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Detection and Partial Characterization of *Polerovirus* and *Luteovirus* Isolates Associated With Lentil and Chickpea in Ethiopia

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ABSTRACT

The partial nucleotide sequence of the coat protein (CP) gene of Ethiopian isolates of chickpea chlorotic stunt virus (CpCSV, genus *Polerovirus*), beet western yellows virus (BWYV, genus *Polerovirus*), and soybean dwarf virus (SbDV, genus *Luteovirus*) was determined from lentil and chickpea plants showing yellowing, stunting, and reddening symptoms. Comparative sequence analysis of CpCSV isolates obtained from five chickpea and five lentil isolates showed 94.9%–100% and 91.9%–98.7% nucleotide sequence identity with each other and with the reference isolates, respectively. One CpCSV isolate from chickpea (MZ043728) showed a close relationship with isolates of the serotype II while the remaining nine isolates were closely related to isolates belonging to serotype I. Sequence identities of three chickpea BWYV isolates varied from 93.3% to 100% with the reference isolates, and one of them (MZ043727) displayed 100% nucleotide identity with previously reported lentil stunt virus (LStV, genus *Polerovirus*). The chickpea isolates MZ043725 and MZ043726 appear to be identical to each other, whereas the other isolate (MZ043727) was identical to previously identified LStV isolate. The nucleotide sequence of three Ethiopian SbDV isolates had a lower identity with GenBank isolates and their phylogenetic analysis showed that they are clustered separately from the rest of the reference isolates indicating that they are the most divergent. This result generates essential information for further research on legume viruses in Ethiopia. In addition, a detailed study should be conducted in the future to understand the prevalence of LStV and determine the potential yield losses associated with the virus in Ethiopia.

1 | Introduction

Lentil (*Lens culinaris* Medik.) and chickpea (*Cicer arietinum* L.) are among the major legume crops grown in Ethiopia (CSA 2021). Despite their economic importance, biotic and abiotic factors are known to influence the productivity of legume crops in Ethiopia (Nigussie et al. 2019). Viruses have become an important biotic factor affecting yield in major lentil and chickpea-producing areas of Ethiopia (Abraham and Vetten 2022; Ademe et al. 2023). Chickpea chlorotic stunt virus (CpCSV), beet western yellows virus (BWYV) (genus *Polerovirus*, family Solemoviridae), and soybean dwarf virus

(SbDV, genus *Luteovirus*, family Tombusviridae) are important RNA viruses on cool-season food legumes, being responsible for disease epidemics in Ethiopia (Abraham and Vetten 2022; Tadesse et al. 1999). Since their first report in Ethiopia, the prevalence and economic importance of these viral diseases have greatly increased the need for management options. All the three viruses are transmitted by aphids in a circulative and nonpropagative manner, with infection restricted to the phloem (Abraham et al. 2006; Garcia-Ruiz, Holste, and LaTourrette 2021; Luoto et al. 2021; Park et al. 2018).

Chickpea chlorotic stunt virus (CpCSV), beet western yellows virus (BWYV) (genus *Polerovirus*, family Solemoviridae), and soybean dwarf virus (SbDV, genus *Luteovirus*, family Tombusviridae) are not mechanically transmitted, and they can cause stunting, yellowing, and reddening with no or poor pod setting of legume host crops (Makkouk and Kumari 2009; Stone et al. 2024). These symptoms induced by CpCSV, BWYV, or SbDV are not easily distinguished from the ones caused by distinct viruses such as faba bean necrotic yellows virus (FBNYV, genus *Nanovirus*, family Nanoviridae) and chickpea chlorotic dwarf virus (genus *Mastrevirus*, family Geminiviridae) (Kumari et al. 2009). In this scenario, additional diagnostic techniques are necessary to determine the exact causal agent of the disease.

Grain legumes, including lentil and chickpea, play a crucial role in human nutrition and serve as staple crops for low-income farmers in developing and underdeveloped countries and contribute to overall food security and agroecosystem services (Jha et al. 2023). However, viruses are known to inflict a huge yield loss in areas with abundant inoculum source and prevailing weather conditions favoring the buildup of vectors in early growth stage of the crop. During recent studies, it is reported that season and location influence the intensity and prevalence of a virus. In some areas, farmers abandoned lentil production leading to the import of the crop to meet the market demand (Ademe et al. 2023; FAOSTAT 2022). Hence, knowing the identity and diversity of viruses that infect lentil and chickpea is the first step towards developing a crop management program and identifying sources of resistance. Therefore, this study was conducted to obtain a better understanding on the sequence variation of the CpCSV, BWYV, and SbDV isolates collected from lentil and chickpea crops.

2 | Materials and Methods

2.1 | Samples Used

Sixteen samples collected in a previous study (nine chickpea and seven lentil) (Table 1) with yellowing and stunting symptoms that reacted positively with broad-spectrum legume monoclonal antibody (MAb) (5G4) and specific MAbs of CpCSV (a mixture of 1-1G5, 1-3H4 and 1-4B12), BWYV (A5977, Agdia, USA), and SbDV (ATCC PVAS-650, USA) were used in the study (Ademe et al. 2023). Total RNA was extracted approximately from about 0.5 g of the selected 16 dried virus-infected plant tissues following the McKenzie RNA extraction protocol (McKenzie et al. 1997) with the RNeasy Plant Mini-Kit (Cat No. 74904, Qiagen) and stored as solutions in nuclease-free water at −80°C.

2.2 | Molecular Analysis

cDNA synthesis was performed using the M-MLV Reverse Transcriptase Kit (Cat. No. 28025–013, Invitrogen) according to the manufacturer's instructions using the generic reverse primer AS3 (5′- CACGCGTCIACCTATTTIGGRTTITG-3′). Three microliter of total RNA, 1 μL of 10-μM reverse primer, 1 μL of nuclease-free water, and 1 μL of 10-μM dNTPs were heated at 65°C for 5 min. The mixture was cooled on ice for 2 min, and the following reagents were added: 2-μL 5× First-Strand Buffer, 1-μL 0.1-M DTT, and 0.5-μL nuclease-free water. The mixture was incubated at 37°C for 2 min then 0.5 μL of M-MLV RT enzyme was added (final volume 10 μL), followed by a further 50 min at 37°C before deactivating the enzyme at 70°C for 15 min. RT-PCR analysis was performed using the generic reverse primer AS3 and species-specific primers (CpCSV3705F, SbDV3731F, and BWYV3969F) following the manufacturer's instruction (Moukahel et al. 2021; Ademe et al. 2024).

PCR products were sequenced using the Sanger method by a commercial sequencing company (Macrogen). The sequences were compared with sequences available in the GenBank database using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1997). The sequences of 16 Ethiopian virus isolates studied are submitted to GenBank (see Table 1 for accession numbers).

Phylogenetic analysis was performed by aligning the partial coat protein (CP) gene sequences of our Ethiopian isolates and other isolates obtained from the GenBank. Multiple sequence alignments of nucleotide and amino acid sequences were performed using MUSCLE, and the percentage identity matrix was generated using Clustal Omega. Maximum likelihood was used to construct the phylogenetic tree using Kimura 2-parameter model with a Gamma distribution (Aftab et al. 2018) with a bootstrap value of 1000 (Giakountis et al. 2015) using MEGA11 software. Nucleotide pairwise similarities were calculated using SDTv 1.2 (Muhire, Varsani, and Martin 2014). BWYV, CpCSV, and bean leaf roll virus (BLRV, genus *Luteovirus*) were used as an outgroup for phylogenetic analysis of CpCSV, BWYV, and SbDV, respectively.

3 | Results

3.1 | Molecular Diversity

RT-PCR generated amplicons of 566, 276, and 418 bp for CpCSV, BWYV, and SbDV, respectively. The percentage identity matrix showed that the Ethiopian CpCSV isolates had 94.9%–100% nucleotide identity. The result displays that some Ethiopian isolates such as EtLe07-21 (OQ867264) and EtLe13-19 (OQ867260) had 100% nucleotide identity among themselves. However, the other sequenced isolates (MZ043728, MZ043729, MZ043730, MZ043731, and MZ043732) had 94.9%–97.2% nucleotide identity with remaining Ethiopian CpCSV isolates. However, their nucleotide identity varied from 91.9% to 98.7% to the corresponding region of the previously sequenced reference isolates included in this study (Figure 1A). The sequence result presented here

TABLE 1 | Designations and GenBank accessions of the Ethiopian CpCSV, BWYV, and SbDV isolates used in this study.

Isolate code[a]	GenBank accession number	Region	Location	Crop	Virus sequence Blastn_Reference GenBank accessions	Blast similarity (%)
EtLe13-19	OQ867260	North Shewa (Oromia)	Ejersa	Lentil	CpCSV_AY956384	97.24
EtCp01-20	OQ867261	North Shewa (Amhara)	Goshe Bado	Chickpea	CpCSV_AY956384	96.96
EthCp1322-20	OQ867262	North Shewa (Amhara)	Minjar	Chickpea	CpCSV_AY956384	96.96
EthLe2057-20	OQ867263	South Wollo (Amhara)	Legyda	Lentil	CpCSV_AY956384	96.96
EtLe07-21	OQ867264	East Shewa (Oromia)	Debre Zeit	Lentil	CpCSV_AY956384	97.24
EthCp103-18	MZ043728	East Shewa (Oromia)	Denkaka	Chickpea	CpCSV_AY956384	95.08
EthCp413-18	MZ043729	North Shews (Amhara)	Woiramba	Chickpea	CpCSV_AY956384	97.23
EthLe191-18	MZ043730	East Shewa (Oromia)	Abru-Seftu	Lentil	CpCSV_AY956384	96.92
EthCp27-18	MZ043731	East Shewa (Oromia)	Abru-Seftu	Chickpea	CpCSV_AY956384	95.91
EthLe335-18	MZ043732	East Shewa (Oromia)	Kembole	Lentil	CpCSV_AY956384	95.91
EthCp426-18	MZ043733	North Shewa (Amhara)	Woiramba	Chickpea	SbDV_GQ118156	99.68
EthLe450-18	MZ043734	North Shewa (Amhara)	Woiramba	Lentil	SbDV_GQ118156	99.68
EthLe451-18	MZ043735	North Shewa (Amhara)	Abaya-Sakala	Lentil	SbDV_GQ118156	99.68
EthCp16-18	MZ043725	East Shewa (Oromia)	Adadi-Goli	Chickpea	BWYV_LC428357	99.58
EthCp84-18	MZ043726	East Shewa (Oromia)	Denkaka	Chickpea	BWYV_LC428357	99.58
EthCp64-18	MZ043727	East Shewa (Oromia)	Bua-Tengego	Chickpea	BWYV_LC428357	96.58

[a]The last two numbers refer to year of collection.

shows that CpCSV isolates share 94.1%–100% amino acid identity with each other whereas their identity was between 90.1% and 99.1% to the reference isolates used in this study. The chickpea isolate (MZ043728) had higher nucleotide and amino acid identities with isolates previously identified as serogroup II (Figure 1A and Table 2). The phylogenetic tree constructed based on the partial CP gene sequences of 10 isolates from this study and 11 previously sequenced CpCSV isolates from different hosts in GenBank showed diversity among isolates (Figure 1B). The isolates OQ867260, OQ867261, OQ867262, OQ867263, and OQ867262 were closely related to the reference faba bean isolate (NC_008249) from Ethiopia. On the other hand, isolates MZ043731 and MZ043732 were closely related to the Ethiopian chickpea isolate (AY956385) previously described by Abraham et al. (2006). However, isolates MZ043728, MZ043729, and MZ043730 displayed higher identities with each other while having a lower relatedness to other isolates.

The nucleotide sequences of the BWYV isolates MZ043725 and MZ043726 displayed 100% identity with each other but differed slightly (97.0%) from isolate MZ043727. BWYV isolates MZ043725 and MZ043726 displayed the highest nucleotide sequence identity with the radish isolates of Japan (LC428357) and South Korea (OQ625515) origin. On the other hand, they had lowest nucleotide identity (89.6%) with the Azerbaijan (HQ199306) and (US AF473561 and NC_004756) reference isolates. Isolate MZ043727, on the other hand, displayed a 100%

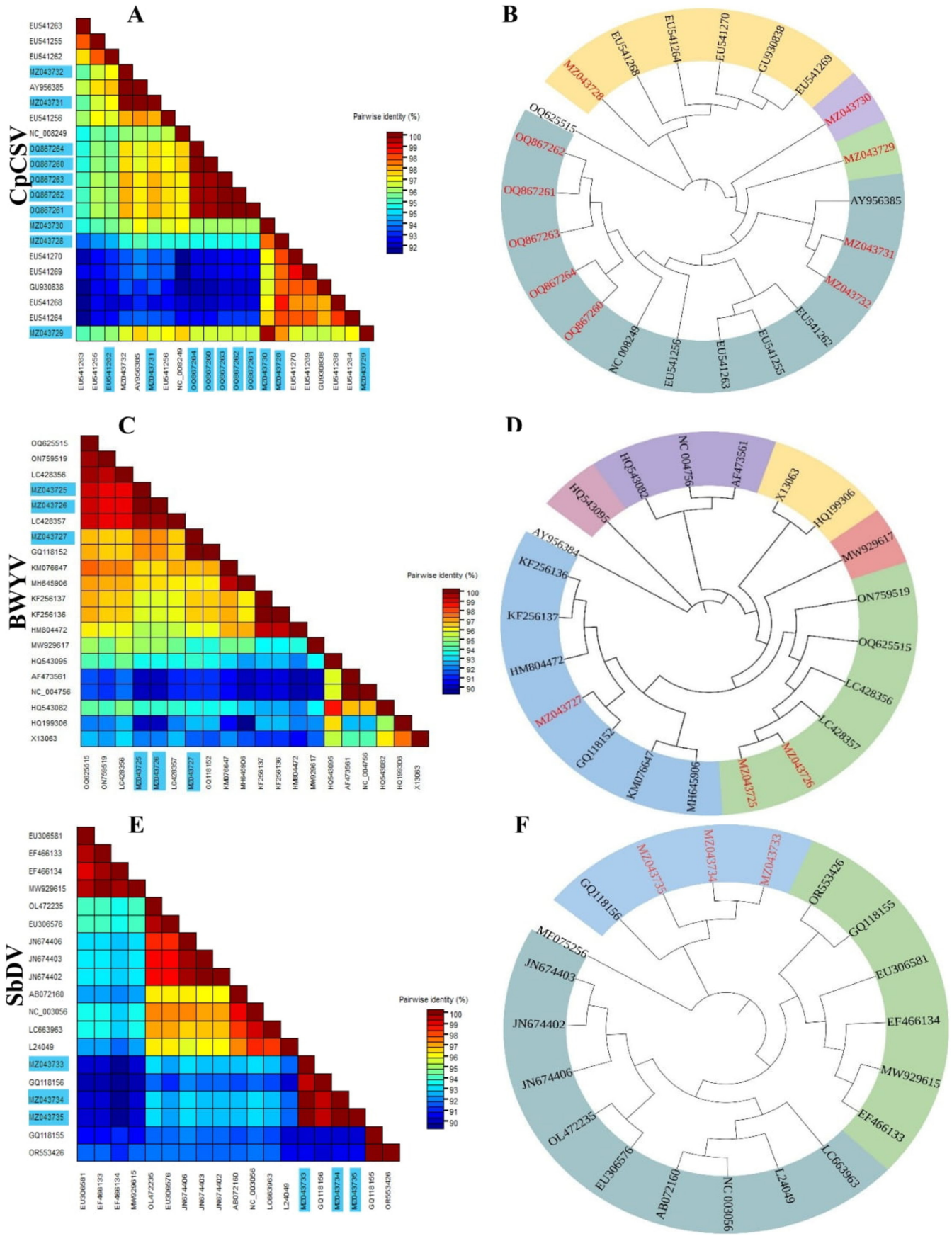

FIGURE 1 | Two-dimensional percentage pairwise similarity plot matrix of chickpea chlorotic stunt virus (CpCSV), beet western yellows virus (BWYV), and soybean dwarf virus (SbDV) generated using Sequence Demarcation Tool (SDT) (A,C,E), and phylogenetic tree of CpCSV, BWYV, and SbDV generated with Mega 11 and viewed in iTOL (B,D,F). Isolates of this study are highlighted.

TABLE 2 | Nucleotide and amino acid sequence identity matrix of Ethiopian chickpea chlorotic stunt virus (CpCSV) isolates obtained from lentil and chickpea.

GenBank accession number	EtLe07-21 (OQ867264)		EtLe13-19 (OQ867260)		EthLe2057-20 (OQ867263)		EthCp1322-20 (OQ867262)		EtCp01-20 (OQ867261)		EthLe191-18 (MZ043730)		EthCp413-18 (MZ043729)		EthCp103-18 (MZ043728)		EthLe335-18 (MZ043732)		EthCp27-18 (MZ043731)	
	na	aa	na	aa	na	aa	na	aa	na	aa	na	aa	na	aa	na	aa	na	aa	na	aa
OQ867264	100.0	100.0	100.0	100.0	99.7	100.0	99.7	100.0	99.7	100.0	96.1	94.1	96.5	94.1	94.9	94.1	97.2	99.1	97.2	99.1
OQ867260	100.0	100.0	100.0	100.0	99.7	100.0	99.7	100.0	99.7	100.0	96.1	94.1	96.5	94.1	94.9	94.1	97.2	99.1	97.2	99.1
OQ867263	99.7	100.0	99.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.1	94.1	96.5	94.1	94.9	94.1	97.5	99.1	97.5	99.1
OQ867262	99.7	100.0	99.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.1	94.1	96.5	94.1	94.9	94.1	97.5	99.1	97.5	99.1
OQ867261	99.7	100.0	99.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.1	94.1	96.5	94.1	94.9	94.1	97.5	99.1	97.5	99.1
NC_008249	97.2	97.5	97.2	97.5	96.9	97.5	96.9	97.5	96.9	97.5	96.9	95.4	97.2	95.4	95.1	95.4	95.9	98.1	95.9	98.1
MZ043730	96.1	94.1	96.1	94.1	96.1	94.1	96.1	94.1	96.1	94.1	100.0	100.0	99.7	99.1	98.2	100.0	96.3	94.9	96.3	94.9
MZ043729	96.5	94.1	96.5	94.1	96.5	94.1	96.5	94.1	96.5	94.1	99.7	99.1	100.0	100.0	97.9	99.1	96.7	94.9	96.7	94.9
GU930838	91.9	90.1	91.9	90.1	92.3	90.1	92.3	90.1	92.3	90.1	96.6	97.2	96.3	96.3	98.2	97.2	93.4	91.4	93.4	91.4
EU541270	92.5	93.9	92.5	93.9	92.8	93.9	92.8	93.9	92.8	93.9	96.9	98.2	96.6	97.2	98.5	98.2	93.4	93.3	93.4	93.3
EU541269	92.5	93.9	92.5	93.9	92.8	93.9	92.8	93.9	92.8	93.9	96.6	98.2	96.3	97.2	98.2	98.2	93.4	93.3	93.4	93.3
MZ043728	94.9	94.1	94.9	94.1	94.9	94.1	94.9	94.1	94.9	94.1	98.2	100.0	97.9	99.1	100.0	100.0	95.0	94.9	95.0	94.9
EU541268	92.2	93.9	92.2	93.9	92.5	93.9	92.5	93.9	92.5	93.9	96.9	98.2	96.6	97.2	98.8	98.2	92.5	93.3	92.5	93.3
EU541264	92.8	93.0	92.8	93.0	93.0	93.0	93.0	93.0	93.0	93.0	97.2	97.2	96.9	96.3	98.5	97.2	93.4	93.3	93.4	93.3
EU541263	95.1	96.5	95.1	96.5	95.4	96.5	95.4	96.5	95.4	96.5	95.1	95.4	95.4	95.4	93.5	95.4	95.6	96.2	95.6	96.2
EU541262	95.7	97.4	95.7	97.4	95.9	97.4	95.9	97.4	95.9	97.4	95.7	94.4	96.0	94.4	94.2	94.4	96.9	97.1	96.9	97.1
EU541255	95.9	98.3	95.9	98.3	96.2	98.3	96.2	98.3	96.2	98.3	95.4	95.4	95.7	95.4	93.9	95.4	96.5	98.1	96.5	98.1
EU541256	96.8	99.1	96.8	99.1	97.1	99.1	97.1	99.1	97.1	99.1	96.6	95.4	96.9	95.4	95.1	95.4	97.5	99.1	97.5	99.1
MZ043732	97.2	99.1	97.2	99.1	97.5	99.1	97.5	99.1	97.5	99.1	96.3	94.9	96.7	94.9	95.0	94.9	100.0	100.0	100.0	100.0
MZ043731	97.2	99.1	97.2	99.1	97.5	99.1	97.5	99.1	97.5	99.1	96.3	94.9	96.7	94.9	95.0	94.9	100.0	100.0	100.0	100.0
AY956385	96.7	98.3	96.7	98.3	96.9	98.3	96.9	98.3	96.9	98.3	96.9	96.3	97.2	96.3	95.4	96.3	100.0	100.0	100.0	100.0

Abbreviations: aa, amino acid; nt, nucleotide.

nucleotide identity with GQ118152 described in Ethiopia as lentil stunt virus (LStV, genus *Polerovirus*). The Ethiopian isolates were found to share lower nucleotide identity with the Australian (HQ543095) and Uzbekistan (MW929617) BWYV isolates of pea and chickpea origin, respectively. The partial BWYV sequence shared 89.7% to 100% nucleotide identity with other isolates from GenBank (Figure 1C and Table 3). The deduced amino acid sequence identity of the Ethiopian BWYV isolates ranged from 97% to 100%. The phylogenetic tree constructed from three partial sequences of Ethiopian isolates from this study and 17 other isolates from GenBank showed great diversity (Figure 1D). The tree shows that MZ043725 and MZ043726 isolates are phylogenetically close to the radish isolate from Japan (LC428357) but appear to be less related to isolate MZ043727. On the other hand, MZ043727 had a close relationship with previously reported reference isolates of lentil (GQ118152) from Ethiopia.

The nucleotide sequence of Ethiopian SbDV isolates showed that isolates MZ043733, MZ043734, and MZ043733 had 100% identity with each other and they have a 99% nucleotide identity to the previously sequenced SbDV isolate (GQ118156), which was identified from faba bean in Ethiopia (Figure 1E and Table 4). Amino acid identities between the newly sequenced Ethiopian isolates and the reference isolate GQ118156 were 100%. On the other hand, they had the lowest amino acid (89.5%) identity with the Syrian reference isolate (GQ118155). Overall, isolates of the present study had 89.5%–100% amino acid identity with other reference isolates from GenBank. The Y-like and D-like sequences were most divergent from the Ethiopian isolates. Overall, these comparisons indicate that Ethiopian SbDV isolates have a lower relationship to the rest of the reference isolates. The tree constructed from three Ethiopian SbDV isolates shows genetic variability with the other reference isolates from GenBank. The three sequenced isolates were most closely related to the previously sequenced Ethiopian isolate used in this study. However, the Y-like isolate (EU306576) was separated from the D-like isolate (EU306581) as both types of isolates were completely different from the isolates of Ethiopian origin, which formed a separate cluster (Figure 1F).

4 | Discussion

Legumes are multipurpose crops grown in Ethiopia. However, several viruses have emerged, re-emerged, and become a threat to legume production worldwide. Since its first report (Abraham et al. 2006), CpCSV has become a major problem in legume producing areas of Ethiopia. In the present work, partial CP sequences of different CpCSV isolates were

TABLE 3 | Nucleotide and amino acid sequence identity matrix of Ethiopian beet western yellows virus (BWYV) isolates obtained from chickpea.

	EthCp16-18 (MZ043725)		EthCp84-18 (MZ043726)		EthCp64-18 (MZ043727)		LStV (GQ118152)	
GenBank accession no.	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**
HQ543095	93.3	98.7	93.3	98.7	93.5	96.3	89.4	87.4
MW929617	94.8	96.1	94.8	96.1	93.5	93.4	93.5	93.4
KM076647	96.3	100.0	96.3	100.0	96.6	97.2	96.4	97.4
MZ043727	97.0	97.4	97.0	97.4	100.0	100.0	100	100
GQ118152	97.1	97.5	97.1	97.5	100.0	100.0	100	100
ON759519	98.8	100.0	98.8	100.0	96.3	97.2	96.6	97.4
LC428356	98.8	100.0	98.8	100.0	96.3	97.2	96.4	97.4
OQ625515	99.2	100.0	99.2	100.0	96.6	97.2	96.7	97.4
MZ043725	100.0	100.0	100.0	100.0	97.0	97.4	97.1	97.5
MZ043726	100.0	100.0	100.0	100.0	97.0	97.4	97.1	97.5
LC428357	99.6	100.0	99.6	100.0	96.3	95.3	96.2	96.4
KF256137	95.4	94.9	95.4	94.9	96.3	94.4	94.8	93.8
HQ199306	89.6	90.9	89.6	90.9	92.3	92.2	91.4	91.8
AF473561	89.6	91.1	89.6	91.1	90.7	89.7	87.8	84.5
KF256136	95.4	94.9	95.4	94.9	96.3	94.4	94.8	93.8
HM804472	95.0	94.9	95.0	94.9	95.7	94.4	94.8	94.3
MH645906	96.3	100.0	96.3	100.0	95.9	97.2	96.0	96.9
HQ543082	93.6	100.0	93.6	100.0	93.2	96.2	89.3	87.8
X13063	91.7	97.5	91.7	97.5	91.3	93.5	87.6	86.5
NC_004756	89.6	91.1	89.6	91.1	90.7	89.7	87.8	84.5

Abbreviations: aa, amino acid; nt, nucleotide.

TABLE 4 | Nucleotide and amino acid sequence identity matrix of Ethiopian soybean dwarf virus (SbDV) isolates obtained from chickpea and lentil.

	EthCp426-18 (MZ043733)		EthLe450-18 (MZ043734)		EthLe451-18 (MZ043735)	
GenBank accession no.	**nt**	**aa**	**nt**	**aa**	**nt**	**aa**
MZ043733	100.0	100.0	100.0	100.0	100.0	100.0
MZ043734	100.0	100.0	100.0	100.0	100.0	100.0
MZ043735	100.0	100.0	100.0	100.0	100.0	100.0
GQ118156	99.4	100.0	99.4	100.0	99.4	100.0
EU306581	90.0	92.5	90.0	92.5	90.0	92.5
LC663963	92.5	91.7	92.5	91.7	92.5	91.7
AB072160	92.5	92.5	92.5	92.5	92.5	92.5
JN674406	92.8	93.3	92.8	93.3	92.8	93.3
JN674403	92.5	93.3	92.5	93.3	92.5	93.3
JN674402	92.5	93.3	92.5	93.3	92.5	93.3
OL472235	92.8	93.3	92.8	93.3	92.8	93.3
EU306576	92.2	93.3	92.2	93.3	92.2	93.3
GQ118155	89.9	89.5	89.9	89.5	89.9	89.5
OR553426	90.3	90.0	90.3	90.0	90.3	90.0
EF466134	89.2	92.4	89.2	92.4	89.2	92.4
EF466133	90.0	92.5	90.0	92.5	90.0	92.5
MW929615	90.0	92.5	90.0	92.5	90.0	92.5
L24049	91.4	90.8	91.4	90.8	91.4	90.8
NC_003056	92.8	91.7	92.8	91.7	92.8	91.7

Abbreviations: aa, amino acid; nt, nucleotide.

phylogenetically analyzed and compared with reference isolates. The result revealed that MZ043728 clustered closely with isolates belonging to serogroup II indicating that the isolate was more similar to it than to some isolates of serogroup I of Ethiopian origin. Diversity among CpCSV isolates was previously analyzed by MAbs raised against CpCSV isolates and placed CpCSV isolates into two distinct serogroups consisting of different geographical origins (Abraham et al. 2009). Group I comprised of Ethiopia and Sudan, whereas Group II consisted of those from Egypt, Morocco, and Syria. This is the first report on the presence of serogroup II isolates in Ethiopia. Overall, the clustering of isolates was related to geographic origin with the exception isolate MZ043728, which may have been recently introduced from other regions.

The amino acid identity between the two Ethiopian BWYV isolates MZ043725 and MZ043726 was 100%, while they shared 97.4% with isolate MZ043727. BWYV tree obtained from the CP alignment shows that the clustering of isolates was not related to geographic origin. Serologically, BWYV has been reported in several legume crops (Ademe et al. 2023; Tadesse et al. 1999). Abraham, Varrelmann, and Vetten (2008) reported that the lentil isolate from Ethiopia (GQ118152) has the closest amino acid identity of 86% with viruses of the BWYV subgroup and proposed it as a new *Polerovirus* named LStV. However, the virus has never been reported outside of Ethiopia so far, but it is likely to occur in other legume-growing regions of the world. Interestingly, isolate MZ043727 collected from chickpea in East Shewa had 100% amino acid identity with the LStV isolate GQ118152 and displayed the lowest amino acid identity (89.7%) with BWYV isolate of US origin. This indicates that in addition to lentil, LStV infects chickpea in Ethiopia. Similarly, LStV isolate displayed a 97.5% amino acid identity with both MZ043725 and MZ043726 isolates. Species demarcation for the genus *Polerovirus* should differ by at least 10% in amino acid sequence identity of any gene product and also have distinct serological and biological properties. In some cases, the taxonomy of the BWYV-like virus group is complex for species differentiation (Yoshida and Tamada 2019). Hence, it will be interesting to determine its complete genome sequence, aphid vectors, natural hosts, and the geographical distribution of LStV to elucidate and validate its accurate taxonomic position as definitive species within the genus *Polerovirus* and understand its impact on legume production in Ethiopia and elsewhere.

The sequence of SbDV isolates obtained from chickpea (MZ043733) and lentil (MZ043734 and MZ043735) were distinct from other SbDV reference sequences. SbDV has been reported

on lentil and faba bean in Ethiopia (Abraham, Varrelmann, and Vetten 2008; Tadesse et al. 1999), but this is the first report of natural infection of SbDV in chickpea in Ethiopia. CP sequence and phylogram of the Ethiopian SbDV isolates have identical sequences among themselves and formed a distinct cluster different from the reference isolates. Authors reported that strains with the same symptom type in soybean are closely related to the same aphid transmissibility type, suggesting the presence of a certain distance in the relationship between D and Y strains (Terauchi et al. 2001). The phylogenetic analysis strongly supported three separate clades defining the Y, D, and Ethiopian isolates. These findings show that there is genetic variability among Ethiopian isolates and other reference isolates. Besides our findings, previous reports on SbDV showed that the virus has been reported worldwide (Abraham et al. 2007; Abraham, Varrelmann, and Vetten 2008; Stone et al. 2024).

Identification of viruses or their strains is crucial in epidemiological studies because isolates may differ in their pathogenicity, host range, vector specificity, and geographical distribution (Abraham, Varrelmann, and Vetten 2012). Viruses have high genetic variability and adaptation to new environments due to their rapid replication, generation of large populations, and high mutation rates (Rubio, Guerri, and Moreno 2013). Understanding genetic variability is crucial for understanding virus epidemiology, emergence, and developing efficient and durable disease control strategies. CpCSV, BWYV, and SbDV were previously reported to be among the viruses found in chickpea and lentil (Abraham et al. 2006; Ademe et al. 2023; Tadesse et al. 1999), whereas SbDV and BWYV were not frequently detected compared with CpCSV. However, due attention should be given to these viruses as crop loss depends on the virus strain, time of occurrence, weather conditions, and host. This information indicated that these viruses deserve attention when developing strategies to improve legume production in Ethiopia and control diseases. In general, persistently aphid-transmitted viruses such as CpCSV, BWYV, and SbDV represent a major impediment to legume production in many parts of the world, especially in countries of West Asia and North Africa (Makkouk and Kumari 2009) including Ethiopia. Because no effective pesticides are available to treat virus-infected plants, the most feasible and cheapest management option is to use virus-resistant crop varieties. Identification of causative viruses and their genetic diversity is critical for implementing effective virus resistance programs. Genetic variability among BWYV isolates has been reported in different parts of the world. However, such information is lacking in Ethiopia. Thus, this study provides baseline information for further study on the diversity of the virus in the country.

Author Contributions

Anteneh Ademe: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft, writing – review and editing. **Safaa G. Kumari:** data curation, funding acquisition, methodology, resources, supervision, writing – review and editing. **Abdulrahman Moukahel:** data curation, investigation, methodology. **Tesfaye Alemu:** supervision, writing – review and editing. **Adane Abraham:** methodology, supervision, writing – review and editing. **Yetsedaw Aynewa:** data curation. **Demsachew Guadie:** methodology, supervision, writing – review and editing. **Seid Ahmed:** funding acquisition, investigation, methodology, resources, supervision, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data not directly published in this paper may be requested from the corresponding author.

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