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Research Article

IDENTIFICATION OF *TOBACCO RATTLE VIRUS* RESISTANT POTATO CULTIVARS IN AZAD JAMMU AND KASHMIR, PAKISTAN

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ABSTRACT

Tobacco rattle virus (TRV) poses a significant challenge to potato production, particularly in the hilly and mountainous terrains of Azad Jammu and Kashmir (AJK), Pakistan. To address this issue, the present study aimed to evaluate the resistance of ten potato cultivars, Line A, Kurado, Desiree, Bataina, Fleminco, Line B, Pomola, Ronaldo, Rodalph, and Margrata, against TRV. The study was conducted in two districts, Poonch and Sudhnoti, where the *in vivo* effects of TRV were assessed based on yield and quality characteristics. The research investigated the impact of TRV on plant growth and yield parameters using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) techniques, highlighting the role of soil-borne nematodes as vectors. The findings suggested that none of the tested cultivars exhibited complete resistance to TRV, though Desiree and Fleminco demonstrated moderate insensitivity. These results provided valuable insights into TRV management and emphasized the need for further research on resistant germplasm. The study concluded that TRV and its vector posed significant threats to potato production in AJK, with no tested germplasm exhibiting complete resistance. These findings may aid the potato industry in selecting cultivars that help mitigate the financial burden of tuber necrosis caused by TRV. This research represents the first comprehensive screening of potato germplasm against TRV in AJK, underscoring the severity of the virus as a hazard in hilly regions. Future studies involving broader germplasm screening are recommended to validate and strengthen these findings, ultimately facilitating the development of TRV-resistant potato varieties.

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INTRODUCTION

Potato (*Solanum tuberosum* L.), which originated in South America, belongs to the family Solanaceae (Camire et al., 2009). It is cultivated in tropical and subtropical regions and is the most important vegetable crop and staple food of the world, ranking third after rice and wheat (Okonya et al., 2021). According to Ali and Karasev (2022), the potato crop is susceptible to various plant pathogens, including nematodes, bacteria, fungi, viruses, and viroids (Nisa et al., 2022). However, viral diseases are particularly challenging to control due to their rapid multiplication, often leading to significant economic losses (Jeffries et al., 2005; Gondal et

al., 2024; Azeem et al., 2025; Tariq-Khan et al., 2025). More than fifty viruses and one viroid are known to infect potatoes (Kreuze et al., 2020).

Soil-borne viruses, such as *tobacco rattle virus* (TRV), play a crucial role in disease incidence. TRV is transmitted by several species of the genera *Trichodorus* and *Paratrichodorus* and is a major concern alongside other viruses. Classified under the genus *Tobravirus* in the family *Virgaviridae*, TRV has the potential to infect multiple crops, including spinach, beans, beets, and peppers, as well as ornamental plants like lilies, tulips, and marigolds (Adams et al., 2012; King et al., 2012). This virus has been reported in many potato-growing regions worldwide, including New Zealand, parts of Central and South America, China, Japan, and Canada (Jeffries, 1998; Gieck et al., 2007).

TRV causes corky ring-spot (CRS) disease, which severely affects potato production in Europe, particularly in commercial seed potato-producing regions that serve as major sources of virus dissemination (Koenig et al., 2016). In the United States, TRV incidence has been reported in new areas, including Minnesota, North Dakota, Michigan, and Wisconsin (Halterman et al., 2012), as well as in parts of Asia (Katoch et al., 2004; He and Chen, 2016). In Pakistan, the virus has recently been identified in the highlands of Khyber Pukhtunkhwa (Hazara and Malakand Divisions) and the northeastern regions of Azad Jammu and Kashmir (AJK) (Haneef et al., 2021).

Corky ring-spot (CRS) disease in potatoes is caused by *tobacco rattle virus* (TRV), a single-stranded RNA virus with a positive-sense genome and a rigid, rod-shaped structure. TRV is classified into two types, M type and NM type, which can be distinguished based on genome structure. The M type consists of two RNA molecules, RNA-1 and RNA-2, whereas the NM type contains only a single genomic RNA (RNA-1) and lacks RNA-2, which encodes the coat protein, making serological detection impossible (Nicolaisen et al., 1999; Robinson, 2003). The TRV RNA-1 has a conserved genome organization of approximately 6,800 nucleotides (Nicolaisen et al., 1999; Xu and Nie, 2006; MacFarlane, 2010).

The lengths of TRV particles vary depending on the specific isolate, with short particles measuring 48-114 nm and long particles ranging from 180-197 nm, both with a diameter of 21-25 nm (Hamed et al., 2012). Infected tubers exhibit internal necrosis, characterized by rust-colored or brown arcs, concentric rings, and browning of tuber tissues, which eventually dry out and form a cork-like texture. Externally, concentric circles or arcs appear on the tuber skin, resembling symptoms caused by Potato virus Y (PVY). In certain cultivars, necrotic symptoms may intensify during storage. The severity of symptoms varies depending on the cultivar, field conditions, location, environmental factors, and the time of year (Yellareddygari, 2018). The virus can remain asymptomatic in infected tissues and is only detectable when symptoms become evident (Charlton, 2006).

TRV spreads naturally or mechanically through soil-borne stubby root nematodes belonging to the genera *Paratrichodorus* and *Trichodorus* (Boutsika et al., 2004; Charlton et al., 2010; Sahi et al., 2016), which are present in infected soils (Agrios, 2005). TRV infestation can severely damage potato crops, with additional losses caused by tuber necrosis symptoms. Once a field is infested with TRV, eradicating the virus is extremely difficult due to its extensive host range and the persistence of its vector. Field treatments with nematicides, applied before or at planting time, may help in managing TRV by reducing vector populations. Moreover, breeding for resistance in cultivars is a promising approach (Brown et al., 2007; Ingham et al., 2000, 2007; Ghazala and Varrelmann, 2007).

The most effective methods for controlling TRV under natural conditions include the use of resistant potato germplasm, seed potato certification programs, and diagnostic methods utilizing viral nucleic acid detection (Ghazala and Varrelmann, 2007). Conventional breeding for genetic resistance is effective but is time-consuming and labor-intensive (Sahi et al., 2016). Several cultivated potato varieties exhibit resistance to TRV-induced CRS disease (Dale et al., 2004; Brown et al., 2009). Breeding programs commonly utilize parental materials with CRS resistance, and nearly 20% of all potato cultivars show some level of resistance to TRV (Brown et al., 2009).

CRS resistance was first reported in British potato varieties, such as Bintje (Harrison, 1968). Since then, resistance has been identified in cultivars from New Zealand (Fianna and Karaka), as well as in Europe, North America, and Poland (Cisa) (Brown et al., 2000, 2009). However, breeding for TRV resistance is challenging due to the complex inheritance of resistance traits (Brown et al., 2009). TRV-tolerant potatoes allow the virus to accumulate while preventing the development of the characteristic ‘corky’ texture in tubers. Recent findings suggest that CRS-resistant cultivars do not reduce nematode vector populations, indicating that these

plants are not resistant to nematodes (Brown et al., 2000). Furthermore, the absence of symptoms in tubers aligns with the inability to detect TRV using RT-PCR, reinforcing the idea that CRS resistance is linked to resistance against TRV infection (Brown et al., 2009).

Currently, managing CRS disease involves planting virus-insensitive cultivars alongside the application of non-selective pesticides to reduce nematode populations in potato fields (Yellareddygari et al., 2018). To prevent the disease, crop rotation with non-host crops, such as alfalfa and Scotch spearmint, can be employed as a sustainable management strategy (Mojtahedi et al., 2002; Boydston et al., 2004).

Viral diseases are becoming a serious concern for agricultural and horticultural crops in Pakistan due to a lack of virus research facilities, the widespread presence of vectors, the unavailability of resistant germplasm, and the import of seeds and propagative material without proper quarantine measures. Moreover, small landholdings and the continuous cultivation of the same germplasm, particularly in potatoes, which are propagated vegetatively, further increase inoculum pressure.

In AJK, the study area revealed that farmers continue to cultivate old, obsolete potato cultivars and rely on their own contaminated seed tubers for planting. As a result, the incidence of viruses, including soil-borne viruses, remains a significant issue in Pakistan, with AJK being particularly affected. The recent introduction of soil-borne viruses, such as PMTV (Arif et al., 2013) and TRV (Haneef et al., 2021), has further exacerbated the situation.

Effective virus management in the affected areas requires proper diagnosis and screening of available cultivars. Therefore, the objective of this study was to evaluate the resistance levels of commercially cultivated potato cultivars by assessing their response to TRV under naturally infested field conditions.

MATERIALS AND METHODS

Potato seed collection

Potato seed tubers from ten commercially grown potato cultivars viz. Flamenco, Desiree, Line A, Bataina, Margrata, Kuroda, Line B, Ronaldo, Rudolph, and Pomola were obtained from the Directorate of Agricultural Research, Department of Agriculture, Muzaffarabad, Azad Jammu and Kashmir.

Field screening of potato germplasm against TRV

Two naturally infested fields with TRV and its vectors, *Trichodorus* and *Paratrichodorus*, were identified in Chotagala and Trarkhel, located in the districts of Poonch and Sudhnoti. These fields were selected for screening potato genotypes for resistance against TRV. The cultivars were evaluated based on various biological yield parameters.

A field experiment was conducted to identify TRV resistance in potato cultivars using a Randomized Complete Block Design with five replications. The tubers were planted with a spacing of 30 cm plant-to-plant (P×P) and 60 cm row-to-row (R×R). Standard agronomic practices were followed throughout the cropping season. The crop was assessed for TRV symptoms on potato foliage using keys reported by Nicolaisen et al. (1999) and Macfarlane (2010). Disease assessment was conducted based on characteristic symptoms, DAS-ELISA, and RT-PCR to determine each cultivar's response to natural TRV infection (Arif et al., 2014).

Vegetative growth data, including plant height and the number of stems per plant, were recorded. Yield parameters, such as the number of tubers per plot and tuber size, were also documented. Representative samples of five plants per experimental plot (a total of 25 plants per treatment) were collected for both vegetative and yield evaluations.

Potato cultivars were categorized into four sensitivity groups, insensitive, moderately insensitive, moderately sensitive, and sensitive, based on the scale proposed by Yellareddygari et al. (2018), with slight modifications (Table 1). Qualitative and quantitative traits of potato cultivars under TRV infestation were evaluated following the methodologies described by Dale et al. (2000) and Duarte et al. (2011).

Table 1. Modified scale for *Tobacco rattle virus* infestation in tubers for potato cultivar resistance evaluation (Yellareddygariet al., 2018).

Scale	Description
Insensitive	incidence of tuber necrosis is <20 percent
Moderately insensitive	incidence of tuber necrosis is 20 to 30 percent
Moderately sensitive	incidence of tuber necrosis is 30 to 40 percent
Sensitive	incidence of tuber necrosis is incidence >40%

Detection of TRV in plants and potato tubers using DAS-ELISA

TRV was detected using DAS-ELISA with ELISA kits provided by AC Diagnostics, Inc. (USA). The procedure was primarily based on the method outlined by Clark

and Adams (1977), with additional instructions provided by the kit manufacturer. The standardized protocol for detecting TRV in potato tubers is detailed below:

The TRV-specific antibody (AC Diagnostics, Inc., USA) was diluted 1:200 in a coating buffer (pH 9.6), and 100 µl of the diluted solution was added to each well of an ELISA plate. The plates were then incubated for 2-3 h at 37°C in a humid chamber or for 4 h at ambient temperature. Following incubation, the plates were washed 3-4 times with 1X PBST, dried, and loaded with 100 µl of each sample, prepared at a 1:10 (w/v) dilution in an extraction buffer (pH 7.3). The plates were subsequently incubated overnight at 4°C or for 23 h at room temperature.

After the second incubation, the plates were washed 6-8 times with washing buffer and then dried using paper towels. Next, 100 µl of enzyme conjugate, diluted 1:200 in a conjugate buffer (pH 7.3), was added to each well and incubated at room temperature (21°C-24°C) for 2-3 h in a humid chamber. The plates were then washed 6-8 times with washing buffer.

A 100 µl aliquot of PNP substrate solution (1 mg/ml, prepared in a substrate buffer at pH 9.8) was added to each well and incubated in the dark at room temperature (24°C-25°C) for 1 h. The reaction was evaluated either by visual inspection or by measuring absorbance at 405 nm after 1-2 h at room temperature or overnight at 4°C. A sample was considered positive if its A405 nm value was at least three times higher than that of virus-free controls. Mean values were calculated from replicated experiments. A scale described by Arif et al. (2014) was used to determine the severity of TRV infection in potato tubers (Table 2).

Table 2. Scale for evaluating the severity of *Tobacco rattle virus* in potato tubers based on ELISA.

Scale	Description	Mean A_{405nm} value (2h)[1]
0	No reaction apparently healthy	0.140
1	Positive (mild visible reaction)	0.389
2	Positive (moderate visible reaction)	0.750
3	Positive (strong reaction)	1.250
4	Positive (severe intense reaction)	1.750

[1]Average value of A_{405nm} after 2h of incubation with substrate buffer at room temperature (Arif et al*.,* 2014).

Detection of TRV using RT-PCR

RNA extraction and cDNA synthesis

Total RNA was extracted from the leaves and tubers of five plants from each of the following cultivars: Flamenco, Line A, Bataina, Kuroda, Margrata, Desiree, Line B, Ronaldo, Rudolph, and Pomola. The extraction was performed using the PureLink™ RNA Mini Kit (Ambion Life Technologies, USA), following the manufacturer's instructions.

For complementary DNA (cDNA) synthesis, the FIREScript RT cDNA synthesis kit (Solis BioDyne, Estonia) was used according to the manufacturer's protocol, as described by Xu and Nie (2006).

Viral genome amplification using polymerase chain reaction

The viral genome was amplified using polymerase chain reaction (PCR), specifically targeting the ORF4 region of TRV RNA1. This region encodes a cysteine-rich protein believed to play a role in viral RNA replication or silencing suppression. Amplification was carried out using the primer set F2 and R2, known to amplify the genome of all reported TRV isolates (Xu and Nie, 2006). Primers A and B were used to modify the forward primer F2 (5'-GACGTGTGTACTCAAGGGTT-3') and the reverse primer R2 (5'-CAGTCTATACACAGAAACAGA-3') (Robinson, 1992). These modifications were based on RNA1 sequence alignment of multiple known TRV strains (Xu and Nie, 2006).

A 463 bp genomic region was amplified using a 50 µl PCR reaction mixture consisting of 2 µl of cDNA as the template, 5 µl of 10× PCR buffer for *Taq* polymerase, 3 µl of $MgCl_2$, 5 µl of a dNTP mixture (2 mM), 1 µl of each primer (F2 and R2), and 1 µl of *Taq* DNA polymerase. Nuclease-free water was added to adjust the final volume to 50 µl. PCR was initiated with an initial denaturation at 94°C for 5 min, followed by 34 cycles of denaturation at 94°C, annealing at 56°C, and extension at 72°C for 1 min. A final extension step was performed at 72°C for 5 min. After completion, the tubes were stored in a refrigerator until further processing.

Gel electrophoresis was performed using a 2% agarose gel to separate the amplified products. The gel was stained with ethidium bromide and visualized using a transilluminator.

Detection of the nematode vector

Nematode identification was conducted by processing randomly collected soil samples from the root zone of ten potato cultivars from both experiments, following the

protocol of Whitehead and Hooper (2008). Soil samples were collected at a depth of 21-30 cm. A composite sample of 1 kg (three subsamples per replication per cultivar) was placed in plastic bags and stored at 4°C.

Nematodes were extracted using the tray method and the modified Baermann funnel technique (Whitehead and Hemming, 1965). Extracted nematodes were viewed and counted under a stereomicroscope, and population densities were determined according to the protocol by Whitehead and Hooper (2008). Identification was performed using a morphological key developed by Decreamer and Baujard (1998).

Detection of TRV in the nematode vector

To confirm the presence of virulent nematodes, ten nematodes per sample were collected using a dropper and placed into pre-autoclaved Eppendorf tubes. A 2 ml extraction buffer was added to each tube, followed by centrifugation at 8,000-10,000 rpm for 10 min. The pellet was resuspended in 0.5 ml of extraction buffer, frozen at -80°C for 30 min, and then thawed on ice before use in DAS-ELISA.

Statistical analysis

Data collected from field experiments at both locations were analyzed using Statistix 8.1 software (Steel and Torrie, 1980). Means were compared using the Least Significant Difference (LSD) test at a significance level of $P = 0.05$. Graphs were generated using GraphPad software. Cultivars were ranked or grouped based on virus-induced tuber necrosis incidence ratings observed at both sites.

RESULTS

Response of potato cultivars to TRV and its nematode vector in naturally infested fields

TRV and its nematode vector have primarily been reported in potato-growing regions across three districts, Poonch, Sudhnoti, and Bagh, of AJK, located in northeast Pakistan. The virus was found to be prevalent at both experimental locations in AJK based on symptom characteristics. A brief description of the reactions of cultivars to field infections by TRV and its nematode vector at two different locations is provided in Table 3.

The foliar canopy remained asymptomatic, whereas tubers exhibited symptoms such as raised external necrotic rings on the tuber surface and internal manifestations, including corky ring spots, multiple necrotic lines, and flecks in the tuber flesh. Cultivars Desiree and Flamenco showed no external symptoms (Table 3). However, symptomatic tubers were found across all cultivars, including Desiree, Margrata, Flamenco, Line A, Bataina, Kuroda, Line B, Ronaldo, Rudolph, and Pomola. Internal symptoms such as necrotic flecks, internal browning, and brown arcs were prominently observed in all varieties at harvest, 12-14 weeks after planting.

The presence of TRV in all examined potato varieties was confirmed using RT-PCR and the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) in randomly selected leaves and tubers, as well as in isolated nematode vectors from both experimental sites. The response of potato cultivars at Chotagala (district Poonch) closely resembled that observed at Trarkhel (district Sudhnoti). Although TRV was detected in all tested potato cultivars, including Desiree and Flamenco, no internal symptoms were observed in their tubers. Notably, ELISA values for Flamenco and Desiree were relatively lower than those of other cultivars (Table 3). RT-PCR further corroborated these findings by amplifying a 463-bp fragment of the 16 kDa protein gene in selected samples from each of the ten potato varieties cultivated at both locations. When evaluated in naturally infested fields at Chotagala and Trarkhel, all ten potato cultivars exhibited varying degrees of susceptibility to TRV, ranging from moderate tolerance to high sensitivity.

Potato cultivar response to TRV and its nematode vector at different locations

The impact of TRV in terms of internal symptoms (tuber necrosis) was recorded (Figure 1). The incidence and severity ranged from 26.0% to 52.0% and 27.0% to 48.3%, respectively (Table 4). The highest incidence of tuber necrosis induced by TRV was observed in the cultivars Ronaldo, Bataina, Line B, and Pomola, while Desiree and Fleminco exhibited the lowest incidence (Figure 2).

At Chotagala in district Poonch, the analysis of internal tuber symptoms revealed significant differences in the incidence ($P < 0.01$) and severity ($P < 0.01$) of tuber necrosis caused by TRV. In the second trial, conducted at Trarkhel in district Sudhnoti, significant differences were observed in the incidence ($P = 0.0008$) and severity ($P < 0.0001$) of tuber necrosis among different cultivars. The incidence and severity of TRV-induced tuber necrosis ranged from 26.0% to 48.0% and 26.7% to 48.8%, respectively. Cultivar Desiree exhibited the lowest incidence of tuber necrosis, whereas Ronaldo, Bataina, Line B, and Pomola had an incidence exceeding 40.0% (Table 4). LSD test results revealed that only three cultivars, Desiree, Fleminco, and Margrata, showed the least necrosis symptoms.

Table 3. Response of potato cultivars against *Tobacco rattle virus* in naturally infested fields.

Potato cultivar	Experiment 1[a]							Experiment 2[b]						
	Symptomology[c]		ELISA[d]			RT-PCR[e]		Symptomology		ELISA			RT-PCR	
	External	Internal	Leaf	Tuber	Nematode	Leaf	Tuber	External	Internal	Leaf	Tuber	Nematode	Leaf	Tuber
Bataina	CRS	NF	0.860	0.412	0.337	+	+	NR	ML	0.337	1.285	0.860	+	+
Desiree	NS	NS	0.670	0.219	0.337	+	+	NS	NS	0.219	0.362	0.412	+	+
Fleminco	NS	NS	0.219	0.378	0.412	+	+	NR	NS	0.412	0.337	0.387	+	+
Kurado	CRS	ML	0.387	1.062	1.285	+	+	CRS	ML	1.285	0.412	0.337	+	+
LineA	CRS	NF	0.337	0.412	0.860	+	+	NR	IB	0.860	0.412	0.362	+	+
LineB	NR	ML	0.387	0.387	0.412	+	+	CRS	NF	0.412	0.378	0.337	+	+
Margrata	NR	IB	0.412	0.337	0.387	+	+	NR	IB	0.387	0.337	0.860	+	+
Pomola	CRS	NF	1.285	0.362	0.337	+	+	CRS	NF	0.337	0.412	0.337	+	+
Rodalph	NR	ML	0.860	0.337	0.362	+	+	CRS	ML	0.362	0.387	0.412	+	+
Ronaldo	CRS	ML	0.412	1.285	0.337	+	+	CRS	NF	0.337	0.412	1.285	+	+

[a] Experiment conducted at Chotagala of district Poonch.
[b] Experiment conducted at Trarkhel of district Sudhnoti.
[c] No symptom (NS) Corky ring spots (CRS) Necrotic rings (NR) on surface of tuber, Necrotic flecks (NF), Multiple lines (ML) or Internal browning (IB) of tuber flesh.
[d] Mean $A_{405\ nm}$ values of five replicates.
[e] Total RNA was extracted from pooled samples of potato cultivars used in the experiment and + indicates PCR positive, producing 463 bp band, while – indicates no reaction.

In the first experiment, the highest incidence of TRV-induced tuber necrosis was recorded in cultivar Ronaldo, followed by Line B and Bataina. The highest severity was also observed in Ronaldo, whereas cultivar Desiree exhibited the lowest severity (Figure 3).

The ranking of cultivars based on their susceptibility to TRV-induced tuber necrosis, as determined from trials at two different sites (Table 4), indicated that Ronaldo, Bataina, Line B, and Pomola were highly susceptible. Margrata, Line A, Rodalph, and Kurado were classified as moderately susceptible, while Fleminco and Desiree were categorized as moderately resistant. No cultivar was found to be completely resistant.

Growth parameters

A significant difference ($P < 0.001$) was observed among different potato cultivars. At Chotagala, the maximum plant height (46.26 cm) was recorded in Margrata, while the minimum (23.58 cm) was observed in Pomola. At Trarkhel, the minimum plant height (25.60 cm) was recorded in Pomola and Line B, whereas the maximum (45.2 cm) was found in Fleminco and Desiree (Table 5; Figure 4).

In experiment 1, the highest number of stems per plant was recorded in the cultivar Desiree, while Line B had the fewest stems per plant. In experiment 2, the highest number of stems per plant was observed in Margrata, whereas Ronaldo and Line B had the fewest (Figure 5). Cultivars were categorized into four groups based on the LSD test at $\alpha = 5\%$. Results from both experiments indicated that the potato cultivars Desiree and Margrata performed best, while Pomola and Line B were the most severely affected by TRV and its nematode vector.

Yield parameters

The effect of TRV and its associated nematodes on tuber size and yield was assessed after harvest. Yield results indicated that Kurado produced the highest number of tubers per plot, while Ronaldo had the lowest at both locations (Figure 6). Although Kurado produced the highest number of tubers, the tubers were smaller compared to those of other cultivars.

Figure 1. Photograph showing characteristics symptoms of necrosis in potato tuber induced by *Tobacco rattle virus*.

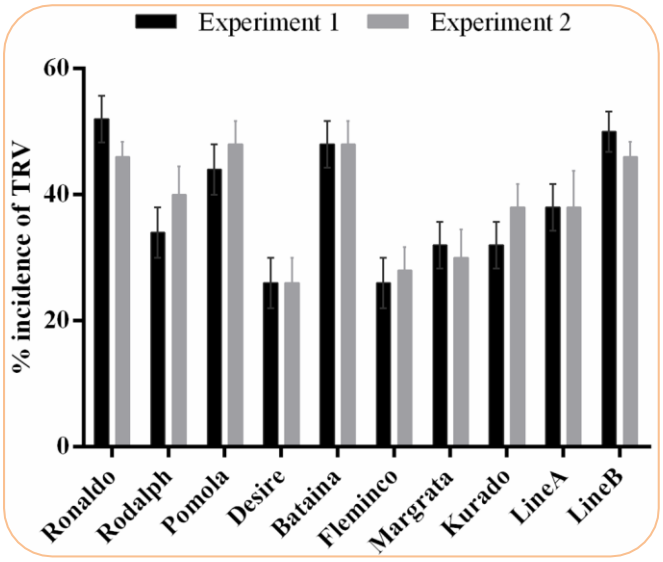

Figure 2. Assessment of potato cultivars at two locations on the basis of incidence of TRV induced tuber necrosis in 2022. Experiment 1: conducted at Chotagala of district Poonch. Experiment 2: conducted at Trarkhel of district Sudhnoti

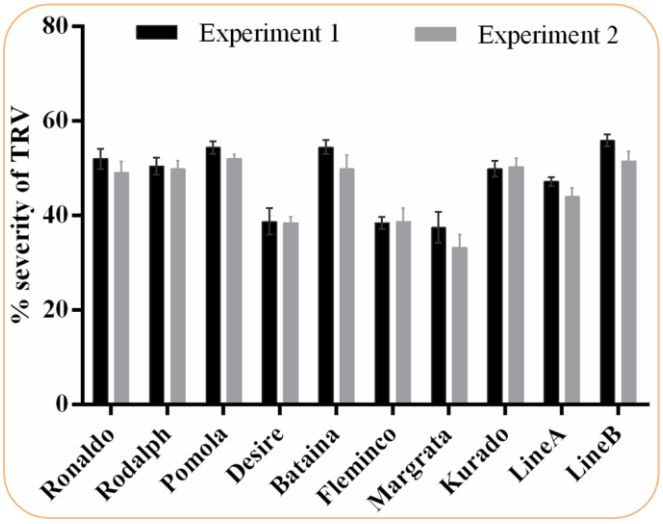

Figure 3. Assessment of potato cultivars at two locations on the basis severity of TRV induced tuber necrosis in 2022.

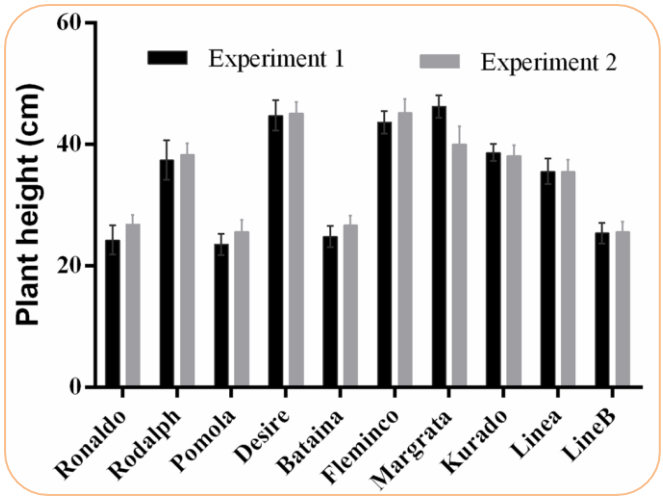

Figure 4. Assessment of potato cultivars at two locations on the basis of plant height in 2022.

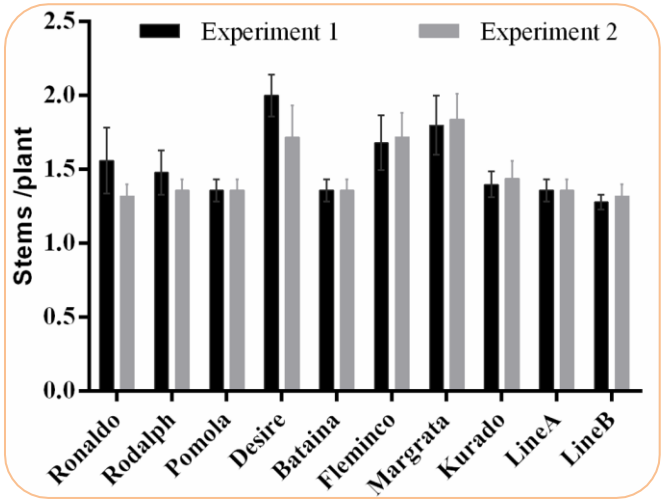

Figure 5. Assessment of potato cultivars at two locations on the basis of number of stems per plant in 2022.

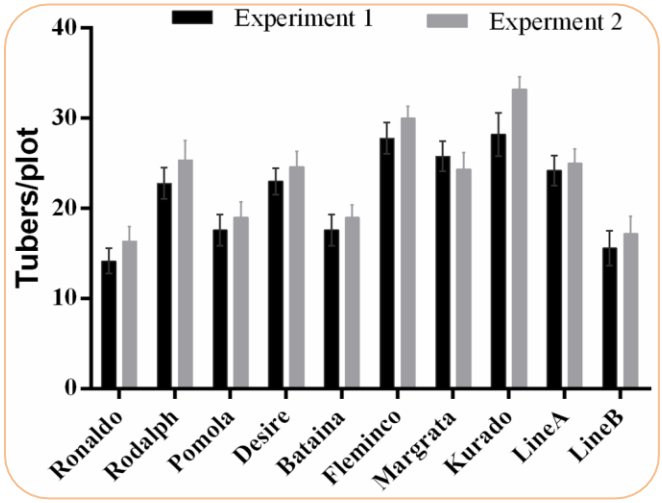

Figure 6. Assessment of potato cultivars at two locations on the basis of number of tubers per plot in 2022.

Table 4. Mean incidence and severity of *Tobacco rattle virus* induced tuber necrosis in 10 cultivars, under natural field conditions during 2022.

Potato cultivar	Experiment 1[a]		Experiment 2[b]		Sensitivity[c]
	% Incidence	% Severity	% Incidence	% Severity	
Bataina	48.0ab	39.7bc	48.0a	39.8bc	S
Desiree	26.0e	27.0d	26.0d	26.9e	MI
Fleminco	26.0e	27.1d	28.0cd	26.7e	MI
Kurado	32.0de	39.3bc	38.0abc	36.6cd	MS
LineA	38.0bcd	31.4cd	38.0abc	30.7 de	MS
LineB	50.0a	46.1ab	46.0a	45.9ab	S
Margrata	32.0de	29.7d	30.0bcd	28.1e	MS
Pomola	44.0abc	47.0ab	48.0a	47.9ab	S
Rodalph	34.0cde	39.9b	40.0ab	37.5cd	MS
Ronaldo	52.0a	48.3a	46.0a	48.8a	S
LSD value @ 0.05	10.757	8.2967	11.615	8.3346	

Means followed by the same letters are not significantly different (P < 0.05).

[a] Experiment conducted at Chotagala, district Poonch.

[b] Experiment conducted at Trarkhel, district Sudhnoti.

[c] Sensitivity classification: Tuber necrosis incidence <20% is ranked as insensitive (I); 20-30% as moderately insensitive (MI); 30-40% as moderately sensitive (MS); and >40% as sensitive (S).

Table 5. Assessment of growth parameters of ten potato cultivars under natural field conditions during 2022.

Potato cultivars	Experiment 1[a]		Experiment 2[b]	
	Plant height (cm)	No. of stems plant^{-}	Plant height (cm)	No. of stems plant^{-}
Bataina	24.880d	1.36cd	26.70c	1.36c
Desiree	44.780a	2.00a	45.12a	1.72ab
Fleminco	43.660ab	1.68abc	45.20a	1.72ab
Kurado	38.680bc	1.40cd	38.12b	1.44bc
LineA	35.600c	1.36cd	35.54b	1.36c
LineB	25.420d	1.28d	25.60c	1.32c
Margrata	46.260a	1.80ab	40.00ab	1.84a
Pomola	23.580d	1.36cd	25.68c	1.36c
Rodalph	37.440c	1.48bcd	38.34b	1.36c
Ronaldo	24.300d	1.56bcd	26.88c	1.32c
LSD value @ 0.05	5.9302	0.3869	5.9075	0.3306

Means followed by the same letters are not significantly different (P < 0.05).

[a] Experiment conducted at Chotagala, district Poonch.

[b] Experiment conducted at Trarkhel, district Sudhnoti.

Data analysis (Table 6) showed a significant difference (P < 0.01) in potato yield among different cultivars under natural disease conditions. At Chotagala, yield results revealed that Kurado differed significantly from Rodalph, Bataina, and Ronaldo, while no significant difference was found between Kurado, Desiree, Fleminco, and Line A in terms of tuber production. At Trarkhel, Kurado produced the highest number of tubers, followed by Fleminco, Rodalph, Line A, Desiree, Margrata, Bataina, Pomola, Line B, and Ronaldo, with significant differences (P < 0.05), except for Fleminco.

Cultivar Desiree had the highest number of large tubers, with an average tuber weight of 52.7 g, while Bataina had the smallest tubers, averaging 32.7 g in the Chotagala

experiment. However, in experiment 2, Fleminco (57.1 g) showed the highest average tuber weight, whereas Ronaldo had the lowest (32.1 g) (Figure 7). Desiree and Fleminco had similar average tuber weights, but both differed significantly from all other cultivars.

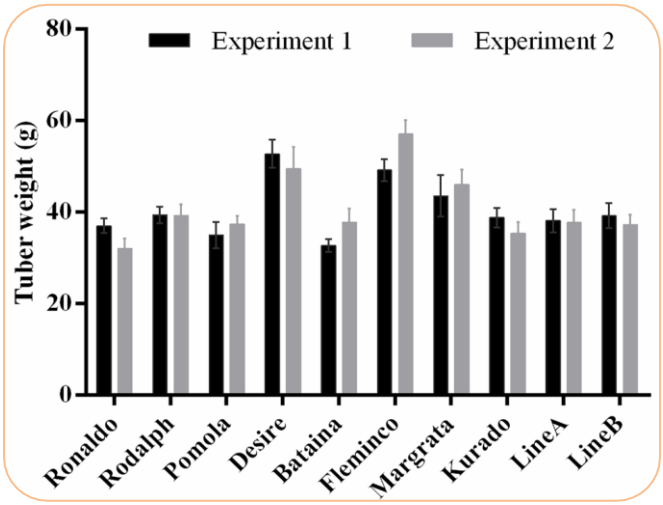

Figure 7. Assessment of potato cultivars at two locations on the basis of weight of tubers per plot in 2022.

Further analysis revealed that cultivars Kurado, Line A, Line B, Ronaldo, and Rodalph had small, statistically insignificant differences in tuber weight but were significantly different from Fleminco, Desiree, and Margrata (Table 6). At Trarkhel, there was variation among cultivars in average tuber weight. The majority of cultivars (Rodalph, Line A, Kurado, Line B, Ronaldo, and Pomola) were grouped together, showing no significant differences ($P < 0.01$). Fleminco and Desiree were not significantly different from each other, but Margrata was significantly different from Fleminco. Rodalph and Bataina were significantly different from Desiree, Fleminco, and Margrata but not from each other or from the remaining cultivars (Table 6). The growth and yield parameters of ten potato cultivars tested against TRV and its associated nematodes under natural conditions indicated that Desiree, Fleminco, and Margrata performed the best. Cultivars Rodalph, Kurado, and Line A showed moderate performance, while Ronaldo, Bataina, Line B, and Pomola were the most severely affected.

Table 6. Assessment of yield parameters of ten potato cultivars under natural field conditions during 2022.

Potato cultivar	Experiment 1[a]		Experiment 2[b]	
	No. of tubers	Weight of tubers	No. of tubers	Weight of tubers (g)
Bataina	17.6cd	32.72d	19.0d	37.82cd
Desiree	23.0ab	52.78a	24.6c	49.60ab
Fleminco	27.8ab	49.24ab	30.0ab	57.10a
Kurado	28.2a	38.82cd	33.2a	35.46d
LineA	24.2ab	38.18cd	25.0c	37.74d
LineB	15.6d	39.22cd	17.2d	37.30d
Margrata	25.8ab	43.60bc	24.4c	46.06bc
Pomola	17.6cd	35.00d	19.0d	37.38d
Rodalph	22.8bc	39.44cd	25.4bc	39.28cd
Ronaldo	14.2d	37.06cd	16.4d	32.10d
LSD value @ 0.05	5.2423	7.5505	4.9628	8.2720

Means followed by the same letters are not significantly different ($P < 0.05$).
[a] Experiment conducted at Chotagala, district Poonch.
[b] Experiment conducted at Trarkhel, district Sudhnoti.

DISCUSSION

The northeastern region of Pakistan, AJK, consisting of mid- and high-altitude lands, has long been a key hub for potato seed production in the country. The presence of both the virus and its vector necessitated an evaluation of commercially grown potato cultivars in the region (Haneef et al., 2021). This study found that none of the tested cultivars exhibited resistance to the virus. Instead, most were classified as either moderately sensitive or highly sensitive to necrosis caused by TRV.

Farmers have been cultivating the same potato cultivars for many years, and the high mutation rate of TRV is believed to have contributed to the loss of resistance against the virus. Moreover, the lack of proper

quarantine measures and the use of uncertified seed may have further reduced germplasm resistance. The presence of the virus, along with a high nematode vector population, has also contributed to the increased incidence of TRV. Once a nematode becomes viruliferous, it remains so for many years, continuously transmitting the virus to new crops.

Environmental factors, such as heavy rainfall and sandy soils, further support nematode proliferation, which in turn exacerbates virus incidence and increases the susceptibility of potato cultivars. Fluctuations in environmental conditions influence the expression of TRV-induced symptoms in tubers under both *in vivo* and *in vitro* conditions (Mojtahedi et al., 2001).

Among the ten cultivars tested, Rodalph, Kurado, Line A, and Margrata were classified as moderately sensitive to TRV-induced tuber necrosis, whereas Ronaldo, Bataina, Line B, and Pomola were classified as sensitive. The cultivars Desiree and Fleminco were categorized as moderately insensitive. Interestingly, none of the evaluated cultivars were found to be completely insensitive to tuber necrosis caused by TRV.

The moderately insensitive cultivars, Desiree and Fleminco, exhibited lower ELISA values and a lower incidence of tuber necrosis compared to the other cultivars. Similar findings have been reported in British potato varieties, where viral incidence was significantly lower in resistant genotypes than in susceptible ones, regardless of the presence or absence of symptoms (Xenophontos et al., 1998; Brown et al., 2000). Previous studies identified only two cultivars, Ciklamen and Bintje, as comparatively resistant to TRV-induced tuber necrosis. However, research conducted in Washington reported that none of the tested potato cultivars exhibited insensitivity to the virus (Yellareddygariet al., 2018).

It has been previously established that TRV resistance in some potato cultivars is regulated by a single resistance gene (Barker and Dale, 2006; Ghazala and Varrelmann, 2007). In addition to tuber necrosis, the impact of TRV on plant growth (e.g., plant height and stem count per plant) and yield (e.g., tuber number and tuber weight) was also examined. Different potato cultivars responded differently to TRV infection. Desiree, Fleminco, and Margrata exhibited better growth and yield under natural conditions, whereas Ronaldo, Pomola, Line B, and Bataina were significantly affected by the virus.

In the case of cultivar Kurado, a high proportion of smaller, lower-grade tubers (characterized by reduced size but increased numbers) suggested that this performance was a result of its genetic potential. Otherwise, Desiree performed best in terms of agronomic traits and had the lowest disease incidence, consistent with the findings of Duarte et al. (2011).

Factors influencing disease outcomes extend beyond the genotype of the potato cultivar itself. Differences in virus isolates can affect cultivar susceptibility (Mojtahedi et al., 2001), although this has not always been observed (Robinson et al., 2004). Cultivars exposed to TRV isolates exhibited varied responses, including reduced production quality, characterized by a noticeable decrease in tuber size, an increased number of tubers, and varying degrees of necrosis. Some symptomless yet deformed tubers and others with visible necrotic symptoms were observed. In this study, all tested cultivars exhibited these symptoms upon exposure to TRV isolates, similar to findings reported by Dale et al. (2000, 2004) and Xenophontos et al. (1998).

Additionally, Crosslin et al. (1999) and Dale et al. (2000) noted that systemic TRV infections in certain cultivars did not always result in characteristic symptoms in tuber flesh. Infected seeds without visible symptoms may serve as a reservoir of TRV for weeds and other crops, particularly in soils containing compatible vector species, which play a critical role in shaping TRV epidemiology in potato fields.

CONCLUSION

The present study demonstrated that TRV and its associated vectors are common in the main potato-growing districts (Poonch, Sudhnoti, and Bagh) of AJK. To prevent the spread of the virus, properly certified virus-free seed tubers should be used for planting in uninfected soil. Moreover, strict adherence to quarantine regulations at the national level is essential, particularly in regions free from virus inoculum, to minimize the future dissemination of soil-borne viruses and their vectors.

RT-PCR using the primer pair F2-R2 provides a rapid and reliable method for accurately differentiating TRV isolates. In field screening under natural conditions, among the 10 potato cultivars tested, Desiree and Fleminco performed best and were classified as moderately resistant.

Both the virus and its vector negatively impact the growth and yield of potato crops in the region. To

mitigate the economic losses caused by the disease, growers should prioritize cultivating the less susceptible cultivars Desiree and Fleminco, which exhibit lower tuber necrosis induced by TRV. Further research is needed to identify the resistance genes that regulate the host response to TRV infection and to determine optimal marketing strategies for post-harvest potato storage.

AUTHORS' CONTRIBUTIONS

NH and MA designed the study; NH prepared the materials, collected and analyzed the data; AS helped in the identification of virus; MTK assisted in nematode collection and identification. MA and AA supervised the studies; NH and MTK wrote the manuscript; All the authors proofread and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUSTAINABLE DEVELOPMENT GOALS TARGETED

SDG 2: Zero Hunger
SDG 3: Good Health and Well-being
SDG 9: Industry, Innovation and Infrastructure
SDG 12: Responsible Consumption and Production
SDG 13: Climate Action

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