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Research

Detection of six potato viruses using double antibody sandwich ELISA from in vitro, screen house and field grown potato crops in Ethiopia

Lemma Tessema[1,2] · Rogers Kakuhenzire[2] · Ebrahim Seid[1] · Shiferaw Tafesse[3] · Yitagesu Tadesse[1] · Kasaye Negash[1] · Margaret McEwan[4]

Received: 25 July 2023 / Accepted: 23 November 2023
Published online: 19 February 2024

Abstract
Virus infection in seed potato reduces yield, and the problem is exacerbated when an early-generation seed is affected. The prevalence of six key potato viruses, PVY, PVX, PLRV, PVA, PVS, and PVM, was assessed among decentralized seed multipliers such as individuals, farmer seed group cooperatives, private companies, and agricultural research centers that produce early generation seed in six major potato growing districts in Ethiopia. A total of 262 leaf samples were randomly collected from potato plants and analyzed using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for six major potato viruses. Potato virus prevalence was calculated as the proportion of samples that tested positive for the viruses against the total number of samples tested. The prevalence of infection with at least one of the six viruses was 98.2%. Among the samples analyzed, 17.2% had a single viral infection with one of the six viruses while the majority had multiple infections. The ELISA tests confirmed presence of latent virus infection in early generation seeds from the three EGS producers and in different seed classes. This result indicates that virus infection is widespread in the country, limiting potato production. To address this issue, it is critical to develop a robust system that prevents viral infection build-up and spread in the seed system through regular seed quality assurance and certification, particularly for early generation seed.

Article Highlights

- Most of the materials including seed potato nuclear stocks were infected with potato viruses beyond the acceptable levels more so in being found in breeder seed.
- Viruses are likely to occur throughout the country's seed potato system and also becoming to the second most important potato diseases next to bacterial wilt for EGS production in Ethiopia.
- Unless intervention is made on seed potato systems, viruses are now threatening the livelihoods of already vulnerable smallholder potato farmers in Ethiopia.

Keywords DAS-ELISA · Decentralized seed multipliers · Early generation seed · Potato viruses · QDS · Virus prevalence

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42452-023-05619-x.

✉ Lemma Tessema, lematessema@gmail.com | [1]Ethiopian Institute of Agricultural Research, P.O. Box 2003, Addis Ababa, Ethiopia. [2]International Potato Center (CIP), Ethiopia Office, c/o ILRI, Gurd Sholla, P.O. Box 5689, Addis Ababa, Ethiopia. [3]Global Green Growth Institute (GGGI), Ethiopia Office, P.O. Box 1302, 1110 Addis Ababa, Ethiopia. [4]International Potato Center (CIP), Regional Office for Africa, ILRI Campus, Uthiru, P.O. Box 25171, 00603 Nairobi, Kenya.

1 Introduction

Potato is an important food crop in the fight against hunger, malnutrition, and poverty particularly in developing countries [1–3]. Research advances have allowed for a significant increase in potato yield while addressing devastating diseases over the past 60 years. However, maintaining potato yield stability has remained a global problem because of gradual genetic erosion, loss of genetic purity and seed degeneration by accumulation of pathogens in seed tubers [2, 4–6]. Today, due to increasing global trade and a changing climate, plant pests and diseases pose a greater threat to food security than ever before [7–9].

Viruses are one of the most significant biotic constraints in global potato production, affecting both tuber yield and quality [10, 11]. More than 50 viruses and one viroid have been identified as infecting potatoes around the world, causing varying degrees of loss depending on the virus type, strain, seed system, and crop production practices [10]. Virus infection in potatoes is critical because it causes seed degeneration and a gradual but long-term decrease in yield, especially in agricultural systems where the use of certified seed is limited [12–14].

Foliar virus symptoms in potato can sometimes be mild or latent and hard to observe, unlike bacterial and fungal disease symptoms, and thus receive little attention from farmers and researchers [15]. However, its impacts are most likely responsible for the low yields of potato in Ethiopia when all production practices have been strictly observed. Studies on potato viruses in the country are generally rare and assessment in seed is lacking. The detection and surveillance of viral diseases, as well as the extent of yield reduction caused by potato viruses have not been well documented in the country. Nevertheless, the presence of major six potato viruses which have a worldwide distribution (PLRV, PVY, PVX, PVS, PVM and PVA) were first reported in some parts of the country more than 30 years ago [16].

In another study, five potato viruses; PLRV, PVY, PVX, PVS, PVM, were described in Central Ethiopia in 1993 from the reports of Bekele and Berga (1996) as cited by Tessera et al. [17] where PLRV and PVY were the most prevalent viruses. The study by Bekele et al. [12] reported potato virus prevalence up to 100 percent for five major potato viruses (PVS, PVX, PVM, PLRV, and PVY) except PVA in Amhara region, one of the regional states in the country.

The prevention and management of plant diseases largely depend on accurate identification of pathogens. Rapid and specific detection tools like serology are fast and easy-to-use and have been widely used in field studies [18–20]. One of the most effective management strategies for viruses and other diseases in potato production is to use resistant varieties [21]. However, a given variety may not be resistant to all key potato viruses and some must be managed by other means especially seed sanitation via inspection and certification to prevent disease accumulation and transmission [22, 23]. Current knowledge on the distribution, disease prevalence, and epidemiology of the viruses naturally infecting potato crops in Ethiopia is limited, though essential for crop protection and sustainable potato production. Furthermore, the importance of viral diseases in potato production is also given less attention in the country so far and efforts to quantify yield reduction due to seed degeneration are not well documented. Planting virus-infected seed potato might be one of the causes of low potato yields [13]. Monitoring the occurrence and distribution of important potato viruses is needed to prevent their spread and yield reduction [24]. Thus, this study was conducted to assess the prevalence of six key potato viruses (PVY, PVX, PLRV, PVA, PVS, and PVM) in major potato-growing districts of Ethiopia mainly among seed potato and early generation seed producers in order to provide a basis for further research for viral infections and their management approaches, especially in the seed potato system.

2 Materials and methods

2.1 Materials

Potato (*Solanum tuberosum* L.) leaf samples were obtained from potato grower farmers, private companies, and the Holetta research center following the international/national, and/or institutional guidelines with permission from individual farmers and institutions.

2.2 Study area and sampling

A survey was conducted in farmers' fields producing quality declared seed (QDS) and ware potato in six major seed potato growing districts, two private seed growers (Solagrow plc. and Wagnose tissue culture laboratory) and Holetta agricultural research Centre in 2021 and 2022 in Ethiopia. The study districts were selected from Oromia and Southern Nations, Nationalities, and People's (SNNP) regions, which are major potato growing regions in Ethiopia [25] and were selected based on their potential for potato production (Fig. 1). Sample size and sampling districts are indicated in Table 1.

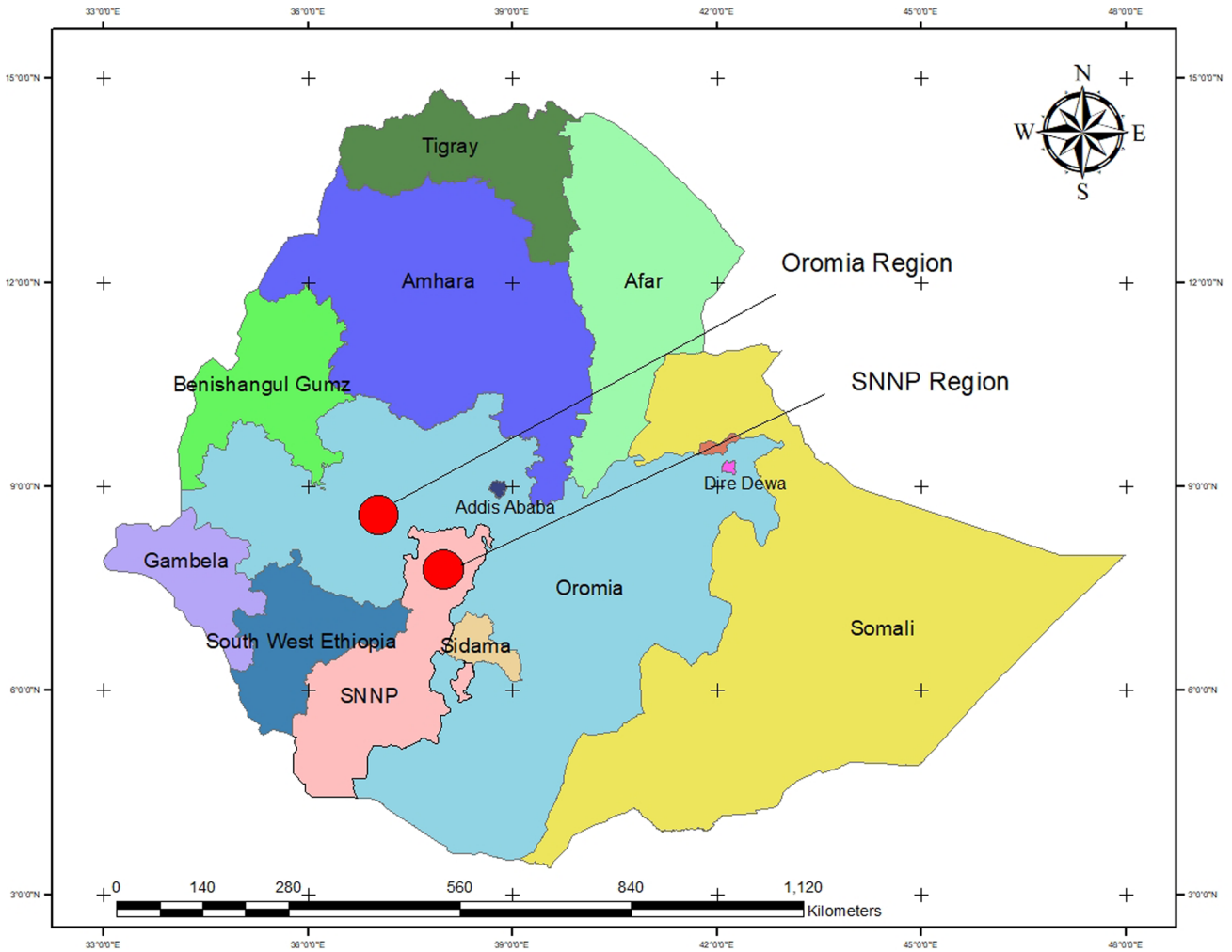

Fig. 1 Location map of the studied potato growing regions in Ethiopia

Table 1 Sampling districts and sample size collected for potato viruses detection in Ethiopia in 2021/22

Number	Sampling area/district	Sample size	Sampling year
1	Jeldu	44	2021
2	Wolmera	33	2021
3	Wenchi	31	2021
4	Degam	31	2021
5	Gumer	24	2021
6	M/Azernet	7	2021
7	Holetta Agricultural Research Centre	47	2022
8	Private seed producers	45	2022
	Total	262	

Sampling was addressed by individual decentralized seed multipliers (I-DSM), farmer seed group cooperatives (FSGCs), and ware potato growers. Early generations (EGS) was also assessed from Holetta ARC, Solagrow plc and Wagnos tissue culture. Five samples (tested as one composite) were taken randomly from within each farm and tissue culture laboratory without bias on symptomatic or asymptomatic plants. For samples collected in the tissue culture laboratory, whole tender plantlets (8 in-vitro plantlets per jar) were removed from culture jars and transferred to numbered sample bags used as one sample for virus detection. Samples from early generation seed tubers were collected under screenhouse grown potato crop at Holetta and Solagrow plc. Sampled plants were 35 to 60 days old after planting. A simple random sampling method was employed along each study district, which was selected by their potato production potential, especially for seed (Table 1). At each sampled field, the purpose of the potato crop, seed source and number of generations the seed has been recycled, the geo-reference, and the name of the farmers were recorded.

2.3 Sample preparation and processing

Potato leaf samples were collected in numbered plastic bags, placed in ice boxes, and transported to the Holetta Research Centre pathology laboratory, where they were stored at 4 °C for three days before being analyzed and consecutive ELISA diagnosis was undertaken following the procedure indicated in study of Onditi et al. [14]. Direct double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used to detect potato virus infections [26]. The International Potato Centre (CIP) provided the antibodies for PVY, PVX, PLRV, PVS, PVM, and PVA in a kit along with relevant reagents and a user's instruction manual from Lima, Peru. The ground leaf samples of 100 µl was loaded in triplicates in the ELISA plate including extraction buffer as a control. Virus-infected samples in micro-titration plates were identified by yellow-orange color development due to a phosphatase enzyme breaking down the phosphate substrate. The relative virus titer was determined using a Multiscan ELISA plate reader (Multiscan FC; SkanIt Software) by measuring the optical density (OD) or light absorbance value of each well at a wavelength of 405 nm. Virus-infected samples based on the light absorbance values were determined using a positive–negative threshold (*T*) per virus set at the mean of five healthy plus three standard deviations ($T = \overline{X} + 3\delta$). Any sample whose mean optical density exceeded the positive–negative threshold for each analyzed virus was declared infected [27].

2.4 Data analysis

The prevalence of a given potato virus was calculated as the proportion of samples that tested positive for that virus against the total number of samples that were analyzed per test. The significance of sample source and virus type were tested using general linear regression and means compared by protected least significant difference at 5% probability for the first set of samples. The data for subsequent sample sets were processed separately and collated by producer category, seed class and districts where the samples were collected. The samples collected from EGS and TC laboratory were calculated separately from filed samples.

3 Results

3.1 Prevalence of the six viruses in Ethiopia

The prevalence of potato viruses was significantly ($P \leq 0.05$) affected by the district where the sample was collected and the virus type but not the producer type. (Table 2). There was no significant ($P > 0.05$) interaction between the virus and potato producer type (Table 1). The district from which the samples were collected was highly significant ($P \leq 0.001$) and contributed 11.1% to the observed variability. However, a high proportion (76.3%) of the variation could not be accounted for analysis of variance model variables.

Data aggregation across districts and potato producers revealed that PVX was the most prevalent virus, while PLRV was the least registered potato virus (Fig. 2).

According to an assessment of virus prevalence by district source, Wenchi and Wolmera had the highest occurrence of all viruses combined, while Jeldu and Degam had the lowest (Fig. 3). Degam district, where seed potato recycling was the major constraint for most potato farmers is, unfortunately, less infected than the other six districts this might be due to less insect pressure in the area.

Table 2 Analysis of variance for the prevalence of potato viruses affected by sample source and virus type in 2021

Source of variation	d.f	Sum of squares	Mean squares	Variance ratio	F-prob	Contribution (%) to total SS
District	5	3554.9	711	4.22	0.001	11.1
Producer type	2	545.7	272.8	1.62	0.202	1.7
Virus	5	2633.2	526.6	3.13	0.01	8.2
Producer type × virus interaction	10	851.1	85.1	0.51	0.884	2.7
Residual	145	24,431.3	168.5			76.3
Total SS	167	32,016.1	191.7			100.0

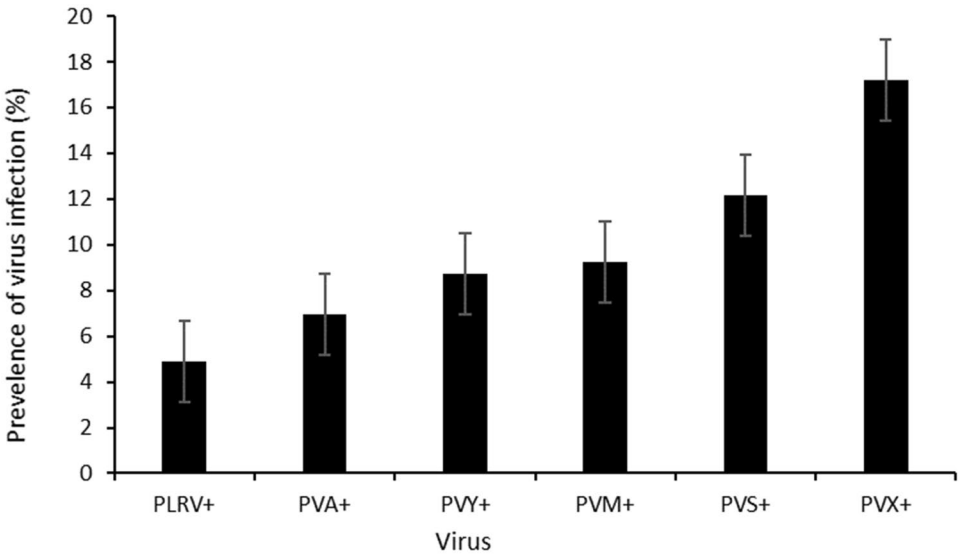

Fig. 2 Aggregated prevalence of potato viruses from six districts in Ethiopia in July 2021

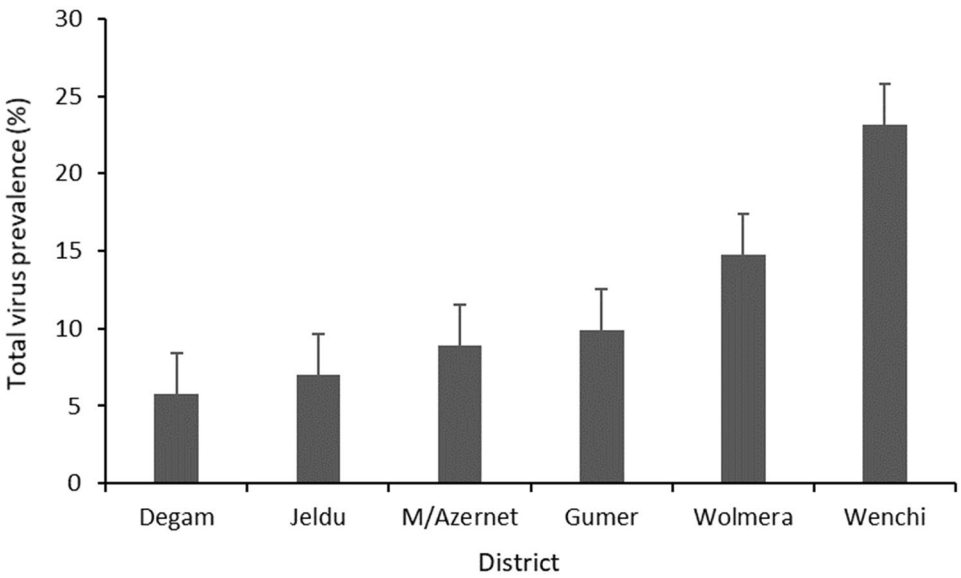

Fig. 3 Aggregate prevalence of key potato viruses in selected potato growing districts in Ethiopia in 2021

The analysis and aggregation of virus prevalence by district revealed that, with the exception of PVS in Degam and PLRV in Mirab Azernet, all of the potato viruses assessed were present in all six study districts (Fig. 4). Wenchi had the highest levels of virus prevalence among the districts studied, while Degam had the lowest (Fig. 4). In Wenchi, which had the highest aggregate virus prevalence, there were low levels of PLRV and PVA when compared to other districts

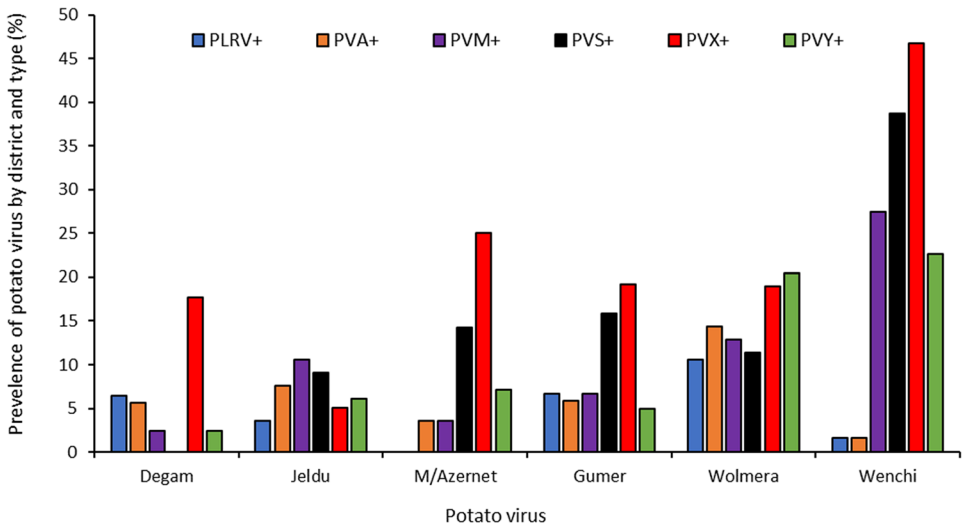

Fig. 4 Prevalence of key potato viruses in selected districts in Ethiopia in July 2021

(Fig. 4). Wolmera and Gumer districts had the highest prevalence of all six viruses, with Wolmera having the highest occurrence of PLRV and PVY (Fig. 4). Despite having the second lowest aggregate virus prevalence, the Jeldu district had a relative similar prevalence of the six viruses, with high levels of PVM and PVS (Fig. 4).

Assessment of viruses' prevalence by potato producer types showed little differences among samples collected from I-DSM, FSGCs, and ware potato growers (Fig. 5). The I-DSM has a lower PVS infection than PVA and PVY. In the FSGC, PVM is slightly lower than PVY, whereas in the ware group PVM and PVY are essentially equal (Fig. 5). The occurrence of all 6 viruses between I-DSM and FSGC showed with various prevalence percentages in Fig. 5 indicating both are supplied at the same seed class and source but grown by different categories of produces however, the later registered a very high prevalence of PVX while I-DSM had slightly equal PLRV and PVM infections (Fig. 5).

Only 1.8% of the samples were virus-free, 17.2% had one of the six viruses tested, and 81% had multiple viral infections (Table 3). PVX had the highest prevalence of single viral infection, while PVS had the lowest (Table 2). The most common types of multiple viral infections were double and triple viral infections (Table 3).

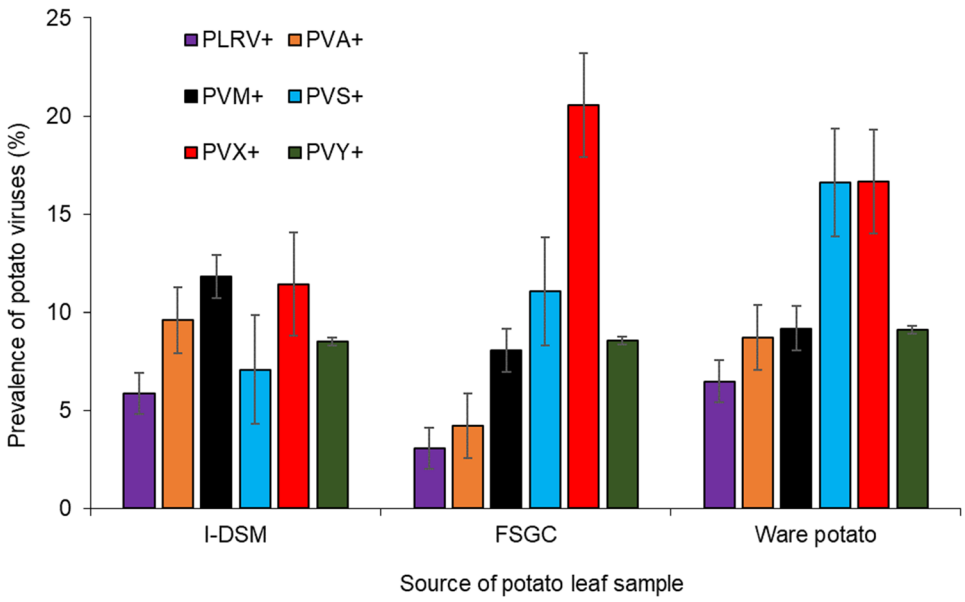

Fig. 5 Prevalence of latent virus infection in potato leaf samples from individual decentralized seed multipliers (I-DSM), farmer seed group cooperatives (FSGCs), and ware potato from six selected districts in 2021

Table 3 Prevalence of six major potato viruses in six districts in Ethiopia from seed and ware potato fields in 2021

Infection level	Virus combination	Frequency	Percent
0	Virus free	3	1.8
1	PVA	2	1.2
	PLRV	2	1.2
	PVM	3	1.8
	PVS	1	0.6
	PVX	19	11.2
	PVY	2	1.2
	Sub-total	29	17.2
2	PVA + PVM	1	0.6
	PVA + PVS	1	0.6
	PVA + PVX	6	3.5
	PLRV + PVM	1	0.6
	PLRV + PVX	3	1.8
	PVM + PVS	2	1.2
	PVM + PVX	2	1.2
	PVS + PVX	10	5.9
	PVX + PVY	10	5.9
	Sub-total	36	21.3
3	PVA + PVM + PVS	4	2.4
	PVA + PVM + PVX	2	1.2
	PVA + PVM + PVY	2	1.2
	PVA + PVS + PVX	4	2.4
	PVA + PVX + PVY	2	1.2
	PLRV + PVA + PVY	1	0.6
	PLRV + PVM + PVX	1	0.6
	PLRV + PVM + PVY	2	1.2
	PLRV + PVS + PVX	2	1.2
	PLRV + PVS + PVY	1	0.6
	PLRV + PVX + PVY	1	0.6
	PVM + PVS + PVX	14	8.2
	PVM + PVS + PVY	3	1.8
	PVM + PVX + PVY	1	0.6
	PVS + PVX + PVY	7	4.1
	Sub-total	47	27.9
4	PVA + PVM + PVS + PVX	4	2.4
	PVA + PVM + PVS + PVY	4	2.4
	PVA + PVM + PVX + PVY	3	1.8
	PVA + PVS + PVX + PVY	1	0.6
	PLRV + PVA + PVM + PVS	1	0.6
	PLRV + PVA + PVS + PVX	1	0.6
	PLRV + PVA + PVS + PVY	1	0.6
	PLRV + PVM + PVS + PVX	3	1.8
	PLRV + PVS + PVX + PVY	2	1.2
	PVM + PVS + PVX + PVY	8	4.7
	Sub-total	28	15.5
5	PVA + PVM + PVS + PVX + PVY	4	2.4
	PLRV + PVA + PVM + PVS + PVX	2	1.2
	PLRV + PVA + PVM + PVS + PVY	6	3.5
	PLRV + PVA + PVM + PVX + PVY	5	2.9
	PLRV + PVM + PVS + PVX + PVY	2	1.2
	Sub-total	19	11.2

Table 3 (continued)

Infection level	Virus combination	Frequency	Percent
6	PLRV + PVA + PVM + PVS + PVX + PVY	8	4.7
	Total	170	100.0

3.2 Prevalence of viral infections in potato early generation seed and breeding stock

Assessment of viral infections in potato breeding stock and early generation seed revealed a high prevalence of PLRV in potatoes grown in both open fields and screen houses at Holetta (Table 4). Other viruses, other than PVA, were present in at least 50% of the analyzed samples (Table 3). Similarly, the prevalence of PLRV and PVY in breeding stock in open fields, greenhouses, and other potato experimental plots was very high, signaling a degeneration in the development or multiplication chain in early planting stock (Table 3). Other than PVA, the prevalence of all assessed viruses was greater than 30%, posing a threat to the seed system, especially given the high prevalence of PLRV and PVY (Table 4).

The assessment of virus infection at the Holetta agricultural research center, Wagnos tissue culture, and Solagrow PLC, which are the primary sources of early generation seed in the Oromia and SNNP regions, revealed a high prevalence of PLRV at Solagrow and Wagnos tissue culture (data not shown). Viral infections were discovered in both tissue culture and greenhouse grown stocks at Holetta ARC. Only PLRV was discovered at Wagnos tissue culture and in materials grown in a rustic greenhouse for mini-tuber production (data not shown), whereas tissue culture samples were virus-free (data not shown). All of the samples obtained at Solagrow were obtained from potato apical cuttings that were being rooted for open-field transfer for mini-tuber production, and they tested positive for the presence of PLRV, PVA, and PVX (data not shown, see Supplementary Table 1).

4 Discussion

This study revealed a high prevalence of viruses in seed potato systems in Ethiopia. Except for PVS in Degam and PLRV in Mirab Azernet, all six tested potato viruses were found in the assessed districts, implying that any clean seed introduced in any of the districts would be susceptible to virus infection, particularly among farmers using own-saved or uncertified seed. The most common virus in the surveyed districts was PVX, which was easily transmitted mechanically, through contact with contaminated objects, or by being carried in infected seed. The high prevalence of PVX in the sampled districts could be attributed to producers' lack of knowledge about appropriate management practices for preventing mechanically transmitted viruses, or to the possible planting of PVX-infected seed [28] due to unavailability of pathogen tested seed tubers in Ethiopia [29]. Potato leaf roll virus had the lowest prevalence across the districts, most likely due to the high altitude of central Ethiopia, where virus transmitting aphid vectors are scarce [8]. Previous studies, however, had found a high prevalence of PLRV ranging from 16 to 32% in central Ethiopia [17], most likely due to differences in the potato crops surveyed, districts surveyed, and months of the year when the assessments were conducted.

The most common viruses in this study (PVS, PVM, and PVY) are non-persistently transmitted by aphids, whereas PVX, the most prevalent virus, is mechanically spread [30]. Aside from PLRV and PVY, which are only transmitted by aphids, and

Table 4 Levels of infection of six major potato viruses in seed potato and germplasm at Holetta Research Centre in Ethiopia in September 2021

Seed stage/cycle	Sample source	Samples analyzed	Percent virus infection (%)					
			PLRV+	PVA+	PVM+	PVS+	PVX+	PVY+
Early generation seed	Open field	7	100.0	0.0	42.9	14.3	28.6	57.1
	Screen houses	12	83.3	0.0	58.3	50.0	50.0	50.0
	Total	19	89.5	0.0	52.6	36.8	42.1	52.6
Germplasm	Breeding	12	25.0	8.3	0.0	50.0	8.3	33.3
	Research	4	50.0	0.0	0.0	50.0	50.0	50.0
	Screen houses	12	83.3	0.0	50.0	33.3	0.0	91.7
	Total	28	53.6	3.6	21.4	42.9	10.7	60.7
	Grand total	47	68.1	2.1	34.0	40.4	23.4	57.4

PVX, which can only be transmitted mechanically, the high prevalence of PVS, PVM, and PVA is not surprising because they can be transmitted by both aphids and mechanically when healthy plants come into contact with contaminated surfaces. Thus, potato viruses transmitted infrequently by aphids or mechanically were more common in the country than viruses transmitted entirely by aphids, such as PLRV. On the other hand, virus transmission mode could affect virus population as well as its evolution [31].

Furthermore, the current study revealed that only a small percentage of samples (1.8%) were virus-free. Most of the samples had two or more viral infections and, in such circumstances, the degenerative effects of the viruses will be high especially where PLRV, PVY or both are involved [13]. This could be the reason farmers are reporting low potato yields despite following most of the recommended agronomic practices in survey districts (Tessema 2022, personal communication) [5].

Individual decentralized seed multipliers, farmer seed group cooperatives, and ware potato farmers had similar prevalence for some potato viruses and comparably different prevalence for some viruses across sampling districts. Moreover, recent studies reported that potato virus Y, a damaging potato pathogen can be transmitted between potato plants by aphid feeding, by wounding, or via tubers (mother to daughter plants) [31]. This is not surprising given that seed potato virus indexing, and seed certification are not common practices in Ethiopia and farmers in sampling districts have limited seed replacement options [29]. The viral infections discovered on farms could have come from primary seed sources in early generation seed that had not been tested for health. To prevent viral infection among later-generation seed multipliers such as decentralized seed multipliers, Ethiopia must implement and enforce latent virus indexing and seed certification among early seed potato producers.

The impact of virus infection on potato depends on a variety of factors, including virus species, strains, infection severity, vector pressure, climate dynamics, plant nutrition, and mixed infections [32, 33]. The high prevalence of PLRV, PVY, and other aphid-transmitted viruses in field environments with potential aphid infestation is likely to accelerate the spread of aphid-borne viral infections, resulting in rapid and severe seed potato degeneration under bulking and low progeny crop yield [34]. When viral infections occur in combination, the degenerative effect is likely to be exacerbated [14], as observed in this study, where more than 80% of the samples from different districts with high potential to produce seed were infected by more than one virus at the same time. The synergism effect of most potato viruses occurring in multiple infections exacerbates potato crop economic and yield loss [10].

The high prevalence of viral infections in the districts of Wenchi, Wolmera, and Jeldu must be considered in context. Farmers in Wenchi reported limited seed replacement [29], which could explain the high virus incidence despite the district's low PLRV prevalence. The high prevalence of all viruses in the Wolmera district, where Holetta ARC is the primary source of breeders and early generation seed, must be noted. Clean breeders' seed is predisposed to early viral infections in the seed bulking chain due to high virus pressure near a key seed production facility, insect vectors could be responsible for virus infestation under screen house conditions [35]. It is not surprising, then, that samples collected at Holetta ARC contained high levels of viral infections. All six viral infections were present in Jeldu, which is a major source of seed potato for most other districts in Ethiopia's central highlands, albeit in low numbers in most cases that might be seed growers are not replacing their seed frequently or poor agronomic practices they are employing [28]. It is critical to note that the Jeldu district is a popular source of potato planting material in Ethiopia, and a low prevalence of degenerative potato viral infections would be ideal. The low infections, on the other hand, provide a source of inoculum to newly acquire clean seed as well as other areas that would obtain seed from these districts.

The high prevalence of viral infections at the farm level could be related to the sources of seed, most potato producer farmers are not accessing seed tubers with known health status [29]. In Ethiopia, seed potato of fair quality is purportedly obtained from decentralized seed multipliers either as individual or farmer group cooperatives who are expected to produce quality declared seed. There was little or no difference in the prevalence of potato virus infections among ware and seed potato producers except in very fine details. Similarly, the prevalence of viral infections was very high among early generation seed producers, especially with PLRV in both breeders and pre-basic seed. Logically, infections found at the farm level can be traced backward to early-generation seed producers where both internal and external seed quality control is not strictly followed with the consequent transfer of infection to the farm level [36].

At Wagnos Biotech, samples collected from the screen house were infected with PLRV whereas the mother plants in from the tissue culture laboratory were clean. This means that the infection observed in the vegetative crop in the screen house did not originate from the TC laboratory as a latent infection. At Holetta ARC, there were viral infections in both TC and screen house stocks as breeders and basic seeds. This calls for the cleaning of stocks in tissue culture while materials in screen houses should be reconsidered if they must be allowed to continue in the seed bulking chain. It is important also to recognize that these viral infections even at low levels were found in nuclear (breeders) stocks. These infections

would probably be higher and more severe by the time the seed reaches the farmers through the seed bulking cycles and likely have a high negative impact on the yield of progeny potato crops [36].

5 Conclusions

The ELISA tests confirmed presence of latent virus infection in early generation seeds from the three EGS producers and in different stock classes. Infections occurred in single or multiple attacks particularly at Holetta ARC where all the six viruses were present although PLRV and PVA, respectively had the highest prevalence above 5%, which is the minimum standard requirements for pre-basic, basic or C1 seed class according to the Ethiopian Standards Agency. The rest of the viral infections occurred at less than 4% however, it is important to recognize that these infections even at low levels were found in breeders seed that is supposed to be completely free of all manner of viral infections. These infections though at low levels were in nuclear (breeders) stocks should not be taken lightly because (i) infection would be severe by the time the seed reaches to ware potato farmers through the bulking stages and (ii) it would pose a threat as sources of new infections (iii) both EGS and QDS seed classes are also infected beyond the acceptable level. Thus, uninterrupted multiplication of such infected stock and future distribution to farmers will likely have a negative impact on ware potato yield. This calls for cleaning of materials especially in tissue culture while stocks under screenhouses should be reconsidered if they must be allowed to continue in the seed bulking chain. Additionally, further research studies need to be conducted to provide more comprehensive information on potato virus prevalence, distribution, and control methods considering the relevance and impact of these six potato viruses for sustainable potato production in Ethiopia.

Acknowledgements This work was supported by ISSD Africa, and Seeds of Change projects implemented by the International Potato Center (CIP) in Ethiopia. We also acknowledge the Ethiopian Institute of Agricultural Research (EIAR), Holetta Agricultural Research Centre particularly the plant pathology laboratory for their kind support in virus diagnosis. Finally, we are thankful to Mr. Mulatu Workie, National Agricultural Biotechnology Department of microbial biotechnology laboratory for his assistance in reading the ELISA plates for relative viral infection load quantification.

Author contributions RK, ST, and LT contributed to the conception and design of the work. LT, ST, ES, KN, and YT collected samples from districts and seed companies. LT, ES, and ST conducted the laboratory diagnosis. RK and LT contributed to the acquisition, analysis, interpretation of the result, and drafting of the manuscript. MM and RK contributed to the language edition. All authors read and approved the final version of the manuscript.

Funding The authors have no relevant financial or non-financial interests to disclose.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The author declares no conflict of interest.

Ethical approval Not applicable.

Informed consent Not applicable.

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