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ESTIMATION OF GENETIC DIVERSITY IN *TERMINALIA ARJUNA* GENOTYPES REPRESENTS THE DIFFERENT AGROCLIMATIC REGIONS OF INDIA

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A measure of genetic diversity among nine populations of *Terminalia arjuna* collected from different agro-climatic regions was estimated by the fingerprints developed by Random Amplified Polymorphic DNA (RAPD) primers. Out of thirty primers, eleven primers produced total of 78 bands with 7.09 loci per primer and 77.6 percentage of polymorphism.A piece of good polymorphic information content (PIC) was given by the all 11 primers among the populations (mean PIC = 0.23). Higher sum of square (SS=3149.08), variance components (VC= 12.02), variance (partitioned) percentage (V%= 96.75) and fixation index (F_{ST}= 0.326) for sub-populations (states) to total population (germplasm) was observed within the population. The highest polymorphism was estimated in the population collected from Madhya Pradesh (MP) followed by Jharkhand(JH)(P%=89.7) while lowest in the population collected from Odisha (OD) (P%=53.8). The highest allele number (Na), Effective allele number (Ne), gene diversity (GE) and Shannon's information index (I) was estimated for the population belonging to Madhya Pradesh (Na=1.89, Ne= 1.69, GE=0.38, and I=0.55). Maximum and minimum Nei's genetic distance was among the accessions of Assam (AS) –Uttar Pradesh (UP) and Andhra Pradesh (AP) –Madhya Pradesh States (AP-UP= 0.3136 and AP-MP= -0.0086). Unweighted pair group method with arithmetic mean(UPGMA) dendrogram based on Nei's genetic distance of RAPD markers for clustering shows that Assam and Madhya Pradesh accessions are highly diverse and genetically dissimilar whereas Madhya Pradesh-Maharashtra accessions are less diverse and show genetic similarities.

Keywords: AMOVA,Molecular markers,PIC,RAPD,*Terminalia arjuna*

India is one of the countries which is gifted by nature with full of medicinal plants in the world and the second-largest exporter next to China. According to an estimate, approximately 25,000 plants used to make the plant-based formulations and in folk medicine used by the local peoples of India.Market Research Future (MRFR) projects that the Global Herbal Medicine Market will capture a CAGR of 5.88% between 2018 and reach upto 1, 29,689.3 million USD in 2023. The contribution of India in the global herbal market is USD 63 billion (i.e., 0.2%).

Recently, the use of molecular techniques to access the genetic variation and conservation of plant genetic resources in plants is significantly increasing. To understand the function of a specific gene and its action, construction of linkage map, reveal the association of a gene with a trait, molecular marker technology is a suitable approach. Molecular technology plays a critical role to understand the evolution of genetically related groups of organism and species and give information about the variation within and between the species or populations.

Terminalia arjuna [Roxb. Ex DC] Wight & Arnotbelong to the family Combretaceae, distributed throughout the greater part of the Indian peninsula along rivers, streams, ravines and dry watercourses, found in the sub-Himalayan tract. Arjun bark is used as anti-oxidant, antibiotic (Perumal *et al*. 1998) astringent, cooling, aphrodisiac cardiotonic (Karthikeyan *et al*. 2003),fractures, ulcers, spermatorrhea, leucorrhea, diabetes, cough, tumor, excessive perspiration, asthma, inflammation, hypocholesteremia(Miller 1998)and skin disorders (Dwivedi *et al*. 1994, Raghavan *et al.* 2006) also shows anti-mutagenic activity(Scassellati-Sforzolini et al. 1999).

MATERIALAND METHODS

Mapping populations were obtained from All India germplasm of arjun (*Terminalia arjuna*) established at Central Tasar Research and

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Training Institute (CTRTI), Nagri, Ranchi that maintains superior arjun accessions from nine states, viz., Andhra Pradesh, Assam, Chhattisgarh, Jharkhand, Maharashtra, Madhya Pradesh, Orissa, Uttrakhand and Uttar Pradesh altogether representing five agro-climatic zones, viz., Eastern plateau and hill (EPH) regions, Southern plateau and hills (SPH) region, Eastern Himalayan (EH) Region**,** Western Himalayan (WH) region and Central plateau and hills (CPH) region (Table 1).

DNA Isolation: Young fully expanded healthy leaves collected from 140 genotypes were used for extraction and purification of genomic DNA, following CTAB or SDS method with slight modification (Doyle *et al*. 1990; Deshmukh *et al*. 2007). The quantity of the genomic DNA was also determined by Nanodrop spectrophotometer.

PCR reaction for RAPD markers: A reaction mixture was preparedcontained sterile water (molecular grade, ultrapure), all the four deoxynucleotide triphosphates (dNTPs), Taq DNA polymerase with 1X Taq buffer A purchased from Himedia and the RAPD primers from GeNoRmie, Chennai (Table 2). To the master mix was added 50ng template genomic DNA was added for in reaction mixture for RAPD. A negative control without any template DNA was also taken. PCR was established for all the primers with all the genotypes. Amplification of the RAPD primers was carried out using one primer at a time across all sampled genotypes. The electrophoresis was performed at 80-100 volts for 3h and monitored the migration of dye till the end of the plate. The run gel was viewed and its photograph recorded under the Gel Documentation system. The scoring of bands of RAPD markers was made from the recorded gel photograph. The amplified products were scored 1 for the presence and 0 for the absence of bands.

Data analysis

Basic genetic parameters: RAPD markers are dominant markers. At Hardy-Weinberg equilibrium, the relative frequency ($P_{0)}$ of the recessive allele (scored as 0) and the relative frequency (P_1) of the dominant allele (scored as 1) at a locus have been calculated as follows:

$$P_0 = \sqrt{\frac{n0}{N}}$$

Where n_0: the number of samples showing the absence of the band (null allele); N: the total number of sampled genotypes.

Gene Diversity: Often indicated as Nei's heterozygosity or expected heterozygosity. It was calculated by Nei's (1973) information index, using POPGENE version 1.31 (Yeh *et al*. 1997).

Shannon's Information Index: Shannon's information index (*I*), was calculated by the equation given by Shannon and Weaver (Shannon *et al*. 1949). It was calculated by using POPGENE version 1.31(Yeh *et al.* 1997).

Percentage polymorphic loci (PPL): PPL was regardless of allele frequencies of scored markers

$$PPL = \frac{Np}{Np+Nm} \times 100$$

Where Np: the number of polymorphic loci;
Nm: the number of monomorphic loci

Polymorphic information content (PIC): Polymorphic information content (PIC) or average heterozygosity was calculated as per the formula of Roldan- Ruiz *et al.* (2000).

PIC = 2fi (1 - fi),
Where, fi is the frequency of the amplified allele
1 - fi is the frequency of null allele

Observed number of alleles per locus (n_0)

The observed numbers of alleles per locus were calculated by using the following equation

$$n_0 = \sum n / L$$

Where $\sum n$: the total number of observed alleles at all

Table1: Sample collection locations and number of accessions of *T. ajuna.*

S. No.	Site of collection	Name of population	No. of Sample
1	Andhra Pradesh	AP	19
2	Assam	AS	1
3	Chhattisgarh	CG	19
4	Jharkhand	JH	48
5	Maharashtra	MH	33
6	Madhya Pradesh	MP	2
7	Odisha	OD	4
8	Uttar Pradesh	UP	6
9	Uttrakhand	UK	8

Table 2: Compositions of PCR reaction mixture and PCR cycling profiles used for RAPD.

Component	Quantity	PCR parameter	Specification
Total reaction volume (µl)	20	No. of cycles	40
Template DNA (ng)	50	Initial denaturation (°C)	94/5 min
Primer (pM)	20	Denaturation (°C)	94/1 min
dNTP (mM)	5	Annealing (°C)	38/1min
$MgCl_2$ (mM)	2.5	Extension (°C)	72/3 min
10X PCR Buffer	1X	Final extension (°C)	72/7 min
Taq polymerase (U)	1	Soaking temperature(°C)	4
Double distilled (µl) water up to	20		

Table 3. Various estimates for RAPD primers as amplified across 140 *T. arjuna* accessions

Primers	Primer codes	Loci	AF	GD	PIC	RP
RAPD	OPJ20	8	0.68	0.39	0.30	10.68
	OPP02	5	0.57	0.48	0.37	5.34
	OPP03	8	0.62	0.46	0.35	9.14
	OPP08	10	0.72	0.37	0.29	12.41
	OPP10	8	0.76	0.33	0.27	11.37
	OPP12	4	0.98	0.03	0.02	7.88
	OPP15	6	0.87	0.20	0.18	8.08
	OPP17	4	0.85	0.19	0.15	6.83
	OPT09	10	0.77	0.32	0.25	13.57
	OPT18	8	0.70	0.37	0.29	9.00
	OPW01	7	0.95	0.08	0.07	11.93

AF- Allele frequency, GD- Nei's gene diversity, PIC- polymorphic information content.

Table 4: Estimates of various population genetic parameters obtained for *T. arjuna* accessions belonging to different states, employing RAPD primers.

State	Population genetic estimates				
	Polymorphism (%)	Na	Ne	GD	I
RAPD markers					
AP	83.3	1.833	1.575	0.325	0.476
CG	80.8	1.808	1.579	0.324	0.472
JH	89.7	1.897	1.634	0.358	0.523
MH	88.5	1.885	1.688	0.376	0.541
MP	89.7	1.898	1.696	0.381	0.550
OD	53.8	1.539	1.390	0.216	0.315
UK	74.4	1.745	1.590	0.322	0.463
UP	60.3	1.603	1.453	0.252	0.364
Average	77.6	1.776	1.576	0.319	0.463

Na – Observed allele number, Ne- Effective allele number, GD-gene diversity, I- Shannon's information index.

Table 5: Analysis of molecular variance (AMOVA) for genetic variability partitioning within and among populations for sampled *T. arjuna* accessions under RAPD systems.

Source of variation	SS	VC	V%	F_{ST}
RAPD				
Accessions among populations	281.335	0.405	3.264	0.0326
Accessions within population	3149.079	12.019	96.735	

SS - Sum of squares, VC – Variance components, V% - Variation (partitioned) percentage,
F_{ST} – fixation index for sub-populations (states) to total population (germplasm).

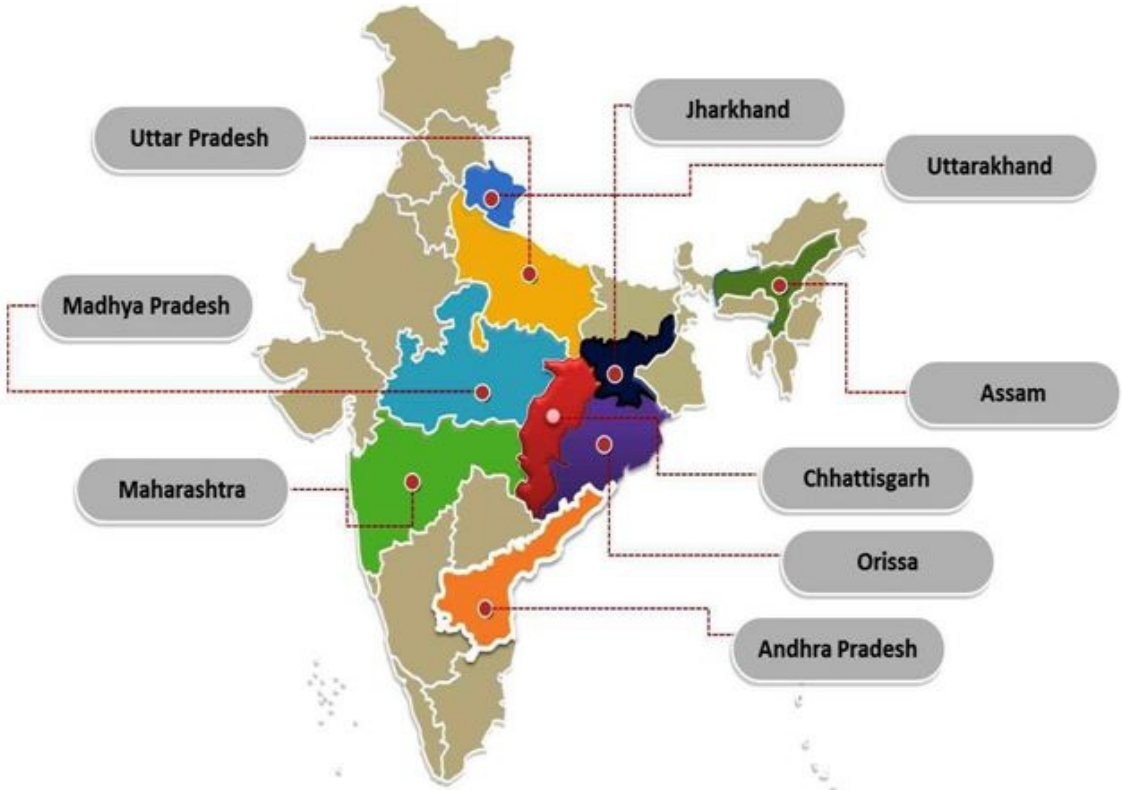

Figure 1: Map showing the state-wise location of the sampled mapping population of *Terminalia arjuna.*

loci; L: the number of observed loci

Average heterozygosity (H_{av}): Average heterozygosity (Hav) was acquired by taking the average of PIC values obtained for all the markers and is calculated as:

$H_{av} = \sum[2fi\,(1\text{-}fi)] / N$

Marker index (MI): MI was obtained by multiplying the average heterozygosity (H_{av}) with MR(Powell *et al*. 1996).

$MI = H_{av} \times MR$

Where H_{av} = Average heterozygosity

MR= Multiplex ratio

Similarity and dissimilarity matrix of Jaccard's coefficient : For the calculation and the representation, the pairwise similarity of the different genotypes Jaccard's similarity coefficient was applied by using the formula

$$GSij = a/a + b + c$$

Where GSij represents the genetic similarity between two individuals 'i' & 'j'. 'a' is the number of polymorphic bands shared by 'I' & 'j'. 'b' is the number of bands present in 'i' and absent in 'j' and 'c' is the number of bands present in 'j' and absent in 'i' (Jaccard, 1908).

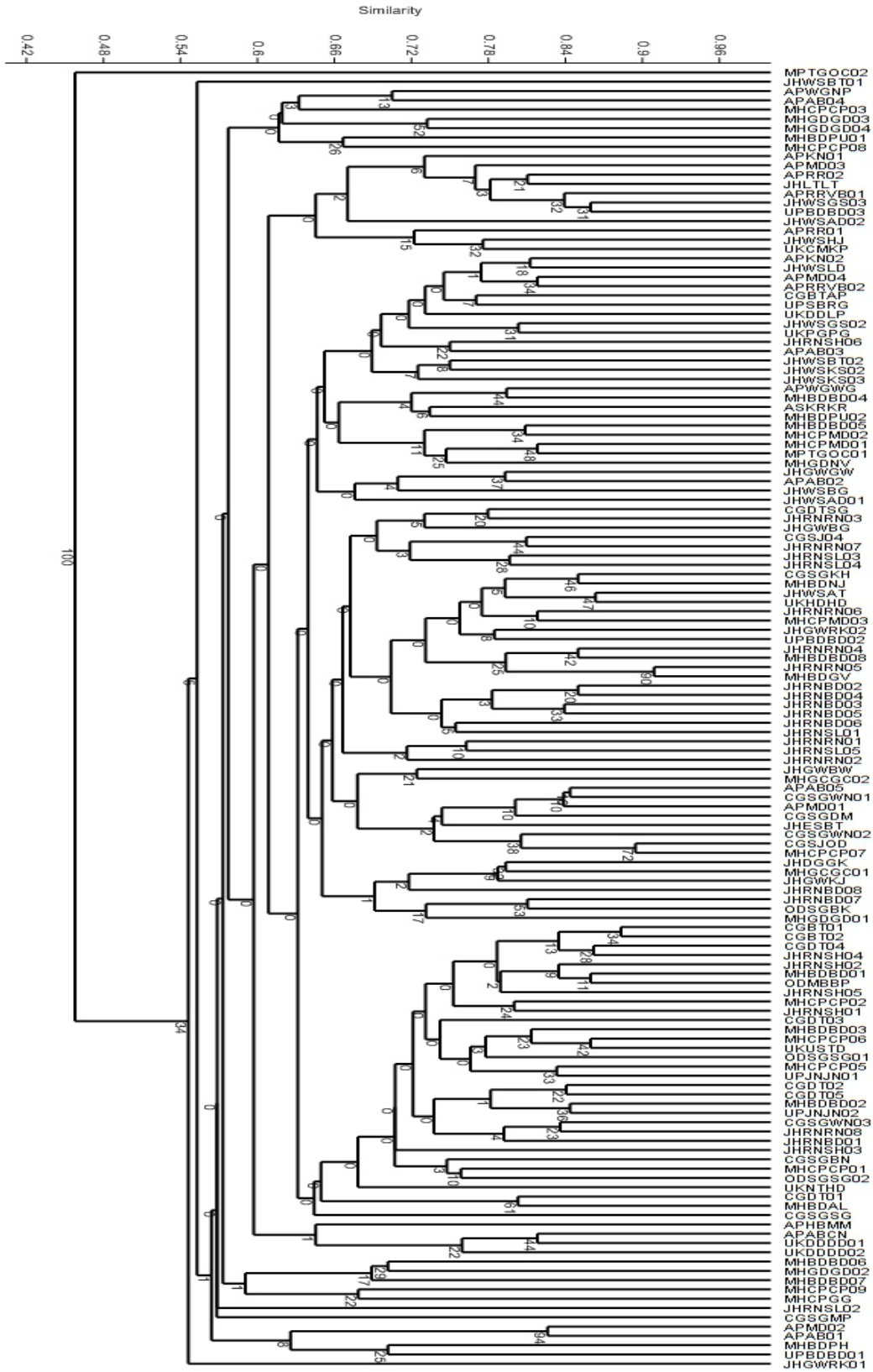

Figure.2. A. UPGMA dendrogram based on Nei's genetic distance of RAPD markers for clustering of *T. arjuna*140 accessions belonging to nine states. Bootstrap values are shown on nodes and coefficient scale below the graph.

Cluster analysis and construction of the phenotypic dendrogram: The Unweighted pair group method of mathematical averages (UPGMA) was used for the graphical demonstration of the cluster. The NTSYS-pc V. 2.11w software was used for the analysis of binary matrix to calculate the similarity values and generate the dendogram.The degree of confidence was evaluated through WINBOOT software developed by Yap and Nelson (Yap *et al.* 1995)) was used for the Bootstrap analysis at the nodes of the dendrogram.

Analysis of molecular variance (AMOVA): To access the partitioning of genetic variation among and within the populations, AMOVA was performed with the help of POPGENE version 1.31 (Yeh *et al*.1997).

RESULTS

140 *Terminalia arjuna* accessions of germplasm bank were subjected to genomic DNA extraction and RAPD marker amplification. A very quality genomic DNA of 140 accessions as visualized on agarose gel was obtained for molecular characterization of the germplasm using parameters of population genetics. Initially, 30 RAPD primers were screened for consistent and reproducible genomic DNA amplification. Only 11 RAPD primers could produce clear, sharp and reproducible bands and were retained for the present investigation. 11 RAPD primers amplified 7438 bands of which 6598, i.e. 88% bands were polymorphic and the amplified band size range was 200-6200bp. The detailed results about the marker systems and consequent estimates of population genetics for the germplasm are given below:

Performance of RAPD primers: 11 RAPD primers altogether detected 78 loci of which 80% loci were polymorphic, thus amplifying 7.09 loci/ primer. Primer OPP08 and OPT09 detected the maximum number of loci, whereas Primer OPP 12 and OPP17, the least number of loci (Table 3).

Allele frequency (AF) and Nei's gene diversity (GD): Among RAPD primers, the highest value for AF was recorded in Primer OPP12 and lowest in Primer OPP02. (Table 3). As for individual primers, the GD value was

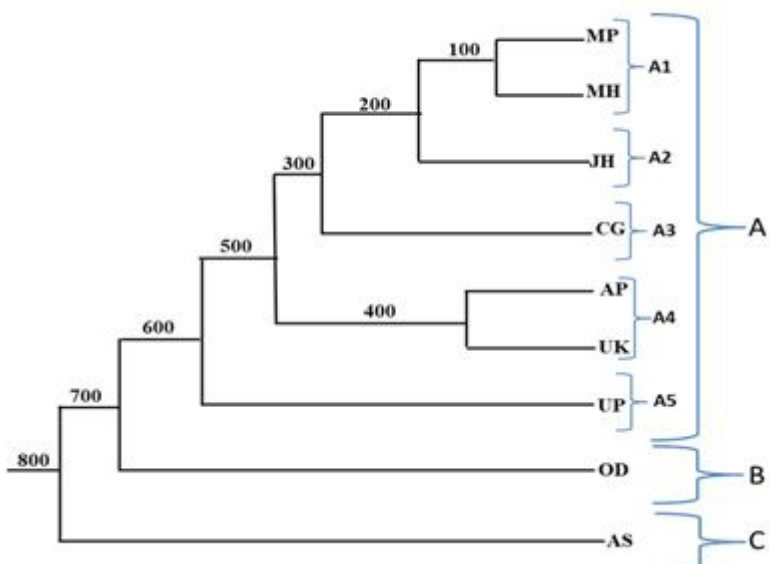

Figure 2. B. UPGMA dendrogram based on Nei's genetic distance of RAPD markers for clustering of *T. arjuna* populations belonging to nine states. Bootstrap values are shown on nodes.

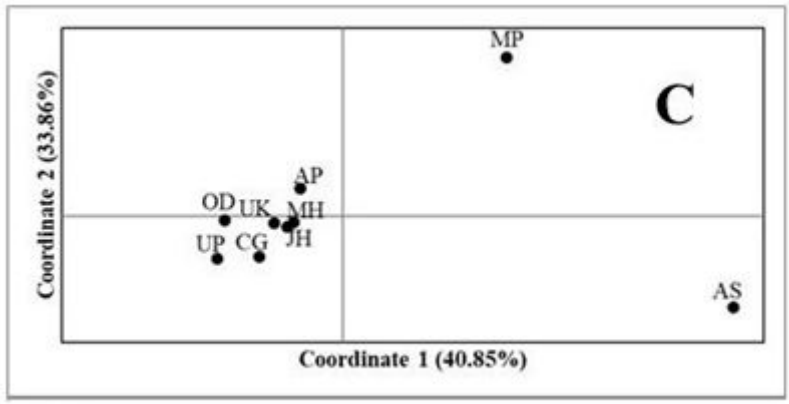

Figure 3:Clustering of accessions belonging to nine states obtained for RAPD markers on two coordinates, employing principal coordinate analysis (PCoA).

obtained the highest in Primer OPP02 and the lowest in Primer OPP12. (Table3).

Polymorphic information content (PIC) and Resolving Power: RAPD Primer OPP02 had the highest value and Primer OPP12 the lowest value for PIC. (Table 4.3.1).In RAPD, RP value was the highest for Primer OPT09 and whereas the lowest RP value estimated for the primer OPP02. Primer OPT09 had a154% higher RP value than Primer OPP02. (Table3).

Population genetic estimates: RAPD markers amplified from the genomic DNA of *T. arjuna* accessions were used for different estimates about thepopulation genetics of the germplasm (Table 4).

Polymorphism (%): With RAPD markers, the accessions from JH and MP states registered maximum polymorphism (%), whereas the accessions from the OD state had the lowest value for polymorphism (%) (Table 4).

Observed allele number (Na) and Effective allele number (Ne): The average value for Na was estimated at 1.78 with RAPD markers. State-wise, the Na value was maximum in accessions from JH and MP and the lowest in accessions from OD and UP with RAPD markers (Table 4).Na values were always higher than Na values and followed the reverse trend to the latter. The average Ne value was estimatedat 1.58. State-wise, Ne value was the highest in MP and the lowest Ne value was recorded in OD state with RAPD markers (Table 4).

Gene diversity (GD), Shannon's information index (I) and Nei's genetic distance and population clustering: The highest GD was recorded in MP state and lowest in the OD state with RAPD markers. The average values of I followed a similar trend like those of GD. Similarly, the highest value for I was obtained in MP state and lowest in OD with RAPD markers.Nei's genetic distance matrices were obtained for RAPD markers that were subjected to UPGMA and PCoA clustering of populations (Table 4).

Genetic distance matrices and RAPD marker supported UPGMA dendrogram: Maximum and minimum Nei's genetic distance was among the accessions of AS-UP and AP-MP states by RAPD markers.RAPD markers assigned accessions to two unequal clusters supported by very high bootstrap value, i.e. 800 or 80%. The first large cluster contained accessions from eight states and the second cluster with accessions of Assam (AS) state only. The large cluster further split into two unequal sub-clusters supported by high bootstrap value, i.e. 700 or 70%. Large sub-

cluster comprised accessions from seven states and the small sub-cluster included accessions from Orissa (OD) state only. The large sub-cluster further bifurcated into two groups supported by high bootstrap value, i.e. 600 or 60%. The large group contained accessions from six states. The small group contained accessions from Uttar Pradesh (UP) only. The large group showed further separation into two sub-groups with adequate bootstrap value, i.e. 500 or 50%. One sub-group contained accessions from four states (MP, MH, JH and CG) and the other sub-group with accessions from two states (AP, UK). Further separation of the cluster into minor groups was not supported by adequate bootstrap value, i.e. <500 or 50% (Fig.2 A, B).

Principal co-ordinate analysis (PCoA) : Nei's genetic distance matrices obtained by RAPD markers were used for clustering by PCoA. The RAPD markers based genetic distance matrix made four clusters, three clusters comprising accessions from a single state, i.e. AS, MP, and OD and the fourth largest cluster with accessions from the remaining six states distributed among two coordinates cumulatively contributing to 75.68% separation (Fig.3).

Germplasm genetic differentiation and Fixation indices: An individual (genotype/ accession) in a population (germplasm) occupies various hierarchies arising from allelic fixation measured by fixation indices or receives an assignment to different genetic groups arising from ancestral genome sharing measured by population structure.

Three fixation indices were computed for the genetic characterization of the germplasm population. Total heterozygosity of the germplasm population, i.e. Ht was 0.41; whereas the average heterozygosity for sub-populations (states), i.e. Hs was 0.29. The depression in the heterozygosity due to migration, i.e. gene flow denoted by Nei's G_{ST} was 0.28 which was equivalent to 28% arising from gene flow (Nm) of 1.24.

Analysis of Molecular Variants (AMOVA) AMOVA was performed to investigate the partitioning of genetic variability within and among populations. In RAPD markers systems, the bulk of the genetic variability was partitioned to the accessions within the population that was to the tune of 96.73 %. On the contrary, the partitioning of genetic variability to accessions among populations was 3.264 %. This indicated very less differentiation of populations that were also confirmed by a very low value of F_{ST}, an index denoting the degree of population differentiation (Table 5).

DISCUSSION

A species survives through various populations, which become discrete in space and time due to the combined influence of intrinsic genetical changes and extrinsic geo-climatic selection forces. The intrinsic genetical changes are perpetually brought by sexual re-combinations, mutations, migrations, inbreeding, admixing, etc. and generate variability and differentiation. The extrinsic geo-climatic selection forces are in fact drivers for adaptation and conservation of specific genetic pattern (s) of the population. Therefore, the dataset for populations needs to be analyzed taking these considerations.

Although the collected genotypes belong to sampled population subset (s), they subscribe membership to various extinct and extant populations of the same species through their constituting diverse loci and consequential alleles, which have been assembled and fixed in the genome due to intrinsic genetical changes and extrinsic geo-climatic selection forces. Thus, the allelic frequencies in the collected genotypes may help detect various hierarchical levels of obscure populations. F statistics introduced by Wright (1965), and subsequently modified to accommodate multiple loci (Nei 1973, Wright 1978), employs allelic frequencies of multiple loci to

resolve hierarchies of populations and introduces F_{ST} as one of the F statistic components for partitioning experimented genetic diversity within and between the populations.

The genetic structure and within and among populations genetic variation is important to estimate the effective population size of an organism for its genetic improvement, management and conservation program.
Our estimate indicates that the germplasm bank harbors adequate Nei's gene diversity. However, the obtained Nei's gene diversity value of *T. arjuna* germplasm bank is incomparable with those published in the literature on two counts. First, the previous investigations include only a very minuscule number of genotypes. Secondly, the previous investigators have not computed Nei's gene diversity values. *T. chebula*, an allied species of *T. arjuna*, has been found to have higher values of Nei's gene diversity (Ranjini,*et al*., 2015). Nevertheless, the observed diversity value in *T. arjuna* is comparable to that of another tropical tree, teak (*Tectona grandis*) (Changtragoon *et al.* 2000; Nicodemus*et al*. 2003; Ansari *et al*. 2012) that often dominantly co-exists with the former. The information is valuable for genetic improvement of the species, for *T. arjuna* meta-population with the level of observed gene diversity provides an opportunity to make the selection of the adequate number of elite genotypes for superior traits of economic worth and their incorporation in a breeding-cum-selection program for further genetic improvement and enhancing the pharmaceutical yield.

Nei's gene diversity of *T. arjuna* accessions belonging to different locations is variable. RAPD markers detect maximum gene diversity in accessions belonging to Jharkhand and Madhya Pradesh. This is expected as the meta-population of a species develops pockets of high and low gene diversity depending upon geo-climatic variables. Ideally, a pocket of accessions existing in heterogeneous geo-edaphic and environmental conditions retains high gene diversity to adapt to fluctuating conditions. On the other hand, a pocket of accessions in less heterogeneous geo-edaphic and environmental conditions tends to retain less gene diversity. In the present investigation, accessions exhibiting high and low Nei's gene diversity belong to a very large Eastern Plateau and Hill agro-climatic zone that includes several small agro-climatic entities such as Bundelkhand region (Madhya Pradesh), Eastern Vidharba (Maharashtra), Central, South and Western Plateau (Jharkhand) and North and Central Plateau (Odisha). It appears that the agro-climatic zone is highly heterogeneous with some pockets of less heterogeneity. As a result, there exist variations in Nei's gene diversity across locations of the sampled accessions. This is the first study on *T. arjuna* that is, however, in agreement, to a great extent, with similar investigations carried out on teak (*T. grandis*) in India (Ansari *et al*. 2012). Further, Locations harboring *T. arjuna* accessions of maximum Nei's diversities may be screened for selection of diverse elite trees to be incorporated for the genetic improvement program.

There exists adequate genetic distance among *T. arjuna* accessions across paired locations detected by RAPD markers that have utilized for clustering of locations employing UPGMA co-phenetic tree and principal co-ordinate analysis. RAPD markers with high resolving power detect more clusters. In all situations, *T. arjuna* accessions belonging to all locations, except those of Assam, Madhya Pradesh, Uttar Pradesh cluster together. On the other hand, the accessions from Assam, Madhya Pradesh and Uttar Pradesh remain outliers and discrete. The clustering has been well supported by high bootstrap values. As for individual accessions, the detected clusters exhibit excessive mixing with all locations, indicating adequate gene flow across *T. arjuna* meta-population in India. Nemali *et al.* 2015 (Nemali *et al.* 2015) have also detected clustering patterns among ten elite genotypes of *T. arjuna*. In a related species of the genus, i.e. *T. chebula*, a

clustering of 12 genotypes has been obtained (Ranjini et al. 2015). Accessions from diverse clusters may be accommodated in a breeding program to create variability and obtain heterosis for the trait of the economic importance in *T. arjuna*.

For population hierarchies, F_{ST} values between populations by RAPD, markers have been found very low, indicating high gene flow and low differentiation. The F_{ST} value in *T. arjuna* accessions is comparable to those in *T. bellerica* populations [25]. A similar range of F_{ST} has also been recorded in *Torreya jackii*(Li *et al*., 2007).Wright (1965)identifies four population genetic differentiation groups based on F_{ST} values. A range of 0.05-0.15 F_{ST} to which *T. arjuna* accessions belong indicates moderate genetic differentiation of population. Nei's G_{ST} value, an analog to F_{ST}, is high, i.e. 0.28 in *T. arjuna* accessions. Nei's G_{ST} value is supposed to depreciate the recurrence of inbreeding (selfing) in the population. RAPD markers being dominant ones are unable to detect and deduct inbreeding within the population and thereby provide an overestimation of Nei's G_{ST}. Therefore, F_{ST} values obtained from AMOVA are more reliable than Nei's G_{ST} value when dominant markers such as RAPD are employed due to their inability to distinguish heterozygous loci from homozygous dominant loci in the meta-population. It is inferred that the sampled *T. arjuna* accessions depict moderate population genetic differentiation.

As mentioned above, Low F_{ST} also indicates the ease of gene flow indicates. As a result, *T. arjuna* accessions appear to have freely traded gene exchange that points out the existence of ineffective geophysical barriers in locations of meta-population of *T. arjuna*. However, teak (*Tectona grandis*) that co-exists with *T. arjuna* in the same habitats also exhibit comparable ranges of the population differentiation in various investigations based on RAPD markers(Ranjini *et al.* 2015, Narayanan *et al.,* 2007). AMOVA analysis also allocates maximum genetic variability to accessions within populations (locations) and a minuscule fraction (< 7%) of genetic variability to among populations due to the free exchange of gene flow. This is expected as *T. arjuna* is a cross-pollinated species. In other cross-pollinated species, a similar situation prevails, e.g. *Tectona grandis*(Nicodemus *et al*. 2003, Shrestha *et al*. 2005, Kumar 2011), *Torreya jackii*(Li *et al*. 2007).

CONCLUSION

Population differentiation is moderate with adequate gene flow among detected population clusters within meta-population. A large proportion of genetic variability remains among accessions within the population and a very small fraction, i.e. <7% of genetic variability, among populations due to outcrossing mating systems in the species. it may be argued that the former randomly covers the whole genome and is more likely to detect associated loci compared to the former that targets hypervariable non-coding regions. RAPD markers efficiently detect gene diversity in the germplasm bank and make reliable clusters of sampled accessions congruently verified by UPGMA and PCoA. Accessions of Assam, Madhya Pradesh, Maharashtra and Odisha are discrete and the remaining accessions of other locations make a separate cluster.Estimates of gene diversity parameters and population differentiation are comparable to those of other forest species, e.g. *T. grandis* co-existing in the same habitat. This is a preliminary study based on molecular marker association with the arjunolic acid and less number of dominant markers having been used. It requires to expand and include more diverse accessions from all available locations/ agro-climatic zones employing more informative co-dominant markers.The identified accessions with high arjunolic acid content in bark may be incorporated in the genetic improvement of *T. arjuna*.

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