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Molecular Diagnosis of Maize Streak Virus (MSV) Infecting Dry Cereals (Maize, Sorghum, Millet) in Burkina Faso

Mahamadou Dao[1,2], Emmanuel Kabore[1], Sidnooma Véronique Zongo[2,3], Thierry K. Dakouo[1], Lassina Ivo[1], Bakary Ouattara[1], Drissa Sereme[1], Jacques Simpore[2,3]*

[1]Virology and Plant Biotechnology Laboratory, Institute of Environment and Agricultural Research (INERA), Ouagadougou, Burkina Faso
[2]Molecular Biology and Genetics Laboratory, University Joseph Ki-Zerbo, Ouagadougou, Burkina Faso
[3]Pietro Annigoni Biomolecular Research Center (CERBA), Ouagadougou, Burkina Faso
Email: *simpore93@gmail.com

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Abstract

The maize streak caused by the maize streak virus (MSV) is a threat to maize cultivation in Burkina Faso. To achieve this, a phytosanitary survey was conducted between 2020 and 2022 in the three agro-climatic zones of Burkina Faso. A total of 600 samples were collected, of which 73% were taken from maize, 10% from sorghum, 13% from millet, and 5% from wild grasses (*Paspalum* sp., *Brachiaria* sp.). Total DNA from the samples have been extracted at Cetyl Trimethyl Ammonium Bromide (CTAB), before being amplified by Polymerase Chain Reaction (PCR). Specific MSV-F/MSV-R primers were used to amplify the capsid protein gene. After aetmplification and visualization using a gel reader MS UVDI 129-0323, bands of approximately 1000 base pairs were observed. A prevalence of 45.71% was obtained for maize, 37.5% for sorghum, and 31.25% for millet. These results indicate that the observed streak symptoms are those of MSV, thus confirming its presence in maize, sorghum, and millet in Burkina Faso, with a predominance in the Sudano-Sahelian zones. The objective of this work was to detect by molecular analysis and assess the prevalence of streak virus infecting dry cereals in Burkina Faso. A study on the genetic diversity of MSV would be necessary to identify the different existing strains in order to develop management strategies for the disease.

Keywords

Maize, Streak, Virus, Diagnosis, PCR, Prevalence, Burkina Faso

1. Introduction

In Burkina Faso, a Sahelian country where the economy relies primarily on agriculture, which employs nearly 86% of the active population, sorghum, millet, and maize are the main food crops [1]. Their contributory share to national production represents 90.16% of cereal production, with 15.13% for millet, 35.26% for sorghum, and 39.77% for maize [1]. Despite this importance, these cereals are subject to many constraints: attacks by pathogens, the most important of which are viruses. Among these constraints, viruses play a crucial role due to their specificity. Previously overlooked or poorly understood because of their often-atypical symptoms, viral diseases have become a major concern for global agriculture in general and for that of Burkina Faso in particular. Among many others, the Maize Streak Virus (MSV) is the most commonly encountered virus affecting cereals in Africa. This disease was first reported in 1900s in South Africa by Fuller, MSV remains a significant constraint to maize production in most regions of sub-Saharan Africa [2] [3]. The disease has also been identified in Nigeria [4], Ethiopia [5], Côte d'Ivoire [6], Ghana [7], and Burkina Faso [8]. The disease is also present on the adjacent islands of Madagascar, Mauritius, Réunion, São Tomé and Príncipe, as well as in Egypt and Yemen [9]. MSV belongs to the genus *Mastrevirus*, family Geminiviridae. The distinctive symptom of the disease is chlorosis, white streaks, and lesions along the veins [10]-[12]. MSV is persistently transmitted by twenty-two known species of leafhoppers from the genus Cicadulina, of which eighteen are present in Africa [13]. It is likely the most destructive viral disease of maize [14]-[16]. Crop losses are estimated at 100% depending on the cultivar and the timing of the infection [15] [17]-[20]. In 1983, the losses incurred by this disease amounted to 30% of maize production in Burkina Faso. While the impacts of MSV on maize yields in Burkina Faso are still low or not well understood, it remains evident that they could be detrimental if agroecological conditions become favorable. Numerous studies conducted worldwide have highlighted viruses infecting cereals and proposed management strategies based on the use of insecticides against the vectors to limit the spread of the disease in the field.

However, in Burkina Faso, little data exists on viruses infecting dry cereals. Nevertheless, Traoré (1993) and Konaté (1994) conducted studies on the epidemiology and variability of MSV in the Sudanian-Sahelian zone, respectively. Unfortunately, these data are not only based on the ELIZA method but are also limited to the Sudanian-Sahelian zone. It is clear that, apart from rice viruses, data on dry cereals remain fragmented and outdated. So, it is important to have knowledge of cereal viruses to develop and propose effective control strategies against this virus to producers. The objective of this work is to molecularly identify the virus responsible for streak disease in cereal crops in Burkina Faso, map its geographical distribution, and estimate its prevalence.

2. Materials and Methods

2.1. Study Area

Burkina Faso has three climatic zones: the Sahelian zone in the north (arid climate,

less than 600 mm of rainfall per year), the Sudano-Sahelian zone in the center (semi-arid climate, 600 to 900 mm of rainfall per year), and the Sudanian zone in the south (subhumid climate, over 900 mm of rainfall per year). These zones are characterized by the duration and volume of precipitation, as well as varying temperatures. The study was conducted in these three climatic zones and involved 30 provinces spread across fourteen regions of the country (see Figure 1).

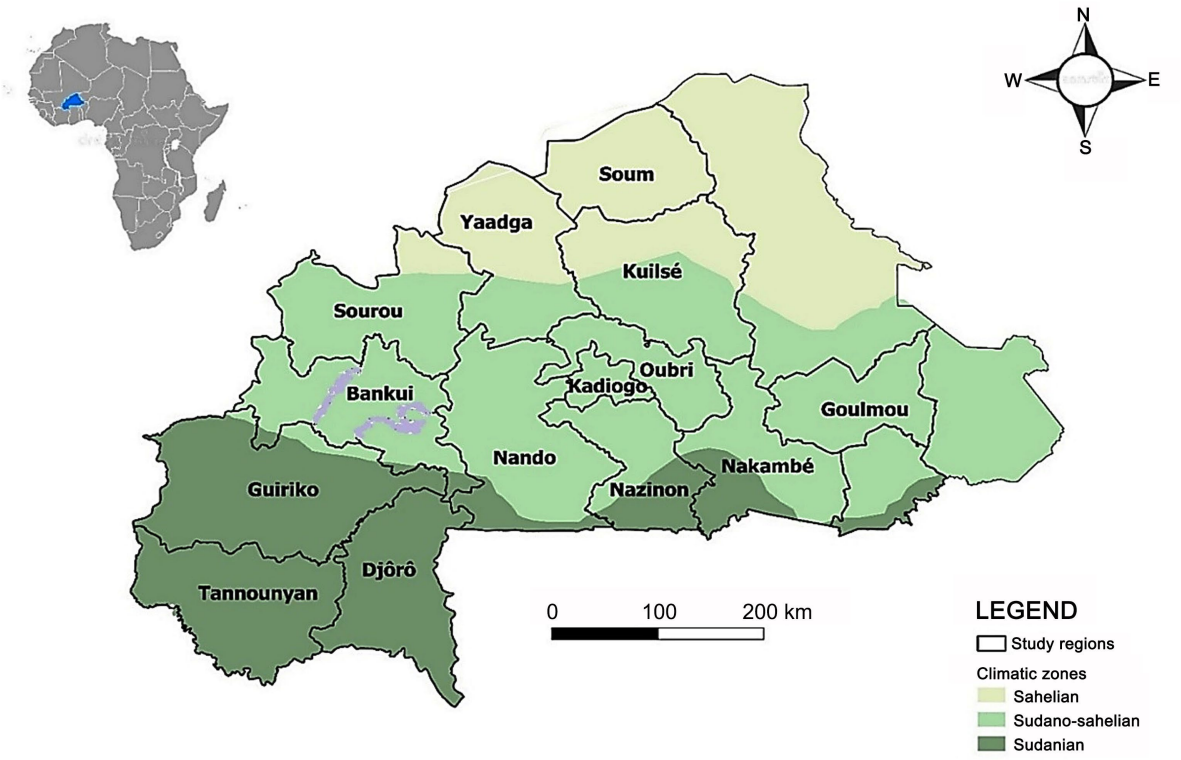


Figure 1. Location map of the surveyed areas and sample collection sites.

2.2. Plant Material

The plant material consisted of leaf fragments showing fine chlorotic streaks parallel to the veins, typical symptoms of MSV. These leaf fragments were collected from maize, sorghum, millet, and from weedy grasses (*Paspalum* sp., *Brachiaria* sp.) in maize farms. Asymptomatic maize leaves were also sampled to be used as control samples.

2.3. Sampling

A survey followed by sample collection was conducted between 2020 and 2022 in maize, millet, and sorghum farms in 36 locations across Burkina Faso (Figure 2). Sampling was done using the "W" method, which involves walking through the field in a zigzag or "W" pattern that covers the entire plot. Samples were collected along this path from plants displaying characteristic MSV symptoms. Samples were also taken under trees if the field had trees. The number of samples collected depended on the number of symptomatic plants observed. The collected samples

were labeled, with key information such as code, date, host, and GPS coordinates recorded on each label. The samples were transported to the laboratory in a cooler with ice and stored in a freezer at −20˚C.

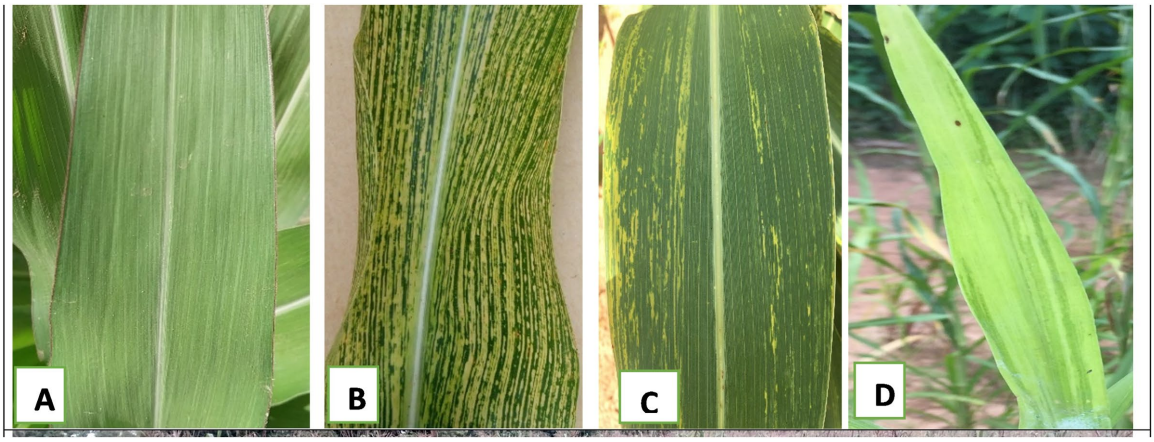


Figure 2. (A) Healthy maize leaf; (B) Maize leaf showing streak symptoms; (C) Sorghum leaf showing streak symptoms; (D) Millet leaf showing streak symptoms.

2.4. Extraction of Nucleic Acids (DNA)

Total DNA was extracted following the protocol of Permingeat *et al.* (1998), modified for extraction with Cetyl Trimethyl Ammonium Bromide (CTAB). Indeed, CTAB is a detergent that, during extractions, helps preserve the integrity of DNA during cell lysis. The extracted DNA was quantified using a NanoDrop spectrophotometer (NanoDrop One/OneC).

2.5. Amplification of Nucleic Acids

Virus amplification was performed by PCR using the specific primers MSV-F 5'-TGAAGGCTCGRCAAGGCAGAT-3' and R 5'-TTCRATGTTYTGCCCGCCGAG-3' [5], chosen to amplify the capsid protein (CP) gene along its entire length. Amplification of the CP gene allows for the differentiation of viral species due to its unique structural sequence. Indeed, the capsid protein (CP or V1 for Viral Coat Protein) gene is a conserved and characteristic genomic region used by several authors for virus identification and detection by PCR. The procedure involved preparing a reaction mixture with a total volume of 25 µl. For this purpose, a mix composed of 2 µl DNA, 0.75 µl of each specific forward and reverse primers for MSV at 10 mM, 6 µl de Blend Master Mix (10 Mm $MgCl_2$) 5X and 20.5 µl water was prepared. The amplification program consisted of the following steps: an initial denaturation at 94˚C for 2 minutes followed by 35 cycles of denaturation at 94˚C for 30 seconds, annealing at 61˚C for 30 seconds, extension at 72˚C for 1 minute, and a final extension at 72˚C for 10 minutes (Table A1).

2.6. Agarose Gel Electrophoresis

To analyze the PCR products obtained after amplification, agarose gel electropho-

resis was performed at 100 V for 30 minutes. The gel was prepared in a 0.5x TAE buffer and melted by microwave heating. After cooling, approximately 2 µl of ethidium bromide (EtBr) was added to the agarose solution. The gel was then poured into pre-prepared casting trays. Upon polymerization, the gel-containing support was placed in an electrophoresis tank filled with 0.5x TAE buffer. PCR products (10 µl) were mixed with loading dye and loaded into the gel wells. A molecular weight marker was loaded in parallel to assess band sizes. After electrophoresis, DNA fragments were visualized using a UV transilluminator gel reader MS UVDI 129-0323. Bands matching the expected size are indicative of positive samples.

2.7. Sequencing and Bioinformatic Analysis of Sequences

The PCR products were sequenced, and the sequences were edited and aligned using BioEdit molecular analysis software. Sequences of the MSV-A strain originating from nine African countries were downloaded from the NCBI database using GenBank accession numbers to study the phylogenetic relationships between the isolates.

2.8. Calculation of Prevalence

The prevalence of MSV was assessed by region and by crop type. It refers to the ratio between all positive samples and the total number of samples tested. Prevalence was calculated using the following formula:

$$\text{Prevalence}\ (\%) = \frac{\text{Positifs samples}}{\text{Tested samples}} \times 100$$

3. Results

3.1. Sample Collection

A total of 600 samples, including 584 symptomatic and 16 asymptomatic, have been from 150 smallholder fields in Burkina Faso. The symptoms observed on the crops mainly included fine longitudinal chlorotic streaks on the leaves and plant growth retardation (Table 1). The samples are classified by crop type, with 420 on maize, 60 on sorghum, 74 on millet, and 30 on weeds (Table 1). 90% of the samples were collected during the rainy season in smallholder farms and 15% during the dry season in vegetable growing areas.

Table 1. Proportion of samples collected by region and by speculation.

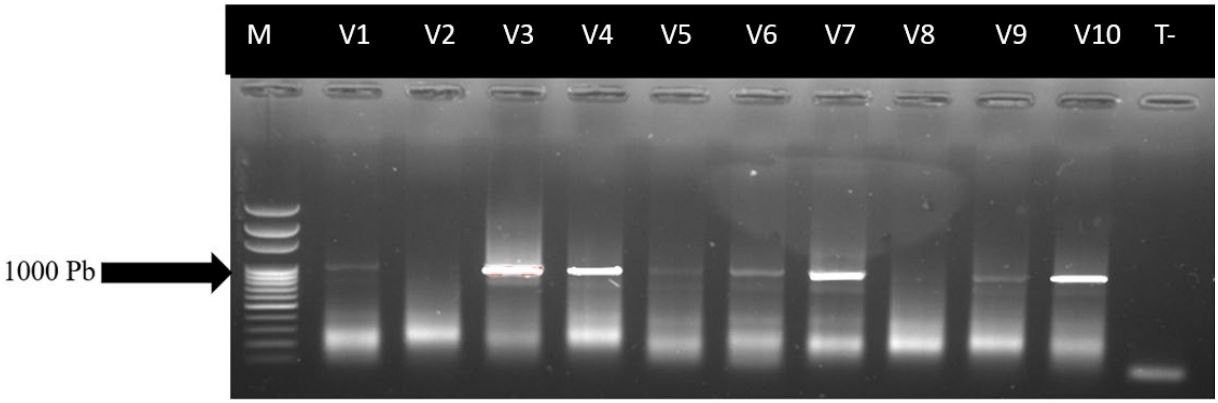
Regions	Samples collected	Maize	Sorghum	Millet	Wild grasses	Healthy leaves
Bankui	55	36	7	8	2	2
Sourou	10	10	0	0	0	0
Kadiogo	55	40	5	4	4	2
Kuilsé	60	28	10	10	5	2
Nakambé	40	20	10	8	0	2

Continued

Nazinon	60	40	5	7	5	0
Nando	60	48	4	8	6	2
Tannounyan	55	40	4	5	4	2
Goulmou	10	10	0	0	0	0
Guiriko	80	68	6	4	0	2
Yaadga	20	20	0	0	0	0
Oubri	40	30	5	5	0	0
Djôrô	45	30	4	5	4	2
Soum	10	0	0	10	0	0
Total	**100%**	**70%**	**10%**	**12.3%**	**5%**	**2.7%**

3.2. MSV Detection

The electrophoretic migration profiles of the virus diagnosed through molecular analysis (PCR) serve as evidence of the presence of MSV in the tested samples (Figure 3). The MSVF/R primer pairs used for MSV detection produced amplicons of approximately 1000 bp in the tested samples and the positive control, while no amplification was observed in the negative control (healthy leaves). The amplification was characterized by strong bands in samples V3, V7, and V10 (T+), and weak bands in samples V1, V4, V5, V6, and V9. The remaining samples, particularly V2, V8, and T−), which did not show bands of the required size, are declared negative in the test. Raw images of some gels are included in Figure A1.



Legend: M = 100 bp DNA ladder size marker, T+ = Positive control (V10 sample), T− = Negative control.

Figure 3. Electrophoretic migration profiles on gel of the diagnosed virus.

Figure 4 presents the phylogenetic tree constructed from the nucleotide sequences of MSV isolates from Burkina Faso and those from eight other African countries available in Genbank (NCBI). Analysis of the tree shows that our isolates are integrated within the group of MSV-A strain isolates from the eight countries.

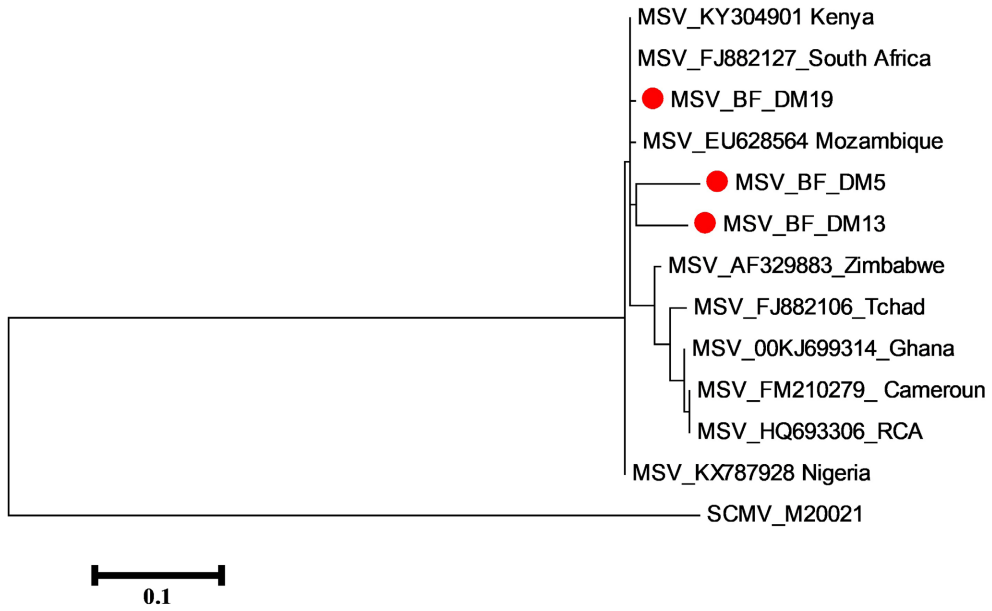


Figure 4. Maximum likelihood phylogenetic tree (constructed with the nucleotide substitution model J-C). The robustness of the trees was assessed with 1000 bootstrap samples. Viral isolates found in this study are indicated with red dots. The other isolates are from eight African countries available in GenBank. SCMV is used as the root of the tree.

3.3. Prevalence of MSV

MSV was detected in samples showing fine chlorotic streaks aligned along the veins, which are typical MSV symptoms (Table 1). A high prevalence (45.71%) of the virus was found in the analyzed maize samples. Other crops such as sorghum and millet also tested positive for the disease, with respective prevalences of 37.5% and 31.25%, as well as 25% of the analyzed weed samples (Figure 5).

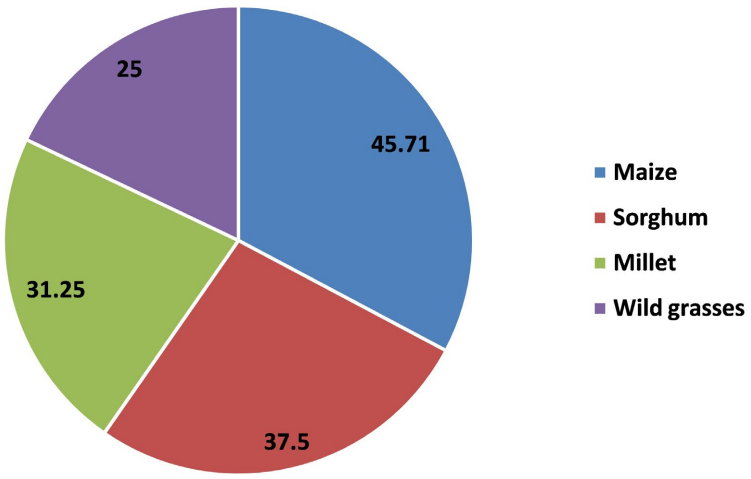


Figure 5. Proportion of cereal and host weed samples testing positive for MSV.

The proportion of infected hosts varies from one area to another. Most samples from the Sudanian-Sahelian zone regions have prevalences exceeding 50%, while

those from the Sudanian zone have less than 50% (Figure 6). The highest rates were recorded in the Nakambé (60%) and Yaadga (56%) regions, while the lowest rates were obtained in the Goulmou (20%) and Nando (23%) regions.

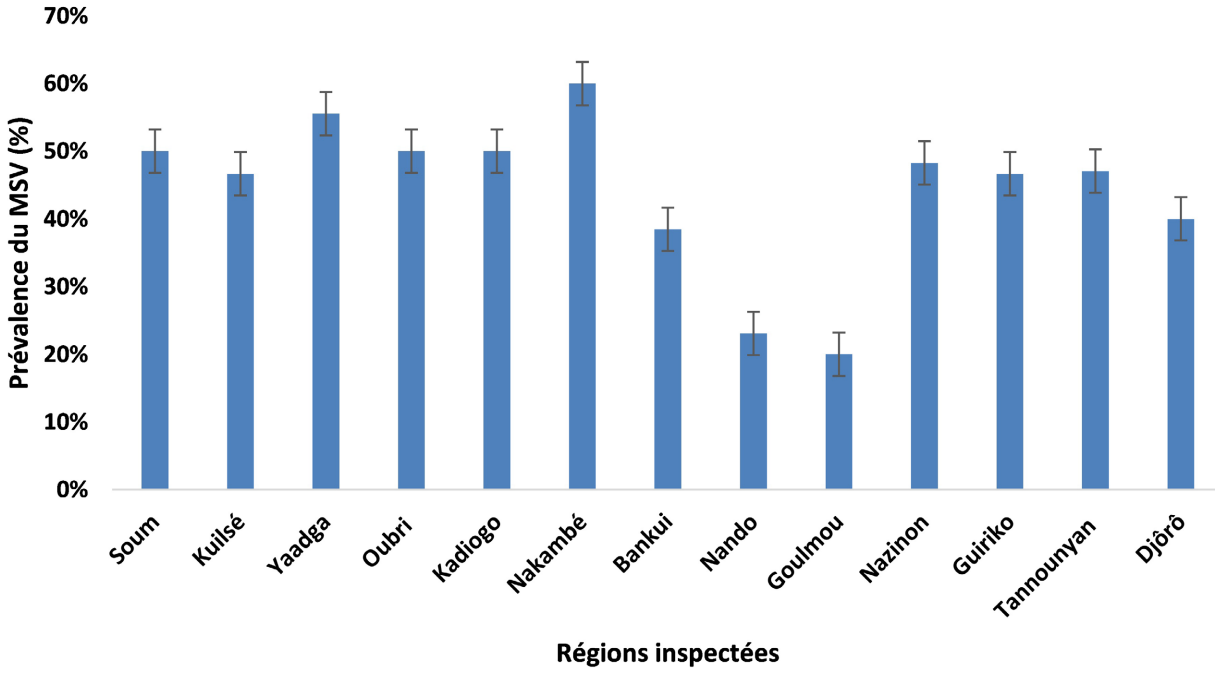


Figure 6. Prevalence of MSV by inspected regions. The error bars represent the standard error.

4. Discussion

This study is preliminary and has allowed for the amplification of the MSV capsid protein gene, extracted from samples collected in Burkina Faso. Symptom–PCR concordance confirmed MSV infection. Analysis of phylogenetic relationships with sequences from other African countries revealed its belonging to the MSV-A strain. The virus was detected in 42.78% of the samples analyzed. The remaining 57.22% could be attributed to the virus being present in low concentration in these samples or to infection by other viruses inducing similar symptoms, given that the primers used were specific to MSV. The Digitaria Streak Virus (DSV) is a specific case that causes streak symptoms on the leaves of plants in the genus *Digitaria*. It is distinct from MSV but serologically related to some of its strains [21] [22]. The detection of MSV in maize, millet, and sorghum indicates that these cereals, like most *Poaceae*, serve as hosts in which the inoculum of the disease can persist or from which the virus can spread. These results corroborate those of [11] [23]-[25], which confirm that MSV is associated with a wide range of indigenous grass species in Africa, as well as various cereal crops, including wheat, oats, sugarcane, millet, rice, barley, rye, and sorghum. Similarly, the ELISA test results used by [8] to detect the virus in samples showed the presence of MSV in 36 species of wild grasses [8]. Similar findings were reported by [26] during a study on virus detection, epidemiology, and observations on some resistant maize varieties in Burkina

Faso. However, the ELISA test was not sensitive enough to distinguish MSV from Digitaria Streak Virus (DSV).

At the sub-regional and African level, the presence of MSV in all major agroecological zones of Burkina Faso aligns with the findings of [20], who concluded that MSV is a widespread viral disease across tropical and subtropical Africa. In the same vein, [27] argue that MSV had a geographic range covering the entire sub-Saharan Africa. This geographic distribution of MSV, according to [13], is attributed to the presence in the environment of *Cicadulina* species, the main vectors of the disease.

Regarding the prevalence and geographic distribution of MSV in Burkina Faso, the study confirmed its presence in all localities where samples were collected (**Figure 4**), with a higher proportion (45.71%) in maize samples. This confirms that maize is the main host of MSV and that all agroecological zones in Burkina Faso are thus infected. The works of [28] had already shown that not only was MSV present in all agroecological zones of Burkina Faso, but also, and importantly, that it was present in the main maize production areas. According to [29], maize production thrives better in areas characterized by annual rainfall between 750 and 1750 mm for highlands and 500 mm for lowlands. In Burkina, these include the Sudanian-Sahelian and Sudanian zones, characterized by an annual rainfall exceeding 600mm. The high prevalence of MSV in these zones could be explained by their favorable agroecological conditions for the development of host plants and vector insects. These hypotheses are in line with those of [30], who affirm that the size of Cicadulina populations is mainly determined by the abundance of host grasses. This abundance of grass, in turn, is primarily limited by rainfall.

The promotion and development of dry season cereal production (rice and maize) lead to the overlapping of seasons. This situation could contribute to the survival of the MSV vector insect. Due to the insect's flight performance, the virus can effectively circulate in the environment. The detection of the virus in samples collected during the dry season in developed areas could be explained by this phenomenon. This assertion aligns with the findings of [30] and [31], who confirm that changes in farming practices, including irrigation implementation, lead to maize streak epidemics in the dry season. The low prevalence of MSV in the dry season could be attributed to the low humidity levels that hinder the development of Cicadulina during this period. Similarly, [32] notes that humidity is a limiting factor for the complete development of the insects. The excessive and unregulated use of insecticides against vegetable crop pests could also explain the low survival rate of leafhoppers and, consequently, the low prevalence of MSV on cereals grown in irrigated areas during the dry season. These ideas align with those of [33], who argue that agricultural practices and the use of pesticides aimed at reducing the number of vectors are viable means of managing the disease.

5. Conclusion

This work presents the first study on the prevalence and distribution of MSV on maize in Burkina Faso. The virus was identified through molecular analysis of the

C2 region of the capsid protein. Like maize, the virus was detected on sorghum, millet, and host weeds. Sample collection was conducted in 150 fields across 30 provinces and 14 regions of Burkina Faso. The presence of the virus was revealed in all localities where samples were collected, with a higher proportion (45.71%) of maize samples. This preliminary work will pave the way for a study on the genetic diversity of the virus in Burkina Faso, contributing to the establishment of sustainable control strategies for the benefit of research centers and maize producers.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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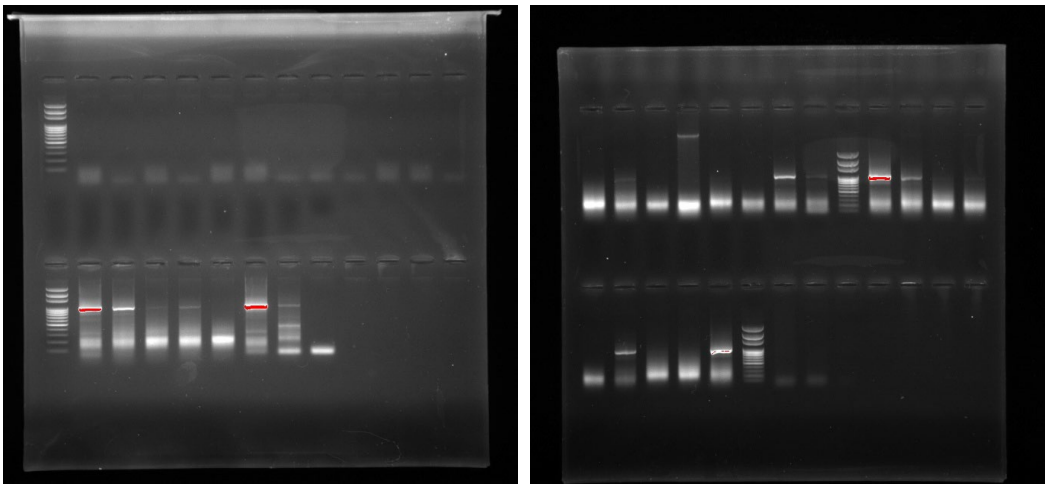
Appendix

Table A1. Concentrations of the reagent medium and PCR program.

Concentrations of the reactive medium		
Sample number	For 1	For 9
DNA	2	-
Master mix	6	54
F	0.75	6.75
R	0.75	6.75
H_2O	20.5	184.5
Volume of mix to distribute/Tube	28	
Final Volume	30	
PCR program		
PCR Conditions	94˚C for 2 mn	
	94˚C for 30 s	
	61˚C for 30 s	35 cycles
	72˚C for 1 mn	
	72˚C for 10 mn	



NB: The red color is a defect related to the camera of the gel reader MS UVDI 129-0323.

Figure A1. Raw photos of some gels.