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Molecular Characterization of Maize Streak Virus Reveals the First Occurrence of Strain H in Burkina Faso

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Abstract

Aims: Maize is a major staple food in Sub-Saharan Africa but its production is severely affected by maize streak disease caused by *Maize streak virus* (MSV). Despite the importance of the disease for food security, information on the genetic diversity of MSV isolates in West Africa remains limited. This study aimed to characterize the genetic diversity of MSV isolates collected from maize and wild grasses in Burkina Faso and

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to determine their phylogenetic relationships, with particular emphasis on identifying previously unreported virus strains.
Study Design: This was a molecular characterization study based on PCR detection, DNA sequencing, and phylogenetic analysis of MSV isolates.
Place and Duration of Study: The study was conducted in Burkina Faso using MSV isolates collected from maize and wild grasses during field surveys over two years.
Methodology: Leaf samples exhibiting symptoms of maize streak disease were collected from maize and wild grasses. MSV was detected by polymerase chain reaction (PCR), and six PCR products of sufficient quality were subjected to Sanger sequencing. The obtained sequences were analyzed for nucleotide composition and pairwise nucleotide identity. Principal component analysis (PCA) was performed to assess genetic relationships among isolates, and phylogenetic relationships were inferred using the Maximum Likelihood (ML) method together with reference MSV sequences retrieved from GenBank.
Results: Six MSV isolates were successfully sequenced and analyzed. Pairwise nucleotide identity and phylogenetic analyses revealed a clear genetic structure, separating the isolates into distinct evolutionary groups. Most isolates clustered within MSV lineages previously described in the country. Interestingly, isolates obtained from *Brachiaria lata* (Schumach.) C.E. Hubb. formed a well-supported cluster belonging to MSV strain H, representing the first report of this strain in Burkina Faso. PCA further confirmed the genetic differentiation among the identified groups.
Conclusion: This study provides the first molecular evidence of *Maize streak virus* strain H in Burkina Faso and expands current knowledge of the genetic diversity of MSV in the country. The detection of strain H in *Brachiaria lata* emphasizes the epidemiological importance of wild grasses as reservoirs of emerging MSV variants and provides valuable information for strengthening virus surveillance and developing sustainable maize disease management strategies.

Keywords: Burkina Faso; genetic diversity; Maize streak virus; MSV-H; phylogenetic analysis; wild grasses.

1. Introduction

Maize streak virus (MSV; family *Geminiviridae*, genus *Mastrevirus*) is the causal agent of maize streak disease, a serious threat to maize production across sub-Saharan Africa. It causes severe disease by inducing streak symptoms and physiological disorders in plants leading to significant yield losses (Bosque-Pérez, 2000; Mushayi et al., 2025; Shepherd et al., 2010). Because maize is a major staple food and an important cash crop in sub-Saharan Africa (Erenstein et al., 2022), MSV poses a significant threat to food and nutrition security in the region. In Burkina Faso, maize is one of the country's major staple cereals, alongside sorghum and millet. Recognizing its strategic importance for food security and agricultural development, the Government has identified maize as a priority crop under the recent Presidential Initiative for Agricultural Production and Food Self-Sufficiency, which aims to increase agricultural production and strengthen national food self-sufficiency.

Maize streak virus has a single-stranded circular DNA of approximatively 2.7 kb (Fiallo-Olivé et al., 2021). This genome comprises four open reading frames (ORF): two in the viral-sense (V1 and V2) encoding the movement protein (MP) and the Capsid Protein (CP), respectively. The other two ORFs (C1 and C2) which overlapped, are in the complementary sense and encode replication-associated proteins Rep and RepA. The two pairs of ORFs are separated on the one hand by a large intergenic region (LIR) containing the origin of replication and the bidirectional promoter and on the other hand by a small intergenic region (SIR) containing the transcription termination signals (Martin & Shepherd, 2009; Shepherd et al., 2010).

Maize streak virus is transmitted in a persistent manner by several leafhoppers of the genus *Cicadulina* (family *Cicadellidae*) (Mushayi et al., 2025), with *C. triangula* being the most reported in Burkina Faso (Konaté & Traoré, 1992). Various factors contribute to the transmission mechanism and viral dynamics, including agroecological factors which play a role in MSV emergence (Alegbejo & Banwo, 2005; Mbong et al., 2021). Furthermore, many studies have highlighted the crucial role of wild *Poaceae* species in the epidemiology of the disease, as a broad range of grasses have been identified as MSV hosts (Konaté & Traoré, 1992; Kraberger et al., 2017; Mushayi et al., 2025). They serve as reservoirs and secondary hosts, facilitating transmission to maize and contribute to the overall genetic diversity of the virus (Varsani et al., 2008).

Genetic diversity of MSV is well documented since different MSV strains (or genotypes) have been described (Martin et al., 2001; Shepherd et al., 2010; Varsani et al., 2008). These include the predominant genotype, MSV-A, which is adapted to maize and is widely distributed throughout Africa, including Burkina Faso and its neighboring countries. Ten additional genotypes, designated MSV-B to MSV-K, have also been identified and are primarily associated with wild grasses. Most of these genotypes have been reported from southern Africa. In contrast, information on the molecular diversity of MSV in West Africa remains limited where only a few isolates have been characterized. In Burkina Faso, molecular data are particularly scarce. Apart from the recent detection of the MSV-G genotype infecting rice in the country during surveys of rice yellow mottle disease (Fouad et al., 2025), previous studies have been limited to small-scale surveys involving relatively few maize samples (Kraberger et al., 2017; Monjane et al., 2011; Varsani et al., 2008). Consequently, the diversity, distribution, and evolutionary relationships of MSV populations circulating in the country remain poorly understood. Given the increasing importance of maize production and the recurrent occurrence of maize streak disease, a comprehensive assessment of the genetic diversity of MSV is essential to improve our understanding of maize streak disease epidemiology and to support the development of effective disease management strategies, including resistance breeding and molecular surveillance.

Therefore, the present study aimed to characterize the genetic diversity of MSV isolates collected from maize-growing regions of Burkina Faso using molecular and phylogenetic analyses, and to determine their relationships with previously described MSV genotypes reported across Africa.

2. Material and Methods

2.1 Virus Samples

A total of 10 leaf samples were collected from symptomatic plants of maize (*Zea mays* L., and wild grasses including *Brachiaria lata* (Schumach.) C.E. Hubb., *Eleusine indica* (L.) Gaertn. and *Digitaria sp*. Most symptoms were severe but moderate and mild symptoms were also observed, particularly on maize (Fig. 1). Samples were coded BF1-BF5 and BF9 for maize, BF6 and BF7 for *B. lata*, BF8 for *Digitaria sp* and BF10 for *E. indica*, respectively (Table 1).

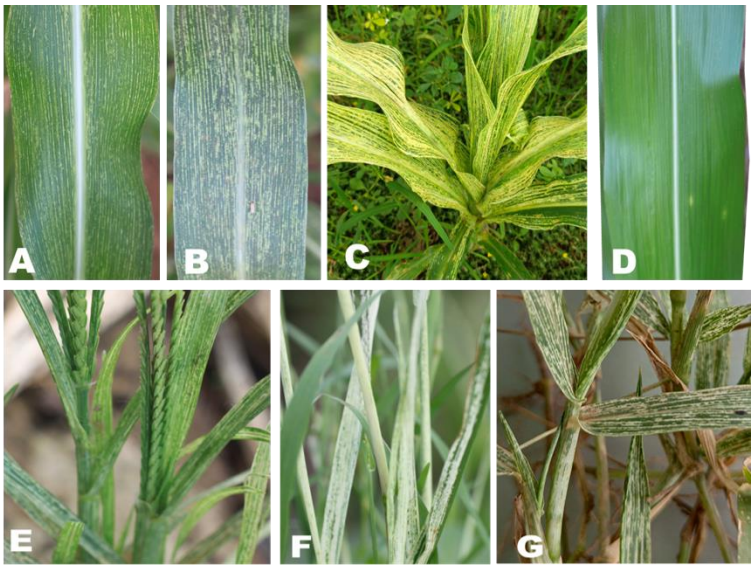

Fig. 1. Symptoms of maize streak disease on maize (A–C), *Eleusine indica* (E), *Digitaria* sp. (F), and *Brachiaria lata* (G). (D) Healthy maize leaf

2.2 DNA Extraction

Leaf tissues were ground using sterile mortars and pestles. Total DNA was extracted using the Solarbio® nuclear extraction kit (Solarbio Science & Technology Co., Beijing, China), following the manufacturer's

instructions. Briefly, samples were ground in the lysis buffer, and then RNA and proteins were removed by treatment with RNase A and proteinase K, respectively. The DNA was then bound to a silica-based column, followed by washing twice with ethanol. Finally, the purified DNA was eluted in nuclease free water. The quality and concentration of the purified DNA were assessed using a SPECTROstar Nano spectrophotometer (BMG LABTECH) (Koetsier & Cantor, 2019).

Table 1. Characteristics of *Maize streak virus* (MSV) isolates collected in Burkina Faso

MSV isolate	Plant species	Location	longitude	latitude
BF1	*Zea mays* L.	Saaba	-1.417324	12.363090
BF2	*Zea mays* L.	Saaba	-1.417334	12.362986
BF3	*Zea mays* L.	Orodara	-4.905144	10.973656
BF4	*Zea mays* L.	Bama	-4.381509	11.364403
BF5	*Zea mays* L.	Toussiana	-4.623279	10.825950
BF6	*Brachiaria lata* (Schumach.) C.E. Hubb.	Ouaga-Nioko II	-1.510189	12.417602
BF7	*Brachiaria lata* (Schumach.) C.E. Hubb.	Kamboinsé	-1.547950	12.456722
BF8	*Digitaria sp.*	Bendatoega	-1.655085	12.495964
BF9	*Zea mays* L.	Bendatoega	-1.656841	12.496951
BF10	*Eleusine indica* (L.) Gaertn.	Pont-Nazinon	-2.008866	12.194538

2.3 Polymerase Chain Reaction (PCR)

Maize streak virus was detected by PCR using degenerated forward (5'-TTGGVCCGMV GATGTASAG-3') and reverse (5'-CAAAKDTCAGCCTCCG-3') primers (Tembo et al., 2020). These primers allow the amplification of a ~1300 bp segment of the genome spanning Rep A gene, LIR and the 5' half of MP gene. Each amplification was done in a total reaction volume of 25 µL containing 12.5 µL of GoTaq® G2 Master Mix 2X (PROMEGA), 2.5 µL of each primer at 10 µM, 5 µL of DNA extract at 10 ng/µL and 2.5 µL of nuclease free water. PCR program (Tembo et al., 2020)consisted of a denaturation step of 5 min at 95°C following by 30 cycles, each of 95°C for 45 sec, 54°C for 30 sec and 72°C for 90 sec. A final elongation step of 10 min at 72°C was added. After amplification, PCR products were analyzed on 1 % (w/v) agarose gel electrophoresis in Tris-Borate-EDTA buffer and stained with ethidium bromide.

2.4 DNA Sequencing and Data Analysis

PCR products were sent to Macrogen Europe (Amsterdam, The Netherlands) for sequencing using the Sanger method. Each sample was sequenced in both directions yielding two readings per base and leading to sequence accuracy > 99.9%. Forward and reverse sequence reads were assembled into consensus contigs using the SeqMan module of the Lasergene software package (DNASTAR, Madison, WI, USA). Resulting sequences were edited using the EditSeq module and used to query the GenBank nucleotide database (NCBI) using the BLASTn algorithm to identify and retrieve similar sequences especially those representing the MSV reference strains (Table S1). Sequence alignments were done using the MUSCLE program (Edgar, 2004) implemented in MEGA. All sequences were trimmed to the same length (964 nucleotides) to enable consistent alignment. Pairwise nucleotide identity percentages were calculated using SDT v.1.3 (Muhire et al., 2014) and the nucleotide composition was analyzed using principal component analysis (PCA) with the R software (R Core Team, 2025). Finally, a phylogenetic tree was reconstructed by the Maximum Likelihood (ML) method based on the HKY+G substitution model determined by the model selection tool in MEGA X (Kumar et al., 2018).

3. Results

3.1 Molecular Detection of MSV in Maize and Grass Hosts

PCR amplification of DNA extracts produced a single fragment of approximately 1,300 bp in all tested samples (Fig. 2). The amplicons showed similar electrophoretic mobility and comparable band intensity, confirming the successful amplification of the target genomic region. No non-specific products or extensive smearing were detected, demonstrating the specificity and consistency of the PCR reactions across all samples

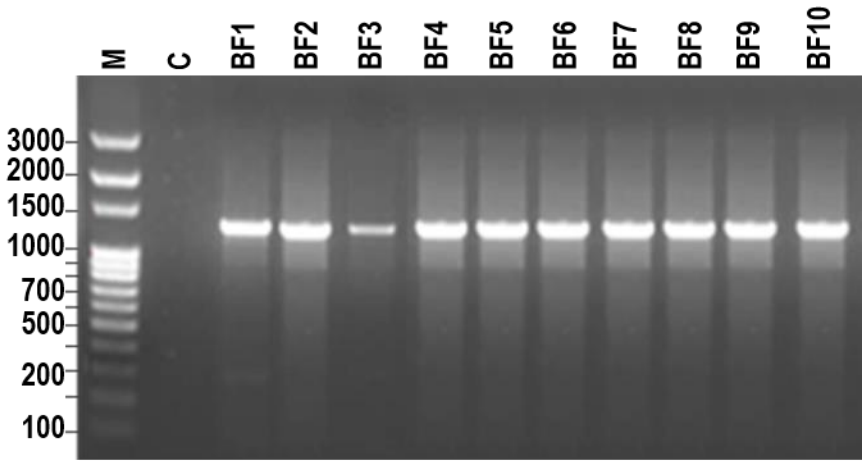

Fig. 2. Agarose gel electrophoresis of PCR-amplified MSV isolates from *Zea mays* (BF1-BF5, BF9), *Brachiaria lata* (BF6 and BF7), *Digitaria sp* (BF8) and *Eleusine indica* (BF10). M: 100-bp DNA ladder; C: negative control

3.2 PCA Analysis of Nucleotide Composition

Six MSV isolates (BF1-BF5 from maize and BF6 form *B. lata*) were successfully sequenced yielding sequence lengths of 950-1200 nucleotides. Sequences from the other isolates were too short (~300 nucleotides) or unexploitable. The sequences were deposited in GenBank under accession numbers PZ692172-PZ692177. Principal component analysis based on the nucleotide composition of the generated and reference sequences yielded the results illustrated in Fig. 3. The first principal component (PC1), explaining 50.2% of the total variance, separated sequences with relatively high G and T contents from those enriched in A and C. The second principal component (PC2), accounting for 35.8% of the total variance, further discriminated against the sequences according to their T content. Along with the PC1 axis, sequences BF1 to BF5 grouped with the MSV-A reference sequences, indicating a similar nucleotide composition. In contrast, BF6 occupied a distinct position, reflecting its relatively higher G and T content and suggesting greater divergence from the remaining isolates.

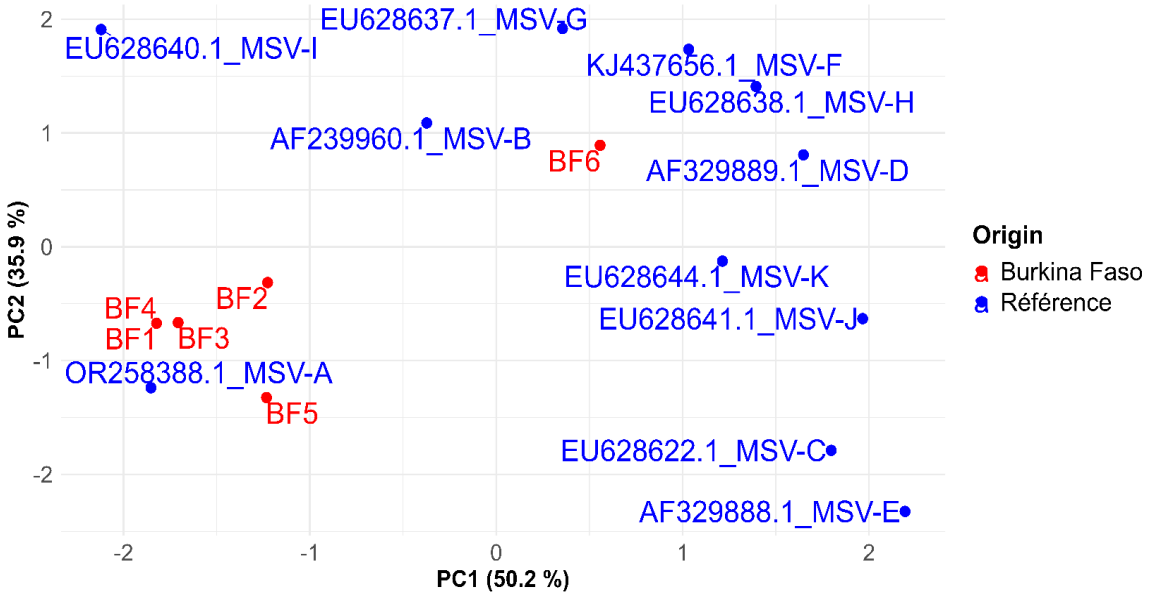

Fig. 3. Principal Component Analysis (PCA) based on the nucleotide composition of MSV isolates showing the clustering of sequenced MSV isolates BF1-BF6 with references sequences

3.3 Nucleotide Sequence Identity

Fig. 4 shows the pairwise nucleotide identity matrix of the MSV isolates. BF1, BF2, BF3, BF4 and BF5 exhibited a high degree of sequence conservation, with a mean nucleotide identity of 98.4 ± 1.05%. Within this group, nucleotide identity ranged from 97.2% (between BF5 and either BF1 or BF4) to 100% (between BF1 and BF4). In contrast, BF6 was substantially more divergent, sharing only 83.7–85.0% nucleotide identity with the other isolates. The lowest identity was observed between BF6 and BF5 (83.7%), whereas the highest (85.0%) was recorded with BF1, BF2 and BF4.

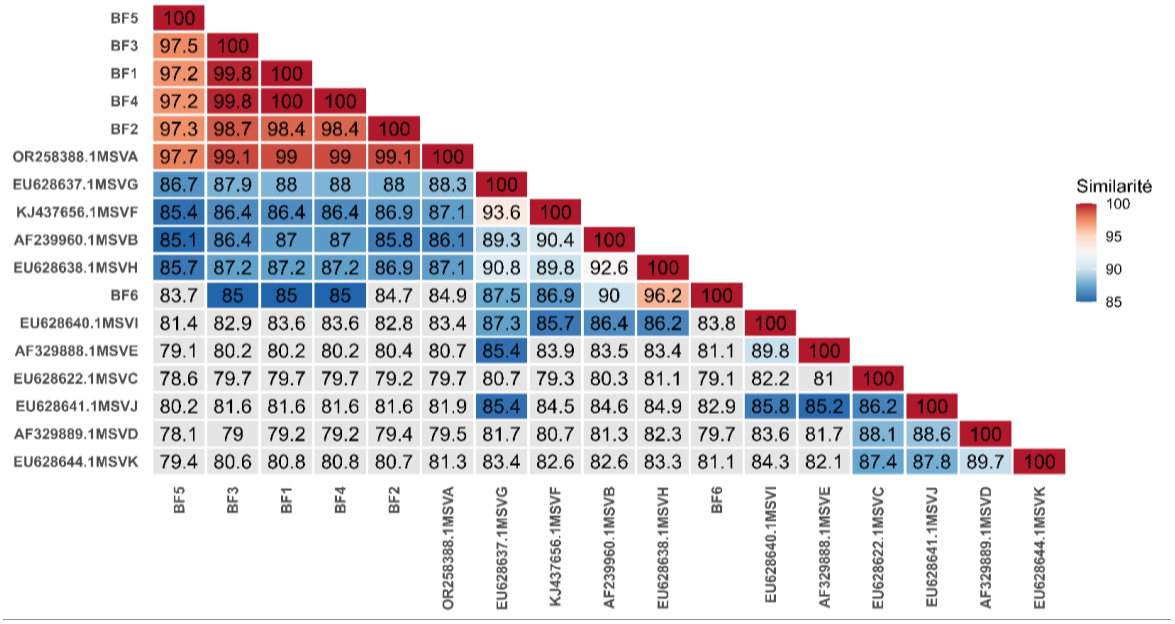

Fig. 4. Pairwise nucleotide sequence identity matrix

3.4 Phylogenetic Relationship among Sequences

The phylogenetic reconstruction of the MSV isolates and representative reference sequences from GenBank is shown in Fig. 5. The resulting maximum-likelihood tree resolved the recognized MSV genotypes into well-supported clades, with bootstrap support values ranging from 83% to 100%. Five isolates (BF1–BF5) clustered within the MSV-A genotype. Within this genotype, BF1, BF3 and BF4 formed a well-supported subclade (bootstrap = 99%), with BF1 and BF4 showing the closest relationship (bootstrap = 99%). In contrast, BF6 clustered within the MSV-H genotype alongside reference isolate EU628638.1, supported by a bootstrap value of 100%. Previously reported Burkina Faso isolates retrieved from GenBank were distributed between the MSV-A and MSV-G genotypes.

4. Discussion

Although typical streak symptoms were observed in the field, molecular confirmation was essential because several viruses infecting maize can produce similar symptoms, making visual diagnosis unreliable (Thottappilly et al., 1993). PCR amplification using MSV-specific primers (Tembo et al., 2020) successfully detected the virus in all symptomatic samples, producing the expected amplicon of approximately 1,300 bp. These results confirmed that the observed symptoms were associated with MSV infection. These findings are consistent with previous studies from Zambia (Tembo et al., 2020), Cameroon (Mbong et al., 2021), and Burkina Faso (Dao et al., 2026), which also demonstrated the reliability of PCR-based detection for MSV.

Nucleotide composition is an important genomic feature that influences genome stability, physicochemical properties and viral evolution while contributing to genome signatures that can distinguish viral groups and reflect evolutionary divergence (Gibbs & Ohshima, 2010; Van Hemert et al., 2016; Zhao et al., 2024). In the

present study, principal component analysis of nucleotide composition separated the generated isolates into two distinct groups, with BF1–BF5 clustering alongside MSV-A reference sequences and BF6 occupying a distinct position. Although nucleotide composition alone is insufficient to infer phylogenetic relationships, the observed separation of BF6 suggests substantial genomic divergence from the MSV-A isolates. Such differences in nucleotide composition are thought to arise through long-term mutation patterns and host-driven selective pressures, which can influence codon usage, genome stability and adaptation to different hosts (Van Hemert et al., 2016; Zhao et al., 2024).

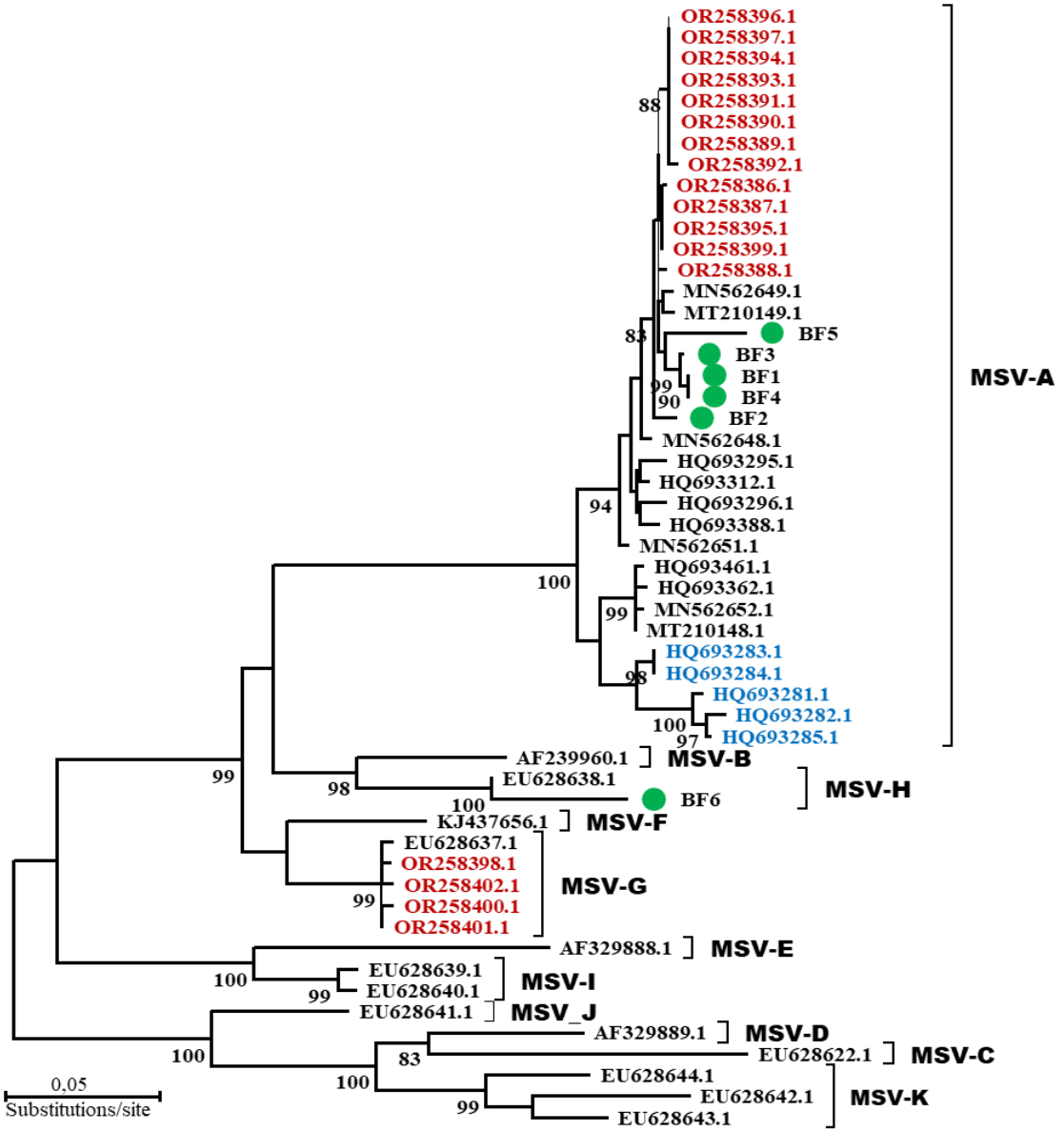

Fig. 5. Maximum-likelihood phylogenetic tree of MSV isolates based on partial genomic sequences

Sequences from this study are marked with green dots. Sequences in red and blue correspond to those previously described in Burkina Faso by Monjane et al.*, (2011) and Fouad* et al., *(2025), respectively. Numbers at the nodes represent the bootstrap percentages (1,000 replicates) and values below 70 % are not shown*

Pairwise nucleotide identity analysis further demonstrated the close genetic relationship among BF1–BF5, which shared a mean nucleotide identity of 98.4%, while BF6 exhibited substantially lower nucleotide identities (83.7–85.0%) with these isolates. According to ICTV taxonomic guidelines, species and strain (genotype) demarcation within the genus *Mastrevirus* is primarily based on pairwise nucleotide identity calculated from

complete genome sequences (Fiallo-Olivé et al., 2021; Muhire et al., 2014). Although the present study compared only a partial genomic region, the high nucleotide identity among BF1–BF5 is consistent with their assignment to the same genotype, whereas the substantially lower identities between BF6 and the remaining isolates support its differentiation from the MSV-A group. These comparisons should therefore be interpreted evidence of genetic relatedness (Duffy & Seah, 2010) rather than taxonomic assignments based on ICTV criteria.

Phylogenetic reconstruction clarified MSV isolates relationships by assigning BF1–BF5 to the MSV-A genotype, the most widespread and economically important genotype infecting maize in sub-Saharan Africa (Dao et al., 2026; Martin et al., 2001; Monjane et al., 2011; Shepherd et al., 2010; Varsani et al., 2008). The use of partial genomic sequences to distinguish MSV strains is consistent with the approach adopted by Tembo et al. (2020). The predominance of MSV-A among the maize isolates analyzed in this study is therefore consistent with previous reports from Africa. In contrast, BF6 clustered within the MSV-H genotype together with reference isolate EU628638.1. To our knowledge, this study provides the first molecular evidence, based on partial genomic sequences, of an MSV-H isolate infecting *Brachiaria lata* in Burkina Faso. In West Africa, MSV-H had previously been reported only from Nigeria, where it was identified in the wild grass *Setaria barbata* (Lam.) Kunth (Varsani et al., 2008). The detection of MSV-H in *Brachiaria lata* further emphasizes the role of wild grasses as natural reservoirs of MSV diversity and highlights their potential importance in the emergence and maintenance of genetically distinct virus populations. These findings underscore the need to include alternative grass hosts in future MSV surveillance to improve our understanding of virus diversity and epidemiology in the region.

5. Conclusion

This study provides molecular evidence of the presence of Maize streak virus in symptomatic maize and wild grass samples collected in Burkina Faso. Molecular analysis showed that the sequenced maize isolates BF1 to BF5 were closely related and clustered within the MSV-A genotype, which is widely associated with maize infection in sub-Saharan Africa. In contrast, isolate BF6 from *Brachiaria lata* was genetically distinct from the maize isolates and grouped with the MSV-H reference strain in the phylogenetic analysis. This result provides evidence for the occurrence of an MSV-H-like isolate in Burkina Faso and suggests that wild grasses may contribute to the maintenance of MSV diversity. The findings support the importance of molecular surveillance of both cultivated maize and alternative grass hosts. However, additional sampling, complete genome sequencing and broader geographic coverage are needed to confirm the distribution, host range and epidemiological significance of MSV-H in the country.

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Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of Grammarly and ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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