could be popularized
short term, if their virus resistance phenotype is confirmed.

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