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Genetic Differentiation among Populations of *Venturia inaequalis* in Kashmir: A North-Western State of India

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ABSTRACT

Apple scab, caused by *Venturia inaequalis*, is one of the most damaging diseases worldwide on apple and is currently managed mainly by scheduled applications of fungicides. The aim of the present study was to understand the pathogen population structure in Jammu and Kashmir which is important for breeding and deployment of resistant cultivars. Twenty-seven isolates of *V. inaequalis* were sampled from commercial apple growing areas to estimate differences in pathogen populations using Random Amplified Polymorphic DNA (RAPD) 10 -and 20-mer primers. RAPD data analysis grouped 27 isolates in to three major clusters accommodating 10, 10 and 7 isolates each. The categories did not follow any geographic or source cultivar pattern. Allele frequencies among the three populations varied from 0.00 to 1.00. The average genetic diversity within each population (H_S) over all loci studied was 0.21, 0.23 and 0.20 in Ganderbal, Pulwama and Srinagar, respectively thereby showing high genetic diversity within each population. The average genetic differentiation at a single locus among all sampled populations (G_{ST}) was 0.12. The overall gene flow (Nm) was 3.54 while the gene flow values at a single locus ranged from 0.70 to 69.32. Pair-wise genetic differentiation values (F_{ST}) among all loci were low, thereby indicating high diversity among the three populations. Analysis of molecular variance (AMOVA) obtained after clustering the isolates at district level showed highly significant genetic differentiation among populations with 5.46 and 94.54% genetic variability recorded among and within district populations, respectively. Population genetic analysis of *V. inaequalis* is the first molecular analysis of this pathogen from the India and especially from Jammu and Kashmir, a north-western Himalayan state of India.

Key words: Apple scab, diversity, *Malus* x *domestica*, random amplified polymorphic DNA, population structure

INTRODUCTION

Apple (*Malus* x *domestica*) grows well in the hilly regions of India at altitudes ranging from 1500 to 2700 m asl. The agro-climatic conditions of Jammu and Kashmir state of India are suitable for growing different varieties of apple which constitute about 91% of its total fruit production. However, the crop, as also the ornamental *Malus* spp., suffer quantitative and qualitative losses on account of frequent epidemics of scab caused by a fungal pathogen *Venturia inaequalis* (Cooke) G. Wint. which has been associated since 1930 with the crop in Jammu and Kashmir (Nath, 1935)

where its epiphytotics have been a regular feature since 1973 (Joshi *et al*., 1973). Reliance on six to eight fungicide foliar sprays and the use of scab-resistant cultivars are the main strategies used for scab control (Ellis *et al*., 1998; Koeller, 1994). However, these strategies have encountered difficulties owing to evolution of fungicide-resistant strains and new resistance-breaking phenotypes. As a consequence, scientists determined the genetic basis for resistance to several fungicides (Jones *et al*., 1987) and characterized new races (Parisi and Lespinasse, 1996), leading to better understanding of the resistance mechanism to develop anti-resistance control strategies. Variability in *V. inaequalis* has been assessed using a standard differential set of cultivars, allowing consistent comparison of data among different research groups for common race spectrum. Eight pathotypes of *V. inaequalis* have been identified worldwide (Benaouf and Parisi, 2000; Bus *et al*., 2005; Heaton *et al*., 1991; MacHardy *et al*., 2001; Parisi *et al*., 1993). However, categorization of the fungus isolates into physiologic races based on reaction on differentials depends on environmental conditions, spore count and the inoculation conditions that may vary from one laboratory to another. In order to overcome this problem, application of molecular markers has proved useful in assessing variability at genotypic level which allows direct comparison between pathogenic phenotype and genotype. These markers are almost unlimited in number and are not affected by the environment (Williams *et al*., 1990). The distinct pattern obtained for each individual can be taken as the specific fingerprint that describes and identifies them. A high level of genetic diversity has been reported in *V. inaequalis* (Guerin and Le Cam, 2004; Melounova *et al*., 2004; Schnabel *et al*., 1999; Tenzer and Gessler, 1997, 1999). *Venturia inaequalis* isolates obtained from different apple cultivars planted in the same orchard in the UK differed significantly in their virulence characteristics (Barbara *et al*., 2008) as well as at the molecular level, based on AFLP analysis, whereas the isolates from different cultivars or regions in China did not (Xu *et al*., 2008). Analysis of microsatellite profiles of *V. inaequalis* samples from five continents suggested that the fungus originated in Central Asia and is now well established worldwide displaying high within-population diversity (Gladieux *et al*., 2008). More information on within-population variability is needed to understand better the forces acting on the host pathogen co-evolution.

In India, no studies have been carried out on the population structure of *V. inaequalis*. Hence, the present investigations were carried out to determine how variability in the causal fungal pathogen, *V. inaequalis*, is structured in the Kashmir valley with an objective to determine the population structure of the fungus using RAPD 10-and 20-mer primers.

MATERIALS AND METHODS

Twenty-seven isolates of *V. inaequalis* were collected during June to August 2009 and 2010 from commercial apple growing areas of seven districts viz., Anantnag, Pulwama, Shopian, Kulgam, Ganderbal, Srinagar and Baramulla of the Jammu and Kashmir state of India and their single spore isolations made on water agar medium. The germinated single spores were transferred on to the Potato Dextrose Agar slants, incubated at 20°C and maintained for further use.

Total genomic DNA of each isolate was extracted using CTAB method (Murray and Thompson, 1980). Initially six isolates of *V. inaequalis* were screened with forty-six 10-mer and twelve 20-mer RAPD primers (Operon Technology) for polymorphism. The primers (OPA-03, OPA-04, OPA-13, U1, U5 and U9) showing maximum consistency of polymorphism among the 2 samples of DNA preps from isolates were selected for RAPD profiling of all the isolates. PCR was carried out

in 0.2 mL PCR tube with 25 μL reaction volume containing 1 X buffer (20 mM Tris-HCl pH 8.0; 50 mM KCl, MBI, Fermentas Life Sciences), 1.5 mM $MgCl_2$, 2.0 μL dNTP mix (0.2 mM, MBI, Fermentas Life Sciences), 1 U of *Taq* DNA polymerase (MBI, Fermentas Life Sciences, 5 U μL^{-1}), 2 μL of DNA tempelate (20 ng), 5 pmols of primer and 17.3 μL of sterilized distilled water. The reaction mixture was vortexed and centrifuged in a microfuge (Thermo Scientific, Thermo electron Corporation). Amplifications were performed using thermal cycler (Whatman Biometra, T-Gradient, Goettingen, Germany) programmed for initial denaturation at 94°C for 5 min followed by 40 cycles at 94°C for 1 min, 37/55°C (37°C for deca-mer and 55°C for twenty-mer) for 1 min, 72°C for 2 min and a final extension at 72°C for 5 min using fastest ramp time between transitions. The amplified PCR products were resolved by electrophoresis using 1.2% (w/v) agarose gel in 1X Tris borate EDTA buffer (0.5 M Tris, 0.05 M boric acid, 1 mM EDTA, pH 8.0). The gels were stained with ethidium bromide (0.5 μg mL^{-1}). DNA ladders of 100 bp and Lambda DNA/EcoR1+Hind-III Marker (MBI Fermentas Life Sciences) were used as markers. The gels were run at 80 V for one and a half hour using Consort Power Pack system (Consort EV 215) and the gel images captured using a combination of Ultracam Digital Imaging (A 650 Canon Camera) and Electronic UV Transilluminator (Ultra.Lum.Inc, 1480 N, Claremont Boulevard, Claremont, CA).

Bands that could be scored univocally for their presence and absence were included in the analysis. The binary matrices were analyzed by DARwin 5.0 (Perrier *et al*., 2003) and the dissimilarity coefficients used to construct dendrogram using neighbor joining programme, selecting the unweighted pair-group arithmetic mean method (UPGMA). The dendrogram with best fit to dissimilarity matrix based on cophenetic value (COPH) chosen from Tree Fit Criterion Dialog Box. The alleles frequencies, total genetic diversity (H_T) and genetic diversity within (H_S) and among (G_{ST}) population were estimated and used to calculate standard population genetic statistics using POPGENE (version 1.31, Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada). Heterogeneity in allele frequencies among samples was tested using the likelihood ratio chi-square statistic 'G^2' (Brown, 1996; Hamelin *et al*., 1994). Pair-wise genetic differentiation (G_{ST}) among sub-populations was estimated using Nei's coefficient of differentiation 'G_{ST}' (Nei, 1973). The population structure was evaluated using the analysis of molecular variance (AMOVA) model in the ARLEQUIN ver 3.0 software (Excoffier *et al*., 2005) and fixation indices F_{ST} analogous to F_{ST} (Hudson *et al*., 2002) estimated. The analysis used a hierarchical structure in which all the isolates belonging to each district were considered as a population except the isolates from district Baramulla and Budgam which could not be included in the analysis owing to paucity of sufficient number of isolates. The isolates from district Shopian were merged with those from district Pulwama on account of their similar topographical and geographical features. The statistical significance of the total and pair-wise fixation indices was estimated by comparing the observed distribution with the null distribution generated by 10,000 permutations of the data matrix. Multiple tests of the same null hypothesis were subject to table-wide sequential Bonferroni correction to avoid elevated Type I error.

RESULTS

Of the six isolates of *V. inaequalis* screened with 10-mer and 20-mer primer, three 10-mers (OPA-03, OPA-04, OPA-13) and three 20-mers (U1, U5 and U9) giving consistent banding patterns were selected for RAPD analysis of 27 isolates of *V. inaequalis*. The number of bands obtained with different primers ranged from 8 to 14, of which 8 to 12 were polymorphic. In all, a total of 25 consistently reproducible RAPD bands were obtained with all the primers of which 22 (88%) were polymorphic. Cluster analysis of the RAPD bands generated dendrograms with a cophenetic

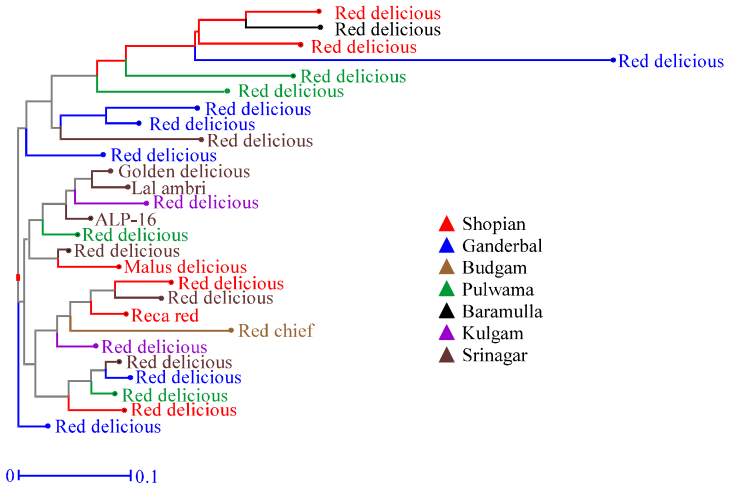

Fig. 1: Dendrogram of 27 isolates of *V. inaequalis* generated by UPGMA. Scale at the bottom depicts the dissimilarity obtained using neighbour joining in DARwin 5.0

correlation coefficient of 0.84 which substantiate the accuracy of dendrogram. The dendrogram analysis of 27 isolates revealed a high genotypic diversity within *V. inaequalis* populations. Three major clusters were obtained using neighbor joining method in DARwin5.0. Cluster I and III accommodated 10 isolates each, where as cluster II contained 7 isolates. Perusal of the dendrogram (Fig. 1) further reveals that the isolates could not be categorized either on the basis of their geographical distribution or the cultivar from which isolated.

Three populations of *V. inaequalis* based on 24 isolates collected from district Ganderbal, Pulwama and Srinagar subjected to population genetic analysis revealed 20 out of 25 loci as significantly different (χ^2 test, p = 0.05) among all the populations (Table 1). Allele frequencies among the populations varied from 0.00 to 1.00. The allele frequency in Ganderbal and Pulwama populations ranged between 0.00 and 1.00, whereas it ranged between 0.12 and 1.00 in Srinagar population (Table 1). The average genetic diversity within each population (H_S) over all loci studied was 0.21, 0.23 and 0.20 in Ganderbal, Pulwama and Srinagar, respectively, thereby showing high genetic diversity within each population (Table 1).

The average genetic differentiation at a single locus among all sampled populations (G_{ST}) was 0.12, even though the value at single locus varied from 0.007 to 0.42 (Table 1). The lower G_{ST} values of 0.007, 0.02, 0.03, 0.05 and 0.0.7 were found at L11, L1, L3, L4, L5, L9, L14, L16 and L25 loci, respectively (Table 1). To obtain the information on the loci under immigration, gene flow (Nm) was calculated across loci. The overall gene flow (Nm) was 3.54, while the gene flow values at a single locus ranged from 0.70 to 69.32. The results obtained show an Nm value greater than 1 indicating little differentiation.

In order to obtain information regarding diversity within populations, pair-wise genetic differentiation values (F_{ST}) among all the loci were calculated (Table 2). Most of the values obtained between a pair of populations were low thereby indicating high diversity among the populations. Value higher than 0.10 (i.e., 0.12) was found only for populations between Ganderbal and Srinagar. To elucidate the diversity existing within each population, analysis of molecular variance (AMOVA) was performed to ascertain the percent diversity among the populations

Table 1: Allele frequencies, population diversity and gene flow estimates of RAPD markers in three populations of *V. inaequalis* collected from three locations of Kashmir valley

	Allele frequencies in *V. inaequalis* populations**			Genetic diversity			
Locus name	Ganderbal	Pulwama	Srinagar	Total (H_T)	Within (H_S)	Among (G_{ST})	Gene flow (Nm)
L1*	0.66	0.60	0.75	0.44	0.43	0.02	28.75
L2*	0.00	0.00	0.12	0.08	0.08	0.09	5.25
L3*	0.33	0.20	0.37	0.42	0.41	0.03	18.44
L4*	0.66	0.40	0.62	0.50	0.46	0.05	8.46
L5*	0.33	0.20	0.50	0.45	0.42	0.07	6.99
L6*	0.50	0.60	0.87	0.45	0.41	0.11	3.97
L7	1.00	1.00	1.00	0.00	0.00	***	***
L8*	1.00	0.75	1.00	0.15	0.12	0.18	2.25
L9*	0.60	0.33	0.62	0.50	0.46	0.07	6.66
L10*	0.80	0.55	0.87	0.38	0.34	0.09	4.62
L11*	0.16	0.22	0.25	0.33	0.33	0.007	69.32
L12*	0.60	0.88	0.87	0.33	0.30	0.10	4.22
L13	1.00	1.00	1.00	0.00	0.00	***	***
L14*	0.00	0.20	0.12	0.19	0.18	0.07	6.60
L15*	0.00	0.30	0.62	0.43	0.30	0.30	1.13
L16*	0.00	0.20	0.12	0.19	0.18	0.07	6.60
L17*	0.00	0.30	0.75	0.45	0.26	0.42	0.70
L18*	0.00	0.10	0.12	0.14	0.13	0.04	11.39
L19*	0.83	1.00	1.00	0.10	0.09	0.12	3.75
L20*	0.83	1.00	1.00	0.10	0.09	0.12	3.75
L21*	0.83	1.00	1.00	0.10	0.09	0.12	3.75
L22	1.00	1.00	1.00	0.00	0.00	***	***
L23	1.00	1.00	1.00	0.00	0.00	***	***
L24*	0.50	0.90	1.00	0.32	0.23	0.29	1.21
L25*	1.00	0.90	1.00	0.06	0.06	0.07	6.75
Mean	0.21	0.23	0.20	0.24	0.21	0.12	3.54
Standard Deviation	0.213	0.19	0.19	0.032	0.026		

*χ^2 values were significant at $p<0.05$ **frequency of RAPD band (Plus allele)

Table 2: Pair wise genetic differentiation (F_{ST}) between three *V. inaequalis* populations

Population	Ganderbal	Pulwama
Pulwama	0.01	-
Srinagar	0.12	0.06

Table 3: Summary of analysis of molecular variance (AMOVA) of three *V. inaequalis* populations from different regions of Kashmir valley

Source of variation	df	Sum of squares	Variance component	Percentage of variation	F_{ST}	p-value
Among districts	2	7.742	0.154	5.46	0.054	<0.001
Within district	21	55.967	2.665	94.54		
Total	23	63.709	2.819			

(Table 3). The analysis revealed highly significant (F_{ST} = 0.054, $p<0.001$; 50175 permutations) genetic differentiation, with 5.46 and 94.54% of the genetic variability occurring among and within district populations, respectively.

DISCUSSION

Scab (*V. inaequalis*) is globally the most important disease in apple '*Malus X domestica*' (MacHardy, 1996; Gupta, 1990; Verma and Sharma, 2003) and was recorded in Jammu and Kashmir state of India first in 1930 (Nath, 1935), experiencing sporadic occurrence and affecting indigenous cultivar 'Ambri'. The frequent epiphytotics of the disease since 1973, with devastating effects on apple production in the region, made it a house hold name (Gupta, 1990; Joshi *et al*., 1973) leading to its declaration as a disease of national importance. Even then, diminutive work on assessing the population structure of the causal fungus has been carried out in India. An attempt to ascertain the population structure of *V. inaequalis* at molecular level was, therefore, made and can be considered as the first molecular analysis of this pathogen in India.

RAPD (10 and 20 mer primers) profiling of 27 *V. inaequalis* isolates of Kashmir in India revealed high level of diversity without congruence of the phenogram with either the geographic location or the apple cultivars from which isolates were obtained. The high genetic differentiation between isolates may be due to the history of apple cultivation in the region and the vertical and horizantal expansion of host density increased over years of production. Until 50 years ago, apples were grown in the meadow system where trees were planted throughout the landscape often with wide spaces between trees. Splash dispersal of pathogen conidia is mainly important for dispersal within or between neighboring trees, whereas wind mediated long-distance dispersal of its airborne ascospores or infected leaf litter determine recurrence/re-infection. Higher the tree density, higher the probability of occurrence of the disease. The intensive cultivation with the use of cost intensive inputs have further aggravated the situation. However, low-input apple cultivation in house gardens or on meadows could serve as reservoirs for *V. inaequalis*, preventing effects of genetic drift. This could also be a reason why re-colonization of newly planted apple trees apparently occurs quite quickly, since there has never been a scab-free orchard in the valley.

The isolates from Red delicious cultivar collected from various commercial apple growing areas during the present study differed significantly among and within populations and were much diverse than expected, suggesting, therefore, that several evolutionary forces might be acting on the pathogen. Firstly the different cultivars viz., Golden Delicious, Red Delicious, Ambri, Cox orange Pippien, Shereen, Firdous etc. present in our orchard ecosystem might have exerted selection pressure on *V. inaequalis*. Secondly, the sexual recombination may be the major reason for creating high genetic diversity.

The low values of H_S, G_{ST} and pair-wise genetic differentiation among the three populations of *V. inaequalis* from the valley indicate that the pathogen maintains high genetic and genotypic diversity which is close to that observed in Central Asia and Europe (Gladieux *et al*., 2008; Guerin *et al*., 2007; Guerin and Le Cam, 2004; Melounova *et al*., 2004; Tenzer *et al*., 1999; Tenzer and Gessler, 1997, 1999). Maintenance of high genetic diversity by the fungus in this part of the world gives an indication about the probable introduction of fungus from Central Asia, the centre of origin of *V. inaequalis* (Gladieux *et al*., 2008) and Europe as most of the growers cultivate Red Delicious cultivar along with the cultivars from European origin. Gladieux *et al*. (2008) observed lower levels of variation in *V. inaequalis* from non-Central Asian populations and suggested that these populations have lost alleles in the process of movement, arrival and establishment outside their native range. Contrary to this, the present study clearly shows that the fungus is highly diverse in the Kashmir valley and all three populations were far from being clonal and none displayed extreme reduction as did *Phytophthora infestans* (Goodwin *et al*.,1994), *P. ramorum* (Ivors *et al*., 2006), *Sphaeropsis sapinea* (Burgess *et al*., 2001), *Ustilago scitaminea*

(Raboin *et al*., 2007), *Magnaporthe grisea* (Zeigler, 1998), *Ceratocystis fimbriata* f. *platani* (Engelbrecht *et al*., 2004) and *Fusarium circinatum* (Wikler and Gordon, 2000). Rather, the variability observed in *V. inaequalis* samples could be compared with that reported for the cereal pathogens *Rhynchosporium secalis* or *Stagonospora nodorum* outside their centre of origin (Stukenbrock *et al*., 2006; Zaffarano *et al*., 2006).

Loci with G_{ST} values higher than 0.1 indicate that the loci might be under selection pressure or else linked to loci under selection in some populations. However, *V. inaequalis* undergoes an annual sexual stage allowing crossovers and recombine allele combinations generating new haplotypes. Selection can thus act on individual loci without affecting drift or gene flow at unlinked loci. Looking at the differentiation between each pair of populations, high F_{ST} values (F_{ST}>0.1) observed during the study between populations from Srinagar and Ganderbal only with small geographic distance gives the clue that very frequent short distance gene flow occurs in the Jammu and Kashmir and the dispersal over longer distances occurs frequent enough to counteract genetic differentiation due to genetic drift or local selection.

The high genetic diversity between *V. inaequalis* isolates of Kashmir with no congruence of the phenogram with the geographic locations or the apple cultivars is suggestive of the occurrence of frequent sexual recombinations in the pathogen and / or exertion of selection pressure or by either cultivation of scab resistant cultivars or the application of foliar fungicidal sprays against the disease. There is immediate need to further characterize and quantify this variability in the pathogen to ultimately base on the breeding programmes for evolving durable resistant apple cultivars. Adoption of holistic approach such as integrated disease management strategy for containing this epidemic disease shall help in easing out selection pressure on the pathogen to avoid further increase in its genetic divergence in the region.

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REFERENCES

Barbara, D.J., A.L. Roberts and X.M. Xu, 2008. Virulence characteristics of apple scab (*Venturia inaequalis*) isolates from monoculture and mixed orchards. Plant Pathol., 57: 552-561.

Benaouf, G. and L. Parisi, 2000. Genetics of host–pathogen relationships between *Venturia inaequalis* races 6 and 7 and Malus species. Phytopathology, 90: 236-242.

Brown, J.K.M., 1996. The choice of molecular marker methods for population genetic studies of plant pathogens. New Phytol., 133: 183-195.

Burgess, T., B.D. Wingfield and M.J. Wingfield, 2001. Comparison of genotypic diversity in native and introduced populations of *Sphaeropsis sapinea* isolated from *Pinus radiata*. Mycol. Res., 105: 1331-1339.

Bus, V.G., F.N. Laurens, W.E. van De Weg, R.L. Rusholme and E.H. Rikkerink *et al*.,, 2005. The *Vh8* locus of a new gene-for-gene interaction between *Venturia inaequalis* and the wild apple *Malus sieversii* is closely linked to the *Vh2* locus in *Malus pumila* R12740-7A. New Phytol., 166: 1035-1049.

Ellis, M.A., D.C. Ferree, R.C. Funt and L.V. Madden, 1998. Effects of an apple scab-resistant cultivar on use patterns of inorganic and organic fungicides and economics of disease control. Plant Dis., 82: 428-433.

Engelbrecht, C.J., T.C. Harrington, J. Steimel and P. Capretti, 2004. Genetic variation in Eastern North American and putatively introduced populations of *Ceratocystis fimbriata* f. platani. Mol. Ecol., 13: 2995-3005.

Excoffier, L., G. Laval and S. Schneider, 2005. Arlequin version 3.01: An integrated software package for population genetics data analysis. Evol. Bioinform. Online, 1: 47-50.

Gladieux, P., X.G. Zhan, D. Afoufa-Bastien, R.M. Valdebenito Sanhueza, M. Sbaghi and B. Le Cam, 2008. On the origin and spread of the scab disease of apple: Out of Central Asia. PLoS ONE, 3: e1455-e1455.

Goodwin, S.B., B.A. Cohen and W.E. Fry, 1994. Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. Pro. Nat. Acad. Sci., USA, 91: 11591-11595.

Guerin, F., P. Gladieux and B.L. Cam, 2007. Origin and colonization history of newly virulent strains of the phytopathogenic fungus *Venturia inaequalis*. Fun. Gen. Biol., 44: 284-292.

Guerin, F. and B. Le Cam, 2004. Breakdown of the scab resistance gene *Vf* in apple leads to founder effect in populations of the fungal pathogen *Venturia inaequalis*. Phytopathology, 94: 364-369.

Gupta, G.K., 1990. Apple scab: A review. Indian J. Plant Pro., 18: 157-265.

Hamelin, R.C., R.L. Doudrick and W.L. Nance, 1994. Genetic diversity in *Cronartium quercuum* f.sp. *fusiforme* on loblolly pines in Southern US. Curr. Genet., 26: 359-363.

Heaton, J.B., S.R. Dullahide, L.B. Baxter and A.D. Mcwaters, 1991. Race determination of nine Australian isolates of the apple black spot fungus *Venturia inaequalis*. Aust. Plant Pathol., 20: 139-141.

Hudson, R.R., M. Slatkin and W.P. Maddison, 2002. Estimation of levels of gene flow from DNA sequence data. Genetics, 132: 583-589.

Ivors, K., M. Garbelotto, I.D.E. Vries, C. Ruyter-Spira, B.T. Hekkert, N. Rosenzweig and P. Bonants, 2006. Microsatellite markers identify three lineages of *Phytophthora ramorum* in US nurseries, yet single lineages in US forest and European nursery populations. Mol. Ecol., 15: 1493-1505.

Jones, A.L., E. Shabi and G.R. Ehret, 1987. Genetics of negatively correlated cross-resistance to a N-phenylcarbamate in benomyl-resistant *Venturia inaequalis*. Can. Plant Pathol., 9: 195-199.

Joshi, N.C., A.G. Malik, M.H. Kaul and S.K. Anand, 1973. Some observations on the epidemic of scab disease of apple in Jammu and Kashmir during 1973. Indian Phytopath., 28: 288-289.

Koeller, W., 1994. Chemical control of apple scab-status quo and future. Norwegian J. Agric. Sci., 17: 149-170.

MacHardy, W.E., 1996. Apple Scab: Biology, Epidemiology and Management. The American Phytopathological Society, St. Paul, MN., ISBN: 0890542066.

MacHardy, W.E., D.M. Gadoury and C. Gessler, 2001. Parasitic and biological fitness of *Venturia inaequalis*: Relationship to disease management strategies. Plant Dis., 85: 1036-1051.

Melounova, M., P. Vejl, P. Sedlak, A. Reznerova, M. Tesarova, J. Blazek and J. Zoufala, 2004. The variability of *Venturia inaequalis* CKE. races in the Czech Republic and the accumulation of resistance genes in apple germplasm. Plant Soil Environ., 50: 416-423.

Murray, M.G. and W.F. Thompson, 1980. Rapid isolation of high molecular weight DNA. Nucleic Acids Res., 8: 4321-4325.

Nath, P., 1935. Studies in the diseases of apples in Northern India II. A short note on apple scab due to *Fusicladium dendritium*. Ful. J. Indian Botanical Soc., 14: 121-124.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA., 70: 3321-3323.

Parisi, L., Y. Lespinasse, J. Guillaumes and J. Krüger, 1993. A new race of *Venturia inaequalis* virulent to apples with resistance due to the *Vf* Gene. Phytopathology, 83: 533-537.

Parisi, L. and Y. Lespinasse, 1996. Pathogenicity of *Venturia inaequalis* strains of race 6 on apple clones (*Malus* sp.). Plant Dis., 80: 1179-1183.

Perrier, X., A. Flori and F. Bonnot, 2003. Data Analysis Methods. In: Genetic Diversity of Cultivated Tropical Plants. Seguin, X. Perrier and J.C. Glaszmann (Eds.). Enfield, Science Publishers, Montpellier, pp: 43-76.

Raboin, L.M., A. Selvi, K.M. Oliveira, F. Paulet and C. Calatayud, 2007. Evidence for the dispersal of a unique lineage from Asia to America and Africa in the sugarcane fungal pathogen *Ustilago scitaminea*. Fun. Gen. Biol., 44: 64-76.

Schnabel, G., E.L. Schnabel and A.L. Jones, 1999. Characterization of ribosomal DNA from *Venturia inaequalis* and its phylogenetic relationship to rDNA from other tree fruit *Venturia* species. Phytopathology, 89: 100-108.

Stukenbrock, E., S. Banke and B. McDonald, 2006. Global migration patterns in the fungal wheat pathogen *Phaeosphaeria nodorum*. Mol. Ecol., 15: 2895-2904.

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

Tenzer, I. and C. Gessler, 1999. Genetic diversity of *Venturia inaequalis* across Europe. Eur. J. Plant Pathol., 105: 545-552.

Tenzer, I., S. Degli-Ivanissevich, M. Morgante and C. Gessler, 1999. Identification of microsatellite markers and their application to population genetics of *Venturia inaequalis*. Phytopathology, 89: 748-753.

Verma, K.D. and J.N. Sharma, 2003. Scab and Marssonina blotch: Threatening disease of apple and their management. Annu. Rev. Plant Pathol., 2: 207-220.

Wikler, K. and T.R. Gordon, 2000. An initial assessment of genetic relationships among populations of *Fusarium circinatum* in different parts of the world. Can. J. Bot., 78: 709-717.

Williams, J.G., A.R. Kubelik, K.J. Livak, J.A. Rafalksi and S.V. Tingey, 1990. DNA polymorphism amplified by arbitrary primers are useful genetic markers. Nucleic Acids Res., 18: 6531-6535.

Xu, X., J. Yang, T. Vijay, R. Anthony and J. Barbara Dez, 2008. Population variation of apple scab (*Venturia inaequalis*) isolates from Asia and Europe. Plant Dis., 92: 247-252.

Zaffarano, P.L., B.A. McDonald, M. Zala and C.C. Linde, 2006. Global hierarchical gene diversity analysis suggests the fertile crescent is not the center of origin of the barley scald pathogen *Rhynchosporium secalis*. Phytopathology, 96: 941-950.

Zeigler, R.S., 1998. Recombination in *Magnaporthe grisea*. Ann. Rev. Phytopathol., 36: 249-275.