Table 10.2 Composition of PCR Master Mix Standardized in Prioritized Species

Component	*Q. semecarpifolia*	*T. wallichiana*	*M. esculenta*	*R. arboreum*	*B. utilis*
Taq Buffer	1X	1X	1X	1X	1X
$MgCl_2$	2.40 mM	3.00 mM	1.50 mM	3.00 mM	3.00 mM
dNTPs	0.20 mM	0.20 mM	0.20 mM	0.25 mM	0.25 mM
Forward primer	0.20 μM	0.26 μM	0.25 μM	0.60 μM	0.50 μM
Reverse primer	0.20 μM	0.26 μM	0.25 μM	0.60 μM	0.50 μM
Taq DNA Polymerase	0.65 U	1.00 U	0.80 U	0.50 U	1.00 U
Template DNA	10.00 ng	20.00 ng	40.00 ng	37.50 ng	20.00 ng
Total Volume	15.00 μl	15.00 μl	15.00 μl	15.00 μl	20.00 μl

Table 10.3 PCR Profiles Standardized in Prioritized Species

Sr. No.	Step	*Q. semecarpifolia*	*T. wallichiana*	*M. esculenta*	*R. arboreum*	*B. utilis*
1.	Initial denaturation	94°C for 3 min	94°C for 5 min	94°C for 3 min	94°C for 3 min	94°C for 3 min
2.	Denaturation	94°C for 45 sec	94°C for 30 sec	94°C for 30 sec	94°C for 30 sec	94°C for 30 sec
3.	Annealing	Ta for 45 sec	Ta for 30 sec	Ta for 30 sec	Ta for 30 sec	Ta for 30 sec
4.	Extension	72°C for 45 sec	72°C for 45 sec	72°C for 30 sec	72°C for 30 sec	72°C for 30 sec
5.	Repeat 2-4	40X	35X	32 X	32 X	32 X
6.	Final Extension	72°C for 7 min	72°C for 5 min	72°C for 10 min	72°C for 10 min	72°C for 10 min

The annealing temperature was standardized for each primer so as to obtain the amplification products of appropriate size range by performing a gradient PCR where a range of annealing temperatures was used to get the best amplification products for each primer. The primers amplifying the PCR product in the expected size range were screened for polymorphism on a subset of randomly selected genotypes. For final validation, a PCR product of each polymorphic primer pair was sequenced using Sanger's di-deoxy method and analysed for the repeat motifs. Finally, the validated SSR primer pairs with desired repeat motif were used for genotyping of sampled genotypes of all selected species. The PCR products were separated through automated capillary electrophoresis in Lab Chip GX Touch 24 Nucleic Acid Analyzer (Perkin Elmer, USA) along with an internal size standard. The allele sizes were recorded and analysed in Gene Reviewer software (Perkin Elmer). The allelic data showing deviations from the expected periodicity of the repeats were adjusted through allele binning software Tandem version 1.07 (Matschiner and Salzburger, 2009). Deviation from Hardy-Weinberg equilibrium was estimated by χ^2 analysis using Arlequin ver. 3.1 software (Schneider *et al.*, 2000).

10.4.4

Analysis of Genetic Diversity

The estimates of genetic diversity were calculated for each population across all loci in terms of the mean number of alleles per locus (*N*a), effective number of alleles (*N*e), Shannon's information index (*I*), the observed heterozygosity (*H*o), expected heterozygosity (*H*e), and number of private alleles using the program GenAlEx 6.5 (Peakall and Smouse, 2012). Gene flow (*N*m) was calculated using the software PopGen32 (Nei, 1978; Yeh *et al.*, 1999). The number of alleles in a population is an imperative measure of genetic variation, but their estimation is generally complicated by the effects of sample size. Therefore, it was tried to keep uniform sample size in the present study (30 samples per population with a few exceptions). Moreover, to nullify the effect of variation in sample size, allelic richness (Rs) and private allelic richness (PRs) were estimated by the rarefaction method implemented in HP-Rare software (Kalinowski, 2005). Genetic divergence based on allele frequency differences between populations was assessed with Nei's genetic distance (DA) using UPGMA (Unweighted Pair Group Method with Arithmetic mean) clustering in the software POPTREE ver. 2 (Nei 1983; Takezaki *et al.*, 2010). The robustness of dendrogram topologies was further tested by bootstrap resampling (n = 1,000). The covariance matrix of Nei's genetic distance was subjected to multivariate Principal Coordinate Analysis (PCoA) in the program GenAlEx 6.5, and the genetic relationship among sampled populations was spatially depicted over two-dimensional plot based on their pairwise genetic distances. Further, the Mantel test was performed to infer the effect of geographical distances (horizontal and vertical) in distribution of genetic diversity over the space using Nei's genetic distance (Mantel, 1967). The test was conducted with 9,999 permutations using GenAlEx ver. 6.5 software. The analysis of molecular variance (AMOVA) was conducted to understand the partitioning of variance within and among the populations using GenAlEx 6.5. The genetic differentiation is an important phenomenon for detecting the degree of disturbances, extent of gene flow, structuring among populations, etc., which is calculated as F_{ST}. The significance of F_{ST} was calculated by performing 10,000 allele permutations.

10.4.5

Analysis of Population Genetic Structure

The underlying genetic structure of populations of different species was reconstructed using the Bayesian model-based clustering approach implemented in the program STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). Ancestry model with admixture was run with the assumption of correlated allele frequencies, and the posterior probability [Pr(X/K] was calculated for each simulation [Estimated Ln Probability of Data, LnP(D)]. The simulations were run with 10 iterations for each K value (in our case 1–10) with 5,00,000 Markov Chain Monte Carlo (MCMC) sampling runs after a burn-in period of 5,00,000 iterations. The optimal K value capturing major structure among sampled populations was determined through the method introduced by Evanno *et al.*, (2005) using Structure Harvester (Earl and Von Holdt, 2012). The resultant data of replicated STRUCTURE runs were further analyzed by collating into a matrix (the Q-matrix) of individual membership coefficients and population ancestry components using CLUMPP 1.1.2 software (Jakobsson and Rosenberg, 2007). The corresponding Q-matrices were graphically displayed by using DISTRUCT 1.1 (Rosenberg, 2004). After determining the optimum K clusters, an arbitrary threshold of proportional membership coefficient ($Q \geq 0.80$) was used to assign populations and/ or genotypes to one group while the populations below threshold ($0.2 < Q < 0.8$) were considered to be admixed.

10.4.6

Landscape Genetics Approach for Spatial Representation of Genetic Diversity and Population Structure

The inverse distance weighted (IDW) interpolation function implemented in the software ArcGIS 9.3 software (ESRI, Redlands, CA, USA) was used to show the geographic patterns of genetic parameters like allelic richness and private allelic richness. The Q values obtained for K clusters were spatially interpolated using the inverse distance weighted (IDW) function implemented in ArcGIS 9.3 as described by Murphy *et al.* (2008). Georeferenced genetic data and geographical data were organized in an ESRI ArcGIS 9.3 geo-database. The overlay technique was used for interpolation of genetic parameters to elaborate genetic surface maps (Holderegger *et al.*, 2010). The diversity maps depicting the population distribution in proportion to their estimated measures were developed in ArcGIS.

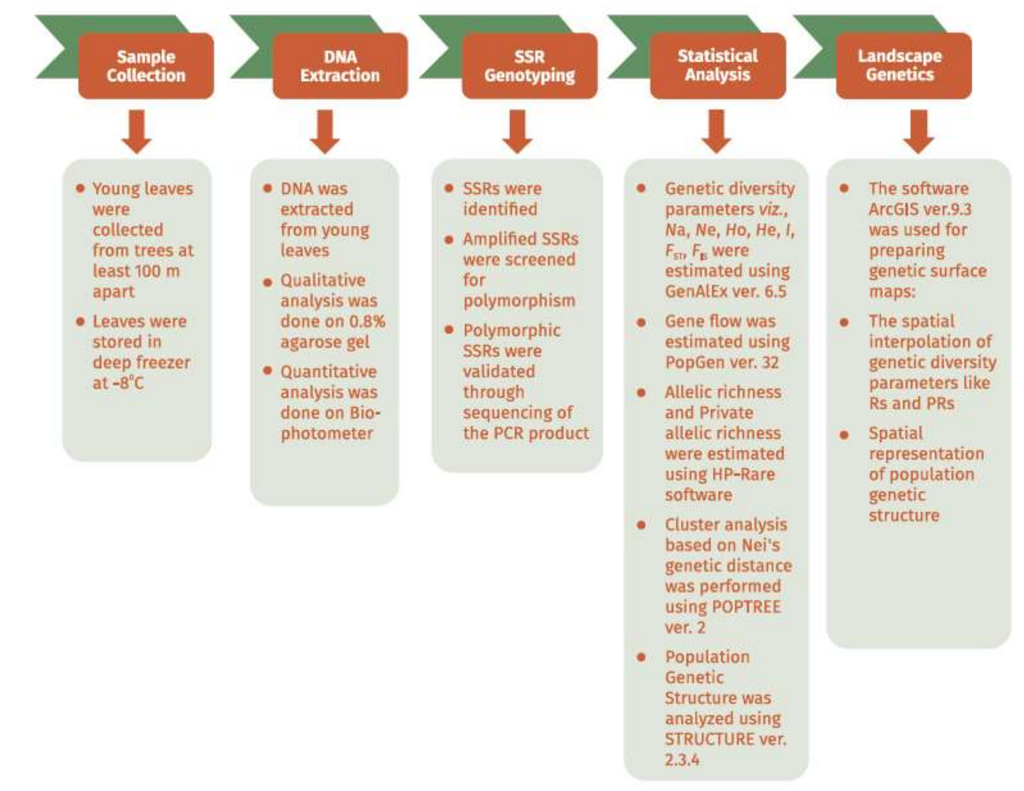

Fig. 10.1 Approach Adopted for Molecular Characterization of Prioritized FGR Species

10.5

Research Findings

The molecular characterization using SSR markers revealed a deep insight into (a) cross-species transferability of SSRs; (b) genetic diversity across sampled populations; (c) spatial representation of genetic diversity; (d) genetic relationship among the populations; (e) genetic differentiation among the sampled populations; and (f) population structure for each of the five prioritized FGR species. Results on these six aspects in case of each studied FGR species are presented below one by one:

10.5.1

Quercus semecarpifolia

The findings of molecular characterization in *Q. semecarpifolia* are described below:

10.5.1.1

Cross-Species Transferability of SSRs

The cross amplification of the selected SSR primer pairs revealed highest (37.5 per cent) transferability for primers of *Q. mongolica*; out of 40 SSRs, 15 were positively amplified and 13 of these were polymorphic. Besides, 2 of the 5 tested primer pairs of *Q. myrsinifolia* were positively amplified but not found polymorphic, whereas the SSRs of other two species namely, *Q. petraea* and *Q. rubra* could not be successfully transferred in the target oak species. The expected repeat motifs of 10 polymorphic SSR primer pairs were finally confirmed after sequencing of their PCR products obtained in *Q. semecarpifolia*. A total of 332 alleles were generated when subjected to PCR amplification with 10 SSR primer pairs in 718 genotypes of 24 populations. The SSR loci were found to be highly polymorphic.

10.5.1.2

Genetic Diversity Across Sampled Populations

The maximum number of alleles (*Na*) was observed in the population QS15 from Munsiyari, Pithoragarh FD (12.5) and minimum in QS07 from Auli, Nanda Devi FD (7.9). The overall allelic richness (Rs) was recorded in the range of 6.71 (QS07) to 9.77 (QS15) with the mean value of 8.373. The populations QS15 from Munsiyari in Pithoragarh FD and QS20 from Nag Tibba in Mussoorie FD showed their genetic distinctness having maximum number of private alleles i.e., 7 and 8, respectively. The observed (*Ho*) and expected heterozygosity (*He*) were recorded in the range of 0.382 (QS22- Kunjkharak, Nainital FD) to 0.672 (QS02- Chopta, Kedarnath WD); and 0.629 (QS05- Lokhandi, Chakrata FD) to 0.814 (QS24- Himkhola, Pithoragarh FD) with a mean of 0.551 and 0.721, respectively. The highest value of genetic diversity ($He = 0.814$) was observed in population QS24 from Himkhola, Pithoragarh FD which was closely followed by population QS19 from Mundhola, Chakrata FD ($He = 0.811$). The Shannon's Information index (*I*) was recorded in the range of 1.445 (QS08- Yamunotri, Upper Yamuna Barkot FD) to 2.01 (QS19- Mundhola, Chakrata FD) with a mean of 1.749. The within population inbreeding coefficient (F_{IS}) varied from -0.035 (QS07) to 0.472 (QS24). Populations were on an average inbred with a mean inbreeding coefficient of $F_{IS} = 0.262$, indicating a significant ($p < 0.001$) excess of homozygotes relative to Hardy-Weinberg expectations. Majority of the populations were depicted with excess of homozygotes, except the populations QS07, Auli, Nanda Devi FD and QS08, Yamunotri, Upper Yamuna Barkot FD wherein a slight excess of heterozygote was recorded though they were non-significant with respect to deviation from Hardy-Weinberg equilibrium.

10.5.1.3

Spatial Representation of Genetic Diversity

The populations QS15 (Munsiyari, Pithoragarh FD), QS11 (Bhukki Top, Uttarkashi FD), QS19 (Mundhola, Chakrata FD), QS21 (Pinswar, Rudraprayag FD); and QS23 (Narayan Ashram, Pithoragarh FD) showed high level of allelic richness and the information could be of high significance in identifying the conservation units for the species (Plate 10.1a).

a

Quercus semecarpifolia

Populations rich in number of alleles (Rs):

QS15 (Munsiyari, Pithoragarh FD)- 9.77
QS11 (Bhukki Top, Uttarkashi FD)- 9.65
QS19 (Mundhola, Chakrata FD)- 9.59
QS21 (Pinswar, Rudraprayag FD)- 9.29
QS23 (Narayan Ashram, Pithoragarh FD)- 8.69
Maximum Rs: QS15 (Munsiyari, Pithoragarh FD)- 9.77
Minimum Rs: QS07 (Auli, Nanda Devi FD)- 6.71
Average Rs: 8.373

b

Populations rich in unique alleles (PRs)

QS15 (Munsiyari, Pithoragarh FD)- 0.69
QS24 (Himkhola, Pithoragarh FD)- 0.53
QS11 (Bhukki Top, Uttarkashi FD)- 0.52
QS19 (Mundhola, Chakrata FD)- 0.48
QS09 (Raditop, Upper Yamuna Barkot FD)- 0.43
Maximum PRs: QS15 (Munsiyari, Pithoragarh FD), QS20 (Nagtibba, Mussoorie FD)- 0.69
Minimum PRs: QS07 (Auli, Nanda Devi FD), QS08 (Yamunotri, Upper Yamuna Barkot FD)- 0.05
Average PRs: 0.321

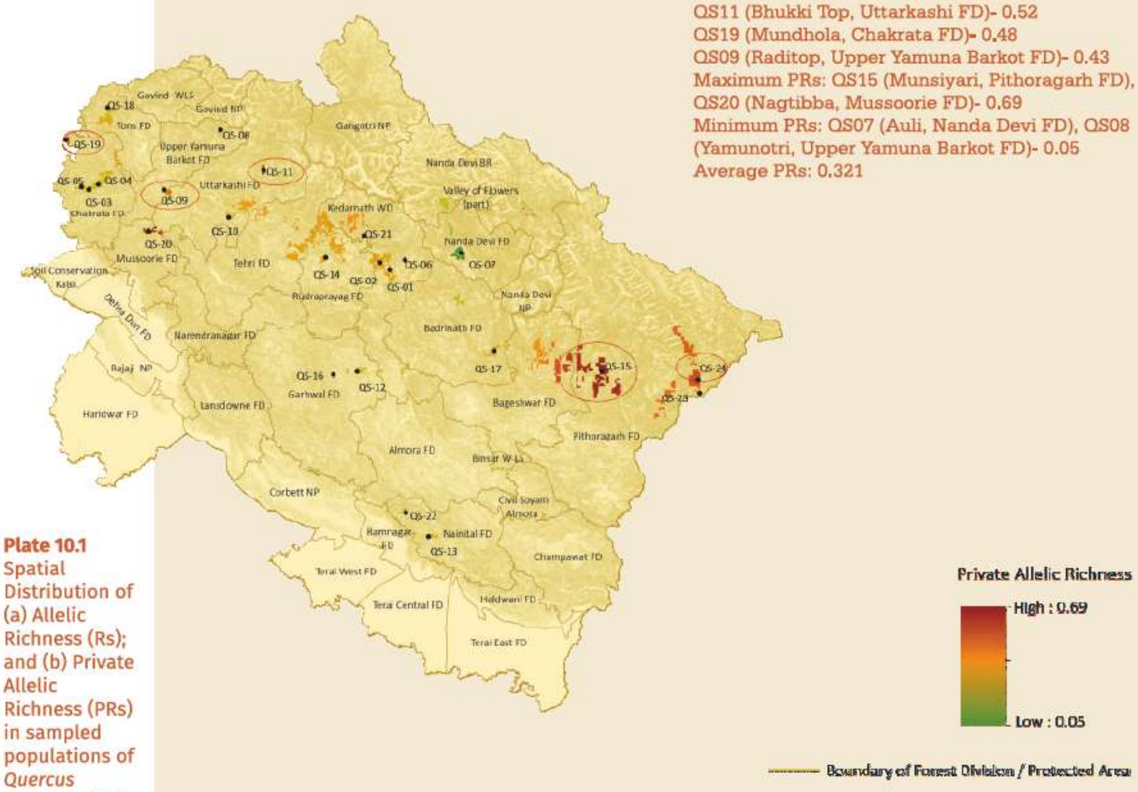

Plate 10.1 Spatial Distribution of (a) Allelic Richness (Rs); and (b) Private Allelic Richness (PRs) in sampled populations of *Quercus semecarpifolia*

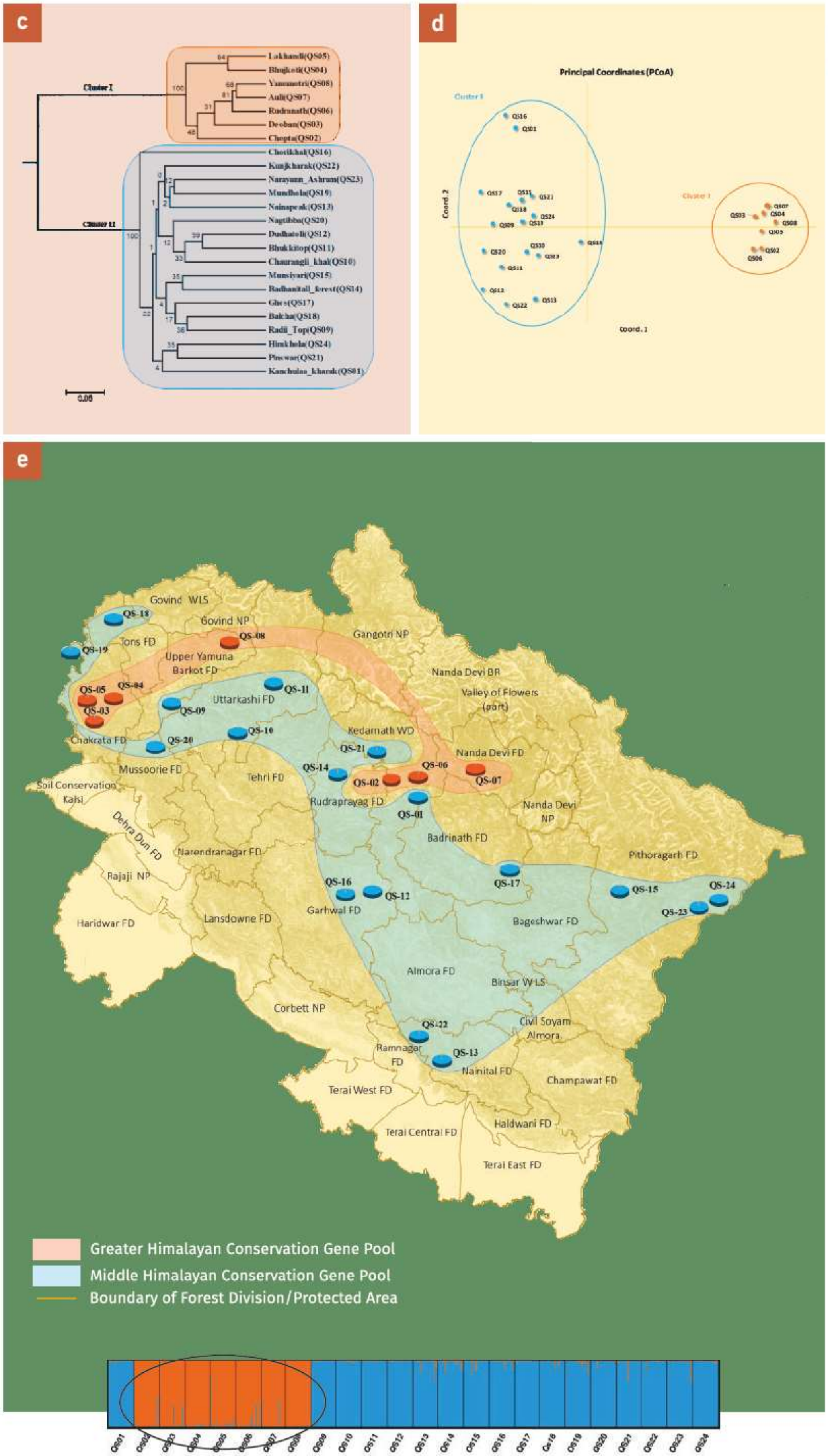

Plate 10.1
(c) UPGMA Dendrogram; (d) Plot of Principal Coordinate Analysis; (e) IDW Interpolation of the Membership Coefficient Values (Q) and Corresponding Bar Diagram (Dark Orange = Cluster -1 and Blue = Cluster-2) in sampled populations of *Q. semecarpifolia*

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The populations including QS15 (Munsiyari, Pithoragarh FD), QS20 (Nagtibba, Mussoorie FD), QS24 (Himkhola, Pithoragarh FD), QS11 (Bhukkitop, Uttarkashi FD), QS19 (Mundhola, Chakrata FD), and QS09 (Radi Top, Upper Yamuna Barkot FD) were found to be rich in presence of unique alleles (Plate 10.1b). Pithoragarh was identified to grasp maximum proportion of private alleles, and hence could be considered at top priority in conservation program.

10.5.1.4

Genetic Relationship Among the Populations

UPGMA based dendrogram clustered the populations into two major clusters *viz.*, Greater Himalayan Conservation (GHC) Gene Pool and Middle Himalayan Conservation (MHC) gene pool (Plate 10.1c). Interestingly, first major cluster comprised the populations of upper Himalayan range including three populations from Chakrata FD (QS03- Deoban, QS04- Bhujkoti and QS05- Lokhandi); two populations from Kedarnath FD (QS02- Chopta and QS06- Rudranath); and one population each from Nanda Devi FD (QS07- Auli) and Upper Yamuna Barkot FD (QS08- Yamunotri) which were most natural with relatively low level of genetic diversity (Average $He = 0.654$). Whereas, all other populations were grouped in second major cluster irrespective of varied geographical distances between them and depicted higher genetic diversity (Average $He = 0.748$).

The PCoA plot conspicuously separated the sampled populations in two major groups with huge proportion (65.53 per cent) of the total genetic variation accounted by its first principal coordinate, and clustering pattern was in concurrence to the UPGMA dendrogram (Plate 10.1d). Clustering of distantly separated populations in a single group, was further supported by Mantel's test, which showed no significant correlation between the geographic distance and genetic distance.

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10.5.1.5

Genetic Differentiation Among the Sampled Populations

AMOVA analysis revealed 84 per cent of the variation to be confined within populations and 16 per cent of the variation attributed to differences among populations ($P < 0.001$). The value of Wright's fixation index ($F_{ST} = 0.156$; $p = 0.01$) indicate the presence of moderate level of genetic differentiation among the groups. A moderate gene flow among the experimental populations ($Nm = 2.236$) further supported moderate differentiation. Pairwise F_{ST} values (indicating the genetic relatedness between two sampled populations), varied from 0.013 to 0.221. The populations QS05 (Lokhandi, Chakrata FD) and QS12 (Dudhatoli, Garhwal FD) were observed to be most distant while the populations QS04 (Bhujkoti) and QS05 (Lokhandi) both from Chakrata FD were genetically the most similar.

10.5.1.6

Population Genetic Structure

Based upon the second order statistics (ΔK) calculated as per Evanno *et al.* (2005) optimal K value was determined to be two (K = 2) for the meta-population of *Q. semecarpifolia*. As per the inferred ancestries (Q-matrix), seven populations belonged to the cluster I (dark orange) and seventeen populations were clearly assigned to cluster II (blue) and with a membership coefficient value greater than threshold ($Q \geq 0.80$) (Plate 10.1e). The structure analysis revealed that the populations grouped in major Cluster I of UPGMA dendrogram shared a common ancestral gene pool as depicted in orange-coloured bars in bar plot. Rest of the populations grouped together in second major cluster.

10.5.2

Myrica esculenta

The findings based on molecular characterization in *M. esculenta* are described below:

10.5.2.1

Cross-Species Transferability of SSRs

The SSR markers available in *M. rubra* were tested for their cross-amplification in *M. esculenta*, which revealed transferability up to 92 per cent. Out of 25 tested SSRs, 23 were positively amplified in *M. esculenta* and 10 of these were found polymorphic. The expected repeat motifs of 10 polymorphic SSR primer pairs were finally confirmed after sequencing of their PCR products. A total of 565 alleles were generated in 688 individuals of 23 populations when subjected to PCR amplification with 10 SSR primer pairs. The SSR loci were found to be highly polymorphic.

10.5.2.2

Genetic Diversity Across Sampled Populations

Maximum number of alleles (*N*a) was observed in the population ME16 from Shitla Khet, Almora FD (21.3) and minimum from ME01 (Rudranath, Kedarnath WD). The overall allelic richness (Rs) was recorded in the range of 10.84 (ME01- Rudranath, Kedarnath WD) to 15.06 (ME17- Bhowali, Nainital FD) with the mean value 13.446. All the studied populations showed the presence of private alleles, and the population ME17 from Bhowali, Nainital FD showed its genetic distinctness with maximum number (24) of private alleles. The observed heterozygosity (*H*o) was recorded in the range of 0.753 (ME11- Adwani, Garhwal FD) to 0.923 (ME17- Bhowali, Nainital FD) with a mean of 0.836. Similarly, the expected heterozygosity (*H*e) ranged between 0.834 (ME09- Peethsen, Garhwal FD) and 0.897 (ME15- Takula, Almora FD) with a mean of 0.866. Total gene diversity was high in *M. esculenta* and the highest value of genetic diversity (*H*e = 0.897) was observed in population ME15 from Takula, Almora FD which was closely followed by population ME18 from Mayali, Rudraprayag FD (*H*e = 0.892). The Shannon's Information index (*I*) was recorded in the range from 2.177 (ME01) to 2.639 (ME17) with a mean of 2.458. The within-population inbreeding coefficient (F_{IS}) varied from - 0.013 (ME17) to 0.168 (ME11) with a mean of 0.074.

10.5.2.3

Spatial Representation of Genetic Diversity

The diversity maps generated for allelic richness (Rs) and private allelic richness (PRs) have displayed spatial distribution of genetic diversity across the demographic regions/ districts of its natural distribution range. The information of diversity hot spots or the regions with maximum private alleles could be immensely useful in identifying the conservation units for the species. For instance, most populations of the Kumaon region namely, ME17, ME16, ME15, and ME08 showed higher allelic richness, and therefore, the region could be prioritized for conservation (Plate 10.2a).

In addition, a map generated for distribution of private alleles highlighted the populations/ regions rich in presence of unique alleles. The population ME17 from Bhowali, Nainital FD was identified to grasp maximum proportion of private alleles followed by ME16 from Shitla Khet, Almora FD and hence could be considered at top priority in conservation program (Plate 10.2b).

10.5.2.4

Genetic Relationship Among the Populations

The UPGMA based dendrogram clustered the populations into two major groups *viz.*, Cluster I and Cluster II (Plate 10.2c) but with a very low bootstrap value (27). The UPGMA clustering was further supported by the principal coordinate analysis. The PCoA plot (Plate 10.2d) conspicuously separated the sampled populations in two major groups with just 23.96 per cent of the total genetic variation accounted by its first principal coordinate, and clustering pattern was in concurrence to the UPGMA dendrogram. Clustering of distantly separated populations in a single group was further supported by Mantel's test, which showed no significant correlation between the geographic distance and genetic distance. Given to the above facts, it could be envisaged that horizontal geographical distance plays a minor role in shaping the distribution of genetic diversity among populations of *M. esculenta*.

a

Myrica esculenta

Populations rich in number of alleles (Rs):

ME17 (Bhowali, Nainital FD)- 15.06

ME16 (Shitla Khet, Almora FD)-14.98

ME15 (Takula, Almora FD)- 14.64

ME08 (Sandev, Pithoragarh FD)- 14.47

Maximum Rs: ME17 (Bhowali, Nainital FD)- 15.06

Minimum Rs: ME01 (Rudranath, Kedarnath WD)- 10.84

Average Rs: 13.446

b

Populations rich in unique alleles (PRs):

ME17 (Bhowali, Nainital FD)- 1.70

ME16 (Shitla Khet, Almora FD)- 1.24

ME18 (Mayali, Rudraprayag FD)- 0.95

ME08 (Sandev, Pithoragarh FD)- 0.94

Maximum PRs: ME17 (Bhowali, Nainital FD)- 1.70

Minimum PRs: ME 04 (Karnaprayag, Kedarnath WD)- 0.22

Average PRs: 0.587

Plate 10.2 Spatial Distribution of (a) Allelic Richness (Rs); and (b) Private Allelic Richness (PRs) in sampled populations of *Myrica esculenta*

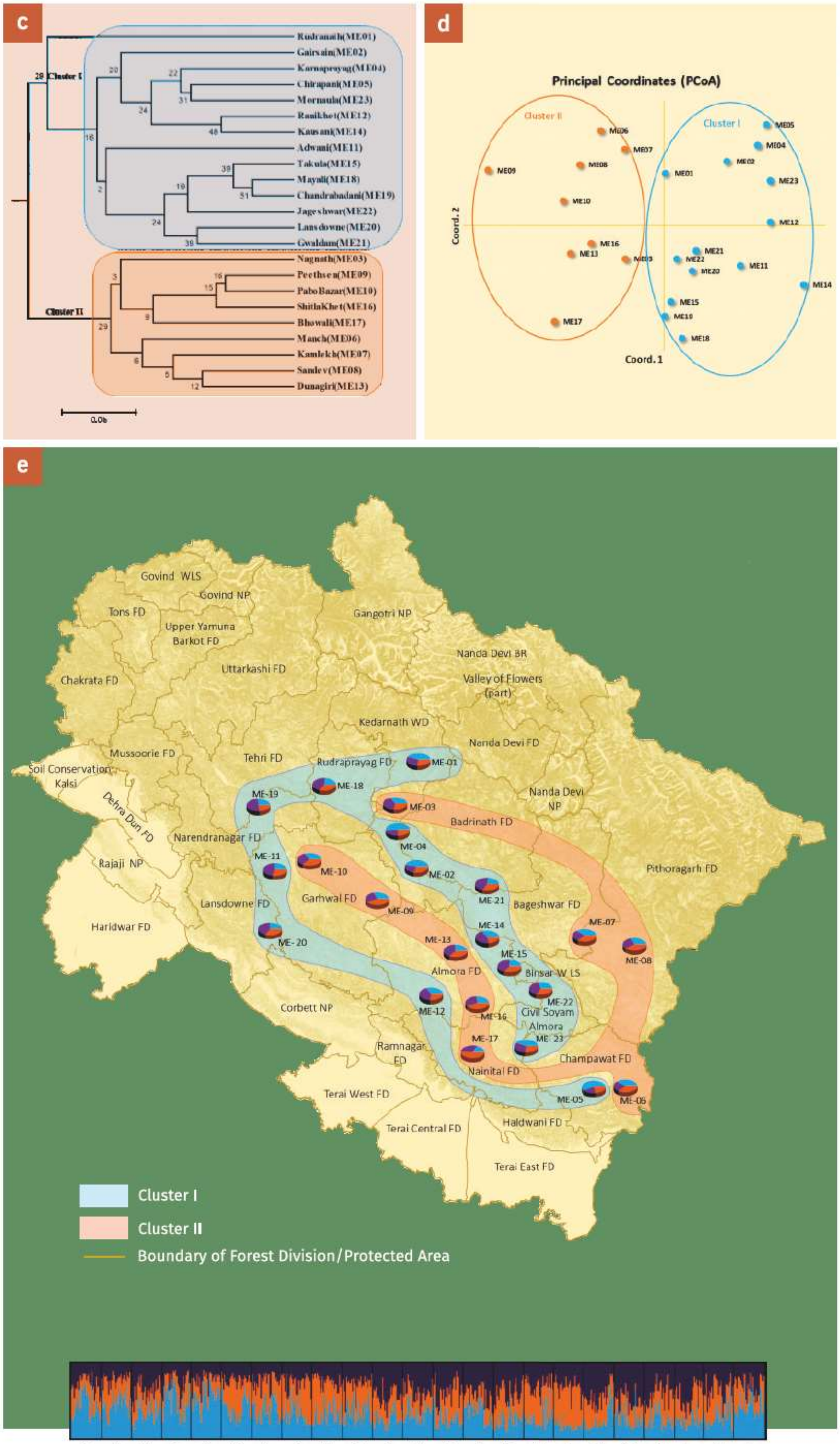

Plate 10.2
(c) UPGMA Dendrogram; (d) Plot of Principal Coordinate Analysis; (e) IDW Interpolation of the Membership Coefficient Values (Q) and Corresponding Bar Diagram (Blue = Cluster -1, Dark Purple = Cluster-2 and Dark Orange = Cluster-3) in sampled populations of *Myrica esculenta*

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10.5.2.5

Genetic Differentiation Among the Sampled Populations

Considering entire range as meta-population, the AMOVA analysis was carried out without assuming any hierarchical structure. It revealed high genetic variance (96 per cent) confined within the populations, and only 4 per cent variance among the populations. Variance estimates were based on 999 permutations. Accordingly, the difference between the individuals within the populations was statistically significant ($P < 0.01$). The results of F statistics calculated as per the Weir and Cockerham (1984) and Nei (1987) revealed low genetic differentiation ($F_{ST} = 0.038$; $p = 0.001$) among populations, which was further supported by high gene flow recorded among experimental populations ($Nm = 6.137$). Pairwise F_{ST} values indicated the genetic relatedness between two sampled populations, and the values were significantly varying from 0.012 to 0.068. Based on the pairwise F_{ST} values, the populations ME09 (Peethsen, Garhwal FD) and ME14 (Kausani, Almora FD) were observed to be most distant while the populations ME18 (Mayali, Rudraprayag FD) and ME19 (Chandra Badni, Tehri FD) were most similar.

10.5.2.6

Population Genetic Structure

Based upon the second order statistics (ΔK) calculated as per Evanno *et al.* (2005), optimal K value was determined to be three ($K = 3$) for the meta-population of *M. esculenta*. It indicated that three sub-clusters captured entire variability among sampled populations, and the underlying hierarchical structure could be best explicated with $K = 3$. However, none of the populations could be clearly defined by any of the inferred clusters, and the populations showed admixed ancestry with lower proportional membership coefficient ($0.2 < Q < 0.8$). Considering a high level of gene flow and genetic admixture (Plate 10.2e), the entire meta-population could be considered as single gene pool. Further, the inferred ancestry of each sampled population was depicted as pie charts at their spatial position in the map.

10.5.3

Taxus wallichiana

The findings based on molecular characterization of *T. wallichiana* are given below:

10.5.3.1

Population Genetic Structure

Out of 59 SSRs tested, 23 primer pairs (19 from *T. wallichiana* and 4 from *T. baccata*) showed positive amplification within the expected size range. A total of 14 SSRs showed polymorphism out of which 10 SSRs (9 from *T. wallichiana* and 1 from *T. baccata*) were validated through sequencing of PCR product. A total of 258 alleles were generated by PCR amplification with 10 SSR primer pairs in 583 individuals of 21 populations.

10.5.3.2

Genetic Diversity Across Sampled Populations

The maximum number of alleles (*N*a = 11.1) was observed in the population TW19 from Har ki Dun, Govind WLS and minimum (*N*a = 6.8) in TW06 from Harshil, Uttarkashi FD. The overall allelic richness (Rs) was recorded in the range from 6.29 (TW06- Harshil, Uttarkashi FD) to 9.41 (TW21- Baling, Pithoragarh FD) with the mean value of 7.501. Most populations except TW06 (Harshil, Uttarkashi FD) and TW16 (Ghangaria, Nanda Devi FD) contained substantial number of private alleles, where highest number of private alleles (11) were recorded for population TW12 from Mundhola, Chakrata FD. The observed (*H*o) and expected heterozygosity (*H*e) were recorded in the range from 0.253 (TW06- Harshil, Uttarkashi FD) to 0.771 (TW21- Baling, Pithoragarh FD) and 0.623 (TW06- Harshil, Uttarkashi FD) to 0.804 (TW21- Baling, Pithoragarh FD) with a mean of 0.424 and 0.725, respectively. The highest value of genetic diversity (*H*e = 0.804) was observed in population TW21 from Baling, Pithoragarh FD which is closely followed by population TW12 from Mundhola, Chakrata FD (*H*e = 0.768). The Shannon's Information index (*I*) was recorded in the range from 1.378 (TW06) to 1.984 (TW21) with a mean of 1.669. The mean inbreeding coefficient (F_{IS}) was recorded as 0.440, which indicated a significant level of inbreeding at various loci.

10.5.3.3

Spatial Representation of Genetic Diversity

The diversity maps displayed a pictorial view of the distribution of genetic diversity across the demographic regions/ districts of its natural range of distribution. For instance, populations or the regions capturing high allelic richness were spatially visualized, where the population TW21 from Baling, Pithoragarh FD showed highest allelic richness. Further, three other populations namely, TW19 (Har Ki Dun, Govind WLS), TW18 (Mornaula, Nainital FD) and TW15 (Himkhola, Pithoragarh FD) have also been identified to possess high allelic richness (Plate 10.3a).

In addition, a map generated for distribution of private alleles has displayed that the populations TW12, TW21, TW18 and TW19 contained relatively higher private alleles (Plate 10.3b). The regions or the populations identified here could be considered at top priority in conservation program.

10.5.3.4

Genetic Relationship Among the Populations

The UPGMA based dendrogram and PCoA, clustered majority of the populations into single major group (Plate 10.3c) except the population from Baling, Pithoragarh FD (TW21), which was observed to be most distant from all other populations. The genetic relatedness among spatially distant populations was the result of adequate genetic exchange through outcrossing. The results of PCoA supported the UPGMA based dendrogram showing no distinct clustering and population TW21 as an outlier (Plate 10.3d).

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a

Taxus wallichiana

Populations rich in number of alleles (Rs):

TW21 (Baling, Pithoragarh FD)- 9.41

TW19 (Har Ki Dun, Govind WLS)-8.76

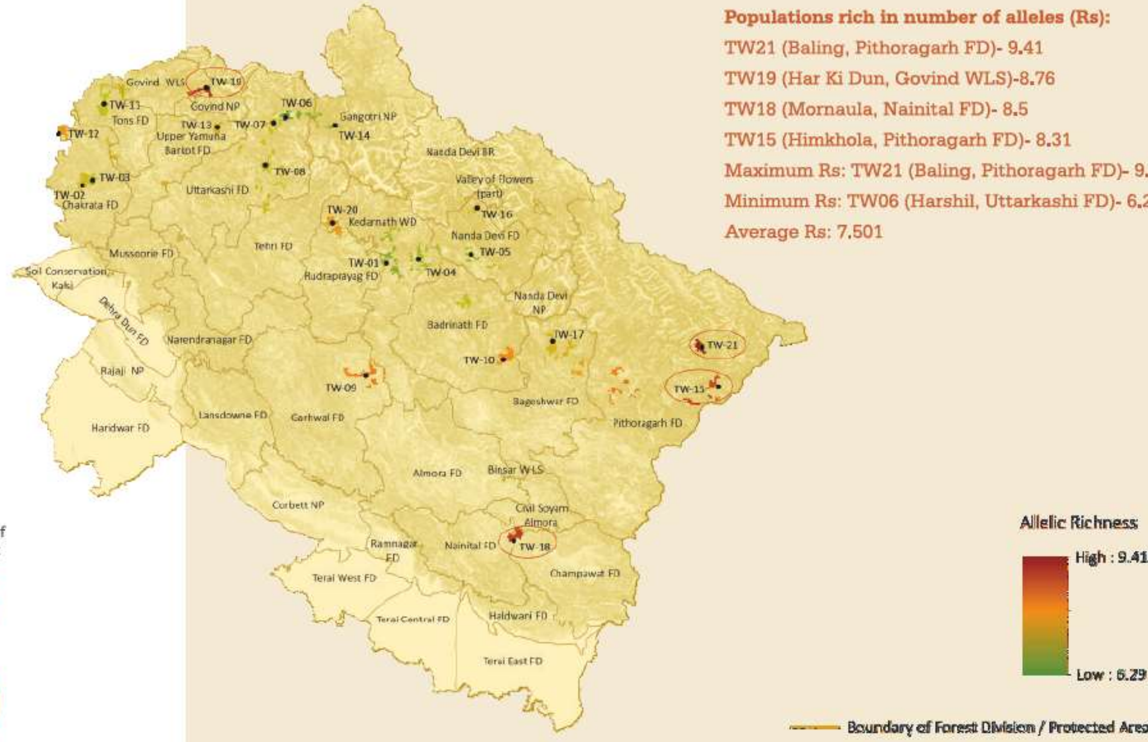
TW18 (Mornaula, Nainital FD)- 8.5

TW15 (Himkhola, Pithoragarh FD)- 8.31

Maximum Rs: TW21 (Baling, Pithoragarh FD)- 9.41

Minimum Rs: TW06 (Harshil, Uttarkashi FD)- 6.29

Average Rs: 7.501



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b

Populations rich in unique alleles (PRs):

TW12 (Mundhola, Chakrata FD)- 0.92

TW21 (Baling, Pithoragarh FD)- 0.78

TW18 (Mornaula, Nainital FD)- 0.67

TW19 (Har Ki Dun, Govind WLS)- 0.46

Maximum PRs: TW12 (Mundhola, Chakrata FD)- 0.92

Minimum PRs: TW06 (Harshil, Uttarkashi FD)- 0.00

Average PRs: 0.312

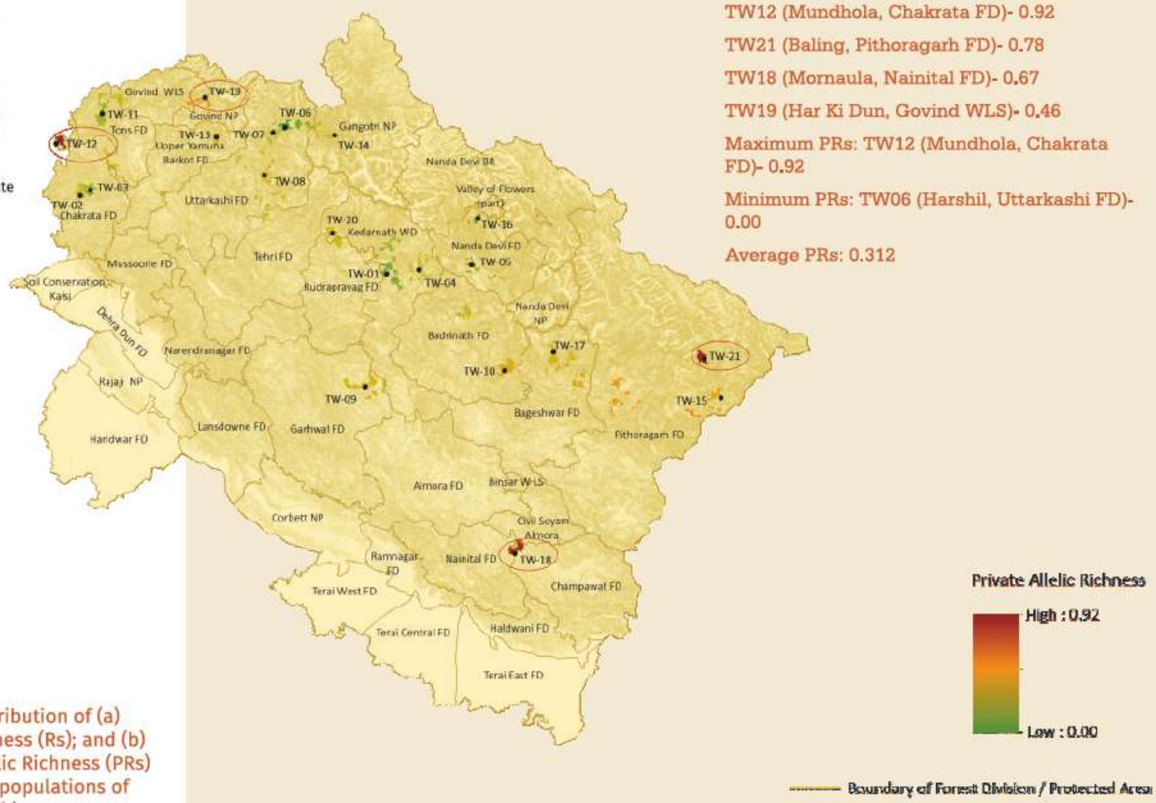

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Plate 10.3
Spatial Distribution of (a) Allelic Richness (Rs); and (b) Private Allelic Richness (PRs) in sampled populations of *Taxus wallichiana*

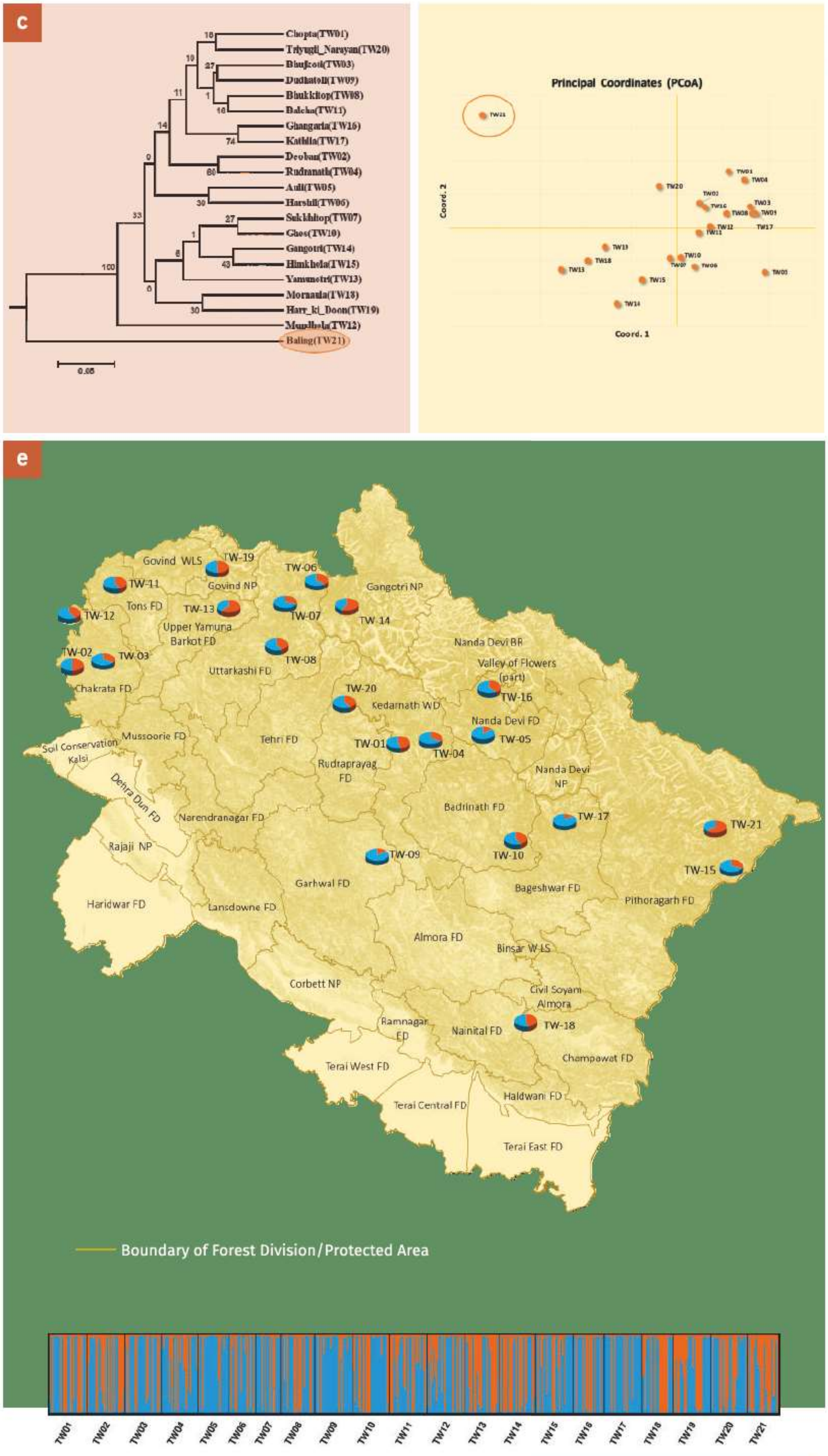

Plate 10.3
(c) UPGMA Dendrogram; (d) Plot of Principal Coordinate Analysis; (e) IDW Interpolation of the Membership Coefficient Values (Q) and Corresponding Bar Diagram (Dark Orange = Cluster-1, and Blue = Cluster-2) in sampled populations of *Taxus wallichiana*

Conservation of Forest Genetic Resources

National Program for Conservation and Development of Forest Genetic Resources

Pilot Project

10.5.3.5

Genetic Differentiation Among the Sampled Populations

On account of the wide and gregarious distribution in its natural range, whole range was considered as a meta-population and AMOVA analysis was carried out without assuming any hierarchical structure. It revealed high genetic variance (93 per cent) to be confined within the populations and only 7 per cent variance among the populations. Variance estimates were based on 999 permutations. Accordingly, the difference between the individuals within the populations was statistically significant ($P < 0.01$). The results of F statistics calculated as per the Weir and Cockerham (1984) and Nei (1987), revealed low genetic differentiation ($F_{ST} = 0.068$; $p < 0.001$) among the populations. A high gene flow among the experimental populations ($N\text{m} = 3.6$) further supported low differentiation. Pairwise F_{ST} values indicated the genetic relatedness between two sampled populations, and the values significantly varied from 0.024 to 0.163. Based on the pairwise F_{ST} values, the populations TW05 (Auli, Nanda Devi FD) and TW21 (Baling, Pithoragarh FD) were observed to be most distant while the populations TW07 (Sukkhi Top, Uttarkashi FD) and TW10 (Ghes, Badrinath FD) were genetically most similar.

10.5.3.6

Population Structure

Based upon the second order statistics (ΔK) calculated as per Evanno *et al.*, (2005), optimal K value was determined to be two ($K = 2$) for the meta-populations of *T. wallichiana*. It indicated that two sub-clusters captured entire variability among sampled populations, and the underlying hierarchical structure could be best explicated with $K = 2$. The clusters defined by Bayesian admixture analysis were observed to be in full agreement with the pattern generated by other methods, *viz.*, UPGMA and PCoA. As per the inferred ancestries (Q-matrix), none of the populations could be clearly assigned to any one of the two defined clusters and displayed admixed ancestry with lower proportional membership coefficient ($0.2 < Q < 0.8$). Structure analysis revealed that almost all the populations shared two ancestral gene pools as depicted in bar plot (Plate 10.3e) and showed a high level of admixed ancestry except the populations TW05 (Auli, Nanda Devi FD), TW09 (Dudhatoli, Garhwal FD) and TW17 (Kathlia, Bageshwar FD) which derived their ancestry majorly from the second gene pool. Further, the inferred ancestry of each sampled population was depicted at their spatial position in the map.

10.5.4

Betula utilis

The findings based on molecular characterization of *B. utilis* are described below:

10.5.4.1

Cross-Species Transferability of SSRs

The cross amplification of the SSR primers from *Betula alnoides* (Guo *et al.*, 2008) in *Betula utilis* revealed (68.42 per cent) transferability. Out of 19 SSRs, 13 were positively amplified and 12 of these were polymorphic. 15 SSR primers developed for *B. utilis* (Wani *et al.*, 2019) were also screened for polymorphism in sampled populations out of which 9 were polymorphic. The expected repeat motifs of 16 polymorphic SSR primer pairs were finally confirmed after sequencing of their PCR products obtained in *B. utilis*. A total of 552 alleles were generated when subjected to PCR amplification with 16 SSR primer pairs in 297 genotypes of 11 populations. The SSR loci were found to be highly polymorphic.

10.5.4.2

Genetic Diversity Across Sampled Populations

The maximum number of alleles (*N*a) was observed in the population BU10 from Triyuginarayan, Rudraprayag FD (12.438) and minimum in BU05 from Munsiyari, Pithoragarh FD (7.625). The overall allelic richness (Rs) was recorded in the range from 7.58 (BU05) to 10.79 (BU10) with the mean value of 9.403. A total of 221 private alleles were distributed among 11 populations. The population BU10 from Triyuginarayan in Rudraprayag FD showed its genetic distinctness having maximum number of private alleles (40). The observed (*Ho*) and expected heterozygosity (*He*) was recorded in the range from 0.413 (BU02- Gangotri, Uttarkashi FD) to 0.758 (BU10- Triyuginarayan, Rudraprayag FD) and 0.734 (BU05- Munsiyari, Pithoragarh FD) to 0.828 (BU10- Triyuginarayan, Rudraprayag FD) with a mean value of 0.609 and 0.785, respectively. The highest value of genetic diversity ($He = 0.828$) was observed in population BU10 which was closely followed by population BU06 from Himkhola, Pithoragarh FD ($He = 0.818$). The Shannon's Information index (*I*) was recorded in the range from 1.654 (BU05) to 2.124 (BU10) with a mean of 1.924. The within-population inbreeding coefficient (F_{IS}) varied from 0.119 (BU06) to 0.484 (BU02). Populations were on an average inbred with a mean inbreeding coefficient of $F_{IS} = 0.271$, indicating a significant ($P < 0.001$) excess of homozygotes relative to Hardy-Weinberg expectations.

10.5.4.3

Spatial Representation of Genetic Diversity

The map generated for visualising the distribution of allelic richness showed the highest level in BU10 (Triyuginarayan, Rudraprayag FD), BU06 (Himkhola, Pithoragarh FD), and BU11 (Darma Valley, Pithoragarh FD) which was closely followed by BU01 (Rudranath, Kedarnath WD) (Plate 10.4a). Similar pattern was observed in the map generated for highlighting the populations/ regions rich in presence of private alleles (Plate 10.4b). The population BU10 (Triyuginarayan, Rudraprayag FD), BU11 (Darma Valley, Pithoragarh FD), BU01 (Rudranath, Kedarnath WD) and BU06 (Himkhola, Pithoragarh FD) reflected the presence of highest number of private alleles besides having highest allelic richness.

10.5.4.4

Genetic Relationship Among the Populations

UPGMA based dendrogram clustered 7 populations (BU03, BU04, BU05, BU06, BU07, BU08 and BU10) into one cluster (Plate 10.4c). All the populations were grouped irrespective of varied geographical distances between them and depicted higher genetic diversity (Average $He = 0.785$). The UPGMA clustering was further supported by the principal coordinate analysis (PCoA). The PCoA plot (Plate 10.4d) conspicuously separated the sampled populations into two major groups with huge proportion (21.07 per cent) of the total genetic variation accounted by its first principal coordinate, and clustering pattern was in concurrence to the UPGMA dendrogram. Clustering of distantly separated populations in a single group was further supported by Mantel's test, which showed no significant correlation between the geographic distance and genetic distance.

a

Betula utilis

Populations rich in number of alleles (Rs):

BU10 (Triyuginarayan, Rudraprayag FD)- 10.79

BU06 (Himkhola, Pithoragarh FD)-10.23

BU11 (Darma Valley, Pithoragarh)- 10.04

BU01 (Rudranath, Kedarnath WD)- 10.00

Maximum Rs: BU10 (Triyuginarayan, Rudraprayag FD)- 10.79

Minimum Rs: BU05 (Munsiyari, Pithoragarh FD)- 7.58

Average Rs: 9.403

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b

Populations rich in unique alleles (PRs):

BU10 (Triyuginarayan, Rudraprayag FD)- 2.23

BU11 (Darma Valley, Pithoragarh)- 2.05

BU01 (Rudranath, Kedarnath WD)- 1.68

BU06 (Himkhola, Pithoragarh FD)-1.49

Maximum PRs: BU10 (Triyuginarayan, Rudraprayag FD)- 2.23

Minimum PRs: BU04 (Bamni village, Nanda Devi BR)- 0.46

Average PRs: 1.216

Plate 10.4
Spatial Distribution of (a) Allelic Richness (Rs); and (b) Private Allelic Richness (PRs) in sampled populations of *Betula utilis*

Plate 10.4
(c) UPGMA Dendrogram; (d) Plot of Principal Coordinate Analysis; (e) IDW Interpolation of the Membership Coefficient Values (Q) and Corresponding Bar Diagram (Blue = Cluster-1, Green = Cluster-2, Orange = Cluster-3, and Purple = Cluster-4) in sampled populations of *Betula utilis*

10.5.4.5

Genetic Differentiation Among the Sampled Populations

Considering entire range as a meta-population, the AMOVA analysis was carried out without assuming any hierarchical structure. It revealed high genetic variance (92 per cent) to be confined within the populations, and only 8 per cent variance among the populations. Variance estimates were based on 999 permutations. Accordingly, the difference between the individuals within the populations was statistically significant ($P < 0.01$). The results of F statistics calculated as per the Weir and Cockerham (1984) and Nei (1987) revealed low genetic differentiation ($F_{ST} = 0.081$; $p = 0.01$) among the populations, which was further supported by high gene flow recorded among the experimental populations ($Nm = 2.968$). Pairwise F_{ST} values indicated the genetic relatedness between two sampled populations, and the values significantly varied from 0.023 to 0.132. Based on the pairwise F_{ST} values, the population BU02 (Gangotri, Uttarkashi FD) was observed to be most distant from BU05 (Munsiyari, Pithoragarh FD) and BU11 (Darma valley, Pithoragarh FD) while the populations BU06 (Himkhola, Pithoragarh FD) and BU08 (Pindari, Bageshwar FD) were found to be most similar.

10.5.4.6

Population Structure

Based upon the second order statistics (ΔK) calculated as per Evanno *et al.*, (2005), optimal K value was determined to be four ($K = 4$) for the meta-populations of *B. utilis*. It indicated that four sub-clusters captured entire variability among sampled populations, and the underlying hierarchical structure could be best explicated with $K = 4$. The clusters defined by Bayesian admixture analysis were observed to be in full agreement with the pattern generated by other methods, *viz.*, UPGMA and PCoA. As per the inferred ancestries (Q-matrix), 6 populations could be clearly assigned to one of the four defined clusters with membership coefficient value higher than 0.8 and 5 populations displayed admixed ancestry with lower proportional membership coefficient ($0.2 < Q < 0.8$). Structure analysis revealed that 5 out of 11 populations shared the four ancestral gene pools as depicted in bar plot and showed a high level of admixed ancestry whereas, the populations BU01, BU02, BU03, BU04, BU10 and BU11 derived their ancestry majorly from a single gene pool. Further, the inferred ancestry of each sampled population was depicted at their spatial position in the map (Plate 10.4e).

10.5.5

Rhododendron arboreum

The findings based on molecular characterization of *R. arboreum* are described below:

10.5.5.1

Screening of SSRs

A total of 38 SSR primers from *R. arboreum* (Choudhary *et al.*, 2014) were screened for polymorphism. Out of 38 primers, 21 showed positive amplification but only 18 primers produced amplicons in the expected size range. When screened on a random subset of samples, 15 primers were found to be polymorphic. A total of 330 alleles were amplified when 810 genotypes of 27 populations were subjected to PCR amplification with 10 polymorphic SSR markers. All the loci were found to be highly polymorphic.

10.5.5.2

Genetic Diversity Across Sampled Populations

The maximum number of alleles (*N*a = 16.4) was observed in the population RA19 from Badhanital in Rudraprayag FD and minimum (*N*a = 9.8) in RA05 from Mohankhal in Kedarnath WD and RA09 from Chirapani, Champawat FD. The overall allelic richness (Rs) was recorded in the range from 7.89 (RA07, Chirapani, Champawat FD) to 12.69 (RA19, Badhanital, Rudraprayag FD) with the mean value of 10.862. Most populations except RA01- Kanchula Kharak, Kedarnath WD; RA06- Gairsain, Kedarnath WD; RA07- Chirapani, Champawat FD; and RA13- Peethsen, Garhwal FD contained substantial number of private alleles, where highest number of private alleles (19) was recorded in population RA18 from Chirbatiya, Tehri FD. The observed heterozygosity (*Ho*) was recorded in the range from 0.447 (RA01- Kanchula Kharak, Kedarnath WD and RA04- Budher, Chakrata FD) to 0.905 (RA16- Dunagiri, Almora FD) and expected heterozygosity (*He*) ranged from 0.753 (RA04, Budher, Chakrata FD) to 0.878 (RA08- Siutal, Champawat FD) with a mean value of 0.707 and 0.837, respectively. The highest value of genetic diversity (*He* = 0.878) was observed in population RA08 from Siutal in Champawat FD, which was closely followed by population RA22 from Dhanaulti, Narendranagar FD (*He* = 0.873). The Shannon's Information index (*I*) was recorded in the range from 1.771 (RA04) to 2.396 (RA22) with a mean value of 2.180. The mean inbreeding coefficient (F_{IS}) was recorded as 0.180, which indicated a significant level of inbreeding at various loci.

10.5.5.3

Spatial Representation of Genetic Diversity

The diversity maps generated for allelic richness (Rs) and private allelic richness have displayed spatial distribution of genetic diversity across its natural distribution range. The information of diversity hot spots or the regions with maximum private alleles could be immensely useful in identifying the conservation units for the species. For instance, the population RA19 (Badhanital, Rudraprayag FD) showed the maximum allelic richness followed by RA22 (Dhanaulti, Narendranagar FD), RA11 (Chaurangi Khal, Uttarkashi FD), RA23 (Ghes, Badrinath FD) and RA16 (Dunagiri, Almora FD), respectively and therefore, these populations could be prioritized for conservation (Plate 10.5a). In addition, a map generated for distribution of private alleles reflected the populations/ regions rich in unique alleles. The population RA18 from Chirbatiya, Tehri FD was identified to grasp maximum proportion of private alleles followed by RA24 (Gwaldam) from Badrinath FD, RA19 (Badhanital) from Rudraprayag FD, RA16 (Dunagiri) from Almora FD and RA23 (Ghes) from Badrinath FD (Plate 10.5b). These populations could be considered at top priority in conservation program.

10.5.5.4

Genetic Relationship Among the Populations

The UPGMA based dendrogram clustered the populations into two major clusters *viz.*, Cluster I and Cluster II (Plate 10.5c). The UPGMA clustering was further supported by the principal coordinate analysis. The PCoA plot conspicuously separated the sampled populations into two major groups with (38.73 per cent) of the total genetic variation accounted by its first principal coordinate, and clustering pattern was in concurrence to the UPGMA based dendrogram (Plate 10.5d). Clustering of distantly separated populations in a single group was further supported by Mantel's test, which showed no significant correlation between the geographic distance and genetic distance. Therefore, it could be envisaged that horizontal geographical distance plays a minor role in shaping the distribution of genetic diversity among populations of *R. arboreum*.

a

Rhododendron arboreum

Populations rich in number of alleles (Rs):

RA19 (Badhanital, Rudraprayag FD)- 12.69

RA22 (Dhanaulti, Narendranagar FD)- 12.44

RA11 (Chaurangi Khal, Uttarkashi FD)- 12.35

RA23 (Ghes, Badrinath FD)- 11.89

RA16 (Dunagiri, Almora FD)-11.83

Maximum Rs: RA19 (Badhanital, Rudraprayag FD)- 12.69

Minimum Rs: RA07 (Chirapani, Champawat FD)- 7.89

Average Rs: 10.864

b

Populations rich in unique alleles (PRs):

RA18 (Chirbatiya, Tehri FD)- 1.61

RA24 (Gwaldam, Badrinath FD)- 0.99

RA19 (Badhanital, Rudraprayag FD)- 0.82

RA16 (Dunagiri, Almora FD)- 0.8

RA23 (Ghes, Badrinath FD)- 0.74

Maximum PRs: RA18 (Chirbatiya, Tehri FD)- 1.61

Minimum PRs: RA07 (Chirapani, Champawat FD)- 0.01

Average PRs: 0.442

Plate 10.5
Spatial Distribution of (a) Allelic Richness (Rs); and (b) Private Allelic Richness (PRs) in sampled populations of *Rhododendron arboreum*

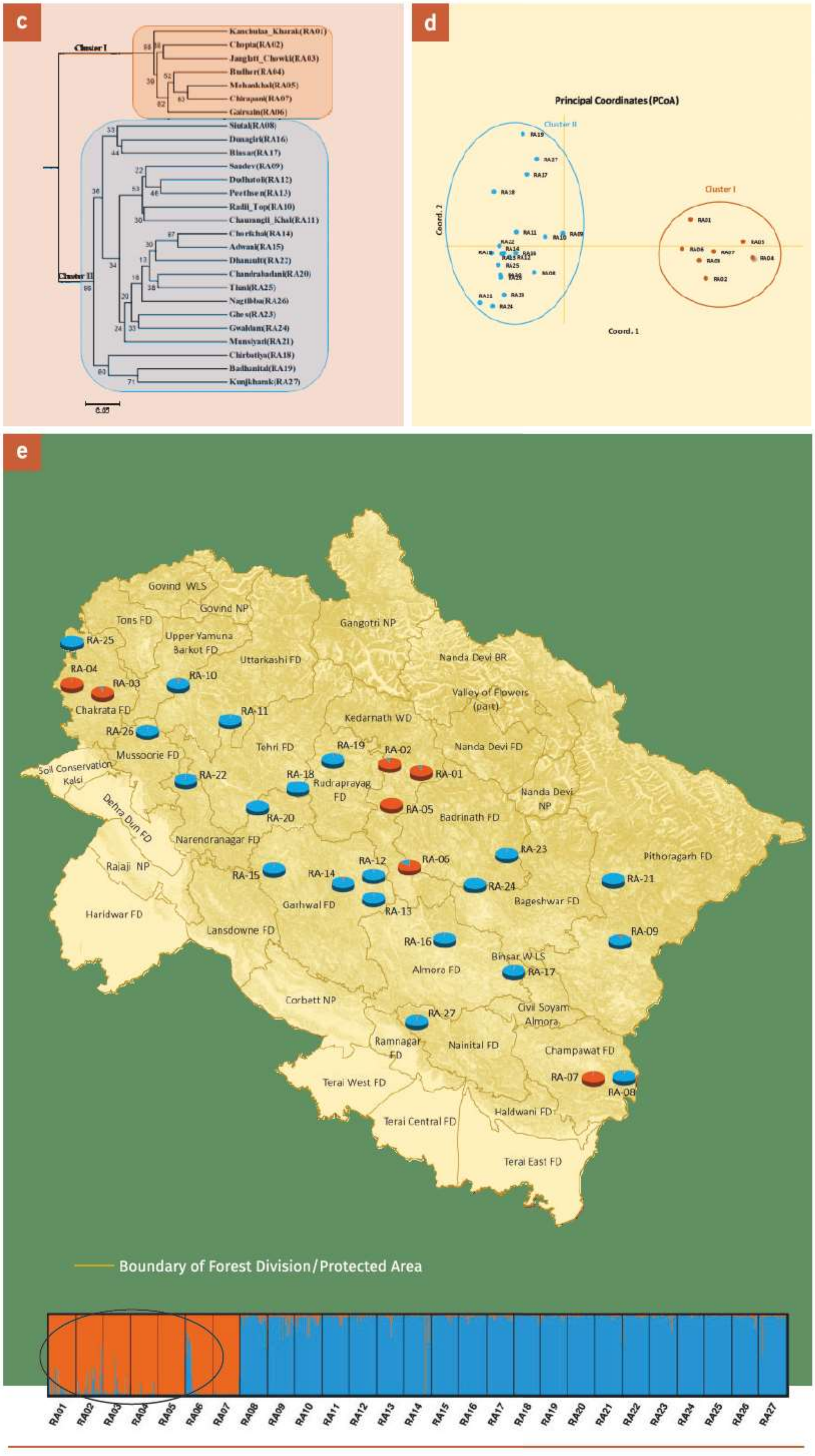

Plate 10.5
(c) UPGMA Dendrogram; (d) Plot of Principal Coordinate Analysis; (e) IDW Interpolation of the Membership Coefficient Values (Q) and Corresponding Bar Diagram (Orange = Cluster-1, and Blue = Cluster-2) in sampled populations of *Rhododendron arboreum*

10.5.5.5

Genetic Differentiation Among the Sampled Populations

On account of the wide and gregarious distribution in its natural range, whole range was considered as a meta-population and AMOVA analysis was carried out without assuming any hierarchical structure. It revealed high genetic variance (94 per cent) to be confined within the populations and only 6 per cent variance among the populations. Variance estimates were based on 999 permutations. Accordingly, the difference between the individuals within the populations was statistically significant ($P < 0.01$). The results of F statistics revealed low genetic differentiation ($F_{ST} = 0.064$; $p < 0.01$) among the populations. A high gene flow among the experimental populations ($Nm = 3.132$) further supported low differentiation. Pairwise F_{ST} values indicated the genetic relatedness between two sampled populations, and the values significantly varied from 0.012 to 0.092. Based on the pairwise F_{ST} values, the populations RA04 (Budher, Chakrata FD) and RA26 (Nag Tibba, Mussoorie FD) were observed to be most distant while the populations RA14 (Chorikhal) and RA15 (Adwani) from Garhwal FD were found to be genetically most similar.

10.5.5.6

Population Structure

Based upon the second order statistics (ΔK) calculated as per Evanno *et al.*, (2005), optimal K value was determined to be two ($K = 2$) for the meta-populations of *R. arboreum*. It indicated that two sub-clusters captured entire variability among sampled populations, and the underlying hierarchical structure could be best explicated with $K = 2$. The clusters defined by Bayesian admixture analysis were observed to be in full agreement with the pattern generated by other methods, *viz.*, UPGMA and PCoA. As per the inferred ancestries (Q-matrix), all the 27 populations could be clearly assigned to one of the two defined clusters with membership coefficient value higher than 0.8 and none of the populations displayed admixed ancestry. Structure analysis revealed that 7 populations (RA01 to RA07) shared the first ancestral gene pool as depicted in orange colour in the bar plot (Plate 10.5e) whereas, the remaining populations RA08 to RA27 derived their ancestry majorly from the second gene pool (blue). Further, the inferred ancestry of each sampled population was depicted in their spatial position in the map.

10.6

Discussion

Molecular marker-based population genetic analysis provides crucial information required for the conservation and management. Among several marker technologies, SSRs are preferentially used for genetic studies in plants due to their abundance in the genome, their high polymorphism, reproducibility and co-dominance (Jarne and Lagoda, 1996). Unlike other random primer-based marker techniques, SSRs have been developed from the unique flanking sequence of microsatellite repeat loci, which therefore restrict their utilization to the species having their own sequence information (Zane *et al.*, 2002; Squirrell *et al.*, 2003). The development of SSR markers is still laborious and costly, principally in organisms with large and complex genomes (Rai and Ginwal, 2018). It has been well established that the flanking regions of microsatellite marker loci share a high level of homology among taxonomically related taxa (Saha *et al.*, 2004; Sharma *et al.*, 2008), and thus SSR markers developed in one species can often be used to cross-amplify in other related species (Shekar *et al.*, 2021). Hence, cross-transferability is the rapid and cost-effective approach for developing locus specific SSR markers in related species (Barbara *et al.*, 2007) and has been successfully utilized in several forestry species (Isagi and Suhandono, 1997; Aldrich *et al.*, 2003). In the present study, SSR resources were identified by surveying published literature and the previously reported SSRs were tested for the sampled individuals of target species. Further, cross-transferability approach was employed in the species lacking their own SSR markers, such as *Q. semecarpifolia*, *B. utilis* and *M. esculenta*. Each successfully amplified SSR markers were evaluated for their polymorphic potential and highly polymorphic SSRs were further confirmed for the expected repeat motifs by sequencing their amplicons. Finally, the populations of all five species were subjected to genotyping with at least ten highly polymorphic SSRs displaying reproducible banding pattern.

10.6.1

Quercus semecarpifolia

As SSR markers were not available in *Q. semecarpifolia*, SSR resources of other *Quercus* species such as *Q. mongolica* (Ueno and Tsumura, 2008; Ueno *et al.*, 2008; Mishima *et al.*, 2006), *Q. myrsinifolia* (Isagi and Suhandono, 1997), *Q. petraea* (Steinkellner *et al.*, 1997), and *Q. rubra* (Aldrich *et al.*, 2003) were tested for their cross-transferability to *Q. semecarpifolia*. Out of four tested oak species, positive amplification was obtained only with the SSRs of *Q. mongolica* var crispula and *Q. petraea*, and polymorphic SSRs could only be identified in *Q. mongolica*. The 10 robust nuclear SSR markers identified through cross-species amplification were employed for genotyping.

Understanding intraspecific genetic variability is one of the key requirements for evaluating adaptive or evolutionary potential of a tree species against the prevailing environmental or anthropogenic changes, as well as designing long-term conservation programs. This is perhaps the first baseline information of population genetics in high altitude oak *Q. semecarpifolia*. The various diversity measures revealed high genetic diversity (*He*: 0.629 to 0.814; Rs: 4.19 to 5.68) in the studied populations, which was observed to be similar to other studied oaks of the world, *viz., Q. myrsinifolia* (Isagi and Suhandono, 1997), *Q. mongolica* (Mishima *et al*., 2006), *Q. glauca* (Lee *et al*., 2006), *Q. serrata* (Kitamura *et al*., 2017), *Q. petraea* (Lupini *et al*., 2019), and *Q. rubra* (Pettenkofer *et al*., 2020), etc. Similarly, high genetic diversity was also reported in three Mexican (*Q. candicans, Q. crassifolia* and *Q. castanea*) (Oyama *et al*., 2018), and one Serbian (*Q. robur*) oak species (Kesi´c *et al*., 2021). In a broader perspective, the *Q. semecarpifolia* possess higher genetic diversity in its populations than the other dominant tree species of the Indian Himalayas, *viz., Pinus roxburghii* (Ginwal *et al*., 2010), *Taxus wallichiana* (Poudel *et al*., 2014), *Pinus kesiya* (Rai and Ginwal 2018), and *Cedrus deodara* (Ginwal *et al*., 2020).

However, this situation cannot be maintained for long if local and landscape-scale anthropogenic pressures are not checked. This is because the gradual loss of private alleles and increasing inbreeding, as found in the present case, can affect the sustainability and existence of *Q. semecarpifolia* populations, if kept small and isolated for many generations. Maximum genetic diversity was detected within the sampled populations, which may be due to ample gene flow in large contiguous populations of the heterogeneous environment in the mountain regions. The oak forest consists of gregarious patches of micro-habitats which are unceasingly affected by anthropogenic activities and climate change, such as recurrence of forest fires, reduced regeneration, frost, tourism, pilgrimage, Cordyceps collection, and illicit felling in its range of occurrence in the Himalayan region (Singh, 2018). These disturbances may affect the regeneration, pollen and seed dispersal and successional status of the species. This temporal heterogeneity is especially strong in temperate forests (Wright, 1976). Forest trees are deeply exposed to this heterogeneity because of their immobility and longevity.

The geospatial interpolation of allelic richness (Rs) and private allelic richness (PRs) using the IDW function has highlighted the area of conservation importance, which could be prioritized in future conservation plans. The population or regions harboring high allelic richness are genetically most potent against future climate or human driven disturbances. The populations rich in private alleles are the reservoir of unique set of germplasm, which need to be preserved for their long-term sustenance. High allelic richness was exhibited by the populations located in the eastern region of Uttarakhand i.e., Pithoragarh district extending towards Nepal. Whereas, maximum allelic richness was recorded in the population QS19 from Mundhola, Chakrata FD, extending towards another Himalayan State i.e., Himachal Pradesh. These maps have been generated by combining the population genetic data with spatial statistical tools and provided critical information required for conservation and management. Moreover, the gene pools with private alleles which are very important from conservation point of view could be geographically identified. Findings of the present study indicated that the main center of genetic diversity for the Himalayan brown oak is located in the eastern part of Uttarakhand State, i.e., the Himalayan Range at border of India and Nepal.

The mean observed heterozygosity (*Ho* = 0.551) was recorded as lower than the mean expected (*He* = 0.721) indicating heterozygotes deficit in the populations that could be a consequence of inbreeding (F_{IS} = 0.262) or many other factors associated with population decline. In congruence, positive values of Wright's inbreeding coefficient or heterozygote deficiency was also observed in three Mexican oaks, namely *Q. candicans, Q. crassifolia*, and *Q. castanea* (Oyama *et al*., 2018). Being a later successional species with poor colonizing habit, achieving good regeneration has been remained a matter of concern for *Q. semecarpifolia*. Recently, a considerable decline in the number of saplings and complete absence of seedlings was observed for this species (Negi and Negi, 2021). Besides, masting event (long fruiting cycle of 8 - 10 years), low seed viability, vivipary and sensitivity to shade (Singh and Singh 1986; Negi and Naithani 1995), are other important causes affecting population dynamics and genetics, which may potentially be influenced by climate change in the Himalayas (Chakraborty *et al*., 2018). In addition, observed deviation of homo and heterozygotes in the populations could also be attributed to the assertive mating caused by spatial clustering or coincidence in flowering time among related groups of trees (Lemes *et al*., 2003). It further indicates that alleles within populations were not united at random and mating between close relatives may play an important role in determining the genetic structure of these populations. Moreover, the effective population size is not being maintained at several pockets of distribution range due to overexploitation and habitat destruction. Habitat fragmentation and isolation of *Q. semecarpifolia* populations may have led to an increased inbreeding. Moreover, the majority of seed dispersal occurs between nearest-neighbour populations during mast seed year due to their large size and precocious germination. Similar observations have also been made by various other authors (Allaye Kelly *et al*., 2004; Ueno *et al*., 2002) who attributed positive F_{IS} values to the populations' sub structuring and inbreeding. Moreover, there was a limited gene flow (*N*m = 1.25) among populations and notable proportion of diversity was harbored within populations.

The UPGMA-based dendrogram distinguished the populations of the Upper Himalayan Region from rest of the populations with a very high bootstrap support indicating the authenticity of present findings. In order to look into the association between the geographical and genetic distance between the populations, Mantle's test was conducted which revealed no significant relationship between geographic distance and shared allele distance among the populations which is quite similar to the results obtained in *Q. mongolica* (Ueno *et al.*, 2008) and *Q. crispula* (Ohsawa *et al.*, 2007). Given to the above facts, it could be envisaged that horizontal geographical distance played a minor role in shaping and distribution of genetic diversity among sampled populations of *Q. semecarpifolia*. In *Q. mongolica* var crispula, no significant isolation by distance was observed among the populations along either horizontal or vertical axes. Thus, it can be concluded that these populations are derived from some common ancestors during the process of evolution. More significantly, the results of Principal Co-ordinate analysis support differentiation of the populations of the Upper Himalayan Region from rest of the populations. The genetic relatedness among spatially distant populations was the result of adequate genetic exchange through outcrossing. Surprisingly, grouping of relatively high-altitude populations in a separate cluster indicated the presence of some sturdy physical or ecological barrier which restricted their genetic admixture, and the populations were not able to enrich their diversity further.

Populations from the Upper Himalayan Region are marginal populations in their range of distribution. Many of the populations *viz.*, QS2, QS6, QS8, QS15, and QS24 form the sub alpine timber line in western Himalaya. Literature indicated that forests in upper areas of western and central Himalayan regions are vulnerable to projected impacts of climate change (Chaturvedi *et al.*, 2011; Gopalakrishnan *et al.*, 2011; Joshi *et al.*, 2012; Shrestha *et al.*, 2012). The potential habitat of *Q. semecarpifolia* is predicted to shrink to 40 % and 76 % with 1°C and 2°C increase in temperature, respectively (Saran *et al.*, 2010). Interestingly, in another study, an altitudinal shift or upslope movement of *Q. semecarpifolia* was observed in response to climate change where topography and climatic factors played a significant role (Shekhar *et al.*, 2022). There is a prevalent assumption that geographically peripheral populations harbour lower genetic diversity and higher genetic differentiation than core populations as a result of higher genetic drift, fragmentation, and isolation, and disturbance regimes in peripheral populations (Pandey and Rajora, 2012; Lesica and Allendorf, 1995; Brown *et al.*, 1996; Eckert *et al.*, 2008).

The SSR markers revealed a high level of intra population variability. This characteristic is common to other *Quercus* species also like *Q. microcarpa*, *Q. robur* and *Q. petraea*, and it is probably due to the mating system of this genus (Aljorna *et al.*, 2007). Further, investigation of genetic admixing among the populations was carried out through the structure analysis. As expected, seven sampled populations from the Upper Himalayan Region were clearly grouped in one cluster with Q values ≥ 0.8. Spatial mapping of the genetic structure highlighted the presence of two major gene pools of *Q. semecarpifolia* in Uttarakhand. The grouping was in agreement with the results of UPGMA based clustering as well as Principal Coordinate Analysis. The first smaller cluster included seven populations (QS02 to QS08) from the Upper Himalayan Region which coincided exactly with the Cluster I in UPGMA-based dendrogram. This shows strong evidence for the common ancestry of these seven populations. Hereafter, for better understanding, Cluster-I located in the Greater Himalayas, particularly in the upper catchments of Yamuna, Bhagirathi, Alaknanda, Mandakini, and Pinder rivers has been named as the 'Greater Himalayan Conservation (GHC) Gene pool' of *Q. semecarpifolia*. The second larger cluster incorporating the remaining 17 sampled populations (QS01, and QS09 to QS24) predominantly located in the Lesser or Middle Himalayas has been named as the 'Middle Himalayan Conservation (MHC) Gene pool'. The results of population genetic structure, analysis of genetic relatedness, and PCoA clearly indicated that there must be some geographical constraints due to which seven populations of the Cluster I are isolated from rest of the 17 populations of Cluster II. The delineated area of Cluster I (GHC Gene pool) is predominantly formed in the upper stretches of the Himalayan rivers (Yamuna, Bhagirathi, Alaknanda, Mandakini, and Pinder) within the Greater Himalayas and the area is surrounded on all the sides by steep mountains, deep gorges, and high-altitude valleys. Hence, the overall genetic diversity in this region of Cluster I was observed to be lower as expected because of lesser genetic exchange with the neighboring populations. Being large sized seed, aerial transmission is limited and the dispersal is largely affected by the topography and the pollen movement could be presumed as the primary mode of gene flow. Similar evidences were also reported in *Q. aquifolioides* occurring at Tibetan Himalayas, where seed dispersal was found to be extremely limited and the gene flow was primarily mediated by extensive pollen movement (Du *et al.*, 2016).

Results of the present study indicated that the topographic features such as mountain ranges and high-altitude valleys impact the patterns of gene flow in *Q. semecarpifolia*. These high mountain ranges disconnect the populations to several hundred miles causing hindrances in the smooth genetic exchange between populations. Migration of alien genes through gene flow will change the genetic composition of the receiving population. This can specifically impact the resilience, adaptability, fitness, and fertility of small populations. The peripheral and isolated populations are at greater risk of diversity loss and thus likely to be more constrained in their ability to tolerate rapid climate change. Hence, there is a strong coincidence between the genetic barrier detected in the study and the actual geographical landscape barriers which could act as obstacles for the gene flow thus separating the populations of the Upper Himalayan Region from rest of the populations in Cluster II. No significant barrier has been observed in the lower region, and hence, the populations from this region showed admixed ancestry as expected because of a free geneflow across the populations in large contiguous range.

In addition to the topological barriers present between the two *Q. semecarpifolia* lineages, significant differences in climatic variables have been detected (Shankhwar *et al.*, 2022) some of the climatic variables studied here may be creating an ecological barrier. The climatic differences found between the lineages are apparently sufficient to keep both lineages separated to a high degree, as indicated by the nuclear data. This kind of ecological niche partitioning may have given rise to some degree of differential adaptation to their respective environmental conditions and would enforce differentiation among the populations. This, with sufficient time gap, might ultimately lead to reproductive isolation (Liu *et al.*, 2013; Rieseberg & Burke, 2001; Nosil *et al.*, 2009; Thorpe *et al.*, 2010; Wagner *et al.*, 2012), resulting in genetic divergence. Meng *et al.*, (2017) have reported occurrence of two lineages viz., cold highlands and warm lowlands in Quercus sect. Heterobalanus in the Himalaya-Hengduan Mountains (HHM) region of China that have diverged as a consequence of local adaptation to diverging climates since the late Miocene. Du *et al.*, (2017) detected three distinct genetic lineages in *Q. aquifolioides* with adjacent but non-overlapping distributions in Eastern Himalayan ranges of south-west China. In congruence to the present study, similar results were obtained in the study conducted on three Mexican oaks (*Q. candicans, Q. crassifolia*, and *Q. castanea*), where resistance analyses showed connectivity among almost all the populations but barrier analysis revealed genetic breaks limiting gene flow across some populations (Oyama *et al.*, 2018).

10.6.2

Myrica esculenta

The microsatellite markers were not available in *M. esculenta*. Thus, SSRs available in closely related *M. rubra* (Terakawa *et al.*, 2006 and Jiao *et al.*, 2012) were tested for cross-amplification in *M. esculenta* which revealed high (92 per cent) transferability. The success of cross-transferability depends upon the evolutionary distance between the source and the target species and the results indicated that there is higher genomic homology between both the species. The key marker characteristics calculated in populations of *M. esculenta* (*N*a = 6-33; *He* = 0.60 - 0.95) were found to be higher than *M. rubra* (*N*a = 13.7 - 21.3, *He* = 0.834 - 0.897) in which the SSRs were originally identified (Terakawa *et al.*, 2006).

The study has displayed high genetic diversity in the 23 sampled populations of *M. esculenta* distributed in western Himalayas, as evidenced by the high values of calculated expected heterozygosity (*He* = 0.866), and allelic richness (Rs = 13.446). Similar to *Q. semecarpifolia*, the diversity of *M. esculenta* was also higher than the other companion species in similar habitat in the Indian Himalayas (given in previous section). Though no primary data is available in *M. esculenta*, results are comparable to the earlier studies carried out in *M. rubra* (Chinese bayberry) in China. For instance, the expected heterozygosity of 0.718 and 0.65 were recorded in two independent studies carried out in 29 accessions (Jia *et al.*, 2015), and 213 accessions of *M. rubra* in China (Xie *et al.*, 2011), respectively. Similar to *M. rubra*, this species also possesses high level of heterozygosity in its genome that could be ascribed to the obligatory cross-pollinated breeding behavior ensured by dioecious floral structure.

M. esculenta is a socio-economically important species of Lesser Himalayas, which has higher ethnobotanical potential. In the natural forest, it is growing in mixed forest dominated by Rhododendron but sporadic occurrence was also observed under the gregarious pine forest. It is popular for its edible fruit, and therefore, a good number of trees could be seen in the vicinity of human settlements. Despite of heavy extraction of fruits no significant impact was observed on the genetic diversity and structure. Mean value recorded for inbreeding coefficient in current study is near to zero (F_{IS} = 0.074) and was also comparable to its close relative Chinese bayberry (F_{IS} = 0.083) (Jia *et al.*, 2015). It indicates that both the species encompassed equal proportion of homo and heterozygotes in their populations due to abundant gene flow without any major obstacle. Hence, the populations could be presumed under HWE.

The mating system and the extent of pollen flow are the two most important genetic features that determine the genetic structure of plant populations, and both are crucial for the design of conservation strategies (Marchelli *et al.*, 2012). *M. esculenta* is an important dioecious anemophilous tree species of the Himalayan region. The genetic structure of this species is highly influenced by its long-distance movement of pollen by wind, and seeds by animals and human beings. The result may fit with the general observation that woody perennial and outbreeding species maintain most of their variation within populations (Hamrick *et al.*, 1992). The local communities residing in the vicinity of the forests are mostly depending on the forest products for income. They gather fruits from the trees and take them to different locations for sale and share among their relatives and friends. This results in low seedling availability and poor regeneration in the natural habitats of *M. esculenta*. This way the migration of germplasm takes place and is very prominent in this species. It appears unlikely that habitat fragmentation in this genus will result in reproductive isolation. Results of the present study, along with a growing number of studies indicating high levels of pollen movement in temperate trees suggest that among many issues faced by conservation biologists, reproductive isolation of fragmented stands may not be of great concern (Burczyk and Chybicki, 2004; Fore´ *et al.*, 1992; Schuster and Mitton, 2000; Craft and Ashley, 2007). Spatially isolated stands of wind-pollinated trees are not likely to be reproductively isolated or suffer losses of genetic diversity (Craft and Ashley, 2007).

The geospatial interpolation of allelic richness (Rs) and private allelic richness (PRs) resulted in the spatial demarcation of populations or areas of conservation importance. The sampled populations from the districts of Nainital, Almora, and Pithoragarh (ME17, ME16, ME15, and ME08) showed the maximum allelic richness. Based on the ground survey, *M. esculenta* populations distributed in the Almora, Nainital, and Pithoragarh districts of Kumaon region were healthier and more gregarious than the population from Rudraprayag district in Garhwal region. Based on the model-based prediction, highest area was estimated for Almora district (approximately 104 ha), which was followed by Pauri and Champawat (Shankhwar *et al.*, 2019). Overall, it has more uniform distribution in Kumaon than the Garhwal region of Uttarakhand, and therefore, the region was expected to possess more genetic diversity. Moreover, some additional populations *viz.*, ME17 (Bhowali, Nainital FD) and ME16 (Shitla Khet, Almora FD) of same region were also identified to possess significant proportion of private alleles. Therefore, the entire Kumaon region could be considered as genetically diverse zone for future conservation programs.

The UPGMA based dendrogram and PCoA plot separated the populations into two distinct clusters but the clustering pattern cannot be explained by topographical, spatial, or climatic features. As evident with the high and long-distance gene flow through natural or manual means, the genetic exchange has taken place randomly across the distribution range. As a result, distantly separated populations clustered together and geographically close populations separated in the distinct cluster. However, boot strap support was too low to be trusted, and therefore, the level and pattern of genetic admixing was further deciphered through the structure analysis. The AMOVA results have indicated that majority of the total variation existed within the populations with fixation index (F_{ST}) 0.038. Based on the Wright's (1978) interpretation, there was little or no genetic differentiation among populations of *M. esculenta*, and all studied populations may be considered as a single meta-population. The low differentiation among populations was commonly explained as a result of long-distance gene dispersal either by pollen or by seed (Yao *et al.*, 2007), which stands true in case of *M. esculenta*, where the rate of gene flow was recorded as significantly high ($Nm = 6.137$). According to Hamrick *et al.*, (1995), gene flow estimates for con-specific populations sampled miles apart varied over species from very low ($Nm << 0.5$) to very high (Nm> 5.0). McDermott and McDonald (1993) indicated that if $Nm > 1$, there will be little differentiation among populations and migration counter-balance the effect of genetic drift. A similarly low degree of genetic differentiation based on microsatellites has been found among other tree species with fragmented populations (White *et al.*, 1999, 2002; Bacles *et al.*, 2005).

Results were further supported by the STRUCTURE analysis through Bayesian model-based clustering, where no significant structuring was obtained. Based on the plot of posterior probabilities of each predicted K values, three optimum sub clusters were predicted to capture entire genetic variability. However, none of the populations could have been defined by any inferred cluster with proportional membership coefficient > 0.8. It indicates that three predicted gene pools are genetically well interconnected due to high level of genetic admixing across the spatially separated populations. Therefore, the sampled populations from distant geographical areas represent a single meta-population with admixed ancestry from three gene pools, and historical levels of gene flow have been very high despite the highly fragmented distribution of stands found in the western Himalayas. Findings also indicated that geological landforms, topography, climatic variation and anthropogenic activities have not adversely affected the genetic processes of populations. Some of the theories claim that admixture, reflecting allele sharing, can result from incomplete lineage sorting of historically contiguous populations (Huang *et al.*, 2015). It is thus suggested that all *M. esculenta* individuals in history were likely a continuously distributed large population which shared only three gene pools connected by extremely high gene flow.

10.6.3

Taxus wallichiana

High medicinal and socio-ecological significance of *T. wallichiana* have resulted in its over-exploitation and indiscriminate extraction from natural forests, and have led the species to the edge of extinction. In addition to anthropogenic factors, regeneration failure caused by poor seed germination, slow growth, and consumption of fruits by birds, monkeys, rats, etc., are other factors associated with population decline of species in its natural habitat (Paul *et al.*, 2013). Globally, several populations in forest areas are severely declined and they will become extinct in the near future, if proper conservation measures are not initiated. Based on the recent assessment in 2010, the population status of *T. wallichiana* was recorded as continuously declining and fragmented in natural habitat, and therefore, listed as 'Endangered' under criteria A2acd of the IUCN Red List of Threatened Species (Thomas and Farjon, 2011). Some studies have also demonstrated its sensitivity against threat of the global warming. In view of these facts, it is one of the important tree species demanding urgent conservation measures. Unfortunately, inadequacy of the data and baseline knowledge required for conservation are the major hurdles before forest managers or conservationists.

Genotyping with ten highly polymorphic SSRs generated a mean of 8.771 alleles per loci, and exhibited mean observed and expected heterozygosity as 0.424 and 0.725, respectively. All the marker characteristics were observed in accordance to their parental literature where these have been published. For instance, Cheng *et al.*, (2015) have described their SSRs with a mean allelic number of 8.44, and characterized with similar values of mean observed (*Ho* = 0.246) and expected heterozygosity (0.698). In comparison to other study by Gajurel *et al.* (2013), better results were obtained with high number of alleles and high level of polymorphism in present study. Surprisingly, many of these SSR loci demonstrated high and positive values of inbreeding coefficient indicating severe heterozygote deficiency in the sampled populations. However, this has not been calculated in their source literature but similar level of inbreeding has been recorded in various studies conducted for different populations with different SSR markers.

Overall high genetic diversity was recorded in the studied populations. The mean observed and expected heterozygosity of the populations were recorded as 0.424 and 0.725, respectively. Similarly, mean allelic richness ranged from 6.29 to 9.41. Huge deviation was noticed between observed and expected heterozygosity indicating significant departure of the populations from HWE. High positive value of inbreeding coefficient (F_{IS} = 0.440) detected in the present study indicated the existence of severe inbreeding among the populations. Inbreeding refers to the mating among closely related individuals which results in an excess of homozygotes in the population. In the long term, it can lead to the deleterious consequences with reduced fitness of the individuals and extinction of populations. Inbreeding is obvious in the threatened species where the populations are facing severe decline and fragmentation. In the threatened species, populations are generally small and isolated where random mating cannot be fully realized across the populations, which ultimately results in inbreeding (Neaves *et al.*, 2015).

Couple of studies have been performed for characterizing populations of different *Taxus* species worldwide, and results of the present study were found to be in high congruence to many of these. For instance, 13 geographically disjunct populations of *T. wallichiana* var. *mairei* in southern China were characterized with a moderate genetic diversity (*He* = 0.538) and genetic differentiation (F_{ST} = 0.159). Noticeably, severe inbreeding (F_{IS}=0.290) and bottleneck effect was also detected in most of these studied populations (Zhang and Zhou, 2013). Similarly, high genetic diversity was recorded in the populations of English yew (*T. baccata*) in different independent studies; first was done in two remnant populations of Poland (Chybicki *et al.*, 2011) and the second study was performed in 24 populations of Cantabrian-Atlantic Region in Northern Spain (*Ho* = 0.599, *He* = 0.664) (Maroso *et al.*, 2021). In the earlier study by Chybicki *et al.* (2011), a substantial deficiency of heterozygotes was detected in both populations, as compared with Hardy-Weinberg expectations. In contrary to results of the present study, low genetic diversity was reported in the populations of *T. chinensis* (*Ho* = 0.107, *He* = 0.121) and *T. wallichiana* (*Ho* = 0.095, *He* = 0.109) distributed in Vietnam (Vu *et al.*, 2017).

Spatial distribution of allelic and private allelic richness has demarcated the genetically diverse regions and the populations encompassing unique alleles. Except some populations, center of diversity seems to be skewed towards Kumaon region, where the population of Pithoragarh FD (TW21- Baling) was richest in allelic diversity. In the Garhwal region, a population located in Govind WLS and NP (TW19- Har ki Dun), followed by population from Nainital FD (TW18- Mornaula) possess higher allelic diversity. Another population of this region, such as TW15 (Himkhola, Pithoragarh FD) also possessed relatively higher allelic diversity. Based on the ocular observations during field surveys, these populations were also recognized as healthier and relatively larger. Looking at the population status and the threat perception, above stated different FDs in two regions need to be prioritized for in situ conservation. Also, these populations may be treated as source for seed collection or selection of planting material for the establishment of *ex situ* plantations or field gene banks. Further, Fig 10.15 showed the genetically distinct populations, where the population TW21 (Baling, Pithoragarh FD) and TW12 (from Mundhola, Chakrata FD) were marked to have significant proportion of private alleles. Besides, several populations, such as Kanchula Kharak (Kedarnath Wildlife Sanctuary) and Kalamuni (Munsiyari, Pithoragarh FD) were extremely smaller and degraded. Due to suboptimal sample size, these were not included in this study but the allelic diversity of these populations needs to be assessed on a priority and alleles of these smaller populations could be rescued by infusing their seeds into a large sized population with broad genetic base.

The UPGMA and PCoA-based clustering revealed that majority of the populations are genetically interrelated and most populations were segregated into single major group except the population from Baling, Pithoragarh FD (TW21), which is observed to be most distant from all other populations. As revealed by the diversity hotspots, TW21 is the population endowed with significant allelic and private allelic richness; hence, it is genetically distant and separated out from the major cluster in the dendrogram. Despite the wide geographical distance, all other populations form a single cluster with high boot strap support because the species is obligatory cross-pollinated and the estimated gene flow was also found adequate. Also, the clustering pattern of UPGMA dendrogram was found to be highly consistent with the spatial clustering demonstrated by the PCoA plot.

Owing to the wider distribution and high genetic relatedness, whole range of sampled populations of *T. wallichiana* was considered as a meta-population, and AMOVA analysis was carried out without assuming any hierarchical structure, which revealed most genetic variance to be confined within the populations. Consequently, very little genetic differentiation was recorded among the studied populations of western Himalayas. The results were further supported by the structure analysis where the entire genetic variability was divided into two gene pools. However, none of the populations could be clearly assigned to any one of the two inferred clusters and displayed admixed ancestry with lower proportional membership coefficient (< 0.8). It indicated that there was no clear-cut clustering and sufficient genetic exchange has been taking place across the geographically disconnected populations. Overall, the results generated with different analysis were consistent and can be trusted with high confidence.

Comparatively, the genetic differentiation recorded in this study was lower than the other populations of this species investigated from eastern and southern China (Zhang and Zhou, 2013; Luo *et al*., 2021) and Vietnam (Vu *et al.*, 2017). Similarly, it was also found lower than the European Yew *T. baccata* (Maroso *et al*., 2021), as well as other out-crossing species in general (Nybom, 2004). In most of the cases, high genetic differentiation in Yew has been associated with the habitat shrinkage and fragmentation, reduced population size, over extraction, etc., which were observed as minimal in the populations of western Himalayas. Moreover, the level of gene flow detected in this study was slightly better than these studies, and this is the reason why the genetic differentiation is very low. As observed during field tours, most sampled populations were large sized and preserved in the protected areas in association with other companion tree species of temperate region, such as *R. arboreum, Abies pindrow, Q. semecarpifolia, Picea smithiana*, etc., where the anthropogenic interventions were prohibited. As mentioned in the foregoing section, some populations which were highly declined and disturbed were not sampled. Therefore, the healthy and undisturbed population distribution over wide geographical span is the main cause of high genetic diversity, low genetic differentiation, high gene flow, lack of structuring, and high genetic relatedness among populations.

The present investigations confirmed that the genetic structure of natural populations of the Himalayan yew are still intact and not significantly affected by regeneration failure, anthropogenic causes, and environmental factors, etc. The credit should be given to the efforts of forest managers who effectively managed its habitat because similar results were also obtained in other studied species of same region in the western Himalayas.

10.6.4

Betula utilis

The Himalayan Region embodies the highest and most diverse tree-line or timber-line over the world, which forms an ecotone between montane forest and alpine shrub. *B. utilis* is one of the principal timberline species in the western Himalayas of Uttarakhand. Being in the forefront of the struggle for survival, worldwide timberline species attracted the attention of researchers for various investigations like impact of climate change on various physiological or genetic processes. However, the Himalayan timberline has been inadequately studied compared to the European counterparts due to difficult and inaccessible terrain (Shi and Wu, 2013). Generally, alpine species are highly vulnerable to the global warming, landscape fragmentation, and limited distribution range at top of the mountains. Intraspecific genetic variation ensures the adaptive and evolutionary capability of the species to respond against selective pressure imposed by anthropogenic activities and the long-term impact of changing climate. In order to estimate their adaptive potential, it is crucial to evaluate an overall genetic diversity and understand the population genetic processes triggered by the selection pressure imposed by changing environmental conditions.

The SSRs available in *B. alnoides* (Guo *et al*., 2008) were tested for their cross-species transferability in sampled populations of *B. utilis*. Owing to the high genomic homology, 68.42 per cent SSRs of *B. alnoides* were successfully cross-amplified with good level of polymorphism. Some SSRs reported in *B. utilis* (Wani *et al.*, 2019) were also screened for polymorphism in the sampled populations which successfully amplified with high level of polymorphism as expected. Finally, 16 highly polymorphic and reproducible SSRs were used in the present study for genotyping.

Results showed a high level of genetic diversity in studied populations of Western Himalayas. The mean value of key diversity measures, such as allelic richness, observed and expected heterozygosity was recorded as 9.403, 0.609, and 0.785, respectively. The diversity hotspots with high allelic richness were identified for prioritization in future conservation programs. Over the distribution range, most populations showed high allelic diversity but some populations with remarkable diversity parameters were depicted in two populations of Garhwal region, namely BU-10 (Triyuginarayan, Rudraprayag FD) and BU01 (Rudranath, Kedarnath WD) and two populations from Kumaon region *viz.*, BU06 (Himkhola, Pithoragarh FD) and BU11 (Darma valley, Pithoragarh FD). Besides, the populations encompassed with private alleles are also important for the conservation point of view but surprisingly most of the private alleles were also captured by these same populations.

Positive inbreeding coefficient indicated a heterozygote deficiency in most populations. Alpine landscapes are highly heterogeneous in environmental conditions, altitudinal gradient, temperature variation, topographical features, high mountain ridges, steep valleys, varied sunlight incidence, etc., which have considerable control over geneflow (Körner, 2003). Restricted gene flow in geographically isolated populations could lead to a strong genetic differentiation as compared to the geographically connected populations at subalpine or lower mountain ranges (Till-Bottraud and Gaudeul, 2002). Several other factors, such as population size and mating system, could also play a profound role in shaping the genetic diversity and differentiation (Hartl and Clark, 1997). Usually, out-crossing species possess a high genetic diversity within the populations and low population differentiation, and vice versa in self-pollinated species (Hamrick and Godt, 1996, 1997; Nybom, 2004).

Being a timberline species, genetic differentiation and gene flow are the important genetic characteristics to be investigated in *B. utilis*. Present study showed low genetic differentiation (F_{ST} = 0.081; p = 0.01) with relatively high gene flow (*N*m = 2.968) among the studied populations. Due to adequate genetic exchange among populations of continuous distribution range, most genetic variance was observed within the populations. Low genetic differentiation could be attributed to its outcrossing nature, continuous geographical span, and high wind flow at mountain tops. Flowers in *B. utilis* are monoecious with female and male catkins in same tree, pollination is mainly wind-driven, and the seeds are winged. Wind plays a major role in seed or pollen dispersal, and seeds can be blown away along the surface of the snow as well as up to the height of 80 m from the mother tree (Anonymous, 2010). Therefore, long-distance gene flow takes place across the geographically separated populations of *B. utilis*. Also, the distribution pattern of *B. utilis* is reported as more continuous and extensive in the western and central Himalayan regions (Bobrowski *et al.*, 2017).

The UPGMA dendrogram and PCoA plot have displayed two major clusters with high boot strap support, where eight populations were grouped together in single cluster, showing high level of genetic exchange in most of the distribution range. However, second major cluster was formed by two populations, BU02 (Gangotri, Uttarkashi FD) and BU09 (Har-Ki-Dun, Govind NP) which were located at the western edge of the Uttarakhand Himalayan Range connecting to Himachal Pradesh in the west and China in the north. Surprisingly, a population BU11 (Darma Valley, Pithoragarh FD), located at most extreme edge of Eastern Uttarakhand near Nepal border, did not group with any other defined clusters and behaved as outlier. As evident from the diversity map (Fig. 10.25), this population appeared as most diverse and unique, possessing significant allelic and private allelic richness. Surprisingly, high level of structuring was detected in the studied populations with four predicted gene pools, which was mostly found in concurrence to the genetic clustering. Out of 11 studied populations, six were clearly assigned to one of the four defined clusters with membership coefficient value higher than 0.8 and other five showed admixed ancestries. Such abrupt clustering and structuring pattern observed here indicated a differential gene flow across the range, may be due to the presence of some sturdy geographical or ecological barrier.

Overall, the sampled populations have been presumed to be under pressure due to over-collection from specific localities for its papery bark or fuel wood. For instance, Gangotri region of Uttarakhand remained as the most vulnerable for manual collection of bark by pilgrims, which might have led to reduced regeneration or population decline. Though damage caused by the ongoing anthropogenic activities may not be detectable at present but the impact could be pronounced in long term, if the adverse conditions prevailed for longer. Over the period, populations continuously accumulate the genetic changes in response to the selection pressure. As evident by genetic clustering and structuring, three populations, BU02 (Gangotri, Uttarkashi FD), BU10 (Triyuginarayan, Rudraprayag FD), and BU11 (Darma Valley, Pithoragarh FD), have displayed significant genetic distinctness with differential genetic exchange. It indicated that the populations have adopted significant genetic change in response to the selection pressure. For instance, severe population decline was recorded at some locations in the Himalayan Ranges but the degree of threat varied among the States or Countries. Based on threat perception, the species has been assigned different threat status categories in different States or Countries e.g., species has been declared as Critically Endangered in Jammu and Kashmir, India (Anonymous, 2010), whereas it is assigned as the Least Concern category by the IUCN Red List of Threatened Species (Shaw *et al.*, 2014).

10.6.5

Rhododendron arboreum

Rhododendron species are the 'keystone element' of the Himalayan mixed forest extended from 1,500 m to 3,300 m amsl, providing ecological and livelihood services to the hill dwelling communities (Bhandari *et al.*, 2020; Singh and Chatterjee, 2021). *R. arboreum* is one of the dominantly occurring species in north-western Himalayas and support diversity of various life forms, such as native, endemic, and rare endangered plants, insect and bird pollinators, etc. (Singh *et al.*, 2009). Despite the widespread distribution, this species is experiencing substantial degree of threat due to various factors including habitat degradation and fragmentation, population shrinkage, anthropogenic activities, climate change, etc. (Mamgain *et al.*, 2017; Bhandari *et al.*, 2020). A recent study has predicted the upward shift of *R. arboreum* in the western Himalayas in relevance to future climate change scenarios (Veera *et al.*, 2020). Also, the populations occurring at high altitude are relatively stable and healthy than the middle or lower altitude region due to increasing anthropogenic activities in lower accessible forests.

Nevertheless, it has been listed in the Least Concern category of the IUCN Red List of Threatened Species (BGCI and IUCN, 2018).

Generally, out crossing plant taxa possess high within population genetic diversity than the self-pollinating plants (Nybom, 2004). Besides population size, extent of distribution range also plays a key role in shaping genetic diversity and population structure in forest tree species. For instance, plant taxa with widespread populations possess higher genetic diversity with lesser genetic differentiation than the narrowly distributed taxa. In agreement to these facts, current SSR marker analysis revealed a high genetic diversity (Ho = 0.707; He = 0.837; Rs = 10.862) in the sampled populations of *R. arboreum*. AMOVA analysis revealed high genetic variance within the populations indicating low level of genetic differentiation. High genetic diversity and lesser genetic differentiation could be attributed to the gregarious and inter connected population distribution in wider geographical range of the Himalayas. Locally, the species spreads through its root suckers, but it relies on sexually produced seeds for long-distance dispersal. A detailed mechanism and extent of gene flow, pollination ecology, and breeding biology are not adequately studied in *R. arboreum* but out-crossing has been reported as the primary mechanism of sexual reproduction (Ng and Corlett, 2000). Several studies have demonstrated extensive pollen movement in wind and insect-pollinated trees like *Quercus* spp. (Dow and Ashley 1998; Streiff *et al.*, 1999), *Pithecellobium elegans* (Chase *et al.*, 1996) and *Magnolia obovate* (Isagi et *al.*, 2000), *Rhododendron metternichii* (Kameyama *et al.*, 2001), etc.

The populations with high allelic richness were visualized in the distribution map (Fig 10.26), which showed high genetic diversity in most populations. For conservation implications, four populations *viz.*, RA19 (Badhanital, Rudraprayag FD), RA22 (Dhanaulti, Narendranagar FD), RA11 (Chaurangi Khal, Uttarkashi FD), and RA23 (Ghes, Badrinath FD) are recommended in Garhwal region while the population RA16 (Dunagiri, Almora FD) is recommended for *in situ* conservation in Kumaon region. In addition, five populations were identified to possess significant proportion of private alleles namely, RA18 (Chirbatiya, Tehri FD), RA24 (Gwaldam, Badrinath FD), RA19 (Badhanital, Rudraprayag FD), RA16 (Dunagiri, Almora FD), and RA23 (Ghes, Badrinath FD), and which could be prioritized in future conservation programs.

In a SNP marker-based study on *R. canescens* (a native species to the southeastern United States), high genetic diversity, low genetic differentiation, low inbreeding coefficient and significant gene flow was recorded (Yadav *et al.*, 2019). Wang *et al.*, (2019) have investigated wild *R. simsii* populations from the Dabie Mountains (Central China), and reported high level of genetic diversity (He: 0.64 – 0.79) with 84.34 per cent of this genetic variation within the populations. Contrary to these findings, in the present study, moderate level of genetic differentiation (F_{ST} = 0.156) was reported with relatively low level of gene flow (Nm = 1.352). Present study findings were also found in agreement with the earlier SSR-based studies where high genetic diversity was reported for the *R. arboreum* populations of Arunachal Pradesh (Bandali, 2008) and Himachal Pradesh (Choudhary *et al.*, 2014; Sharma *et al.*, 2020). The estimated number of migrants per generation among populations was reported as 3.132 which are high enough to overcome differentiation among populations by genetic drift (Wright, 1949). Despite the small sized and light weighed seed, limited seed dispersal is reported in most *Rhododendron* species (Ng and Corlett, 2000; Yang *et al.*, 2020; Cao *et al.*, 2022). Though it is not well studied in *R. arboreum* but if true the high gene flow would have been achieved by the continuous interconnected distribution range.

In the situations of endangering and endemism, genetic diversity drastically declined and the population showed high genetic differentiation, but recorded remarkably good diversity with low to moderate genetic differentiation in the Chinese endangered *Rhododendron* species, namely *R. longipedicellatum* (Cao *et al.*, 2022) and *R. protistum* var. *giganteum* (Wu *et al.*, 2017). Similarly, in *R. brachycarpum* occurring in fragmented geographic range of East Asia, a good level of genetic diversity was reported for the populations of Korea and Japan (He=0.556 - 0.626) (Polezhaeva *et al.*, 2021). However, the diversity was quite low for the small and isolated (peripheral) populations of Russia (He=0.100 - 0.369) with a high inbreeding coefficient (F_{IS}=0.471 - 0.526). Also, strong population differentiation (F_{ST}=0.356) and structuring was detected in the studied populations.

Results of the present study showed that the genetic relationship was in accordance with different clustering methods, namely UPGMA dendrogram, PCoA plot, and structure analysis. All the populations have been clearly divided into two distinct groups with high boot strap support. However, clustering pattern was not observed in coherence to their spatial distribution indicating differential pattern of gene flow irrespective of their geographical distance. Further, strong genetic structure was observed in *R. arboreum* populations where all the populations were clearly defined by two inferred genetic clusters without any admixture. The genetic relationship was not correlated with geographic distances between the populations indicating lesser role of geographic constraints in genetic structuring. Probably, populations may not follow the isolation by distance rule in the species with a large contiguous distribution area (Hutchison and Templeton, 1999; Wang *et al.*, 2019).

10.7

Key Messages and Priority Actions

- Maintenance of genetic diversity is one of the main objectives of conservation programs (Frankham *et al.*, 2010; Allendorf *et al.*, 2013; Oldenbroek, 2017), and it is aimed at maximizing either expected heterozygosity or allelic diversity (López-Cortegano *et al.*, 2019). However, maximization of allelic diversity has been reported to be more efficient in maintaining the genetic diversity of subdivided populations than maximization of expected heterozygosity because the former maintains a larger number of alleles and better control of inbreeding (López-Cortegano *et al.*, 2019). Hence, priority can be given to the populations with higher allelic diversity and private alleles for conservation programs (Petit, El Mousadik, and Pons, 1998).

- Overall, high genetic diversity was recorded in all five studied Himalayan tree species which indicated their high adaptive and evolutionary potential. The genetic information and the genetic diversity hotspots identified in the present study will serve as the guiding principles for the future conservation programs. On the basis of molecular characterization, particularly allelic richness and private allelic richness, the following genetic diversity hotspots in case of five targeted species have been identified:

  (i) ***Q. semecarpifolia*** – QS09 (Radi Top, Upper Yamuna Barkot FD), QS11 (Bhukkitop, Uttarkashi FD), QS15 (Munsiyari, Pithoragarh FD), QS19 (Mundhola, Chakrata FD), QS21 (Pinswar, Rudraprayag FD), QS23 (Narayan Ashram, Pithoragarh FD), and QS24 (Himkhola, Pithoragarh FD)

  (ii) ***M. esculenta*** – ME08 (Sandev, Pithoragarh FD), ME15 (Takula, Almora FD), ME16 (Shitla Khet, Almora FD), ME17 (Bhowali, Nainital FD), and ME18 (Mayali, Rudraprayag FD)

  (iii) ***T. wallichiana*** – TW12 (Mundhola, Chakrata FD), TW15 (Himkhola, Pithoragarh FD), TW18 (Mornaula, Nainital FD), TW19 (Har ki Dun, Govind WLS and NP), and TW21 (Baling, Pithoragarh FD)

  (iv) ***B. utilis*** – BU01 (Rudranath, Kedarnath WD), BU06 (Himkhola, Pithoragarh FD), BU10 (Triyuginarayan, Rudraprayag FD), and BU11 (Darma Valley, Pithoragarh FD)

  (v) ***R. arboreum*** – RA11 (Chaurangi Khal, Uttarkashi FD), RA16 (Dunagiri, Almora FD), RA18 (Chirbatiya, Tehri FD), RA19 (Badhanital, Rudraprayag FD), RA22 (Dhanaulti, Narendranagar FD), RA23 (Ghes, Badrinath FD), and RA24 (Gwaldam, Badrinath FD)

  Above identified hotspots are recommended as the prioritized *in situ* conservation sites among sampled populations in order to ensure the maintenance of high genetic diversity among five targeted species. These hotspots need to be taken into consideration while planning for *in situ* conservation (e.g., establishment of forest gene banks), any type of *ex situ* conservation (e.g., field gene banks) including germplasm storage, rescue of inbred populations, or even the production of starting material for propagation.

- The populations identified with unique set of private alleles (with high values of PRs) in each of the five prioritized FGR species are of utmost importance from the perspective of conservation as the rare alleles contained in these populations could gradually be lost, if such populations continue to deteriorate. Hence, such populations with rare private alleles need to be firstly, effectively protected and conserved; and secondly, small populations in such cases need to be augmented by reforestation and enrichment planting using the germplasm of its own population so as to maintain their unique characteristics based on private alleles.

- Select sampled populations of the two targeted species *viz.*, *Q. semecarpifolia* and *T. wallichiana* showed substantial inbreeding (higher values of F_{IS}) and require immediate conservation attention. Augmented gene flow from neighboring genetically diverse populations needs to be considered as a way of increasing the fitness and adaptive potential of these inbred populations. The sampled populations of *Q. semecarpifolia* and *T. wallichiana* across different sampled forest sub-types in the present study revealed as such 'good' regeneration (see section 3.6.4.2). However, the Chapter 12 on standardization of propagation techniques and production of starting material amply highlighted that *T. wallichiana* is dioecious i.e., with separate male and female plants and they differ in their reproductive capacity. Collected fresh seeds failed to germinate away from their natural environment, and the six studied populations in the State yielded low seedling survival with the best storage duration of 42 months. The use of seeds proved to be unreliable option for raising nursery stock in the species. Instead, branch cuttings especially taken from male trees of *T. wallichiana* were found to have higher sprouting, rooting, and survival percentage than those from female trees. Identified inbred populations in case of both the species need careful field level observations so as to diagnose the causative factors for inbreeding. It is also possible that in these two species, either populations are of small size or their seeds have some issues on account of reproductive biology, phenology, local environmental and biotic conditions, restricting their production as well as germination, recruitment, and establishment.

- Genetic differentiation and gene flow are two another important genetic processes of natural populations which are inversely related but controlled by multitude of factors related to the species and geographical distribution range. As most of the studied species are out-crossing and distributed in contiguous range over large geographical span, the gene flow has been maintained to be sufficient to counter the effect of genetic differentiation. Moderate level of genetic differentiation and structuring were recorded in *Q. semecarpifolia* due to strong physical barriers imposed by the mountain ranges and river systems of Western Himalayas leading to the emergence of two distinct gene pools (GHC and MHC gene pools). Thus, two distinct gene pools need to be maintained as independent reservoirs of alleles so as to conserve the genetic diversity captured in these gene pools in the long run.
- Further, significant genetic structuring was detected in the populations of *Q. semecarpifolia, B. utilis*, and *R. arboreum*, and therefore, conserving any random population will not serve the conservation purpose. By utilizing this important genetic information, the seed or planting material must be collected from the diverse populations identified in each genetic cluster for the establishment of FGCUs as well as *ex situ* field gene banks. By looking on the overall statistics of the genetic data of three high altitude species (*B. utilis*, *Q. semecarpifolia*, and *T. wallichiana*), the populations present in Pithoragarh FD, particularly Darma Valley and Narayan Ashram in Dharchula forest range emerged as the most important reservoirs of allelic diversity, and could be considered at top priority in conservation programs.

10.8

Recommendations and Future Prospects

- Advances in molecular characterization techniques have opened new avenues in the fields of conservation, tree improvement, and commercial utilization of species, besides providing opportunities for deeper insight on genetic diversity existing in a species, and causative factors for genetic erosion. In general, there has been an increasing recognition of the role of genetic diversity for ecological and economic resilience, and greater emphasis to prevent genetic erosion and safeguarding genetic variability. In the wider connotation, safeguarding genetic diversity 'seeks actions *in situ* or *ex situ* which are designed to characterize, slow, halt or reverse genetic erosion, and promote the processes ensuring adaptive potential'. The field and laboratory-based techniques involved in molecular characterization are complex, time consuming, expensive, and requires professionally skilled man power. The Pilot Project facilitated molecular characterization of just five select prioritized FGR species which provided a deep insight in formulating future strategies for their conservation. It covered five ecologically and economically important species in a vast Himalayan State and provided desired hands-on experience to deal with field level assessments and sequential laboratory investigations. Certainly, present contribution is meagre in view of the vast diversity of FGR. Although, the overall process of characterization may appear time consuming and slow, against the unprecedented loss of forest biodiversity, but modern genetic tools definitely provide an edge over the conventional means of assessing genetic diversity based on morphological characteristics in a vast and difficult State like Uttarakhand. In view of this, there is an urgency to replicate similar efforts to assess genetic diversity of prioritized FGR species, particularly species which play vital role as 'ecosystem engineers' or keystone species, instead of species listed under red list categories as they are poor predictors of genetic erosion owing to small population size.
- Translocation of germplasm from distant locations and gene mixing across genetically different clusters (e.g., GHC and MHC gene pools identified in case of *Q. semecarpifolia*) may lead to loss of distinct genetic base of the populations which otherwise might be playing an important role in local adaptation. As far as possible, introduction of genes is recommended between neighboring populations or populations within an identified genetic cluster. Consequences of gene mixing on allele frequency shifts and how such processes could be facilitated to rescue genetically impoverished populations (e.g., select identified inbred populations in case of *T. wallichiana*) or populations under severe threat of extinction are some of the emerging areas for further investigations.
- Assessment on species viability as a genetic conservation unit (population size, and number of reproductive and unrelated individuals) is a prerequisite for molecular characterization of a species.
- Present study has successfully identified genetic diversity hotspots for the targeted FGR species of higher conservation significance and provided desired pathways for establishment of their FGCUs and *ex situ* 'field gene banks'.

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FUNGAL CHARACTERIZATION OF FGR

11

Kainthola, C., Saxena, J., Pandey, V.V., Tyagi, K.,
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Forests are one of the most important global ecosystems, performing multifarious functions from maintaining healthy catchment, to wildlife habitat, environmental amelioration, carbon sequestration and sink, nature tourism, and raw material to forest-based industries through harvesting of timber and non-timber forest products. Appreciating and mitigating a wide range of threats to these vital forest resources is therefore of paramount importance for ecological and societal needs. Forest pests (insects, nematodes, rodents and other animals) and pathogenic (disease causing) microorganisms including bacteria, viruses, and most commonly, fungi are amongst the most known and obvious threats to forest health, with their impacts amplified by current issues such as enhanced biotic disturbance, climate change and global trade (Allen *et al.*, 2010; Wingfield *et al.*, 2016; Linnakoski and Forbes, 2019). Globally, enhanced frequency and severity of insect outbreaks and pathogen infestations, particularly fungal diseases have led to the loss of significant forest areas and reduced productivity, and urgently calls for the priority need for rigorous research not only to understand the underlying causes, but also to appreciate their impacts and develop measures for mitigation (Roy *et al.*, 2014; Linnakoski and Forbes, 2019). Forest pests and pathogens have understandably attracted strong research attention in India and other parts of the world, particularly in countries where forestry has been a major economic industry. Fungal infection is a common ailment for forestry trees. Fungal diseases of prominent and commercially important trees in India have been documented by Bakshi (1976) while Shankhwar *et al.*, (2021) have studied fungal infestations and diversity in the Greater Himalayas. As a part of the Pilot Project on FGR in Uttarakhand, studies based on fungal characterization of five prioritized tree species *viz.*, *Rhododendron arboreum, Taxus wallichiana, Quercus semecarpifolia, Myrica esculenta* and *Betula utilis* formed an integral part of the Component on FGR Characterization.

11.1

Fungal Pathogens and Diseases Associated with Tree species

Fungi are filamentous microorganisms that lack chlorophyll and hence must obtain nutrients from living hosts or organic matter. Fungi, once considered to be plants are now classified in their own kingdom separate from both plants and animals. Varying estimates for global diversity of fungi exist. According to BSI (2021), 72,000 species of fungi have been identified and described globally, but much more than this species of fungi may exist worldwide. As stated earlier in Chapter-1, in India, fungal species accounts for nearly 30 per cent of floral diversity among which 4,100 are endangered and 580 are threatened species (MoEFCC, 2019). Not all fungi are pathogenic; in fact, most are obligate saprophytes meaning they can only feed on organic material. These fungi play vital role in decomposing dead plant material and recycling nutrients. Some fungi are facultative pathogens, meaning that they can live on dead plant material, but they can also attack living plants and cause disease. Other fungi are obligate pathogens that can only survive on a living host plant. Fungi are infectious and transmissible, meaning they can spread from one host plant to infect another, and thus cause disease. They are not disease themselves. Fungi cause disease and they can result into sustained disruption of physiological or structural functions of a plant that results in damage to cells or tissues, reduced growth or vitality or economic losses or even death. A disease is the resulting interaction between a host, pathogen and environment and can occur under certain environmental conditions only. Plant pathogens are host specific, and have only one or a few suitable host species while some can attack hundreds of plant species. Trees and other plants have evolved structural and chemical defenses such as thick bark, waxy leaf coatings, root secretions and antimicrobial toxins that prevent infections. Generally, plants have 'pre-formed' defense mechanism in place and provide general protection from pathogens. However, certain pathogens have developed virulence factors (e.g., enzymes that degrade plant tissues; special structures that can pierce plant cells; or specialized metabolic pathways that can neutralize host toxins) that enable them to overcome general plant defenses. In response, plants have capacity to detect pathogen and can initiate powerful induced defenses. However, pathogens continue to adapt induced plant defenses or develop virulence factors. Some plants are resistant to specific pathogen while susceptible plants are vulnerable to pathogen attack. A plant can be resistant to one pathogen but susceptible to another. Pathogens have specific environmental requirements to complete their lifecycle. Several fungi produce spores within a very narrow temperature range only while some spores can spread only in splashing rain, high winds or through a specific insect vector. The disease cycle includes: (a) transmission- movement of the pathogen from one host plant to another, (b) infection- an act of the pathogen entering the host, (c) colonization- invasion of plant tissues by the pathogen, (d) parasitism- when spreading pathogen begins to feed on plant tissues, (e) symptom development- occurs in response to the damage caused by the pathogen, (f) reproduction- necessary for the pathogen to complete its lifecycle, and (g) transmission- the reproductive structures (spores) move to the other host. Identification of 'weak points or vulnerabilities' in the disease cycle is the first and key step in designing control or management strategies.

Insect pests carry out variety of injuries in the plant due to their feeding habits, which are often introduced from carriers, competitors and predators in their new environments, and target healthy trees without evolved resistance (Linnakoski and Forbes, 2019). The nature of fungal-insect interactions is diverse, ranging from incidental associations in shared habitats to co-evolved obligate nutritional mutualisms (Hulcr and Stelinski, 2017). Introduced invasive pathogens may pass to native insect species to vector, and invasive insect pests may become vectors for native or already established invasive pathogens (Haack, 2006; Wingfield *et al.*, 2016). Novel beetle-fungus interactions include some of the most important invasive species affecting forest ecosystems, such as the Dutch elm disease and beech bark disease (Ploetz *et al.*, 2013; Santini and Faccoli, 2013; Cale *et al.*, 2017). Tree resistance is a critical parameter when considering the negative impacts of insect pests and pathogens. Environmental disturbances, especially those associated with climate change, such as droughts, floods, storms, and elevated temperatures, are particularly concerning. These events can cause physical damage to trees (Allen *et al.*, 2010), enhancing the ease of colonization by insects and their accompanying fungal pathogens, while prolonged stress may also impair the ability of trees to direct resources toward defense and repair (Bolton, 2009).

Different fungal species could be associated with different plants and vary with respect to the geographical locations and plant parts. Many are present on the plant surface or as endophytes causing no harm to the plants. However, reduction in plant vigour due to different reasons such as aging, abiotic and biotic disturbances leads to predisposition of various fungi which results in diseases. An innocuous fungal species may turn pathogenic when encountered with favourable conditions. Diseases are major biological determinants of forest productivity affecting nurseries, plantations and natural forests adversely. At nursery level, they cause heavy damage to seedlings and saplings, resulting in huge reduction in quantity and quality of planting stock. In plantations and other germplasm repositories, diseases cause major problems, thus leading to less biomass production and loss of valuable germplasm collections. Natural forests are also not unaffected by various biotic and abiotic factors. Under environmentally suitable conditions, forests (both natural and man-made) are expected to grow healthy. However, under adverse conditions of growth which include edaphic, climatic and biotic factors, and in a locality where a disease is present, forests may become diseased. Infections in trees develop over time with favourable environment, virulence of the pathogens and susceptibility of the hosts. Fungi induce a wide range of diseases in plants. They cause seed rots, seedling damping-off, root rots, foliage diseases, cankers, vascular wilts, diebacks, galls and tumors, trunk rots, and decays of ageing trees.

Due to drastic changes in the ecosystem owing to natural or human induced factors, catastrophic losses occur in the event of an outbreak of a disease. Thus, there is an utmost need to record the associated fungal species with highly valuable tree species. With the change in climatic conditions over a long time period, the fungal species may turn pathogenic or become more virulent, though this needs further studies in this direction. As a part of the project, different symptomatic expressions associated with fungi were studied.

11.1.1

Earlier Reports of Fungal Species

Trees of high altitudes have always attracted the attention of foresters and botanists urging them to unleash new dimensions in various fields of study. *Rhododendron arboreum*, an important forestry species has extensive medicinal and commercial uses as it exhibits anti-inflammatory, hepato-protective, anti-diarrheal, anti-diabetic, anti-oxidant properties due to presence of flavonoids, saponins, tannins and other phytochemicals (Srivastava, 2012). *Quercus semecarpifolia* is one of the dominant trees of Himalayan forests and play an important role in maintaining ecosystem stability as it protects soil fertility, watershed and local biodiversity (Shrestha, 2003). *Taxus wallichiana*, an evergreen tree, has been used by the native populations for treating their common ailments and the leaves and bark are a prime source of taxol, a potent anticancer drug (Juyal *et al.*, 2014). *Myrica esculenta*, a well-recognized tree species of ayurvedic pharmacopeia, is widely used to treat several asthmatic, inflammatory and gastrointestinal disorders due to its multidimensional pharmacological and therapeutic effects (Kabra *et al.*, 2019). *Betula utilis*, a long-lived Himalayan tree species, possess anti-microbial, anti-inflammatory, anti-cancerous, anti-oxidant and anti-HIV activities and its bark is antiseptic and carminative (Farooq *et al.*, 2017).

Despite of unimaginable therapeutic and ecological importance, these tree species also suffer from various fungal diseases which suppress their physiological activities. Table 11.1 illustrates some pathogenic, endophytic, saprophytic and other associated fungal species reported earlier with respect to selected FGR species.

Table 11.1 Fungal Species Reported by Earlier Workers on Selected FGR Species

Sr. No.	Host plant	Disease/ Association	Fungal Species	References
1.	*Rhododendron arboreum*	Ectomycorrhiza	*Gyroporus castaneus*	Lakhanpal *et al.* (1985)
		Leaf spot	*Phomopsis ericaceana*	Gaur *et al.* (1986)
		Ectomycorrhiza	*Amanita fulva* *Russula heterophylla*	Bhatt *et al.* (1988)
		Sooty mold	*Leptoxyphium fumago* *Flagellospora penicilloides*	Singh *et al.* (1990) Sati *et al.* (1990)
		Root rot	*Armillaria* sp.	Moorman (2016)
		Stem canker	*Phytopthora* sp. *Botryosphaeria dothidea*	
		Leaf gall	*Exobasidium vacinii* *E. japonicum*	
		Leaf spot	*Cercospora handelli* *Colletotrichum gloeosporoides* *Pestalotiopsis* sp.	
		Leaf blight	*Cylindrocladium scoparium*	
		Web blight	*Rhizoctonia solani*	
2.	*Quercus semecarpifolia*	Heart rot	*Fomes senex* *Hymenochaete rubigiosa* *Polyporus gilvus*	Anonymous (1950)
		Root rot	*Armillaria mellea* *Ganoderma lucidum*	Bagchee *et al.* (1954)
		Saprophytic growth	*Trametes gibbosa*	
		Leaf spot	*Gloeosporium quercinum* *Phyllosticta maculiformis*	Phillips and Burdekin (1982)
		Saprophytic growth	*Irpex lacteus*	Sarbhoy and Agarwal (1987)
		Ectomycorrhiza	*Russula fuelitii*	Saini and Atri (1989)
		Anthracnose	*Apiognomonia* sp.	Moorman (2016)
		Leaf scotch	*Xylella fastidiosa*	
		Wilt	*Bradziella fagacearum*	
		Saprophytic growth	*Daldinia concentrica* *Fomes fomentarius* *Trichaptum biforme*	Shankhwar *et al.* (2021)

Sr. No.	Host plant	Disease/ Association	Fungal Species	References
3.	*Taxus wallichiana*	Leaf spot	*Atopospora taxi* *Chaetopyrina negii* *Alternaria alternata* *Colletotrichum gloeosporoides* *Pestalotiopsis guipini* *Phomopsis juniperivora*	Muller (1958) Srivastava (1974) Mirski (2012)
		Leaf blight	*Cladosporium macrocarpum* *Fusarium avanaceum*	
4.	*Myrica esculenta*	Important fungal reorts lacking		
5.	*Betula utilis*	Endophytes	*Alternaria* sp., *Aspergillus* sp., *Fusarium* sp., *Mortierella* sp., *Penicillium* sp., *Phoma* sp., *Pythium* sp., *Trichoderma* sp.	Rattan *et al.* (2017)

11.1.2

Signs and Symptoms: Key to Identification of Fungal Diseases

Signs refer to the evidences of diseased conditions in the trees which include vegetative or fruiting structures of the disease-causing organisms which usually develop at or near the points of infection, for e.g., fruit bodies, epicormic branches, exudation, rust, etc. whereas symptoms are the apparent expressions of disease processes in the plant e.g., root rot, heart rot, butt rot, leaf spots, leaf blight, wilt, canker, decay, necrosis, witches'-broom, etc. When a plant becomes infected, it takes time for symptoms to develop (Bakshi, 1976).

Fruiting structures or sporophores or fructifications of fungi commonly develop on diseased plants and are important signs of the disease. Epicormic branching is a result of limited growth of dormant or adventitious buds due to adverse conditions or infection, thus, causing clusters on the main stem. They may also appear as Witches'- broom. Exudations could result either due to normal physiological process or caused by microbial infections. Rust disease often appears as powdery mass of golden, yellow, orange, brown or black pustules on upper/ lower surface of leaves. In root rot, mycelia spread over the host's roots by the invasion of fungal pathogen in internal tissues. Heart rot leads to decay of wood at the center of the trunk and branches when heartwood becomes exposed through openings in the bark. The moist, poorly protected undersurface of tree trunk above the root is attacked by the fungus, affecting the roots along with is called butt rot. Leaf spots are discolored diseased area/ lesions caused by action of microorganisms often promoted by prolonged wet and humid conditions. Leaf blight is sudden and severe yellowing or drying of plant parts caused by fungal or bacterial infections and is most apt to occur under moist conditions. Pathological wilting is often caused due to the blockage of vascular tissues by pathogens or their metabolites, thus causing hindrance in the ascent of sap. Cankers are localized lesions on woody stems formed as a result of killing of bark tissues exposing the wood from beneath. Decay results in breaking down of trunk or root tissues by fungal infections in the heartwood or sapwood thus causing loss of mechanical strength and could eventually lead to wind throw. Necrosis is death of the affected tissues of plant usually during early stages of attack resulting in discoloration or shot holes.

11.2

Materials and Methods

Five prioritized FGR species and their different sampled populations formed the material for fungal characterization. Broadly, studies on fungal characterization included: (a) field surveys and sampling, (b) isolation and identification of the fungal pathogens, and (c) preservation of fungal cultures. Details of these activities are elaborated below:

11.2.1

Survey and Sampling

Surveys in different forest areas of Uttarakhand were undertaken periodically in the natural distribution range of selected tree species. Methodology of survey and sampling of fungal diseases of those tree species was designed with modification (Bakshi, 1977) and all the required parameters (*viz.*, tree health, any kind of infection and injuries, Disease Intensity %, etc.) for individual infected tree were recorded on the disease survey data sheet. Sampling was mainly carried out from March to July during 2016-2019 to record the outbreak of fungal diseases and sporophores.

The core natural forests in the State were surveyed for recording symptomatologic expressions and collection of affected tissues of the five targeted species which were found to be associated with various fungal species, of which some were pathogenic and some were saprophytic in nature. The diseases were identified on the basis of signs and symptoms. For exploration of the fungal organisms, logs, living trees and forest floor were examined. The sampling of the fungal fruit bodies and tissues from diseased tree parts (leaves, stem, roots, etc.) was carried out. Samples were placed in individual paper bags with their serial number, dried (except for foliar samples) in a dry, warm place in order to prevent molds developing on the samples (Sharma, 2012). After recording disease symptomatology, incidence and severity, sampling of infected plant parts and fungal fruit bodies were performed for pathogen isolations, pure culturing and determination of taxonomic identity of fungal species.

11.2.2

Isolation and Identification of the Fungal Pathogens

Diseased samples were collected from different field locations and placed in brown paper bags. Diseased tissue samples were surface sterilized with 0.1 per cent sodium hypochlorite solution and placed on potato dextrose agar (PDA) plates in aseptic conditions. The plates were incubated at 25±1°C in a BOD incubator for 3-5 days. The sample pieces started showing mycelial growth after 3-5 days of incubation. The pure cultures of these isolated fungal species were then maintained at 4°C in a refrigerator. The taxonomic identities of different fungal isolates were determined on the basis of morphological and microscopic characteristics with the help of standard manuals, monographs and taxonomic keys such as Gilman (1957), Barnett and Hunter (1972), Booth (1971), Ellis (1971 and 1976), Sharma (2012), etc.

11.2.3

Altitudinal Measurements of the Locations

The altitude of the field locations of disease sampling for all the prioritized species was recorded by GPS Held-Hand Device and vapour pressure for the same was extracted using ArcGIS software.

11.2.4

Preservation of Fungal Cultures

The preservation and maintenance of stock/ pure cultures are required for microbiological and pathological purposes. Paraffin oil preserves fungal cultures by covering actively growing mycelium in the agar slants. This technique has long been recognized as an effective means of extending the longevity of the common agar slant. In addition to reducing the effects of drying, the reduced availability of oxygen slows the fungal metabolic rate. The pure fungal cultures were preserved in paraffin oil and were deposited in the National Type Culture Collection (NTCC) at Forest Pathology Discipline, Forest Protection Division, FRI, Dehra Dun.

11.3

Research Findings

Research findings on fungal characterization of five prioritized species are summarized below on the basis of: (a) signs and symptoms of pathogenic and saprophytic fungi recorded in case of different studied populations of each of the five species and associated fungal species observed in the field situation, and (b) disease intensity in sampled populations of each species with respect to altitude and vapour pressure. In addition, microscopic and morphological characteristics of isolated fungal species, and a comparative account on occurrence of fungi in five species are also presented.

11.3.1

Fungal Species Recorded in Five Species

The five investigated species with reference to signs and symptoms and disease intensity in sampled populations are described below one by one:

11.3.1.1

Rhododendron arboreum

Signs, symptoms and associated fungal species recorded on varied sampled populations of *Rhododendron arboreum* are summarized in Table 11.2. In the forests of *R. arboreum* (Fig. 11.1.), *Ganoderma applanatum* was found to be causing butt rot in the live trees in Nag Tibba (RA 26) and Kanchula Kharak (RA01), respectively whereas, *Fomes fomentarius* was recorded as heart rot pathogen

in the live trees in Munsiyari (RA 21). *Chaetomium globosum* was found as root rot pathogen on live trees in the forests of Kanchula Kharak (RA 01). Some saprophytic fungal species (*Aspergillus niger*, *Cladosporium* sp., *Phomopsis* sp., and *Heterobasidion annosum*) were also recorded on *R. arboreum* in Budher (RA 04); Binsar (RA 17) and Nag Tibba (RA 26). Regarding foliage infection; leaf spots and blight, bud blight and tip blight were some common symptoms recorded on *R. arboreum* (Fig. 11.2.). The associated fungal species isolated were *Alternaria alternata, A. phragmospora, A. triticina, Cladosporium cladosporioides, C. herbarum, Curvularia lunata, Exobasidium vacinii, Fusarium* sp., *F oxysporum, Gliocladium* sp., *Graphium* sp., *Hansfordia* sp., *Nigrospora sphaerica, Phomopsis* sp., *Pithomyces* sp., *Phyllosticta* sp., *Sordaria fimicola* and *Verticillium* sp. with disease severity ranging from 2-40 per cent in the forests of Kanchula Kharak, Chopta, Mohankhal, Chirapani, Raditop, Chaurangi Khal, Chorikhal, Binsar, Munsiyari, Dhanaulti, Gawaldam, Tiuni, Nagtibba and Kunjkharak. Open wounds on the live trees in Chaurangi Khal and anthropogenic activities were recorded in Tiuni forests (Table 11.2).

As per the results shown in Fig. 11.3, the altitudinal range for *R. arboreum* populations varied from 1,566 to 3,849 m amsl. With the increase in altitude, vapour pressure relatively decreased from 1.41 to 0.49 mm of Hg. A positive relation was observed regarding the disease intensity shown by fungal species and the elevation of the populations. Heart and root rot caused by different fungal species were recorded at higher altitudes at around 2,522-3,027 m amsl with foliar disease intensity varying from 10-30 per cent. However, at lower elevations, saprophytic growth and higher foliar disease intensity (upto 40 per cent) was recorded.

Based on fungal characterization of 27 populations across the state of Uttarakhand, twelve populations *viz.*, RA 03 Janglat Chowki (2,346 m), RA 06 Gairsain (2,014 m), RA 08 Suital (1,566 m), RA 09 Dev Dhula (1,765 m), RA 12 Dudatoli (2,400 m), RA 13 Peethsen (2,272 m), RA 15 Adwani (1,889 m), RA 16 Dunagiri (2,066 m), RA 18 Chirbatiya (3,849 m), RA 19 Badanital (2,455 m), Chandrabadni Temple (1,927 m) and RA 23 Ghes, Dewal (2,210 m) appeared to be superior as no disease incidence was recorded on them. No heart and root rot pathogens were recorded from RA 02 Chopta, Gopeshwar (2,815 m), RA 07 Chirapani (1,799 m), RA 10 Raditop (2,146 m), RA 11 Chaurangi Khal (2,207 m), RA 14 Chorikhal, Bharsar (2,289 m), RA 22 Dhanaulti (2,367 m), RA 24 Gawaldam (1,708 m), RA 25 Tiuni (2,061 m) and RA 27 Kunjkharak, Pangot (2,218 m) but 5-40 per cent intensity of leaf spots was observed.

Table 11.2
Signs, Symptoms and Associated Fungal Species Recorded on *R. arboreum*

Population	Location	Trees' Health	Root	Stem	Foliage (Symptom Intensity)	Remarks
RA 01	Kanchula Kharak, Gopeshwar	Unsound	Root infection (*Chaetomium globosum)*	Heart rot (*Ganoderma applanatum)*	Leaf spots (*Phyllosticta* sp.): 15-30%	Nil
RA 02	Chopta, Gopeshwar	Unsound	Nil	Nil	Leaf blight (*Alternaria alternata*): 5-10%; Bud blight (*Pithomyces* sp.)	Nil
RA 03	Janglat Chowki	Healthy	Nil	Nil	Nil	Nil
RA 04	Budher	Unsound	Nil	Saprophyte (*Heterobasidion annosum*)	Nil	Nil
RA 05	Mohankhal, Nagnath	Unsound	Saprophytic growth	Nil	Leaf spots (*Phomopsis* sp.): 25-30%	Nil
RA 06	Gairsain	Healthy	Nil	Nil	Nil	Nil
RA 07	Chirapani	Unsound	Nil	Nil	Saprophytic growth (*Fusarium* sp.): 5-15%	
RA 08	Siutal	Healthy	Nil	Nil	Nil	Nil
RA 09	Dev Dhula	Healthy	Nil	Nil	Nil	Nil
RA 10	Raditop	Unsound	Nil	Nil	Leaf spots (*Alternaria triticina*): 20-30%	Nil
RA 11	Chaurangi Khal	Unsound	Nil	Nil	Leaf spots (*Cladosporium herbarum; Sordaria fimicola*): 20-40%	Open wounds
RA 12	Dudatoli	Healthy	Nil	Nil	Nil	Nil
RA 13	Peethsen	Healthy	Nil	Nil	Nil	Nil

Population	Location	Trees' Health	Root	Stem	Foliage (Symptom Intensity)	Remarks
RA 14	Chorikhal, Bharsar	Unsound	Nil	Nil	Leaf spots (*Cladosporium cladosporioides*); Leaf blight (*Phomopsis* sp.): 30-35%	Nil
RA 15	Adwani	Healthy	Nil	Nil	Nil	Nil
RA 16	Dunagiri	Healthy	Nil	Nil	Nil	Nil
RA 17	Binsar	Unsound	Nil	Saprophytic growth (*Aspergillus niger*)	Leaf spots (*Curvularia lunata*): 20-40%	Nil
RA 18	Chirbatiya	Healthy	Nil	Nil	Nil	Nil
RA 19	Badanital	Healthy	Nil	Nil	Nil	Nil
RA 20	Chandrabadni Temple	Healthy	Nil	Nil	Nil	Nil
RA 21	Munsiyari	Unsound	Nil	Heart rot (*Fomes fomentarius*)	Leaf blotch (*Verticillium* sp.): 5-15%	Nil
RA 22	Dhanaulti	Unsound	Nil	Nil	Leaf blight (*Fusarium* sp.): 5-10%	Nil
RA 23	Ghes, Dewal	Healthy	Nil	Nil	Nil	Nil
RA 24	Gawaldam	Unsound	Nil	Nil	Leaf tip blight (*Nigrospora sphaerica*); Leaf gall (*Exobasidium vacinii*): 25-30%	Nil
RA 25	Tiuni	Unsound	Nil	Nil	Leaf blight (*Alternaria phragmospora*; *Fusarium oxysporum*): 30-35%	Anthropogenic activities
RA 26	Nagtibba, Nainbagh	Unsound	Butt rot (*Ganoderma applanatum*)	Saprophytic growth (*Cladosporium* sp.)	Leaf blotch (*Hansfordia* sp.): 2-10%	Nil
RA 27	Kunjkharak, Pangot	Unsound	Nil	Nil	Leaf blight (*Gliocladium* sp., *Graphium* sp.): 10-25%	Nil

Conservation of Forest Genetic Resources

Establishment of Center of Excellence on Forest Genetic Resources (CoE-FGR)

Fig. 11.1
Signs, Symptoms, Pathogenic and Saprophytic Fungi Recorded on *Rhododendron arboreum*: (A) Heart and Butt Rot, (B-C) Heart Rot, (D) Anthropogenic Activities, (E) Saprophytic Growth of *Cladosporium* sp., (F) *Ganoderma applanatum*, (G) *Fomes fomentarius*, (H) *Heterobasidion annosum*, (I) Saprophytic Growth of *Aspergillus niger*, (J) Leaf Blight by *Alternaria alternata*, (K) *Phomopsis* Leaf Spot, (L) Leaf Spots Caused by *Curvularia lunata*


E
F
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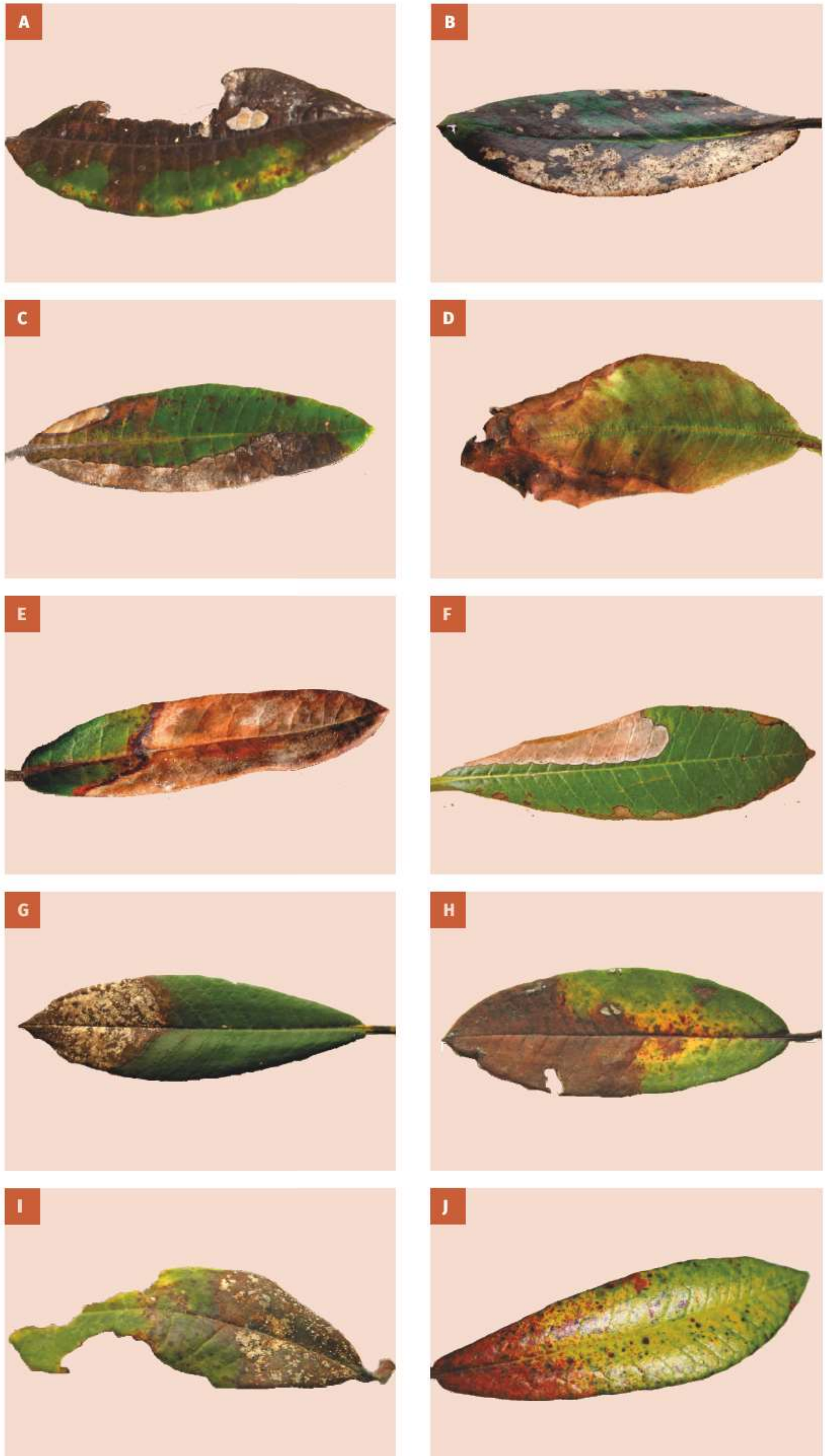
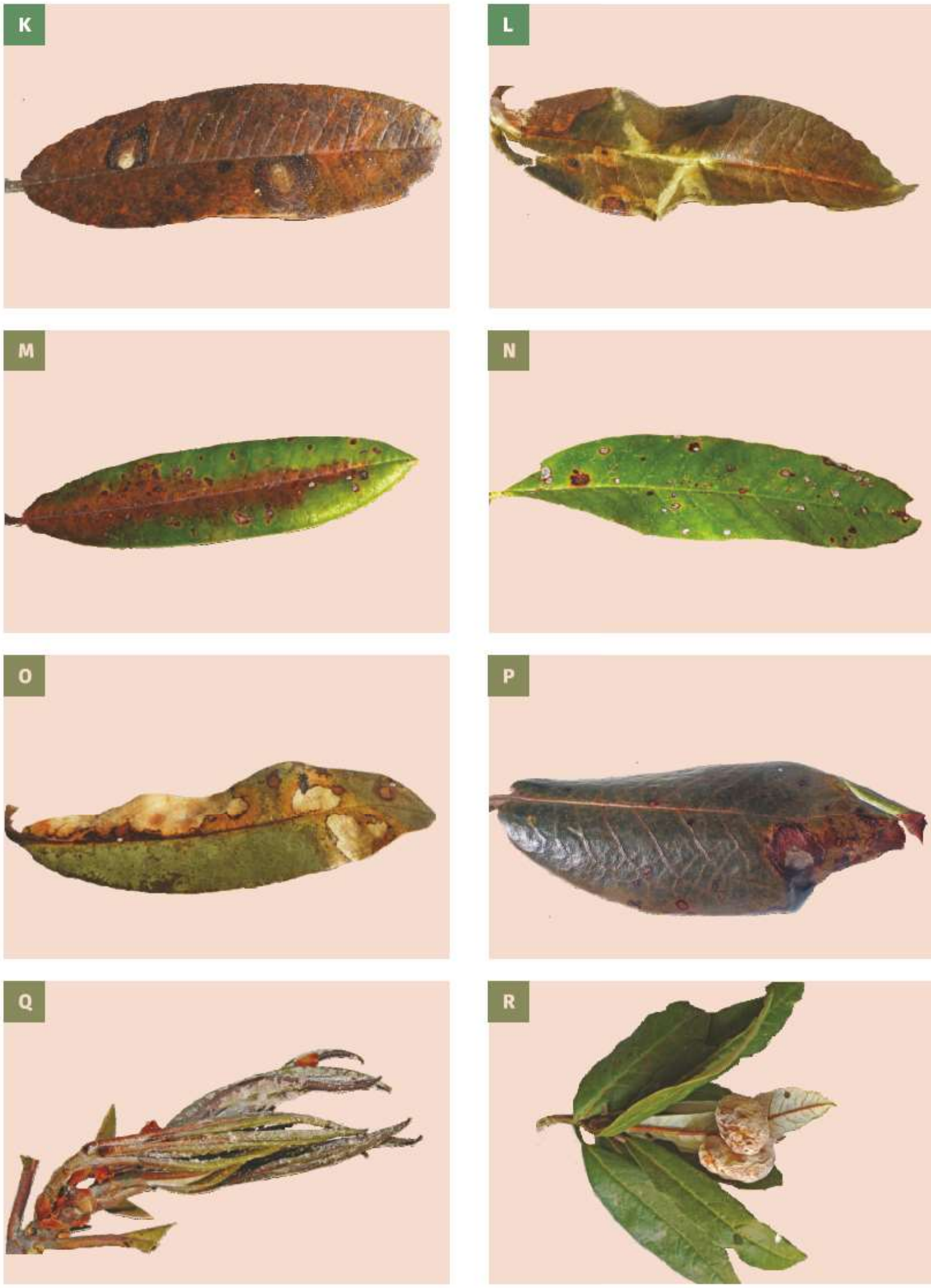
Fig. 11.2 Foliar Problems of *R. arboreum*: (A-I) Leaf Blight, (J-P) Leaf Spots, (Q) Bud Blight, and (R) Leaf Gall





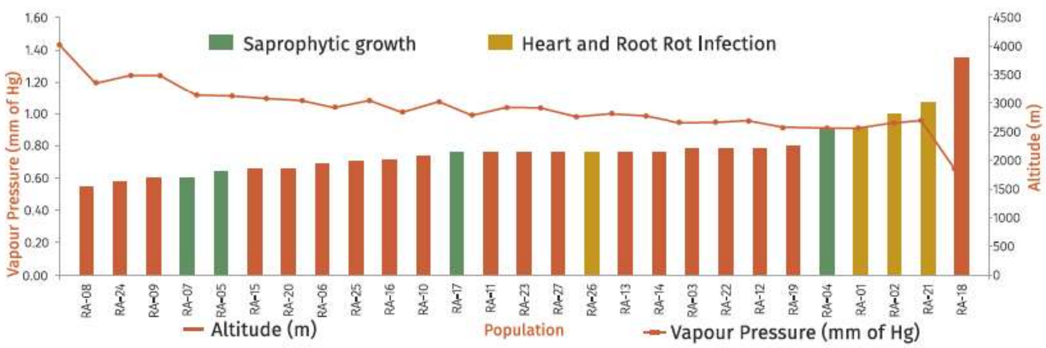


Fig. 11.3 Different Populations of *Rhododendron arboreum* with Respect to Altitude and Vapour Pressure

11.3.1.2

Quercus semecarpifolia

Signs, symptoms and associated fungal species recorded on varied sampled populations of *Quercus semecarpifolia* are summarized in Table 11.3. In *Q. semecarpifolia* forests, *Ganoderma lucidum* was recorded as the major pathogen causing root rot in the live trees in Kanchula Kharak, Gopeshwar (QS 01); Lokhandi, Chakrata (QS 05) and Munsiyari, Pithoragarh (QS 15) forests. *G. applanatum* was recorded as heart rot pathogen in forests of Raditop, Barkot (QS 09). Other fungal species *viz.*, *Agaricus* sp., *Heterobasidion annosum, Trametes cubensis, T. versicolor* and *Acremonium* sp. were found occurring as saprophytes on *Q. semecarpifolia* in Chopta, Gopeshwar (QS 02); Bhujkoti, Chakrata (QS 04); Rudranath, Gopeshwar (QS 06); Mundhola, Tiuni (QS 19) and Kunjkharak, Pangot (QS 22) (Fig. 9.4). *A. alternata, A. chlamydospora, A. phragmospora, C. herbarum, C. lunata, Fusarium* sp., *Graphium* sp., *Humicola* sp., *Hymenula* sp., *Macrophomina* sp., *Pestalotiopsis* sp., *N. sphaerica, Phomopsis* sp. and *Verticillium* sp. were isolated from infected foliar tissues (leaf spots and blight, marginal leaf blight and necrosis) with 2-45 per cent disease severity in the forests of Chopta, Bhujkoti, Raditop, Chaurangi Khal, Dudhatoli, Munsiyari, Chorikhal, Mundhola, Pinswar, Kunjkharak and Himkhola (Fig. 11.5). Mistletoe and other epiphytic plant growth on live tree of *Q. semecarpifolia* were recorded in forests of Raditop and Dudhatoli. Open wounds on the live trees and anthropogenic activities were also recorded in forests of Kunjkharak and Mundhola (Table 11.3).

The results in Fig. 11.6 depicted the altitude variation of all the sampled populations of *Q. semecarpifolia* which varied from 2,218 to 3,233 m amsl. With the increase in altitude, vapour pressure relatively decreased from 1.08 to 0.69 mm of Hg. Major heart and root rot pathogens (*G. lucidum* and *G. applanatum*) were recorded at different altitudes (2,579 m, 2,626 m, 2,699 m and 3,027 m) with foliar disease intensity varying from 10-25 per cent. Higher foliar disease intensity was recorded at 2,513 m (upto 45 per cent).

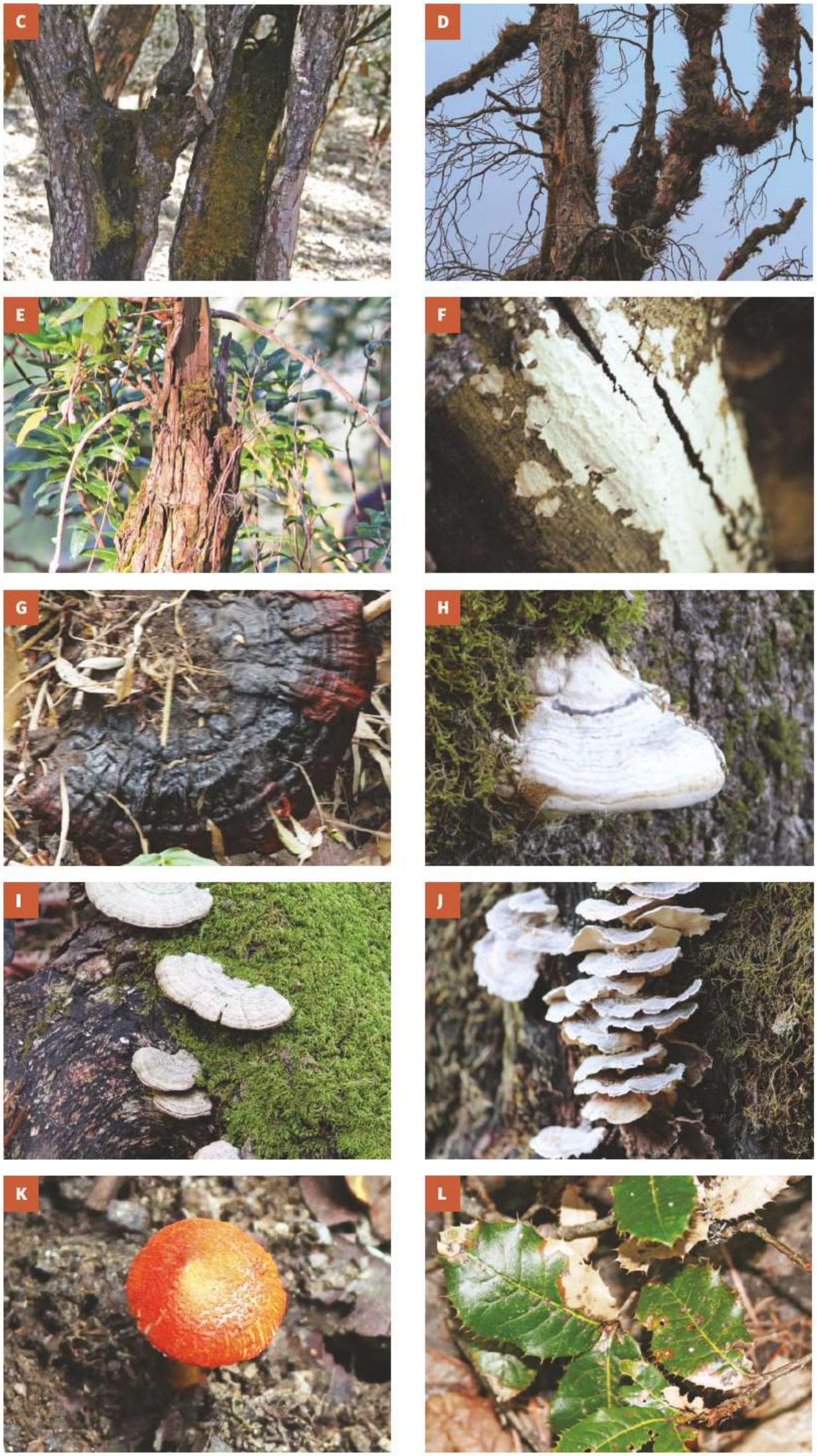
Based on fungal studies on 24 sampled populations of *Q. semecarpifolia*, ten populations were found to be relatively healthy. Trees in QS 03 Deoban, Chakrata (2,675 m), QS 07 Auli, Joshimath (3,029 m), QS 08 Yamunotri (3,203 m), QS 11 Bhukkitop, Bhatwari (2,874 m), QS 13 Nainapeak, Nainapeak (2,494 m), QS 14 Badanital forest, Jakholi (2,354 m), QS 17 Ghes, Dewal (2,486 m), QS 18 Balcha, Tiuni (2,759 m), QS 20 Nagtibba, Nainbagh (2,557 m) and QS 23 Narayana Ashram, Dharchula (2,788 m) were observed to be healthy with no disease symptoms. *Ganoderma applanatum* was recorded at QS 09 Raditop, Uttarkashi (2,626 m) causing heart rot in the live trees along with 10-25 per cent foliar disease intensity. No heart and root diseases were recorded at QS 10 Chaurangi Khal, Uttarkashi (2,568 m), QS 12 Dudhatoli, Thalisain (2,538 m), QS 16 Chorikhal, Bharsar (2,513 m), QS 21 Pinswar, Buda Kedar (2,280 m) and QS 24 Himkhola, Dharchula (3,037 m), but the trees were infected with foliar diseases at these locations with disease intensity ranging from 2-45 per cent.

Table 11.3 - Signs, Symptoms and Associated Fungal Species Recorded on *Quercus semecarpifolia*

Population	Location	Trees' Health	Root	Stem	Foliage (Symptom Intensity)	Remarks
QS 01	Kanchula Kharak, Gopeshwar	Unsound	Root rot (*Ganoderma lucidum*)	Nil	Sooty mold	Nil
QS 02	Chopta, Gopeshwar	Unsound	Nil	*Phanerochaete* sp.; Saprophyte (*Agaricus* sp.)	Leaf spots (*Alternaria alternata*): 35-40%	Nil
QS 03	Deoban, Chakrata	Healthy	Nil	Nil	Nil	Nil
QS 04	Bhujkoti, Chakrata	Unsound	Nil	Sap rotter (*Trametes versicolor*)	Marginal leaf blight (*Humicola* sp.); Leaf stain (*Verticillium* sp.): 10-20%	Nil
QS 05	Lokhandi, Chakrata	Unsound	Root rot (*Ganoderma lucidum*)	Nil	Nil	Mechanical injury
QS 06	Rudranath, Gopeshwar	Unsound	Nil	Saprophyte (*Heterobasidion annosum*)	Nil	Nil
QS 07	Auli, Joshimath	Healthy	Nil	Nil	Nil	Nil
QS 08	Yamunotri	Healthy	Nil	Nil	Nil	Nil

Population	Location	Trees' Health	Root	Stem	Foliage (Symptom Intensity)	Remarks
QS 09	Raditop, Barkot	Unsound	Nil	Heart rot (*Ganoderma applanatum*)	Necrosis (*Fusarium* sp.); Leaf spot (*Nigrospora sphaerica*): 10-25%	Mistletoe overgrowth
QS 10	Chaurangi Khal, Uttarkashi	Unsound	Nil	Nil	Leaf spots (*Cladosporium fusiforme*, *Phomopsis* sp.): 15-35%	Nil
QS 11	Bhukkitop, Bhatwari	Healthy	Nil	Nil	Nil	Nil
QS 12	Dudhatoli, Thalisain	Unsound	Nil	Nil	Leaf spots (*Alternaria* sp.; *Hymenula* sp.): 5-10%	Epiphytic growth
QS 13	Nainapeak, Nainital	Healthy	Nil	Nil	Nil	Nil
QS 14	Badhanital forest, Jakholi	Healthy	Nil	Nil	Nil	Nil
QS 15	Munsiyari, Pithoragarh	Unsound	Root rot (*Ganoderma lucidum*)	Nil	Leaf spots (*Nigrospora* sp., *Verticillium* sp.): 15-25%	Nil
QS 16	Chorikhal, Bharsar	Unsound	Nil	Nil	Leaf spots and blight (*Alternaria alternata*): 30-45%	Nil
QS 17	Ghes, Dewal	Healthy	Nil	Nil	Nil	Nil
QS 18	Balcha, Tiuni	Healthy	Nil	Nil	Nil	Nil
QS 19	Mundhola, Tiuni	Unsound	Nil	Sap rotter (*Trametes cubensis*)	Leaf spots: (*Alternaria chlamydospora*; *Phomopsis* sp.; *Macrophomina* sp.): 15-35%	Anthropogenic activities
QS 20	Nagtibba, Nainbagh	Healthy	Nil	Nil	Nil	Nil
QS 21	Pinswar, Budha Kedar	Unsound	Nil	Nil	Leaf blight (*Graphium* sp.; *Cladosporium herbarum*) 2-15%	Nil
QS 22	Kunjkharak, Pangot	Unsound	Nil	Saprophytic growth (*Acremonium* sp.)	Leaf blight (*Alternaria phragmospora*); Leaf spots (*Pestalotiopsis* sp.): 15-30%	Open wounds
QS 23	Narayan Ashram, Dharchula	Healthy	Nil	Nil	Nil	Nil
QS 24	Himkhola, Dharchula	Unsound	Nil	Nil	Leaf spots (*Curvularia lunata*; *Alternaria chlamydospora*): 30-35%	Nil

Fig. 11.4
Signs, Symptoms, Pathogenic and Saprophytic Fungi Recorded on *Quercus semecarpifolia*: (A) Heart Rot, (B-C) Root Rot, (D) Mistletoe Overgrowth, (E) Anthropogenic Activities, (F) *Phanerochaete* sp., (G) *Ganoderma lucidum*, (H) *Ganoderma applanatum*, (I) *Trametes cubensis*, (J) *Trametes versicolor*, (K) *Agaricus* sp., and (L) Foliar Infection by *Alternaria alternata*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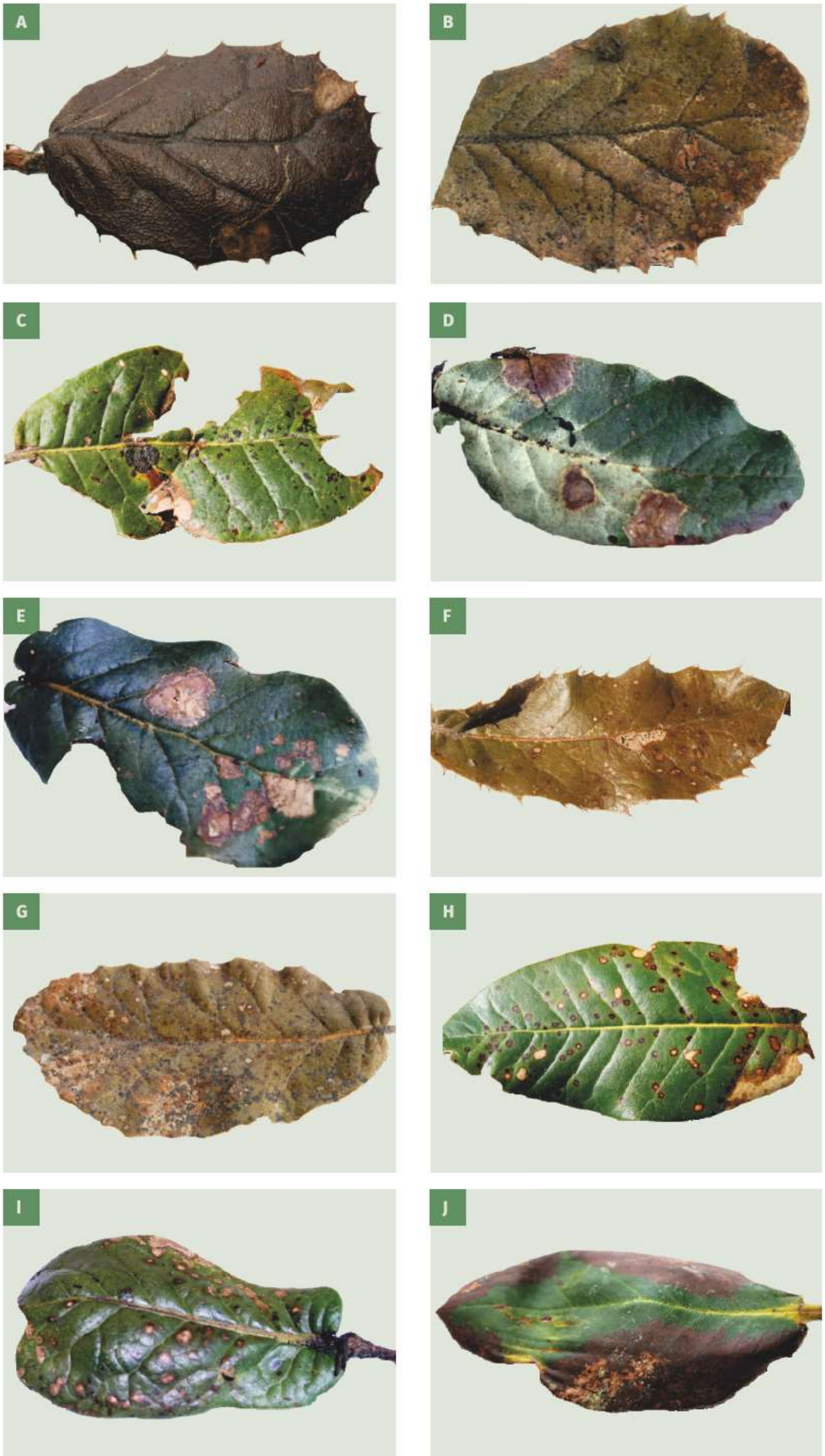

Fig. 11.5 Foliar Problems of Q. *semecarpifolia* : (A-I) Leaf Spots, (J-N) Leaf blight, and (O) Bud Blight

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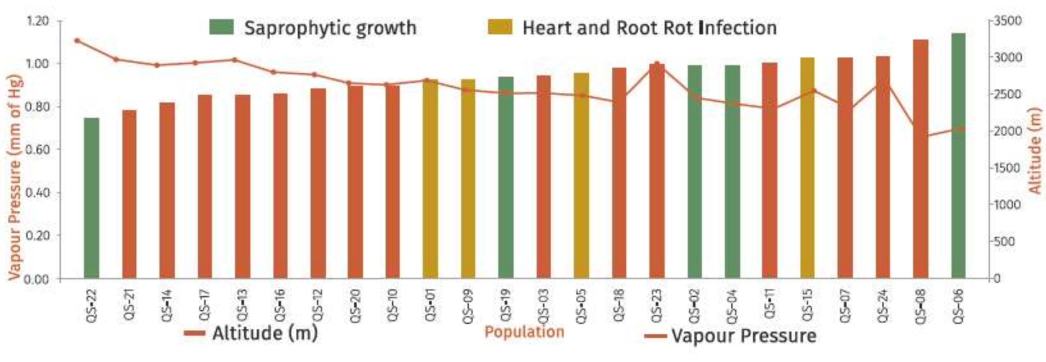


Fig. 11.6 Different Populations of *Quercus semecarpifolia* with respect to Altitude and Vapour Pressure

11.3.1.3

Taxus wallichiana

Signs, symptoms and associated fungal species recorded on varied sampled populations of *Taxus wallichiana* are summarized in Table 11.4. *G. lucidum* was recorded as heart rot and root rot pathogen in the live trees of *T. wallichiana* in Chopta (TW 01) and Himkhola, Pithoragarh (TW 15) FDs, respectively. Other saprophytes recorded were *Auricularia auricula*, *Marasmius* sp. and *Xylobolus subpileatus* on dead logs of the host species in the forests of Harshil (TW 06) and Yamunotri (TW 13) FDs. *Aspergillus nidulans* and *Rhizopus* sp. were found in association with the root region of *T.*

wallichiana occurring as saprophytes in Deoban (TW 02) and Dudhatoli (TW 09) FDs (Fig. 11.7). Die-back symptoms in trees were also recorded in Balcha forests. Foliage infections such as leaf spots and blight, leaf tip blight, mycelial overgrowth and twig blight and spots were also recorded with disease intensity ranging from 10-45 per cent in the forests of Deoban, Rudranath, Harshil, Bhukkitop, Dudhatoli, Yamunotri, Gangotri, Kathlia and Triyugi Narayana (Fig. 11.8). The associated fungal species having effect on the plant part were *A. alternata, A. phragmospora, A.niger, C. cladosporioides, Curvularia chlamydospora, C. pallescens, Fusarium* sp., *N. sphaerica, Penicillium chrysogenum, Pestalotiopsis* sp. and *Phomopsis* sp. Anthropogenic activities were also recorded in the forests of Balcha and Himkhola (Table 11.4).

As per the results shown in Fig. 11.9, the altitudinal range of all the 21 sampled populations of *T. wallichiana* varied from 2,123 to 3,349 m amsl. With the increase in altitude, vapour pressure gradually decreased from 1.2 to 0.7 mm of Hg. Heart and root rot diseases were recorded at two locations with an altitude of 2,815 m (Chopta) and 3,037 m (Himkhola) with no foliar disease. However, maximum foliar disease intensity was recorded at Dudhatoli (2,400 m) which ranged in 30-45%.

A comparison of 21 sampled populations of *Taxus wallichiana* for fungal diseases revealed that nine populations *viz.*, TW 03 Bhujkoti (2,830 m), TW 05 Auli (3,029 m), TW 07 Sukkhitop (2,829 m), TW 10 Ghes (2,462 m), TW 12 Mundhola (2,657 m), TW 16 Ghangaria (3,077 m), TW 18 Mornaula (2,123 m), TW 19 Har ki Doon (3,163 m) and TW 21 Baling, Darma Valley (2,748 m) were found totally free from disease. Two populations: TW 1 Chopta (2,815 m) and TW 15 Himkhola (3,037 m) showed presence of heart and root rot with no foliar disease.

Table 11.4 - Signs, Symptoms and Associated Fungal Species Recorded on *Taxus wallichiana*

Population	Location	Trees' Health	Root	Stem	Foliage (Symptom Intensity)	Remarks
TW 01	Chopta	Unsound	Nil	Heart rot (*Ganoderma lucidum*)	Nil	Nil
TW 02	Deoban	Unsound	Saprophytic growth (*Rhizopus* sp.)	Nil	Twig and leaf spots (*Cladosporium cladosporioides*): 25-30%	Open wounds
TW 03	Bhujkoti	Healthy	Nil	Nil	Nil	Nil
TW 04	Rudranath	Unsound	Nil	Nil	Mycelial overgrowth (*Aspergillus niger, Penicillium chrysogenum*): 15-25%	Nil
TW 05	Auli	Healthy	Nil	Nil	Nil	Nil
TW 06	Harshil	Unsound	Nil	Saprophyte (*Auricularia auricula*)	Leaf spots (*Nigrospora sphaerica*.): 10-35%	Nil
TW 07	Sukkhitop	Healthy	Nil	Nil	Nil	Nil
TW 08	Bhukkitop	Unsound	Nil	Nil	Leaf blight (*Fusarium* sp.); Leaf spots (*Alternaria phragmospora*): 25-35%	Nil
TW 09	Dudhatoli	Unsound	Saprophytic growth (*Aspergillus nidulans*)	Nil	Leaf spots (*Pestalotiopsis* sp., *Alternaria alternata*): 30-45%	Open wounds
TW 10	Ghes	Healthy	Nil	Nil	Nil	Nil
TW 11	Balcha	Unsound	Nil	Die-back	Nil	Illicit lopping
TW 12	Mundhola	Healthy	Nil	Nil	Nil	Nil
TW 13	Yamunotri	Unsound	Nil	Saprophyte (*Xylobolus subpileatus*)	Twig blight and spots (*Curvularia chlamydospora*): 30-40%	Nil
TW 14	Gangotri	Unsound	Nil	Nil	Leaf blight (*Alternaria alternata*): 15-25%	Nil
TW 15	Himkhola	Unsound	Root rot (*Ganoderma applanatum*)	Nil	Nil	Anthropogenic activities
TW 16	Ghangaria	Healthy	Nil	Nil	Nil	Nil
TW 17	Kathlia, Pindari glacier	Unsound	Nil	Nil	Leaf tip blight (*Fusarium* sp.): 10-15%	Nil
TW 18	Mornaula	Healthy	Nil	Nil	Nil	Nil
TW 19	Har ki Doon	Healthy	Nil	Nil	Nil	Nil

Population	Location	Trees' Health	Root	Stem	Foliage (Symptom Intensity)	Remarks
TW 20	Triyugi Narayana	Unsound	Saprophytic growth (*Marasmius* sp.)	Nil	Leaf spots (*Curvularia pallescens, Phomopsis* sp.): 20-35%	Nil
TW 21	Baling, Darma Valley	Healthy	Nil	Nil	Nil	Nil

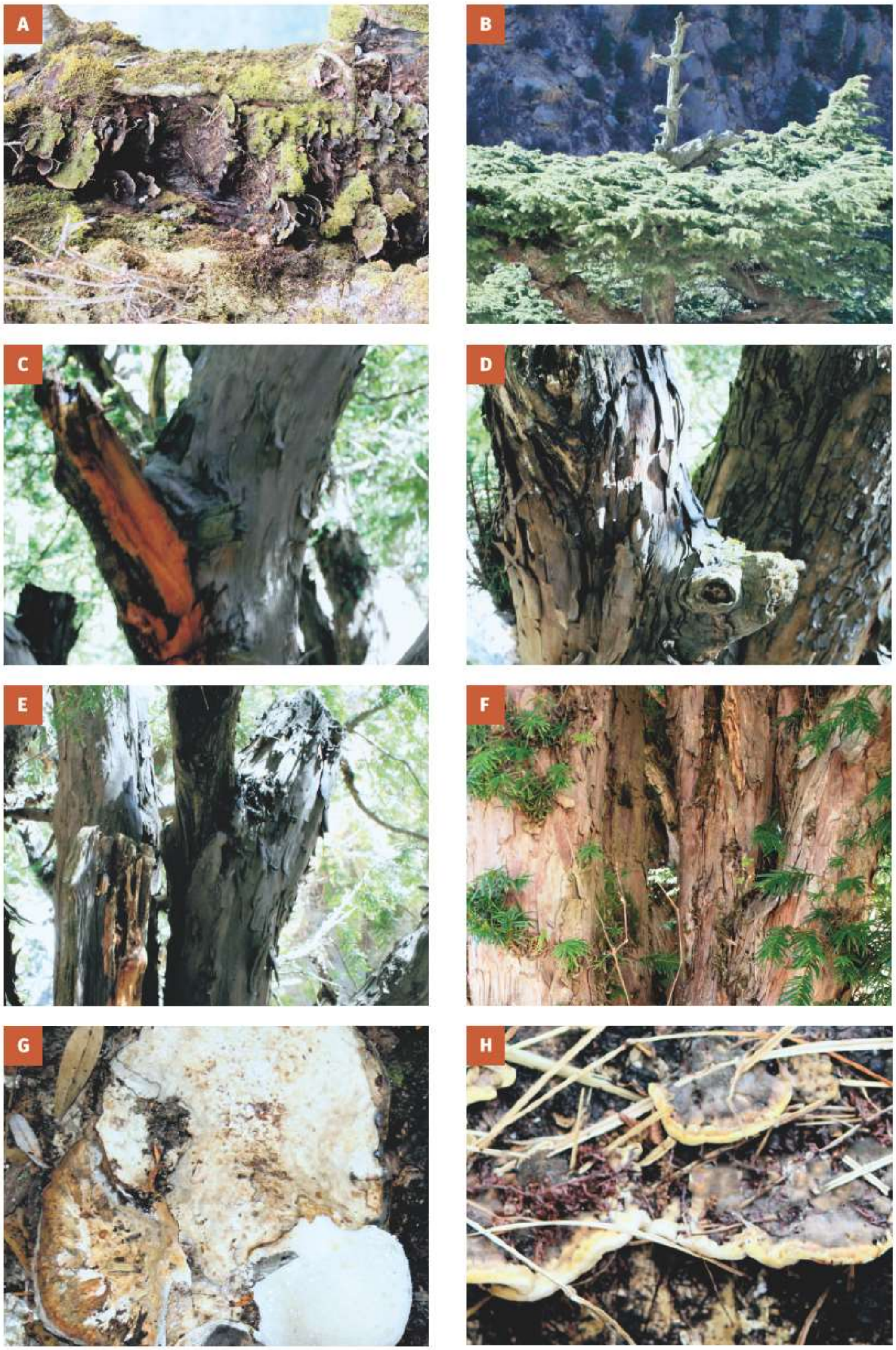
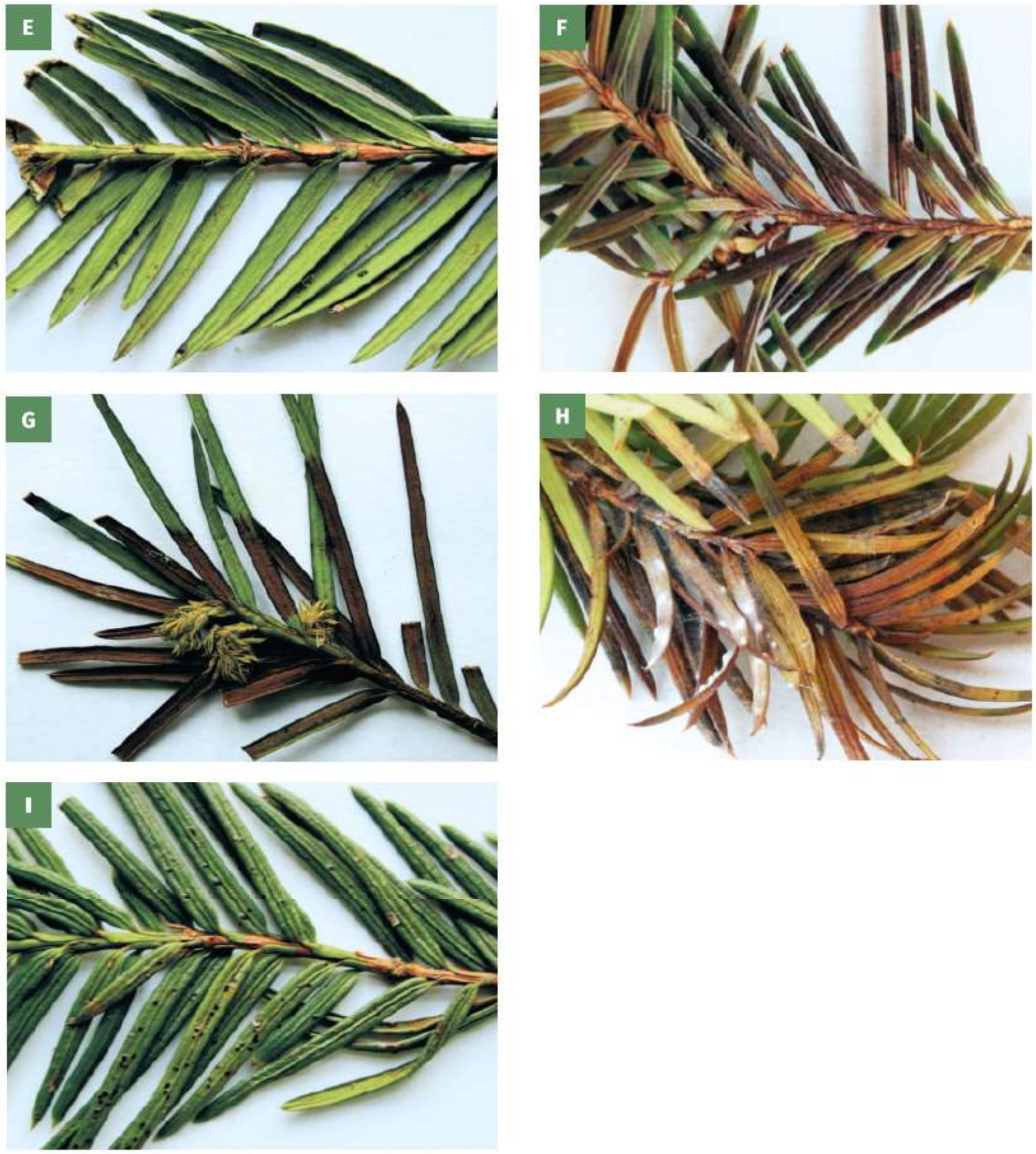


Fig. 11.7
Signs, Symptoms, Pathogenic and Saprophytic Fungi Recorded on *Taxus wallichiana*: (A) Saprophytic Growth of *Xylobolus subpileatus*, (B) Die-back by *Fusarium* sp., (C-F) Anthropogenic Activities, (F) Open Wounds, (G) *Ganoderma applanatum*, (H) *Ganoderma lucidum*, (I) Saprophytic Growth by *Trametes versicolor*, (J) *Marasmius* sp., (K) *Auricularia auricula*, and (L) Leaf Blight by *Pestalotiopsis* sp.

Fig. 11.8 Foliar Problems of *T. wallichiana*: (A-C) Leaf Spots, (D-E) Leaf Blight, (F-H) Saprophytic Growth on Leaves, and (I) Leaf Gall



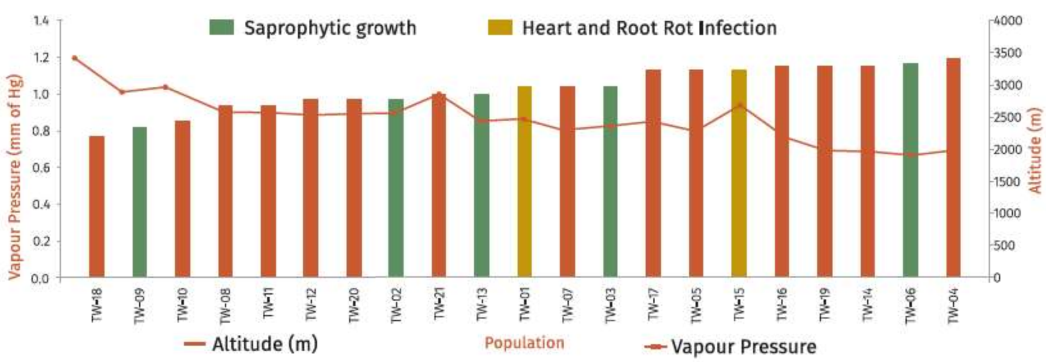


Fig. 11.9 Different Populations of *Taxus wallichiana* with respect to Altitude and Vapour Pressure

11.3.1.4

Myrica esculenta

Signs, symptoms and associated fungal species recorded on varied sampled populations of *Myrica esculenta* are summarized in Table 11.5. In *M. esculenta*, no root infection was recorded during forest surveys. As saprophytes, *Fomitopsis pinicola, Trametes hirsuta, Polyporus squamosus, Tylopilus alboater* and *Aspergillus nidulans* were recorded in the forests of Rudranath, Gopeshwar (ME 01); Manch (ME 06);

Adwani (ME 11); Chandra Badni (ME 19) and Jageshwar (ME 22) (Fig. 11.10). Leaf gall symptoms which were suspected to be infected by aphids were also recorded in the forests of Nagnath and DevDhula causing upto 5-50% infection (Fig. 11.11). Limited foliar infections (leaf spots, leaf blight, leaf rust and leaf blotch) were recorded in the forests of *M. esculenta*. Few fungal species *viz.*, *Alternaria fasciculata*, *A. triticina*, *C. pallescens*, *N. sphaerica* and *Puccinia* sp. were recorded causing foliar infections with 5-25% disease severity in the forests of Pabo Bazar, Kausani, Bhowali and Jageshwar. Epiphytic growth on live trees of *M. esculenta* was also recorded in the forests of Kausani. Anthropogenic activities were also observed in the forests of Chirapani, Adwani and Mayali in large scale (Table 11.5).

The results presented in Fig. 11.12 depicted the altitudinal range of all the 23 sampled populations of *M. esculenta* ranging from 1,454 to 2,422 m amsl. With the increase in altitude, vapour pressure gradually decreased from 1.39 to 0.99 mm of Hg. Maximum foliar disease intensity was recorded at Bhowali, Nainital (2,081 m elevation) which ranged from 40-60%. No root and stem diseases were recorded in all the populations.

Based on fungal studies on 23 sampled populations of *M. esculenta* across the pilot demonstration State of Uttarakhand, it was revealed that twelve populations *viz.*, ME 02 Gairsain (2,422 m), ME 04 Karnaprayag (1,530 m), ME 05 Chirapani (1,799 m), ME 07 Kamlekh (1,889 m), ME 12 Ranikhet (1,596 m), ME 13 Dunagiri (1,732 m), ME 15 Takula (1,454 m), ME 16 Shitla-Khet (1,648 m), ME 18 Mayali (1,693 m), ME 20 Lansdowne (1,644 m), ME 21 Gwaldam (1,695 m) and ME 23 Mornaula (2,123 m) were healthy as no disease incidence was recorded. As stated earlier, no root and stem diseases were recorded on any of the sampled populations. Notably, population ME 17 Bhowali (2,081 m) showed 45-60% foliar disease intensity which was the highest amongst all the sampled/studied populations.

Table 11.5 - Signs, Symptoms and Associated Fungal Species Recorded on *Myrica esculenta*

Population	Location	Trees' Health	Root	Stem	Foliage (Symptom Intensity)	Remarks
ME 01	Rudranath, Gopeshwar	Unsound	Nil	Saprophyte (*Fomitopsis pinicola*)	Nil	Nil
ME 02	Gairsain	Healthy	Nil	Nil	Nil	Nil
ME 03	Nagnath	Unsound	Nil	Nil	Leaf gall:45-50%	Nil
ME 04	Karnaprayag	Healthy	Nil	Nil	Nil	Nil
ME 05	Chirapani	Unsound	Nil	Nil	Nil	Anthropogenic activities
ME 06	Manch	Unsound	Nil	Saprophyte (*Trametes hirsuta*)	Nil	Nil
ME 07	Kamlekh	Healthy	Nil	Nil	Nil	Nil
ME 08	DevDhula	Unsound	Nil	Nil	Leaf gall:5-10%	Nil
ME 09	Peethsen	Healthy	Nil	Nil	Nil	Nil
ME 10	Pabo Bazar	Unsound	Nil	Nil	Leaf spots and blight (*Alternaria triticina*, *Curvularia pallescens*): 5-20%	Nil
ME 11	Adwani	Unsound	Nil	Saprophyte (*Aspergillus nidulans*)	Nil	Anthropogenic activities
ME 12	Ranikhet	Healthy	Nil	Nil	Nil	Nil
ME 13	Dunagiri	Healthy	Nil	Nil	Nil	Nil
ME 14	Kausani	Unsound	Nil	Nil	Leaf blotch (*Alternaria fasciculata*): 5-10%	Parasitic plant growth
ME 15	Takula	Healthy	Nil	Nil	Nil	Nil
ME 16	Shitla-Khet	Healthy	Nil	Nil	Nil	Nil
ME 17	Bhowali	Unsound	Nil	Nil	Leaf rust (*Puccinia* sp.): 40-60%	
ME 18	Mayali	Unsound	Nil	Nil	Nil	Illicit lopping
ME 19	Chandra Badni	Unsound	Nil	Saprophyte (*Tylopilus alboater*)	Nil	Nil
ME 20	Lansdowne	Healthy	Nil	Nil	Nil	Nil
ME 21	Gwaldam	Healthy	Nil	Nil	Nil	Nil
ME 22	Jageshwar	Unsound	Nil	Saprophyte (*Polyporus squamosus*)	Leaf spots (*Nigrospora sphaerica*): 15-25%	Nil
ME 23	Mornaula	Healthy	Nil	Nil	Nil	Nil

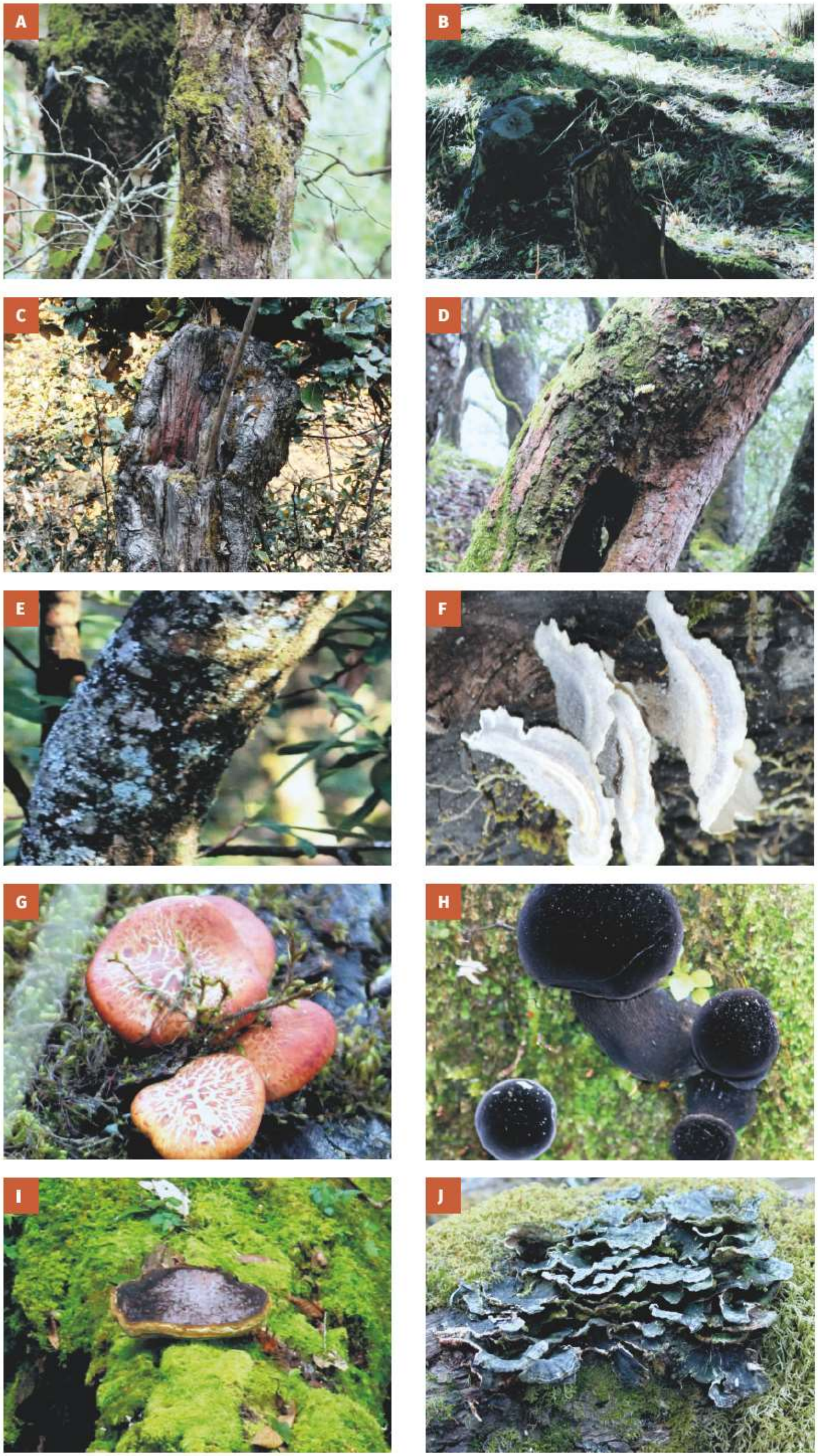


Fig. 11.10.
Signs, Symptoms, Pathogenic and Saprophytic Fungi Recorded on *Myrica esculenta*: (A) Parasitic Plant Growth (B-C) Anthropogenic Activities (D) Open Wound (E) Saprophytic Growth by *Aspergillus nidulans* (F) *Trametes versicolor* (G) *Polyporus squamosus* (H) *Tyropilus alboater* (I) *Fomitopsis pinicola* (J) *Trametes hirsuta* (K) Leaf Spot by *Curvularia pallescens* (L) *Alternaria fasciculata* leaf blight

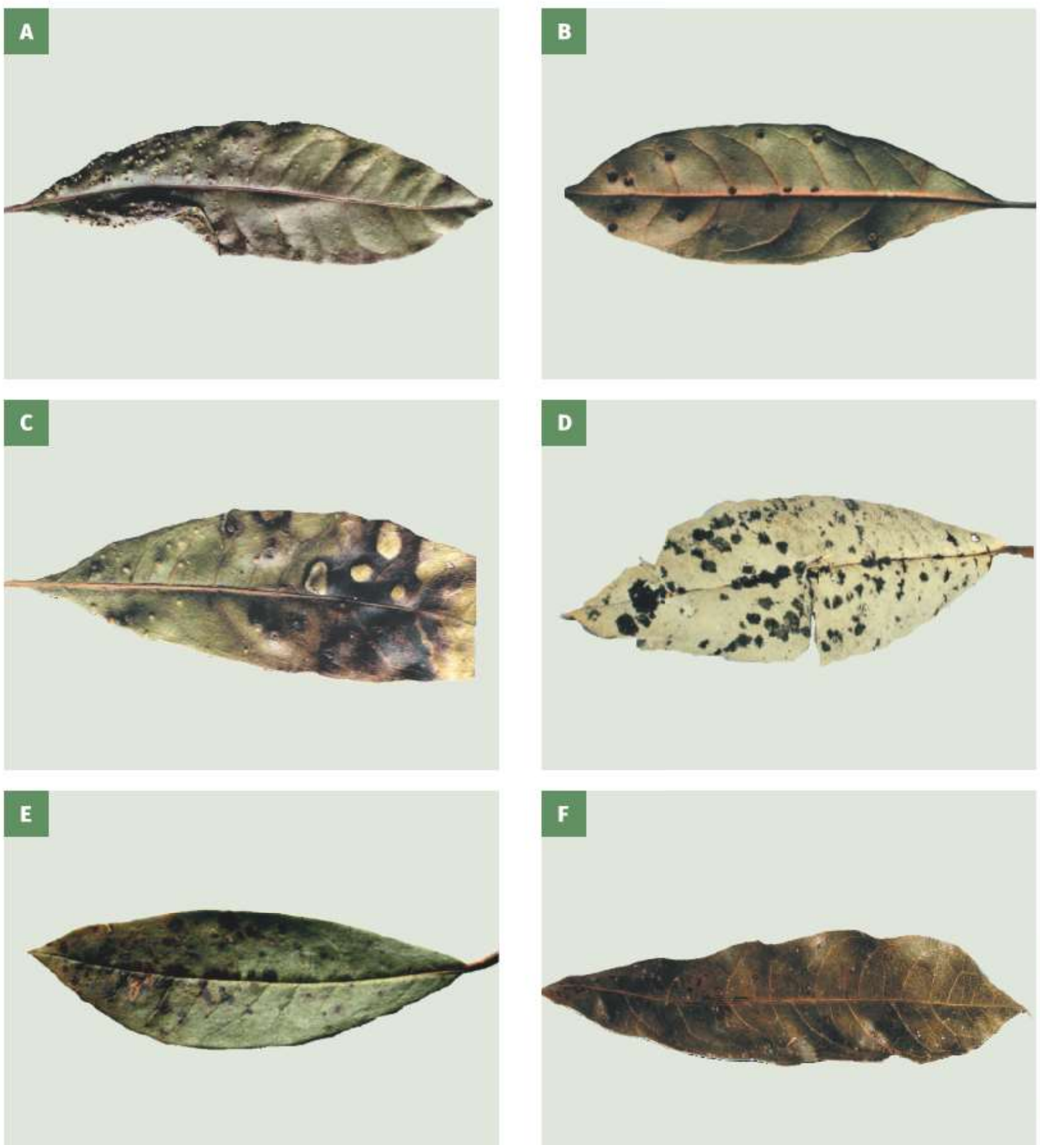
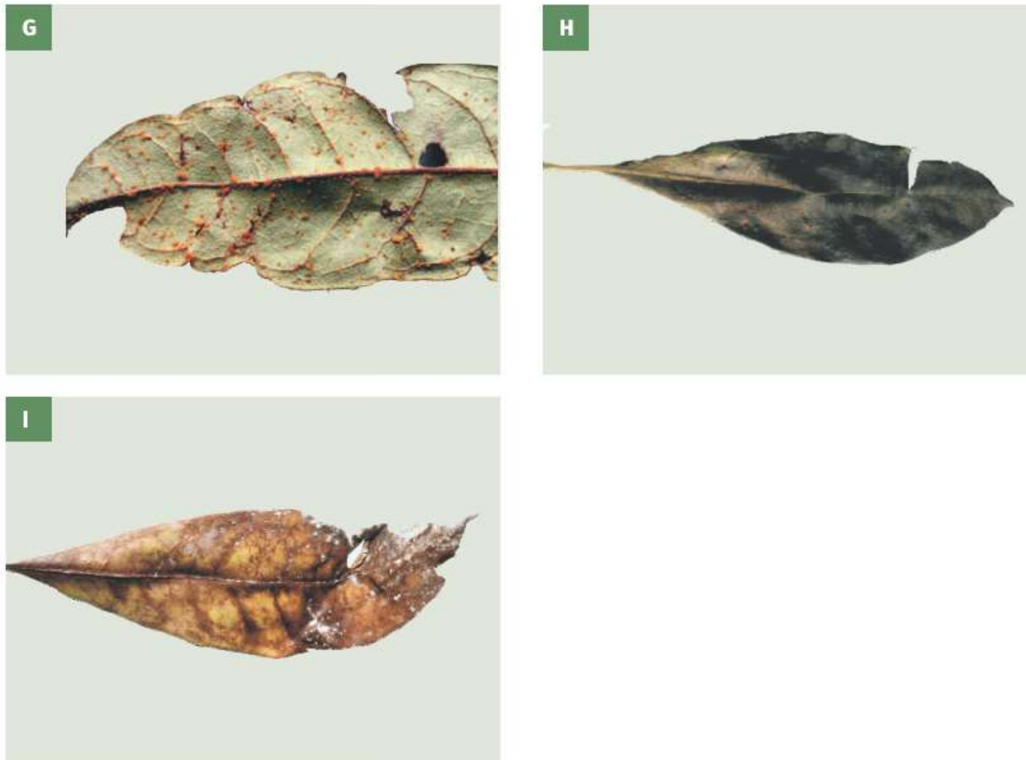


Fig. 11.11 Foliar Problems of *Myrica esculenta*: (A-C) Leaf Gall, (D–F) Leaf Spots, (G) Leaf Rust, (H) Leaf Blotch, and (I) Saprophytic Growth on Leaves



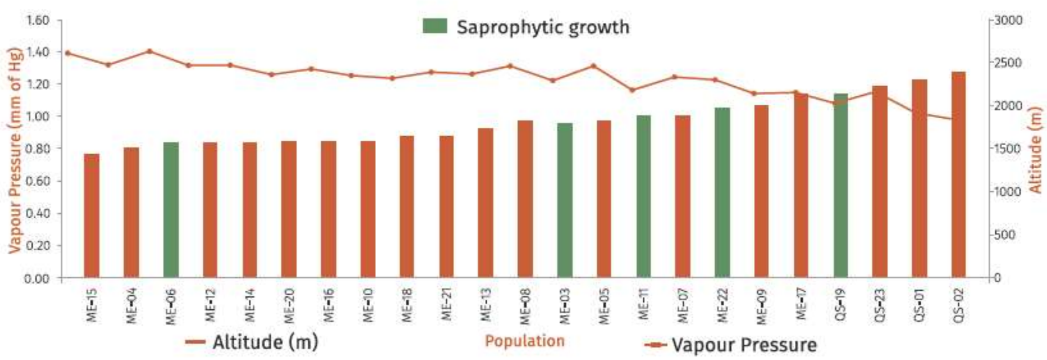


Fig. 11.12 Different Populations of *Myrica esculenta* with Respect to Altitude and Vapour Pressure

11.3.1.5

Betula utilis

Signs, symptoms and associated fungal species recorded on varied sampled populations of *Betula utilis* are summarized in Table 11.6. In the sampled forests of *B. utilis* (Fig. 11.13), *Russula bravipes* and *Pycnoporus sanguineus* were recorded on dead trees in Munsyari-2, Milam glacier (BU 05) and Pindari (BU 08) FDs respectively. No root infection was recorded in the trees of *B. utilis* whereas, 5-50% disease severity was recorded for foliar infections caused by various fungal species *viz.*, *Alternaria rosae, A. triticina, Cladosporium fusiforme, Fusarium* sp., *Lacellina* sp., *Mortierella* sp., *Pestalotiopsis* sp. and *Puccinia* sp. (Fig. 11.14) which were found associated with symptoms such as leaf rust, leaf spot and blight and twig blight in forests of Rudranath, Harshil, Niti, Munsiyari, Pindari and Triyugi Narayana. Mechanical injury was also recorded in the live trees in Harshil (Table 11.6).

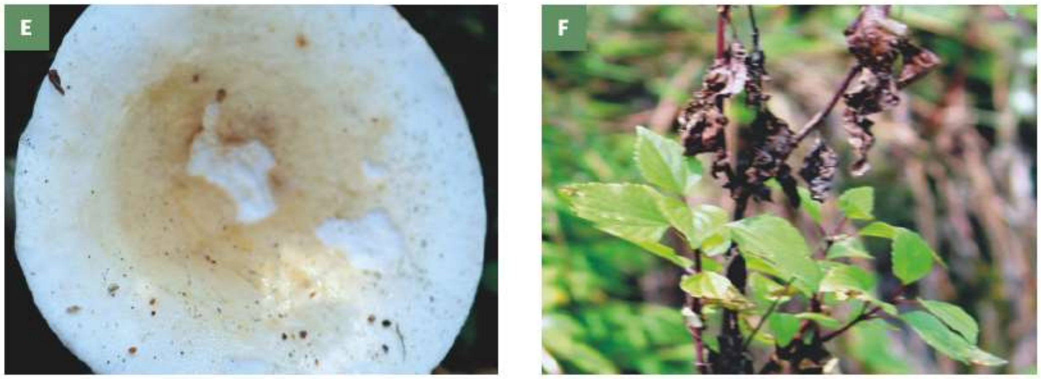
As per the results presented in Fig. 11.15, the altitudinal range of all the 11 sampled populations of *B. utilis* varied from 1,179 to 3,745 m amsl. With the increase in altitude, vapour pressure gradually decreased from 1.46 to 0.54 mm of Hg. Maximum foliar disease intensity was recorded at RA 10 Triyugi Narayan, Rudraprayag (3,168 m elevation) which ranged from 20-50 per cent. No root and stem diseases were recorded in all the populations.

A comparison of 11 populations of *B. utilis* sampled across its distributional range in Uttarakhand revealed that five populations *viz.*, BU 04 Bamni village, Badrinath (3,262 m), BU 06 Himkhola, Dharchula (3,656 m), BU 07 Hemkund Sahib, Valley of Flowers (3,178 m), BU 09 Har ki Doon (3,401 m) and BU 11 Darma Valley (3,148 m) were free from any disease but notably, higher foliar disease intensity (20-50%) was observed at BU 10 Triyugi Narayan (3,168 m). About 2-15 per cent foliar disease intensity and saprophytic fungal growth (*Russula bravipes* and *Pycnoporus sanguineus*) on the trees of population BU 05 (Munsiyari-2, Milam Glacier) and BU 08 (Pindari) was recorded.

Table 11.6 - Signs, Symptoms and Associated Fungal Species Recorded on *Betula utilis*

Population	Location	Trees' Health	Root	Stem	Foliage (Symptom Intensity)	Remarks
BU 01	Rudranath, Gopeshwar	Unsound	Nil	Nil	Leaf spot (*Lacellina* sp.): 5-10%	Nil
BU 02	Harshil	Unsound	Nil	Saprophytic growth	Leaf rust (*Puccinia* sp.); Leaf blight (*Cladosporium fusiforme*): 30-40%	Mechanical injury
BU 03	Niti	Unsound	Nil	Nil	Leaf blight (*Alternaria triticina, Pestalotiopsis* sp.): 20-40%	Nil
BU 04	Bamni village, Badrinath	Healthy	Nil	Nil	Nil	Nil
BU 05	Munsyari-2, Milam glacier	Unsound	Nil	Saprophyte (*Russula brevipes*)	Twig blight (*Fusarium* sp.):2-10%	Nil
BU 06	Himkhola, Dharchula	Healthy	Nil	Nil	Nil	Nil
BU 07	Hemkund Sahib, Valley of Flowers	Healthy	Nil	Nil	Nil	Nil
BU 08	Pindari	Unsound	Nil	Saprophyte (*Pycnoporus sanguineus*)	Leaf spot (*Alternaria rosae, Mortierella* sp.): 10-15%	Nil
BU 09	Har ki Doon	Healthy	Nil	Nil	Nil	Nil
BU 10	Triyugi Narayana	Unsound	Nil	Nil	Leaf rust (*Puccinia* sp.): 20-50%	Nil
BU 11	Darma Valley	Healthy	Nil	Nil	Nil	Nil

Fig. 11.13
Signs, Symptoms, Pathogenic and Saprophytic Fungi Recorded on *Betula utilis*: (A-B) Mechanical Injury, (C) *Pycnoporus sanguineus*, (D-E) *Russula brevipes*, (F) Leaf Blight by *Alternaria triticina*, and *Pestalotiopsis* sp.



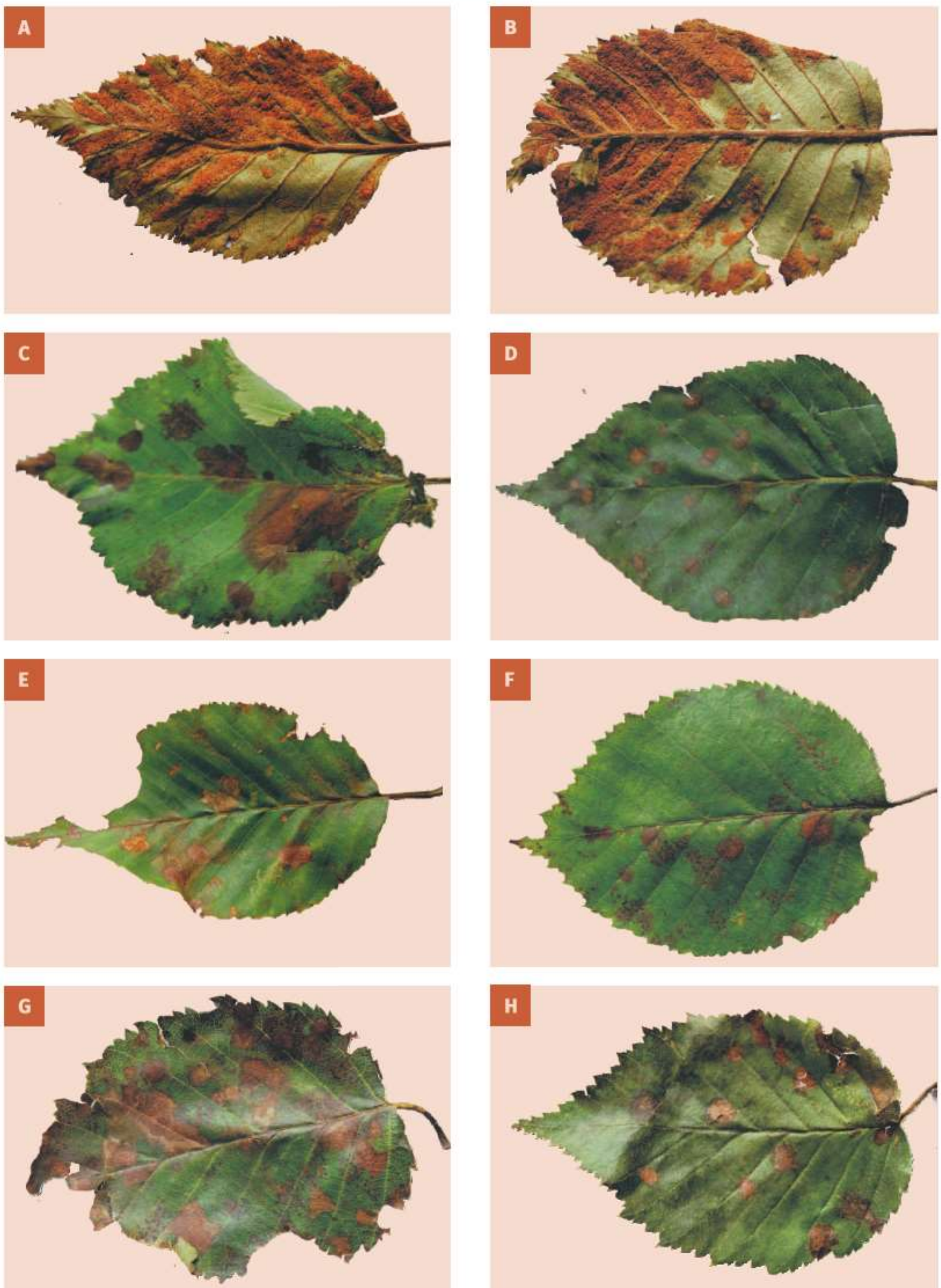
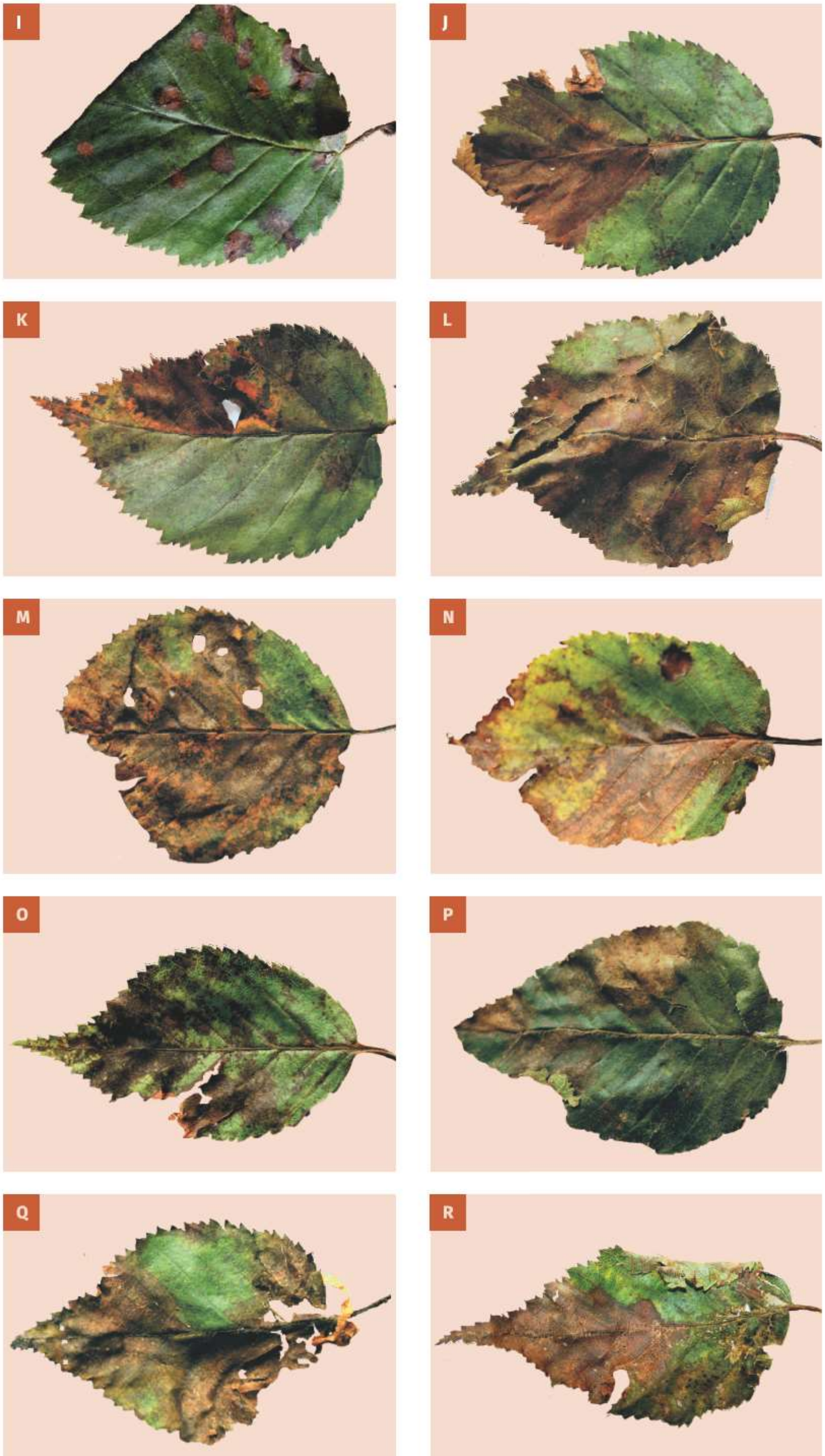


Fig. 11.14 Foliar Problems of *B. utilis*: (A–B) Leaf Rust, (C–I) Leaf Spots, and (J–R) Leaf Blight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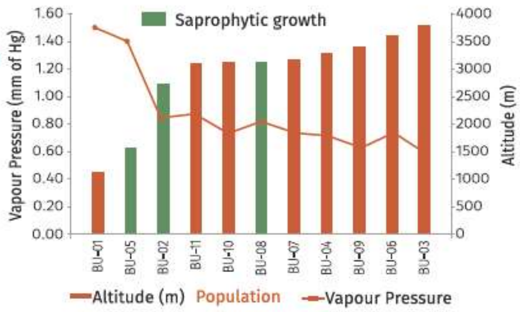


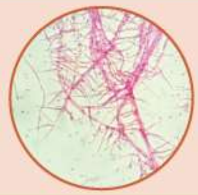
Fig. 11.15 Different Populations of *Betula utilis* with respect to Altitude and Vapour Pressure

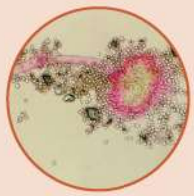
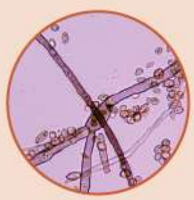
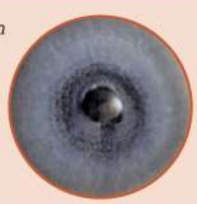
11.3.2

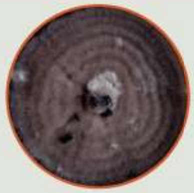
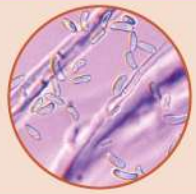
Microscopic and Morphological Characteristics of Isolated Fungal Species

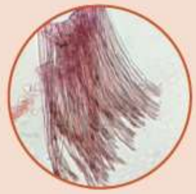
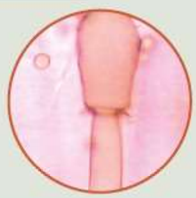
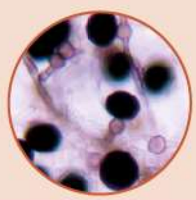
Altogether, 52 species (35 microscopic and 17 macroscopic) of fungi were recorded during the present study on fungal characterization of five prioritized species and the pure culture plates and microphotographs of the isolated fungal species are presented in Table 11.7 and Table 11.8.

Table 11.7 - Microscopic and Morphological Characteristics of Isolated Fungal Species

Sr. No.	Fungal Species	Fungal Colony	Microscopic View	Characteristic Features
1.	*Acremonium* sp.			The colony is white in colour. Mycelium is prostrate and slender. Conidiophores are upright; conidia are hyaline, single celled and is borne singly and apically.
2.	*Alternaria alternata*			The colony is effuse, deep grayish olive to olivaceous black. Conidiophores arise singly; branched, flexuous, pale to mid olivaceous brown, smooth. Conidia are formed in long, branched chains, obclavate, ellipsoidal with a short cylindrical beak, golden brown in colour, smooth, with upto 8 transverse and longitudinal septa. The dimensions of different types of conidia are 59.93 μ x 9.22 μ, 27.66 μ x 13.83 μ, and 5.8 μ x 5.8 μ.
3.	*Alternaria chlamydospora*			The colony is effuse, brownish olive to black. Chlamydospores are multi-celled, vary in shape and size and are golden brown in colour. Conidiophores are pale brown in colour. Conidia are swelled and varied in shape, golden brown in colour, smooth and verruculose, ranged from 20-60 x 6-30 μ with a short beak (2-5 μ thick).
4.	*Alternaria fasciculata*			The colony is effuse, dark blackish brown to black. Conidiophores are solitary or fasciculate. Conidia are solitary or in chains, fusiform to obclavate, rostrate, smooth or verruculose, pale or mid pale golden brown, with transverse or longitudinal septa, beak much shorter than the body and ranged from 4-6 μ x 7-8 μ.
5.	*Alternaria phragmospora*			The colony is effuse, yellowish olive. Conidiophores are simple, pale brown in colour, upto 55 μ long and 3.5 μ thick. Conidia are catenate, cylindrical, light gray in colour, smooth with 3-7 transverse septa ranging from 25-50 μ x 5-12 μ in dimensions.
6.	*Alternaria rosae*			The colony is amphigenous, brown. Conidiophores are simple or branched, straight or flexuous, minutely verruculose, upto 80 μ long and 7-10 μ thick. Conidia are obclavate rostrate, golden brown in colour with 7-14 transverse and 1-3 longitudinal septa, ranging from 20-35 μ x 10-12 μ in dimensions.

Sr. No.	Fungal Species	Fungal Colony	Microscopic View	Characteristic Features
7.	*Alternaria triticina*			The colony is discrete, dark black brown to black. Conidiophores are occasionally branched, upto 30 μ long and 3-6 μ thick. Conidia are solitary, obclavate, golden brown, smooth, 20-90 μ x 9-30 μ thick.
8.	*Aspergillus nidulans*			The colony effuse, often white to pale green. Mycelium is partly immersed, partly superficial. Conidiophores are macro- and mono-nematous, straight or flexuous, colourless, usually smooth covered with phialides. Conidia are catenate, dry, acrogenous, spherical, smooth, verruculose or echinulate ranging from 3-5 μ in diameter.
9.	*Aspergillus niger*			The colony is effuse, colourless to black mass with heavy growth. Mycelium is partly immersed and partly superficial. Conidiophores are found with a foot cell, straight and sometimes flexuous, non-septate, 200-400 μ in length, light brown in colour, smooth, covered with phialides on the head. Conidia are catenate, acrogenous, spherical in shape, black-colored, smooth, arranged spirally ranging from 2.5-4 μ in diameter.
10.	*Chaetomium globosum*			The colony is grey to brown with granular growth. Ascomata is globose to ostiolate with erect to coiled ascomatal hairs. Asci is evanescent, clavate to slightly fusiform. Ascospores are limoniform, bilaterally flattened, brown in colour ranging from 9-12 μ x 6-8 μ in dimensions.
11.	*Cladosporium cladosporioides*			The colony is effuse, constricted, olive brown to black. Conidiophores are macro- and micronematous, 2-5 μ thick, pale brown in colour and smooth. Two types of conidia are found scattered, limoniform (11.6 X 5.8 μ) and cylindrical (20.3 X 5.8 μ), which are pale olivaceous brown in colour and smooth.
12.	*Cladosporium fusiforme*			The colony is amphigenous, effuse, grayish olive and velvety. Conidiophores are macronematous, erect, straight, nodose, pale to mid brown, smooth upto 180 μ long and 5-6 μ thick. Conidia arise from terminal swellings in simple or branched chains, cylindrical or oblong rounded at the ends, ellipsoidal, pale to mid brown, 12-32 x 6-9 μ in dimensions.
13.	*Cladosporium herbarum*			The colony is effuse, olive green and velvety. Conidiophores are mostly macronematous, flexuous, geniculate, often nodose, pale to olivaceous brown in colour, smooth, upto 250 μ long and 3-6 μ thick. Conidia are long, often branched chains, ellipsoidal or oblong rounded at the ends, olivaceous brown, 5-23 x 3-8 μ in dimensions.
14.	*Curvularia chlamydospora*			The colony is black, hairy and velvety. Conidiophores are mono-nematous, often geniculate, straight, brown and usually smooth. Conidia are solitary, acro-pleurogenous, simple, curved, obovoid, pale to brown in colour and 45-66 x 18-28 μ in dimensions.

Sr. No.	Fungal Species	Fungal Colony	Microscopic View	Characteristic Features
15.	*Curvularia lunata*			The colony is sub-floccose to dark grayish olive. Hyphae are septate and much branched, dark olive in colour, 3-4 μ in diameter. Conidiophores are erect, more than 100 μ long and 3 μ in diameter. Conidia are borne on tip, found in whorls and scattered, curved, 3 septate, brown in colour, 27.66 μ X 13.83 μ in dimensions.
16.	*Curvularia pallescens*			The colony is grayish, cottony and partly immersed. Conidiophores are macronematous, flexuous, nodose, usually smooth. Conidia are branched, often curved, clavate, ellipsoidal and grayish in colour ranging from 17-32 x 7-12 μ in dimensions.
17.	*Fusarium* sp.			The colony is cottony and whitish yellow. Conidiophores are variable, slender, branched irregularly bearing a whorl of phialides. Conidia are hyaline; macroconidia are 3-septate, curved or bent at the pointed ends, hyaline, microconidia are single celled, ovoid or oblong, borne singly or in chains and hyaline.
18.	*Fusarium oxysporum*			The colony is purple coloured and cottony. Mycelium is partly submerged in the medium. Conidiophores are hyaline, sparingly branched, macroconidia are oval to cylindrical, pointed at end, 3-5 septate, occasionally curved, 7-14 x 3.2-4 μ, microconidia are usually absent. Chlamydospores are terminal and intercalary, spherical to oval, cream to pale brown in colour.
19.	*Gliocladium* sp.			The colony is pale peach and cottony. Conidiophores are septate, the upper portion bears penicillate branches. Conidia are hyaline or peach coloured, single celled, produced apically.
20.	*Graphium* sp.			The colony is brownish and submerged. Synnemata is tall, dark, bearing a rounded, terminal mass of conidia embedded in mucus, simple and hyaline. Conidiophores are produced in abundance bearing oblong conidia.
21.	*Hansfordia* sp.			The colony is yellowish brown with white margins. Conidiophores are hyaline to brown, erect to repent, lower part unbranched, irregular, flexuous, denticulate. Conidia are single celled, hyaline, globose, ovoid or fusoid.
22.	*Humicola* sp.			The colony is white and floccose. Conidiophores are erect, straight, sometimes septate, simple, shortly branched, brown. Conidia are single, apical, sub-globose, brown and single celled.

Sr. No.	Fungal Species	Fungal Colony	Microscopic View	Characteristic Features
23.	*Hymenula* sp.			The colony is white with brown exudations. Sporodochia are somewhat flattened or discoid, light coloured. Conidiophores are hyaline, sparingly to moderately branched bearing terminal conidia. Conidia are hyaline, single celled, ovoid to oblong.
24.	*Lacellina* sp.			The colony is pale white and cottony. Setae are erect, tall, brown and simple. Conidiophores are shorter, paler and simple. Conidia are single celled, brown, globose or ovoid and produced at or near the apex in acropetallous chains.
25.	*Macrophomina* sp.			The colony is grayish brown and floccose. Hyphal branches are formed at right angles. Microsclerotia are jet black in colour, smooth and round to oblong or irregular.
26.	*Mortierella* sp.			The colony is brownish and submerged with a lighter margin. Conidiophores produces a large number of conidia in sporulating layers. Basipetal chains are formed in conidiation. Conidia are uninucleate, ovoid to cylindrical and measured 9.1-10.1 x 2.4-2.9 μ in dimensions.
27.	*Nigrospora sphaerica*			The colony is at first white, later turns black from middle forming two zones, floccose with superficial mycelium. Conidiophores are semi-nematous, branched, flexuous, colourless to light brown, and smooth. Conidia are solitary, acrogenous, broadly ellipsoidal, shining black in colour, smooth, aseptate, 18μ long and 13.83μ wide.
28.	*Penicillium chrysogenum*			The colony is felty and olive green The culture turned to brown after few days. Conidiophores are around 300 μ long. Conidia are elliptical to globose in shape, 3-4.5 μ in dimensions.
29.	*Pestalotiopsis* sp.			The colony is floccose white. Acervuli are dark, discoid or cushion-shaped and subepidermal. Conidiophores are short, simple and light brown in colour. Conidia are dark, several celled with hyaline, pointed end cells, ellipsoid to fusoid with two or more hyaline, apical appendages.
30.	*Phomopsis* sp.			The colony is pale white with exudations. Pycnidia are dark, ostiolate, immersed, erumpent and nearly globose. Conidiophores are simple. Conidia are hyaline, single celled, of two types, ovoid to fusoid alpha conidia and filiform, curved or bend stylospores beta conidia.

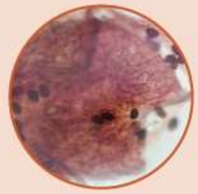
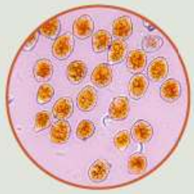
Sr. No.	Fungal Species	Fungal Colony	Microscopic View	Characteristic Features
31.	*Phyllosticta* sp.			The colony is brown and submerged. Pycnidia are dark, ostiolate, lenticular to globose, erumpent or with a short beak piercing the epidermis. Conidiophores are short or obsolete. Conidia are small, one celled, hyaline, ovoid to elongate.
32.	*Pithomyces* sp.			The colony is grayish, punctiform and velvety. Conidiophores are micronematous, branched, flexuous, subhyaline, smooth or verruculose. Conidia are solitary, pleurogenous, simple, ellipsoidal, pyriform, echinulate to verruculose.
33.	*Rhizopus* sp.			The colony is grayish having rapid growth. Sporangiophores are present in groups from nodes directly above the rhizoids. Sporangia are apophysate, columellate, multi-spored, generally globose. Sporangiospores are globose to ovoid, single celled, brown and striate.
34.	*Sordaria fimicola*			The colony is pale at first turning to dark brown to black. Hyphae are thin-walled, septate and branched. Ascomycota are glabrous covered with flexuous colourless hairs, pear-shaped or obpyriform with central ostiole. Ascocarps are composed of several layers. Asci with eight uniseriate ascospores are arranged obliquely growing from the bottom of the perithecium. Ascospores are olivaceous brown, aseptate, granular and ellipsoidal.
35.	*Verticillium sp.*			The colony is floccose white which later became creamish. Conidiophores are septate, hyaline, verticillately branched having 2-5 phialides at the nodes. Conidia are formed in abundance, cylindrically oval in shape, small in size, hyaline, 11.56-13.83 μ long and 4.23-4.61 μ wide.

Table 11.8 - Macroscopic Fungal Species Recorded on Five Prioritized FGR Species

Sr. No.	Fungal Species	Fungal Colony	Characteristic Features
1.	*Agaricus sp.*		Basidiocarps are perennial. Cap is 2.5-14 cm, convex to nearly round, flat, dry with small scales. Gills are free from the stem, close, pinkish to reddish. Stem is 2-7 cm long, 1-2.5 cm thick, with small scales with a thin ring. Flesh is white and firm. Basidia mostly two spored. Cheilocystidia 20-30 μ long, clavate, smooth, thin walled, hyaline. Spores are 6-8 x 5-6 μ in dimensions, ellipsoid, smooth, thick walled, brown.
2.	*Auricularia auricula*		Basidiocarps are perennial, wavy and irregular, often more or less ear-shaped, elliptical, cup-shaped, thin, brown to red. Flesh is scattered, poorly defined, gelatinized. Hyphae is 2-3 μ wide, medullary zone not differentiated. Basidia are 65-75 x 4-5 μ, cylindric, transversely 3-septate. Spores are 12-14 x 4-5.5 μ, allantoids, smooth and hyaline.

Sr. No.	Fungal Species	Fungal Colony	Characteristic Features
3.	*Fomes fomentarius*		Basidiocarps are perennial, sessile, ungulate, upto 250 mm wide, 500 mm broad, 80-120 mm thick at base, woody hard, pileus smooth, sulcate, darkening to blackish grey to brown, margin obtuse, finely velutinate, cuticle present, 1-3 mm thick. Hyphal system trimitic, generative hyphae thin walled, septate, clamped, 2-3.5 μ wide, skeletal hyphae thick walled, aseptate, 3-6 μ wide, cystidia absent, cystidioles thin walled, fusoid, 25-40 x 4-7.5 μ. Basidia with swollen base, 4-sterigmate, 20-35 x 7-10 μ with a basal clamp, spores cylindrical, hyaline, smooth, 12-17.5 x 4-7 μ in dimensions.
4.	*Fomitopsis pinicola*		Basidiocarps are perennial, sessile, reflexed or resupinate, woody, applanate, upto 300 mm wide, 50 mm broad and 50-80 mm thick near base, upper surface with a sticky reddish brown resinous layer, laccate, smooth with red margin. Hyphal system trimitic, generative hyphae thin walled, with clamps, 2-4 μ wide, skeletal hyphae thick walled, hyaline, non-septate, branched, basidia short clavate, 4-sterigmate, 15-20 x 5-7.5 μ, with a basal clamp, spores cylindric-ellipsoid, hyaline, smooth, 6-9 x 3-3.5 μ in dimensions.
5.	*Ganoderma applanatum*		Basidiocarps are perennial, sessile, woody to corky, applanate, 140-300 mm broad, 80-120 mm wide, grayish to brownish to reddish black, warty tuberculate, pores circular. Hyphal system trimitic, generative hyphae inconspicuous, thin walled, with clamps, 2-4 mm wide, occasionally branched. Cystidia absent, basidia clavate, 4-sterigmate, 18-35 x 8-10 μ, with a basal clamp; spores ovoid, truncate at distal end, bitunicate, exspore hyaline, smooth, endospore echinulate, brown, 7-9.5 x 4.5-7.5 μ in dimensions.
6.	*Ganoderma lucidum*		Basidiocarps are annual, centrally stipitate, pileus reniform, upto 150 mm broad, 10-20 mm thick, upper surface yellowish brown to almost reddish black, glossy and shiny, smooth, concentrically sulcate, crust thin and margin obtuse. Hyphal system trimitic, generative hyphae hyaline, thin walled, branched, 3-4.5 μ wide, skeletal hyphae yellowish brown, solid, arboriform, spores 8.5-11 x 6-7 μ, ovoid to truncate, brown, smooth and guttulate.
7.	*Heterobasidion annosum*		Basidiocarps are annual to perennial, sessile, effused-reflexed or often resupinate, pileus dimidiate to long, narrow and shelf-like, irregular in shape, imbricate or single, 100-250 mm long, 40-80 mm broad and 10-40 mm thick, upper surface glabrous, brown, rough and irregular, concentrically zonate. Hyphal system dimitic, generative hyphae hyaline, thin walled, simple septate with occasional branching, 2.5-4 μ, skeletal hyphae hyaline, thick walled, non-septate, branched, 3-5 μ wide, basidia clavate, 4-sterigmate, 15-20 x 5-6 μ, spores sub-globose to ovoid, hyaline, echinulate, 4.5-5.5 x 3.5-4.8 μ in dimensions.
8.	*Marasmius sp.*		Basidiocarps are annual. Cap is 1-3.5 cm wide, convex, smooth or somewhat wrinkled. Gills are attached to the stem, distant, pale at first, becoming tan or brown. Stem is 3-8 cm long, upto 3 mm thick, equal, dry, straightly or slightly curved, reddish brown with whitish basal mycelium. Flesh is thin, insubstantial and pale. Cystidia prominent, variously shaped but often fusiform, 20-110 μ long and 3 μ thick. Spores are 7-10 x 3-5.5 μ in dimensions, smooth, elliptical and inamyloid.
9.	*Polyporus squamosus*		Basidiocarps are annual, stipitate, pileus circular, 150-180 mm wide, 10-30 mm thick, solitary, upper surface pale buff with thin pellicle, azonate, margin concolorous. Hyphal system dimitic, generative hyphae thin walled, clamped, 2.5-3.5 μ in diameter, cystidia absent, basidia clavate with a narrow base, 4-sterigmate, 30-40 x 7-8 μ, with a basal clamp, spores broadly cylindrical, hyaline, smooth 10.5-12.5 x 4.5-5.5 μ in dimensions.

Sr. No.	Fungal Species	Fungal Colony	Characteristic Features
10.	*Puccinia* sp.		Yellowish-orange uredinia found mostly on the lower surface of the leaves, were ovate in shape, verrucose, 9.5-12 μm in diameter.
11.	*Pycnoporus sanguineus*		Basidiocarps are annual, sessile, in imbricate clusters, thin, applanate, 50-80 mm long, 20-40 mm wide and 3 mm thick, pileus surface orange red to salmon buff, concentrically zonate. Hyphal system trimitic, generative hyphae thin walled, hyaline, with clamps, branched, 2.5-3.5 μ wide, skeletal hyphae thick walled, hyaline, non-septate, 2-5 μ wide, conspicuous, branched, 2-3 μ wide, cystidia absent, basidia clavate, 4-sterigmate, 11-15 x 4-6 μ, with a basal clamp, spores cylindric, slightly curved, hyaline, smooth, 5-6 x 2-2.5 μ in dimensions.
12.	*Russula bravipes*		Basidiocarps are perennial. Cap is 6-20 cm wide, convex, shallowly vase-shaped, suede like, creamy to peach coloured. Gills are attached to the stem, crowded, whitish. Stem is 3-4 cm long, 1.5-3 cm thick, sturdy with white basal mycelium. Flesh is whitish, sometimes discolored brown. Pleuro- and cheilocystidia 35-50 x 7.5-10 μ, fusiform, cylindric or subclavate, thin walled, hyaline. Spores are broadly ellipsoid to sub-globose, 7-10 x 5-7 μ in dimensions.
13.	*Trametes cubensis*		Basidiocarps are annual, sessile, applanate, rigid to corky, 50-80 mm long, 100-120 mm wide and 10-15 mm thick at the base, upper surface velutinate, glabrous, cream to tan. Hyphal system trimitic, generative hyphae hyaline, with clamps, 1-3 μ wide, skeletal hyphae abundant, thick walled, hyaline, 3-6 μ wide, solid, 2-4 μ wide, cystidia absent; basidia clavate, 4-sterigmate, 10-15 x 4-5 μ with a basal clamp, spores cylindric, slightly bent, hyaline, 7-9.5 x 3-3.5 μ in dimensions.
14.	*Trametes hirsuta*		Basidiocarps are annual, sessile, effused, pileus applanate, upper surface hirsute, zonate, margins yellowish brown, tomentose. Hyphal system trimitic, generative hyphae thin walled, with clamps, 2.5-5 μ wide, skeletal hyphae thick walled, sinuous, hyaline, non-septate, branched, 3-6 μ wide, hyphal pegs present, basidia clavate, 4-sterigmate, 15-22 x 4-5 μ, with a basal clamp, spores cylindric, hyaline, smooth, 6-9 x 2-2.5 μ in dimensions.
15.	*Trametes versicolour*		Basidiocarps are annual, sessile, reflexed, in large imbricate clusters, upper surface tomentose, sharp concentric zones of brown, buff and reddish shades. Hyphal system trimitic, generative hyphae thin walled with clamps, 2-3 μ wide, skeletal hyphae thick walled, non-septate, 4-5 μ wide, cystidia absent, basidia clavate, 4-sterigmate, 12-20 x 4-5 μ, with a basal clamp, spores cylindrical, slightly curved, hyaline, smooth, 5-6 x 1.5-2 μ in dimensions.
16.	*Tylopilus alboater*		Basidiocarps are perennial. Cap is 4-9.5 cm wide, convex, dark gray to black. Stem is 5-7 cm long, 1.5-2 cm thick, enlarging towards base, finely reticulate near the apex. Flesh is thick and white to pinkish turning slowly grayish black. Hymenial cystidia 25-42.5 x 7.5-12.5 μ, widely fusiform, smooth, thin walled. Spores are 8-11.5 x 3.5-5 μ in dimensions, long-ellipsoid to sub fusiform, smooth and hyaline.
17.	*Xylobolus subpileatus*		Basidiocarps are thick, perennial, woody, effused, upto 400 mm broad, 150 mm long and 5 mm thick, pileus large, irregular, upper surface concentrically sulcate, zonate, cinnamon to blackish brown, margin paler and acute. Hyphal system dimitic, generative hyphae upto 3 μ wide, septate, branched, skeletal hyphae upto 4 μ wide, aseptate, unbranched, cystidia present, incrusted, clavate-cylindrical, sub-hyaline to brownish, moderately thick walled, gloeocystidia absent, basidia 15-22.5 x 3-4.5 μ, clavate-cylindrical, 4-spored; spores 4-5 x 2-3 μ, hyaline, smooth and shortly apiculate.

11.4

New Fungal Records from FGR Tree Species from Uttarakhand

Several fungal species were found to be reported earlier, either parasitic or in association on selected FGR species (*R. arboreum, Q. semecarpifolia, T. wallichiana, M. esculenta* and *B. utilis*). However, a total of 72 isolates belonging to 52 fungal species were identified causing various diseases in prioritized FGR tree species (Table 11.9). In the present study,

22 fungal species and one leaf gall insect were recorded on *Rhododendron arboreum viz., Alternaria alternata, A. phragmospora, A. triticina, Aspergillus niger, Chaetomium globosum, Cladosporium cladosporioides., C. herbarum, Curvularia lunata, Exobasidium vacinii, Fomes fomentarius, Fusarium oxysporum, Fusarium* sp., *Ganoderma applanatum, Gliocladium* sp., *Graphium* sp., *Hansfordia* sp., *Heterobasidion annosum, Nigrospora sphaerica, Phomopsis* sp., *Pithomyces* sp., *Phyllosticta* sp., *Sordaria fimicola* and *Verticillium* sp. Out of these, *Exobasidium vacinii* and *Phomopsis* sp. were reported earlier causing leaf gall and leaf spot respectively (Table 11.1), 21 fungal species are new fungal records on *R. arboreum* (Table 11.9).

22 fungal species and one *Phanerochaete* sp. were recorded on Quercus *semecarpifolia viz., Acremonium* sp., *Agaricus* sp., *Alternaria alternata, A. chlamydospora, A. phragmospora, Cladosporium fusiforme, C. herbarum, Curvularia lunata, Fusarium* sp., *Ganoderma applanatum, Ganoderma lucidum, Graphium* sp., *Heterobasidion annosum, Humicola* sp., *Hymenula* sp., *Macrophomina* sp., *Nigrospora sphaerica, Pestalotiopsis* sp., *Phanerochaete* sp., *Phomopsis* sp., *Trametes cubensis, T. versicolor* and *Verticillium* sp. Out of these, *Ganoderma lucidum* was reported earlier causing root rot (Table 11.1), 22 fungal species are new fungal records on *Q. semecarpifolia* (Table 11.9).

18 fungal species were recorded on *Taxus wallichiana viz., Alternaria alternata, A. phragmospora, Aspergillus nidulans, A. niger, Auricularia auricula, Cladosporium cladosporioides, Curvularia chlamydospora, C. pallescens, Fusarium* sp., *Ganoderma applanatum, G. lucidum, Marasmius* sp., *Nigrospora sphaerica, Penicillium chrysogenum, Pestalotiopsis* sp., *Phomopsis* sp., *Rhizopus* sp. *and Xylobolus subpileatus.* Out of these, *Altenaria alternata, Fusarium* sp., *Pestalotiopsis* sp. and *Phomopsis* sp. were reported earlier causing leaf spot and leaf blight diseases (Table 11.1), 14 fungal species are new fungal records on *T. wallichiana* (Table 11.9).

10 fungal species were recorded on *Myrica esculenta viz., Alternaria fasciculata, A. triticina, Aspergillus nidulans, Curvularia pallescens, Fomitopsis pinicola, Nigrospora sphaerica, Polyporus squamosus, Puccinia* sp., *Trametes hirsuta* and *Tylopilus alboater* and all of them are new fungal records on *M. esculenta*.

10 fungal species were recorded on *Betula utilis viz., Alternaria triticina, A. rosae, Cladosporium fusiforme, Fusarium* sp., *Lacellina* sp., *Mortierella* sp., *Pestalotiopsis* sp., *Puccinia* sp., *Pycnoporus sanguineus* and *Russula bravipes*. Out of these, *Fusarium* sp. and *Mortierella* sp. were recorded earlier as endophytes (Table 11.1), 8 fungal species are new fungal records on *B. utilis* (Table 11.9).

Table 11.9 - Occurrence of Fungal Species in Selected FGR Species

Sr. No.	Fungal Species	*Rhododendron arboreum*	*Quercus semecarpifolia*	*Taxus wallichiana*	*Myrica esculenta*	*Betula utilis*	Occurrence
1.	*Acremonium* sp.	-	+*	-	-	-	1
2.	*Agaricus* sp.	-	+*	-	-	-	1
3.	*Alternaria alternata*	+*	+*	+	-	-	3
4.	*Alternaria chlamydospora*	-	+*	-	-	-	1
5.	*Alternaria fasciculata*	-	-	-	+*	-	1
6.	*Alternaria phragmospora*	+*	+*	+*	-	-	3
7.	*Alternaria rosae*	-	-	-	-	+*	1
8.	*Alternaria triticina*	+*	-	-	+*	+*	3
9.	*Aspergillus nidulans*	-	-	+*	+*	-	2
10.	*Aspergillus niger*	+*	-	+*	-	-	2
11.	*Auricularia auricula*	-	-	+*	-	-	1
12.	*Chaetomium globosum*	+*	-	-	-	-	1
13.	*Cladosporium cladosporioides*	+*	-	+*	-	-	2
14.	*Cladosporium fusiforme*	-	+*	-	-	+*	1
15.	*Cladosporium herbarum*	+*	+*	-	-	-	2
16.	*Curvularia chlamydospora*	-	-	+*	-	-	1
17.	*Curvularia lunata*	+*	+*	-	-	-	2
18.	*Curvularia pallescens*	-	-	+*	+*	-	2
19.	*Fomes fomentarius*	+*	-	-	-	-	1
20.	*Fomitopsis pinicola*	-	-	-	+*	-	1

Sr. No.	Fungal Species	*Rhododendron arboreum*	*Quercus semecarpifolia*	*Taxus wallichiana*	*Myrica esculenta*	*Betula utilis*	Occurrence
21.	*Fusarium* sp.	+*	+*	+*	-	+	4
22.	*Fusarium oxysporum*	+*	-	-	-	-	1
23.	*Ganoderma applanatum*	+*	+*	+*	-	-	3
24.	*Ganoderma lucidum*	-	+	+*	-	-	2
25.	*Gliocladium* sp.	+*	-	-	-	-	1
26.	*Graphium* sp.	+*	+*	-	-	-	2
27.	*Hansfordia* sp.	+*	-	-	-	-	1
28.	*Heterobasidion annosum*	+*	+*	-	-	-	2
29.	*Humicola* sp.	-	+*	-	-	-	1
30.	*Hymenula* sp.	-	+*	-	-	-	1
31.	*Lacellina* sp.	-	-	-	-	+*	1
32.	*Macrophomina* sp.	-	+*	-	-	-	1
33.	*Marasmius* sp.	-	-	+*	-	-	1
34.	*Mortierella* sp.	-	-	-	-	+	1
35.	*Nigrospora sphaerica*	+*	+*	+*	+*	-	4
36.	*Penicillium chrysogenum*	-	-	+*	-	-	1
37.	*Pestalotiopsis* sp.	-	+*	+	-	+*	3
38.	*Phomopsis* sp.	+*	+*	+	-	-	3
39.	*Phyllosticta* sp.	+*	-	-	-	-	1
40.	*Pithomyces* sp.	+*	-	-	-	-	1
41.	*Polyporus squamosus*	-	-	-	+*	-	1
42.	*Puccinia* sp.	-	-	-	+*	+*	2
43.	*Pycnoporus sanguineus*	-	-	-	-	+*	1
44.	*Rhizopus* sp.	-	-	+*	-	-	1
45.	*Russula brevipes*	-	-	-	-	+*	1
46.	*Sordaria fimicola*	+*	-	-	-	-	1
47.	*Trametes cubensis*	-	+*	-	-	-	1
48.	*Trametes hirsuta*	-		-	+*	-	1
49.	*Trametes versicolor*	-	+*	-	-	-	1
50.	*Tylopilus alboater*	-	-	-	+*	-	1
51.	*Verticillium* sp.	+*	+*	-	-	-	2
52.	*Xylobolus subpileatus*	-	-	+*	-	-	1
	Total	22	22	18	10	10	

(*) New Fungal Records on FGR Species from Uttarakhand; (+) Species Recorded; (-) Species Not Recorded

11.5

Preservation of Fungal Cultures in Mineral Oil (Paraffin Oil)

The pure fungal cultures were preserved in paraffin oil and were deposited in the National Type Culture Collection (NTCC) at Forest Pathology Discipline, Forest Protection Division, Forest Research Institute, Dehra Dun (Fig. 11.16 - Fig. 11.20).

Fig. 11.16 Preserved Fungal Cultures from Diseased Leaf Samples of *Rhododendron arboreum*

Fig. 11.17 Preserved Fungal Cultures from Diseased Leaf Samples of *Quercus semecarpifolia*

Fig. 11.18 Preserved Fungal Cultures from Diseased Leaf Samples of *Taxus wallichiana*

Fig. 11.19 Preserved Fungal Cultures from Diseased Leaf Samples of *Myrica esculenta*

Fig. 11.20 Preserved Fungal Cultures from Diseased Leaf Samples of *Betula utilis*

11.6

Important Findings

Alternaria spp., *Fusarium* sp. and *Nigrospora sphaerica* were recorded for maximum number of times causing foliar diseases with disease intensity of upto 45 per cent, followed by *Phomopsis* sp., *Pestalotiopsis* and *Curvularia* spp. on the tree species. Foliar pathogens tend to be deeply influenced by weather and their sporulation and infection are affected by change in climatic conditions because they often occur within a narrow range of temperature and their spore release usually coincide with periods of precipitation and regions of wet winters (Broadmeadow and Ray, 2005). *Ganoderma* spp. (*G. applanatum* and *G. lucidum*) were also found frequently causing heart and root rot, thus leading to death of the trees. Studies by La porta *et al.* (2000) depicted that low temperature is highly correlated with sporulation rate in *Ganoderma* spp. and *Heterobasidion* spp., major root and butt rot pathogens of important forestry species, whose spore production increases above 5°C, usually between 10-20°C.

Due to shorter time duration of the life cycle and multiplication, the evolutionary changes and adaptation to newer climatic conditions and hosts, the fungi are capable of creating high biodiversity within short time span as compared to their hosts including plants. Many factors could be responsible for the emergence of newer pathogens of host tree species at higher altitude on account of frequent weather fluctuations. One of the major reasons could be climate change that produce a confluence of factors acting together to drive the emergence of new pathogens. However, this needs to be substantiated with experimentations and climatic data over a long time period along with molecular validation of the phylogenetic correlations. Plant diseases are highly influenced by the environment; a susceptible host will not be infected by a virulent pathogen if the environmental conditions are not conducive for disease. On the other hand, a host mediated evolution may allow the microbe to venture newer tree species. The change in CO_2 concentrations, temperature and water availability have detrimental effects on plant health and may favour microbes and predisposing factors involved in disease development. Thus, there is an urgent need for further scientific investigations with emergence of new fungal records and in view of severe limitations to pathogenicity testing with respect to high altitude tree species besides the need for essential management interventions and approaches for the conservation of rare, endangered, threatened and important tree species.

11.6.1

Key Messages and Recommendations

- The baseline data of the association of different fungal species with respect to prioritized FGR species *viz., R. arboreum, Q. semecarpifolia, T. wallichiana, M. esculenta* and *B. utilis* has been prepared under this component.
- Out of the populations sampled for each prioritized FGR species, eleven populations (RA 03, RA 06, RA 08, RA 09, RA 12, RA 13, RA 15, RA 16, RA 18, RA 19 and RA 23) for *R. arboreum*; ten populations (QS 03, QS 07, QS 08, QS 11, QS 13, QS 14, QS 17, QS 18, QS 20 and QS 23) for *Q. semecarpifolia*; nine populations (TW 03, TW 05, TW 07, TW 10, TW 12, TW 16, TW 18, TW 19 and TW 21) for *T. wallichiana*; twelve populations (ME 02, ME 04, ME 05, ME 07, ME 12, ME 13, ME 15, ME 16, ME 18, ME 20, ME 21 and ME 23) for *M. esculenta* and five populations (BU 04, BU 07, BU 09, BU 11 and BU 16) for *B. utilis* were found healthy and superior for in situ conservation.
- The future disease surveys in the studied sites will give valuable information when compared to the baseline data generated in the present study regarding the spread of the existing diseases or incidences of newer health problems of the tree species.
- A monitoring of the changing climatic conditions could have discreet effect on the tree health in future and early recognition of the same may result in the timely mitigation of the problem.
- With increased green-house gases, the nutritional uptake would get modified resulting in nutritional deficiencies especially with respect to micronutrients which could become a limiting factor for the tree health and growth. Needless to say, the nutritional imbalances may affect the tree vigor adversely and may work in favor of the pathogens. This need to be monitored carefully and the soil status could provide an insight in future trend predictions. The key lies in employing the data generated in this study as a reference point and building up the observations with regular surveys for devising timely remedial measures against the emerging physiological, entomological and pathological problems.
- Any kind of interventions/ disturbances in natural or protected forests should be minimized in order to avoid anthropogenic consequences.

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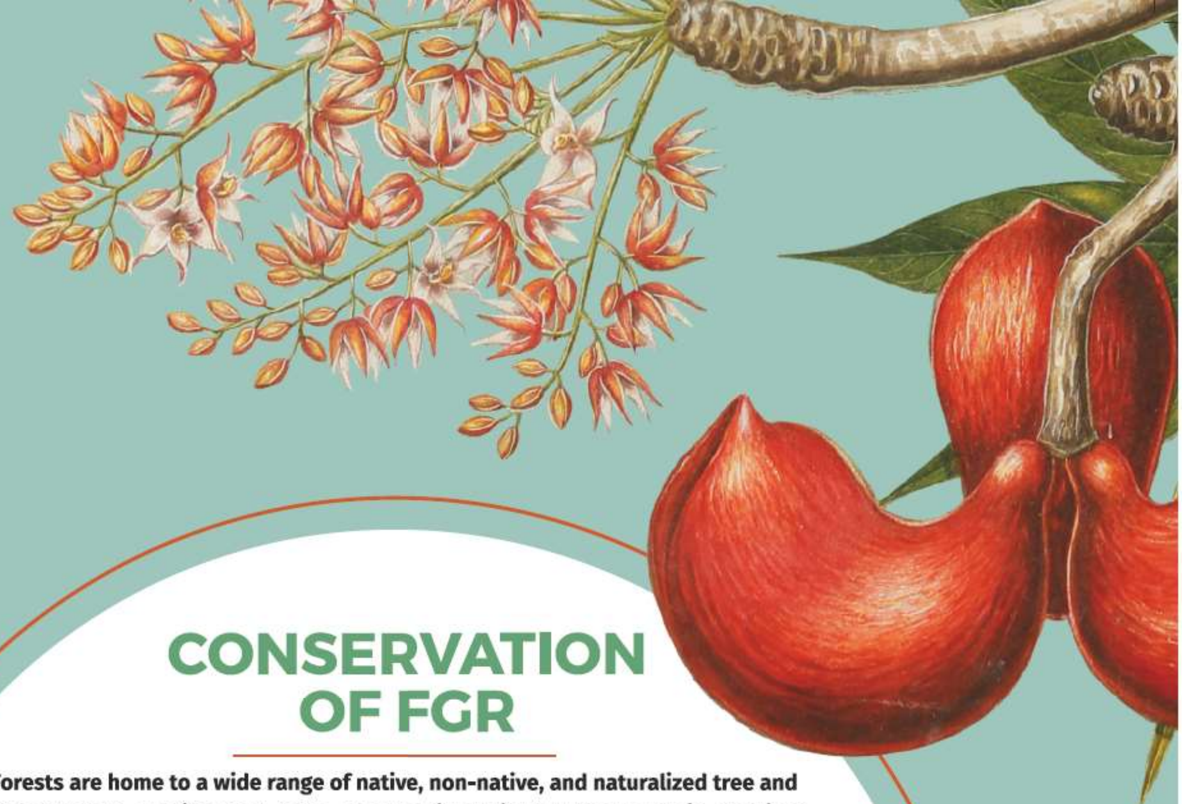
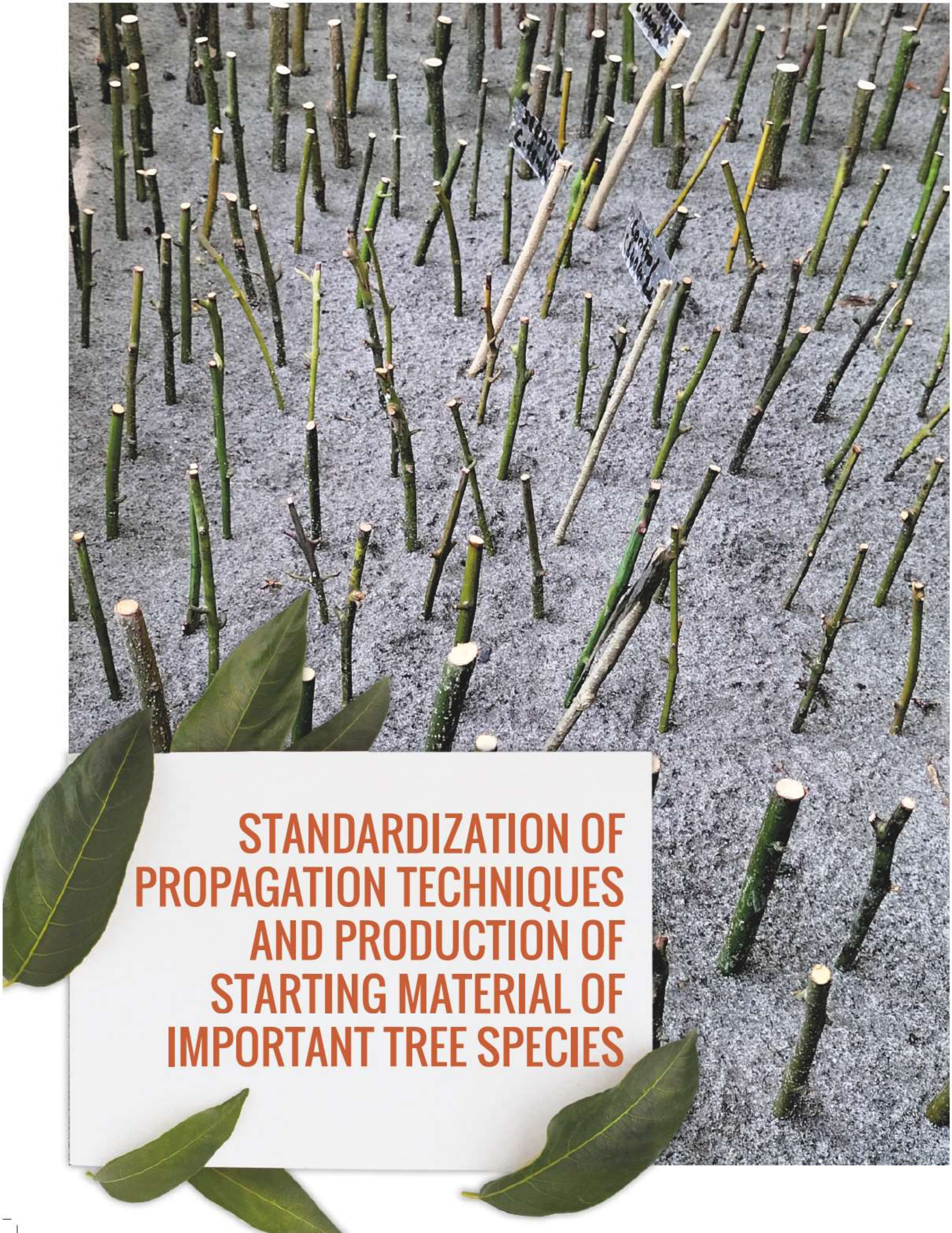
PART 05



CONSERVATION OF FGR

Forests are home to a wide range of native, non-native, and naturalized tree and other forest species that have adapted in various ways to their growing environments. Owing to enhanced biotic activities, many tree and other woody species persist as small, semi-natural, fragmented populations. FGR can be broadly conserved in situ or *ex situ*, and dynamically or statically. Thus, generally, conservation is along two axes. The first axis relates to the 'location' of conservation (*in situ*, at the site of the population, or *ex situ*, in another location). The second axis describes the objective of conservation, which may be static, to conserve the existing genetic diversity of the population, or dynamic, to conserve the evolutionary potential of the population. In addition, circa situm conservation or preservation of remnant trees in farmlands has significant role in conservation. The Part V includes the following three Chapters, dealing with propagation techniques, establishment of forest gene banks (*in situ*), field gene banks (*ex situ*), and *circa situm* conservation.



STANDARDIZATION OF PROPAGATION TECHNIQUES AND PRODUCTION OF STARTING MATERIAL OF IMPORTANT TREE SPECIES

12

Kumar, D., Jabeen, S., Kumar, S. and Sanawar, M.

Forests and forest-based biodiversity are under intense stress owing to changes in land use, depleting forest cover, unsustainable harvest, enhanced biotic pressure, and climate change. Efforts are underway to reverse the effects of degradation through reforestation, afforestation, and plantation outside the forest in areas such as wastelands, community lands, private lands, agricultural fields, and urban spaces for increasing the forest and tree cover. In addition, plantations of economically important forest species are being raised so as to satisfy the growing demand for wood, fuelwood, small timber, and wide array of NTFPs. This requires genetic improvement of forest tree species and development of fast and economical methods for production of starting material of important tree species, propagation, and raising superior stock. Worldwide, plantations of the economically important forest species including agroforestry plantations are usually established with seedlings, either by entire transplanting of nursery stock or through clonal propagation. Plants can be propagated by sexual (through generation of seeds) or asexual (through multiplication of vegetative parts) means. Clonal propagation refers to the process of asexual reproduction by multiplication of genetically identical copies of individual plants and it can be achieved by various means viz., grafting, rooting of cuttings, coppicing, or in vitro micropropagation. As stated earlier, often in case of numerous trees and other FGR species, propagation and planting by seed is

difficult in case of recalcitrant seeds or species producing small quantities of seeds. Thus, in such instances as well as in research, and genetic improvement of undomesticated forest tree species, vegetative propagation methods have been developed and used for centuries. Generally, plantations are highly productive and with further improvement in genetic composition of planting stock by appropriate application of biotechnological approaches, an additional productivity enhancement can be envisioned. Further, wild populations exhibit a wide variation in important product characteristics (e.g., fruit quality, bole straightness, biomass). Hence, individuals within a population that produce a high quality of the desired product or services are being selected so as to propagate such individuals vegetatively to 'capture' the genetic variation expressed, which may otherwise get lost or diluted during sexual propagation. Varied vegetative propagation methods in trees have been described by Libby (1974), Bhatnagar (1974), Jaenicke and Beniest (2002), Park *et al.* (2016), and ICRAF (2020).

12.1

Need for Propagation Technology of Tree Species of Concern

Loss of within-species variability, resulting into genetic erosion is an important dimension of forest degradation. It is imperative to conserve within-species variation, multiply the rare, endangered and threatened germplasm, and augment its population in natural stands and conservation areas besides raising plantations outside the forest areas. Efforts are being made at the international, national as well as local levels to conserve genetic diversity and augment it by propagating and planting species of concern. This calls for reliable protocols for propagation of priority species. One of the Components of the Pilot Project envisaged developing technology for conservation of genetic diversity of priority tree species of concern *viz., Taxus wallichiana, Rhododendron arboreum, Myrica esculenta, Cinnamomum tamala*, and *Diploknema butyracea*. These species are multipurpose and highly important culturally and commercially, and serve as source of livelihood for the local people. There is an imminent threat to these species in the State because of over-exploitation, habitat destruction, and inadequate regeneration. The Component of the project aims at standardizing nursery techniques for these priority species and eventually developing field gene banks for the species. Field gene banks are *ex situ* conservation areas where genetic variation is maintained away from the original location because samples of a species, subspecies or variety are transferred and conserved as living collections. Conservation in field gene banks is particularly necessary in perennial plants including species producing recalcitrant seeds, species producing little or no seeds, species that are preferably stored as clonal material, and species that have a long-life cycle to generate breeding and/ or planting material (Tao, 2001). Even if advancements take place in technology for conservation of recalcitrant seeds, still there will remain problems and uncertainties due to long regeneration cycle of perennial species (Hawkes, 1982).

12.2

Need for Producing Starting Material of Important Tree Species

Genetically superior and diverse germplasm is used as a base material for propagating planting materials for use in raising plantations for various purposes and on farm and other *circa situm* plantings. The deployment of germplasm with variability is an essential component of many FGR and biodiversity conservation programmes, research, and developments as well as in environmental restoration programmes. Also, there are new requirements like creating carbon sinks to fight climate change events. India has to meet the Nationally Determined Contributions (NDC) target of 2.5 to 3 billion tonnes of additional carbon sinks by 2030. Out of this NDC, 1.92 billion tonnes will be met from natural forests and the rest from Trees Outside the Forest (TOF). Gera (2019) documented that it is essential that the productivity of forestry plantations is enhanced both in terms of quantity of wood and its quality in view of the country's global obligations and commitments, and to meet the domestic demand of 153 million m^3 of timber and wood by the year 2020.

Trees Outside Forest (TOF) which constitute all trees grown outside the natural forest area under different systems like agroforestry, social forestry and farm forestry are the main source of wood and timber in India. The country ranks first in the extent of plantation area for *Eucalyptus* (3 million ha), Teak (2.6 million ha) and *Casuarina* (0.8 million ha) and another 1-2 million ha of plantations of other tree species (ICFRE, 2019). There is necessity of increasing productivity of tree plantations to meet the future demand for wood products and environmental services. Hence, the demand for quality planting material has increased manifold in the country in the recent past. However, the greatest bottleneck in the expansion of area is the non-availability of genuine and quality planting material in adequate quantity from reliable sources. Lack of technical protocols of multiplication of elite germplasm limits its area expansion in the country. Improved quality planting material is the prerequisite for agroforestry, orchard plantation as well as raising of rootstocks.

The impact of investment in forestry research can be realized only if farmers/ planters have better access to high quality seed and planting materials of the improved varieties at reasonable price and at their production sites. Use of quality seeds and planting material of forestry species for the recommended zones can ensure higher productivity and easy availability of the produce. In recent past, a good number of tree genotype suited to different agro-climatic zones has been developed. However, those seed and planting materials are still in short supply. Tree breeding programmes have the potential to improve the production of planted forests and trees in a sustainable way and are necessary to meet growing global demand for forest products and services. More extensive development of forest industries based on planted forests using diverse, improved tree germplasm has the potential to meet a large proportion of the world's wood requirements and relieve pressures on natural forests. Development of clones and varieties is a dynamic process for different end uses and is considered as an important output of the long-term tree improvement programmes. ICFRE institutes have contributed significantly in this field and have released 47 new varieties/ clones of nine taxa possessing different adaptability and end-use characters and suitable for planting in different parts of the country during the last decade. These new clones/ varieties belong to *Casuarina* (19), *Eucalyptus* (15), *Dalbergia sissoo* (1), *Melia dubia* (10), and *Rauvolfia serpentina* (2). Further to meet the ever-increasing demand of wood and industrial raw material, and to increase the income of the farmers/ growers, it is essential that more research emphasis is given in this field. Clonal approach should also form an important constituent of ecological restoration with identification and deployment of clones having adaptability growth and productivity at degraded sites.

Many of these new varieties/ clones are now widely planted by farmers, forest departments, forest development corporations, and wood-based industries. Some of the varieties/ clones have been licensed to industries and private nurseries for commercial propagation and supply to the tree growers. The licensing to commercial organizations located at different parts of the country ensures the large-scale multiplication and supply of the new varieties/ clones to farmers and other tree growers leading to increase in the area of cultivation.

The germplasm and plant materials produced for deployment in conservation programme may not be genetically improved. Programmes for FGR and biodiversity conservation require enrichment of diversity in remnant populations, or introduce characteristics vital to the survival of threatened species. For productive purposes, improved material from genetic improvement and selection programmes enables the delivery of larger amounts of desired benefits with fewer inputs, or better growth under less favourable environmental conditions. Plant materials used for commercial and utility plantings and plantations are largely derived from high-quality, improved, source-identified seeds and plants.

12.3

Objectives

In view of the above, the following two objectives were set-forth relevant to the task of standardization of propagation protocols and production of starting material of priority/ important tree species and their distribution.

(i) Standardize propagation techniques of priority tree species.

(ii) Produce starting material of important tree species.

12.4

Material and Methods

The study on standardization of propagation techniques of priority tree species was carried out in Lesser-Known Tree Species (LKTS) Nursery, Silviculture and Forest Management Division, Forest Research Institute, Dehra Dun and Kaddukhal Nursery, Narendranagar FD, Rishikesh. Five species *viz., Taxus wallichiana, Rhododendron arboreum, Myrica esculenta, Cinnamomum tamala* and *Diploknema butyracea* were taken up for standardization of propagation techniques through seed, branch cutting, and air layering. The broad methodology for propagating plants in the nursery through seed, cuttings, and air layering is described hereunder. Specific modifications employed for different species along with species-specific experiments are later elaborated under the respective species.

12.4.1

Propagation Through Seeds

Fruits were collected from randomly selected medium-sized trees in natural populations of the species and brought to the laboratory. Fruits were dried in shade. Seeds were extracted from the fruits and dried in shade for two to three weeks. The dried seeds were stored in plastic containers at ambient temperature.

For raising nursery, the seeds were soaked in tap water for about 18 h. Nursery beds were prepared having sand:soil:FYM in 2:1:1 ratio. Seeds soaked in water were sown at 5 to 6 cm distance in rows 10 cm apart. Seedlings were pricked out when they had two pairs of leaves and were planted in containers having sand:soil:FYM in 1:1:1 ratio. Irrigation and weeding were done as per requirement till the plants reached suitable size for outplanting.

12.4.2

Propagation Through Branch Cuttings

Branches were collected from medium-sized randomly selected trees in natural populations of the species and brought to the laboratory. Semi-hardwood cuttings of 20 to 22 cm length and 5 to 15 mm diameter were made with sharp secateurs. The branch cuttings were treated with auxin in powder formulation and planted in rooting media in high humidity environment. Data on sprouting, rooting, and survival were recorded after 1, 3, and 6 months of planting of cuttings, respectively. Rooted cuttings were planted in nursery containers and reared in the nursery for hardening.

12.4.3

Propagation Through Air Layering

Air layering was carried out on semi-hardwood shoots of medium-sized trees growing in natural populations of the species. Bark was removed from the part of the branch at the place of air layering and cambium was exposed. Auxin powder was sprinkled on the treated part of the branch and covered with moistened moss. A plastic sheet was wrapped all over the moss. Data on rooting of air layered shoots were recorded after 8, 12, and 16 weeks of air layering. The rooted section of the shoot was detached from the mother branch. Plastic sheet was removed and the rooted section of the shoot was planted in nursery container filled with sand:soil:FYM in 1:1:1 ratio. The plants were reared in the nursery for hardening.

12.4.4

Species-wise Treatments and Experiments

The list of populations studied and experiments conducted, keeping in view the available literature, is presented in Table 12.1. The modifications employed in methodology for different species and experiments are summarized below:

(a) *Taxus wallichiana*: Dark brown mature fruits covered with arils (possessing red colour) were collected during September-October. The depulped seeds were rubbed by hand to remove the sticky material followed by washing in running tap water. Seeds treated with 0 and 400 ppm gibberellic acid (GA_3) were sown in nursery in the month of March.

Branch cuttings were collected from trees of 5 to 25 cm diameter at breast height (dbh) from the selected populations during March (Table 12.1). Branch cuttings were treated with 0 to 5,000 ppm indole butyric acid (IBA). The cuttings were planted in mist chamber. In order to study the effect of sex of tree on propagation through cuttings, cuttings were planted in non-mist propagation chamber (Fig. 12.1). During air layering, IBA was applied at six different concentrations ranging from 0 to 5,000 ppm to study the effect of IBA concentration.

(b) *Rhododendron arboreum*: Fruits (capsules) of *R. arboreum* were collected during December. Seeds of different populations were treated with 0, 150, and 300 ppm GA_3 and sown in nursery in the month of March (Table 12.1). Branch cuttings were collected from trees of 15 to 25 cm diameter during March. The cuttings were treated with 0 to 5,000 ppm concentrations of IBA planted for rooting (Fig. 12.2). Root suckers were collected from the source populations of seeds, and root cuttings of 15 cm length were made from root suckers. Leaves were cut to half size to reduce transpiration. Root cuttings were treated with 0 to 4,000 ppm IBA and planted in the rooting media. Air layering was performed in the natural stands of the species using semi-hardwood shoots and IBA, indole acetic acid (IAA) and naphthalene acetic acid (NAA) at 0 to 4,000 ppm concentration.

Table 12.1 Populations of Different Species Studied and Experiments Conducted

Sr. No.	Species and Populations Studied	Experiments Conducted
1.	*Taxus wallichiana*	
	Chakrata (Dehra Dun), Kaddukhal (Tehri Garhwal), Dyara Bugyal (Uttarkashi), Joshimath (Chamoli), Jageshwar (Almora) and Munsiyari (Pithoragarh)	(a) Performance of varied source populations and effect of GA_3 concentration on seedling survival (b) Effect of plant diameter on vegetative propagation through cuttings (c) Effect of IBA concentration and media on rooting of cuttings (d) Effect of IBA concentration and propagation environment on rooting of cuttings (e) Effect of sex of tree on vegetative propagation of cuttings (f) Performance of varied source populations on vegetative propagation success through cuttings

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Sr. No.	Species and Populations Studied	Experiments Conducted
2.	***Rhododendron arboreum***	
	Chakrata (Dehra Dun), Kaddukhal (Tehri Garhwal), Khirsu (Pauri Garhwal), Bhowali (Nainital), Jageshwar (Almora) and Didihat (Pithoragarh)	(a) Effect of GA_3 application on seedling emergence and seedling survival of different populations (b) Effect of IBA concentration on propagation through branch cuttings (c) Performance of varied source populations and effect of different IBA concentrations on propagation through root cuttings (d) Effect of month, auxin type and auxin concentration on air layering success (e) Effect of different IBA concentrations on air layering success in different source populations
3.	***Myrica esculenta***	
	Chakrata (Dehra Dun), Khirsu (Pauri Garhwal) and Bhowali (Nainital)	(a) Effect of scarification on seedling emergence and survival (b) Effect of auxin and other supplements on propagation through branch cuttings
4.	***Cinnamomum tamala***	
	Chakrata (Dehra Dun), Bhowali (Nainital), Jageshwar (Almora) and Munsiyari (Pithoragarh)	(a) Effect of method of depulping of seeds on seedling emergence and survival (b) Effect of different IBA concentrations on propagation through branch cuttings (c) Performance of varied source populations and effect of different IBA concentrations on propagation through cuttings (d) Effect of month and varied IBA concentrations on air layering success
5.	***Diploknema butyracea***	
	Pithoragarh	(a) Effect of maturity stage of mother tree on propagation through cuttings (b) Effect of different methods of air layering and varying IBA concentrations on vegetative propagation success

Fig. 12.1 Cuttings of *Taxus wallichiana* Planted in Non-Mist Propagation Chamber

Fig. 12.2 Cuttings of *Rhododendron arboreum* Planted in Mist Chamber for Root Induction

(c) *Myrica esculenta*: Mature fruits (drupes) of *M. esculenta* were collected during the month of May. The fruits were depulped and the extracted seeds were stored at ambient temperature for four weeks. Seeds were then subjected to different scarification treatments and sown in the nursery.

Branch cuttings were collected from trees of the above-said populations during the end of February. The cuttings were treated with 0 to 4,000 ppm IBA, NAA, 0 to 5 per cent Captan (a fungicide) and 0 to 5 per cent Sucrose, and planted in humid environment for rooting. Air layering was performed in the natural stands of the species using semi-hardwood shoots. IBA was applied at 0 to 4,000 ppm concentration. The plants were affected by monkeys/ human beings in the three years of trials and rooting was not obtained.

(d) *Cinnamomum tamala*: Mature fruits (capsules) of *C. tamala* were collected during the month of November. The fruits were dried in air and the extracted seeds were stored during winter. Seeds were depulped by different methods and sown in the nursery during March.

Branches were collected from mature trees of the species in the above populations and cuttings were made. The cuttings were treated with 0 to 4,000 ppm IBA and planted in humid environment for rooting. Air layering was performed in the natural stands from March to May using IBA at 0 to 4,000 ppm concentration.

(e) *Diploknema butyracea*: Seeds were collected during July and immediately extracted from the fruits after depulping. Freshly extracted seeds were sown in the nursery to produce juvenile plants for study on vegetative propagation.

Cuttings were collected from juvenile plants (two-year-old plants in FRI nursery) and mature trees. The germplasm of both the plant material belonged to Pithoragarh. Cuttings were treated with 2,000 ppm IBA and planted in humid environment. Air layering was performed on 8-year-old plants of this species. FRI-wire technique and conventional air layering technique were used in presence of 0 to 2,000 ppm IBA.

The work on production of starting material of important tree species was carried out in the Silviculture and Forest Management Division, and the Genetic and Tree Improvement Division of FRI, Dehra Dun.

12.5 Experimental Findings

The data obtained from the experiments were statistically analysed and are presented below:

12.5.1 *Taxus wallichiana*

12.5.2 Propagation Through Seeds

In order to examine the feasibility of enhancing seedling emergence and survival in the species, an experiment was conducted in which seeds of six different populations stored for varying lengths of period up to 42 months were subjected to 400 ppm GA_3 and compared with control (without Ga_3). The research findings are summarized in Table 12.2. The data revealed that population and GA_3 concentration did not have any significant influence on seedling survival (i.e., plant per cent). However, seedling survival increased as seed storage duration increased. Seedling emergence was not observed till 12 months of seed storage, beyond that seedling emergence was recorded in small numbers. When averaged over populations, the highest seedling survival was 1 per cent achieved with 400 ppm GA3 treatment of 42-month stored seeds, while 0.84 per cent seedling survival was noticed without application of GA_3 in seeds stored for an equal length of time. Seeds stored for 42 months upon treatment with 400 ppm GA_3 recorded 1.33 per cent seedling survival while other populations registered 0.67 to 1 per cent seedling survival (Fig. 12.3).

Table 12.2 - Effect of Population and GA_3 Concentration on Seedling Survival in *Taxus wallichiana*

	Different Source Populations and GA_3 Concentration													
Duration of Seed Storage (months)	**Chakrata (Dehra Dun)**		**Kaddukhal (Tehri Garhwal)**		**Dyara Bugyal (Uttarkashi)**		**Joshimath (Chamoli)**		**Jageshwar (Almora)**		**Munsiyari (Pithoragarh)**		**Mean**	
	0 ppm	4 ppm	0 ppm	400 ppm	0 ppm	400 ppm	0 ppm	400 ppm	0 ppm	400 ppm	0 ppm	400 ppm	0 ppm	400 ppm
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.07
24	0.33	0.33	0.00	0.33	0.00	0.67	0.00	0.67	0.00	0.00	0.00	0.33	0.06	0.39
30	0.67	1.00	0.33	0.67	0.33	0.67	0.67	0.67	0.67	0.67	0.33	0.67	0.50	0.73
36	0.67	1.00	0.67	0.67	0.67	1.00	1.00	1.32	1.00	1.00	0.67	0.67	0.78	0.94
42	1.00	1.33	0.67	1.33	0.67	0.67	1.00	1.00	0.67	1.00	1.00	0.67	0.84	1.00

$CD_{0.05}$ for Population: Non-significant
$CD_{0.05}$ for GA_3 Concentration: Non-significant
$CD_{0.05}$ for Duration of Seed Storage: 0.39
$CD_{0.05}$ for Duration of Population x GA_3 Concentration x Seed Storage Duration: Non-significant

Fig. 12.3 Plants of *Taxus wallichiana* Raised Through Seeds

12.5.3

Propagation Through Cuttings

Branch cuttings collected from plants of different diameters exhibited significant variation in sprouting, rooting, and survival (Table 12.3). Response of cuttings significantly declined with increase in stem diameter of the plants from where cuttings were collected (Fig. 12.4). Branch cuttings of *T. wallichiana* plants from 5 to 10 cm dbh, and 10 to 15 cm dbh yielded 75.00 and 73.33 per cent rooting, respectively while plants of 15 to 25 cm diameter recorded 35 to 55 per cent rooting. Plants of 5 to 10 cm diameter recorded maximum 75 per cent rooting and 73.33 per cent survival while the corresponding values for cuttings belonging to >20 cm dbh plants were 35 and 25 per cent, respectively.

Plant Diameter (cm)	Sprouting (%)	Rooting (%)	Survival (%)
5-10	93.33	75.00	73.33
10-15	90.00	73.33	71.67
15-20	66.67	55.00	41.67
>20	65.00	35.00	25.00
$CD_{0.05}$	12.46	10.88	10.20

Table 12.3 Effect of Plant Diameter on Vegetative Propagation of *Taxus wallichiana* through Cuttings

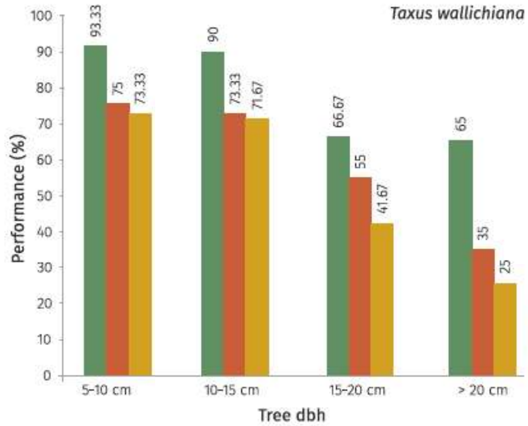

Fig. 12.4 Performance of Cuttings of *Taxus wallichiana* Belonging to Trees of Different Diameters

Table 12.4 and Fig. 12.5 show the response of branch cuttings of *T. wallichiana* to different rooting media and IBA concentrations in the mist chamber. Rooting media x IBA concentration had significant interaction effect on rooting and survival of cuttings while sprouting was not significantly affected. Rooting and survival of cuttings increased from below 40 per cent without IBA application to above 58 per cent with IBA application regardless of the media used (Fig. 12.6). Effect of interaction between media x IBA concentration was not significant. Gravel proved slightly better than sand and vermiculite. Overall, the best rooting and survival (76.67 per cent and 71.67 per cent, respectively) were obtained when cuttings were treated with 5,000 ppm IBA and planted in gravel media. This is in contrast to 35.00 per cent rooting and 33.33 per cent survival recorded with planting of cuttings without IBA in vermiculite. Survival of cuttings in sand was 31.67 per cent when IBA was not used.

Media and IBA Concentration	Sprouting (%)	Rooting (%)	Survival (%)
Media: Sand			
Control	70.00	38.33	31.67
2,000 ppm	78.33	68.33	61.67
3,000 ppm	75.00	66.67	63.33
4,000 ppm	83.33	70.00	65.00
5,000 ppm	81.67	63.33	58.33
Mean	**77.67**	**61.33**	**56.00**
Media: Vermiculite			
Control	66.67	35.00	33.33
2,000 ppm	65.00	68.33	61.67
3,000 ppm	71.67	66.67	58.33
4,000 ppm	80.00	73.33	66.67
5,000 ppm	83.33	68.33	68.33
Mean	**73.33**	**62.33**	**57.67**
Media: Gravel			
Control	73.33	36.67	35.00
2,000 ppm	78.33	75.00	66.67
3,000 ppm	81.67	73.33	68.33
4,000 ppm	86.67	73.33	71.67
5,000 ppm	88.33	76.67	71.67
Mean	**81.67**	**67.00**	**62.67**
$CD_{0.05}$ for Media	Non-significant	Non-significant	Non-significant
$CD_{0.05}$ for IBA Concentration	Non-significant	13.40	11.04
$CD_{0.05}$ for Media x IBA Concentration	Non-significant	Non-significant	Non-significant

Table 12.4 Effect of IBA Concentration and Media on Rooting of *Taxus wallichiana* Cuttings in Mist Chamber

Fig. 12.5 Mean Performance of Cuttings of *Taxus wallichiana* Planted in Three Different Rooting Media in Mist Chamber

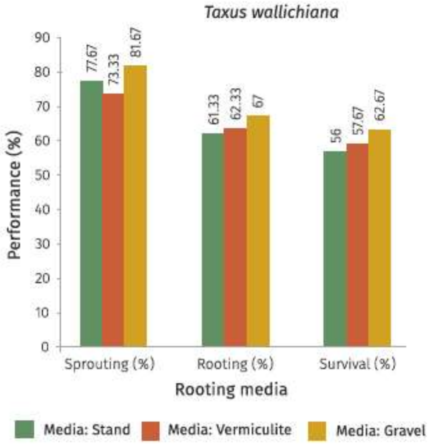

Fig. 12.6 Rooting in Cuttings of *Taxus wallichiana* Raised in the Rooting Medium of Sand After Treatment with IBA

The data on performance of branch cuttings in relation to propagation environment and IBA concentration are presented in Table 12.5. In the present experiment, all the cuttings were planted in sand which is cheaper and more readily available than vermiculite. The cuttings were found to sprout, root, and survive significantly better in non-mist propagation chamber than in mist chamber (Fig. 12.7). The best per cent values of sprouting, rooting, and survival were: 91.67, 81.67 and 76.67, respectively when cuttings were treated with 4,000 ppm concentration. Nevertheless, response of 5,000 ppm was quite close to that recorded for 4,000 ppm concentration. In contrast, the maximum rooting and survival in mist chamber were 68.33 and 60 per cent, respectively.

Table 12.5 Effect of IBA Concentration and Propagation Environment on Rooting of *Taxus wallichiana* Cuttings Using Sand Media

Propagation Environment and IBA Concentration (ppm)	Sprouting (%)	Rooting (%)	Survival (%)
Propagation Environment: Mist Chamber			
Control	71.67	41.67	30.00
2,000	76.67	66.67	58.33
3,000	73.33	68.33	60.00
4,000	80.00	66.67	58.33
5,000	81.67	68.33	56.67
Mean	**76.67**	**62.33**	**52.67**
Propagation Environment: Non-mist Propagation Chamber			
Control	76.67	40.00	36.67
2,000	81.67	76.67	70.00
3,000	80.00	78.33	73.33
4,000	91.67	81.67	76.67
5,000	91.67	80.00	76.67
Mean	**84.67**	**71.67**	**67.00**
$CD_{0,05}$ for Propagation Environment	5.47	7.35	7.99
$CD_{0,05}$ for IBA Concentration	Non-significant	10.85	13.54
$CD_{0,05}$ for Media x IBA Concentration	Non-significant	Non-significant	Non-significant

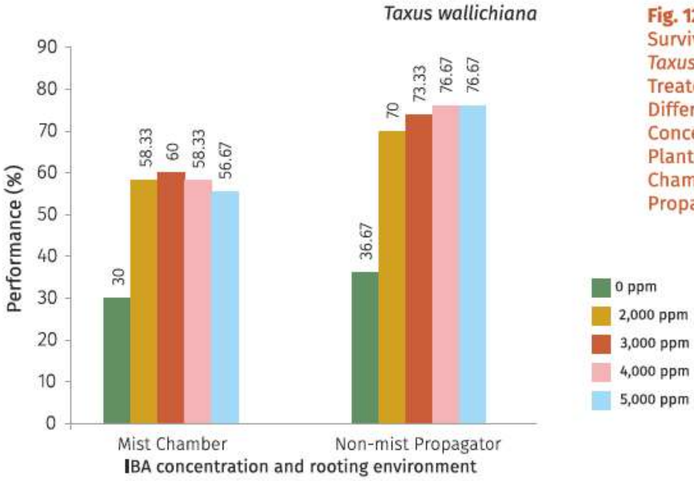

Fig. 12.7 Survival of Cuttings of *Taxus wallichiana* Treated with Five Different IBA Concentrations and Planted in Mist Chamber and Non-Mist Propagator

Cuttings of male plants and female plants of *T. wallichiana* were collected from the forest and tested for vegetative propagation ability in non-mist propagation chamber by using t-test (Table 12.6). The rooting and survival of cuttings from male plants was higher than cuttings obtained from female plants, although sprouting percentages of the two were not significantly different. The survival after 5 months was nine per cent higher taking success rate of cuttings from female plants as 100 per cent.

Table 12.6 Effect of Sex of Tree on Vegetative Propagation of Cuttings of *Taxus wallichiana* Planted in Non-mist Propagation Chamber

Treatments	Sprouting (%)	Rooting (%)	Survival After Three Months (%)	Survival After Six Months (%)
Male	93.33	80.00	76.67	61.67
Female	90.00	71.67	70.00	56.67
t-test$_{0.05}$	Non-significant	Significant	Significant	Significant

Table 12.7 provides information about the effect of IBA on performance of cuttings belonging to six different populations in the State. Rooting and survival of the cuttings were not found to vary significantly with the populations of cuttings although significant difference was observed among populations in respect of sprouting percentage with application of IBA. The range of variation in sprouting of IBA-treated cuttings was 66.67 to 75.00 per cent.

Table 12.7 - Performance of Varied Source Populations and Effect of Different IBA Concentrations on Vegetative Propagation of *Taxus wallichiana* through Cuttings

IBA Conc.	Populations						Mean	CD$_{0.05}$
	Chakrata (Dehra Dun)	Kaddukhal (Tehri Garhwal)	Dyara Bugyal (Uttarkashi)	Joshimath (Chamoli)	Jageshwar (Almora)	Munsiyari (Pithoragarh)		
Control								
Sprouting (%)	71.67	68.33	66.67	65.00	68.33	63.33	67.22	NS
Rooting (%)	38.33	36.67	31.67	35.00	36.67	33.33	35.28	NS
Survival (%)	33.33	31.67	30.00	28.33	28.33	31.67	30.56	NS
IBA 4,000 ppm								
Sprouting (%)	75.00	71.67	70.00	73.33	66.67	68.33	70.83	7.95
Rooting (%)	66.67	61.67	65.00	63.33	58.33	61.67	62.78	NS
Survival (%)	61.67	60.00	58.33	56.67	55.00	60.00	58.61	NS

NS - Non-Significant

12.5.4

Propagation Through Air Layering

Shoots of *T. wallichiana* responded favourably to application of IBA when propagation was attempted through air layering (Table 12.8). Rooting occurred around eight weeks of air layering operation and maximum 66.67 per cent rooting was observed in 16 weeks with application of 5,000 ppm IBA. A mere 11.67 per cent rooting was observed when IBA was not applied. There was no significant difference in plant response with respect to IBA application from 2,000 ppm to 5,000 ppm.

Table 12.8 Effect of IBA Concentration on Air Layering Success of *Taxus wallichiana*

IBA Concentration	Rooting (%)			Root Length (cm)	Survival (%)
	8 Weeks	12 Weeks	16 Weeks	16 Weeks	5 months
Control	0.00	6.67	11.67	3.69	6.67
IBA 1,000 ppm	3.33	13.33	45.00	5.69	31.67
IBA 2,000 ppm	6.67	18.33	63.33	6.98	36.67
IBA 3,000 ppm	6.67	25.00	65.00	6.63	35.00
IBA 4,000 ppm	10.00	25.00	63.33	6.21	38.33
IBA 5,000 ppm	8.33	30.00	66.67	6.24	40.00
$CD_{0.05}$	NS	10.38	12.84	NS	10.57

12.6 *Rhododendron arboreum*

12.6.1 Propagation Through Seeds

Seedling emergence increased with application of GA_3 (Table 12.9). In general, the seedling emergence was in the order: control <150 ppm <300 ppm. Highest 72.75 per cent seedling emergence was observed in seeds from Khirsu treated with 300 ppm GA_3. Survival per cent of 8.50 to 24.75 was recorded from these seeds, implying that only 8.50 to 24.75 per cent seeds led to production of seedlings (Fig. 12.8). Significant differences were found among populations in respect of seedling emergence at 150 ppm GA_3. However, the differences were less than 15 per cent. Difference among populations was not found to be significant from the standpoint of survival.

Table 12.9 Effect of GA_3 Application on Seedling Emergence and Seedling Survival of Different Populations in *Rhododendron arboreum*

Ga_3 Concentration and Seedling Parameter	Populations						Mean	$CD_{0.05}$
	Chakrata (Dehra Dun)	Kaddukhal (Tehri Garhwal)	Khirsu (Pauri Garhwal)	Bhowali (Nainital)	Jageshwar (Almora)	Didihat (Pithoragarh)		
Control								
Emergence (%)	50.25	52.75	57.75	46.75	49.75	52.25	51.58	NS
Survival (%)	10.00	13.75	17.25	8.50	12.00	14.75	12.71	NS
150 ppm								
Emergence (%)	63.25	68.25	69.25	65.50	58.25	66.50	65.17	8.65
Survival (%)	13.25	12.75	16.75	12.75	16.50	20.00	15.33	NS
300 ppm								
Emergence (%)	68.75	70.25	72.75	67.75	60.75	71.75	68.67	NS
Survival (%)	15.00	24.75	17.75	23.75	19.50	12.75	18.92	NS
$CD_{0.05}$ for Emergence	8.24	6.58	9.22	10.93	8.55	7.13	7.69	
$CD_{0.05}$ for Survival	NS	6.49	NS	8.46	NS	NS	8.54	

NS - Non-significant

12.6.2 Propagation Through Cuttings

(a) Branch Cuttings:

Application of auxin in the form of IBA up to 5,000 ppm led to root induction in the branch cuttings of *R. arboreum* which otherwise proved hard to root and did not produce roots without IBA application (Table 12.10). There was no significant difference in sprouting percentage but rooting and survival of the cuttings generally increased as IBA concentration increased. The greatest values of 6.67 per cent rooting (with 4,000 ppm IBA) and 5.83 per cent survival (with 5,000 ppm IBA) were recorded.

Table 12.10 Effect of IBA Concentration on Propagation of *Rhododendron arboreum* Through Branch Cuttings

IBA Concentration	Sprouting (%)	Rooting (%)	Survival (%)
Control	7.50	0.00	0.00
2,000	7.50	0.83	0.00
3,000	8.33	2.50	2.50
4,000	10.83	6.67	5.00
5,000	9.17	5.83	5.83
$CD_{0.05}$	Non-significant	3.27	3.49

Fig. 12.8 Plants of *Rhododendron arboreum* Raised Through Seeds

(b) Root Cuttings: Root cuttings yielded fair response in *R. arboreum*. The values of rooting and survival of root cuttings varied in the range of 21.75 to 38.00 per cent, and 6.50 to 27.25 per cent, respectively (Table 12.11). Differences in response among 0, 2,000, and 4,000 ppm IBA concentrations were generally significant for rooting as well as survival per cent. Variations amongst populations too were not significant in most of the IBA concentrations.

Table 12.11 Effect of IBA Application on Propagation of Different Populations in *Rhododendron arboreum* through Root Cuttings

GA_3 Concentration and Seedling Parameter	Populations: Chakrata (Dehra Dun)	Kaddukhal (Tehri Garhwal)	Khirsu (Pauri Garhwal)	Bhowali (Nainital)	Jageshwar (Almora)	Didihat (Pithoragarh)	Mean	$CD_{0.05}$
Control								
Rooting (%)	30.75	33.25	38.00	28.50	30.25	31.75	32.08	NS
Survival (%)	21.00	23.75	27.25	18.50	22.00	24.75	22.88	NS
2,000 ppm								
Rooting (%)	25.50	31.25	33.50	31.25	21.75	29.00	28.71	4.99
Survival (%)	13.25	12.75	16.75	12.75	6.50	10.00	12.00	NS
4,000 ppm								
Rooting (%)	29.50	30.00	33.75	32.50	27.50	31.75	30.83	NS
Survival (%)	15.00	14.75	17.75	13.75	9.50	12.75	13.92	NS
$CD_{0.05}$ for Rooting	3.22	NS	NS	9.27	NS	NS	-	-
$CD_{0.05}$ for Survival	3.27	NS	NS	4.01	NS	NS	-	-

NS - Non-significant

12.6.3

Propagation Through Air Layering

Table 12.12 shows the behaviour of *R. arboreum* when shoots are subjected to air layering in different months using different types and concentrations of auxin. Comparison of months indicated that May was the most favourable month for carrying out air layering, followed by April and March in decreasing order with mean rooting percentages of 38.57, 24.05, and 11.43, respectively over a period of 16 weeks from air layering operation. Root length also followed similar trend.

Comparison within a month revealed that IBA proved most effective followed by IAA and NAA in diminishing order while no rooting was observed without application of any of the three types of auxins. Overall highest rooting percentage (73.33) was registered for 4,000 ppm IBA in the month of May, followed by 56.67 per cent rooting obtained with 2,000 ppm IBA in the same month (Fig. 12.9).

Table 12.12 Effect of Month, Auxin Type, and Auxin Concentration on Air Layering Success in *Rhododendron arboreum*

Month of Air Layering, Auxin Type and Concentration	Rooting (%)			Root Length (cm)
	8 Weeks	12 Weeks	16 Weeks	
March				
Control	0.00	0.00	0.00	0.00
IBA 2,000 ppm	0.00	1.67	18.33	3.05
IBA 4,000 ppm	0.00	6.67	21.67	3.98
IAA 2,000 ppm	0.00	1.67	11.67	2.93
IAA 4,000 ppm	0.00	1.67	16.67	3.20
NAA 2,000 ppm	0.00	0.00	3.33	1.09
NAA 4,000 ppm	0.00	5.00	8.33	3.01
Mean	**0.00**	**2.38**	**11.43**	**2.47**
April				
Control	0.00	0.00	1.67	2.27
IBA 2,000 ppm	0.00	15.00	31.67	4.87
IBA 4,000 ppm	3.33	21.67	46.67	5.29
IAA 2,000 ppm	0.00	6.67	18.33	1.89
IAA 4,000 ppm	1.67	5.00	28.33	3.12
NAA 2,000 ppm	0.00	1.67	8.33	2.68
NAA 4,000 ppm	0.00	6.67	33.33	4.25
Mean	**0.71**	**8.10**	**24.05**	**3.35**
May				
Control	0.00	0.00	3.33	2.54
IBA 2,000 ppm	0.00	21.67	56.67	3.64
IBA 4,000 ppm	8.33	33.33	73.33	5.91
IAA 2,000 ppm	0.00	10.00	31.67	3.25
IAA 4,000 ppm	3.33	13.33	48.33	4.13
NAA 2,000 ppm	0.00	5.00	15.00	2.54
NAA 4,000 ppm	0.00	16.67	41.67	3.55
Mean	**1.67**	**14.29**	**38.57**	**3.89**
$CD_{0.05}$ for Month	0.90	3.51	9.32	1.11
$CD_{0.05}$ for Month x Auxin Type x Auxin Concentration	2.68	9.25	11.73	2.89

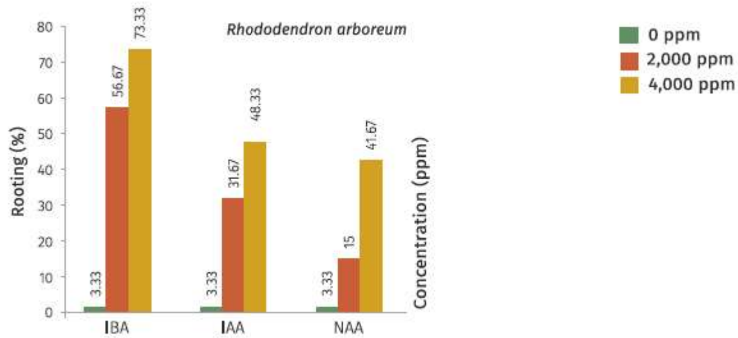

Fig. 12.9 Rooting After Air Layering of *Rhododendron arboreum* in the Month of May with IBA, IAA, and NAA at Different Concentrations

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The findings of experiment on air layering at three populations in the State are summarised in Table 12.13. Air layering yielded negligible success when it was not supported by exogenous IBA application. Application of 2,000 ppm and 4,000 ppm IBA powder during air layering resulted in root induction; the latter concentration produced better results as judged from rooting and plant survival at all the three sites. Highest 75 per cent rooting and 61.67 per cent survival of rooted shoots were observed in Chakrata when 4,000 ppm IBA was used. Differences across sites were significant and the rooting ranged from 48.33 to 75 per cent with 4,000 ppm IBA concentration and 40 to 58.33 per cent with 2,000 ppm IBA. Survival was more than 33.33 per cent for all the IBA concentrations.

Table 12.13 Effect of IBA Concentration on Air Layering Success in Different Populations of *Rhododendron arboreum*

IBA Concentration	Places of Air Layering			Mean	$CD_{0.05}$
	Chakrata (Dehra Dun)	Kaddukhal (Tehri Garhwal)	Bhowali (Nainital)		
Control					
Rooting (%)	1.67	0.00	0.00	0.56	0.98
Survival (%)	0.00	0.00	0.00	0.00	Non-significant
2,000 ppm					
Rooting (%)	58.33	40.00	48.33	48.89	Non-significant
Survival (%)	50.00	33.33	35.00	39.44	10.08
4,000 ppm					
Rooting (%)	75.00	48.33	55.00	59.44	11.68
Survival (%)	61.67	40.00	38.33	46.67	13.56

12.7 *Myrica esculenta*

12.7.1 Propagation Through Seeds

The effect of scarification on the seeds of *M. esculenta* is presented in Table 12.14. Treatment of seeds with concentrated H_2SO_4 for two minutes resulted in 40 per cent seedling emergence (Fig. 12.10), and 36.50 per cent survival. Scarification of radicle end of the seeds with sand paper followed by soaking in water was less effective while control seeds recorded the lowest seedling emergence (18.50 per cent) and survival (17.00 per cent).

Table 12.14 Seedling Emergence and Survival in *Myrica esculenta* in Response to Scarification Treatments of Seeds

Scarification Treatment	Seedling Emergence (%)	Survival (%)
Control	18.50	17.00
Soaking in water for 24 h at room temperature	23.00	19.50
Scarification of radicle end of the seeds with sandpaper followed by soaking in water for 24 h at room temperature	26.00	24.50
Scarification of radicle end of the seeds with sandpaper, followed by soaking in cooling warm water 24 h at room temperature	28.50	26.00
Concentrated H_2SO_4 treatment for two minutes	40.00	36.50
$CD_{0.05}$	5.60	6.88

Fig. 12.10 Seedling Emergence from *Myrica esculenta* After Pretreatment with Sulphuric Acid

12.7.2
Propagation Through Cuttings

Branch cuttings of *M. esculenta* were subjected to various auxin and supplement treatments. When planted in mist chamber, low rooting was observed; the highest rooting (18.33 per cent) and survival (13.33 per cent) were observed in NAA 2,000 ppm + 5 per cent Captan + 5 per cent Sucrose (Table 12.15, Fig. 12.11).

Table 12.15 Response of Branch Cuttings of *Myrica esculenta* to Auxin and Supplements

Treatment	Sprouting (%)	Rooting (%)	Survival (%)
Control	16.67	0.00	0.00
IBA 2,000 ppm	21.67	13.33	6.67
IBA 4,000 ppm	20.00	10.00	3.33
NAA 2,000 ppm	23.33	13.33	5.00
NAA 4,000 ppm	23.33	16.67	8.33
NAA 2,000 ppm + 5% Captan + 5% Sucrose	28.33	18.33	13.33
NAA 4,000 ppm + 5% Captan + 5% Sucrose	26.67	15.00	5.00
$CD_{0.05}$	Non-significant	11.21	9.94

12.8
Cinnamomum tamala

12.8.1
Propagation Through Seeds

The data pertaining to seedling emergence from the seeds extracted by various depulping treatments is shown in Table 12.16. Seeds depulped prior to sowing exhibited greater seedling emergence and survival in comparison with seeds not depulped. Depulping of seeds with hand or cow dung proved better registering 74.44 to 78.33 per cent seedling emergence, and 67.22 to 68.89 per cent survival (Fig. 12.11). Depulping by soaking in water was less successful.

Fig. 12.11 Plants of *Cinnamomum tamala* Raised Through Seeds

Table 12.16 Effect of Method of Depulping of Seeds on Seedling Emergence and Survival in *Cinnamomum tamala*

Method of Depulping	Seedling Emergence (%)	Survival (%)
Control (seeds with pulp)	8.33	7.22
Depulped seeds (depulped with hand)	78.33	67.22
Depulped seeds (depulped with water)	40.56	33.89
Depulped seeds (depulped with cow dung)	74.44	68.89
$CD_{0.05}$	11.15	13.64

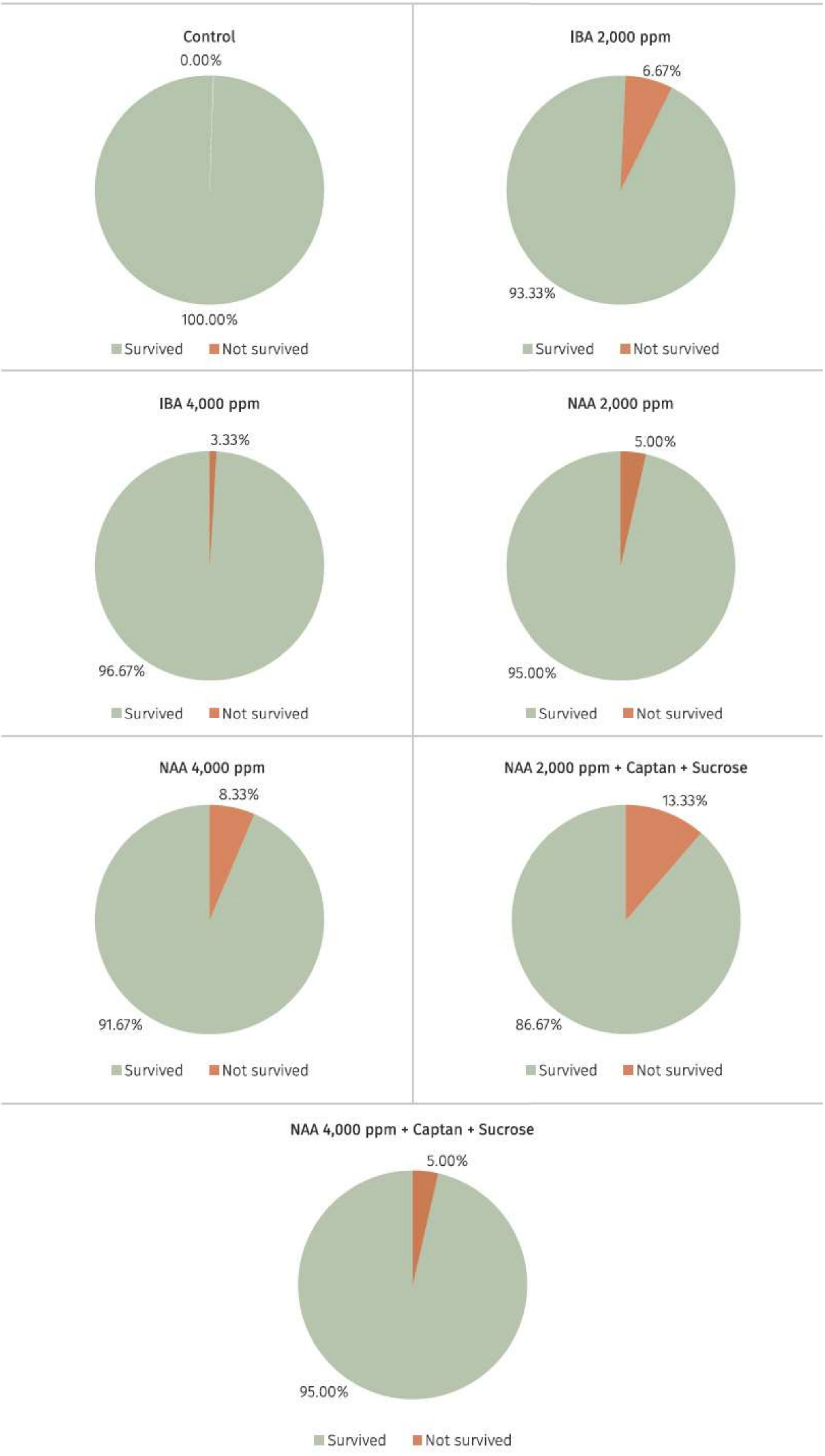

Fig. 12.12 Percentage Survival of Branch Cuttings of *Myrica esculenta* in Response to Various Concentrations and Types of Auxin and Supplements

12.8.2

Propagation Through Cuttings

Table 12.17 shows data about effect of IBA concentration on propagation of *C. tamala* through cuttings. Despite sprouting of cuttings, rooting, and development of plants failed to occur without application of IBA. IBA concentrations of 3,000 to 5,000 ppm were more effective than control or 2,000 ppm in root induction and plant production. Greatest rooting and cutting survival (32 and 26 per cent, respectively) were achieved with 5,000 ppm IBA.

Table 12.17 Effect of IBA Concentration on Propagation of *Cinnamomum tamala* Through Cuttings

IBA Concentration (ppm)	Sprouting (%)	Rooting (%)	Survival (%)
Control	38.67	0.00	0.00
2,000	46.67	17.33	14.67
3,000	50.67	28.00	25.33
4,000	48.00	22.67	18.67
5,000	40.00	32.00	26.00
$CD_{0.05}$	Non-significant	9.45	8.34

The effect of IBA concentrations and variation in source populations on propagation of C. tamala through cuttings is reflected in Table 12.18. The data indicate that IBA 4,000 ppm generally produced slight increase in sprouting, rooting, and survival of cuttings. However, different populations had negligible effect on rooting and survival of cuttings. Greatest survival (21.67 per cent) was observed in cuttings collected from Bhowali (Nainital) and subjected to 4,000 ppm IBA.

Table 12.18 Performance of Source Populations and Effect of Different IBA Concentrations on Propagation of *Cinnamomum tamala* through Cuttings

IBA Concentration	Chakrata (Dehra Dun)	Bhowali (Nainital)	Jageshwar (Almora)	Munsiyari (Pithoragarh)	Mean	$CD_{0.05}$
Control						
Sprouting	38.67	33.67	47.67	43.33	40.84	NS
Rooting	18.33	15.00	28.33	23.00	21.17	NS
Survival	13.33	10.67	20.33	14.33	14.67	NS
IBA 4,000 ppm						
Sprouting	35.00	51.00	49.67	53.33	47.25	13.54
Rooting	16.67	33.67	21.00	24.67	24.00	NS
Survival	16.00	21.67	15.33	14.67	16.92	NS

NS - Non-significant

Fig. 12.13 *Cinnamomum tamala* Tree Showing Air Layered Shoot

12.8.3

Propagation Through Air Layering

The effect of month of air layering and IBA concentration on the success of air layering in *C. tamala* are presented in Table 12.19. The data on rooting success at 16 weeks after air layering suggest that May and July were the most successful months for carrying out air layering procedure of vegetative propagation. The mean rooting success was 54.07 to 56.30 per cent during this period. However, interaction data revealed that air layering during July with 4,000 ppm IBA recorded the highest i.e., 86.67 per cent rooting which was closely followed by 82.22 per cent rooting recorded for air layering during May with 4,000 ppm IBA (Fig. 12.13 and 12.14).

Treatments	Rooting (%) 8 Weeks	Rooting (%) 12 Weeks	Rooting (%) 16 Weeks	Root Length (cm)	Survival (%) 5 months
March					
Control	0.00	0.00	4.44	4.11	2.22
IBA 2,000 ppm	0.00	24.44	37.78	4.52	28.89
IBA 4,000 ppm	2.22	33.33	60.00	4.69	48.89
Mean	**0.74**	**19.26**	**34.07**	**4.44**	-
May					
Control	0.00	2.22	6.67	4.97	0.00
IBA 2,000 ppm	24.44	51.11	73.33	5.36	8.89
IBA 4,000 ppm	26.67	46.67	82.22	5.91	22.22
Mean	**17.04**	**33.33**	**54.07**	**5.41**	-
July					
Control	2.22	8.89	15.56	4.87	8.89
IBA 2,000 ppm	4.44	71.11	66.67	5.09	44.44
IBA 4,000 ppm	6.67	73.33	86.67	5.32	57.78
Mean	**4.44**	**51.11**	**56.30**	**5.09**	-
September					
Control	4.44	6.67	11.11	4.09	6.67
IBA 2,000 ppm	24.44	51.11	62.22	5.99	46.67
IBA 4,000 ppm	40.00	42.22	73.33	5.72	51.11
Mean	**22.96**	**33.33**	**48.89**	**5.27**	-
November					
Control	17.78	4.44	11.11	3.85	0.00
IBA 2,000 ppm	13.33	26.67	42.22	4.21	8.89
IBA 4,000 ppm	20.00	44.44	48.89	4.18	22.22
Mean	**17.04**	**25.19**	**34.07**	**4.20**	-
$CD_{0.05}$ for Month	7.68	11.97	12.09	NS	-
$CD_{0.05}$ for Month x IBA Concentration	9.07	10.22	11.53	NS	-

Table 12.19 Effect of Month and IBA Concentration on Air Layering Success in *Cinnamomum tamala*

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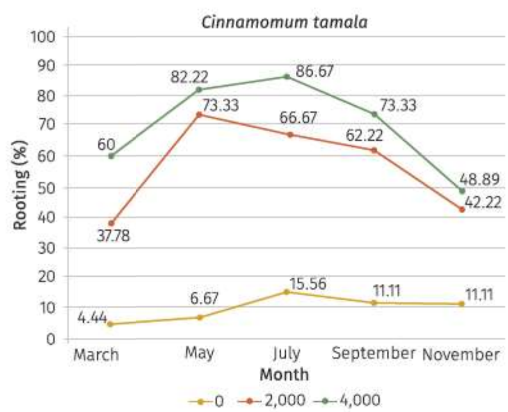

Fig. 12.14 Rooting Percentage of *Cinnamomum tamala* After Air Layering on Treatment with Three Different IBA Concentrations in Five Different Months

12.9 *Diploknema butyracea*

12.9.1 Propagation Through Seeds

Propagation through seeds was found to be very simple and easy and the method yielded high rate of success without any pre-sowing treatment or special nursery operation. Seedling survival was found to be 85-90 per cent (Fig. 12.15).

Fig. 12.15 Plants of *Diploknema butyracea* Raised from Seeds in the Root Trainers

12.9.2

Propagation through cuttings

Juvenile cuttings recorded 66.67 per cent rooting against 2.50 per cent rooting in mature cuttings (Table 12.20). Mature cuttings gave a very poor survival of 1.67 per cent while 60.83 per cent survival was recorded for juvenile cuttings (Fig. 12.16).

Table 12.20 Effect of Maturity Stage of Mother Tree on Propagation of *Diploknema butyracea* through Cuttings

Maturity Stage	Sprouting (%)	Rooting (%)	Survival (%)
Juvenile	79.17	66.67	60.83
Mature	6.67	2.50	1.67
$CD_{0.5\%}$	4.65	3.58	4.04

Fig. 12.16 Rooted Cuttings of *Diploknema butyracea* Ready for Planting in Container

12.9.3

Propagation Through Air Layering

Without IBA application, rooting was 15 to 18 per cent in FRI-wire technique and conventional technique of air layering. However, rooting increased with application of IBA during air layering by both the methods. When IBA was applied at 2,000 ppm concentration, the conventional air layering technique yielded 33.33 per cent rooting which was significantly less than rooting percentage of 48.33 in FRI-wire technique (Table 12.21; Fig. 12.17 and 12.18). Survival of plants was also in order of rooting percentage.