



Research article

Identification of yam mosaic virus as the main cause of yam mosaic diseases in Ethiopia

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ABSTRACT

Yam (*Dioscorea* spp.) is a staple food crop with cultural, nutritional and economic significance for millions of small-scale farmers in sub-Saharan Africa. While various virus-like symptoms such as mosaic and chlorosis are frequently observed in yam fields in Ethiopia, little information is available on the prevalence, distribution, and molecular characteristics of viruses causing these symptoms. The aim of this study was to investigate the incidence and distribution of yam viruses and determine the primary cause of yam mosaic diseases (YMD) in Ethiopia. Both symptomatic (n = 280) and asymptomatic (n = 110) yam leaf samples were collected and tested for potyviruses using ACP-ELISA. In addition, the symptomatic leaf samples were screened for yam mosaic virus (YMV), yam mild mosaic virus (YMMV), and cucumber mosaic virus (CMV) by DAS-ELISA. Subsequently, total RNA was extracted from 130 leaf samples comprising 94 symptomatic and 36 asymptomatic samples representing the different study areas. The representative RT-PCR amplicons (n = 6) were Sanger sequenced. The ACP-ELISA and DAS-ELISA results showed 9.2%, and 12.9% YMV infection, respectively, while the RT-PCR analysis showed 28.5% YMV positivity rate. Both CMV and YMMV were not detected in any of the samples tested. Thus, YMV is confirmed as the primary cause of YMD in Ethiopia. YMV isolates from Ethiopia shared 92–93% nucleotide identity among themselves and 85–99% with other YMV isolates from the GenBank. Phylogenetic analysis revealed that YMV isolates from Ethiopia, South America, and west-central Africa have the most recent common ancestor, while isolates from China and Japan are clustered as sister groups. This study enhances our understanding of YMV's genetic diversity and provides valuable information regarding the first report of YMV in Ethiopia.

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1. Introduction

Yams (genus *Dioscorea*, family *Dioscoreaceae*) are among the top ranking and most economically important root crops cultivated in tropical and sub-tropical regions of the world [1,2]. Ethiopia is the fifth largest yam producer worldwide and Ethiopian endemic yam species are among the top yielders [3,4]. There are six farmer preferred yam species (*D. rotundata*, *D. abyssinica*, *D. bulbifera*, *D. praehensilis*, *D. cayenensis*, and *D. alata*) commonly grown in the southern and south western regions of Ethiopia [5]. They are cultivated by subsistence farmers, particularly in major growing areas namely, Dawuro, Gamo, Gofa, Jimma, Keficho, Wolaita, and Sidama zones [5,6].

Yam production is adversely affected by several constraints including biotic stresses such as pests and diseases, mainly viruses, bacteria and fungi, as well as abiotic stresses like decline in soil fertility [7–9]. Different species of viruses belonging to the genera *Aureusvirus*, *Badnavirus*, *Cucumovirus*, *Macluravirus*, *Potyvirus*, *Potexvirus* and *Tobamovirus* have been reported threatening yams production worldwide [10–14]. These viruses have high genetic and serological variability, making their diagnosis and management challenging [14].

Potyviruses (family *Potyviridae*) are the largest genus of RNA plant viruses having single-stranded, positive-sense RNA (ssRNA (+)) genomes [15]. The virions are flexuous and filamentous particles, 680–900 nm long and 11–20 nm wide. The genome size ranges from 9.4 to 11.0 kb, with a monopartite organization [16,17].

Yam mosaic virus (YMV), yam mild mosaic virus (YMMV), and Japanese yam mosaic virus (JYMV) are yam infecting potyviruses recognized by the ICTV [8,14]. They are naturally spread in a non-persistent manner via various aphid species (*Aphis craccivora*, *A. gossypii*, and *Rhopalosiphum maidis*) [15,18], through vegetative propagation of infected plant material, or mechanically by sap to other yam plants or indicator plants, like *Nicotiana benthamiana* [19,20]. On the other hand, cucumber mosaic virus (CMV) (genus, *Cucumovirus*, family *Bromoviridae*) has a global distribution and a very wide host range [21]. It can be transmitted mechanically by sap and by various species of aphids, similar to potyviruses [20].

Yam mosaic virus-infected yam plants usually exhibit a variety of symptoms including chlorosis, mosaics, mottles, stripes, leaf deformation, and stunting [21,22]. YMV can cause severe yield losses in *D. rotundata*, the most important food yam in many parts of the world [23,24]. In sub-Saharan Africa, where 97% of global yam production takes place [4,25], YMDs threaten food security and

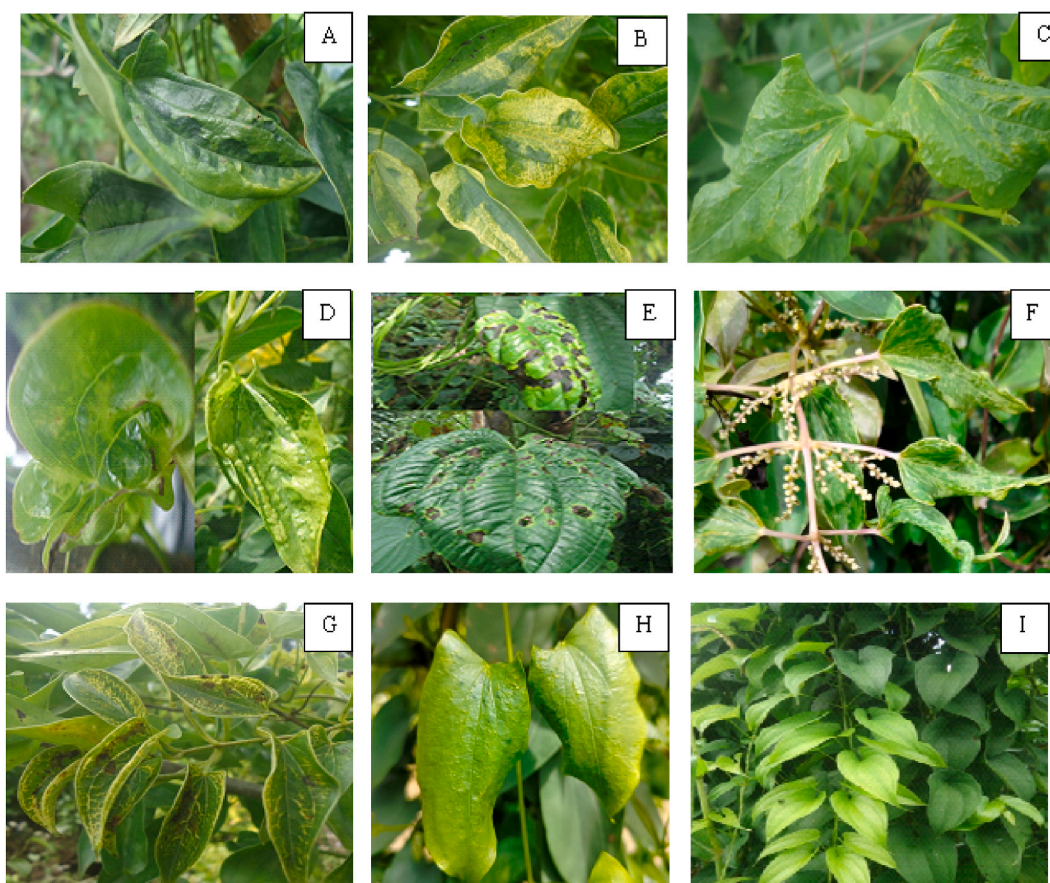


Fig. 1. Yam plant showing different kinds of virus-like symptoms from which YMV is identified in Ethiopia. (A. Mosaics, B. Chlorosis, C. Mild mosaics, D. Leaf deformation, E. Mosaics and puckering, F. Mottles, G. Vein chlorotic spotting, H. Leaf discoloration, I. Healthy leaf).

farmers' ability to generate sustainable income [21,22].

Despite the genetic diversity, accessible cropland, and a higher number of released yam varieties, yam production in Ethiopia has declined from an annual production of 200,000 tons in 1993 to 45,000 tons in 2020 [26]. Although there are some virus-like symptoms regularly observed in yam fields in the country, very little is known about the prevalence, distribution, and molecular characteristics of yam infecting viruses. Hence, this study aims to investigate the identity and distribution of virus(es) causing yam mosaic diseases by applying standard diagnostic procedures such as ELISA and PCR. This will facilitate rapid decision making regarding the health status and distribution of yam planting materials in the country.

2. Materials and methods

2.1. Sample collection

Yam fields with 4–6 months old plants were randomly surveyed along main and rural roads in southern Ethiopia at approximately 4–8 km intervals from February to May in 2021 and 2022 cropping seasons. Yam leaf and tuber samples were collected from five major yam growing areas (Dawuro, Gamo, Gofa, Kembata-Tembaro (KT), and Wolaita), and two agricultural research centers (Areka Agricultural Research Center (AARC) and Hawassa Agricultural Research Centers (HARC)). A total of 96 fields from 21 districts were surveyed and symptomatic ($n = 280$) and asymptomatic ($n = 110$) leaf samples were collected (Fig. 1A–I, Supplementary Table 1).

2.2. Serological assay for yam RNA viruses

Initially, all 390 leaf samples (symptomatic, $n = 280$, and asymptomatic, $n = 110$) were tested for potyviruses using an antigen-coated-plate (ACP)-ELISA kit (DSMZ, RT-0573/1) provided by the Leibniz Institute DSMZ Germany Collection of Microorganisms and Cell Cultures (GmbH), following the manufacturer's instructions with minor modification in which 150 μ l potyvirus-specific monoclonal antibody and rabbit anti-mouse conjugate were used instead of 200 μ l [27]. Previously screened virus-free yam leaf samples were used as negative control. The results were visually assessed and the optical density values were measured with a spectrophotometer (DR-200BS Microplate reader (Diatek)) at an absorbance value of 405 nm. A sample was taken as positive if the value of absorbance at 405 nm was greater than twice the mean absorbance reading of the negative control [28,29].

Based on the findings from the ACP-ELISA, only the symptomatic ($n = 280$) leaf samples were tested for YMV, YMMV, and CMV using a DAS-ELISA kit (DSMZ, RT-1142, RT1038, and RT-0929, respectively) [27]. DAS-ELISA was done following the manufacturer's instructions with minor modifications in which 150 μ l of virus-specific IgG and IgG-conjugates were used instead of 200 μ l. The visual inspection and spectrophotometer detection was carried out as indicated previously in ACP-ELISA.

2.3. RNA extraction, RT-PCR analysis

A total of 94 symptomatic samples including all the samples tested positive in ELISA and 36 asymptomatic samples were randomly selected for RNA extraction and RT-PCR analysis representing the different study areas. The total RNA was extracted using the RNeasy® Mini Kit (cat. Nos. 74104 and 74106, QIAGEN) following the manufacturer's instructions with minor modifications. The RNA integrity and quantity was checked by electrophoresis using a 1% agarose gel and NanoDrop 2000 spectrophotometer.

One step RT-PCR was performed using CMV, YMV, and YMMV primer pairs specific to the coat protein and 3' UTRs of the individual viruses (Supplementary Table 2). The total volume of the RT-PCR amplification was 25 μ l consisting of 2 μ l of extracted RNA, 2 μ l of dNTPs mixes (10 mM), 5 μ l of 5 x M. MLV RT buffer, 0.5 μ l of each primer, 0.5 μ l of M-MLV Reverse Transcriptase (RT) (Promega, USA), 1 μ l of Dream Tag DNA polymerase, and 13.5 μ l of nuclease-free water. The one-step RT-PCR amplification was carried out as reported in Silva et al. [30], with minor modifications. The thermal cycling conditions were 50 °C for 10 min for the RT step, and 95 °C for 4.5 min for one cycle, followed by 35 cycles of 95 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min and a final extension of 72 °C for 10 min. The amplified products (10 μ l) were analyzed by agarose gel electrophoresis using 1% (w/v) agarose (Supplementary Fig. 1(a–d)), and bands of the expected size were excised and purified using the QIAquick Gel Extraction Kit (Cat. nos.28704 and 28706 QIAGEN) following on the manufacturer's instructions.

2.4. Sanger sequencing and analysis of YMV isolates

Purified RT-PCR amplicons from YMV positive samples representing different study areas were randomly selected; mixed with forward or reverse primers in separate tubes and Sanger sequenced at Macrogen Inc. (Seoul, South Korea). Out of the 10 samples we sequenced, the quality of the sequences for four of them was not good so we disregarded them from further analysis. Sequences were first analyzed using BLASTn algorithm from the GenBank nucleotide database to determine the virus identities (Supplementary Table 3). The number of amino acid differences per site among sequences was computed in MEGA11 [31]. The coding data was translated assuming a standard genetic code table, and all positions containing gaps and sequences with short lengths or low quality were eliminated. Phylogenetic analysis was carried out using a Maximum likelihood statistical method based on the JTT matrix-based model with 1000 bootstrap replications in MEGA11 software [31] and Ryegrass mosaic virus was used as an outgroup.

3. Results

3.1. Yam infecting viruses detected using ELISA, and RT-PCR

Based on the different mosaic, chlorotic and vein chlorotic spotting symptoms observed (Fig. 1A–I), the plants were suspected of being infected with potyviruses and/or CMV. Therefore, All the 390 samples (symptomatic, n = 280, and asymptomatic, n = 110) were initially screened for potyviruses with ACP-ELISA. Out of these, 36 (9.2%) samples tested positive to potyviruses. Potyvirus positive samples were detected from all the sampling sites, except from HARC (Table 1). All the potyvirus positive samples were exclusively identified in samples exhibiting virus-like symptoms, with none of the asymptomatic samples testing positive. The highest number of potyvirus positive samples came from Wolaita (12 samples) and Gofa (8 samples) zones, while the least number of potyvirus positive samples were collected from Kembata-Tembaro zone (2 samples). Positive results were obtained from various yam cultivars, including *D. rotundata*, *D. alata*, *D. praezensilis*, *D. abyssinica*, and *D. cayenensis*. Most of the positive results were associated with *D. rotundata*, with only one *D. abyssinica* sample from Wolaita tested positive for potyvirus on ACP-ELISA. Notably, all the *D. bulbifera* samples collected from the different zones tested negative for potyviruses.

Since none of the asymptomatic samples tested positive for potyviruses by ACP-ELISA, they were excluded from DAS-ELISA screening. Consequently, all the 280 symptomatic samples were subjected to DAS-ELISA targeting the two common potyviruses (YMV and YMMV) and CMV which are known to cause mosaic and chlorotic symptoms on yam. On YMV specific DAS-ELISA only the 36 (12.9%) samples which previously tested positive for potyviruses in ACP-ELISA also tested positive. However, the remaining 244 samples tested negative. In addition to YMV, all the 280 symptomatic samples were screened for YMMV and CMV using DAS-ELISA, but none of them reacted positively to either virus.

Subsequently, PCR screening was conducted on a total of 130 samples consisting of 94 symptomatic samples including 36 samples that tested positive to YMV on DAS-ELISA, and 36 asymptomatic samples representing different study areas. In the RT-PCR, all the 36 YMV positive samples from DAS-ELISA screening also tested positive using YMV specific primers with the highest number of positive samples identified from Wolaita and Gofa zones in *D. rotundata*. Interestingly, one asymptomatic *D. rotundata* sample collected from

Table 1

Summaries of diseases incidence, severity index, ELISA and RT-PCR positive result for RNA viruses.

Study areas	Yam species	Total samples	Potyviruses positive (%)	DAS-ELISA (n = 280)	Samples selected for RT-PCR test (n = 130)	RT-PCR positive YMV (%)
				YMV(%)		
Dawuro	<i>D. rotundata</i>	21	5(23.8)	5(29.4)	11	5(45.5)
	<i>D. bulbifera</i>	8	0(0.0)	0(0.0)	2	0(0.0)
	<i>D. alata</i>	16	2(12.5)	2(16.7)	5	2(40)
	<i>D. abyssinica</i>	11	0(0.0)	0(0.0)	3	0(0.0)
	Total	56	7(12.5)	7(16.7)	21	7(33.3)
Gamo	<i>D. rotundata</i>	34	3(8.8)	3(12.5)	12	3(25.0)
	<i>D. cayenensis</i>	23	0(0.0)	0(0.0)	7	0(0.0)
	<i>D. praezensilis</i>	14	2(14.3)	2(18.2)	4	2(50)
	<i>D. abyssinica</i>	14	0(0.0)	0(0.0)	4	0(0.0)
	Total	85	5(5.9)	5(7.8)	27	5(18.5)
Gofa	<i>D. rotundata</i>	23	5(21.7)	5(27.8)	8	5(62.5)
	<i>D. bulbifera</i>	8	0(0.0)	0(0.0)	2	0(0.0)
	<i>D. praezensilis</i>	20	2(10.0)	2(14.3)	5	2(40)
	<i>D. abyssinica</i>	13	0(0.0)	0(0.0)	3	0(0.0)
	<i>D. cayenensis</i>	8	1(12.50)	1(16.7)	2	1(50.0)
Total	72	8(11.1)	8(14.6)	20	8(40.0)	
KT	<i>D. rotundata</i>	20	2(10.0)	2(16.7)	9	2(22.2)
	<i>D. abyssinica</i>	11	0(0.0)	0(0.0)	4	0(0.0)
	<i>D. bulbifera</i>	4	0(0.0)	0(0.0)	1	0(0.0)
	<i>D. praezensilis</i>	7	0(0.0)	0(0.0)	2	0(0.0)
	Total	42	2(4.8)	2(7.7)	16	2(12.5)
Wolaita	<i>D. rotundata</i>	24	6(25.0)	6(30.0)	12	7(58.3) ^a
	<i>D. bulbifera</i>	10	0(0.0)	0(0.0)	4	0(0)
	<i>D. alata</i>	22	3(13.6)	3(18.8)	6	3(50.0)
	<i>D. abyssinica</i>	21	1(4.8)	1(6.3)	6	1(16.7)
	<i>D. cayenensis</i>	13	0(0.0)	0(0.0)	4	0(0.0)
	<i>D. praezensilis</i>	14	2(14.3)	2(20.0)	4	2(50.0)
Total	104	12(11.5)	12(15.0)	36	13(36.1)	
AARC	Breeding lines	13	2(15.4)	2(22.2)	4	2(50.0)
HARC	Breeding lines	18	0(0.0)	0(0.0)	6	0(0.0)
Total		390	36(9.2)	36(12.9)	130(33.3)	37(28.5)

KT: Kembata-Tembaro; AARC: Areka agricultural research centre; HARC, Hawassa agricultural research centre, SE, standard error.

^a One asymptomatic sample was RT-PCR positive. “No sample was positive for CMV and YMMV in ELISA and RT-PCR”.

Wolaita zone tested positive for YMV on RT-PCR. On the other hand, none of the 130 samples tested positive for CMV or YMMV on RT-PCR (Table 1).

3.2. Pairwise sequence comparison and phylogenetic analysis

The number of nucleotide differences per site among YMV sequences revealed that the YMV isolates from Ethiopia share 92–93% nucleotide identity among themselves and 85–99% with other YMV isolates from the GenBank. Isolate OQ571882 from Ethiopia shared the highest (99%) nucleotide identity with YMV isolates from France Guadeloupe (AJ244063, and AJ244057), Nigeria (AJ244047), Cameroon (AJ244055), Benin (AJ244049) and France Guiana (AJ244062) (Table 2).

Phylogenetic analysis based on the YMV isolates from Ethiopia together with selected reference sequences from the NCBI GenBank clustered into two major groups (Fig. 2). The first group comprises YMV isolates from Brazil, West Africa, and France Guadeloupe, while the second group includes all the YMV isolates (accession no OQ571881– OQ571886) from Ethiopia sequenced in the current study. The YMV isolates from Ethiopia and the reference sequences from the South America and west-central Africa shared a common ancestor and clustered distinctly from the Japanese yam mosaic virus (JYMV).

4. Discussion

Various types of symptoms were reported in the majority of yam growing areas with leaf mosaics and chlorosis as the most dominant once. The highest YMV positive results were obtained from *D. rotundata* in DAS-ELISA, and RT-PCR. This is in agreement

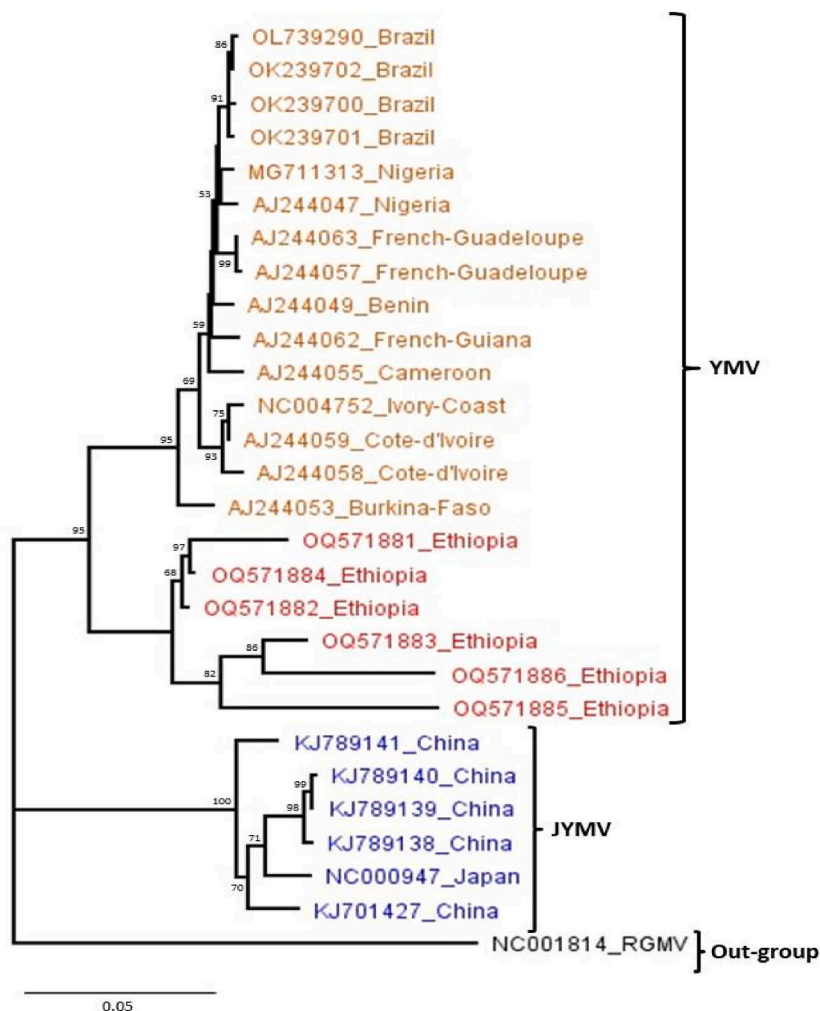


Fig. 2. The evolutionary relationship of YMV isolates from Ethiopia (red), together with representative YMV (orange) and JYMV (blue) isolates from different countries using coat protein nucleotide sequences. *Ryegrass mosaic virus* (RGMV) (light blue) was used as an out-group. The evolutionary tree was generated using the Maximum Likelihood method based on the JTT matrix-based model with 1000 bootstrap replications in MEGA11. Branches <50% bootstrap test (1000 replicate) were removed. The scale bar represent substitution per site.

with previous studies which indicated that *D. rotundata* was the most susceptible cultivar and was reported to be infected by several viruses such as badnaviruses, potexvirus, and potyviruses compared to other yam species [32–34]. This was suggested to be due to the possibility that *D. rotundata* species is more susceptible to viral diseases because it has a low expression of the disease resistance genes (R genes), which play important roles in plant defense against different pathogens [34,35]. No or fewer virus diseases were noticed in yam species, such as *D. bulbifera* and *D. abyssinica* possibly these species have good resistance ability to the viral diseases or its genetic background differ as indicated by Penet et al. [36]; Bekele and Bekele [6]; Agre et al. [37].

Only one asymptomatic sample tested positive to YMV on RT-PCR. Interestingly, this sample produced the mean absorbance reading value less than twice the negative control on both ACP- and DAS-ELISA. This could possibly be due to the presence of a very low virus titer in the samples which could not be detected on ELISA as described by Seal et al. [38].

Globally, YMV is the most prevalent potyvirus, infecting *D. alata*, *D. rotundata*, and *D. cayenensis-rotundata* [14,21]. This study also revealed that YMV is the widespread virus associated with YMD in Ethiopia. The findings of the current study are also consistent with previous studies where a wide distribution of YMV was recorded wherever yams are cultivated [24,39]. Bakayoko et al. [22] reported the prevalence up to 100% YMV in Côte d'Ivoire, while Azeteh et al. [21] reported 37.3% in Cameroon and Njukeng et al. [40] described 31% and 13–73% prevalence in Benin and Cameroon, respectively. The vegetative propagative nature of yams, the lack of a proper yam seed documentation system, and limited knowledge about the diversity of viruses infecting yams have contributed to the introduction and establishment of new viruses in areas where they did not exist before [41]. In Ethiopia, farmers' accesses to planting materials from open markets and gene flow among regions via uncontrolled seed exchange have substantially contributed to the widespread distribution of YMV.

Previous studies reported that CMV and YMMV are not common in yam samples [12,21,32,39]. Similarly, CMV and YMMV were not detected in the samples screened in this study, indicating that these viruses were not contributing to the virus-like symptoms observed in the field. Furthermore, reports from many sub-Saharan African countries indicated that these viruses are not prevalent [21, 22,42] or had a very low prevalence in yam plants [25].

The BLASTn analysis revealed that YMV isolates from Ethiopia shared the highest nucleotide sequence identity with YMV Las-Sc2-17-2, and Al-Sc3-17-1 isolates, reported from *Dioscorea cayenensis-rotundata* from Nigeria [43]. The phylogenetic tree indicates that YMV isolates from Ethiopia, South America and west-central Africa contained the most recent common ancestor (monophyletic groups). This suggests that the YMV isolates from Ethiopia are closely related to those in other sub-Saharan Africa countries, although they make a distinct clade of their own. To learn more about these isolates' and their evolutionary links with other YMV populations, future studies are anticipated to generate and analyze the entire genome sequences of these and other isolates.

In conclusion, this is the first study carried out on yam viruses in Ethiopia. The incidence of virus diseases in yam fields suggests that the disease has the potential to undermine food security in the areas. The outcome of the study will contribute to growers and agricultural research centers in making decisions on the movement of seed materials between regions. This study only assess the status of yam infecting viruses in major yam growing regions and it only screen for YMV, CMV and YMMV. Therefore, future studies should include other yam growing regions in Ethiopia. In addition, samples need to be screened for other viruses in order to understand the status of virus infection in yam in Ethiopia.

Data availability

Sequences described in this paper are under GenBank accession numbers OQ571881 - OQ571886. The other datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Ashebir Gogile: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Misrak Kebede:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Dawit Kidanemariam:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Adane Abraham:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e26387>.

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