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Background

In 2000, the then Faculty of Forestry and Nature Conservation (now college of Forestry, Wildlife and Tourism) of the Sokoine University of Agriculture (SUA) in Morogoro, Tanzania, inaugurated the *Tanzania Journal of Forestry and Nature Conservation*. This development was taken in order to elevate the former publication of the then Faculty of Forestry, *Faculty of Forestry Records*, to a status of an International Journal. The last issue of the *Faculty of Forestry Records* was volume 72 and this Journal took over beginning with volume 73.

Scope

The *Tanzania Journal of Forestry and Nature Conservation* accommodates the current diverse and multidisciplinary approaches towards ecosystem conservation at national and global levels. The journal is published biannually and accepts research and review papers covering technological, physical, biological, social and economic aspects of management and conservation of tropical flora and fauna.

Subscription

The *Tanzania Journal of Forestry and Nature Conservation* (ISSN 2408-8137for e-version and 2498-8129 for print version) is published in June and December and a subscription price for a copy of a single issue is US $ 50.00, inclusive of postage charges. Copies can be ordered from: The Principal, College of Forestry, Wildlife and Tourism, P.O. Box 3009, Chuo Kikuu, Morogoro, Tanzania. Tel. and Fax: +255 23 2604648; E-mail: <*forestry@suanet.ac.tz*>.

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ABOUT THE COLLEGE OF FORESTRY, WILDLIFE AND TOURISM

The College of Forestry, Wildlife and Tourism of SUA attained its present status in July 2017. It started in 1973 as a Division of Forestry in the Faculty of Agriculture of the University of Dar es Salaam. Thereafter, it was elevated to a Faculty of Forestry in 1984 when SUA was established. SUA is located 3 km from the centre of Morogoro Municipality, which is 200km west of Dar es Salaam, along the Tanzania-Zambia highway.

There are six departments in the College formed on the basis of specialisation: Departments of Ecosystems and Conservation, Forest Engineering, Forest Economics, Forest Mensuration and Management, Forest Products and Technology and Wildlife Management.

The Faculty maintains three training forests. The first, covering 848 ha, is located at Olmotonyi on the slopes of Mount Meru near Arusha and is devoted to plantation forest management. The second covering 320 ha is a fully protected virgin rain forest located at Mazumbai in the west Usambara Mountains devoted to montane rain forest management. The third is Kitulangh'alo forest reserve covering 500 ha located near Morogoro and devoted to the management of miombo woodlands. These forests offer practical and research venues for both students and staff.

The College offers 3 three-year undergraduate degrees namely B.Sc. (Forestry), B.Sc. (Wildlife Management) and Bachelor of Tourism Management. So far, these programmes have attracted students from many African countries. Post-graduate programmes: (MSc) both in Forestry and in Management of Natural Resources for Sustainable Agriculture (MNRSA) and PhD in Forestry, are also offered. These programmes are tailored to produce personnel for higher professional positions in forestry, wildlife, natural resource management, tourism management and administration including teaching and research. Graduates find employment in forestry, wildlife, tourism other environmental services in government institutions and private or non-government organizations.

Entry Qualifications for all degree programmes are detailed in the SUA prospectus, but specific information related to forestry, wildlife and tourism management programmes may be obtained from:

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Genetic variation, structure and conservation status of *Terminalia arjuna* L. (Arjun) in Achanakmar Amarkantak Biosphere Reserve (AABR), Central India

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ABSTRACT

Terminalia arjuna has received much attention in the recent years by its remarkable value in medicinal, sericulture and tannin industry, with no prior reported genetic characterization studies.Sixteen genotypes of *T. arjuna* with diverse geographical locations from three natural populations (Achanakmar, Chhaparwa and Lamini) of AABR were selected for this study. Ten Random Amplified Polymorphic DNA (RAPD) markers were used to evaluate the genetic diversity of *T. arjuna.* Among ten RAPD decamer primers only six primers produced the clear and scorable bands. Six RAPD primer combinations generated a total of 515 bands of which 213 bands were polymorphic with 41.34±6.23 polymorphic percentage. The average genetic diversity and polymorphic information content (PIC) of *T. arjuna* in AABR was recorded as 0.193±0.018 and 0.323±0.01 respectively. The dendrogram constructed through UPGMA algorithm based on the binary matrix classified all the 16 genotypes into three major clusters; A, B and C. The population of Lamini was found genetically more diverse (0.263 ± 0.033) and the population of Achanakmar was found less genetically diverse (0.157±0.030). The fixation index values (F) indicated more number of heterozygotes in Lamini population of AABR. The AMOVA confirmed that 8% of the genetic diversity exists between the populations and 92% of the genetic diversity was recorded within the *T. arjuna* populations of AABR. This study provides a new insight into the exploitation of genetically diverse populations of *Terminalia arjuna* from AABR, as a potential resource for future tree breeding programmes.

Keywords: AABR, Conservation, Genetic Diversity, RAPD markers, *Terminalia arjuna* (Arjun).

INTRODUCTION

Achanakmar Amarkantak Biosphere Reserve (AABR) having 3835.51 sq km area is very rich with high diversity of natural flora and fauna. The biosphere reserve lies between $21^0 15'$ to $22^0 58'$ north latitude and $81^0 25'$ to $82^0 05'$ east longitude. It comprises of 1527 species of identified flora and 324 identified fauna. The rich dense forest of biosphere reserve is dominated by Sal tree (*Shorea robusta*) and its associates support a number of ecosystem services. The area is also recognized as "Genetic Express Highway" linking two important biological hot spots namely Western Ghats and Eastern Himalayas and also serves as confluence point for northern and southern type of vegetation in India. This Biosphere Reserve of Central India has a unique pure natural Sal (*Shorea robusta)* forests, but from few decades the biotic and abiotic environmental stress have brought this forest under threat. One of the causes for this threat can be

uneven and low genetic diversity of this natural forest. The quantification of the genetic diversity of this forest has become essential for the forest tree breeders, as it will provide guideline and direction for the conservation of the different forest tree species.

Molecular markers have proven to be invaluable tools for assessing forest trees genetic resources by improving our understanding with regards to the distribution and the extent of genetic variation within and among species in the forest. The development of polymerase chain reaction (PCR) technique has revolutionized the field of molecular biology (Waugh and Powell 1992). Polymerase chain reaction (PCR) derived markers with non-specific primers have been exceedingly popular since they do not require sequence information from target species. Different types of molecular markers are used to evaluate DNA polymorphism. Random amplified polymorphic DNA (RAPD), a PCR based technique is simple, cost-effective and a powerful tool for the analysis of plant genome characterization (Manimekalai and Nagarajan 2006; Ahmad 1999; Williams *et al.* 1990). RAPD being dominant, detect multiple loci distributed throughout the genome and hence strongly preferred for genetic variation studies of forest tree species. This technique has been applied for detecting population genetic variability of different forest tree species like *Tectona grandis* (Changtragoon and Szmidt 2000); *Cocusnucifera* (Manimekalai and Nagarajan 2006); *Butea monosperma* (Vaishali *et al.* 2008); *Terminalia* species (Vishal *et al.* 2009); *Jatropha curcas* (Ikbal *et al.* 2010); *Melia azadarach* (Yulianti *et al.* 2011); *Terminalia bellirica* (Sahu and Koche 2013); *Larixgmelinii* (Zhang *et al.* 2013); *Quercus* species (Kumar and Kumar 2014); *Pongamia pinnata* (Sharma *et al.* 2014) and *Terminalia pallida* (Sasikala and Kamakshamma 2015).

Terminalia arjuna L. (family: Combretaceae) is widespread in India and plays an important role in the sericulture (Orwa *et al.*2009) and tannin industry. It is a remarkable medicinal tree for its important phytochemical presence in different parts of the tree (Prakash *et al.* 2007). *T. arjuna* based phytochemicals are considered as one of the best heart tonic (Singh *et al.* 2004) and can be used on daily bases as tonic for healthy cardiovascular system. The bark of this tree is used for curing many diseases like anthelmintic, alexiteric, styptic, asthma, ulcers and anemia etc. In AABR this forest tree is distributed along the banks of the water ways. Unfortunately, no work has been carried out to unravel the genetic diversity at inter and intra population level of this species in AABR. A report on standardization of DNA isolation for RAPD based genetic diversity analysis of *T. arjuna* was established (Sarwat *et al.* 2006).

An understanding about the population genetic structure of *T. arjuna* can be useful for both its genetic improvement and conservation (Deshwal *et al.* 2005).The major objectives of this study are to analyze genetic diversity within and among populations of *T. arjuna* in AABR using RAPD markers, to establish population genetic structure in AABR, and to identify the nuclear zones of genetic diversity which could act as reservoir for development of conservation priorities in *T. arjuna.* The present investigation supports in future the development of genetic maps in *Terminalia,* which are highly useful tool in breeding, and also may provide information on the inheritance of features crucial for increase in active chemical content, tannin and the resistance against biotic and abiotic stress.

MATERIALS AND METHODS

Material Collection:

A total of sixteen genotypes of *T. arjuna* of diverse geographical locations in AABR were selected for studying genetic diversity as described in Table 1 and Figure 1. Trees were selected from the same girth class. The fresh Juvenile leaf samples of fully grown trees of *T. arjuna* were collected. The samples were stored on dry ice, lyophilized using standard procedure and stored at -20ºC till DNA isolation.

Table 1. Details of the Accessions (Genotypes) of *Terminalia arjuna* in Achanakmar Amarkantak Biosphere Reserve (AABR) used for the analysis of genetic diversity.

S. No	Accession No	Source	Latitude	Longitude	Altitude(m)
01	A1	Achanakmar	$22^0$19'39'' N	$81^0$56'14''E	322
02	A2	Achanakmar	$22^0$19'46'' N	$81^0$56'23''E	319
03	A3	Achanakmar	$22^0$19'49'' N	$81^0$56'28''E	311
04	A4	Achanakmar	$22^0$19'76'' N	$81^0$57'12''E	325
05	A5	Achanakmar	$22^0$19'45'' N	$81^0$46'26''E	318
06	B1	Chhaparwa	$22^0$26'26'' N	$81^0$44'87''E	406
07	B2	Chhaparwa	$22^0$26'44'' N	$81^0$46'08''E	402
08	B3	Chhaparwa	$22^0$26'27'' N	$81^0$45'37''E	400
09	B4	Chhaparwa	$22^0$27'17'' N	$81^0$45'07''E	403
10	B5	Chhaparwa	$22^0$27'09'' N	$81^0$45'24''E	402
11	B6	Chhaparwa	$22^0$27'24'' N	$81^0$45'30''E	410
12	C1	Lamini	$22^0$33'89'' N	$81^0$45'87''E	542
13	C2	Lamini	$22^0$35'73'' N	$81^0$46'78''E	554
14	C3	Lamini	$22^0$35'92'' N	$81^0$46'68''E	534
15	C4	Lamini	$22^0$36'02'' N	$81^0$46'66''E	540
16	C5	Lamini	$22^0$36'28'' N	$81^0$46'66''E	541

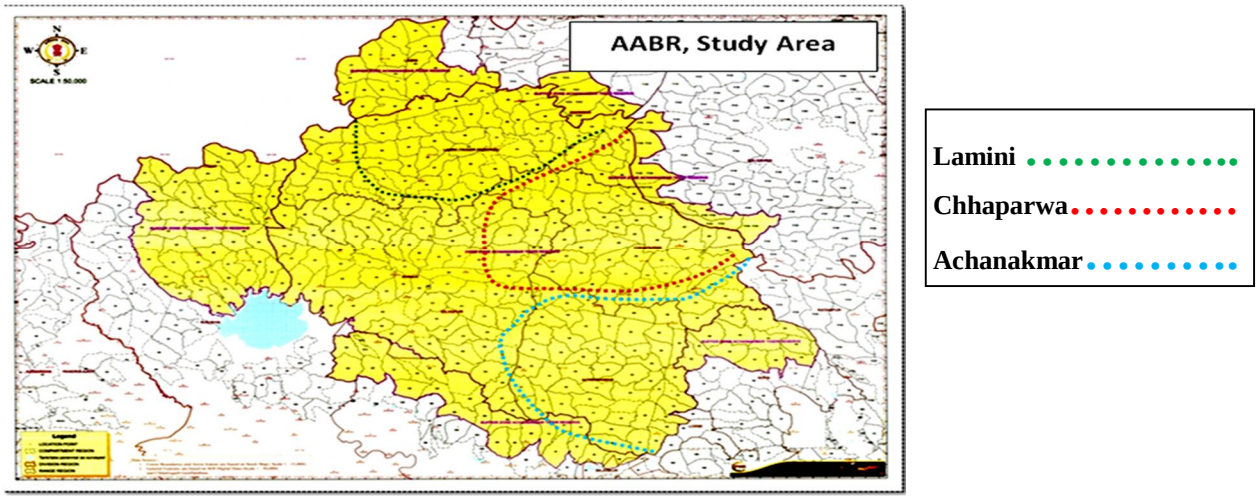

Figure 1. Approximate geographical locations of sampled populations of Achanakmar Amarkantak Biosphere Reserve (AABR)

Source: State Forest Department Chhattisgarh, India.

DNA Isolation

Genomic DNA were extracted from lyophilized leaf material of *T. arjuna* following modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (Sarwat *et al.* 2006), and stored at -20^0C

until for further use. DNA was quantified using agarose gel electrophoresis, employing serial dilutions of uncut DNA size marker as standard.

The following solutions and solvents were used for the present study e.g. CTAB Buffer (1.4M NaCl, 100 mMTris-HCl pH 8.0, 20mM EDTA pH 8.0, 2% CTAB; added β-mercaptoethanol before use), Chloroform: IsoAmyl alcohol (24:1),Isopropanol and Ethanol 70% and 96%(Sarwat *et al.* 2006)

RAPD Analysis

The RAPD reaction and procedures were carried out as described by Williams *et al*. 1990.The RAPD analysis was performed in a 15 µl volume of reaction mixture containing 1 X *Taq* Polymerase buffer (with 25 mM MgCl2), 0.6 units of *Taq* DNA Polymerase, 5 mM dNTPs, 10 mM of random decamer primer ,and 15 ng of total genomic DNA. Amplifications were carried out using a DNA thermal cycler (Mastercycler gradient, Biorad) with the following sequence: One cycle at 94ºC for 2 min, 36ºC for 2 min and extension at 72ºC for 2 min, 29 cycles of denaturation at 94ºC for 1 minute, primer annealing at 36ºC for 1 minute and extension at 72ºC for 1 minute and final extension at 72ºC for 10 minutes. This reaction was stored at 8ºC till loaded on gel. The products were size fractionated on 1.2% agarose gel and gel was photographed on Gel Documentation System.

Data Analysis

The results of PCR-RAPD reaction were processed in binary system where '1' denotes presence of band and '0' the absence of a particular amplification product. The number of polymorphic and monomorphic amplification products were determined for each primer and for 16 *T. arjuna* genotypes. The binary matrix was analyzed for the calculation of different genetic diversity parameters by GenALEx version 6 software. The Marker index was calculated by using the formula MI = PPLx PIC**,** Where PPL is Percentage of Polymorphic Loci and PIC is Polymorphic Information Content for each RAPD marker. The polymorphism of amplification product (P), the mean number of effective alleles (Ne), the (Na) number of different alleles (Hartl and Clark 1989). The (h) Nei's genetic diversity index (Nei 1973), and (I) Shanons information index (Shanon and Weaver 1949) were calculated by GenALEx version 6 and POPGENE version1.31 software packages. The genetic dissimilarity of 16 accessions of *T. arjuna* genotypes was assessed by Jaccard's dissimilarity index (Jaccard 1908). A Dendrogram based on RAPD data representing the genetic relationship among 16 genotypes of *T. arjuna* was prepared by UPGMA method using NTSYSpc (Numerical Taxonomy System, 2.02 version) application. Polymorphic Information Content (PIC) as power of marker discrimination was obtained by using the formula PIC = $1-\sum p_i^2$ where p_i is the frequency of i^{th} allele at a given locus (Anderson *et al.* 1993).Analyses of molecular variance (AMOVA) values determine the partition of total genetic variation at inters and intra population level in AABR.

RESULTS

Ten random decamer primers were employed for RAPD and among ten RAPD decamer primers only six primers produced the clear polymorphic reproducible and scorable bands (Figure 2). These bands were selected for further genetic diversity analysis and other four primers were discarded. The six RAPD primer combinations generated a total of 515 bands of which 213 were polymorphic and 302 bands were monomorphic (Table 2).

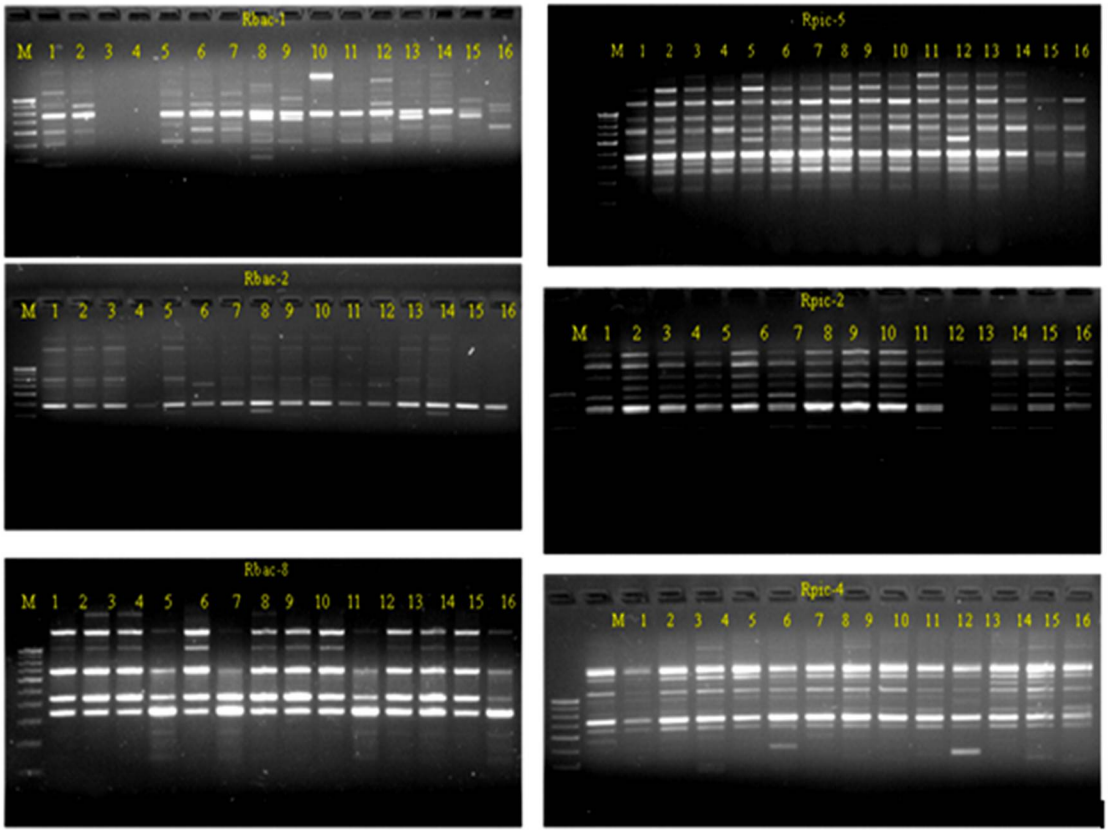

Figure 2. RAPD profile of sixteen accessions of *T. arjuna*, Molecular weight of 100bp DNA ladder. Lane 1-5(Achanakmar accessions),Lane 6-11(Chhaparwa accessions) and Lane 12-16 (Lamini accessions)

Table 2. Polymorphic percentage and Polymorphic Information Content (PIC) detected by six RAPD primers used in the study.

S.No	Primer	Sequence(5'-3')	Total bands	Poly bands	Mono bands	Polymorphism (%)	PIC
1	RBAC-1	**AAAGCTGCGG**	50	22	28	44.00	0.481
2	RBAC-2	**AAACGCGTCG**	90	39	51	43.30	0.377
3	RBAC-8	**ACCACCCACC**	67	28	39	41.79	0.382
4	RPIC-5	**ACCGCCTATG**	61	24	37	39.02	0.337
5	RPIC-2	**AATCGGGCTG**	93	37	56	39.38	0.390
6	RPIC-4	**ACACACGCTG**	92	38	54	41.30	0.401
	Total		515	213	302	249.19	2.368
	Average		**85.83**	**35.5**	**50.34**	**41.53**	**0.395**

The mean number of amplified bands per primer was 85.84 with an average polymorphic percentage recorded as 41.53%. Rbic-5 primer amplified the highest number of polymorphic (49) and total bands (123), but the highest percentage of polymorphism was detected by Rbac-1 (44%).In contrast the lowest percentage of polymorphism was recorded by Rbac-2 (39.78%). All primers used showed a wide range of amplicons, with 100bp. The values of Marker Index (MI) of RAPD markers

used for the detection of polymorphism were recorded as 10.74±0.07.

Table 3. Jaccard's genetic similarity (below diagonal) and dis-similarity (above diagonal) index of sixteen *T. arjuna* accessions of AABR

	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	B6	C1	C2	C3	C4	C5
A1	-	0.262	0.250	0.325	0.262	0.310	0.215	0.315	0.267	0.214	0.357	0.357	0.399	0.171	0.419	0.429
A2	0.738	-	0.179	0.308	0.195	0.205	0.195	0.304	0.167	0.195	0.341	0.310	0.425	0.238	0.405	0.452
A3	0.756	0.821	-	0.244	0.132	0.275	0.262	0.289	0.147	0.175	0.283	0.366	0.369	0.175	0.391	0.440
A4	0.675	0.692	0.756	-	0.217	0.223	0.257	0.326	0.310	0.257	0.278	0.450	0.500	0.300	0.258	0.315
A5	0.738	0.805	0.868	0.783	-	0.250	0.196	0.228	0.167	0.150	0.211	0.342	0.343	0.196	0.366	0.375
B1	0.690	0.795	0.725	0.777	0.750	-	0.080	0.348	0.256	0.244	0.264	0.350	0.475	0.286	0.29	0.298
B2	0.785	0.805	0.738	0.743	0.804	0.920	-	0.298	0.205	0.233	0.293	0.334	0.543	0.273	0.358	0.366
B3	0.681	0.696	0.711	0.674	0.772	0.652	0.702	-	0.156	0.223	0.319	0.556	0.396	0.298	0.378	0.457
B4	0.733	0.833	0.853	0.690	0.833	0.744	0.795	0.844	-	0.163	0.262	0.303	0.381	0.245	0.364	0.445
B5	0.786	0.805	0.825	0.743	0.850	0.756	0.767	0.777	0.837	-	0.250	0.334	0.415	0.191	0.396	0.442
B6	0.643	0.659	0.717	0.722	0.789	0.736	0.707	0.681	0.738	0.750	-	0.400	0.448	0.293	0.425	0.352
C1	0.643	0.619	0.634	0.550	0.658	0.650	0.666	0.644	0.697	0.666	0.600	-	0.315	0.373	0.500	0.530
C2	0.601	0.575	0.631	0.500	0.657	0.525	0.547	0.604	0.619	0.585	0.552	0.685	-	0.375	0.520	0.527
C3	0.829	0.762	0.825	0.700	0.804	0.714	0.727	0.702	0.755	0.809	0.707	0.627	0.625	-	0.358	0.405
C4	0.581	0.595	0.609	0.742	0.634	0.710	0.642	0.622	0.636	0.604	0.575	0.500	0.480	0.642	-	0.182
C5	0.571	0.548	0.560	0.685	0.625	0.702	0.634	0.543	0.555	0.558	0.648	0.470	0.473	0.595	0.818	-

The RAPD data were used to make pairwise comparison of the accessions based on shared and unique amplification products to generate a similarity matrix with NTSYS-PC (version 2.1). The genetic disimilarity matrix (Jaccard's disimilarity index) of 16 *T. arjuna* genotypes were prepared on the basis of binary matrix obtained from RAPD data and presented in Table 3. The genetic distance ranged between 0.530 to 0.080 as per Jaccards dissimilarity index among 16 *T. arjuna* accession of AABR

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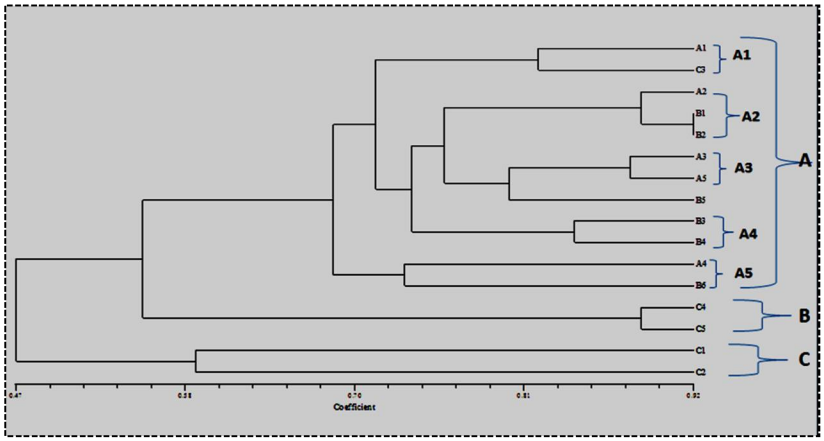

Figure 3. Dendrogram illustrating genetic relationships among 16 *T. arjuna* genotypes, generated by NTSYS pc 2.02 cluster tree analysis.

A Dendrogram of sixteen genotypes of *T. arjuna* was constructed through UPGMA algorithm (Figure 3) based on RAPD data. All 16 genotypes were classified into three major clusters; A, B, and C. The range of similarity was 47% to 92%. The cluster A have maximum number of genotypes (12), Cluster B have two genotypes i.e., C4 and C5 and Cluster C also have two genotypes i.e., C1 and C2. Cluster A was further divided in five sub-clusters A1, A2, A3, A4 and A5. Sub-cluster A1 has only two genotypes A1 and C3, sub-cluster A2 has three genotypes, A2, B1 and B2, sub-cluster A3 has two genotypes A3 and A5, sub-cluster A4 has two genotypes B3 and B4, Sub-cluster A5 has also two genotypes A4 and B5 respectively. The Cluster A was found to be genetically more diverse. The sub clusters B1 and B2 have maximum similarity (92%). The Cluster B has around 56% similarity while Cluster C has around 59% similarity (Figure 3).

Table 4. Mean values ±SE of different genetic parameters of three populations of *T. arjuna* in AABR.

Population	Na	Ne	P (%)	I	H	F
(Pop1) Achanakmar	1.275±0.093	1.247±0.052	37.23±2.36	0.210±0.039	0.157±0.031	0.101±0.027
(Pop2) Chhaparwa	1.392±0.075	1.243±0.050	40.18±3.39	0.214±0.039	0.158±0.030	-0.109±0.001
(Pop3) Lamini	1.490±0.094	1.420±0.056	46.62±1.94	0.346±0.043	0.263±0.033	-0.113±0.004
Average±SD	1.386±0.051	1.303±0.031	41.53±2.24	0.257±0.024	0.193±0.018	-0.107±0.006

Na (No. of Different Alleles); Ne (No. of Effective Alleles);P(%) polymorphic percentage; I (Shannon's Information Index) H (Nei's (1973) gene diversity) and F Wrights *F* inbreeding coefficient(Fixation Index)

The effective number of alleles per population varied from 1.243±0.050 in the population of Chhaparwa to 1.420±0.056 in the population of Lamini, with a mean of 1.303±0.031 (Table 4). The mean number of different alleles across three *T. arjuna* populations of AABR ranged from 1.275±0.093to 1.490±0.094 with an average of 1.386±0.051respectively.Across the three populations the accessions of the population of Lamini showed the highest percentage of polymorphism (46.62%) and it was followed by the accessions of the population of Chhaparwa (40.18%) and Achanakmar (37.23%) respectively (Table 4). The estimates of Nei's genetic diversity (h) varied from 0.157±0.031 in Achanakmar population to 0.263±0.033 of Lamini population with an average of 0.193±0.018. The average value obtained for Shannon's information index(I) was 0.257±0.024.The average fixation index values (F) were found lower than zero for all the three populations studied. The negative values indicated excess of heterozygotes in AABR which confirm the excess out breeding pattern in *T. arjuna.*

Table 5. Inter-population genetic distance shown in below diagonal of three natural populations of *T. arjuna* in AABR.

	Pop1	**Pop2**	**Pop3**	
Pop1	0.000	0.0011	0.0017	**Pop1**
Pop2	0.067	0.000	0.0001	**Pop2**
Pop3	0.155	0.166	0.000	**Pop3**

(**Pop1**: Achanakmar, **Pop2**: Chhaparwa and **Pop3**: Lamini)

	Pop1	**Pop2**	**Pop3**

*Probability values based on 999 permutations are shown as above diagonal

At inter population level the genetic diversity ranged between 0.166 and 0.067 respectively (Table 5). The population of Chhaparwa and Lamini were recorded to be 17% genetically diverse from each other which was the highest value whereas the population of Achanakmar and Lamini were 16 % genetically diverse from each other. The genetic differentiation within and between populations was done by AMOVA. The AMOVA results confirm that the genetic variability within population was large i.e. 92%, whereas genetic variability between populations was only 8% (Table 6). The Table shows that the more genetic variability exists at within population level, whereas differences among populations were lower.

Table 6. Partitioning of total genetic variation among and within-populations of *T. arjuna* in AABR by AMOVA Test using GenAlex 6 software

Source	df	SS	MS	Est. Var.	%Variation	*P-value*
Among Pops	2	17.200	8.600	0.513	08%	0.001
Within Pops	12	72.400	6.033	6.033	92%	0.001
Total	14	89.600		6.547	100%	

DISCUSSION

In recent years, the molecular markers are proven to be useful for genetic diversity analysis (Kesari and Langan 2011), inheritance studies (Lerceteau and Szmidt 1999), gene tagging studies (Negi *et al.* 2000) and gene bank management tool (Hokanson *et al.* 1998). The effectiveness of any tree improvement programme depends upon the nature and magnitude of existing genetic variability and also on the degree of transmission or heritability of traits (Zobel and Telbert 1984) because genetic variation is the fundamental requirement for maintenance and long term stability of forest ecosystems. Monitoring the genetic diversity of forest tree species can improve our knowledge on how forest ecosystem services and goods are being delivered (FAO 2014). In the present study, 16 genotypes representing the AABR of central India were screened for molecular diversity using RAPD technology. Although some leaf morphometric traits of *Terminalia arjuna* in these populations were studied (Wani and Singh 2016), which highlighted a significant variation of accessions in the biosphere reserve.

RAPD markers are the simplest and most common methods used in characterization of genetic similarity and diversity in forest tree species (Rout *et al.* 2009).The percentage of polymorphic loci for *Terminalia arjuna* populations obtained in this study was variable and ranges from 46.62% (Lamini), 40.18% (Chhapparw) and 37.23% (Achanakmar) respectively. These values were found to be lower comparable to the estimates obtained for *Terminalia* using RAPD markers system (69.5%, six different populations of Chittoor district, Andhra Pradesh, India (Sasikala,2015); 90% in six populations of Chhattisgarh, India **(**Sahu and Koche 2013);74.4% in five populations of Karnataka, India (Gowda 2012) and 55.2% three populations of Telangana, India (Gandhi *et al.*2015). The values of marker index and Polymorphic information content (PIC) indicate the adaptability of RAPD markers for genome mapping(Fischer *et al.*2000).The present work confirms that RAPD markers could be used for estimation of genetic relationship, which ultimately help in characterization of *T. arjuna* germplasm.

The genetic diversity of each population was highlighted by the estimates of Shannon index *(I)* and Nei index *(h)* based on the RAPD markers. For both indices, the values were found to be higher for the Lamini population than Chhaparwa and Achanakmar populations of *Terminalia arjuna* in AABR respectively. This result was similar to those of previous genetic diversity analyses of most forestry tree populations (Lee 2006; Nosrati *et al.* 2012). The estimates of Shannon index *(I)* and Nei index *(h)* values in this work are lower than those reported for *Tectona grandis* by Fofana *et al.* (2009), Ansari et al., (2012), and in *Pinus sylvestris* by Cipriano *et al.* (2013). The higher genetic diversity of *T. arjuna* in Lamini population (26%) can be due to the greater protection imposed in the area because the area comes under the center of the Core region of the biosphere reserve. Also the rights and concessions of the forest dwellers are seized in the core region of the biosphere reserves thus more conservation practice of the forest tree species in this area exists. The present report confirms the intensive conservation activities after the area was declared as a biosphere reserve. These genetically diverse stands of *T. arjuna* can be used as the nuclear zones for the future improvement and conservation of this tree. The present report will provide a baseline in selection of different traits and additive traits for making outbreed hybrids from genetically diverse stands of AABR.

The uncontrolled exploitation of *T. arjuna* for its medicinal value by forest dwellers in Chhaparwa and Achanakmar has eroded this genetic resources and caused the narrow genetic diversity in these populations. The over exploitation of the tree species also reduced the size of *Terminalia* population in Chhaparwa and Achanakmar of AABR and the species has adapted to the restricted habitat where they got a perennial water source. The study indicates a significant impact of population size on the level of genetic variation in the *T. arjuna* in AABR. The restricted habitat niche of *T. arjuna* of Chhaparwa and Achanakmar along the banks of the water ways in AABR has restricted its population size which can be a cause of its narrow genetic variability. The low genetic diversity detected in small-sized populations of *T. arjuna* could result in genetic drift, high inbreeding depression and low evolutionary potential (Reed and Frankham 2003), that can consequently result in decreased fruit/seed set (Keller and Waller 2002). Moreover, pollination failure is very common in small tree populations since pollinators are less attracted to the small-sized populations (Jennersten 1988; Andrieu *et al.* 2009).

Pairwise Population Matrix of Nei Genetic distance indicate that the *T. arjuna* population of Lamini was 16% and 17% genetically dissimilar from the populations of Chhapparwa and Achanakmar. However the *T. arjuna* population of Chhaparwa was only 6% genetically dissimilar than the Achanakmar. The reason behind the low genetic variability between Chhaparwa and Achanakmar populations can be correlated with their geographical distance and migration through seeds by the shared water ways of these populations. The results were found similar as in *Jatropha quercus (*Ikbal *et al.* 2010) and *Butea monosperma* (Vaishali *et al.* 2008).

Based on dendrogram of genetic similarity coefficients of *T. arjuna* population of Achanakmar Amarkantak biosphere Reserve, three clusters were classified i.e., Cluster-A, Cluster-B and Cluster-C respectively. The results show that the *T. arjuna* genotypes of Achanakmar and Chhaparwa were closely related. However the *T. arjuna* genotypes of Lamini range were genetically diverse as it represent separate entity in the dendrogram. Due to the high genetic diversity, the accessions from the Cluster-B and Cluster-C provides a better scope for selection of plus tree and elite tree with respect to its functional diversity for its ayurvedic medicinal properties. The cluster analysis showed three main groups for 20 genotypes of *Melia azedarach* in community forests of West Java assessed by RAPD marker system (Yulianti *et al.* 2011).The pattern of genetic diversity of *T. arjuna* genotypes in AABR indicates that these genetic resource are still maintained by evolutionary forces**.** The pattern of the clustering of *T. arjuna* genotypes confirms the fragmentation of the populations, which occurred due to habitat disjunction (Lienert 2004).

In the present study the accessions analyzed show a significant genetic variation, but the distribution of this variation was not homogenous. The genetic differentiation within and among the populations was measured by AMOVA (Table 6). The most of genetic variation in *T. arjuna* were allocated to within-population (92%) rather than among the populations (08%).*T. arjuna* is predominantly an outcrossing species due to andromonoecy promoting outcrossing and having a showy and large number of stamens, which help the plant in attracting the pollinators and consequently increasing outcrossing rate (Zhang and Tan 2008), therefore majority of the total genetic variation resided within accessions. Although small and fragmented populations are expected to have higher levels of among-population genetic diversity as these populations diverges more rapidly than larger populations (Frankham *et al.* 2002). On the other hand, low genetic diversity detected among the populations of *T. arjuna* studied may indicate that fragmentation has recently occurred due to habitat disjunction.

CONCLUSION

The maintenance of genetic diversity in *Terminalia arjuna* is of great importance, as the tree has immense multipurpose uses, but genetically uncharacterized. The accurate identification of high genetically diversified genotypes is essential for tree breeding and improvement programmes, and also it will provide a genetic input for sustainable management of *T. arjuna.* In the present study the result demonstrates that RAPD is a powerful tool for fingerprinting, variability analysis and genome mapping in genotypes of *T. arjuna.* The distribution of high genetic variation in Lamini population verified the existence of diversified genotypes of *T. arjuna*, which could be attributed to the specific adaptation to different regional environmental or experience of a different evolutionary system. The present study

demonstrates that the Achanakmar and Chhaparwa populations harbors less genetic variation, which requires urgent conservation measures to counteract deforestation and climate change. Implementation of a database with all the molecular characterization will help to select the proper genotypes to perform new crosses in order to generate new diversified *T. arjuna* genotypes with more medicinal, sericulture and tannin importance. Further studies are needed to investigate local adaptation of this tree species are in progress, which can be utilized to conserve this species in this region for sustainable delivery of its goods and services.

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