

Tuber Size-Grading and Altitudinal Vector Dynamics as Non-Chemical IPM Strategies against Potato leafroll virus in the North-Western Himalayas

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Article

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Abstract

Potato leafroll virus (PLRV) severely effects potato crop production by degrading seed quality through successive generations. This study evaluates the geo-epidemiological dynamics of PLRV and its primary aphid vector, *Myzus persicae* (Sulzer), across 18 locations featuring diverse altitudinal gradients (270-2,500 m) in Jammu region of North Western Himalayas. Serological validations via Double Antigen Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) revealed that the high-altitude hill farms recorded 77.5% healthy plants compared to lowland plains with 65.6% infected. Quantitative tuber indexing demonstrated a significant inverse relationship between tuber size and viral absorbance metrics (OD_{405}), identifying a prominent viral dilution effect in large-size tubers. The correlation studies revealed an inverse relationship between viral accumulation and yield, while tuber size positively correlated with yield. Weekly vector monitoring fitted with a LOESS model demonstrated a clear, continuous altitudinal suppression of aphid pressure. The findings suggested that integrating high-altitude geographic isolation with tuber size grading protocols can help to reduce the virus spread and produce healthy potato seed tubers for quality seed production.

Introduction

Globally, potato (*Solanum tuberosum* L.) production is heavily constrained by a complex of viral pathogens, among which Potato leafroll virus (PLRV), Potato virus Y (PVY), Potato virus X (PVX) are primary drivers for significant yield reductions and economic losses (Kreuze et al. (2020). PLRV induces distinct physiological abnormalities classified as either primary or secondary symptoms, depending strictly on the crop growth stage at the time of vector inoculation Taliansky et al. (2003). Primary infections target young, apical foliage manifesting as upright growth, marginal leaf rolling and localized chlorosis Kreuze et al. (2020). Conversely, secondary symptoms develop systematically when plants emerge directly from infected seed tubers harvested from prior generations Thomas-Sharma et al. (2016). These secondary symptoms are vastly more descriptive, causing severe stunting, an erect growth habit and pronounced rolling of older lower leaves that turn brittle leathery and chlorotic due to starch accumulation in the phloem Taliansky et al. (2003). Taxonomically, PLRV is a positive-sense single stranded RNA virus belonging to the genus *Polerovirus* within the family *Solemoviridae* a critical classification distinction separating it from potyviruses like PVY ICTV, (2022); Walker et al. (2022). Enzyme linked immunosorbent assay (ELISA) has served as the global diagnostic tool for seed certification and epidemiological tracking as demonstrated by early regional surveillance networks in Iraq and seed indexing initialization in Lebanon. While modern high-throughput molecular techniques like RT-qPCR and digital PCR offer superior sensitivity Boonham et al. (2014). Serological identification via DAS-ELISA remains an internationally accepted diagnostic standard for certifying formal seed stocks and mapping macro epidemiological trends Salazar (1996). Because systemic phytopathogenic viruses cannot be cured using conventional chemical therapeutic agents once field transmission has occurred, the primary aim of management remains the production and multiplication of high grade, virus free seed

tubers Struik (2023). Growing seed potatoes over and over in areas with uncontrolled aphid population leads to a fast buildup of the virus causing yield loss Thomas-Sharma et al. (2016).

Accordingly, the objectives of this study were (i) identification and distribution of PLRV in the various districts of Jammu Division of North Western Himalayas (ii) Estimation of virus concentration in varying sized tubers (iii) Evaluation of growth and yield parameters (iv) Spatial vector population dynamics. (v) Evaluating tuber size-grading as non-chemical, mechanical management strategy to eliminate high viral loads prior to seed multiplication.

Materials and methods

Survey and Sample Collection

Comprehensive field surveys were carried out during the 2022/2023 and 2023/2024 cropping seasons across five districts of the Jammu Division, Jammu, Kathua, Udhampur, Poonch and Rajouri. Potato leaf samples were randomly collected from multiple target fields and the corresponding plants were physically marked with pegs for seasonal monitoring (Table 1). To ensure rigorous epidemiological profiling, leaf samples were harvested from both symptomatic plants and asymptomatic individuals. For each selected plant, three leaves representing top, middle and bottom were harvested directly into specially labelled plastic bags. Collected leaf tissues were transported immediately to the laboratory and maintained overnight in a refrigerator at 4°C prior to serological processing. At the time of crop maturity, three tubers were harvested from each of the marked plants to evaluate downstream viral loading and yield metrics.

Table 1
Geographical coordinates and altitudinal classifications of 18 field survey
locations across the Jammu Division

District	Location	Latitude (°N)	Longitude (°E)	Altitude (m)
Jammu	Ponichak	32.73	74.79	280
	Sobka Thathi	32.85	74.65	275
	Gajansoo	32.74	74.74	285
	Panjore	32.86	74.77	270
Kathua	Mahrin	32.40	75.45	310
	Chadwal	32.44	75.34	290
	Kagri Khokyal	32.45	75.43	325
	Rajbagh	32.42	75.40	340
Udhampur	Basantgarh	32.81	75.54	1,855
	Chenani	33.02	75.28	1,220
	Ramnagar	32.81	75.31	820
	Tikri	32.99	75.10	780
Poonch	Loran	33.84	74.35	2,500
	Mandi	33.79	74.26	1,718
	Gambhir	33.45	74.21	860
Rajouri	Mangnar	33.78	74.12	1,020
	Jhullas	33.72	74.15	890
	Rajouri	33.38	74.30	915

Serological Diagnostics and Quantification

Serological indexing of PLRV was achieved using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA), following standard operational protocols described by Clark and Adams (1977). While high-throughput molecular techniques offer ultra-sensitive detection, DAS-ELISA remains the internationally recognized regulatory gold standard endorsed by EPPO and CIP for large-scale field seed indexing, macro-epidemiological surveillance and high throughput seed potato certification schemes. Certified high-affinity PLRV polyclonal antibodies and conjugated enzyme reagents were sourced directly from Agdia (Elkhart, IN, USA).

Vector Population Dynamics

Weekly monitoring of *Myzus persicae* (Sulzer) populations was conducted across 13 consecutive standard meteorological weeks (SMW 50 to 10) during both cropping seasons. Aphid counts were recorded visually on 100 randomly selected leaves from four different locations including Loran and Chenani from high altitude while Panjore and Mahrin from low lying plain (representing top, middle and bottom canopy positions) to quantify vector density in hilly and plain altitudinal gradients.

Statistical analysis

All the experimental data was subjected to statistical evaluation using R software (version 4.3.1). The temporal dynamics of weekly aphid vector populations across standard meteorological weeks (SMW) were modeled using locally Estimated Scatterplot Smoothing (LOESS).

Results

Distribution and Detection of PLRV

The disease was recorded during the survey carried out during 2022/2023 in five districts of Jammu Division including Jammu, Kathua, Udhampur, Poonch and Rajouri on the basis of symptomatology and confirmed by using serological detection method (Fig. 1). In Jammu district, maximum disease incidence was recorded at Gajansoo (37.60%) followed by Panjore (32.00%), Ponichak (28.00%), Sobka Thathi (21.14%) while the mean percentage of disease incidence was 29.68 per cent. In Kathua district, maximum incidence of PLRV was recorded in Chadwal (44.80%) followed by Kagri Khokyal (42.40%), Mahrin (36.00%) and Rajbagh (33.60%) and the mean percentage of disease incidence was 39.20 per cent. In Udhampur district, maximum incidence was recorded in Basantgarh (33.60%), followed by Tikri (29.60%), Chenani (28.00%) and Ramnagar (25.60%) and the mean percentage was 29.20 per cent. In Poonch district, maximum incidence was recorded in Gambhir (36.00%) followed by Mandi (29.60%) and Loran (28.80%) with mean of 31.46 per cent while in Rajouri district, maximum incidence was recorded in Jhullas (40.00%) followed by Rajouri (39.20%) and Mangnar (24.00%) having the mean of 34.4 per cent. The tubers of various sizes were collected from virus infected plants and brought to the laboratory for identifying the virus and detection of its concentration in various size tubers used by the farmers.

The results showed inverse relationship between tuber size classes and viral concentration (Table 2). The highest concentration of viral titer was clustered within the smallest tuber grouping (< 20 mm), yielding a prominent median OD value of approximately 0.132 (ranging from 0.110 to 0.155) (Fig. 2). As the tuber size class advanced, a pronounced and consistent downward step-shift in viral concentration was observed. At 20–40 mm the median absorbance value dropped sharply to approximately 0.068, while in the 41–55 mm sized tuber it was further suppressed to 0.051. Notable individual fluctuations were recorded in this titer, marked by isolated high titer approaching 0.072 and lower dropping to 0.039. Large, fully mature seed tubers > 55 mm showed the lowest overall viral footprint, highly uniform median

absorbance value of 0.027. The narrow vertical distribution interquartile ranges seen in the 20–40 mm and > 55 mm cohorts indicate high physiological uniformity in viral distribution within these specific fractions indicating that smaller, undersized tubers serve as concentrated reservoirs for PLRV replication.

Table 2
Serological detection of PLRV from different sized tubers of potato in different locations of Jammu Division

District	Location	OD values at 405 nm			
		< 20mm	20-40mm	41-55mm	> 55mm
Jammu	Ponichak	0.1045– 0.1239	0.0675– 0.0789	0.0446– 0.0527	0.0396– 0.0419
	Sobka Thathi	0.0986– 0.1232	0.0586– 0.0675	0.0492– 0.0543	0.0392– 0.0412
	Gajansoo	0.1234– 0.1564	0.0678– 0.0765	0.0698– 0.0735	0.0230– 0.0312
	Panjore	0.0945– 0.1245	0.0636– 0.0755	0.0456– 0.0565	0.0345– 0.0435
Kathua	Mahrin	0.1024– 0.1346	0.0641– 0.0756	0.0453– 0.0526	0.0234– 0.0344
	Chadwal	0.1245– 0.1564	0.0625– 0.0726	0.0455– 0.0586	0.0212– 0.0316
	Kagri Khokyal	0.1235– 0.1456	0.0615– 0.0706	0.0345– 0.0456	0.0215– 0.0326
	Rajbagh	0.1034– 0.1454	0.0561– 0.0676	0.0534– 0.0657	0.0205– 0.0346
Udhampur	Basantgarh	0.1265– 0.1526	0.0612– 0.0766	0.0654– 0.0798	0.0215– 0.0335
	Chenani	0.1046– 0.1269	0.0546– 0.0659	0.0456– 0.0548	0.0145– 0.0269
	Ramnagar	0.1025– 0.1299	0.0526– 0.0639	0.0487– 0.0523	0.0132– 0.0367
	Tikri	0.1456– 0.1646	0.0628– 0.0721	0.0476– 0.0586	0.0215– 0.0362
Poonch	Loran	0.1085– 0.1564	0.0545– 0.0642	0.0463– 0.0554	0.0135– 0.0324
	Mandi	0.1132– 0.1457	0.0515– 0.0623	0.0435– 0.0545	0.0125– 0.0315
	Gambhir	0.1342– 0.1542	0.0678– 0.0742	0.0487– 0.0598	0.0212– 0.0332
Rajouri	Mangnar	0.1013– 0.1243	0.0498– 0.0523	0.0346– 0.0423	0.0105– 0.0313
	Jhullas	0.1345– 0.1675	0.067–0.0723	0.0634– 0.0693	0.0214– 0.0334

Rajouri	0.1436– 0.1656	0.0674– 0.0765	0.0436– 0.0586	0.0214– 0.0354
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Correlation and Regression Analysis

To explore the interconnected relationships between plant productivity, tuber size and viral presence during 2022/2023 a multivariate Pearson correlation analysis was executed (Fig. 3). The matrix revealed a moderate, positive correlation between yield and tuber size ($r = 0.47, p < 0.05$), indicating that improvements in individual plant yield are structurally coupled with an increase in the physical size distribution of the harvested tubers. Crucially, evaluating the direct linear relationship for viral titer (OD_{405}) demonstrated a strong negative correlation with tuber size ($r = -0.92, p < 0.001$), directly reflecting the physiological viral dilution observed in larger tuber fractions and aligning with linear regression model ($R^2 = 0.855$). Furthermore, viral load exhibited a negative correlation with total plant yield ($r = -0.61, p < 0.01$), conforming that secondary PLRV accumulation significantly impairs overall crop productivity.

A simple linear regression analysis was performed using the midpoints of the evaluated tuber size classes (10 mm, 30 mm, 48 mm and 65 mm) against their corresponding PLRV absorbance values (OD at 405 nm) (Fig. 4). The model confirmed a robust, highly significant negative linear relationship between the variables. The total variance of 85.5% was demonstrated between PLRV titer concentration and potato tuber size. The downward slope of red line clearly illustrated that as the tuber size increase from 10 mm to 65 mm, there was predictable, continuous reduction in viral load, shifting from a maximum cluster near 0.155 OD down to a baseline of approximately 0.020 OD .

Geographical Distribution and Density Profiling

The geographical epidemiological mapping of all these 18 locations across the Udhampur, Rajouri, Poonch, Kathua and Jammu districts, revealed that the virus was systematically present across all surveyed regions with highest viral concentration recorded in Udhampur ($OD = 0.1551$, located near $75.13^{\circ}E, 32.90^{\circ}N$) and Rajouri ($OD = 0.1546$, located near $74.31^{\circ}E, 33.26^{\circ}N$). Poonch and Kathua showed a moderate concentration with ($OD = 0.1442$, located near $74.09^{\circ}E, 33.77^{\circ}N$) and ($OD = 0.1405$, located near $75.52^{\circ}E, 32.38^{\circ}N$) respectively. The lowest viral presence was recorded in Jammu ($74.85^{\circ}E, 32.72^{\circ}N$) with an OD value of 0.1399 (Fig. 5).

Interannual Shifts in Crop Performance

The shifting trends in crop performance across the 18 regional evaluation sites, paired data tracking both tubers per hill and yield per plant were analyzed across consecutive production cycles during 2022/2023 and 2023/2024. During 2022/2023 the average number of tubers per hill was recorded low with an average of approximately 5.26 and ranging from a minimum 5.06 to maximum 5.68 and similarly during the 2022/2023 season, average yield per plant (g) was recorded approximately 289.4 g/plant,

ranging from 258.88 g to 307.77 g this was mainly due to the presence of higher concentration of PLRV infestation in the potato tubers during that particular season (Fig. 6, Fig. 7). In 2023/2024 season, farms utilizing larger seed tubers (> 55 mm) exhibited substantially higher yields (811.4 g/plant vs. 289.4 g/plant in 2022/2023). While interannual climatic variations likely contributed to total biomass differences, selecting larger potato seed fractions served as key cultural contributors by lowering initial viral loading which resulted in a massive increase in the number of tubers per hill with an average up to 9.47 ranging between 9.15 and 9.79 tubers per hill and yield was also increased simultaneously with an average of ranging from 709.55 g to 933.33 g. The multi-location comparison of net potato yield (g/plant) analyzed across 18 distinct locations within the Jammu Division during the 2022/2023 and 2023/2024 cropping season revealed that Loran of Poonch district recorded highest yield of 307.77 g/plant and 933.33 g/plant respectively whereas Mahrin of Kathua district recorded the lowest yield of 258.88 g/plant and 709.55 g/plant during both the cropping seasons.

Vector Population Dynamics and Environmental Barriers

To study the relationship between the altitude and virus distribution, four different locations including Loran and Chenani from high altitude while Panjore and Mahrin from low lying plain were selected and the data obtained was analyzed by Chi-square test which revealed that in high altitude (Loran/Chenani) nearly 77.5% plants were found as healthy while the presence of PLRV infection was suppressed to 22.5% in lowland plains (Panjore/Mahrin) which showed a complete reversal with infected samples surged to 65.6% and 34.4% as healthy (Fig. 8).

To study the relationship between the vector population and spatial virus distribution in (Chenani/Loran) high altitude and low lying plain (Panjore/Mahrin) weekly aphid population profiles was monitored across 13 consecutive standard meteorological week (SMW 50 to 10) during both the crop growing seasons and was analyzed using a LOESS (Locally Estimated Scatterplot Smoothing) model which revealed consistent variation in vector pressure based on elevation, with lowland plains exhibiting high population than high altitude farms. The low altitude regions like Panjore and Mahrin recorded 3.67 and 2.50 aphids per plant during the peak vegetative phases (Fig. 9). In contrast, the high-altitude locations like Chenani and Loran recorded a vector count below 2.00 aphids per plant during both the crop cycles.

Discussion

The localized systemic PLRV movement depends mostly on phloem mediated transport channel from source leaves down into developing sink tubers, heavily affecting economic yield Kumar et al. (2023); Peter et al. (2009). Exceptionally high virus presence was observed in tubers of < 20 mm size. In contrast, larger tubers > 55 mm exhibit a prominent virus concentration Kumari et al. (2020). The rapid tissue expansion driven by starch deposition, cell enlargement and rapid tuber volume increase suppresses internal viral replication, significantly reducing the final concentrations Bertschinger et al. (2017). From an integrated pest management perspective, this physiological behaviour offers a practical, highly cost-effective cultural control intervention for small and medium farmers. The consecutive season

trials revealed that adoption of larger seed tubers (> 55 mm) strongly coincided with favourable interannual climatic conditions increased the average yield per plant from 289.4 to 811.4 g. This structural variance along with seasonal variations in precipitation, ambient temperature and localized vector flight timings provides a significant protocol to mechanically eliminate high-risk, seed fractions from tuber production.

Multivariate correlation analysis demonstrated a strong positive correlation between tuber size and yield ($r = 0.47$, $p < 0.05$) indicating that optimal growth environments consistently produce larger, commercially viable seed fractions. Crucially, viral titer (OD_{405}) showed a strong inverse correlation with tuber size ($r = -0.92$, $p < 0.001$, $R^2 = 0.855$). Conversely, secondary PLRV infection negatively impacts total crop yield and shifts tuber size distribution toward smaller fractions Kumar et al. (2020), as high viral titers severely impair phloem transport during starch deposition.

Environmental dynamics explain spatial distribution of maximum viral presence across the region. Intermediate altitudinal regions like Udhampur ($OD_{405}=0.1551$) and Rajouri ($OD_{405}=0.1546$) acting as hot spots for viral accumulation due to optimum climatic conditions that favor vector feeding and development Szittyá et al. (2003). Meanwhile, lowland heat waves in Jammu and Kathua limit long-term replication by reducing vegetative growth and increasing maturity of the crop Chung et al. (2016).

The spatial and altitudinal variations in PLRV distribution and aphid populations provide an empirical validation for the altitude vector escape paradigm within seed potato networks Vučetić et al. (2013). The LOESS smoothing model effectively establishes altitudinal thresholds for aphid flight activity, serving as a decision support tool for seed producers. High-altitude zones (> 1,700 m) naturally suppress vector pressure (< 2.00 aphids/plant), acting as a natural isolation barrier. The higher disease infection rates (65.6%) and constant population peaks of *Myzus persicae* (Sulzer) observed in lowland plains of Panjore and Mahrin regions demonstrate how microclimates accelerate aphid reproduction and flight activity, resulting in secondary viral transmission through the phloem during critical growth stages Eigenbrode et al. (2002). Inversely, the significant suppression of aphid vector population and the proportion of healthy samples (77.5%) in high-altitude hill farms of Loran and Chenani confirms that low ambient conditions create a strong ecological barrier against multiplication and early-season colonization of aphid vector Vučetić et al. (2013).

Conclusion

The study demonstrates that geography, altitude insulation and seasonal vector dynamics are the deciding factors governing seed potato health and productivity. High altitude locations provide exceptional phytosanitary safety boundaries by naturally suppressing aphid populations whereas lowland plain environments face high vector distribution that accelerates virus distribution and limits crop output. Furthermore, interannual weather variability and inverse relationship between tuber size and viral titer indicates that internal viral loads are heavily dictated by physiological growth dynamics. These

findings provide a clear biological and mathematical justification for foundation seed multiplication systems to eliminate the virus from future crop propagation cycles.

Declarations

Author Contribution R.S conceived and supervised the research. R.S and N.K. conducted the research and analysed the data. R.S and S.G drafted the manuscript and all authors read and approved the final draft.

Data Availability The data that support the findings of this study is available from the corresponding author and can be provided upon reasonable request.

Statements and Declarations

Conflict of Interest The authors declare no competing interests.

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Figures

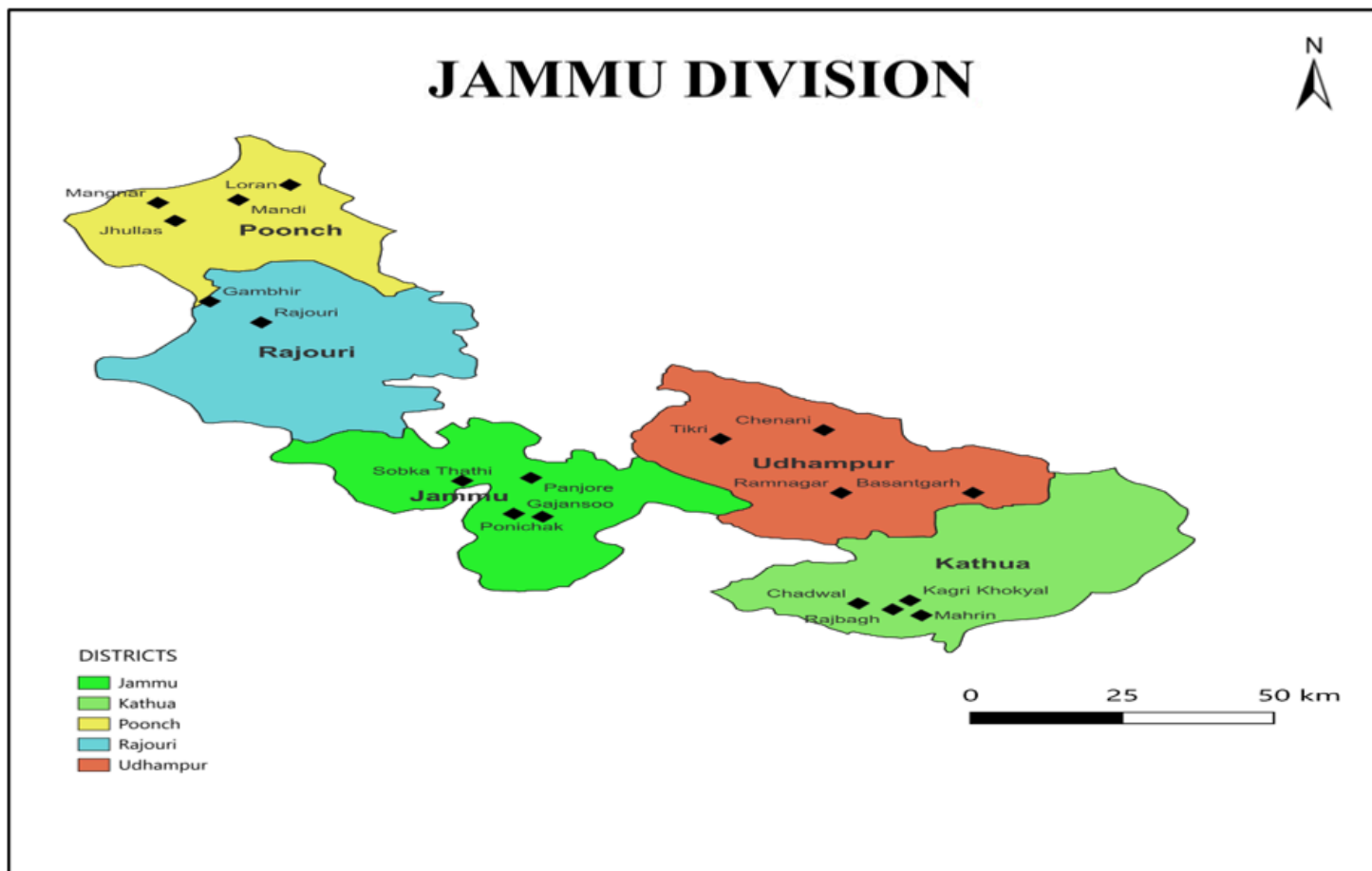


Figure 1

Fig 1. Distribution of PLRV during the growing seasons 2022/2023 and 2023/2024 in Jammu

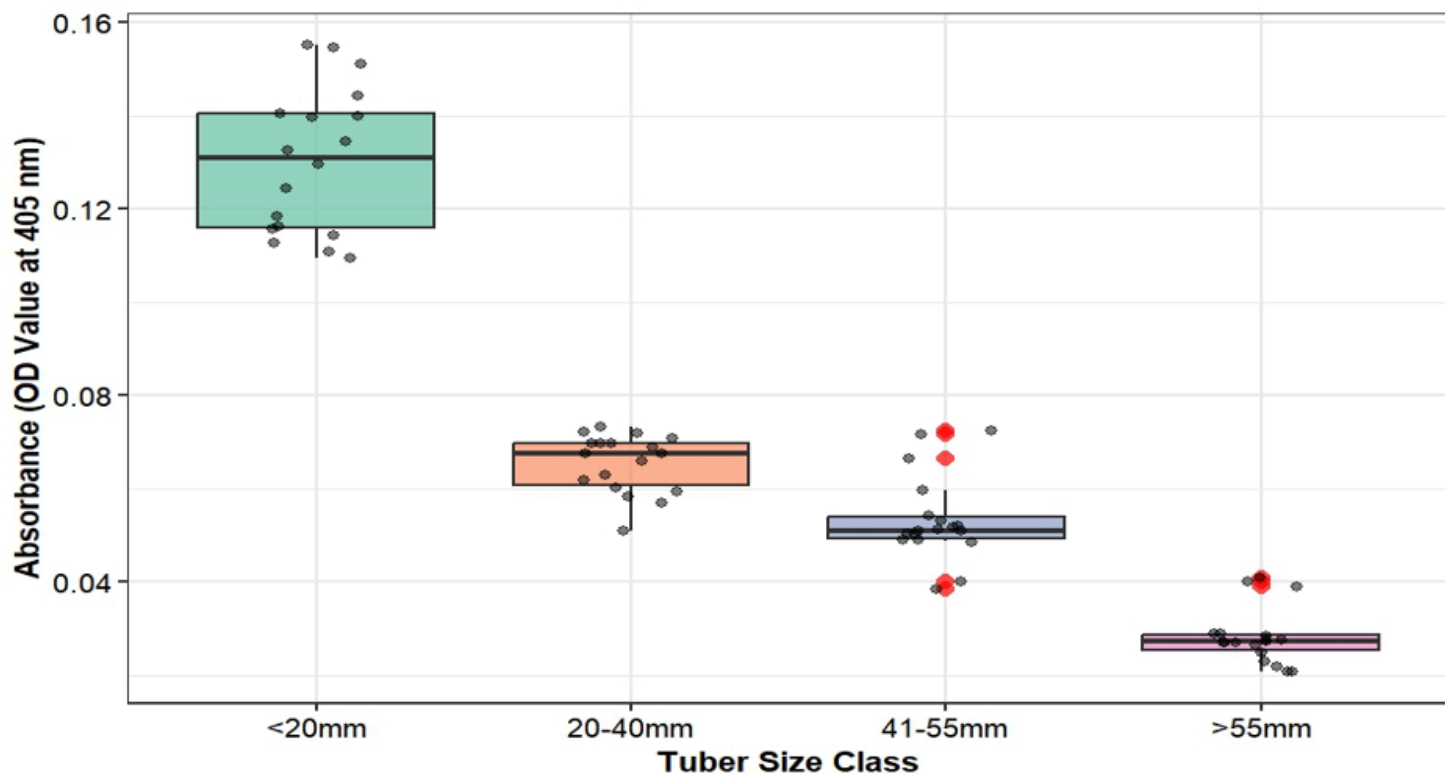


Figure 2

Fig. 2 Distribution of PLRV concentration across tuber sizes

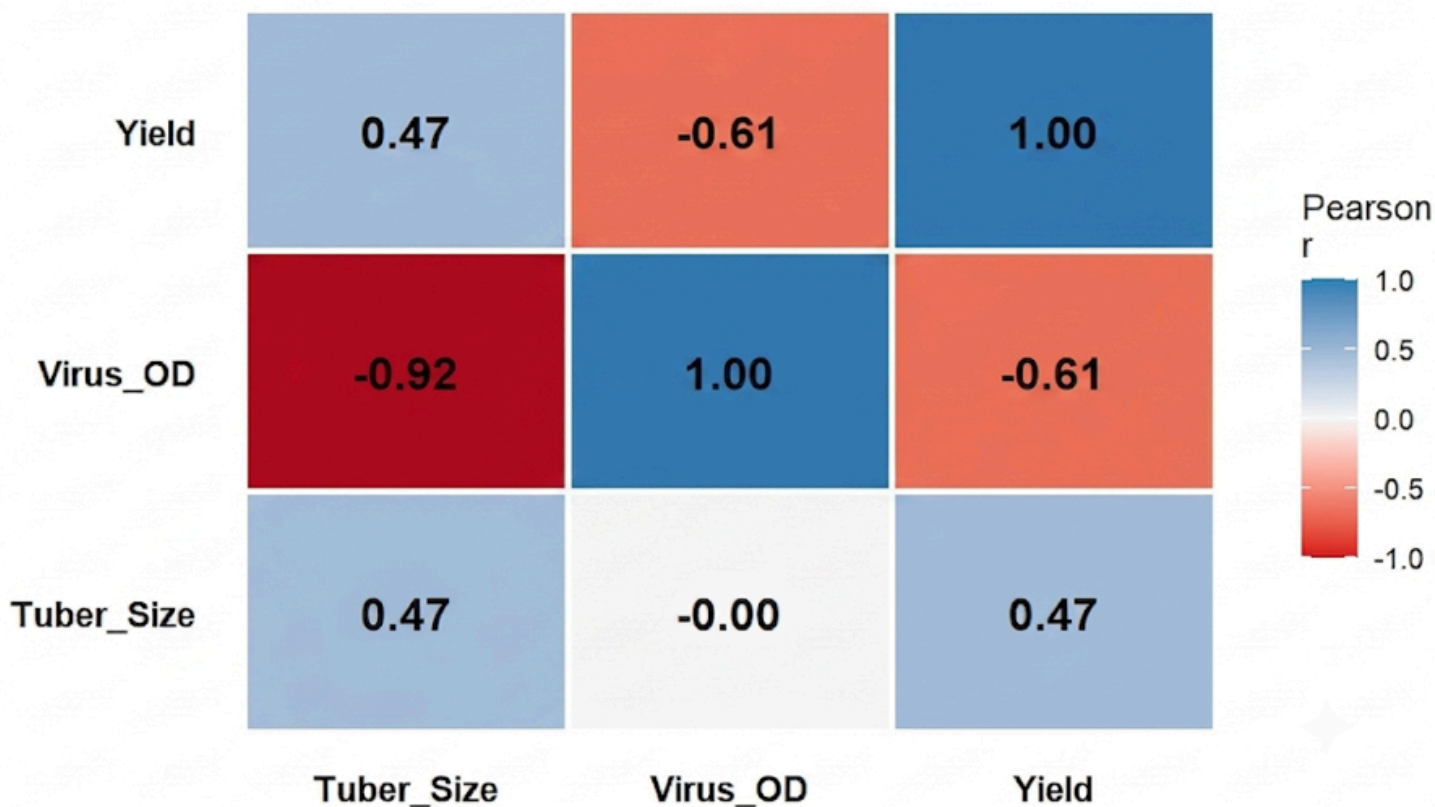


Figure 3

Fig. 3 Pearson correlation matrix heatmap displaying the interactive coefficients (r) among total yield, virus OD and tuber size

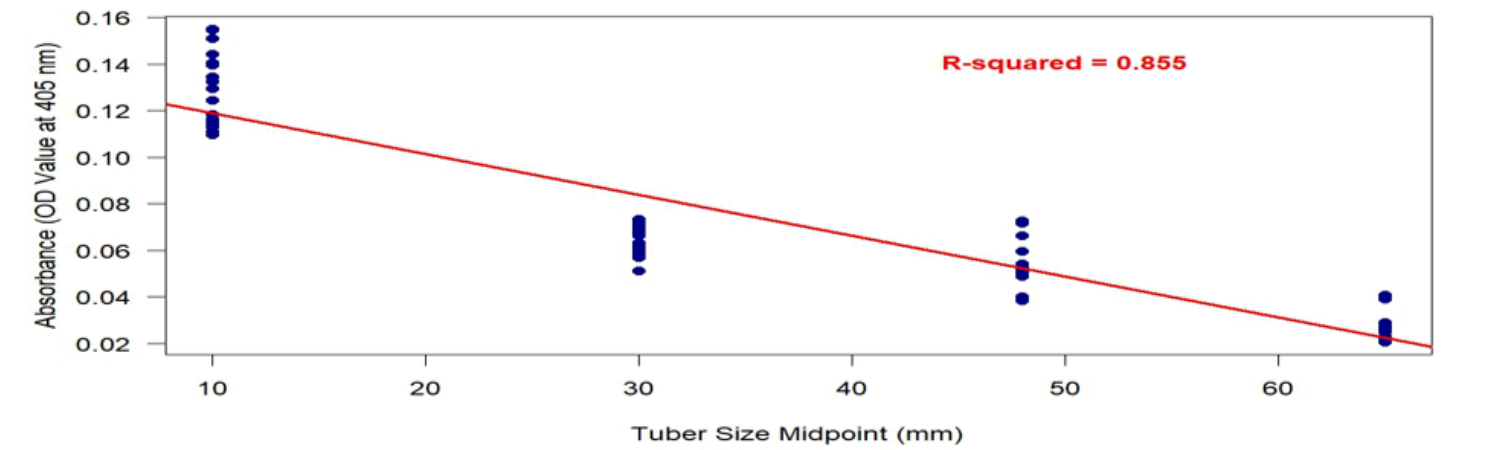


Figure 4

Fig. 4 Linear regression plot illustrating the relationship between tuber size class midpoints (mm) and PLRV absorbance (OD at 405 nm)

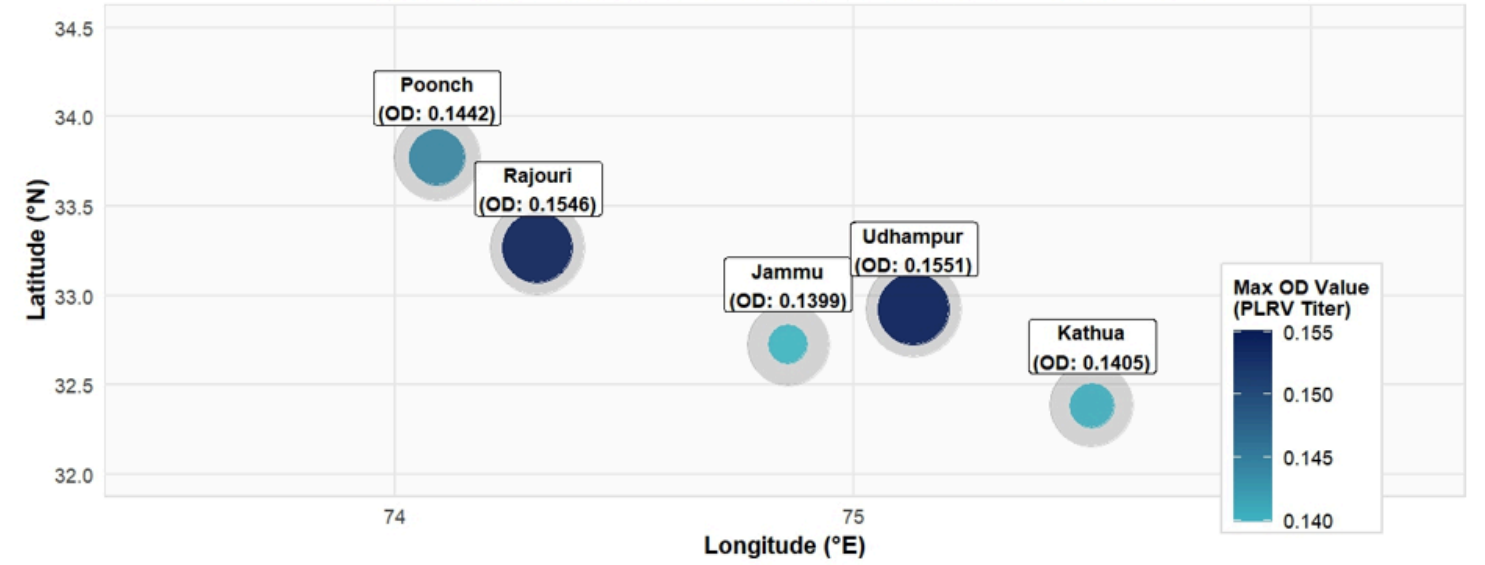


Figure 5

Fig. 5 Geographical epidemiology map of maximum PLRV titer distribution

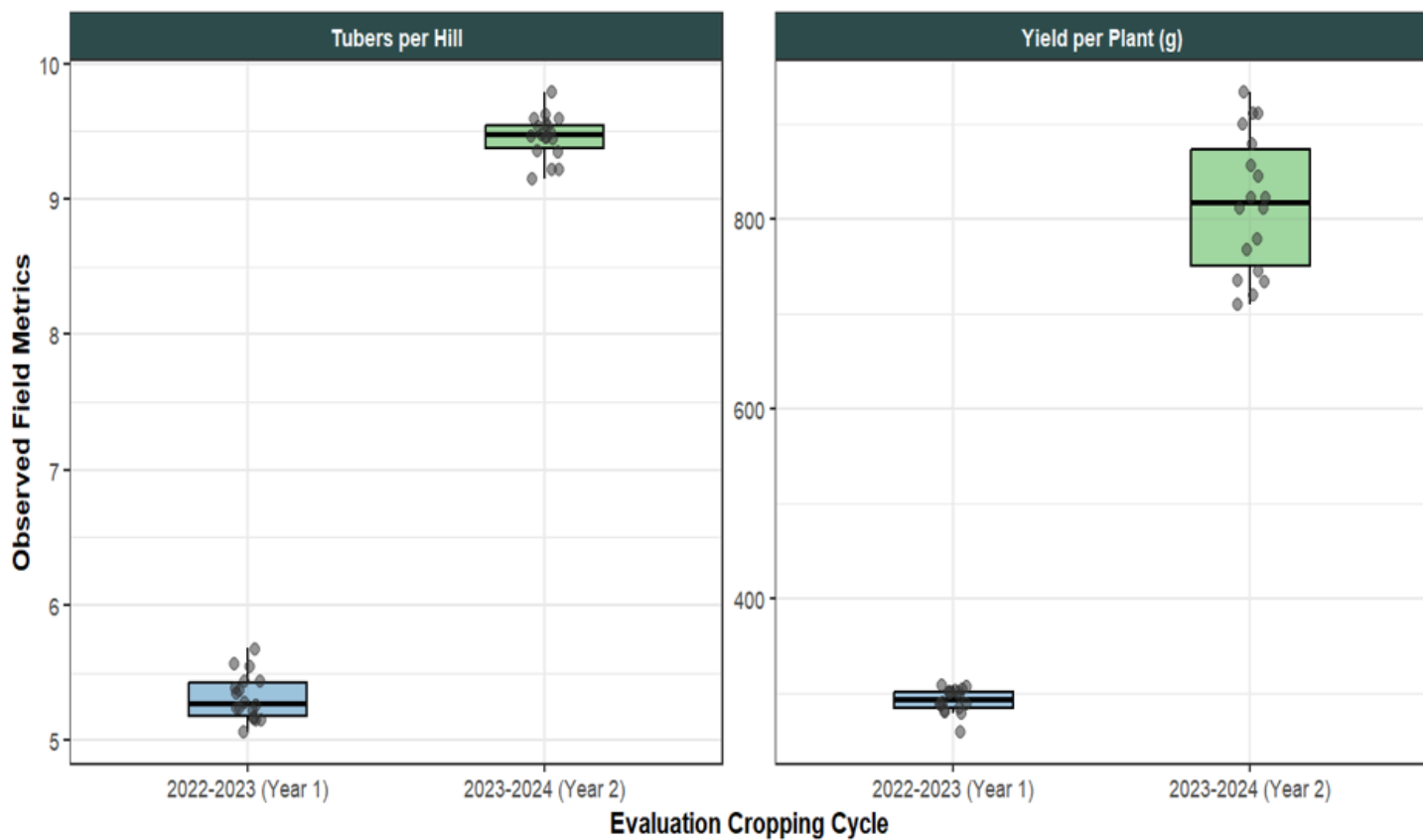


Figure 6

Fig. 6 Interannual comparison of performance components. The boxplots illustrate the distribution of tubers per hill (left panel) and total yield per plant (right panel) during 2022/2023 and 2023/2024

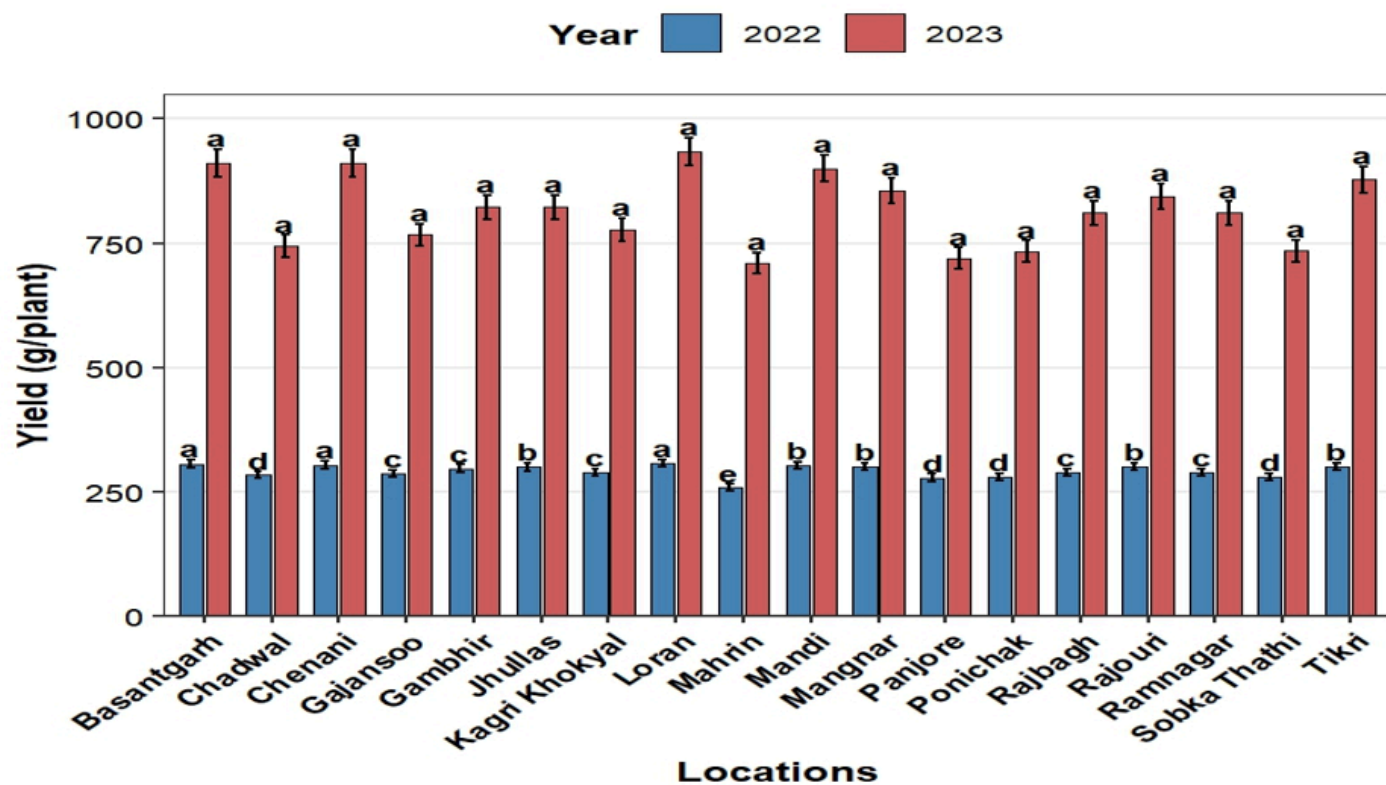


Figure 7

Fig. 7 Spatial comparison of potato yield metrics across 18 locations

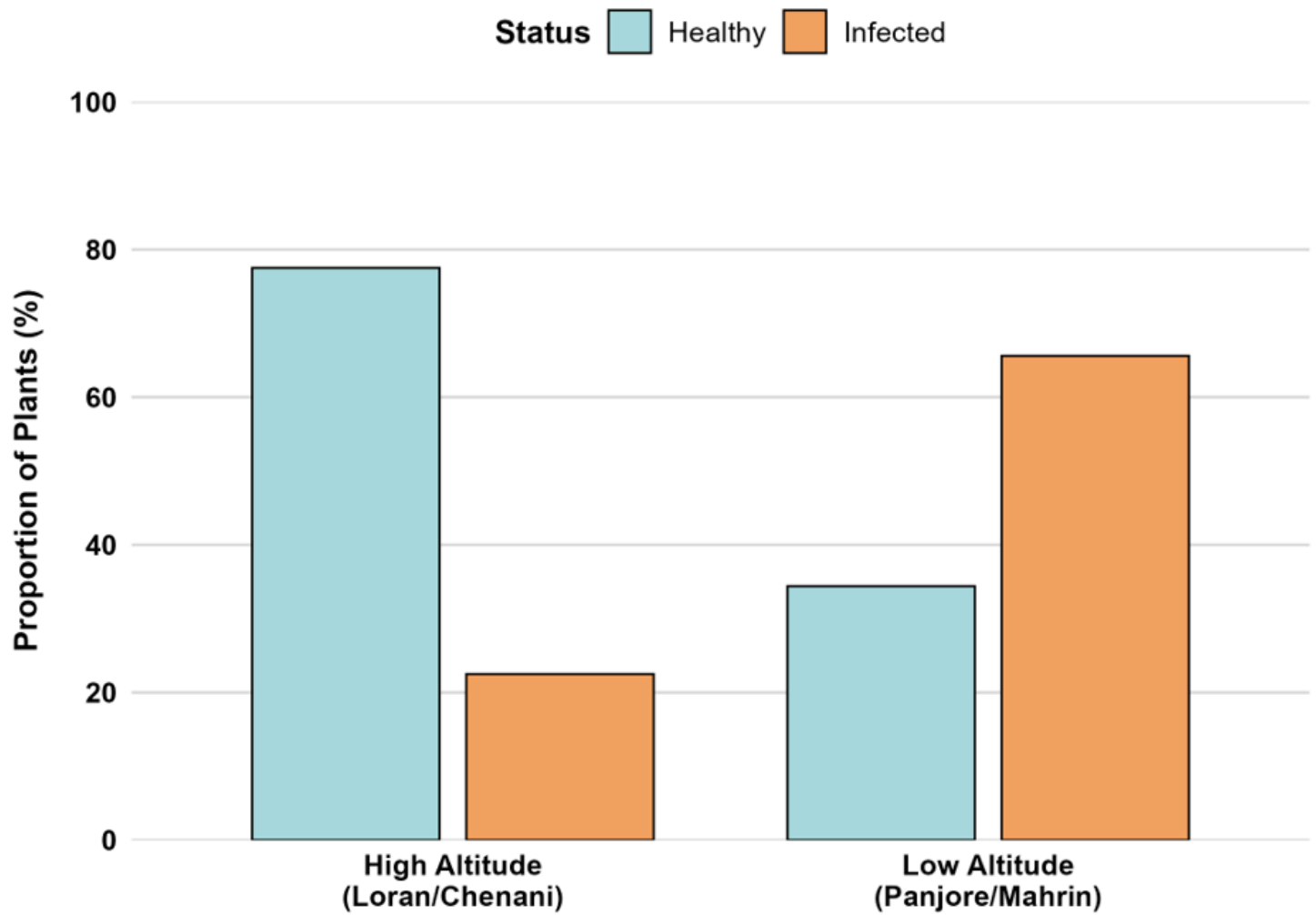


Figure 8

Fig. 8 Proportional distribution of PLRV infection across high and low altitude farm types



Figure 9

Fig. 9 Weekly monitoring profiles of aphid vector population dynamics. Solid lines connect weekly mean field counts for high-altitude locations (Loran and Chenani) versus lowland plain (Mahrin and Panjore). Dashed lines represent fitted LOESS smooth conditional trend trajectories, illustrating a clear, continuous altitudinal suppression of vector pressure