Research Article

Assessment of Strawberry Latent Ring Spot Virus in Commercial Strawberry Growing Fields of Khyber Pakhtunkhwa-Pakistan

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Abstract | Commercially, strawberries are grown under diverse climatic conditions, from tropical and subtropical to slightly temperate regions. In Khyber Pakhtunkhwa (KP), fields in the districts of Mardan and Charsadda are major contributors for commercial strawberry production whereas Swat provides the runner to all the commercial producers in KP as well as in Punjab. Strawberry is also cultivated to some extent in Abbottabad, Mansehra, Haripur, and Peshawar. Strawberries latent ring spot virus (SLRSV) causes symptoms such as mosaic, chlorosis, mottling, deformed leaves, and crinkling, whereas leaf cupping, savoying, necrotic rings, or necrotic spots, and misshapen fruits with small size were also noticed in some plants, posing a threat to strawberry production in the areas. Therefore, two-year surveys (2020-21 and 2021-22) were conducted in these areas to determine the incidence and distribution of SLRSV and to characterize the virus. DAS-ELISA confirmed the presence of SLRSV with incidence higher in the plains compared to hilly areas. The mean incidence of SLRSV in district Swat was 11.7% during the first year; intriguingly, it increased by 3% in the second year (14.7%). Similarly, in district Mardan, the average incidence was 23.3% in the first-year survey, 3.4% lower than the following year's (26.7%). In the district of Charsadda, the average incidence of SLRSV was 23.5%, increasing to 26.8% the following year. Molecular characterization disclosed the sequence identity range of SLRSV (72.49%-91.75%), however, most of the isolates showed identity ranges between 83% and 86%. The nematode vector Xiphinema diversicaudatum has also been extracted and identified from soils of the commercial strawberry fields in Mardan and Charsadda. Sequence analysis confirmed that SLRSV isolates are closely related to the Egyptian SLRSV isolate (MT648777).

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Introduction

Strawberry (Fragaria x ananassa Duchesne), a significant horticultural cash crop in the Rosaceae family with high economic returns (Green, 1971). The climate of Northern Pakistan is very favorable for strawberry cultivation, as the crop receives low chilling temperatures, which enhance productivity. Commercially, the crop is grown in the districts of Abbottabad, Charsadda, Haripur, Mardan, Mansehra, and Peshawar of Khyber Pakhtunkhwa (KP) province (Tariq *et al.*, 2018). The crop is cultivated on 227 ha in Pakistan, producing almost 96 tons of strawberries annually (GOP, 2017). District Swat, primarily

focusing on raising nursery (saplings), provides almost 60 to 70 million saplings/year to other parts of the country. Comparatively, sapling production is a more profitable business than fruit production (Shaukat *et al*., 2018). These exotic cultivars/varieties failed to achieve the targeted production as achieved in other strawberry-producing countries (Mabood, 1994). There are certain constraints in production technology, cultivar selection, and adaptation that directly or indirectly affect production. Therefore, locally adapted cultivars are preferred and ranked as the top priority of the growers. However, there are some important strawberry viruses that pose a threat to strawberry production, which include strawberry vein bending virus (SVBV), strawberry mild yellow edge virus (SMYEV), strawberry latent ring spot virus (SLRSV), strawberry mottle virus (SMoV), tobacco streak virus (TSV), and strawberry crinkle virus (SCV) (Sharma *et al*., 2018). Strawberry latent ringspot virus (SLRSV) is a member of family Secoviridae, genus Nepovirus, which is one of the most important genera of nematode-transmitted viruses. Martin *et al*. (2004) reported SLRSV in cherry from Canada and the US for the first time, and *Xiphenema diversicaudatum* is a major vector transmitting the virus locally under natural field conditions (EPPO/CABI, 1996).

SLRSV poses a threat to a wide range of hosts, including berries, olives, peaches, roses, and other plant species. In Italy, the virus present in an asymptomatic olive plant was detected and confirmed by one-step RT-PCR (Faggioli *et al*., 2002). Using ELISA and RT-PCR protocols, the virus was confirmed from India infecting rose plants (Kulshresthaet *al*., 2004). SLRSV-specific forward and reverse primer pair amplified a band fragment of 497 bp from RNA2 of the SLRSV-encoding region of the coat protein isolated from Canada and the US strawberries (Martin *et al*., 2004; El-Morsy *et al*., 2016), apricot, and peaches (Kumari, 2009). Therefore, SLRSV was identified as the most important strawberry virus and ranked as a high-risk strawberry virus in the area (Martin *et al*., 2004; Martin and Tzanetakis, 2013). The virus could be detected, isolated, and characterized on assay hosts' plants and through DAS-ELISA. SLRSV is prevalent in many countries, causing severe losses in strawberry crops, particularly in Europe (Tang *et al*., 2013). Moreover, one-step RT-PCR was successfully used to detect SLSRV, in addition to DAS-ELISA (Faggioli *et al*., 2002), and it was shown that direct detection can be performed reliably with one-step RT-PCR (Tang *et al*., 2013), which is a reliable, rapid diagnostic technique that enables early diagnosis of SLRSV in strawberries. This assay was easy, quick, and specific, with significant sensitivity.

Little work has been done on incidence, distribution, and especially on characterization of SLRSV infecting strawberries in KP, Pakistan. Proper identification is necessary for the effective integrated approach to control the virus. To overcome the problems associated with the quality, quantity, and perishability of fruit, Pakistan can export it to other countries, including the United States, Europe, and the Middle East. This article focuses on the scientific lexicon of unexplored SLRSV found in commercial strawberry fields in the KP province.

Materials and Methods

Survey for the assessment of strawberry latent ring spot virus

Symptom-based observations led us to conduct extensive surveys of commercial strawberry-growing areas (runners and fruit-producing areas) in Khyber Pakhtunkhwa (KP), including the districts of Mardan, Charsadda, and Swat (Figure 1), during February to May 2020-2021 and 2021-2022. Surveys aimed to determine the incidence and distribution of strawberry latent ring spot virus (SLRSV) infecting strawberry crops. The two-stage clustered sampling technique was used for sample collection, with five fields selected in each district. In each field, 30 random strawberry leaf samples were collected irrespective of the visible symptoms. These samples were stored at -20°C until further processing.

Serological detection of viruses infecting strawberries

All strawberry leaf samples collected during the survey were tested using the double-antibody sandwich ELISA (DAS-ELISA) method to confirm the presence of SLRSV with commercial kits from BIOREBA, Switzerland. The ELISA was performed according to the manufacturer's protocol.

Disease incidence (%) was calculated as the number of positive samples divided by the total number of samples, using the following formula.

$$\%DI = \frac{\text{No. of infected plants}}{\text{Total No. of plants}} \times 100$$

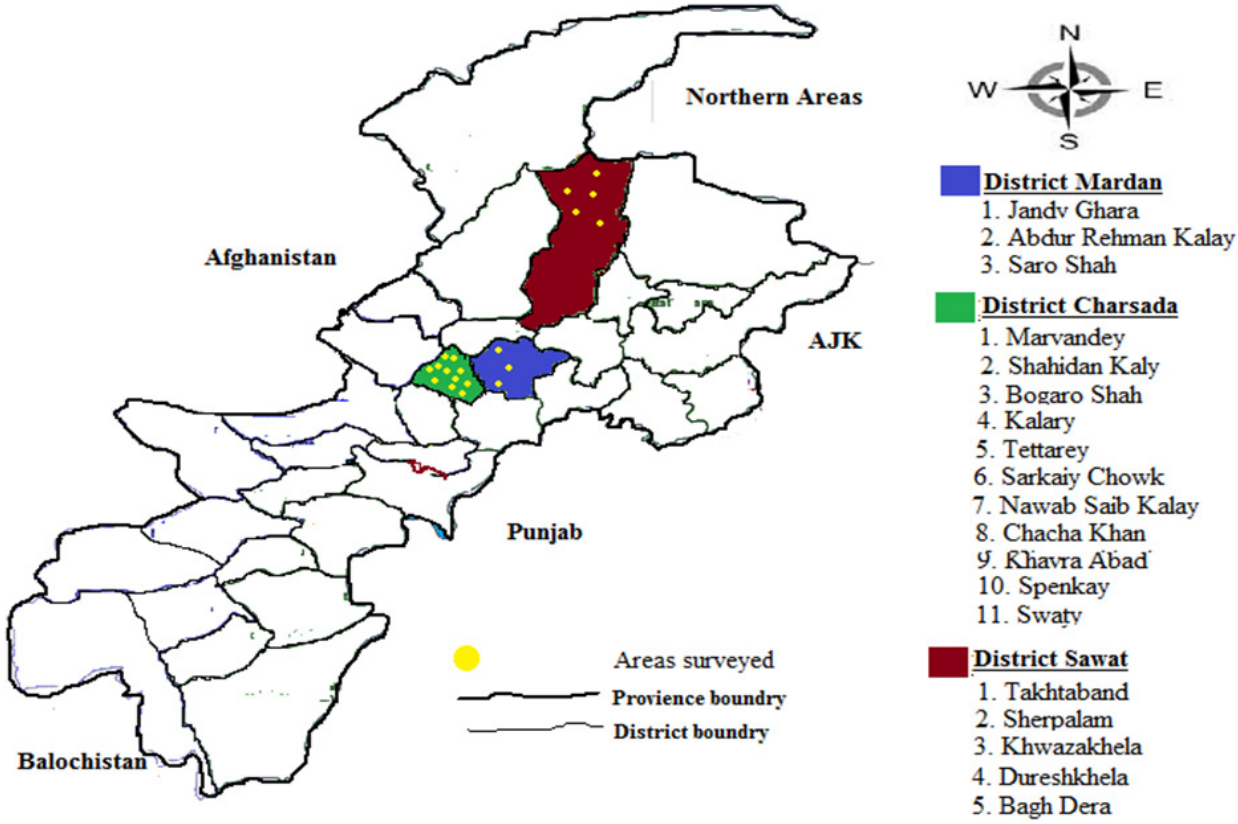


Figure 1: *Strawberries are grown commercially in Swat, Mardan and Charsadda districts of Khyber Pakhtunkhwa, Pakistan as highlighted in the in the administrative boundaries. Major strawberry growing areas surveyed to determine the incidence of SLRSV infecting strawberry. The locations in each district were tagged with yellow dots mentioning the strawberry fields where the samples were collected randomly.*

Nematode extraction and identification

Nematodes are known to transmit strawberry viruses, especially SLRSV (EPPO/CABI, 1996; Sharma *et al*., 2018). J3 and J4 were isolated from a 20 g soil sample using the decanting and sieving method (Southey, 1986) and the tray method (Whitehead and Hemming, 1965). Nematodes were identified using morphological characteristics observed under a compound microscope at 40X magnification, compared with the identification key described by Loof and Luc (1990).

Table 1: *Primer set, their sequences and size of amplicons used in the current study to amplify the coat protein encoding region of the respective viruses infecting strawberry crop.*

SLR SV	CP-F CP-R	497 bp	CCTCTCCAACC TGCTAGACT	(Martin *et al*., 2004)

RNA extraction

Total RNA was extracted from samples, confirmed as SLRSV-positive, by ELISA using the EZ spin column total RNA extraction kit (Bio basic Inc., Canada, as per the manufacturer's instructions).

DNA synthesis

Revert Aid First Strand cDNA synthesis kit (Thermo Scientific) was used to synthesize cDNA according to the manufacturer's protocol.

Polymerase chain reaction (PCR) to amplify coat protein encoding regions

The viral genome in the coat protein regions of the respective virus was amplified using virus-specific primer sets (Table 1). For each 50µl PCR reaction, 1 µl cDNA template, 5 µl 10X PCR buffer, 5µl 2mM dNTPs, 1µl *Taq* DNA polymerase and forward and 1µl of reverse primers (25pmol) were aliquoted in the reaction mixture. The PCR regime comprised of an initial denaturation step for 2 min at 94°C. It was then followed by 34 cycles of denaturation at 94°C for 1 minute, annealing at 55°C (30sec), and an extension step at 72°C (1 min). The final extension step was performed at 72°C for 3 min, after which the tubes were held at -20°C until further processing.

Purification of PCR product

The PCR products were resolved on a 2% agarose gel. The bands after ethidium bromide staining were

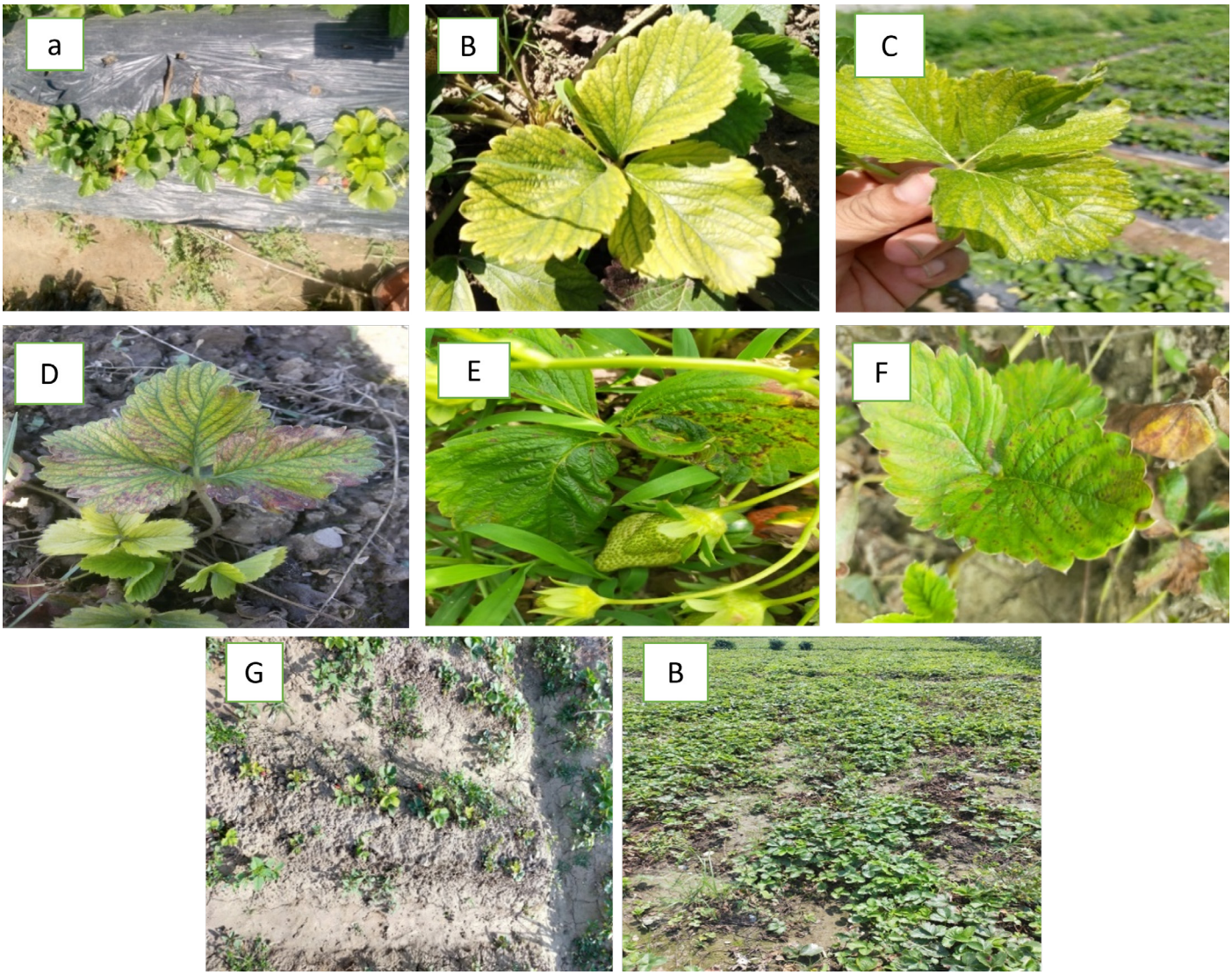


Figure 2: *SLRSV infection led to different types of symptoms on strawberries, observed during the surveys, which are (a) Severe stunting and chlorosis (b) Chlorosis of strawberry leaves (c) strawberry leaves with chlorosis and deformation (d) Strawberry leaves showing mosaic, chlorosis and necrosis of leaves(e) Severe leaf deformation and crinkling with mottling and insect injury(f) Strawberry leaves showing mottling and crinkling of leaves with necrotic spots.(g, h) Strawberry field showing patches of poorly grown plants.*

observed under UV light using a trans-illuminator. The required bands were cut from the gel under UV light in a trans-illuminator. The gel was purified using the SV-GEL extraction kit according to the manufacturer's recommendations.

Sequence analysis of SLRSV

After eluting the DNA of interest from the gel, the samples were sent, along with the virus-specific primers (Table 1), for direct sequencing on an ABI sequencing machine. Each segment was sequenced in both directions. The phylogenetic relationship of Pakistani isolates was established by comparing the obtained sequences with other virus isolates reported in GenBank. The sequences were analyzed using GENETYX ver. 7.0 (GENETYX Co., Tokyo, Japan). Multiple alignments were generated using the neighbor-joining method in the Clustal W program, and phylograms were constructed in Mega X with 1000 bootstrap replicates.

Results

SLRSV in strawberry fields in Khyber Pakhtunkhwa

Surveys revealed that strawberry fields, particularly those in plain areas where strawberries are grown primarily for fruit production, were infected with SLRSV. Symptoms including stunting (Figure 2a), chlorosis (Figure 2b), cupping (Figure 2c), mosaic, mottling (Figure 2d), leaf deformation (Figure 2e), and crinkling (Figure 2f) were observed in fields of the strawberry crop of Khyber Pakhtunkhwa (KP),

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Pakistan. In some strawberry fields, signs of nematode infection were also observed as poorly grown patches (Figure 2g,h).

SLRSV incidence

Data extracted from the surveys (2020-21 and 2021-22) revealed that the average incidence of SLRSV *was 11.7% and 14.7%, respectively,* in district Swat (Table 2). During 2020-21 in the district of Swat, the highest incidence of 15% was recorded in Sherpalam (Shakardara), whereas it was 8.3% in Khwazakhela, the lowest among the surveyed locations. The following year, an increase was observed in the incidence percentage across most fields. In Sherpalam (20%), the incidence of SLRSV increased by 5% compared to the previous observation. Intriguingly, the lowest incidence (11.7%) was recorded at Bagh Dera (Matta) rather than at Khwazakhela this year. However, the incidence of SLRSV at Bagh Dera (11.7%, 2021-22) remained higher than that reported for Khwazakhela (8.3%, 2020-21).

In district Mardan, the mean SLRSV incidence was 20.5% (2020-21), increasing to 23.3% the following year (Table 3). During the first-year survey in the fields of Abdur Rahman Kalay, the incidence was 23.3%, increasing to 26.7% in the second year, while in Saro Shah, the incidence was 18.3% in the first year and increased to 23.3% in the second year. In two-year surveys, the lowest disease incidence was observed in the Saro Shah field, with SLRSV infection rates of 18.3% and 30.0%.

Similarly, in district Charsadda, 23.5% of plants were infected by the virus during 2020-21. However, the number increased to 26.8% in 2021-22 (Table 4). In strawberry fields of Marvandai (Rajar), Khavra Abad (Tangi), and Teetara (Sarkay), the incidence of SLRSV was once again as high as 26.7%, whereas Kalary (Sarkay) was ranked at the top (36.7%) following the year's survey (2021-22). However, in 2 020-21, the lowest incidence (20%) was noted in Shaheedan Kalay (Rajar) and Spenky (Tangi), whereas in 2021-22, the incidence percentage (20%) remained unchanged at Sarkay.

Comparatively, the lowest mean percent incidence was observed in the strawberry fields of the district of Swat, where the land was used for runner production, while the district of Mardan ranked second most affected by the virus. However, the district of Charsadda holds the top position among the surveyed districts, and the maximum incidence was recorded in more districts than Swat and Mardan.

Table 2: *Incidence of SLRSV infecting strawberries in hilly areas of district Swat (Nursery producing areas) in Khyber Pakhtunkhwa, Pakistanduring the year 2020-21*

Area surveyed	SLRSV					
	2020-21			2021-2		
	Samples tested	Samples positive	% Incidence	Samples tested	Samples positive	% Incidence
Takhtaband / Ogday	60	6	10.0	60	8	13.3
Sherpalam (Shakardara)	60	9	15.0	60	12	20.0
Khwazakhela	60	5	8.3	60	8	13.3
Dureshkhela (Matta)	60	8	13.3	60	9	15.0
Bagh dera (Matta)	60	7	11.7	60	7	11.7
Average percent incidence	11.7			14.7		

Table 3: *Incidence of SLRSVinfecting strawberry crops in district mardan of khyber pakhtunkhwa, pakistan during the year 2020-21 and 2021-22.*

Area surveyed	SLRSV					
	2020-21			2021-22		
	Samples tested	Samples positive	% Incidence	Samples tested	Samples positive	% Incidence
JandyGhara	60	12	20.0	60	12	20.0
Abdur Rehman Kalay	60	14	23.3	60	16	26.7
Saro Shah	60	11	18.3	60	14	23.3
Average percent incidence	20.5			23.3		

Table 4: *Incidence of SLRSVinfecting strawberry crops in district Charsadda of Khyber Pakhtunkhwa, Pakistan during the year 2020-21 and 2021-22.*

Area surveyed	SLRSV					
	2020-21			2021-22		
	Samples tested	Samples positive	% Incidence	Samples tested	Samples positive	% Incidence
Marvandey (Rajer)	60	16	26.7	60	18	30.0
Shaheedankalay	60	12	20.0	60	20	33.3
Bogaro shah (Sarkay)	60	13	21.7	60	15	25.0
Kalary (Sarkay)	60	15	25.0	60	22	36.7
Teetara (Sarkay)	60	16	26.7	60	14	23.3
Sarkay chowk	60	14	23.3	60	12	20.0
Nawab saibkalay (Utmanzai)	60	13	21.7	60	17	28.3
Chacha khan bangle	60	15	25.0	60	14	23.3
Khavra abad (Tangi)	60	16	26.7	60	17	28.3
Spenkay (Tangi)	60	12	20.0	60	13	21.7
Swaty	60	13	21.7	60	15	25.0
Average percent Incidence	23.5			26.8		

Table 5: *Percent identities at nucleotide sequence level of Pakistani isolate of SLRSV infecting strawberry crop with other SLRSV isolates reported across the world in GenBank.*

Accession #	Isolate/strain	Host	Country	% Nuc. Identity
MF796988	Lilium 14-010-2	Lilium	Netherlands	85.74
MF796974	Lilium 12-001	Lilium	Netherlands	84.91
MF797006	Robinia 14-027	Robinia	Netherlands	85.71
MF796998	Fragaria 14-023	Chenopodium	Netherlands	72.49
MF797000	Phaseolus 14-024	P. vulgaris	Netherlands	84.91
MN082041	Golden peach NZ	Prunus persica	New Zealand	84.94
KF695118	Iso 3-6	Vaccinium	New Zealand	85.71
HM591198	Blackberry NZ	Rubus sp.	New Zealand	86.12
MZ291960	19SP058	Fragaria	Canada	74.46
AY461735	NCRG-MEN 454	N.A.	USA	90.34
HQ456537	Mint 515	Mint	USA	85.45
AY438666		Mint	USA	90.74
MW854292	DSMZ PV 1314	S. moricatum	Germany	83.50
MT648777	SLRSV-Eg	Fragaria	Egypt	91.75
X77466	Hampshire H	N.A.	UK	85.11
MZ405641	DSMZ PV-0247	N.A.	Scotland	83.70
KF695119	6-6	V. darrowii	New Zealand	86.62
DQ324376	Gorz 2	N.A.	Poland	86.12

** Sequence of these isolates has been used in the phylogenetic analysis as well.*

Vector identification

Dagger nematode, *Xiphinema diversicaudatum* was found in strawberry fields in all the surveyed areas. Its morphological traits, such as long cylindrical bodies (4-6 mm long) that assumed a distinctive J-shaped structure when heat relaxed, served as the basis for identification.

Molecular detection and characterization of SLRSV

Molecular detection by PCR confirmed the presence of the virus in ELISA-positive samples, as evidenced by the formation of a single, crisp band of approximately 500 bp in SLRSV-positive samples. Comparison of the Pakistani SLRSV isolate with globally reported isolates in GenBank showed nucleotide identity

range of 72.49% to 91.75% (Table 5). Sequence analysis showed that the Pakistani SLRSV isolate was extremely like SLRSV isolate reported from Egypt (MT648777) that infected strawberries. Both isolates showed strong sequence similarity to an isolate reported from the USA, indicating they shared a common ancestor. Data analysis showed that the SLRSV genome appears to be evolutionarily stable, and many of these strains or isolates reported worldwide have already undergone the necessary alterations in accordance with their specific agro-ecological circumstances. Most of the isolates show an identity of 83-86%.

Phylogenetic analysis of the SLRSV Pakistani isolate, compared with globally reported isolates using the nucleotide sequence of the coat protein-encoding region, resulted in two clusters; however, the majority of the reported isolates fell into cluster A (Figure 3). Pakistani isolates delineated into cluster A, close

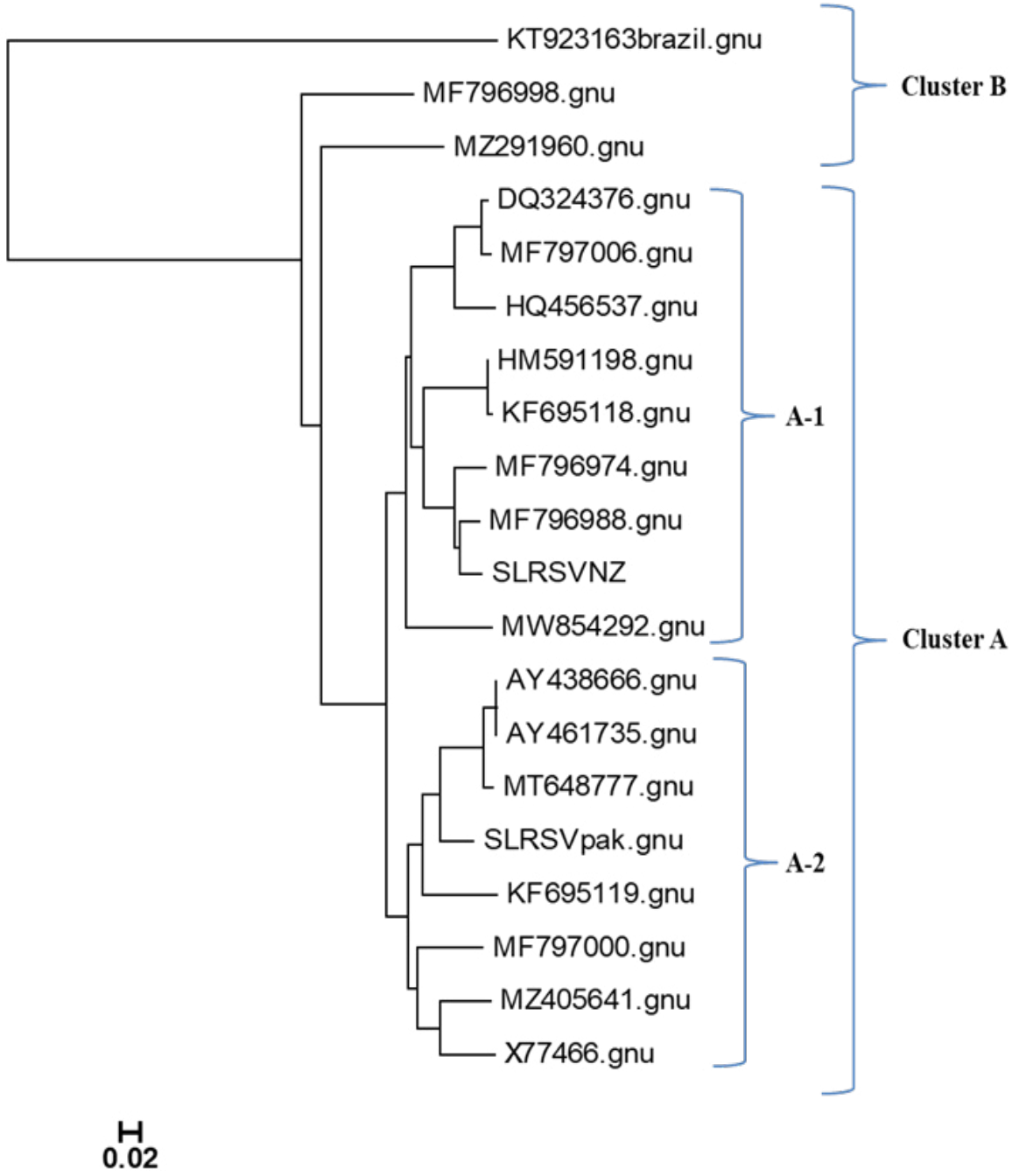


Figure 3: *Representation of phylogenetic relationship of SLRSV isolates in the GenBank with Pakistani isolate (SLRSVpak) based on nucleotide sequence of coat protein encoding region using neighbor joining method with a boot strap value of 1000. A total of two clusters, namely, Cluster A and Cluster B were constructed, whereas Cluster A further divided into two subclades (A-1 and A-2). SLRSVpak grouped in A-2 showing close relationship with isolates with MT648777, AY461735 and AY438666.*

to the isolates reported from Egypt and the USA. No grouping based on geographic distribution or host plant infected could be seen in the phylogram. Cluster A was divided again into two subclades, with the majority of the isolates grouping together in subclade-1, which was further divided into two groups. Subclade-2 comprised of only two isolates: one from Canada infecting strawberry and the other from the Netherlands infecting *Chenopodium* spp. Similarly, Cluster B is also delineated into two subclades. Pakistani isolates, along with isolates reported from the USA, Egypt, and Canada, were in one subclade, while the isolates from the UK, Scotland, and the Netherlands were grouped together in a second subclade. Intriguingly, a great diversity was observed among isolates reported from the Netherlands, infecting different host crops, and they were delineated across all clusters and subclades, although the majority of the isolates were in Cluster A and subclade-1. A similar topology was also observed in a phylogram constructed from amino acid sequences in the coat protein-encoding region (data not shown).

Discussion

The assessment of Strawberry latent ringspot virus (SLRSV) in commercially cultivated strawberries in Khyber Pakhtunkhwa (KP), Pakistan, provided insights into the virus's prevalence, symptomatology, and molecular characteristics. During 2020-21 and 2021-22, extensive surveys highlighted the prevalence of SLRSV in the districts of Swat, Mardan, and Charsadda. The symptoms, including stunting, chlorosis, cupping, mosaic, mottling, leaf deformation, and crinkling, produced by the virus, are consistent with previous reports of SLRSV infections in strawberries (Sharma *et al*., 2018). In addition, the appearance of nematode-induced reduced-growth patches in some areas further complicated the disease scenario, suggesting a potential interaction between nematodes and SLRSV that led to crop loss (Sharma *et al*., 2018).

A significant variation was noticed in the incidence of SLRSV across the surveyed regions. In the district of Swat, the mean incidence was 11.7% during 2020-21, which increased to 14.7% in 2021-22 (Table 2). In Swat, strawberry fields located in the Sherpalam (Shakardara) area were most affected by the virus, where maximum incidence of 15% and 20% was recorded in 2020-21 and 2021-22, respectively, whereas minimum incidence during 2020-21 was observed in Khwazakhela (8.3%) and 11.7% in Bagh Dera (2021-22). In Sherpalam, 5% difference in increase was noticed during two-year surveys, which indicates a potential spread of the virus, possibly due to poor disease management or the free movement of infected propagative material (Martin *et al*., 2013). In district Mardan, the average SLRSV incidence increased from 20.5% to 23.3%, with the highest incidence observed in Abdur Rahman Kalay (26.7%) in 2021-22. Among the districts, the incidence rose from 23.5% to 26.8% in Charsadda, with Kalary (Sarkay) securing the top rank at 36.7% in 2021-22. These results highlighted the escalating threat posed by SLRSV to commercial strawberry production in KP, particularly in regions where the virus is already prevalent. Compared with hilly areas used for nursery production, the disease incidence was higher in plain areas. The vector pressure, weather, and closeness to virus sources all affect the temporal and spatial dispersion of viruses (Grafton-Cardwell *et al*., 1996). Numerous and diverse causes, including variations in the populations of alternative host plants, the presence and effectiveness of vectors, and viral interactions in infected hosts, could all affect shifts in virus incidence (Ullman *et al*., 1991). The prevalence of SLRSV with increased incidence in Charsadda might be due to differences in farming practices, environment, or the evolution of SLRSV to produce more virulent strains, whereas in Swat, where strawberry runners are primarily produced, suggests that poor management strategies used may influence the spread of SLRSV (Tzanetakis *et al*., 2006). The climate on the plains is favorable for both the multiplicity of insect vectors and the spread and multiplication of the virus, and this high vector population leads to an increased prevalence of viral diseases. Chandler cultivar is planted in most Pakistani locations. The poor level of tolerance in. Chandler may be the reason for this widespread viral infection of strawberries in Pakistan. Handa and Sharma (2018) reported that among the various cultivars grown in India, the Chandler cultivar had the highest incidence of viral disease. The weed population was also extremely high across nearly all strawberry-growing regions (Figure 2). At various locations, weeds were nearly overgrowing the strawberry plants (fully covering the strawberry plants). These weeds may serve as alternate host for the virus and the insects that carry it, creating a microclimate favorable for insect fecundity. This may also explain why plains have a higher incidence of viruses in strawberry crops than hilly regions.

The presence of "*Xiphinema diversicaudatum*," commonly known as dagger nematode, extracted and

identified from soil in all surveyed areas, is significant, as it is the only known vector of SLRSV (Taylor and Brown, 1997), contributing to the spread of the virus and highlighting the need for IPM strategies for the control of both SLRSV and its vector. Molecular detection of SLRSV by PCR confirmed its presence of the virus in DAS-ELISA-positive samples. The identity of SLRSV, and the molecular confirmation concured with PCR-based detection of SLRSV has also been reported in other crops (Martin *et al*., 2004; Martin and Tzanetakis 2006).

Phylogeny of SLRSVpak (Figure 3) suggesting a close ancestral lineage with isolates reported from Egypt (MT648777) and the USA (AY461735, AY438666). The isolate shares a high nucleotide sequence identity of 91.75% with the Egyptian isolate, which is a possible indicator of SLRSV introduction into the region or of an evolutionary background. Clustering of SLRSVpak with Egyptian and USA isolates in cluster A supports the common ancestor lineage/ origin hypothesis, which could be driven by the facilitation of free exchange of plant materials that viol*ate* quarantine rules and regulations. El-Morsy *et al*. (2017) also reported an 80-99 % identity range for SLRSV between an Egyptian isolate and isolates reported worldwide. Mazyad *et al*. (2014) have also reported a DNA sequence identity in the range of 80–85 percent range. However, no such evolution based on the host plant infected was observed. It appears that after evolving, isolates reported from various geographic sites are now stable. Although, Tang *et al*. (2013) documented the geographically based evolution of SLRSV reported from various locations worldwide and the grouping of isolates by host in New Zealand. A wide host range of SLRSV isolates, particularly those from the Netherlands, shows diversity, suggesting adaptability to different agroecological conditions (Tzanetakis *et al*., 2006). Phylogenetic trees based on geographic or host-range data do not provide a clear understanding, suggesting significant evolutionary changes that may be influenced by host adaptability and/or environmental pressures (Tang *et al*., 2013).

Conclusions and Recommendations

This article provides significant insight into the SLRSV situation in Khyber Pakhtunkhwa, which poses a serious threat to commercial strawberry-producing areas. The increasing spread of SLRSV, coupled with its nematode vector, suggests the need for extensive virus management protocols, i.e., the use of virus-free planting material, control of nematode vectors, and support for breeding to develop resistant strawberry varieties. Additionally, the molecular and phylogenetic characterization of SLRSV isolates underscores the virus's evolution and worldwide distribution, which could be better to inform future research and management efforts.

Acknowledgments

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Novelty Statement

This research work is conducted for the first time on SLRSV situation in Khyber Pakhtunkhwa, Pakistan. The study suggests the need of virus-free planting material, control of nematode vectors and support for breeding to help contain its rapid spread in strawberry varieties.

Author's Contribution

Both the authors contributed in the research work as well as in the manuscript write-up.

Generative AI and AI-assisted technology statement
The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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