3 Biotech (2023) 13:273
https://doi.org/10.1007/s13205-023-03672-2

ORIGINAL ARTICLE

Genetic footprint of population diversity and genetic structure of *Venturia inaequalis* infecting apple (Malus × domestica Borkh.)

Mohammad Saleem Dar[1] · Mushtaq Ahmad[2] · Nakeeb-Un-Nisa Yetoo[3] · Bhagyshree Bhatt[4] · Suhail Nazir Bhat[5] · Heena Altaf[1] · Sumira Rafiqee[3] · Asha Nabi[1] · F. A. Mohiddin[6] · Abdel-Rhman Z. Gaafar[7] · Sheikh Mansoor[8] · Mehraj D. Shah[2] · Muntazir Mushtaq[4]

Received: 18 February 2023 / Accepted: 13 June 2023 / Published online: 12 July 2023

Abstract

Apple scab instigated by *Venturia inaequalis* impels remarkable losses to apple fruit production. In an effort to comprehend the key mechanisms of evolutionary potential defining *V. inaequalis* population, 132 isolates of *V. inaequalis* from five commercial apple orchards were collected and assayed using 14 microsatellite markers. The average diversity was observed within the individuals of populations based on the Shannon-Wieners index (I) and observed heterozygosity (Ho) was average but considerably lower than expected heterozygosity (He). The genetic differentiation based on FST values was revealed as an average measure of divergence between populations and had varying proportions of gene flow and migration among themselves. Analysis of Molecular Variance (AMOVA) revealed that variance (94%) was dispersed across individuals with a significant (6%) variation between populations from different regions. To examine host specialization within the *V. inaequalis* population, the assignment approach based on K-means of clustering (an unsupervised machine learning approach), revealed that the clustering method supported three clusters at ($K=3$) and three major clusters were also observed in Principle Component Analysis (PCA). Additionally, Nei's genetic distance values, pairwise estimates of genetic differentiation, dendrogram using the neighbor-joining and PCoA revealed the random distribution of *V. Inaequalis* isolates that depicted a high proportion of genotypic diversity within populations and population genetic structure.

Keywords Apple · *Venturia inaequalis* · Population diversity · Genetic structure

✉ Mehraj D. Shah
mehraj547@rediffmail.com

✉ Muntazir Mushtaq
muntazirhuda@gmail.com

1 Division of Plant Pathology, Faculty of Agriculture, SKUAST-Kashmir, Wadura, Sopore, Jammu and Kashmir 193201, India

2 Division of Plant Pathology, Faculty of Horticulture, SKUAST-Kashmir, Shalimar, Srinagar, Jammu and Kashmir 190025, India

3 Division of Genetics and Plant Breeding, FoA, SKUAST-K, Wadura, Sopore, Jammu and Kashmir 193201, India

4 MS Swaminathan School of Agriculture, Shoolini University of Biotechnology and Management Sciences, Bajhol, Solan, Himachal Pradesh 173229, India

5 Division of Fruit Science, Faculty of Horticulture, SKUAST-Kashmir, Shalimar, Srinagar, Jammu and Kashmir 190025, India

6 Mountain Research Centre for Field Crops, SKUAST-Kashmir, Khudwani, Kulgam, Jammu and Kashmir 192101, India

7 Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 11451, Riyadh, Saudi Arabia

8 Department of Plant Resources and Environment, Jeju National University, Jeju 63243, Republic of Korea

Introduction

Venturia is the most destructive genus responsible for the scab of many important fruit crops. The genus encompasses ascomycete fungi and has a haplontic life cycle (Zhang et al. 2015; Dar et al. 2022). It contains more than 50 species that have been characterized based on morphology and host specificity, with the majority being responsible for plant diseases (Sivanesan 1977). The prominent species in the genus are *V. inaequalis* (Cooke) and G. Wint (anamorph Spilocaea), which cause substantial losses to the apple crop globally. In orchards with a severe infection, productivity losses might reach up to 70% (Gupta 1990). It is currently one of the most important diseases economically, affecting yield and quality attributes in temperate areas of countries with freezing and wetting periods. Apple scab infection occurs on a variety of plant parts such as bud scales, young shoots, sepals, petioles, and leaves, as well as on fruits (Vaillancourt and Hartman 2000). The direct losses are caused primarily due to severe infection on pedicel and fruits (Ogawa and English 1991; Dar et al. 2020). The primary inoculum for disease dissemination sexually generated ascospores that are discharged from overwintered leaf litter and asexually generated conidia from overwintering on buds depending on the temperature and weather conditions (Holb et al. 2004a, b, 2005; Passey et al. 2017). The secondary infections from inoculum as a result of asexual conidia produced after primary infection can lead to enormous loss of quantities making fruits unmarketable and their frequent high occurrence can cause early leaf defoliation, diminished growth of the plant, and high yield penalty (MacHardy 1996). The best strategy to obtain high-grade scab-free fruit is through the usage of disease monitoring and forecasting systems, and several rounds of fungicide spray throughout the orchard (Nabi et al. 2023). But the issue, with the continuous use of fungicides, results in the evolution of fungicide-resistant strains. So environment-friendly and low-cost management systems are developed and the evaluation of resistant cultivars for major resistance genes and their deployment through Spatio-temporal for economically sustainable strategy (Mir et al. 2022, 2019; Dar et al. 2022).

The pathosystem follows typical gene for gene interaction and 17 R genes are identified within the *Malus* genus to impart resistance to apple scab caused by *V. inaequalis*. For instance, the presence of the *Rvi6* resistance gene in apples led to the emergence of two populations of *V. inaequalis*: one *virRvi6*, which infects cultivars with this resistance gene, and another, *avrRvi6*, which infects cultivars without it (Leroy et al. 2013; Michalecka et al. 2018; Papp et al. 2022). The population diversity and genetic structure between *avrRvi1* and *virRvi1* of *Venturia* nevertheless remain constant within an agro-ecological region even cultivars are planted with *Rvi1* and non-*Rvi1* genes in the same orchards (Dar et al. 2020).

Different polymorphic markers systems like RAPDs (Tenzer and Gessler 1999), RFLPs, AFLPs, and microsatellites also known as SSRs, are widely used to characterize the population diversity and structure of *V. inaequalis* population. A number of studies on the *V. inaequalis* population in several nations that produce apples from around the world have also been carried out (Tenzer and Gessler 1997; Guerin and Le Cam 2004; Guerin et al. 2004; Gladieux et al. 2008, 2010, 2011; Xu et al. 2013; Michalecka et al. 2018; Mansoor et al. 2019; Dar et al. 2020; Li et al. 2021; Papp et al. 2022; Sokolova et al. 2022; Lu et al. 2022). Delineating the genetic makeup of *V. inaequalis* populations sheds light on evolutionary divergence and lineage histories and helps to predict the emergence of new pathogen races, effective population size, dispersal potential, the likelihood of host range expansion, and virulence of pathogen races (Padder et al. 2011, 2013; Dar et al. 2022). Microsatellite-based genotyping and analyses of population networks could be helpful to delineate the population structure, genetic diversity, and evolution of natural populations (Dar et al. 2020). The characteristics of the natural processes that have influenced the structure of the population are reflected in population demographic metrics (Charlesworth and Charlesworth 2017). Recombination, gene conversion, lineage sorting, and deep coalescence are some examples of population dynamics that might create reticulate connections that can be studied using phylogenetic network studies (Posada and Crandall 2001). To ascertain whether the *V. inaequalis* population in Kashmir represents a single random matting or multiple populations or subdivisions due to geography/host association, so the study undertaken was to determine dynamics in population diversity and genetic structure of the population of fungus *V. inaequalis*.

Materials and methods

Sampling of diseased scab lesions from fruits and leaves and mono-conidial isolation

The most popular varieties viz., Red Delicious, Golden Delicious, Lal Ambri, and Royal Delicious were selected. Leaf and fruit samples showing typical lesions of scab were collected for isolations from April to August 2018 to 2020 from apple orchards of five locations, viz. Tral, Anantnag, Bejbehara, Pulwama, and Srinagar of Jammu and Kashmir (UT) of India. Most of the plantation at these locations manifested severe symptoms on the leaves and fruits (Fig. 1), with the Red Delicious host showing severe infections symptoms, whereas Golden Delicious showing the least. The samples were collected in triplicate and only

Fig. 1 Disease severity of apple scab pathogen on fruits and leaves sampled during experimetal study

one representative sample from a plant was used for isolation. Axenic cultures were obtained, maintained, and purified from 132 samples as described by Padder et al. (2011), Dar et al. (2020), Dar et al. (2015), and Xu et al. (2008). These fungal isolates showed no observable morphological differences that could lead to reliably distinguishing them in the field. For the study of pathogenicity, as described by Dar et al. (2015) in which the cultures were transferred onto the Potato Dextrose Agar (PDA) plates to grow them for 15 days, and then spore suspension was sprayed with an atomizer at the concentration of (2×106 spores/ml) (Fig. 2), and the inoculated varieties were incubated in a growth chamber for 21 days at 20 °C and 90% humidity. The scoring was performed after 21 days and classified using the Chevaliers scale of 0–4 (Chevalier et al. 1991).

Fungal genomic DNA extraction and genotyping

Two mycelial discs of 8 mm diameter from 15-day-old fungal cultures were inoculated into the conical flasks containing 150 cc PDB media (Potato Dextrose Broth) and incubated at 20 °C for 60 days. To characterize isolates with genetic markers mycelium was obtained by filtering it through sterile cheesecloth; agar plugs were then taken out, and the mycelium that was left was dried in an aseptic environment. Using the Cetyl Methyl Ammonium Bromide (CTAB) technique, DNA was isolated from mycelium (Murray and Thompson 1980). For genotyping, the working concentration of genomic DNA was kept at 20 ng/µl by adding NFW (Nuclease-free water). The purity and concentration of the DNA were assessed using an Eppendorf spectrophotometer at 260 nm.

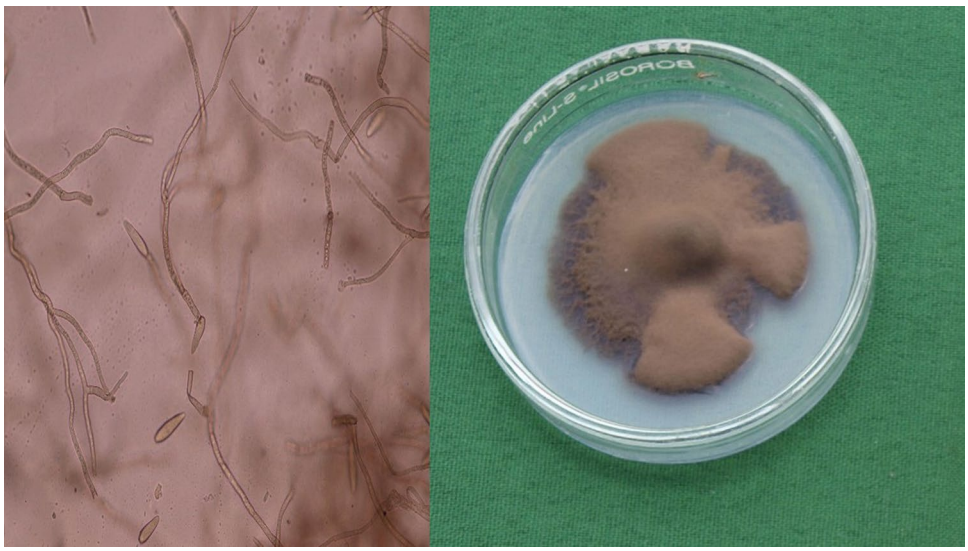

Fig. 2 Mono-conidial isolate of *Venturia inaequalis* with flame shaped conidia and culture plate

To genotype the 132 fungi populations, fourteen SSR primer pairs previously published by (Guerin et al. 2004; Tenzer and Gessler 1997) (Table S1) were employed. A 10 µl final volume was used for the PCR reaction, which contained 5X reaction buffer (2 µl), MgCl2, (3 mM), dNTPs (0.16 mM), Taq polymerase (0.2 µl), each primer (2 µM) and a DNA template (2 µl). PCR amplification was performed in 0.2 ml PCR tubes in a thermal cycler (T-Gradient Whatman Biometra) programmed for initial denaturation at 95 °C for 5 min, then followed by 35 cycles with denaturation, annealing, extension at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min, respectively, with 10 min final extension at 72 °C. Amplified PCR products were resolved at 3.5% Agarose gel, then visualized using the Alfa Imager gel documentation system (Alfa Imager EC, Protein Simple, USA),and the SSR primers showing consistency in polymorphism were photographed. For subsequent examination, the successfully amplified PCR bands were separated on 10% denatured PAGE and visualized using a silver staining procedure (Sambrook and Russell 2001) to examine and score allele the size of the PCR product.

Data analysis

On apple, a recombining population structure was anticipated because it is known that the fungus regularly reproduces sexually on this host's dead fallen leaves during winters (Gladieux et al. 2008; McHardy 1996). The fraction of common alleles and genetic distance (Nei and Chesser 1983) between the populations were assessed using software like GenALEx ver. 6.5 and PopGene (Peakal and Smouse 2012). The spatial distribution of genetic variation was estimated through classical estimates of Nei's (1973) gene flow and differentiation (Nm and Gst) and was also assessed based on Wright's genetic differentiation F-statistics (FST), measuring the population differentiation using the software Arlequin ver. 3.5 (Excoffier and Lischer 2010) and GenALEx ver. 6.5. In addition to the average allelic number per locus, private alleles and gene diversity averages (Nei 1987) were determined. Using PopGene and Arlequin, the heterozygosities such as expected heterozygosity (He) and observed heterozygosity (Ho) for each population were estimated. GenALEx ver. 6.5 and Arlequin ver. 3.5 were used to estimate AMOVA (analysis of molecular variance) to determine the distribution of variation patterns in population substructure at various spatial scales. Haplotype divergence is taken into account by the AMOVA framework (a matrix of squared distances) between all haplotype pairings to calculate variance components and ϕ-statistics (*F*-statistic analogs) (Excoffier et al. 1992). The goal was to estimate an optimal number of clusters that represent the population subdivision, therefore, hierarchical means of clustering was performed to *V. inaequalis* isolate populations and K-means clustering, which is an unsupervised machine learning technique in R software (R Core Team 2018), to assign *Venturia* isolates to a predetermined number of clusters (*K*) (MacQueen 1967) and determine the population ancestries. Given the occurrence of random mating, only one population should exit ($K=1$); nevertheless, if population divergence occurs, (*K*) is anticipated more than 1 and classified *V. inaequalis* isolate into 5 clusters (assume k clusters) fixed a priori. The R software (stats and facto_extra package) (R Core Team 2018) was used to determine k-means clustering using the function *k* means () to generate a graph of the clusters. We used the technical method of deciding *K* (Hartigan and Wong 1979) in which the largest average silhouette width, representing different *K*, indicated the best and ideal cluster number, and function [fviz_cluster() in facto_extra package] visualized the results.

To evaluate the genetic structure in *V. inaequalis* isolates from four hosts, network analyses were undertaken. First, the K2 parameter distances matrix across loci was created (Kimura 1980), and then using MEGA ver. 6.02 (Tamura et al. 2013) to display the resulting phylogeny to identify the ideal genetic cluster in the population. Additional clustering methods to evaluate the genetic structure in *Venturia* isolates from four hosts, plots of the most important axes were created using GenALEx's (PCoA) Principal Coordinate Analysis to verify grouping patterns (GenAlEx ver. 6.5 and Arlequin ver. 3.5 software) were employed. First, the distance matrix that resulted was put via. Principal Component Analysis (PCA) in R software. Second, we conducted a correlation study on different *V. inaequalis* isolate sets. To investigate if this aspect may contribute to distinction, a dataset was found using GenAlEx ver. 6.5 was collected from multiple hosts and visually studied through the Corrplot tool (Wei and Simko 2017) for R software. Additionally, the heatmap3 package (Zhao et al. 2014) for R software was used to show the linear genetic distance across isolates, enabling the utilization of genetic distance and variability measurements in connection to population genetics. GenAlEx ver. 6.5 was used to evaluate the Average (I) Shannon's index, (Np) private alleles, (Na) observed allelic number, (Ne) effective allelic number, Nei's (h) gene diversity, and (uh) unbiased gene diversity, and genetic distance and identity to depict allelic patterns between the isolates of five locations. Allelic patterns were further displayed with (Circlize package) in R (Gu et al. 2014) to better understand the genetic variation features across groups of samples obtained from each location.

Results

Distribution of genetic variation in *V. inaequalis*

During apple scab sampling, differential infections lesions were observed on cultivars viz., Red Delicious, Golden

Delicious, Lal Ambri, and Royal Delicious in Tral, Pulwama, Bejbehara, Anantnag and Srinagar. In totality, 132 *V. inaequalis* isolates were chosen (33 isolates from each variety such as; Red Delicious, Golden Delicious, Lal Ambri, and Royal Delicious to investigate the population diversity and genetic structure. Based on their geographical origin, *V. inaequalis* isolates were divided into five populations, such as Tral, Pulwama, Bejbehara, Anantnag and Srinagar (Table 1). For each isolate, genotype was defined as the allelic combination across 14 SSR-tested loci. The effective allelic number in the Tral, Pulwama, Bejbehara, Anantnag, and Srinagar populations was 5.011, 5.007, 5.058, 5.083, and 6.686 respectively, (Table 1). An estimate of diversity (I) Shannon-Wieners index, across five populations, ranged from 1.583 (Pulwama) to 1.849 (Srinagar), depicting the average diversity of *V. inaequalis* isolates within the populations (Table 1). The within individuals Ho (observed heterozygosity) was comparable for all *Venturia* populations but significantly lower than He (expected heterozygosity) (Table 1). The results based on AMOVA revealed that the maximum variation (94%) was distributed among individuals within a population, and a variation of (6%) was also distributed among regional populations from different areas (Table 2). The *Fst* value displayed medium differentiation between different populations observed (Table 2), which indicates that these five populations have a variable amount of immigration and emigration between each other and distribution of small population substructure at the geographic level with a total $FST = 0.055$ ($P = 0.001$).

Directional and amount of immigration and emigration among regional populations of *Venturia inaequalis*

We estimated genetic diversity indices from five *V. inaequalis* populations from host and location. The overall average Nei's gene diversity for five populations is 0.78. The highest diversity was observed for Srinagar (0.834 ± 0.441) population and the lowest values were observed for Bejbehara (0.733 ± 0.381) population (Table 3). Similarly, the highest average number of private alleles was in the Srinagar population (1.500 ± 0.489), while Bejbehara and Anantnag were having the lowest average number of private alleles (0.143 ± 0.097) (Table 3), there for indicated that is regular movement of planting materials and leads to the reduced number of private alleles. The unbiased estimates of Nei's genetic diversity calculate genetic distance or identity between populations with the assumption that both mutation and genetic drift lead to differences across all loci. The genetic

Table 1 Molecular diversity indices of five populations of 132 *Venturia inaequalis* isolates on the basis of location from different areas using 14 SSR marker data sets

Pop		N	Na	Ne	I	Ho	He	UHe	F
Tral	Mean	22.857	6.643	5.011	1.597	0.468	0.756	0.773	0.386
	SE	0.512	1.239	0.739	0.133	0.117	0.029	0.030	0.157
Pulwama	Mean	23.643	6.500	5.007	1.583	0.479	0.750	0.766	0.402
	SE	0.225	1.203	0.728	0.144	0.117	0.032	0.032	0.148
Bejbehara	Mean	23.143	6.714	5.058	1.598	0.456	0.743	0.760	0.449
	SE	0.718	1.056	0.771	0.148	0.125	0.035	0.036	0.152
Anantnag	Mean	23.571	7.143	5.083	1.603	0.448	0.736	0.752	0.432
	SE	0.309	1.222	0.896	0.147	0.119	0.041	0.042	0.149
Srinagar	Mean	34.500	8.857	6.686	1.849	0.462	0.794	0.806	0.457
	SE	0.552	1.537	1.115	0.164	0.126	0.031	0.032	0.151
Grand mean and SE over loci and pops									
Total	Mean	25.543	7.171	5.369	1.646	0.463	0.756	0.771	0.425
	SE	0.582	0.557	0.383	0.065	0.053	0.015	0.015	0.066

Sample size, *Na* no. of different alleles, *Ne* no. of effective alleles, *I* Shannon's Information Index, *Ho* observed heterozygosity, *He and UHe* expected and unbiased expected heterozygosity, *F* Fixation Index

Table 2 Analysis of molecular variance (AMOVA) for testing homogeneity across 132 *Venturia inaequalis* isolates of five locations

Source of variation	Degrees of freedom	Sum of squares	Variance component	Percentage of variation	*Fst* values
Among population	4	28.215	0.163	6%	0.055 *P* value = 0.001
Within population	127	354.317	2.790	94%	
Total	131	382.532	2.953	100%	

Table 3 Polymorphism survey of five populations of *Venturia inaequalis* collected from different locations based on 14 SSR marker data sets

Population	Percent polymorphism (%)	Average gene diversity	No. of private alleles
Tral	100.00	0.782 ± 0.410	0.571 ± 0.173
Pulwama	100.00	0.758 ± 0.391	0.643 ± 0.427
Bejbehara	100.00	0.733 ± 0.381	0.143 ± 0.097
Anantnag	100.00	0.776 ± 0.402	0.143 ± 0.097
Srinagar	100.00	0.834 ± 0.441	1.500 ± 0.489

distance was highest between Pulwama and Anantnag (0.468) means there is a low genetic identity between them and there will be a low flow of individuals between these two populations (locations). There was lower genetic distance between Pulwama and Bejbehara (0.312) means there is more genetic identity and more flow of individuals between the two populations (Table 4). The pair-wise estimates of differentiation among five regional populations indicated the highest (0.066) genetic differentiation between Pulwama and Anantnag populations, while the lowest genetic differentiation (0.041) was observed between Tral and Srinagar populations. Apart from this, the Srinagar population shared low values of genetic differentiation with other populations (Table 5). The classical estimates of gene flow (Nm) among populations across all loci were greater than 1, depicting regular gene flow i.e., immigration and emigration between populations with different intensities. The highest gene flow (Nm) of (17.047) along with the lowest divergence (Fst) (0.014) were observed for locus VITC2D and the lowest gene flow (1.315) along with the highest divergence (0.160) was obtained for locus Vitc2/16 suggesting the greater and lower flow of genes/individuals within them respectively (Table 6). The average genetic differentiation (Gst) among all samples at a single locus of the population was 0.058 and the values at a single locus varied from − 0.008 (VITC2D) to 0.132 (Vitc2/16) (Table 6).

Table 4 Pairwise comparison of Nei's genetic distance (below) and Identity (above) between five populations of *Venturia inaequalis* collected from different locations based on SSR data sets

Tral	Pulwama	Bejbehara	Anantnag	Srinagar	
0.000	0.640	0.656	0.631	0.704	Tral
0.447	0.000	0.732	0.626	0.644	Pulwama
0.422	0.312	0.000	0.714	0.638	Bejbehara
0.461	0.468	0.336	0.000	0.703	Anantnag
0.351	0.439	0.450	0.353	0.000	Srinagar

Table 5 Pairwise comparison of genetic differentiation between five populations of *Venturia inaequalis* collected from different locations based on SSR data sets

Tral	Pulwama	Bejbehara	Anantnag	Srinagar	
0.000					Tral
0.054	0.000				Pulwama
0.053	0.043	0.000			Bejbehara
0.062	0.066	0.052	0.000		Anantnag
0.041	0.050	0.053	0.046	0.000	Srinagar

Population genetic structure in *Venturia inaequalis*

Using the hierarchical method of clustering, three clusters were obtained viz. cluster I accommodate 70, cluster II 16 & cluster III 46 samples (Fig. 3A) and also in K-means clustering, different patterns of population structure were observed with K ranged from 2 to 5 (Fig. 3B). The augmented pattern of population structure was determined with average silhouette width approaches and the optimal number of K-means assigned *V. inaequalis* isolates into three clusters (Fig. 3C). Likewise, principal coordinate analysis (PCoA) grouped 132 V. *inaequalis* isolates into three clusters. In this analysis, (I) first Pc, (II) second Pc, and 3rd principal coordinate accounts for 4.75, 3.70, and 3.18 percent of the variation, respectively (Fig. 1). A 2D display of allelic distribution in isolates of *V. inaequalis* collected from five regions was observed using PCA (principal component analysis). The most principal significant axis (1 and 2) explained 13.9% and 8.6% of the variance, respectively (Fig. 4).

Cluster analysis based on the SSR data set using the neighbor-joining method in Mega software among five locations with 132 isolates generated dendrogram, presented a high level of genotypic diversity within populations of *V. inaequalis*. There were number of sub-cluster and *V. inaequalis* isolates from each geographic population were distributed randomly in different clusters (Fig. 5), there by indicating a high level of genetic variation. Across all pairwise comparisons of linear genetic diversity, there was a positive and negative correlation relationship between the isolates of each variety/host being tested (Fig. S2A). In general, intraspecific populations were compared with interspecific populations and were significantly visualized through a correlation Heatmap (Fig. S2B). As within interspecific comparisons across all and between populations of *V. inaequalis* isolates from a different location, there was a higher correlation. The isolates having a different host and location origin tend to have membership in these distinct clusters, thereby exhibiting a fraction of shared genetic ancestry. However, this correlation was observed both positively and negatively

Table 6 Various parameters of inbreeding, divergence, differentiation and gene flow among population averaged across all loci

All pops	Locus	Fis	Fit	Fst	Gst	Nm
	1tc1b	− 0.186	− 0.113	0.062	0.049	3.803
	Vitc1/82	− 0.028	0.025	0.052	0.036	4.603
	Vigtg10/95	0.952	0.957	0.120	0.092	1.829
	Vict1/130	− 0.341	− 0.185	0.116	0.107	1.900
	Vitc2/16	0.988	0.990	0.160	0.132	1.315
	Vitc1/2	− 0.104	− 0.032	0.065	0.051	3.588
	1aac4h	0.936	0.943	0.115	0.084	1.918
	Vica9/152	0.989	0.990	0.143	0.114	1.500
	1aac3b	− 0.315	− 0.255	0.046	0.035	5.225
	1tc1g	0.015	0.096	0.083	0.064	2.765
	VITC2D	0.335	0.345	0.014	-0.008	17.04
	VITG9/129	0.743	0.758	0.056	0.029	4.194
	VICA9/134	0.978	0.979	0.034	0.002	7.087
	IAAC4B	1.000	1.000	0.060	0.027	3.939

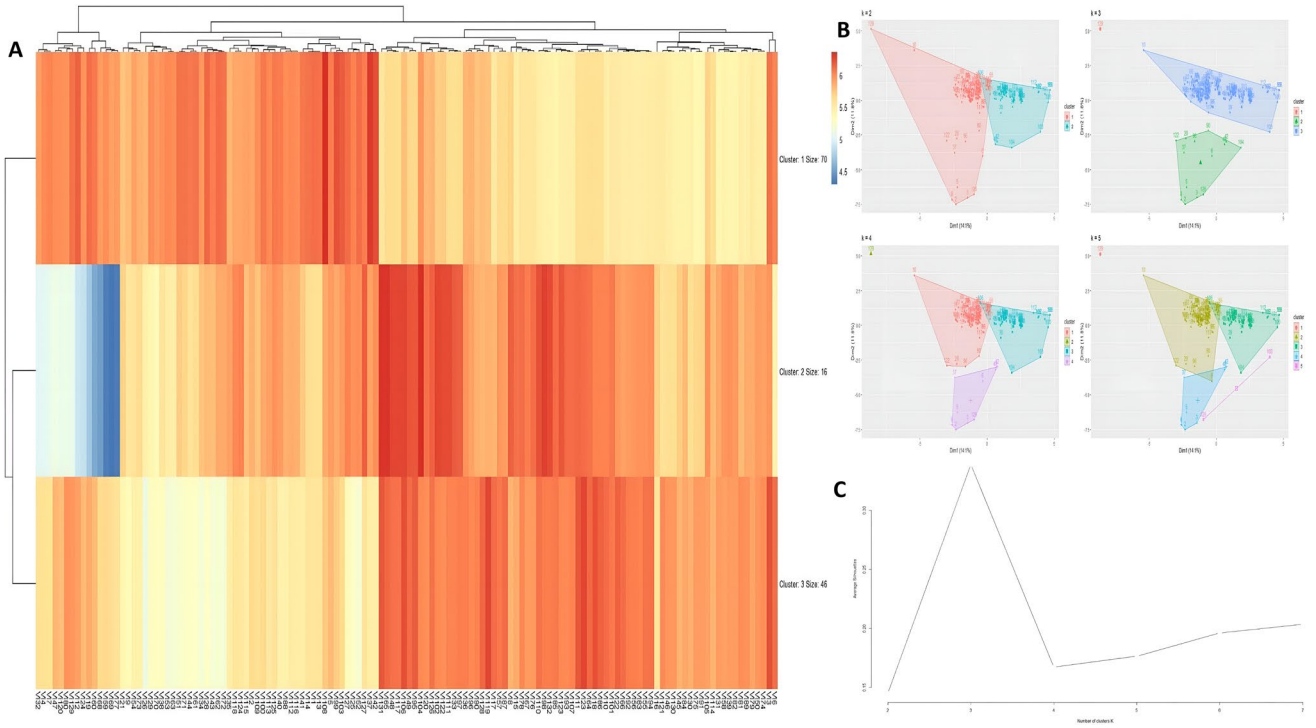

Fig. 3 **A** Hierarchical means of clustering represents three cluster in *Venturia* populations. **B** K-Means is simplest unsupervised learning algorithms that solve the well-known clustering problem and classified *Venturia inaequalis* isolate through a three number of clusters (assume k clusters) fixed a priori. **C** The largest average silhouette width, over different *K*, indicates the best number of clusters, A clustering can be characterized by the average silhouette width of individual entities

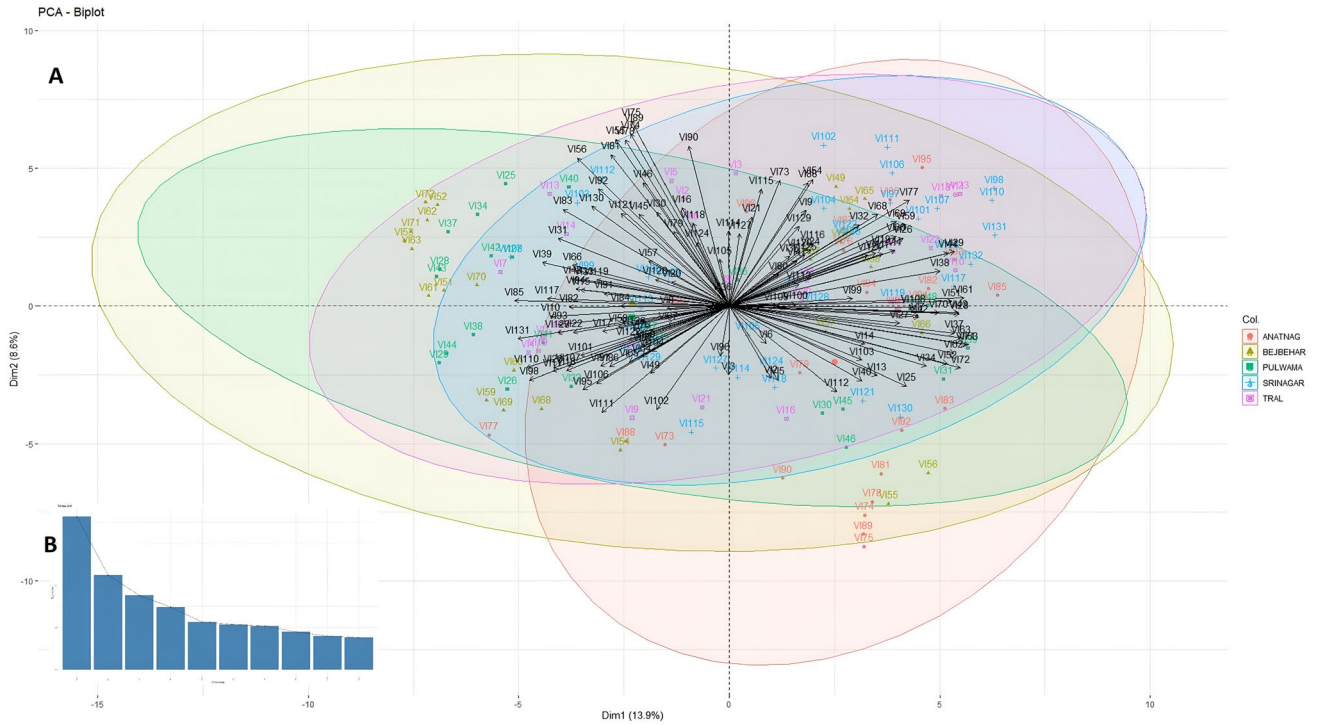

Fig. 4 Inter-class principal component analysis based on allelic profiles at 14 SSR markers for 132 isolates of *Venturia inaequalis* collected on five locations. Points represent genotypes; groups are labeled inside their 95% inertia ellipses. Bar plots represent eigenvalues. The *x*-axis is eigenvector 1 and the *y*-axis is eigenvector 2

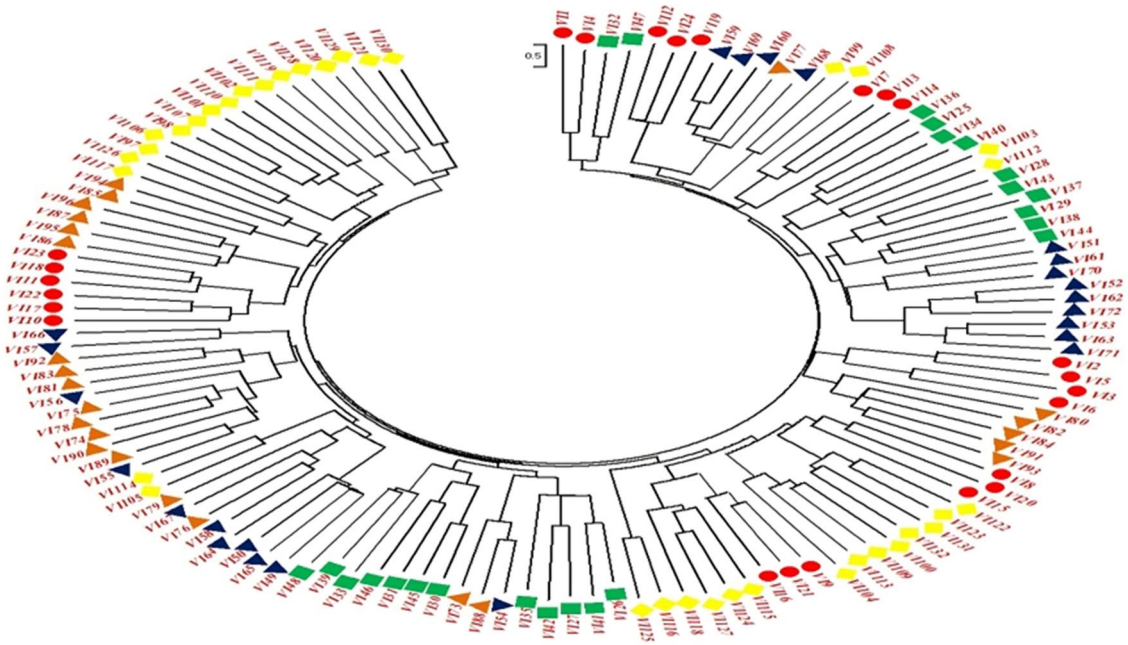

Fig. 5 Network analyses of 132 *Venturia inaequalis isolates* based on 14 markers data set among five locations using GenAlex and visualized through Mega

between all interspecific comparisons. Between more closely related species pairs comparisons were not stronger i.e., "within-clade" comparisons.

Discussion

Apples are typically planted and harvested at the same times in Kashmir's growing regions, and the exchange of apple products as food or planting material is common among and within growing regions. The climate of Kashmir situated in the Northern Himalayan region of India is conducive equally for the cultivation of apples and apple scabs. The introduction of new virulent races frequently undermines the intensive crop protection practices and host resistance, the most successful management tactic.

Genetic diversity

A comparison of the genetic makeup and diversity of spatio-temporally diverse isolates of *V. inaequalis* was done in an effort to comprehend the key evolutionary pathways and potential mechanisms determining the populations of scab pathogens in the Kashmir region. Using 14 microsatellite markers for genotyping, the *V. inaequalis* populations of Kashmir demonstrated a substantial amount of genetic variations within the population and minimal genetic variance across populations (Table 2). Similarly other studies on the genetic diversity of *V. inaequalis* in different countries presented same results (Gladieux et al. 2008; Ebrahimi et al. 2016; Michalecka et al. 2018; Li et al. 2021; Papp et al. 2022; Lu et al. 2022). The gene diversity and average number of private alleles were highest in the Srinagar population) and lowest values in the Bejbehara population (Table 3). The findings of Ebrahimi et al. (2016), Dar et al. 2020, Michalecka et al. 2018, and Meng et al. (2018), Mansoor et al. (2019) for various pathogens are in agreement with the results. Natural selection for local habitat fitness's, reduced immigration and emigration caused by heterogeneity in habitation and geographic barriers, both results in patterns of isolation-by-distance of genetic variation in nature (Feng et al. 2016; Petkova et al. 2016). We draw the conclusion that isolation by distance observed is brought about by geographic barriers, despite the fact that the molecular markers we utilized in the current investigation are neutral. The highest values of Nei's genetic distance were found between Pulwama and Anantnag (0.468) means there is low genetic identity between them and there will be a low flow of individuals between these two populations (locations) due to post-zygotic barriers or unavailability to immigrants. The lowest values of genetic distance were found between Pulwama and Bejbehara (0.312) depicting there is more genetic identity and more flow of individuals between the two populations and are in conformation with Ebrahimi et al. (2016), Dar et al. (2020). Population structure increases the longevity of plant diseases (Postma and van Noordwijk 2005) as it enhances the local subpopulations' capacity for adaptation in agricultural ecosystems (Hanski and Ovaskainen 2000).

In concurrence with previous studies, the present study's population diversity analysis of 132 isolates of *V. inaequalis* using 14 SSR markers indicated a significant degree of variation, indicating that SSR markers are appropriate for analyzing genetic diversity in this disease. This investigation reaffirms that SSR markers have 100% sensitivity for detecting polymorphism in *V. inaequalis* isolates (Gladieux et al. 2011; Leroy et al. 2013; Mansoor et al. 2019; Li et al. 2021; Papp et al. 2022). The literature has demonstrated the use of microsatellite markers for examining the genetic diversities in phyto-pathogenic fungi (Leroy et al. 2013; Xu et al. 2012; Gladieux et al. 2010). SSR markers have a high polymorphism character because of capability to recognize co-dominantly inherited length variation in DNA (repetitive) sequence and subsequently differentiate a sufficient allelic numbers (Matsuoka et al. 2002; Perseguini et al. 2011). Gene diversity indices (0.73–0.83) within and between populations suggest a high degree of genetic variation within and between populations. The genetic diversity indices in *V. inaequalis* groups reflect that the isolates within each population exhibit great genetic diversity and are not very similar to one another. Similar outcomes were attained in our earlier research (Padder et al. 2013; Dar et al. 2019; Mansoor et al. 2019). Furthermore, the pairwise fixation index (PWI) revealed modest genetic location-level differentiation. The differentiation i.e., genetic variations among the five populations indicated the highest variations among Pulwama and Anantnag populations and the lowest between Tral and Srinagar populations (Table 5) and the results were in confirmation with Gladieux et al. (2008). High gene diversity might be attributed to frequently occurring gene flow between populations, sexual recombination, and mutation. Additionally, the pathogen may be under selection pressure from specialized evolutionary forces including genotypes of varieties, regular fungicide sprays, different scab resistance genes, and environmental factors (Xu et al. 2008; Dar et al. 2020). Furthermore, it implies that there is regular gene movement at the cultivar level, most likely as a result of the transit of unhealthy and uncertified planting material. However, the considerable diversity of the *V. inaequalis* from the present study implies that the pathogen retains a significant number of alleles and supports previous findings that the *V. inaequalis* population exhibits medium genetic differentiation (Dar et al. 2020; Gladieux et al. 2008; Leroy et al. 2013; Michalecka et al.2018; Padder et al. 2013; Gladieux et al. 2010; Mansoor et al. 2019). However, the genetic variations

at the local level were just subtly different. It was observed that there is little genetic exchange between isolates of each host plant possibly due to high immigration-resistance selection, or host specificity of cultivars, which is a significant and pervasive barrier to genetic exchange between local and expanding populations.

Genetic structure

Three unique groups of *V. inaequalis* isolates were distinguished using principal coordinate analysis, with the first, second, and third components accounting for 4.75, 3.70 and 3.18 percent of the total variation (Suppli. Fig. 6), respectively. These findings concur with those of Gladieux et al. (2010), Leroy et al. (2013) and Michalecka et al. (2018), Papp et al. (2022), Lu et al. (2022). The assignment approach used in *K* means of clustering was also used to examine any host specialization within the *V. inaequalis* population. At ($K=3$), the clustering method supported three clusters and samples typically belong to three clusters. These clusters could not be distinguished in the PCoA results. This population was split into infected Malus x domestica cultivars based on the absence or presence of the *Rvi1* and *Rvi6* genes, as previously documented in commercial orchards (Dar et al. 2020; Gladieux et al. 2011; Michalecka et al. 2018; Papp et al. 2022). Numerous host selection pressures should have been applied to *V. inaequalis* populations given that several scab genes are found in wild apple species (Bus et al. 2011) as demonstrated by in*Rvi6, Rvi3 or Rvi5*genesby (Michalecka et al. 2018; Gladieux et al. 2011; Papp et al. 2022). The presence of the unknown resistance gene in Lal Ambri (F1 of Red delicious and Ambri), *Rvi1* in Golden Delicious, and various scab genes in Red Delicious & Royal Delicious varieties may have caused an identical split in scab pathogen in Kashmir valley, despite presence of numerous resistance specificities in a diverse genetic background of sampled varieties (Dar et al. 2020). Given the relatively different level of mixing between the two subpopulations, there may be pre and post-zygotic barriers to random matting or panmixis. There was no observable population genetic pattern within any subpopulation. It is, therefore, possible to the hypothesis that there is high virulence cost to produce a potent pre-zygotic barrier strong enough to separate *virRvi1*, F1 virulent and other infecting to various resistance sources, respectively, from subpopulations by itself. Other studies obtained same result from *virRvi6, Rvi3 or Rvi5*loci containing subpopulations (Leroy et al. 2013; Michalecka et al. 2018; Papp et al. 2022). The idea of additional structural resistance components that are shared by accessions from various kinds cannot be completely ruled out by the findings of the present investigation.

The population genetic structure of *V. inaequalis* on apples in Kashmir was confirmed by principal component analysis, and K means clustering of the pathogen. The isolates of five populations formed five major clusters based on principal component analyses and the isolates were randomly distributed randomly in clusters and locations (Fig. 4). The populations most likely resulted from recent mixing events, which are frequently found in hybrid zones when various populations interact by dispersal or by sharing a boundary (Papp et al. 2022; Lu et al. 2022; Dar et al. 2020; Gladeuix et al. 2011; Vaha and Primmer 2006). Gene flow within cropping zones is substantially larger than that between cropping zones due to the human-mediated dissemination of disease through contaminated areas in the former. As a result, the Cluster analysis showed that *V. inaequalis* populations had substantial genotypic diversity and that isolates from each population were randomly dispersed in various clusters (Fig. 5). Padder et al. (2011) obtained similar results in *V. inaequalis*. These results support the notion that pathogen reproductive structures are dispersed across the country, and they provide opportunity of demographic assemblage and stability. They may also hasten the adaptation of *Venturia* to management strategies like the use of resistance specificities and site-specific fungicides application. *V. inaequalis* is not the only organism that exhibits parallel patterns of spatial genetic structure, as other agricultural plant diseases have also been found to do so (Toale et al. 2015; Dar et al. 2020). These results defy logic and cannot be fully elucidated by natural dispersal, supposed to lead to more genomic similarities (lower genetic differentiation) among geographically nearby subpopulations. For locus Vitc2/16, reduced gene flow was detected, leading to higher genetic differentiation. There was less gene flow at locus Vitc2/16, which increased genomic divergence. With an average value of 0.058, the divergence at a single locus among all sample of populations (Gst) varied from − 0.008 (VITC2D) to 0.132 (Vitc2/16) (Table 6). According to Gladieux et al. (2008) fungi in the western Himalayas have maintained a high genetic diversity, indicating that they were probably brought there from central Asia, where *V. inaequalis* originally settled. Gladieux et al. (2008) discovered reduced dissimilarity in isolates of *V. inaequalis* from populations outside of central Asia and proposed that there is lost alleles during the migratory, departure, onset and settlement processes outside of their native area. The adaptation capacity is brought on by the constant genetic exchange from the nearby population or decreased inbreeding depression (Numminen et al. 2016; Richards 2000), which makes it easier

to replace subpopulations with superior genes. This study supports the premise that stringent quarantine regulations should be imposed both inside and outside of national borders to stop the spread of *V. inaequalis* to control this devastating plant disease effectively.

Conclusion

Since plant pathogen's genetic structure and population's high genetic diversity enhance the subpopulation's capacity to adapt in agricultural ecosystems and it was concluded there are three population, one colonize F_1 varieties and another one *Rvi1* varieties and a third to further known or unknown resistance sources. The population diversity and genetic diversity of the *Rvi1*, F_1-virulent, and third other populations are recognized as the host adaptation capacity which is brought on by the constant inflow of new genetic information from nearby subpopulations. Based on the K means of clustering and PCA, analysis of the variance of 132 *Venturia* isolates, is supported for sub-populations or non-random matting which does not make it easier to replace subpopulations with superior genes. The genetic exchange between individuals of two populations living in the same orchard could lead to the formation of hybrid swarms. Such pathogen reproductive strategies could lead to the possibility of subpopulation assemblages, demographic stability, and adaptation of *Venturia* pathogen to management strategies viz., use of resistance specificities and site-specific fungicide application. Keeping in view the economic and nutritional importance of apples in Kashmir valley, regular monitoring of ecological balance, genetic structure, and population diversity is necessary for sustainable disease management and it may be necessary to impose rigorous quarantine regulations both inside and outside of national boundaries to check the emergence of more virulent and fungicide resistant strains.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13205-023-03672-2.

Acknowledgements First author acknowledges Plant Virology and Molecular Pathology Laboratory, Division of Plant Pathology, SKUAST-Kashmir, Shalimar for providing necessary facilities to conduct the experiment. The authors extend their appreciation to the Researchers Supporting Project Number RSPD2023R686, King Saud University, Riyadh, Saudi Arabia.

Author contributions MSD, MA, and MDS contributed to the design of the experiments. MSD performed the experiments. MA and MDS guided in performing the experiments. MSD analyzed the results and wrote the manuscript. MSD, NUN, BB, SNB, HA, SR, AN, FAM, AZG, SM, and MM wrote and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

Bus V, Rikkerink E, Caffier V, Durel C, Plummer K (2011) Revision of the nomenclature of the differential hostpathogen interactions of Venturia inaequalis and Malus. Ann Rev Phytopathol 49:391–413

Charlesworth B, Charlesworth D (2017) Population genetics from 1966 to 2016. Heredity 118(1):2–9. https://doi.org/10.1038/hdy.2016.55

Chevalier M, Lespinasse Y, Renaudin S (1991) A microscopic study of the different classes of symptoms coded by the Vf gene in apple for resistance to scab (*Venturia inaequalis*). Plant Pathol 40(2):249–256

Dar MS, Mohammed I, Sofi TA, Ahanger FA, Shah MD, Ahmad M, Hamid A, Mir AA, Nabi A, Padder BA (2015) Distribution of apple scab race flora and identification of resistant sources against *Venturia inaequalis* in Kashmir. Plant Pathol J 14(4):196–201

Dar MS, Ahmad M, Shah MD, Padder BA, Bhat NA, Khan MA, Ahanger FA, Nabi A, Banoo A (2019) *Venturiacrataegi,* causing scab on *crataegussongarica*: morpho-molecular characterization and a record from India. Appl Biol Res 21(3):274–282z

Dar MS, Padder BA, Ahmad M, Sofi TA, Mir AA, Nabi A, Shah MD (2020) Population structure of *Venturiainaequalis*, a hemibiotrophic fungus, under different host resistance specificities in the Kashmir valley. Arch Microbiol 2020(8):2245–2253

Dar MS, Ahmad M, Nabi A, Shah MD, Padder BA (2022) Multiplex PCR based detection method for Venturia species infecting pome and stone fruits. Indian Phytopathol 75(4):941–950

Ebrahimi L, Fotuhifar KB, Nikkhah MJ, Naghavi MR, Baisakh N (2016) Population genetic structure of apple scab (*Venturia inaequalis* (Cooke) G. Winter) in Iran. PLoS ONE 11(9):e0160737

Excoffier L, Lischer HE (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour 10:564–567

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Feng B, Zhao Q, Xu J, Qin J, Yang ZL (2016) Drainage isolation and climate change–driven population expansion shape the genetic structures of Tuber indicum complex in the Hengduan Mountains region. Sci Rep 6:21811. https://doi.org/10.1038/srep21811

Gladieux P, Zhang XG, Afoufa-Bastien D, Valdebenito Sanhueza RM, Sbaghi M et al (2008) On the origin and spread of the scab disease of apple: out of Central Asia. PLoS ONE 3(1):e1455. https://doi.org/10.1371/journal.pone.0001455

Gladieux P, Zhang XG, Roldan-Ruiz I, Caffier V, Leroy T, Devaux M, Van Glabeke S, Coart ELS, Le Cam B (2010) Evolution of the population structure of Venturia inaequalis, the apple scab fungus, associated with the domestication of its host. Mol Ecol 19(4):658–674

Gladieux P, Guerin F, Giraud T, Caffier V, Lemaire C, Parisi L, Didelot F, Le-Cam B (2011) Emergence of novel fungal pathogens by ecological speciation: importance of the reduced viability of immigrants. Mol Ecol 20(21):4521–4532

Gu Z, Gu L, Eils R, Schlesner M, Brors B (2014) *Circlize* implements and enhances circular visualization in R. Bioinformatics 30(19):2811–2812

Guerin F, le Cam B (2004) Breakdown of the scab resistance gene Vf in apple leads to a founder effect in populations of the fungal pathogen *Venturia inaequalis*. Phytopathol 94:364–369

Guerin F, Franck P, Loiseau A, Devaux M, Le Cam B (2004) Isolation of 21 new polymorphic microsatellite loci in the phytopathogenic fungus *Venturia inaequalis*. Mol Ecol Notes 4:268–270

Gupta GK (1990) Apple scab: a review. Indian J Plant Protect 18:157–265

Hanski I, Ovaskainen O (2000) The metapopulation capacity of a fragmented landscape. Nature 404:55–758. https://doi.org/10.1038/35008063

Hartigan JA, Wong MA (1979) Algorithm AS 136: A K-means clustering algorithm. Appl Stat J R Stat Soc 28:100–108

Holb I, Heijne B, Jeger M (2004a) Overwintering of conidia of *Venturia inaequalis* and the contribution to early epidemics of apple scab. Plant Dis 88:751–757

Holb IJ, Heijne B, Withagen JCM, Jeger MJ (2004b) Dispersal of *Venturia inaequalis* Ascospores and disease gradients from a defined inoculum source. J Phytopathol 152:639–646. https://doi.org/10.1111/j.1439-0434.2004.00910.x

Holb IJ, Heijne B, Jeger MJ (2005) The widespread occurrence of overwintered conidial inoculum of *Venturia inaequalis* on shoots and buds in organic and integrated apple orchards across the Netherlands. Eur J of Plant Pathol 111:157–168

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Leroy T, Lemaire C, Dunemann F, Le Cam B (2013) The genetic structure of a *Venturia inaequalis* population in a heterogeneous host population composed of different *Malus* species. BMC Evol Biol 13:64

Li X, Tao F, Fan S, Li H, Yang J, Gao L (2021) Genetic diversity of *Venturia inaequalis* isolates (Apple scab) in China and UK determined by SSR markers. PLoS ONE 16:e0252865. https://doi.org/10.1371/journal.pone.0252865

Lu Z, Hui N, Wang L, Zheng G, Wang S, Li J (2022) Genetic diversity of *Venturia inaequalis* isolates from the scabs in apple trees in Gansu Province, China, using AFLP markers. PeerJ 10:e14512. https://doi.org/10.7717/peerj.14512

MacHardy WE (1996) Apple scab: biology, epidemiology, and management. American Phytopathological Society, St Paul

MacQueen J (1967) Some methods for classification and analysis of multivariate observations. In: Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability, volume 1: Statistics. University of California Press, Berkeley, pp 281–97. http://projecteuclid.org:443/euclid.bsmsp/1200512992

Mansoor S, Ahmed N, Sharma V, Jan S, Nabi SU, Mir JI, Mir MA, Masoodi KZ (2019) Elucidating genetic variability and population structure in *Venturia inaequalis* associated with apple scab diseaseusing SSR markers. PLoS ONE 14:e0224300. https://doi.org/10.1371/journal.pone.0224300

Matsuoka Y, Mitchell S, Kresovich S, Goodman M, Doebley J (2002) Microsatellites in Zea–variability, patterns of mutations, and use for evolutionary studies. Theor Appl Genet 104:436–450

Meng JW, He DC, Zhu W, Yang LN, Wu EJ, Xie JH, Shang LP, Zhan J (2018) Human-mediated gene flow contributes to metapopulation genetic structure of the pathogenic fungus *Alternaria alternata* from potato. Front Plant Sci 9:198

Michalecka M, Masny S, Leroy T, Pulawska J (2018) Population structure of *Venturia inaequalis*, a causal agent of apple scab, in response to heterogeneous apple tree cultivation. BMC Evol Biol 18:5–20

Mir S, Masoodi KZ, Sakina A, Bhat KM, Padder BA, Murtaza I, Nazir N, Shikari AB (2019) Marker based screening of F1 (Firdous × Gala) mapping population for major scab resistance gene Rvi6 in apple (Malus × Domestica). Int J Curr Microbiol App Sci 8(12):2641–2646

Mir S, Sakina A, Masoodi KZ, Bhat KM, Padder BA, Murtaza I, Nazir N, Bhat ZA, Wani SH, Shikari AB (2022) Mapping of quantitative trait loci for scab resistance in apple (Malus × domestica) variety Shireen. Mol Biol Rep 49(6):5555–5566

Murray M, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4326

Nabi A, Ahmad M, Shah MD, Padder BA, Dar MS, Banday S (2023) First report of Myclobutanil resistance and shift in sensitivity to difenoconazole and flusilazole in North-western Himalyan *Venturia inaequalis* populations. Australas Plant Pathol 52:13–22

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323. https://doi.org/10.1073/pnas.70.12.3321

Nei M (1987) Molecular evolutionary genetics, 1st edn. Columbia University Press, New York

Nei M, Chesser RK (1983) Estimation of fixation indices and gene diversities. Ann Hum Genet 47:253–259. https://doi.org/10.1111/j.1469-1809.1983.tb00993.x

Numminen E, Gutmann M, Shubin M, Marttinen P, Meric G, van Schaik W, Coque TM et al (2016) The impact of host metapopulation structure on the population genetics of colonizing bacteria. J Theor Biol 396:53–62. https://doi.org/10.1016/j.jtbi.2016.02.019

Ogawa JM, English H (1991) Diseases of temperate zone. Tree fruit and nut crops. University of California Publications, California

Padder BA, Shah MD, Mushtaq A, Sofi TA, Ahanger FA, Hamid A (2011) Genetic differentiation among populations of *Venturria inaequalis* in Kashmir: a north-western state of India Asian. J Plant Pathol 5(2):78–83

Padder BA, Sofi TA, Ahmad M, Shah MD, Hamid A, Saleem S, Ahanger FA (2013) Virulence and molecular diversity of *Venturia inaequalis* in commercial apple growing regions in Kashmir. J Phytopathol 161:271–279

Papp D, GangadharappaHarigondra S, Paredes C, Karacs-Végh A, Penksza KT, Jardi I, Papp V (2022) Strong genetic differentiation between generalist populations of *Venturia inaequalis* and populations from partially resistant apple cultivars carrying Rvi3 orRvi5. Diversity 14:1050. https://doi.org/10.3390/d14121050

Passey TAJ, Robinson JD, Shaw MW, Xu XM (2017) The relative importance of conidia and ascospores as primary inoculum of *Venturia inaequalis* in a southeast England orchard. Plant Pathol 66:1445–1451

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in excel. Population genetic software for teaching and research—an update. Bioinformatics 28(19):2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Perseguini JMKC, Chioratto AF, Zucchi MI, Colombo CA, Carbonell SA, Costa Mondego JM, Gazaffi R, Franco Garcia AA, de Campos T, de Souza AP, Rubiano LB (2011) Genetic diversity in cultivated carioca common beans based on molecular marker analysis. Genet Mol Biol 34(1):88–102. https://doi.org/10.1590/S1415-47572011000100017

Petkova D, Novembre J, Stephens M (2016) Visualizing spatial population structure with estimated effective migration surfaces. Nat Genet 48:94–100. https://doi.org/10.1038/ng.3464

Posada D, Crandall KA (2001) Intraspecific gene genealogies: Trees grafting into networks. Trends Ecol Evol 16(1):37–45

Postma E, van Noordwijk AJ (2005) Gene flow maintains a large genetic difference in clutch size at a small spatial scale. Nature 433:65–68. https://doi.org/10.1038/nature03083

R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org/

Richards CM (2000) Inbreeding depression and genetic rescue in a plant meta-population. Am Nat 155:383–394. https://doi.org/10.1086/303324

Sambrook J, Russell D (2001) Molecular cloning: a laboratory manual. Springer, London

Sivanesan A (1977) The taxonomy and pathology of *Venturia* species. Bibl Mycol 59:11–39

Sokolova O, Morocko-Bicevska I, Lacis G (2022) Genetic diversity of *Venturia inaequalis* in latvia revealed by microsatellite markers. Pathogens 11:1165

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2013) MEGA ver. 6.02: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Taole M, Bihon W, Wingfield BD, Wingfield MJ, Burgess TI (2015) Multiple introductions from multiple sources: invasion patterns for an important Eucalyptus leaf pathogen. Ecol Evol 5:4210–4220. https://doi.org/10.1002/ece3.1693

Tenzer I, Gessler C (1997) Subdivision and genetic structure of four populations of *Venturia inaequalis* in Switzerland. Eur J Plant Pathol 103:565–571

Tenzer I, Gessler C (1999) Genetic diversity of *Venturia inaequalis* across Europe. Eur J Plant Pathol 105:515–522

Vaha JP, Primmer CR (2006) Efficiency of model-based Bayesian methods for detecting hybrid individuals under different hybridization scenarios and with different numbers of loci. Mol Ecol 13:63–72. https://doi.org/10.1111/j.1365-294X.2005.02773.x

Vaillancourt LJ, Hartman JR (2000) Apple scab. Plant Health Instr. https://doi.org/10.1094/PHI-I-2000-1005-01

Wei T, Simko V (2017) R Package "Corrplot": visualization of a correlation matrix (version 0.84). https://github.com/taiyun/corrplot

Xu X, Yang J, Thakur V, Roberts A, Barbara DJ (2008) Population variation of apple scab (*Venturia inaequalis*) isolates from Asia and Europe. Plant Dis 92:247–252

Xu X, Harvey N, Roberts A, Barbara D (2012) Population variation of apple scab *(Venturia inaequalis*) within mixed orchards in the UK. Eur J Plant Pathol 135:97–104

Xu X, Harvey N, Roberts A, Barbara D (2013) Population variation of apple scab (*Venturia inaequalis*) within mixed orchards in the UK. Eur J of Plant Pathol 135:97–104

Zhang J, Dou Z, Zhou Y, He W, Zhang X, Zhang Y (2015) *Venturia chinensis* sp. nov., a new venturialean ascomycete from Khingan Mountains. Saudi J of Biol Sci 1:44–57

Zhao S, Guo Y, Sheng Q, Shy Y (2014) Heatmap3: an improved heatmap package with more powerful and convenient features. BMC Bioinform 15(Suppl 10):P16