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Development and Characterization of UGMS markers for Genetic Diversity Analysis in Rhododendron arboreum

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ABSTRACT

Rhododendron arboreum is an ecologically important species growing in Himalayan regions. It is widely popular due to its medicinal potential, edible, sacred & economic value, however very little is known about its genetic diversity. The development and characterization of UGMS markers will provide a major impact on genetic diversity analysis. In present study, the development of EST-SSR markers were design by using some conserved genes in the form of Expressed Sequence Tags (ESTs) of species Rhododendron catawbiense from the NCBI site and used to shows cross-species transferability in genomic DNA of Rhododendron arboreum. Total 7, 92 (615 singletons and 177 contigs) unigenes were predicted from 1,241 publicly available EST database from NCBI (http://www.ncbi.nlm.nih.gov/) site in R.catawbiense by clustering of 2 – 33 random EST sequences. From 50 Unigenes, 36 UGMS primers were designed for the cross amplification approach and six pairs of microsatellite primers were produced clear PCR amplification. The cross related species/genera transferability rate was 16.66% from *R.catawbiense* to *R.arboreum. The polymorphism information content (PIC) ranged from 0.2756* to 0.9212 *with an average of* 0.5765.*The average observed heterozygosity (H_o) was 0.8666 i.e. greater than average expected heterozygosity (H_e) was 0.6792.* The genetic diversity was found with an average of 0.6222 within population of R.arboreum and genetic diversity found with an average of 0.3436 among the population.*This study revealed the insight of abundance & distribution of microsatellite in the expressed region of the Rhododendron arboreum genome.*

Key words: Dendrogram, EST-SSRs, Genetic diversity, Rhododendron arboreum, UGMS.

I. INTRODUCTION

The genus *Rhododendron* belongs to family Ericaceae. The word *Rhododendron* is derived from two Greek words rhodon (rose) & Dendron (tree) meaning Rose tree (Hora, 1981). The genus with attractive & beautiful flowers is represented by 1000 species in the world. They are mostly distributed at higher elevations in the sino-himalayan region with maximum concentrations in western china. In India, the species are mostly confined to the Himalayan region particularly in eastern Himalaya. *Rhododendrons* are mostly used by the local inhabitants of Indo-Himalayan regions due to its aesthetic & sacred values with some medicinal values also. Its anti-inflammatory & hepatoprotective activities are due to some neutraceuticals compounds such as Flavonoids,

Saponins & Phyto-phenols present in it. With the shrinking of green cover almost everywhere, the *Rhododendrons* are also experiencing the impact of disturbed ecological systems. The major threats to *Rhododendrons* are deforestation and unsustainable extraction for firewood and incense by local people. Due to the presence of polyphenols and flavonoids in *Rhododendrons* make it excellent firewood that burns even under wet conditions (Sastry & Hajra, 2010).

Global efforts are being made to conserve the phytodiversity especially of rare, endangered & threatened species (Rodger & Panwar, 1998), which are known to be important component of biodiversity. Due to human interference the natural population of *Rhododendrons* in the entire Himalayan region is gradually diminishing. A set of *Rhododendrons* which are classified as rare/endangered may be wiped out from the biota in the near future, if proper conservation measures are not carried out. Genetic markers are essential tools for the quick detection & characterization of genetic variation in the population. In present study the use of EST-SSR marker were developed for the analysis of genetic diversity in Rhododendron arboreum species. EST-SSRs are derived from the transcripts, where in flanking regions are expected to be more conserved and show homology with the related genera/species. Thus, they tend to show higher level of transferability in cross amplification and possibility of the markers (Varshney et al.,2005). EST-SSRs are expected to be more transferable to closely related genera since they are anchored within more conserved transcribed regions compared to genomic SSRs (Cordeiro et al., 2001). However polymorphism level of EST-SSR markers has beeen lower than geneomic SSRs (Barbara et al., 2007) but EST-SSRs also have a higher probability of being functionally associated with differences in gene expression than genomic SSRs (Guo et al., 1996). It is estimated that 2-5 % of all plant derived ESTs have SSRs (Kantety et al., 2002). Also, one of the most important benefits of using EST based SSRs markers are transferability of these markers among species, because they are from more conserved regions of the genome (Ellis et al., 2007). Molecular tools can play a valuable role for investigating the pattern of genetic diversity in these species, and clarifying demographic and ecological issues early in species management in order to plan long-term conservation or restoration programs (Lande.R., 1988).There is a need to develop the EST-SSR markers related to *Rhododendron arboreum to* conserve such type of great biodiversity from human interference in their ecological niche and to understand its present status.

Research Objectives

❖ To collect the sample & targeting various population of *Rhododendron arboreum* from western Himalayan region.

❖ To study & analysis of various target population of western Himalayan region for their Genetic Diversity by UGMS markers.

II. MATERIALS AND METHODS

2.1. Sample collection and Study Sites

The study sites are located in five different parts of Himachal Pradesh in India. Young, disease free and fresh leaves of *Rhododendron arboreum* from western Himalayan region of Himachal Pradesh were taken as a sample from 47 individual trees. The samples were collected and transported to the laboratory for genomic DNA extraction.

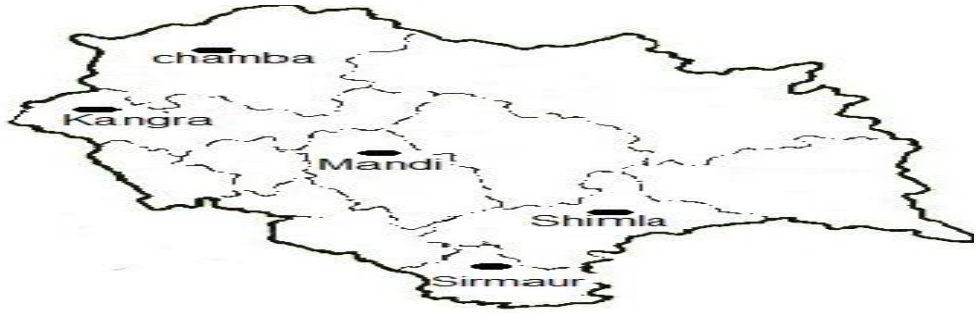

Fig-1: Different locations of Sample collection from 5-different parts of Himachal Pradesh

2.2. DNA isolation

Genomic DNA was extracted from young leaf tissues of each genotype using CTAB method given by Doyle and Doyle (1990). The quality and quantity of DNA were estimated on 0.8% agarose gel and checked by Nano drop 2000 spectrophotometer.

2.3. EST data mining, Unigene prediction and SSR Detection

A total of 1,241 FASTA formatted EST sequences in *Rhododendron catawbiense* were retrieved on March 24, 2012 from the National Centre for Biotechnology Information (NCBI;http://www.ncbi.nlm.nih.gov/entrez) for subsequent data mining. This dataset was scanned and assembled using SeqMan DNA Star lasergene version 10.0 (DNASTAR Inc, Madison, WI) and predicted potential unigenes that contained contigs and singletons from all the EST sequences with parameters (match size: 5, minimum match percentage: 80, match spacing: 150, gap penalty: 0.00, gap length penalty: 0.70, maximum mismatch bases: 15). Further, gaps in the aligned sequences due to limited dataset were removed on the basis of probability function of nucleotide occurring at the particular position using Gene runner. All the unigenes were subsequently searched individually for the presence of SSRs with help of Repeat masker (http://www.repeatmasker.org/cgi-bin/WEBRepeatMasker) and SSRs with a minimum length of ≥ 18 bp (di & tri) and ≥ 15 bp (tetra, penta & hexa) were masked. These parameters were chosen to identify SSRs with high polymorphic rate and these assemblies were used as data on Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/) primer design input site for the designing of primers. The primers were design with following criteria:- (I) nucleotide length of 18 – 22 base pairs, (II) a Tm value of 50°C to 60°C, (III) the 3' end base with a G or C, preferably and (IV) an amplified fragment size of 100 – 350 bp. The formation of secondary structure and primer dimmers were critically monitored to get success of the primers.

The names of the primers were prefixed as RUGMS (*Rhododendron* Unigene derived microsatellite) markers as the source is from *Rhododendron catawbiense* Unigene database.

2.4. PCR amplification

PCR reactions is carried out in a total of 20 µl volume containing 25 ng template DNA, 2.0 µl of each forward and reverse primer, 4.0 µl of 100mM of dNTPs (Himedia), 2.5 µl of Taq DNA polymerase (Himedia), 2.0 µl of 10X PCR buffer (Himedia) and 2.0 µl of 2.5 mM of MgCl2 & 2.5 µl of autoclaved distilled water in each PCR tubes. The amplified products were stored at 4°C. The amplification products of PCR were mixed with denaturing dye (98% Formamide) and run on 7.8% PAGE in 1x TBE buffer. The gels photographs are taken by placing gel slides on X-ray illuminator view box.

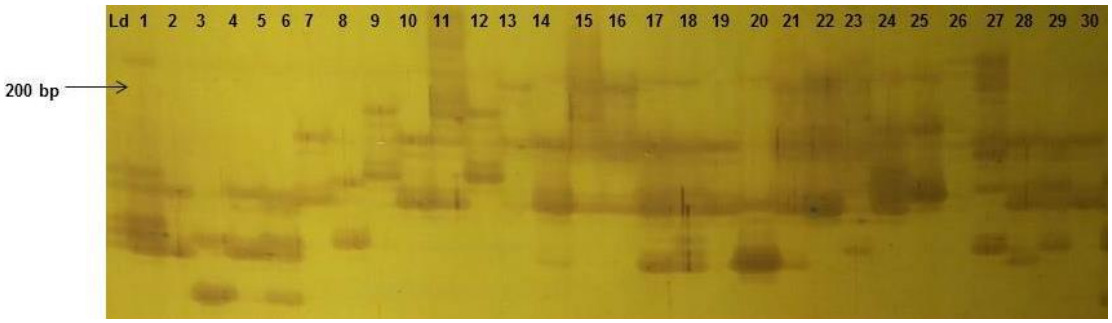

Fig. 2: Amplification profile generated with primer RUGMS-204. Lanes 1- 30 represent sampled individuals of *R. arboreum* as presented; Ld: 100 bp DNA ladder as size standard (Bangalore GeneiTM).

2.5. Data analysis

The DNA fragments size were estimated by comparing with DNA marker (100bp DNA ladder, Himedia) run on the same PAGE gel and DNA fragment size estimated. The visualized intense bands were scored and recorded for statistical analysis. Polymorphism information content (PIC), Number of amplified bands, allele frequencies and expected (H_e) and observed heterozygosity (H_o) was calculated from observed genotypes. The POPGENE software version 1.32 (Yeh et al.,1997) was employed to determine different parameters which include number of polymorphic loci and their percentage, observed number of loci, effective number of alleles (N_e) was estimated using the formula *Ne = Mixi2*, where, *xi* is the frequency of the /th allele for each locus (Crow and Kimura, 1964).

III. RESULTS

3.1. ESTs/Unigenes data set

A total 7, 92 (615 singletons and 177 contigs) unigenes were predicted from 1,241 publicly available EST database *from* NCBI (http://www.ncbi.nlm.nih.gov/) site in *Rhododendron catawbiense* by clustering of 2 – 33 random EST sequences. Non-redundant (NR) sequence data set represented~184.41 kb expressed genome of *Rhododendron catawbiense.*

3.2. Abundance and distribution of SSRs

All 7, 92 potential unigenes were searched for the presence of microsatellites. A total of 177 (22.3%) unigenes containing 50 SSRs with motif length ranging from 2 to 6 bp were identified. One sequence contained six SSRs and three sequences contained two SSRs each. Six SSRs were of compound types. One SSR was detected for every 27.17 kb of the EST sequences. Further analysis of SSR containing Unigene sequence data revealed that majority of them (72%) were perfect repeat and/or class I (≥ 20 nucleotides; nts length). However, remaining 28% (comprising of 17.9% di repeats and 3.3% each of tri repeats, tetra and penta repeats) were found to be of class II types (≥ 12 nts and < 20 nts length). In present study, only class I UGMS markers were selected for primer designing. All the repeats type with their distribution and frequencies were shown in below mention table with their frequencies.

Table no: 1 Showing different types of SSR predictions in 50 unigenes of *Rhododendron catawbiense* & its types of repeats are following:-

S.No.	Type of repeats	S.No. of Repeat Types	Repeats	No. of each Repeats	Total
1	Di-Repeats	1	TG/CA	1	17
		2	GA/TC	16	
2	Tri-Repeats	1	CCA/TGG	6	23
		2	CTG/CAG	3	
		3	TTC/GAA	8	
		4	CCG/CGG	3	
		5	CAT/ATG	1	
		6	TCC/GGA	1	
		7	CGA/TCG	1	
3	Tetra-Repeats	1	CATA/TATG	1	2
		2	TTTC/GAAA	1	
4	Penta-Repeats	1	TTCTC/GAGAA	2	6
		2	CCCCG/CGGGG	1	
		3	TTTAG/CTAAA	1	
		4	GCATG/CGTAC	1	
		5	CAAAA/TTTTG	1	
5	Hexa-Repeats	1	GGAGAA/TTCTCC	1	2
		2	TAAAAA/TTTTTA	1	

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Table no. 2: Marker validation and features of new 6 UGMS markers of *Rhododendron arboretum.* Abbreviations- PIC-Polymorphic information content, H_o-Observed heterozygosity, H_e-Expected Heterozygosity

Locus name	Primer sequence	Repeat motif	Annealing temperature	No. of alleles	Heterozygosity		PIC	Approximate size range(bp)
					H_o	H_e		
RUGMS-619	F5'- CCTTGAATGGAGGTGGAGAA R5'- CCAACCAGCAAATAGGCATT	$(CCA)_{20}$	50°C	5	0.8000	0.7109	0.3463	200-300bp
RUGMS-212	F 5'- TGGCTAGCCCAAGCACTAAT R 5'- CCAAATGGAAATGGGTTTTG	$(TTC)_9$	50°C	5	0.9250	0.6572	0.9212	250-300bp
RUGMS-282	F 5'- TCTACTACTGCGCCAAAGCA R 5'- ATTGGTCCCCTTCTGGTGAT	$(GA)_{11}$	50°C	5	0.8500	0.7012	0.5885	300-400bp
RUGMS-320	F 5'- CTCCGTTGTGTTTGGTTGAG R 5'-TTCTCGAAGATAGGGGCAGA	$(TTTAG)_6$	50°C	5	0.8250	0.7050	0.4999	300-400bp
RUGMS-204	F 5'- GATGCTTCAAAACCCCAAAA R 5'- CCGAAGGGTTCATGAAGAAA	$(CCG)_8$	50°C	4	0.9000	0.6338	0.2756	250-300bp
RUGMS-39	F 5'- GAATTGATTGCTTGGGGAAA R 5'- TTCGGGTTTCAACAGCTAGG	$(CTG)_2$	50°C	5	0.9000	0.6672	0.8279	300-500bp

3.3. UGMS primer designation

Of the 177 NR unigenes containing one or more SSRs, 50 (28.24%) were amenable to design flanking oligonucleotide primer pairs. Thirty six UGMS primer pairs (25 from singletons and 9 from clusters) flanking to different repeat motifs could be designed.

3.4. Cross species Marker evaluation and polymorphism detection

Thirty six primer pairs designed in this study from the EST databases of *R. Catawbiense* were used to amplify DNA from a panel of 47 accessions of *R. arboreum*. Of these, 6 (16.66%) primer pairs produced repeatable and reliable amplifications in at least forty seven accessions of *Rhododendron arboreum*, while 30 (83.33%) primer pairs either completely failed or led to weak amplifications and thus were excluded from further analysis. Marker evaluation details are given in table no: 2.

These six amplified primers were used for the scoring & genotyping of the *R.arboreum* samples. These six primers were further used for the assessment of genetic diversity of *R.arboreum* in western Himalayan region of India. PCR products of the expected size were obtained in all the cases except in one UGMS primer (RUGMS-

39) that had amplified larger size additional amplicons in some cases. Multi-locus amplifications were recorded in case of RUGMS-212, RUGMS-282 and RUGMS-39. Over all, amplification success rate was the maximum in case of RUGMS primer pairs containing tri repeats (66.66%), followed by di-repeat (16.66%) and penta-repeat (16.66%). Six polymorphic primer pairs namely RUGMS-212, RUGMS-282, RUGMS-619, RUGMS-204, RUGMS-320 and RUGMS-39 gave amplification in all the tested genotypes irrespective of species and hence can be utilized as universal markers for molecular analysis in *R.arboreum*. However, these markers need to be validated in a larger panel of *Rhododendron* species. Six primer pairs amplified 29 alleles of which 20 (68.96%) were found to be polymorphic. All the UGMS markers identified in the present study remained highly polymorphic. The number of alleles detected in the present case ranged from 4 to 5 alleles per locus. The UGMS markers namely RUGMS-619, RUGMS-212, RUGMS, RUGMS-282 and RUGMS-39 recorded a maximum of 5 alleles and RUGMS-204 with 4 alleles respectively. A high level of polymorphism has been observed at the species level. The expected heterozygosity (H_e) and observed heterozygosity (H_o) ranged from 0.6338 to 0.7109 (with an average of 0.6783) and 0.8000 to 0.9250 (with an average of 0.8666), respectively (Table 4.4). All the UGMS markers showed a significant departure from Hardy-Weinberg equilibrium (HWE) at $P < 0.001$ level. The polymorphism information content (PIC) ranged from 0.2756 to 0.9212 with an average of 0.5765. There was significant difference in the average PIC values was recorded in UGMS locus harboring different repeat types. Average PIC values ranged from 0.4999 (penta repeats) to 0.9212 (tri repeats). However, an average of 0.5885 PIC values were recorded in RUGMS primers with di repeats respectively (Table 4.4). Of the 6 UGMS primer pairs with PIC values ≥ 0.50, 3 (50%) namely RUGMS-619, RUGMS-320 and RUGMS-204 recorded amplification in 47 accessions were identified as informative and thus would be useful in future marker assisted studies in *Rhododendron.* Further, the others 3 (50%) UGMS primer pairs with average PIC values ranged from above 0.50 to 1.0 were identified, namely RUGMS-212, RUGMS-282 and RUGMS-39 recorded amplification in 47 samples which may categorized as very informative primers after their validation in a larger panel of *Rhododendron* accessions. The approximate size of the PCR amplified bands were ranged from 200bp to 500bp. The highest number of genotypes amplified at locus RUGMS-204 and lowest at locus RUGMS-212 and RUGMS- 39.

3.5. Genetic variation in populations

Four parameters were used to assess the genetic variation within populations and they included number of loci with an average alleles per locus, number and percentage of polymorphic loci, actual or observed number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (H_o), expected heterozygosity (H_e). The values of all these variables were shown in above mention table. The 47 samples analyzed from 5 different populations of *R.arboreum,* 5 different alleles were detected at six loci, with an average of 4.5 alleles per locus. The alleles revealed by SSR markers showed a high degree of polymorphism; with as many as 3 primers out of total 6 primers produced 100% polymorphic bands. A total of 177 bands were obtained from 6 SSR primers, of which 66.66% were polymorphic, with an average of 62.76 bands per primer. The PIC values, derived from allelic diversity and frequency among the genotypes, were not uniform for all of the SSR loci tested. The Polymorphism Information Content (PIC) value is also used as a measure of genetic variation. The population

diversity & allelic variability is indicated by PIC. It's value always between 0 and 1. This is use to assess the diversity of a gene or DNA segment in a population,which will throw light on the evolutionary pressure on the allele & the mutation on the locus might have undergone over a period of time. The PIC value for the SSR loci in present study ranged from 0.2756 to 0.9212. The highest PIC value present at locus RUGMS-212 & lowest PIC value present at locus RUGMS-204. The average PIC value was 0.5765, which show moderate level of genetic diversity within the population. The PIC value refers to the markers informativeness for detecting polymorphism within population. The average of the highest polymorphic loci was 6.66% at locus RUGMS-204 and lowest at RUGMS-212 and 39. The PIC value in present study shows that SSR marker is very informative for measuring polymorphism within the population. In each population, the mean of effective number of alleles (Ne) was 3.13 and observed number of alleles (Na) was 4.83 compared within the population. The mean effective number of alleles is the minimum number of alleles required to maintain the current level of heterozygosity in the population if all allele frequencies were equal. Most populations showed a higher number of observed alleles than the effective number of alleles in the table shown below. The mean effective number of alleles is the minimum number of alleles required to maintain the current level of heterozygosity in the population if all allele frequencies were equal. Most populations showed a higher number of observed alleles than the effective number of alleles.

Over all populations of *R. arboreum* the observed heterozygosity (H_o) was highest (0.92) at locus RUGMS-212 followed by (0.90) at RUGMS-204 & RUGMS-39, (0.85) & (0.82) at locus RUGMS-282 & RUGMS-320 & (0.80) at locus RUGMS -619 loci respectively. Expected heterozygosity (H_e) was highest (0.71) at locus RUGMS-619 followed by (0.705) at locus RUGMS-320, (0.701) at locus RUGMS-282, (0.66) at locus RUGMS-39, (0.65) at locus RUGMS-212 & (0.63) at RUGMS-204 loci respectively. According to our results the observed heterozygosity is greater than the expected heterozygosity in each population. Jain et al. (2000) also reported a high level of observed heterozygosity for *R. arboreum.* The high level of observed heterozygosity could be attributable to predominant outcrossing in the species (Jain et al., 2000). This species is thought to be predominantly pollinated by insects, which supports high outcrossing rates as in other insect pollinated plants (Hamrick and Godt, 1996).

The genetic variation among the populations was also investigated by using four parameters, which included average observed (H_o) and expected heterozygosity (H_e), coefficient of genetic simmilarity and dissimilarity, Nei's genetic identity and genetic distance and allele frequency. The frequency distribution of alleles varied over populations. A few alleles were rare and found in some populations with a frequency as low as 0.05 similarly few alleles were restricted to only one or two populations only. Nei's (1978) standard genetic distances and genetic identity between all pairs of populations ranged highest distance from (0.0065) to (0.7697) in population of chamba and mandi respectively at different locus. The observed heterozygosity (H_o) was highest than expected heterozygosity (H_e) in each population. The average observed heterozygosity (H_o) was 0.8666 and average expected heterozygosity (H_e) was 0.6792. According to present results the genetic variation among the population is also less than genetic variation within population. By using jaccard's coeffcient of simmilarity obtained by using distance matrix table formed by NTSYSpc version 2.1 software (Rohlf , 2000), the level of genetic simmilarity and dissimmilarity were calculated. The genetic diversity was found with an average of

0.6222 within population of R.arboreum and genetic diversity found with an average of 0.3436 among the population. The results revealed that high level of gentic diversity was found within the population of *R.arboreum* was due to out-crossing species. The across related species/genera transferabilty rate from *R.catawbiense* to *R.arboreum* was found 16.66% i.e higher than sugarcane (Cordiero et al., 2001) was 15% only and lower than many studies as in robusta coffee (Hendre et al., 2008) was 92% transferability rate. The lower level of transferability show less homology betweeen the related genera. Hence, within populations were showing higher level of genetic diversity than among the population s.

3.6. Cladistic analysis

A dendrogram was constructed by UPGMA clustering based on Jaccard's coefficient of similarity by using distance matrix table formed by NTSYSpc version 2.1 software (Rohlf , 2000) to visualize the relationships among the 5 populations of R.arboreum. The dendrogram shown four clusters in five populations according to simmilarity coefficient obtain by the jaccard distance matrix. The matrix of similarity was analyzed by the unweighted pair group method using the arithmetic average (UPGMA), as suggested by Sneath and Sokal (1973). The clustering of the SSR based genetic simmilarity (GS) was shown in the dendrogram(fig. 4.6) divided into four main clusters. Which present some degree of simmilarity between them. The cluster I have average genetic simmilarity 0.55 and comprises of 4 different genotypes, cluster II have average genetic simmilarity of 0.45 and comprises of 21 different types of genetypes, cluster III have average genetic simmilarity of 0.39 and comprises of 18 different types of genotypes and cluster IV have an average genetic simmilarity of 0.20 and comprises of 4 different types of gentypes, respectively. Cluster analysis showed a significant genetic variation among the genotypes, with average similarity coefficient ranging from 0.20 to 0.55 respectively. The highest values of Nei's genetic distance were found between the populations of chamba (0.0065) and mandi (0.7697) respectively.

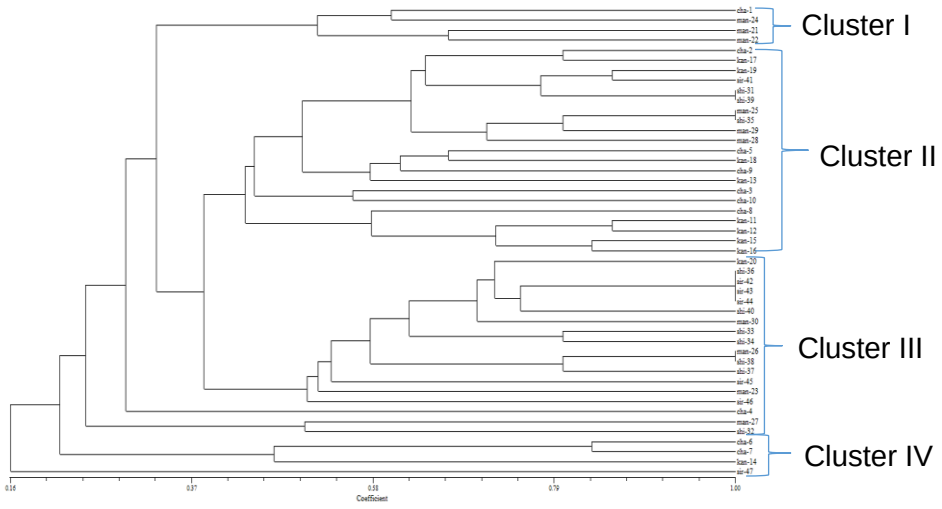

Fig. 2 Dendrogram showing clustering of 47 samples of five populations of *Rhododendron arboreum* **constructed by using UPGMA cluster analysis of** Jaccard's **genetic similarity based on SSR data.**

IV. DISCUSSION

The data collected is used for the assessment of genetic diversity and that is important for the conservation of R.arboreum species in western Himalayan region. EST-SSR has been used to assess the genetic diversity within and among the populations of sugarcane germplasm (Cordeiro et al., 2003), Hordeum vulgare L. (Meszaros et al., 2006), pigeonpea (Datta et al., 2013), Elite Chilli Pepper Lines (Dhaliwal et al., 2013), *Oryza sativa* L. (Garris et al. , 2004), Grapes (Emanuelli et al. , 2013) and many others. In present study, the polymorphic information content (PIC) value ranged from 0.2756 to 0.9212 indicate high level of genetic diversity within population due to geographic isolation of the two species. The highest PIC value present at locus RUGMS-212 & lowest PIC value present at locus RUGMS-204 with an average PIC value of 0.6326. When the PIC value compared to other studies such as in sugarcane by (Singh et al., 2013) the PIC ranged from 0.12 to 0.99, Sesame (Bin et al., 2008) average (PIC) was 0.390, ranged from 0.105 to 0.844, *Camellia Sinensis* (Sharma et al., 2011) average (PIC) was average 0.392, ranged from 0.020 to 0.924 and many others species show low as well as high value than present study. An average PIC value 0.6326 shows that the RUGMS markers used in in the present study are very informative and it also estimate the discriminatory power of the UGMS marker locus to reveal the genetic diversity. According to present study in *R.arboreum* the higher level of genetic diversity was observed within population (H_S = 0.6222) compared to among population (H_T =0.3436). This is in accordance to Jain et al., 2000, which found the genetic diversity of Rhododendron within population (0.40) and between the population (0.18) respectively. When genetic diversity is classified within and among population, selfing species exhibit low levels of genetic diversity within population, but a considerable high genetic diversity among populations (Wang et al., 1996). Limited gene flow between populations of plant species cause genetic differentiation due to increased geographic distance among populations or distant spatial pattern of populations. Alteration and fragmentation of many habitats lead to increased geographic distance which may be the cause of low level of gene flow and high genetic diversity among population (Hou and Lou, 2011).

The conserved genome synteny can facilitate the cross-species transferability among related species/genera. The degree to which synteny can facilitate cross-species analysis of gene function will depend both on the conservation of gene order and contents, as well as on the frequency with which similar traits have a common genetic basis in different species (Tang et al., 2006). The phylogenetically close species will share a higher proportion of microsatellites or other types of markers & show high level of amplification via the PCR process (Parida et al., 2006). In present study, the across related species/genera transferabilty rate from *R.catawbiense* to *R.arboreum* was found 16.66% i.e higher than sugarcane (Cordiero et al., 2001) was 15% only and lower than many studies as in robusta coffee (Hendre et al., 2008) was 92% transferability rate. The lower level of transferability show less homology betweeen the related genera. It show the divergence of the lineage between the R.catawbiense to R.arboreum. The level of synteny between the species depends upon the genomic restrucuring events that occurred since their evolurtionary divergence. Conservation geneticists can identify the cases via the present types of studies in which variation has occur or being lost, investigate the main reasons

behind this type of loss and provide suggestions and recommendations to counter the ultimate effect for their conservation.

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