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Full Length Research Paper

Allozyme diversity in South Western Ghats populations of *Terminalia paniculata* Roth. (Combretaceae)

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Terminalia paniculata **Roth. (Combretaceae) is a tropical tree with a large natural distribution in Western Ghats. The tree is extensively utilized in pharmaceutical, timber tannin, leather and silk industries. However, the species has been overexploited and information on its existing gene pool is currently lacking. The present work was therefore carried out using allozyme markers to assess the genetic diversity within and between populations in order to suggest conservation and management strategies. Six enzyme systems generated 15 loci from four populations which were used to estimate allele frequency, percentage of polymorphic loci, observed heterozygosity (*Ho*) and expected heterozygosity (*He*) and Shannon information index. In the Sasthakovil populations 12 rare alleles, two private alleles (AAT-1C and PGM -2C), and a high level of polymorphism (86.66%) were observed. The levels of heterozygosity observed in all populations were lower than expected from Hardy-Weinberg equilibrium values, except Sasthakovil populations. As a priority, the population at Sasthakovil may be targeted for conservation. This would ensure the conservation of a relatively rich proportion of genetic diversity and presence of private allele representative of that existing in other populations.**

Key words: Allozyme, genetic variation, allele frequency, heterozygosity, homozygosity.

INTRODUCTION

Terminalia paniculata Roth. (Combretaceae) is a tropical tree with a large natural distribution in Western Ghats, India. The tree is extensively utilized in pharmaceutical, timber, tannin, leather and tasar industries (Srivastava, 1993). An extensive plantation of *T. paniculata* has been raised all over India for its utilization. Due to overexploit-tation and anthropogenic pressure on this species, habi-tat fragmentation and loss of the species natural popula-tion is also on the increase especially in highly settled area.

Conservation of plant genetic diversity has recently ge-nerated a lot of interest in the tropics as a result of many years of mismanagement, adverse environment as well as socio-economic changes. Population genetic theory predicts that the decrease in the genetic diversity limits a species ability to keep pace with the changing selection pressure (Young, 1988). Plant species, especially the perennials such as trees, rely on the available genetic diversity for stability and survival under the ever-changing environments. Understanding species popula-tion genetic structure is essential for their conservation, planning and sustainable management (Sun and Wong, 2001). The level of genetic diversity in forest species is a function of both the biological characteristics of the spe-cies and the process of pollination. At the species level, the conserva-tion and the maintenance of genetic varia-tion in forest species and their close relatives is a goal of germplasm collection and seed bank establishment.

Plant genetic resources not only provide basis for life on the earth but most valuable and basic raw materials to human life, which have posed a great threat to the need to conserve these genetic resources. In addition, greater

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levels of genetic variation may buffer genotypes against environmental challenges. Conversely the loss of genetic variability could render population more vulnerable to extinction in cases of habitat perturbation, reproductive bottlenecks (Wright, 1943).

Maintenance of genetic diversity is considered crucial for long term survival and evolutionary responses of populations to changes in the environment (Hunenke, 1991). In addition, genetic erosion would reduce the potential of the species improvement through breeding and selection. Knowledge of allozyme diversity will aid in taking measures on conservation of *T. paniculata.* In the present study, allozyme markers were used to charac-terize genetic diversity within and between populations of *T. paniculata.*

MATERIALS AND METHODS

Allozymes were used as gene markers in determining the genetic diversity of the *T. paniculata.* The six enzyme systems such as aspartate aminotransferase (AAT, E.C. 2.6.1.1), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), phosphoglucose isomerase (PGI, E.C. 5.3.1.9), phospho glucomutase (PGM, E.C. 2.7.5.1), peroxidase (PRX, E.C.1.11.1.7), esterase (EST, E.C. 3.1.1.1) were assayed in four populations of southern Western Ghats, India.

Plant material and extraction of enzymes

Samples of young leaves were collected from the four populations of Courtallam (9^o 15 ' N, 77^o 30' E), Sasthakovil (9^o 21' N, 77^o 48' E), Kodaiyar (8^o 30' N, 77^o 18' E) and Petchiparai (8^o 25' N, 77o 13' E). Fresh samples of young leaves, a small piece taken from each individual, were placed in moist filter papers in a cool ice box during field collections and stored at 4^{o}C until enzyme extraction. Samples of leaf tissue from each individual were ground with 0.5 ml of extraction buffer described in Sun and Wong (2001). The composition of the extraction buffers was as follows: 100 ml Tris-HCl 0.1 mol / L pH 7.0 buffer, 0.6 g PVP (Poly Vinyl Pyrrolidone), 0.0372 g EDTA (ethylene diaminetoral acetic acid), 0.145 g BSA (bovine albumin), 6.846 g saccharose, 0.13 g DIECA (Sodium diethyl carbamate) 100 µl – B – mercaptoethanol.

Separation and staining of enzymes

After grinding, the resultant slurry was absorbed by 4 x 11 mm wicks, cut from Whatman filter paper. Wicks were placed on starch gels after gels had cooled for at least 1h and electrodes were attached. The composition of the starch gels and the separation conditions were as follows:

System I used for separation of alcohol dehydrogenase (ADH) which contains 10.5% starch, 2% sucrose, 1% urea, 0.07% EDTA, electrode buffer 0.06 m/lL histidine citrate at pH 6.2, gel buffer that was a mixture of one part electrode buffer to three parts of water and running conditions applied was 170 mA and time required for separation of enzyme was five to five and half hours.

System II used for separation of aspartate aminotransferase (AAT) and phosphoglucose isomerase (PGI), which contains 10% starch, 4% sucrose, electrode buffer 0.03 m/L, LiOH, 0.2 m/L boric acid at pH 8.1; gel buffer that was mixture of 90% 0.05 m Tris citrate adjusted the pH 8.1 and added 10% electrode buffer, applied 80 mA current for 5 h for enzyme separation.

System III used for separation of esterase (EST) and peroxidase (PRX), which separated by 10% starch + 2% sucrose; electrode buffer 0.3 m/L Boric acid, 0.06 mol/lit NaOH, pH was 8.0; gel buffer to 0.01 m/L tris, at pH 8.5 running conditions 1 - 30 mA. Running time was 5 h.

System IV used for separation of phosphoglucomutase (PGM), which contains 10% starch, 4% sucrose, electrode buffer 0.03 m LiOH, 0.2 m H_3BO_3 , pH at 8.1, gel buffer 0.05 m tris – citrate pH 8.1 and added 10% electrode buffer. Running conditions 80 mA and enzyme separation completed after four to five and half hours of loading.

The staining was carried out with and without substrate for each enzyme systems on replicate slabs of the same gel to test the substrate specificity of the enzymes.

Genetic interpretations of zymograms

A first genetic interpretation of banding patterns was made by simple inspection of zymograms, assuming diploidy. Alleles within loci were designated by alphabets according to their relative position on the gel with the lowest alphabet corresponding to the most anodally migrating allele, and so were loci whenever more than one polymerphic banding zone was present for a given enzyme.

Estimation of allozyme diversity parameters

Allozyme frequency data were analyzed by the software, POPGENE (version 1.31) (Yeh and Boyle, 1997).

Allele frequencies were calculated for all the four populations of *T. paniculata*. These statistics included allele frequencies within a population, rare alleles (the allele frequency below the value 0.05 considered as rare alleles) (Wright, 1978), the percentage of polymorphic loci (*P*) (we considered that a locus was polymorphic when the most common allele occurred at a frequency #0.99 (Nei, 1987), the mean number of alleles per locus (*A*), the mean number of alleles per polymorphic locus (*Ap*), total no. of alleles(*TA*), observed no. of alleles (*na*), effective no. of alleles (*ne*) (Hartl and Clarke, 1989), Shannon's information index (*I*) (Shannon and Weaver, 1949) the observed heterozygosity (*Ho*) and the expected heterozygosity assuming Hardy-Weinberg equilibrium (*He*) (Levine, 1949). The expected heterozygosity is obtained by applying Hardy – Weinberg' law and it been used to compare the observed heterozygosity to identify deviations from a population in equilibrium. Departure from Hardy – Weinberg equilibrium for each population/ polymorphic loci combination was examined by Chi – square test. This test was performed to compare observed and expected genotypic frequencies and 0.05 used to probability level. All measurements were expressed in mean $\pm$ standard error of at least five independent replicates. Statistical analysis of all data was done using the statistical package, Microsoft EXCEL, version 4.0, Microsoft corporations, USA.

RESULTS

Genetic interpretations of phenotypes of the six enzyme systems namely aspartate aminotransferase (AAT), alcohol dehydrogenase (ADH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), peroxidase (PRX) and esterase (EST), visualized in the starch gel provided a total of 15 loci that were consistently scored in all the populations. They are as follows AAT-1, AAT-2, AAT-3, ADH-1, ADH-2, PRX-1, PRX-2, EST-1, PGM-1, PGM-2,

PGM-3, PGM-4, PGI-1, PGI-2, and PGI-3. The observed enzyme bands migrated anodally for all the enzymes.

Allele frequencies

The comparisons of found variations found in the alleles distribution among four populations revealed that the highest percentage of polymorphic loci was found in Sasthakovil (86.66%) population while the lowest in Courtallam (46.66%) population as they have the lowest and highest number of monomorphic loci respectively (Tables 1and 4). The other two populations showed the higher percentage of polymorphic loci. It is quite interesting to note that the Courtallam population has lower genetic multiplicity (1.90) when compared to other populations. However, the mean number of alleles per polymorphic locus of Courtallam population is higher (3.00) than that of other populations. The highest num-bers of total alleles were found in the population Sastha-kovil (35 alleles) and the lowest in Courtallam population (29 alleles). Totally twelve rare alleles were identified from the overall allele frequency (AAT-1C, AAT-2B, AAT-3B, PRX-2B, EST-1C, PGM-1B, PGM-2B, PGM-2C, PGM-4C, PGI-3B, PGI-3C and ADH-2E). Three (ADH-2D, PGI -3D and PGI – 3E) private alleles (alleles not found in any populations) in Courtallam population and absent in Kodaiyar population.

Genic variation statistics

In the present investigation, Sasthakovil population has high mean value of *na* (2.3333 $\pm$ 0.4666) and *ne* (1.4812 $\pm$ 0.1354) than other populations. The mean value of *na* was lower in Courtallam population (1.9333 $\pm$ 0.2555). But in the case of mean value of *ne* was low in Petchiparai population. The gene diversity (*I*) found in the population Sasthakovil (0.3891 $\pm$ 0.0775) was high than other population (Table 2).

Genetic differentiation statistics

The genetic diversity statistics was observed in all population derived from both observed *(Ho)* and expec-ted heterozygosity deviation from Hardy-Weinberg equilibrium. The comparison of the *Ho* to those *He* under Hardy-Weinberg proportions reveals a substantial deficiency of heterozygotes for all the populations.

Genetic diversity estimate for each population are presented in Table 3. For every population the mean *Ho* was lower than *He* at normal conditions. But in the popu-lation of Petchiparai the mean observed heterozygosity (0.2089 $\pm$ 0.0485) was higher than mean expected heterozygosity (0.1971 $\pm$ 0.0451). The mean *Ho* range from 0.1797 to 0.2222 and averaging in all population the *Ho* was 0.2050. The maximum of *Ho* was observed in the Sasthakovil population (0.2222 $\pm$ 0.0516) and low level of *Ho* observed in Courtallam (0.1797 $\pm$ 0.0454). The mean value of *He* was very low in Courtallam (0.1864 $\pm$ 0.0372) than other population and *Ho* was high in Sasthakovil (0.2288 $\pm$ 0.0499).

DISCUSSION

Allele diversity studies of tree species have furthered our understanding of how population genetic architecture is produced by provided insights on patterns of gametic union, gene flow and genetic sub structuring of plant populations (Hamrick et al., 1979). However most studies to date have focused on temperate species, particularly conifers and tropical species have only recently begun to be studied in detail (Runo et al., 2004). Species genetic diversity can be interpreted under three criteria. One is the allelic diversity, which is measured as the total number of alleles at each locus in a population or species. A second factor that also accounts for expected heterozygosity and third factor is the percentage of polymorphic loci present at the species level. Rare alleles (with an allelic frequency less than 0.05 within given population) were more abundant in Sasthakovil populations (12 rare alleles) (Table 1) than in others. The frequency of rare alleles is negatively related to gene flow (Slatkin, 1985). Hence, the private alleles (alleles de-tected only in Sasthakovil population; three private alleles (PGI-3D, PGI-3E and ADH-2D) is congruent with high gene flow.

At the population level, the Courtallam population to be genetically depauperate was compared to the rest of the populations because the size of their populations was probably seriously affected by anthropogenic pressure. The pressure was created due to tourisms and colonization – extinction episodes. However, no information is available regarding the possibility that such processes have affected the *T. paniculata* population of Western Ghats. Diversity estimates obtained with allozymes indicate that these populations have lower allelic diversity and low level of observed heterozygosity (Table 2). These results agree with theoretical models that indicate that these parameters are differently affected by population bottlenecks. Although both allelic diversity and average heterozygosity decrease with a reduction in population size, the loss of allelic diversity is greater than the loss of heterozygosity, when compared to the other population. The levels of heterozygosity observed in all populations were lower than expected from Hardy-Wein-berg equilibrium values, except Sasthakovil populations (Table 3). Two possible explanations can given why the observed heterozygosity is lower than expected heterozygosity values. First, significant amounts of selfing could be occurring within *T. paniculata* populations. However, experimental crossings have shown that *T. paniculata* is strictly self-incompatible.

Second, genetic diversity may be structured in neighborhoods, and mating may mainly take place among

Table 1. Allele frequencies at 15 allozyme gene loci in *T. paniculata* populations.

Locus	Allele	Population			
		Petchiparai	Kodaiyar	Sasthakovil	Courtallam
AAT-1	A	0.9667	1.0000	0.9762	1.0000
	B	0.0333▲	-	-	-
	C	-	-	0.0238*▲	-
AAT-2	A	1.0000	0.9762	0.9762	1.0000
	B	-	0.0238*	0.0238*	-
AAT-3	A	1.0000	0.9762	0.9524	1.0000
	B	-	0.0238*	0.0476*	-
ADH-1	A	0.8333	0.8095	0.8095	0.8261
	B	0.1667	0.1905	0.1905	0.1739
ADH-2	A	0.4000	0.4286	0.4286	0.3913
	B	0.4667	0.3333	0.3333	0.3696
	C	0.1333	0.2143	0.2143	0.2174
	D	-	-	-	0.0217*▲
	E	-	0.0238*	0.0238*	-
PRX-1	A	0.4333	0.2143	0.2143	0.1957
	B	0.1667	0.4048	0.4048	0.5870
	C	0.4000	0.3810	0.3810	0.2174
PRX-2	A	0.9667	0.9762	0.9762	1.0000
	B	0.0333*	0.0238*	0.0238*	-
EST-1	A	0.3333	0.4286	0.4286	0.4565
	B	0.6667	0.5476	0.5476	0.5435
	C	-	0.0238*	0.0238*	-
PGM-1	A	1.0000	0.9762	0.9762	1.0000
	B	-	0.0238*	0.0238*	-
PGM-2	A	0.9333	1.0000	0.9524	1.0000
	B	0.0667	-	0.0238*	-
	C	-	-	0.0238*▲	-
PGM-3	A	1.0000	1.0000	1.0000	1.0000
PGM-4	A	0.8333	0.4762	0.4762	0.8043
	B	0.1333	0.4762	0.4762	0.1739
	C	0.0333*	0.0476*	0.0476*	0.0217*
PGI-1	A	1.0000	1.0000	1.0000	1.0000
PGI-2	A	0.8667	0.9286	0.9286	0.9130
	B	0.0667▲	-	-	-
	C	0.0667	0.0714	0.0714	0.0870
PG1-3	A	0.9333	0.9048	0.9048	0.8696
	B	0.0333*	0.0476*	0.0476*	0.0652
	C	0.0333*	0.0476*	0.0476*	0.0217*
	D	-	-	-	0.0217*▲
	E	-	-	-	0.0217*▲

* rare allele; ▲ private allele

Table 2. Genetic variation statistics for allele distribution in different loci of *T. paniculata* populations.

Populations												
	Petchiparai			Sasthakovil			Kodaiyar			Courtallam		
Locus	*na*	*ne*	*I*	*na*	*ne*	*I*	*na*	*ne*	*I*	*na*	*ne*	*I*
AAT-1	2.0000	1.0689	0.1461	2.0000	1.0488	0.1125	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000
AAT-2	1.0000	1.0000	0.0000	2.0000	1.0488	0.1125	2.0000	1.0488	0.1125	1.0000	1.0000	0.0000
AAT-3	1.0000	1.0000	0.0000	2.0000	1.0998	0.1914	2.0000	1.0488	0.1125	1.0000	1.0000	0.0000
ADH-1	2.0000	1.3846	0.4506	2.0000	1.4459	0.4869	2.0000	1.4459	0.4869	2.0000	1.4032	0.4620
ADH-2	3.0000	2.5281	0.9908	4.0000	2.9302	1.1484	4.0000	2.9302	1.1484	4.0000	2.9636	1.1500
PRX-1	3.0000	2.6627	1.0275	3.0000	2.8179	1.0638	3.0000	2.8179	1.0638	3.0000	2.3253	0.9637
PRX-2	2.0000	1.0689	0.1461	2.0000	1.0488	0.1125	2.0000	1.0488	0.1125	1.0000	1.0000	0.0000
EST-1	2.0000	1.8000	0.6365	3.0000	2.0656	0.7819	3.0000	2.0656	0.7819	2.0000	1.9850	0.6894
PGM-1	1.0000	1.0000	0.0000	2.0000	1.0488	0.1125	2.0000	1.0488	0.1125	1.0000	1.0000	0.0000
PGM-2	2.0000	1.1421	0.2449	3.0000	1.1011	0.2245	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000
PGM-3	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000
PGM-4	3.0000	1.4019	0.5340	3.0000	2.1940	0.8516	3.0000	2.1940	0.8516	3.0000	1.4756	0.5626
PGI-1	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000	1.0000	1.0000	0.0000
PGI-2	3.0000	1.3158	0.4851	2.0000	1.1529	0.2573	2.0000	1.1529	0.2573	2.0000	1.1888	0.2954
PGI-3	3.0000	1.1450	0.2911	3.0000	1.2149	0.3805	3.0000	1.2149	0.3805	5.0000	1.3127	0.5493
Mean ±	2.0000±	1.3679±	0.3302±	2.3333±	1.4812±	0.3891±	2.1333±	1.4678±	0.3614±	1.9333±	1.3769±	0.3115±
S.E	0.1690	0.1109	0.0700	0.4666	0.1354	0.0775	0.1831	0.1371	0.0815	0.2559	0.1192	0.0791

Table 3. Levels of genetic differentiation of *T. paniculata* populations

Locus	Populations							
	Petchiparai		Sasthakovil		Kodaiyar		Courtallam	
	Ho	***He***	***Ho***	***He***	***Ho***	***He***	***Ho***	***He***
AAT-1	0.0667	0.0667	0.0476	0.0476	0.0000	0.0000	0.0000	0.0000
AAT-2	0.0000	0.0000	0.0476	0.0476	0.0476	0.0476	0.0000	0.0000
AAT-3	0.0000	0.0000	0.0952	0.0929	0.0476	0.0476	0.0000	0.0000
ADH-1	0.3333	0.2874	0.2857	0.3159	0.2857	0.3159	0.3478	0.2937
ADH-2	0.4667	0.6253	0.5238	0.6748	0.5238	0.6748	0.4783	0.6773
PRX-1	0.8000	0.6460	0.7619	0.6609	0.7619	0.6609	0.6957	0.5826
PRX-2	0.0667	0.0667	0.0476	0.0476	0.0476	0.0476	0.0000	0.0000
EST-1	0.5333	0.4598	0.4762	0.5285	0.4762	0.5285	0.3913	0.5072
PGM-1	0.0000	0.0000	0.0476	0.0476	0.0476	0.0476	0.0000	0.0000
PGM-2	0.1333	0.1287	0.0952	0.0941	0.0000	0.0000	0.0000	0.0000
PGM-3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PGM-4	0.3333	0.2966	0.6667	0.5575	0.6667	0.5575	0.3478	0.3295
PGI-1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PGI-2	0.2667	0.2483	0.0476	0.1359	0.0476	0.1359	0.1739	0.1623
PGI-3	0.1333	0.1310	0.1905	0.1812	0.1905	0.1812	0.2609	0.2435
Mean ± S.E	0.2089 ± 0.0485	0.1971± 0.0451	0.2222 ± 0.0516	0.2288 ± 0.0499	0.2095 ± 0.0533	0.2163 ± 0.0518	0.1797 ± 0.0454	0.1864 ± 0.0372

Table 4. Genetic variability at 15 allozyme loci in four populations of *T. paniculata*

Population	***P***	***P* (%)**	***A***	(***Ap***)	(***TA***)	***na***	***ne***	***I***	***Ho***	***He***
Petchiparai	10	66.66	2.00	2.50	30	2.0000 ± 0.1690	1.3679 ± 0.1091	0.3302 ± 0.0700	0.2089 ± 0.0485	0.1971 ± 0.0451
Sasthakovil	13	86.66	2.30	2.50	35	2.3333 ± 0.4666	1.4812 ± 0.1354	0.3891 ± 0.0775	0.2222 ± 0.0516	0.2288 ± 0.0499
Kodaiyar	11	73.33	2.10	2.50	32	2.1333 ± 0.1831	1.4678 ± 0.1371	0.3614 ± 0.0815	0.2095 ± 0.0533	0.2163 ± 0.0518
Courtallam	7	46.66	1.90	3.00	29	1.9333 ± 0.2559	1.3769 ± 0.1192	0.3115 ± 0.0791	0.1797 ± 0.0454	0.1864 ± 0.0372

P: No. of polymorphic loci; *P (%)* percentage of polymorphic loci; A**:** Mean no. of alleles per loci; ***Ap* :** Mean no. of alleles per polymorphic loci; *TA*: Total no. of alleles; *na*: Observed no. of alleles; *ne*: Effective no. of alleles; *I*: Shannon Information Index; *Ho*: Observed heterozygosity; *He*: Expected heterozygosity.

genetically related and geographically close indi-viduals. The pollinators of *T. paniculata* (personal data) have shown that pollinators fly nearest inflorescence while they collect the pollen and nectar.

Sakai et al. (1999) described that the genetic structure of the plant species was influenced by nearest – neighbor pollination by pollinators. They concluded that it increases inbreeding, homozygosity and patchiness in the spatial distribution of genotypes. Thus, pollinator behaviour coupled with the less effective specialized seed dispersal mechanisms could be favouring the establishment of neighborhoods of related individuals.

Implications for conservation

The ability of a population to respond to selection is directly related to the level of genetic variation available for relevant adaptive characters (Huenneke, 1991). Therefore it is expected that species with a narrow gene-tic base will not be able to respond well to changes in abiotic or biotic environmental conditions as species with a broad genetic base. The present investigation reveals that the estimate of allelic diversity and average heterozygosity, *T. paniculata* maintains a significant level of genetic variation in the studied regions of the genome. Furthermore, a large proportion of the alleles identified (28 and 34%) occurs at frequencies below 0.05 at Kodaiyar and Sasthakovil (Table 4). Therefore, *T. paniculata* is especially vulnerable to the loss of allelic richness due to fluctuations in population size (Nei et al., 1975).

Data on the distribution of genetic variation among populations have direct implications for the management

of forest tree species, for instance in suggesting what sampling strategies should be adopted to efficiently sample the genetic pool for *ex-situ* conservation (Ceska et al., 1997 and Ferguson et al., 1998) or in deciding which populations should be enhanced and / or pre-served (Kres et al., 1994; Richter et al., 1994). In Cour-tallam population, three private alleles (PGI-3D, PGI-3E and ADH-2D) from all over the allele frequency were detected (Table 1).

Based on the present investigation related to genetic diversity among the populations for a viable *in situ* programme, the genetic resources of *T. paniculata* are best conserved in Sasthakovil and Kodaiyar population. As a priority, the population at Sasthakovil may be targeted for conservation. This would ensure the conservation of a relatively rich proportion of genetic diversity and presence of private allele representative of that existing in other po-

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