

Research Article

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Effects of sweet potato feathery mottle virus, sweet potato chlorotic stunt virus and their co-infection on sweet potato yield in Western Burkina Faso

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Abstract: To determine the effects of sweet potato feathery mottle virus (SPFMV), Sweet potato chlorotic stunt virus (SPCSV) and their co-infection on sweet potato yield, twelve sweet potato varieties were assessed in a hotspot area in Western Burkina Faso. The experiment was carried out in a randomized complete-block design with the twelve varieties in three replications. Data were collected on plant growth parameters, plant virus symptoms and yield parameters. Additional testing for selected sweet potato viruses was done using a nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA) and RT-PCR. SPFMV and SPCSV were the viruses detected in this study. Varieties Djakani and Ligri were virus-free and had the highest average yields out of twelve sweet potato

varieties assessed. Field monitoring indicated that 58% of plants were found to be virus-infected. The results suggest that severe symptoms were associated with sweet potato virus disease (SPVD) and yield reduction. However, the interaction of SPCSV with other viruses, which may result in synergistic negative effects on sweet potato yield and quality, needs further research.

Keywords: Diagnostic; Farako-Bâ; Incidence; Serology; virus; Sweet potato

1 Introduction

Sweet potato (*Ipomoea batatas*) is cultivated and consumed in many tropical and sub-tropical regions, including several countries in Africa (Rey et al. 2012). In Sub-Saharan Africa, sweet potato is the third most important root and tuber crop after cassava (*Manihot esculenta*) and yam (*Dioscorea* spp.) (FAOSTAT 2018). Sweet potato is an important food crop and the orange-fleshed sweet potato (OFSP) has the potential to address malnutrition and vitamin A deficiency among children under five and lactating women populations. OFSP varieties which are rich in beta-carotene, a precursor of vitamin A, and other micronutrients are promoted by several Non-Governmental Organizations, as a candidate to prevent malnutrition in children under five (Kimura et al. 2007) cassava and maize were developed. In orange and salmon-fleshed sweetpotatoes, (all-E. However, sweet potato productivity is limited by viral diseases. Wherever sweet potato is grown, viruses are also present (Valverde et al. 2007). Because sweet potato is vegetatively propagated (by taking cuttings directly from a previous crop or from sprouted tubers), it is prone to the accumulation of viruses and other pathogens (Souto et al. 2003; Cuellar et al. 2015)

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and 46 were found to be positive. All were symptomless in sweet potato and generated leaf curling and/or chlorosis in *Ipomoea setosa*. The five most divergent isolates, based on complete genome sequences, were used to study interactions with Sweet potato chlorotic stunt virus (SPCSV). More than 30 viruses belonging to *Badnavirus*, *Begomovirus*, *Carlavirus*, *Caulimovirus*, *Crinivirus*, *Cucumovirus*, *Ipomovirus*, and *Potyvirus* have been reported as infecting sweet potatoes worldwide (Mukasa et al. 2006; Untiveros et al. 2007; Valverde et al. 2007; Clark et al. 2012; Adikini et al. 2016). From these different virus populations, only three viruses and their co-infection have been formally reported in Burkina Faso, namely sweet potato feathery mottle virus (SPFMV), sweet potato chlorotic stunt virus (SPCSV) and sweet potato leaf curl virus US (SPLCV-US) (Tibiri et al. 2019) genus *Begomovirus*. SPFMV and SPCSV are usually involved in co-infection, resulting in severe symptoms described as sweet potato virus disease (SPVD) causing up to 90% yield losses (Loebenstein 2015). Indeed, plants affected by SPVD are easily recognized by farmers and destroyed from the field preventing their use as planting material for the next cropping (Adikini et al. 2016). However, the single infections which are usually symptomless are difficult to identify. Several studies were carried out in East Africa ((Mukasa et al. 2003; Loebenstein 2015; Adikini et al. 2016) root slips (sprouts, in West Africa (Abidin et al. 2017) and South Africa (Mulabisana et al. 2019) 8 locally bred and four imported, were selected for evaluations and these were graft-infected with different virus combinations: 1 on virus disease incidence on sweet potato yield.

In Burkina Faso, sweet potato is the most important root crop and ranked third after cereals and legumes in overall importance (Dabiré and Belem 2001). Sweet potato production has increased from 12,000t in 1990 to 167,000t in 2013 with a yield of 20t/ha in 2018 (FAOSTAT, 2019). Since 2012, the Institute of Environment and Agricultural Research (INERA) is working to develop OFSP varieties adapted to the agro-ecological context of Burkina Faso (Koala et al. 2013; Somé et al. 2015). Twenty-two varieties of OFSP have thus far been developed by the INERA breeding programme (Somé et al. 2015); five of these have been released in 2014, five are being processed for release and many others are at different stages of selection. In Western Burkina Faso, a significant proportion of the cultivated land is used for sweet potato and it is the main food crop. Because Burkina Faso is now actively developing OFSP as a strategy for food security (Somé et al. 2015) and to address malnutrition, studies were conducted to identify major threats to sweet potato production, including viruses. The relationship between virus diseases and

sweet potato yield losses has not been yet reported in a Burkina Faso context. This study was undertaken (i) to assess in a hotspot area in Western Burkina Faso the effects of virus infection on yield losses on twelve sweet potato varieties, (ii) to determine which virus or group of viruses has a greater effect on the twelve OFSP varieties production, and, then (iii) to identify promising varieties resistant to SPVD.

2 Materials and methods

The study was conducted from July to November 2017 at the INERA station of Farako-Bâ (N 11°5' 36.402" W 4°20' 4.581"), located in Western Burkina Faso. The climate of this locality is Sudano-Guinean type with an alternation of two seasons: a rainy season from June to October and a dry season, from November to May. Western Burkina Faso is the sweet potato production hub and virus hotspot environment. The average rainfall ranges from 900 to 1200 mm. The rainfalls recorded during the experiment times were 120.7 mm, 152.7mm, 118.5 mm and 18.4 mm in July, August, September and October respectively. Minimum/maximum temperatures during crop duration ranged from 26C/32C to 30C/37C. Relative Humidity ranged from 52% to 81% for the same period. The soil is of tropical ferruginous type with low organic matter (<2%) and, predominantly sandy to loamy texture with a low cation exchange capacity (CEC). The mineral reserves are also low, particularly in potassium (Bado 2002).

2.1 Plant materials

A total of twelve sweet potato varieties were used in this study. They are composed of locally bred clones (BF59xCIP-1, BF59xCIP-1-2, BF59xCIP-4, BF64-7, BF77xResisto-5-10, BF77xResisto-5-20), a farmer's variety Djakani, Tiebele-2 released in 2014 and introduced materials (Caromex, Ejumula-2, Ligri and Kb_Pourpre) (Table 1). The varieties BF59xCIP-1, BF59xCIP-1-2, BF59xCIP-4, BF64-7, BF77xResisto-5-10, BF77xResisto-5-20 are OFSP in process for release.

2.2 Field experiment

Planting materials were multiplied in insect-proof net tunnels and in adjacent field plots at the primary multiplication stage at Kamboinsé near Ouagadougou (Burkina

Table 1: RT-PCR diagnostic results of evaluated varieties

Record	Varieties	Score	SPFMV	SPCSV	SPVD
1	BF59xCIP-1-26	8	+	+	+
1	BF59xCIP-1-26	8	+	+	+
1	BF59xCIP-1-26	6	+	+	+
2	BF77xResisto-5-10	5	+	-	-
2	BF77xResisto-5-10	6	+	-	-
2	BF77xResisto-5-10	5	+	-	-
3	BF59xCIP-4	2	-	-	-
3	BF59xCIP-4	7	+	-	-
3	BF59xCIP-4	2	-	-	-
4	BF64-7	7	+	+	+
4	BF64-7	8	+	+	+
4	BF64-7	9	+	+	+
5	BF77xResisto-5-20	5	+	-	-
5	BF77xResisto-5-20	3	-	-	-
5	BF77xResisto-5-20	5	-	+	-
6	Caromex	6	+	+	+
6	Caromex	6	+	+	+
6	Caromex	5	+	+	+
7	Ejumula-2	3	-	-	-
7	Ejumula-2	3	+	-	-
7	Ejumula-2	3	-	-	-
8	BF59xCIP-1	6	+	-	-
8	BF59xCIP-1	1	-	-	-
8	BF59xCIP-1	5	+	-	-
9	Kb Pourpre	9	+	+	+
9	Kb Pourpre	1	-	-	-
9	Kb Pourpre	5	+	-	-
10	Tiebele-2	4	+	-	-
10	Tiebele-2	3	-	-	-
10	Tiebele-2	2	-	-	-
11	Ligri	1	-	-	-
11	Ligri	1	-	-	-
11	Ligri	1	-	-	-
12	Djakani	2	-	-	-
12	Djakani	2	-	-	-
12	Djakani	1	-	-	-

Faso). So, cuttings from each of twelve sweet potato varieties plants were healthy. The experimental design was a randomized complete block design with twelve varieties in three replications. Each variety was planted on one ridge of 4 meters long. The ridges were 1m apart and on each ridge the planting spacing was 30cm. The field was plowed, prepared in ridges, and vines were planted on July 2017. NPK fertilizer (14-23-14) was applied at the dosage of 200Kg. ha⁻¹ 21 days after planting. Weeding was done on the 46th day during the vegetative stage and after according to the need.

2.3 Data collection

Data were collected on plant growth parameters (vine length, vine diameter, soil cover), plant virus symptoms and yield parameters. The virus symptoms were recorded using a scale of 1 to 9 as described by CIP in McEwan *et al.* (2015), and leaf samples were harvested 60 days after planting.

2.4 Yield

Storage root number, storage root weight and fresh vine biomass weight per plot were recorded at harvest at 4 months after planting to compute storage root and biomass yield. The root shape, skin colour, flesh colour, level of root flesh oxidation and growth habit for all cultivars were recorded following the CIP/IBPGR descriptors for sweet potato as described by Huaman (1991). For quantitative traits, a mean from five measurements was obtained (Huaman 1991).

2.5 Virus symptoms collection

Virus symptoms were recorded monthly from each plot using the 1 to 9 scales (McEwan *et al.* 2015), with scale 1 for no virus symptom and the scale 9 for severe virus symptom with stunted plants that are dying (Table 2).

2.6 NCM-ELISA for virus diagnosis

To assess the presence of viruses, a nitrocellulose membrane ELISA (NCM-ELISA) test kit with polyclonal antibodies was used according to the manufacturer protocol. The kit was kindly supplied by International Potato Center (CIP), Sub-Saharan office, Nairobi, Kenya, and was able

Table 2: Viruses symptoms observation scale for sweet potato

Viruses symptoms observation scale	
1	Symptoms free
2	Unclear symptoms
3	Clear virus symptoms under <5% of plants through field
4	Clear virus symptoms from 6 to 15% of plants through field
5	Clear virus symptoms from 16 to 33% of plants through field (Less than 1/3)
6	Clear virus symptoms from 34 to 66% of plants through field (Less than 2/3)
7	Clear virus symptoms from 67 to 99% of plants through field (above 2/3)
8	Clear virus symptoms at 100% of plants (no stunting)
9	Severe virus symptoms at 100% of plants (stunting, plant dying)

to identify the following ten viruses: *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato mild mottle virus* (SPMMV), *Sweet potato latent virus* (SPLV), *Sweet potato chlorotic flecks virus* (SPCFV), *Sweet potato mild speckling virus* (SPMSV), *Sweet potato C6 virus* (SPC6V), *Sweet potato chlorotic stunt virus* (SPCSV), *Sweet potato collusive virus* (SPCV, former *Sweet potato caulimo-like virus*- SPCaLV), *Sweet potato virus G* (SPVG), and *Cucumber mosaic virus* (CMV). This primary identification is based on a visual observation of the membrane color shift in virus-positive samples.

2.7 Nucleic acid extraction, RT-PCR

Total RNA was extracted from 36 selected leaf samples representing one sample per plot (each variety in 3 replications) using a RNeasy® Plant Mini Kit (Qiagen). Reverse transcription (RT) was performed on the extracted RNA using MMLV reverse transcriptase (Promega) and Random hexamer (Promega) as primers at 42°C for 1 h. Then the primers CP1A (5'-GCAGAGGATGTCCTATTGCACACC-3') and CP1S (5'-AGTGGGAAGGCACCATACATAGC-3'), previously described by Prasanth, Heggde (2008) were used for the polymerase chain reaction (PCR) to diagnose the SPFMV. PCR for SPFMV detection was carried out in 50 µl reaction volumes using 2.5 µl of the cDNA and the 0.2 µM of primers CP1A/CP1S. The amplification conditions were 94°C for 3min; then 30 cycles of 94°C for 30s, 56.3°C for 30s and 72°C for 1min; and then 72°C for 10min. Afterwards, the primers CP-F (5'- ATGGCTGATAGCACTAAAGTCGA-3') and CP-R (5'-TCAACAGTGAAGACCTGTTCCAG-3') were used for PCR to diagnose the SPCSV according the protocol describe by Qin et al. (2014). To check all the amplifications status, 10µl of PCR products were electrophoresed

in 1% agarose gels, stained with ethidium bromide and viewed under UV transillumination.

2.8 Data analysis

Data were analyzed using SAS (version 9.4, SAS institute Inc., USA). An analysis of variance was computed for all the collected parameters and the mean comparisons were performed for the quantitative variable to evaluate the level of variability among the varieties and replication using the least significance difference at $p < 0.05$ level. Thereafter, correlation analysis was also computed to estimate the relatedness among variables, especially the relationship between the virus single or co-infection on the storage root and upper ground biomass yield.

2.9 Results

Virus symptoms observed in field: The most frequently observed viral symptoms on field plants (Table 2) were stunting (Figure 1d), leaf distortion (Figure 1b), and either a pale mosaic or vein-clearing (Figure 1a-c). Some of the cultivars also were purpling on lower leaves (Figure 1e).

Regarding the observation of symptoms, the Ligri variety was asymptomatic (Figure 1f), Djakani exhibited a low symptom score, BF59xCIP-1, BF59xCIP-4, Ejumula-2, Tiebele-2, and Caromex exhibited moderate symptoms, while the BF59xCIP-1-26, BF64-7, BF77xResisto-5-10, BF77x-Resisto-5-20 and Kb_Pourpre varieties showed severe symptoms (Figure 1d).

NCM-ELISA revealed the presence of SPFMV and SPCSV, while no other viruses tested were found. RT-PCR showed that 56% of plants were SPFMV infected, 30.55%

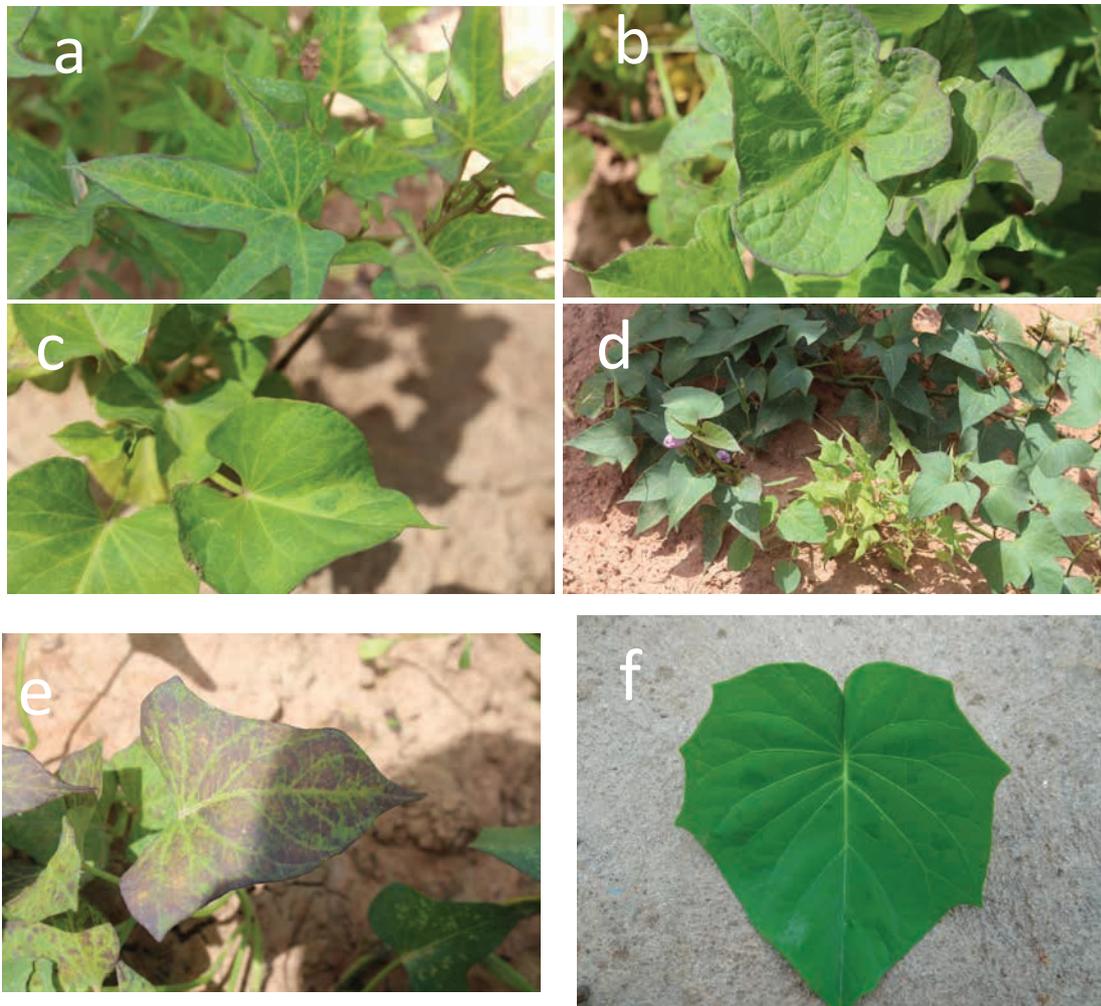


Figure 1: Leaf symptoms on sweet potato plants affected by viruses (a-c) Mild mosaic and vein-clearing. (d) Symptomless plant (extreme left) with severe stunting of growth (extreme right). (e) Purpling spotting in systemically infected leaves. (f) Healthy leaf.

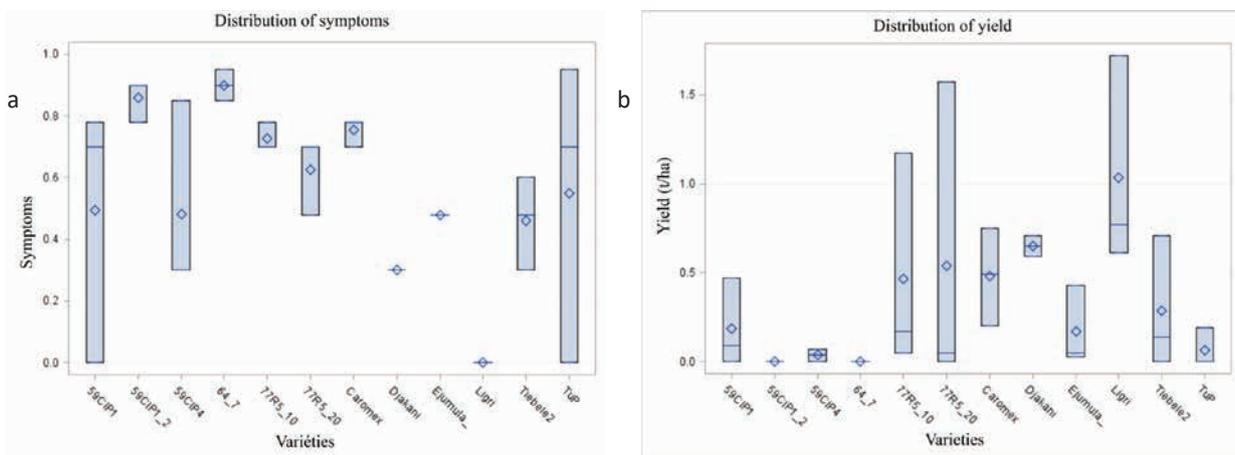


Figure 2: Symptoms and root yield distribution according to varieties

for SPCSV among which 28% were SPFMV+SPCSV infected and confirmed serologically (Table 1). From RT-PCR analyses most of the varieties were SPFMV-positive; one third of varieties was SPCSV-positive including varieties BF59xCIP-1-26, BF64-7, BF77xResisto-5-20, Caromex and Kb_Pourpre, and SPVD-positive on varieties BF59xCIP-1-26, BF64-7, Caromex and Kb_Pourpre. Regarding the total of 36 plants (3 repetitions per variety), 42% were virus-free. Only varieties Djankani and Ligri were found virus-free out of the twelve varieties evaluated (Table 1).

SPFMV and SPCSV distribution was homogeneous for the three replications with an average score of 0.6 (Figure 2). BF59xCIP-1-26, BF64-7 and BF77xResisto-5-10 were SPFMV positive over the extended of the three replications, while BF59xCIP-4, BF77xResisto-5-20, Ejumula-2 and Tiébélé-2 were positive for SPFMV only for one replication. BF59xCIP-1 and Kb_Pourpre were positive only in one replication (Figure 3a, Table 1).

SPCSV infection was less prevalent in the experiment. Only BF59xCIP-1-26, BF64-7 and Caromex were infected with SPCSV over the 3 replications. The varieties BF77xResisto-5-20 and Kb_Pourpre were positive for this virus in 2 replications. While the remaining varieties were SPCSV negative (Figure 3b).

This study showed that SPFMV and SPVD were associated to severe symptoms during the experimentation. However, depending on the variety, SPFMV symptom intensity varied. BF59xCIP-4, Ejumula-2 and Tiébélé-2 varieties showed less symptoms; BF77xResisto-5-20 variety presented moderate symptoms, whereas BF59xCIP-1, BF77xResisto-5-10, BF59xCIP-1-26, BF64-7 and Kb_Pourpre showed severe symptoms. SPCSV in single infection, induces less symptoms on tested varieties (Figure1). Only

BF77xResisto5-20 was found positive for SPCSV in simple infection with moderate symptoms.

Djankani and Ligri were for SPFMV, SPCSV and SPVD negative. However, Djankani variety showed moderate symptoms.

Yield performance: The overall yield performance during this experiment was very low due to poor rainfall (410mm). However, the varieties Ligri and Djankani (average 1t/ha and 0.5t/ha respectively) (Figure 2) that were virus-free had the highest average yields compared to the other varieties evaluated in this study. While BF64-7, BF59xCIP-1-20, BF59xCIP-4 and Kb_Pourpre varieties infected by SPVD had the lowest yields (Figure 2). The average biomass weight ranged between 0.17 and 1.12 t/ha. Varieties Ligri and BF7755-10 were the highest weight with an average of 1.12 and 0.98 t/ha respectively. Data related to the growth parameters, upper ground biomass and root yield were subjected to an analysis of Pearson correlation coefficients with significant positive correlations between symptoms, viruses, upper ground biomass and root yield (Table 3).

The Pearson Correlation analysis (Table 3) showed that symptoms were highly ($P < 0.0001$) and positively correlated with SPFMV, SPCSV and SPFMV+SPCSV infections. SPFMV symptom severity was significantly and negatively correlated with upper ground biomass production and storage root yield.

Our results showed that SPFMV had a higher negative impact on upper ground biomass and root yield compared to SPCSV and SPFMV+SPCSV (Table 3). Out of Caromex, the other varieties BF59xCIP-1-26, BF64-7 and Kb_Pourpre that were in coinfection had a yield of zero tons per hectare (Figure 2b).

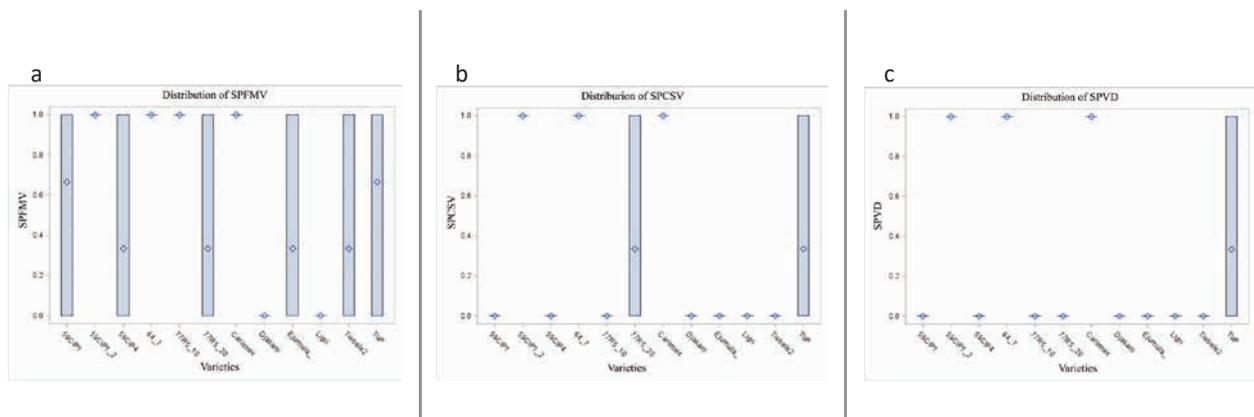


Figure 3: SPFMV, SPCSV and SPVD distribution according to varieties

Table 3: The Pearson correlation coefficients showed significant positive correlations between symptoms, viruses, upper ground biomass and root yield

Coefficients correlation of Pearson, N - 35						
Proba > r sous H0: Rho=0						
	Symptoms	SPFMV	SPCSV	SPFMV+SPCSV	Biomass yield	Yield
Symptoms	1					
SPFMV	0.82526	1				
SPCSV	0.62280 <.0001	0.46193 0.0052	1			
SPFMV+SPCSV	0.61043 <.0001	0.54772 0.0007	0.93420 <.0001	1		
Biomass yield	-0.36245 0.0324	-0.23741 0.1697	-0.28655 0.0951	-0.28864 0.0926	1	
Yield	-0.47612 0.0038	-0.40962 0.0145	-0.27437 0.1107	-0.24420 0.1574	0.81848 <.0001	1

3 Discussion

The rainfall amount and distribution during the experiment was very poor with around 410.3mm of rain being recorded for the minimum rainfall required of 600mm for sweet potato. Therefore, the overall performance of the experiment showed a very low yield. Storage root yield was far lower compared to those reported by Some et al. (2015) on the same station where the yield average was 13.19t/ha and those from Ghana (11.8t/ha) (Abidin et al. 2017) using four varieties. Our study represents the first identification of sweet potato viruses in Burkina Faso and the assessment of their effect on storage root yield. The relatively high prevalence of virus diseases is due to the fact that the experiment was carried out in a virus hotspot environment.

This study's results indicated that viruses constitute a significant constraint to sweet potato production similarly to studies conducted in South Africa, USA and Ghana (Domola et al. 2008; Ling et al. 2010; Abidin et al. 2017). The coinfection involving SPFMV+SPCSV, commonly known as *sweet potato virus disease* (SPVD), has been reported to be the viral disease with the greatest impact on sweet potato yield (Valverde et al. 2007; Rey et al. 2012; Loebenstein 2015). In accordance with our work, other studies reported that coinfection mostly resulted in severe disease symptoms due to a synergistic interaction between SPFMV and SPCSV (Untiveros et al. 2007; Adikini et al. 2016). Several symptoms were observed

during the trial, the most severe were linked to SPVD, revealed by NCM-ELISA tests and confirmed by RT-PCR. The most severe symptoms were negatively correlated with upper ground biomass production and storage root yield which suggests that SPVD is a very fearsome infection for sweet potato production. This result corroborates several studies around the world (Ateka et al. 2004; Mukasa 2004; Clark and Hoy 2006; Njeru et al. 2008; Kim et al. 2017). However, the variety Caromex was SPVD infected but had a yield of 0.5 ton per hectare, among the highest. Caromex variety could be considered as SPVD tolerant. The positive correlation between upper ground biomass and storage root yield to SPVD was also reported by Njeru et al. (2004) in Kenya. Indeed, they had found that single SPFMV and SPCSV infection had no significant effect on biomass contrary to SPVD (Njeru et al. 2004). Likewise, contradictory studies in other countries on the effect of SPFMV on yield of sweet potato cultivars have been reported. Studies have even reported no effects on storage roots and upper-ground biomass yield in comparison with healthy plants (Trenado et al. 2007; Adikini et al. 2016). Some studies reported SPFMV infected plants producing a better yield than the healthy control (Gutiérrez et al. 2003), while others have reported yield reduction of up to 46% (Mukasa, 2004; Njeru et al. 2004; Domola et al. 2008). In this study, the statistical analyses showed the negative correlation between the SPVD and the root yield was not very significant ($P=0.16$).

The varieties that were positive for SPVD and had severe symptoms could be considered as susceptible; however, this needs to be confirmed by further studies. Varieties Djakani and Ligri varieties had no SPVD symptoms and were found to be virus free using NCM-ELISA and RT-PCR, they might be considered as resistant varieties. Abidin et al. (2017) reported fewer virus symptoms on Ligri variety in an experiment conducted in Ghana.

SPFMV was the most prevalent virus detected among evaluated varieties in this study, which is in accordance with many studies that reported that it is the most widespread virus on sweet potato crops in the world (Ateka et al. 2004; Njeru et al. 2008; Maina et al. 2018). As described by Moyer and Salazar (1989), leaf vein clearing is not specific to SPFMV but can indicate the presence of other viral infections. Symptom expression differed with the infecting viruses and varieties. SPFMV-infected plants produced mild symptoms in most of our varieties except Djakani and Ligri varieties (Abidin et al. 2017). This suggests that these varieties are resistant or tolerant to SPFMV infection, although Abidin et al. (2017) concluded that Ligri was infected with SPFMV, nevertheless had mild symptoms. SPCSV was present but did not seem to be very important in sweet potato fields in the western Burkina Faso. Typical symptoms of SPCSV observed were chlorotic spots, purpling and yellowing of the middle and mature leaves, similar to symptoms reported by Adikini et al. (2016).

This study has demonstrated that SPVD and generally virus-infection are serious threats to sweet potato production in Western Burkina Faso as yields are significantly affected. Varieties that were virus-free (Ligri and Djakani) had the highest average yields compared to the infected ones. Based on these results, it has been shown that sweet potato variety yield performance depended on the use of disease-free planting material or the use of SPVD resistant varieties like Ligri or Djakani.

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