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Research article

Identification and genetic diversity analysis of *Memecylon* species using ISSR, RAPD and Gene-based DNA barcoding tools

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ABSTRACT

Background: *Memecylon* species are commonly used in Indian ethnomedical practices. The accurate identification is vital to enhance the drug's efficacy and biosafety. In the present study, PCR based techniques like RAPD, ISSR and DNA barcoding regions, such as 5s, *psb*A-*trn*H, *rpo*C1, *ndh* and *atp*F-*atp*H, were used to authenticate and analyze the diversity of five *Memecylon* species collected from Western Ghats of India.
Results: Phylogenetic analysis clearly distinguished *Memecylon malabaricum* from *Memecylon wightii* and *Memecylon umbellatum* from *Memecylon edule* and clades formed are in accordance with morphological keys. In the RAPD and ISSR analyses, 27 accessions representing five *Memecylon* species were distinctly separated into three different clades. *M. malabaricum* and *M. wightii* grouped together and *M. umbellatum*, *M. edule* and *Memecylon talbotianum* grouped in the same clade with high Jaccard dissimilarity coefficient and bootstrap support between each node, indicating that these grouped species are phylogenetically similar.
Conclusion: Data from the present study reveals that chloroplast *psb*A-*trn*H region could be used as a potential candidate region for identifying *Memecylon* species, and ISSR marker system could be used for estimating genetic diversity since it has high percent polymorphism compared to RAPD marker.

1. Introduction

The family Melastomataceae consists of about 166 genera and more than 4000 species distributed worldwide. The genus *Memecylon* consists of 300–400 species, distributed in the tropical areas of Asia, Africa and America [1]. *Memecylon* species have great importance in traditional medicine practices in India. In Ayurveda and Siddha system of medicine, several *Memecylon* species are used to treat skin disorders, stomach disorders, herpes, chickenpox, leucorrhoea, polyuria, menorrhagia, dysentery, bacterial infections, inflammations, diabetes and also has antimicrobial, hepatoprotective and antipyretic properties [2,3].

There is a taxonomic ambiguity in identification and distinction of *Memecylon* species viz., *Memecylon malabaricum* Clarke and *Memecylon wightii* Thwaites [4,5,6,7,8]. Saldanha [7] stressed the need for clarification regarding the relationship between *Memecylon amplexicaule* var. *malabarica* and *M. wightii*. Vivekanandan [9] treated *Memecylon umbellatum* and *Memecylon edule* Roxb, as separate species. However, Brandis [6], Neginhal [10] and Pullaiah et al. [11] treated *M. umbellatum* as a synonym of *M. edule*. Bhat [12] mentioned that *M. umbellatum* and *M. edule* treated as co-specific in some of the regional floras [13]. Nomenclature status of most of the Indian *Memecylon* species is not clear (www.plantlist.org) and also complexity exists in morphological characters and identification. Therefore, it has become imperative to study the mechanisms of species diversification of *Memecylon* species [14].

In recent years, to authenticate a plant, a number of single loci and combined loci have been used. Based on the performance of seven plastid DNA regions (*atp*F-*atp*H, *mat*K, *rbc*L, *rpo*B, *rpo*C1, *psb*K-*psb*I, and *psb*A-*trn*H), the Plant Working Group of the Consortium for the Barcode of Life recommended the combination of *rbcL* and *matK* as the plant barcode [15]. The study on 5s IGS region of different species of *Vigna* subgenus *Ceratotropis* [16] was found to be phylogenetically informative to detect intragenic relationship. The chloroplast intergenic spacer *psbA-trnH* has been recommended as an ideal DNA barcode candidate [17]. *rpo*C1, *ndh* and *atp*F-*atp*H are used to evaluate plant phylogeny with low taxonomic variation [18]. DNA markers RAPD and ISSR are used to establish the phylogenetic relationship

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Table 1
List of *Memecylon* accessions collected for the study and their Morphological characters.

Sl no.	Accession code	Plant name	Voucher No	Morphological character for identification	References for morphological identification
1	Mu1-Mu10	*M umbellatum*	#IOELP0001a-#IOELP0001j	Leaves petiolate and ovate-elliptic. The inflorescence is distinctly peduncled and branched.	Saldanha [7]
2	Me11	*M. edule*	#IOELP0001h	Leaves ovate, acute at both ends and nerves obscure. The inflorescence is pedunculate and flowers blue.	Hooker [5]
3	Mm12-Mm20	*M. malabaricum*	# IOELP0003 #IOELP0003a-#IOELP0004h	Branchlets subterete, Leaves subsessile and rounded, cordate at base. Inflorescence of subsessile clades.	Saldanha [7]
4	Mw21-Mw24	*M. wightii*	# IOELP0004 #IOELP0004a-#IOELP0004c	Branchlets winged or angled. Leaves subsessile and cordate at base. Flowers pedicellate and fascicled on short tubercles at leafless nodes.	Ramaswamy [8]
5	Mt25-Mt-27	*M. talbotianium*	#IOELP0002 #IOELP0002a #IOELP0002b	Leaves distinctly petiolate, elliptic, attenuate at the base and the tip acute or acuminate. Inflorescence unbranched, at most subsessile.	Saldanha [7]

among *Bacopa monnieri* [19], *Eucalyptus* [20] *Gaultheria fragrantissima* [21] and to identify the genus/species of the plants at different taxonomic levels.

In the current study, nuclear ribosomal 5s and chloroplast *psb*A-*trn*H, *atp*F-*atp*H, *ndh*, *rpo*C1 sequences are used for validation and identification of *Memecylon* species of Western Ghats. RAPD and ISSR are used to understand the genetic diversity within five *Memecylon* species of the Western Ghats namely *M. umbellatum* Burm., *M. malabaricum* Clarke, *M. wightii* Thwaites, *M. edule* Roxb. and *M. talbotianum* Brandis.

2. Materials and methods

Memecylon species plant samples were collected from different regions of Western Ghats, Karnataka, India. Identification of the plant species based on their morphological characteristics was confirmed by plant taxonomist. A total of 27 accessions representing five species namely *M. umbellatum*, *M. edule*, *M. talbotianum*, *M. malabaricum* and *M. wightii* were collected for this study (Table 1).

2.1. DNA extraction, PCR amplification and DNA sequencing

DNA was isolated using CTAB method [22]. The 5s [16], *psb*A-*trn*H [23], *rpo*C1 and *atp*F-*atp*H [24] and *ndh* gene amplifications were performed as per the protocol (Table S1). The amplified products were sequenced (Chromous Biotech, Bangalore).

The sequences were submitted to the GenBank, NCBI (Table 2). The PCR Protocol was optimized by using varying concentrations of template DNA, dNTPs, Taq DNA polymerase and annealing temperature. For RAPD, the DNA was denatured at 94°C for 3 min followed by 40 cycles of denaturation at 94°C to this and annealed at 36°C. For ISSR, the protocol is similar to RAPD except for the annealing temperature which varied between 36° and 50°C. Amplification products were electrophoresed, and gel images were captured using Gel Doc. 2000, BioRad, California, USA.

2.2. Sequence alignment and analysis

The nuclear 5s and chloroplast *psb*A-*trn*H, *rpo*C1, *ndh*, *atp*F-*atp*H sequences of *Memecylon* species and related genera were obtained from GenBank (Appendix 1). The Codon Code Aligner 3.6.1 Clustal Omega was used to align nuclear and chloroplast DNA sequence data. The different gene datasets were first analyzed with J model test [25] using the Akaike information criterion to find the most appropriate model for DNA substitution. A phylogenetic tree was constructed by the maximum likelihood (ML) in RAxML-HPC2 7.2.8 [26] with a rapid bootstrap analysis using a random starting tree and 100 bootstrap replicates searching for the best maximum-likelihood tree.

RAPD, ISSR bands were scored for 27 samples by visual inspection and fragment sizes were estimated with a medium range ruler (Genei). The scored bands ranged from 100–10,000 bp. The presence or absence of bands was scored as diallelic for each assigned locus (1 = band present; 0 = band absent) and compiled into a matrix. RAPD and ISSR data matrix was constructed containing all scorable bands. The dissimilarity matrix was used to construct a dendrogram using the neighbor-joining method (NJ) with 100 bootstrap replicates. These analyses were carried out using the DARwin 5.0.148 [27]. The similarities between matrices based on different marker systems were calculated using the standardized Mantel co-efficient [28] using the NTSYSpc ver. 2.01 program [29]. For each primer combination Percent polymorphisms were calculated [30].

3. Results

The resulted sequences of the 5s, *psb*A-*trn*H, *rpo*C1, *ndh* and *atp*F-*atp*H were subjected for the BLAST search. The sequences were >80% homologous to their respective gene sequences from *Memecylon* species and other closely related genus belonging to the Melastomataceae family such as *Mouriri*, *Pternandra*, *Miconia*, *Conostegia*, *Syzygium*, *Eucalyptus* and other genus. Besides taxanomical identification, BLAST search helped in further validation of identification of the *Memecylon* species collected from the Western

Table 2
List of GenBank accession numbers of NCBI database obtained for nuclear and chloroplast gene regions of *Memecylon* species.

Sl. no.	Name of the plant species	Nuclear gene	Chloroplast genes			
		5s	*psbA-trnH*	*rpoC1*	*atpF-atpH*	*ndh*
1.	*M. umbellatum*	KF934431.1	KJ488997.1	KF918314.1	KJ406529.1	KJ540941.1
2.	*M. edule*	KF934433.1	KJ488998.1	KF918312.1	KJ412915.1	KJ540942.1
3.	*M. talbotianum*	KF530880.1	KJ488996.1	KF918311.1	KJ412917.1	KJ551840.1
4.	*M. malabaricum*	KF934430.1	KJ488995.1	KF912867.1	KJ412914.1	KJ372212.1
5.	*M. wightii*	KF530881.1	KJ488994.1	KF918313.1	KJ412916.1	KJ372211.1

Ghats. Aligned sequence length of nuclear 5s and chloroplast *psb*A-*trn*H, *rpo*C1, *ndh* and *atp*F-*atp*H was 587, 514, 586, 1086 and 878 base pairs, respectively. *psb*A-*trn*H has a higher percentage of the parsimony informative site followed by *atp*F-*atp*H, 5s, *ndh* and *rpo*C1. In the j model test, 24 models were tested. The best fit models with least likelihood score were screened. 5s, *psb*A-*trn*H, *rpo*C1, *ndh*, and *atp*F-*atp*H have best fit model G + I, F81 + G, HYK, GTR + G, GTR + G, GTR + G respectively.

The nuclear 5s and chloroplast *psb*A-*trn*H, *atp*F-*atp*H gene regions have a higher percentage of parsimony informative sites indicating that these regions are highly variable regions in the Melastomataceae family. *rpoC1* and *ndh* gene region are conserved in *Memecylon* species as indicated by low parsimony informative sites.

From the ML analysis of 5s, *ndh*, *psb*A-*trn*H, *rpo*C1 and *atp*F-*atp*H gene regions it is clear that the bootstrap support greater than 50% was observed in the gene region of *psb*A-*trn*H and *rpo*C1 regions, whereas the bootstrap supports less than 50% was observed in other gene regions such as 5s, *atp*F-*atp*H and *ndh* regions. In 5s, *psb*A-*trn*H, *atp*F-*atp*H, and *rpo*C1 it is seen that Western Ghats accessions have been grouped together to form a clade. In the *ndh* sequence, phylogeny disjunct is seen in Western Ghats species, where *M. umbellatum*, *M. talbotianum*, and *M. edule* group together along with *M. edule* of South East Asia being the sister taxa and *M. wightii* and *M. malabaricum* being sister to *Memecylon bakeriaum* of Madagascar. *Memecylon* species was sister to the *Mouriri crassifolia* of South America in marker *psb*A-*trn*H *and atp*F-*atp*H. Monophyly of the *Memecylon* species is observed in 5s, *rpo*C1 and *ndh* analysis (Fig. 1, Fig. 2 and Table 3).

3.1. RAPD, ISSR, combined analysis

A total of 25 RAPD primers were used for all the accessions. Out of 25 primers, 20 generated amplified products. 16 primers which produced

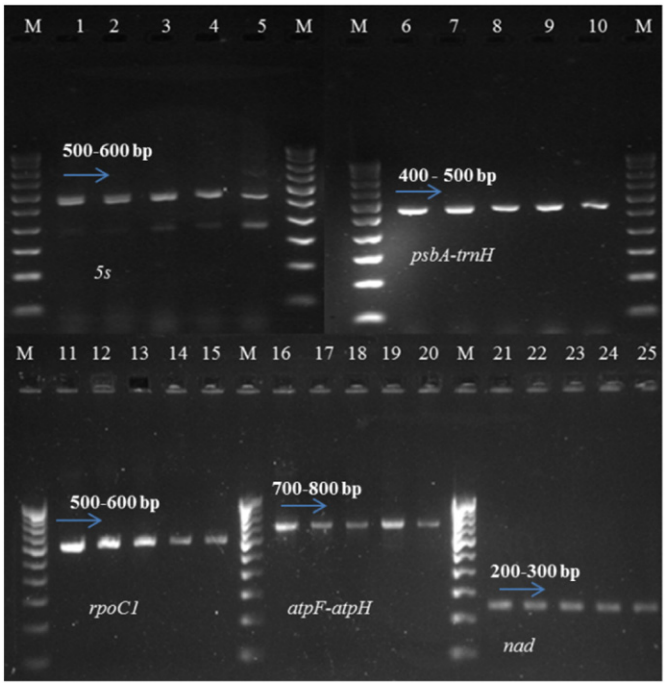

Fig. 2. Amplification of *Memecylon* accessions generated using DNA barcodes such as 5s, *PsbA-trnH rpoC1*, *atpF-atpH* and *ndh* where lane M is 100-1000 bp DNA ladder; lanes 1, 6, 11, 16 and 21 — *M. umbellatum*; lanes 2, 7, 12, 17 and 22 — *M. edule*; lanes 3, 8, 13, 18 and 23 — *M. talbotianum*; lanes 4, 8, 14, 19 and 24 — *M. malabaricum*; lanes 5, 10, 15, 20 and 25 — *M. wightii.*

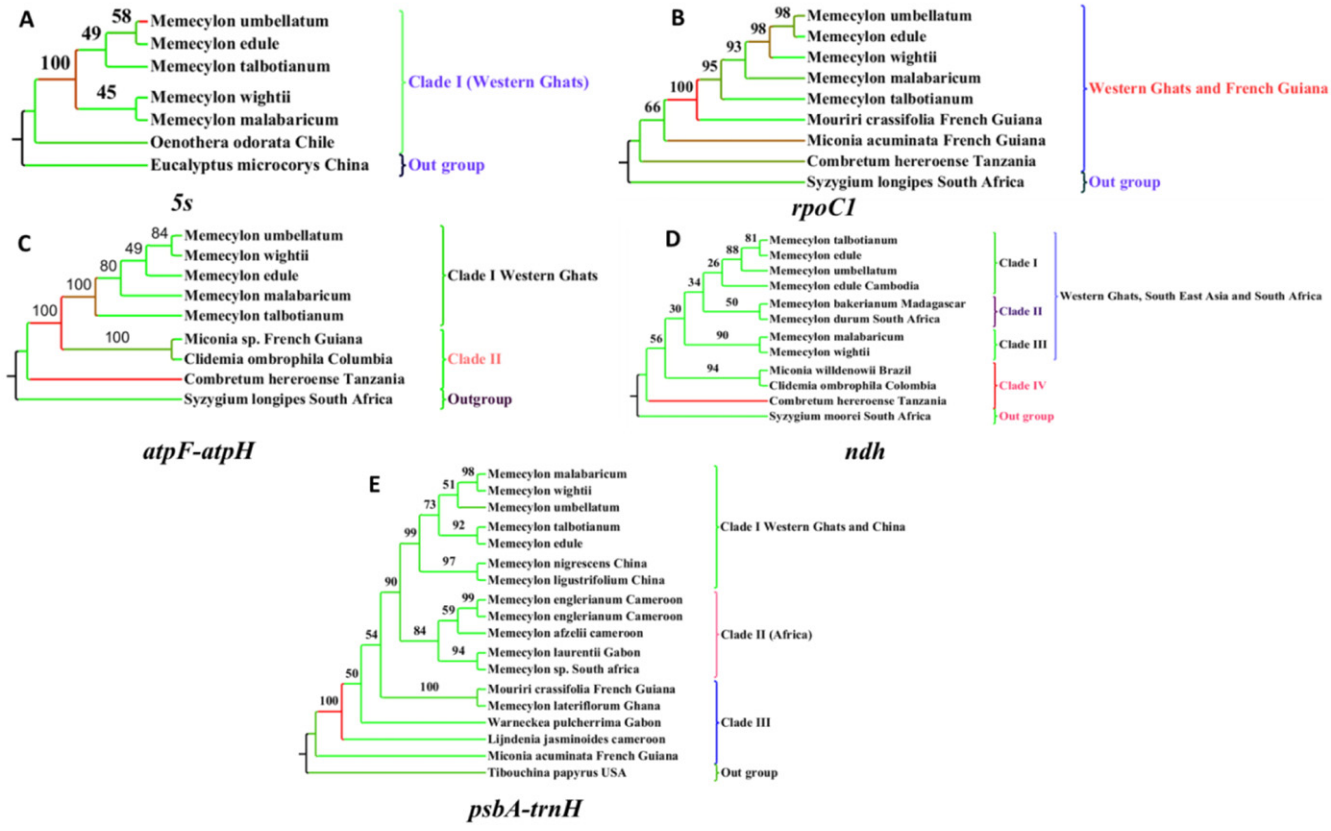

Fig. 1. Phylogenetic trees for the five *Memecylon* species constructed using A — 5s; B — *rpo*C1; C — *atp*F-*atp*H; *D* — *ndh* and E — *psbA-trnH* gene data sets and related sequences obtained from GenBank The tree was rooted with outgroup samples. Bootstrap values higher than or equal to 50% (1000 replicates) are shown at each branches.

Table 3
Sequence length and variation of five candidate sequences of nuclear and chloroplast gene regions.

	5s	*psbA-trnH*	*rpo*C1	*ndh*	*atp*F-*atp*H
Aligned length	587	514	586	1086	878
Parsimony informative sites (in %)	120/587 = 20.44%	134/515 = 26.07%	29/586 = 4.5%	110/1086 = 10.12%	195/878 = 22.55%
Best fit model	G+I	F81+G	HYK	GTR+G	GTR+G
Number of sequences in the alignment	7	18	9	12	9

clear and reproducible bands were selected for further analyses. Similarly, for ISSR analysis 32 primers were initially screened. Of these, 20 primers that produced clearly resolved polymorphic amplified products were used for further analyses.

From RAPD analysis a total of 185 amplicons were produced by 27 *Memecylon* accessions representing five species. Of the 185 amplified bands, 121 were polymorphic, with a mean of 7–8 polymorphic fragments per primer. The percentage polymorphic bands ranged from 69.4–100%. The PIC values, were found in the range from 0.385 to 0.96 with an average 0.86. Similarly, twenty ISSR primers produced, on an average, 308 bands in the accessions examined, of which 211 were polymorphic (Fig. 3 and Table 4).

The unweighted NJ dendrogram resulting from the Darwin program is depicted in Fig. 4**,** Fig. 5 and Fig. 6. Dendrograms based on the RAPD analysis of 27 accessions representing five species of *Memecylon* were grouped into three major clades with an average distance of 0.295 and Jaccard's dissimilarity coefficient ranged from 0.297 to 0.825. Clade I consisted of 13 accessions, which include three species namely, *M. umbellatum*, *M. edule* and *M. talbotianum* (Mu6, Mu13, Mu5, Mu9, Mu4, Mu8, Mu2, Mu7, Mu1, Mu10, Me11, Mt25 and Mt26). Clade II consisted of 10 accessions which include two species namely *M. malabaricum* and *M. wightii* (Mm19, Mm18, Mw23, Mm15, Mw21, Mm20, Mw24, Mw22, Mm17 and Mm14) grouped together with 62–71% dissimilarity. Clade III consisted of three accessions which include two species namely *M. malabaricum* (Mm13 and Mm16) and *M. talbotianum* (Mt27) grouped together with 62–80% dissimilarity. Bootstrap values >86 were observed (Fig. 4).

Dendrogram based on ISSR analysis grouped the accessions into three major clades with an average distance of 0.268 and Jaccard's dissimilarity coefficient ranging from 0.203 to 0.896. Clade I consisted of 10 accessions, which include two species namely, *M. umbellatum* and *M. edule* (Me11) and nine *M. umbellatum* individuals (Mu7, Mu6, Mu9, Mu8, Mu5, Mu4, Mu2, Mu1, Mu3, Mu10 and Me11) grouped together with 26–45% dissimilarity. Clade II consisted of 11 accessions which include *M. malabaricum* accessions (Mm16, Mm15, Mm18, Mm17, Mm12, Mm14, Mm13 and Mm20) grouped together with 73–81% dissimilarity and three individual from *M. wightii* (Mw22, Mw21 and Mw24) grouped together with 49–83% dissimilarity. Clade III consisted of five accessions which include three species namely *M. wightii*, *M. malabaricum* and *M. talbotianum* with one individual from both *M. wightii* (Mw23) and *M. malabaricum* (Mm19) and three individuals of *M. talbotianum* (Mt25, Mt26 and Mt27) grouped together with 50–71% dissimilarity. Bootstrap analysis revealed the very high bootstrap values >71 (Fig. 5).

The NJ analysis of the combined RAPD and ISSR showed three clades. Clade I consisted of total 11 accessions of *M. umbellatum* and *M. edule*, nine *M. umbellatum* accessions (Mu1 to Mu9) and one individual from both *M. umbellatum* (Mu10) and *M. edule* (Me11) grouped together with 32–50% dissimilarity. Clade II consisted of 11 accessions of *M. malabaricum* and *M. wightii*, seven accessions of *M. malabaricum* (Mm12, Mm13, Mm14, Mm15, Mm16, Mm17 and Mm18), three accessions of *M. wightii* (Mw22, Mw24, Mw23) and one accessions of *M. malabaricum* (Mm20) grouped together with 62–67% dissimilarity. In clade III consisted of five accessions of *M. malabaricum*, *M. wightii*, *M. talbotianum*, three individuals of *M. talbotianum* (Mt25, Mt26 and

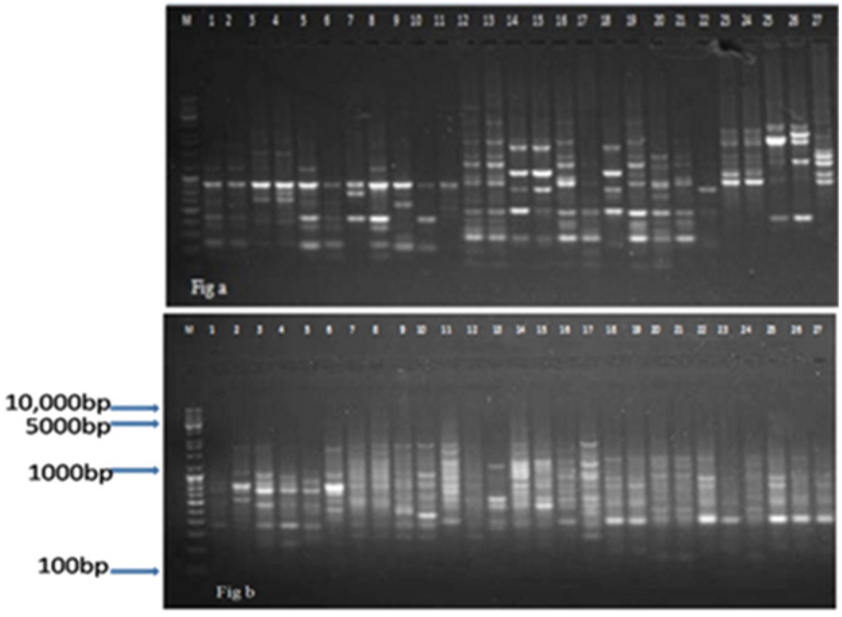

Fig. 3. RAPD and ISSR amplification profiles of *Memecylon* accessions generated using RAPD primer like RFu6 in panel (a), (lanes 1–27) and ISSR primer like (CA) 6AG in panel (b) (lanes 1–27) where lane M is 100–10,000 bp DNA ladder.

Table 4
Summary of genetic diversity obtained through RAPD and ISSR analysis.

Primer type	Sequence (5′-3′)	Amplicon range (Mol. wt)	Annealing temperature (°C)	Total no of bands	Total no of polymorphic bands	% polymorphism	PIC
RAPD analysis							
RFu1	CCTGGGCCAG	600–2500 bp	30	8	6	75	0.838
RFu2	CCTGGGCGAG	300–3000 bp	35	8	5	63	0.851
RFu3	CCTGGGCTGG	200–2500 bp	35	11	7	64	0.893
RFu4	CCTGGGCTAT	300–3000 bp	35	8	3	63	0.385
RFu5	CCTGGGCTTG	400–2500 bp	35	8	8	100	0.97
RFu6	CCTGGGCTAC	600–4000 bp	35	10	5	50	0.899
RFu7	CCTGGGCTTA	400–3000 bp	35	6	4	67	0.82
RFu8	CCTGGGTCGA	300–2500 bp	35	11	3	27	0.918
RFu9	CCTGGGTGCA	100–4000 bp	35	14	8	57	0.926
RFu10	CCTGGCTTGC	400–10,000 bp	35	17	13	76	0.935
RFu11	CCTGGCTTAC	150–8000 bp	35	27	15	56	0.96
RFu12	CCTGGGTTAC	300–6000 bp	35	7	3	43	0.829
RFu13	CGGGGGATGG	100–8000 bp	36	7	4	57	0.857
RFu14	CTCCCTGACC	400–10,000 bp	36	13	11	85	0.91
OPR	GGGGATGGCC	300–4000 bp	36	6	4	67	0.858
OPS	GGGGAAATGG	200–5000 bp	36	24	22	91	0.95
Total				185	121	1041	13.799
Avg				11.56	7.56	69.4	0.86
ISSR analysis							
ISSR1	(GA)6CC	250–5000 bp	40	12	10	83	0.77
2	(CA)6AG	200–10,000 bp	40	17	14	82	0.61
3	(CA)6GT	500–10,000 bp	42	16	16	100	0.93
4	(GACA)4	100–3000 bp	42	10	8	80	0.91
5	(GT)6CC	100–5000 bp	42	5	5	100	0.79
6	(GTG)3GC	100–2500 bp	42	5	3	60	0.79
7	(AG)8T	100–4000 bp	42	12	8	67	0.80
8	(CTC)3GC	150–6000 bp	42	20	14	70	0.94
9	(CA)6GC	150–4000 bp	45	20	9	45	0.94
10	(GA)6GG	150–8000 bp	45	24	20	83	0.95
11	(CA)8A	150–10,000 bp	45	8	6	75	0.87
12	(GGAT)4	200–4000 bp	42	16	12	75	0.90
13	(GAG)3GC	100–3000 bp	47	11	5	45	0.90
14	(AG)8G	100–4000 bp	50	7	3	43	0.85
15	(GA)9C	400–10,000 bp	36	21	13	62	0.92
16	(CT)8GC	150–8000 bp	42	23	11	48	0.95
17	(CA)6 AC	300–6000 bp	42	21	10	48	0.95
18	(CT)8 AC	100–8000 bp	42	26	12	46	0.95
19	(AG)8C	400–10,000 bp	42	9	9	100	0.88
20	(CT)8TC	300–8000 bp	45	25	23	92	0.95
Total				308	211	1404	17.55
Avg				15.4	10.55	70.2	0.87

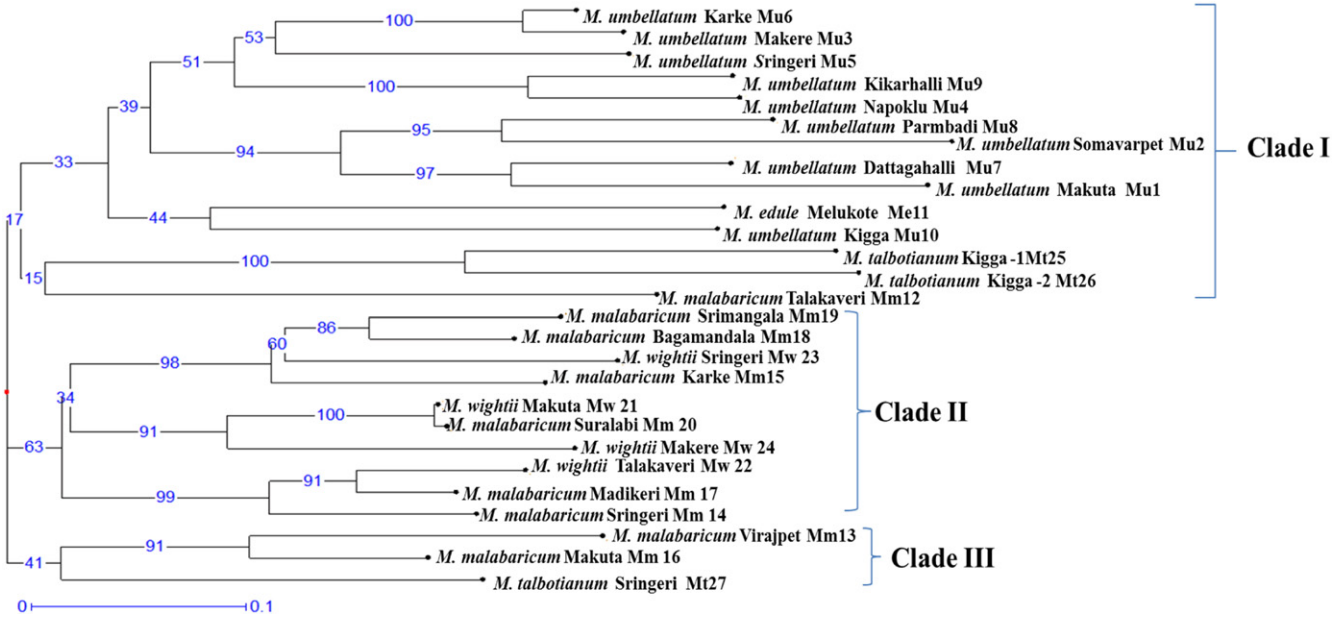

Fig. 4. Dendrogram of 27 *Memecylon* accessions representing five species based on NJ tree with 100 bootstraps clade analysis using the dissimilarity matrix of RAPD markers.

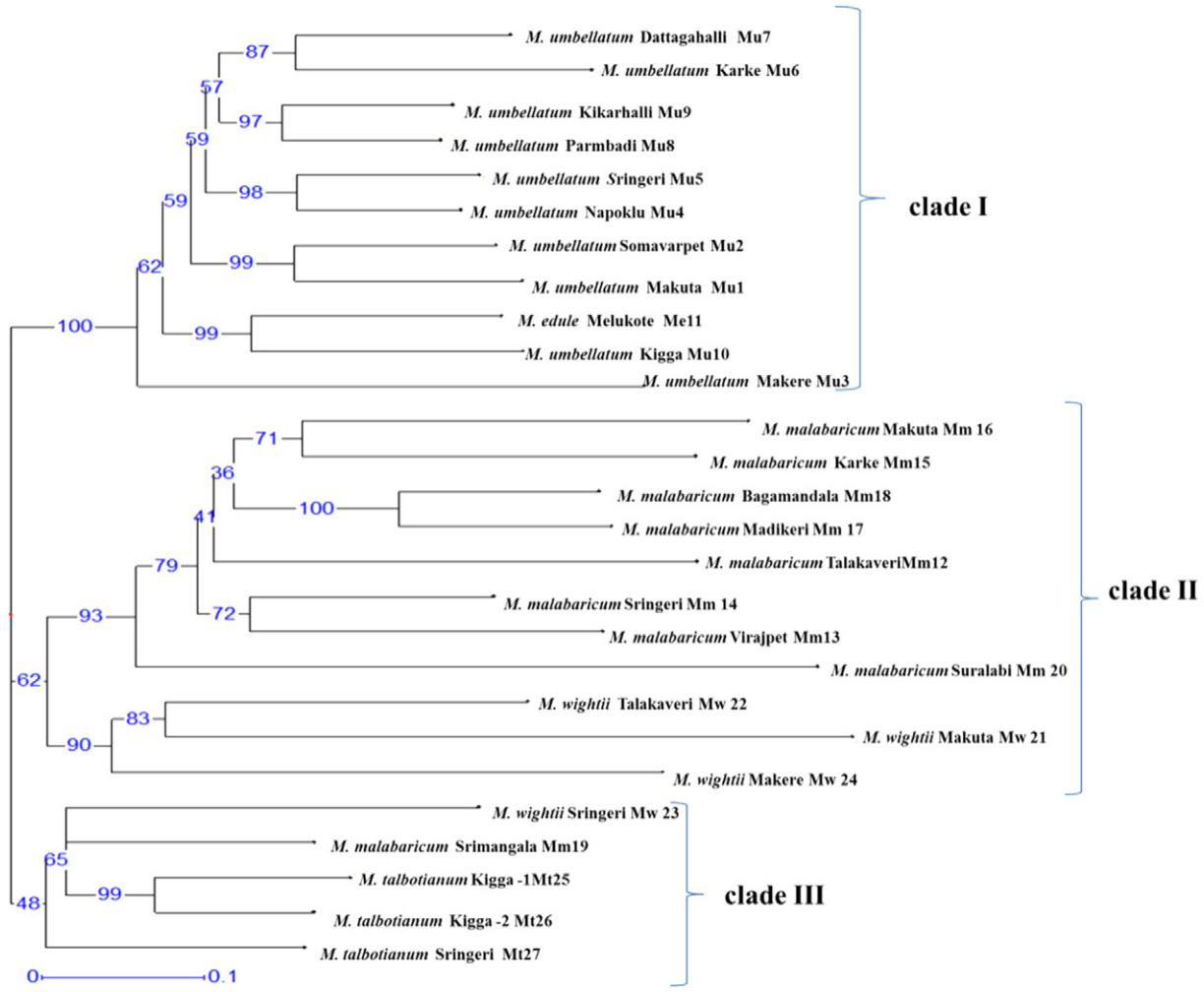

Fig. 5. Dendrogram of 27 *Memecylon* accessions representing five *Memecylon* species based on NJ tree with 100 bootstraps clade analysis using the dissimilarity matrix of ISSR markers.

Mt27) and one individual from both *M. malabaricum* (Mm19) and *M. wightii* (Mw23) grouped together with 55–67% dissimilarity. Bootstrap analysis revealed the very high bootstrap values >50 (Fig. 6).

The percent polymorphisms of ISSR markers scored higher (68.5%) than RAPD (65.4%) and marker index (MI) is 13.39 and 9.89 respectively for ISSR and RAPD. Thus, ISSR is a better marker to evaluate the genetic diversity in *Memecylon* species (Table 5).

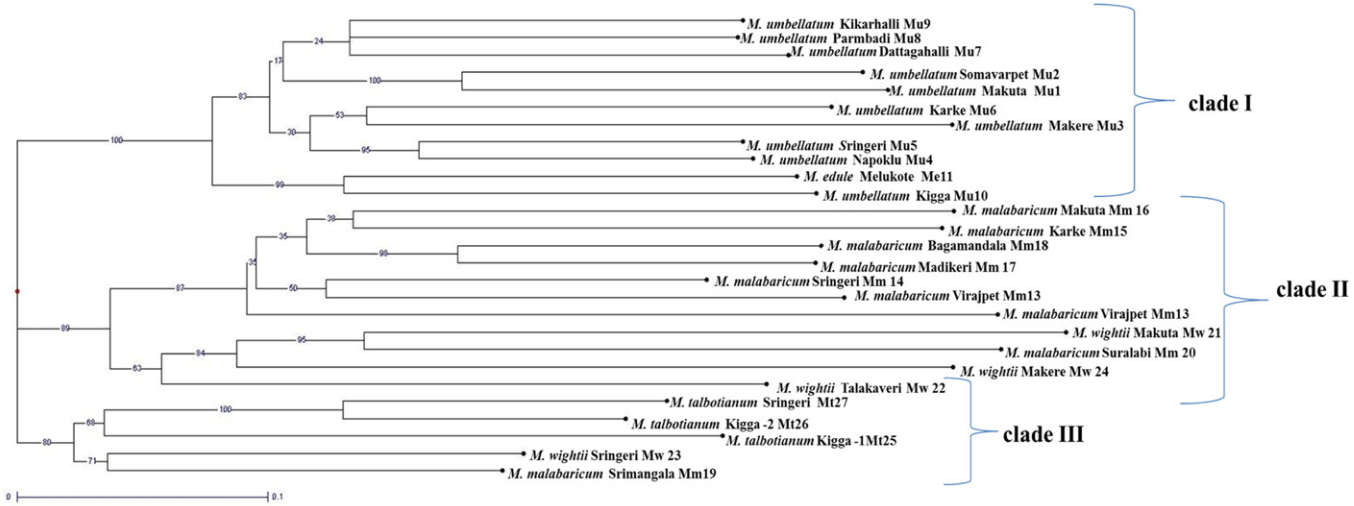

Fig. 6. Dendrogram of 27 *Memecylon* accessions representing five *Memecylon* species based on NJ tree with 100 bootstraps clade analysis using the dissimilarity matrix of a combination of RAPD and ISSR marker.

Table 5
Comparison of RAPD and ISSR molecular markers in evaluating genetic diversity of *Memecylon* species.

Molecular markers	RAPD	ISSR
No of genotypes	27	27
Total no. of bands	185	308
Polymorphic bands	121	211
Total no. of assays	16	20
Percentage polymorphism	65.4	68.5
Multiplex ratio (MR)	11.5	15.4
Average heterozygosity (Hav)	0.86	0.87
Marker index	9.89	13.39

The Mantel test between RAPD and ISSR Jaccard's dissimilarity matrices gave $r^2 = 0.145$, showing low correlation between these markers based on dissimilarities. Grouping of genotypes within groups was not similar in RAPD and ISSR derived dendrogram when compared, whereas the pattern of a grouping of the genotypes remained more or less the same in ISSR and combined data of RAPD and ISSR (Fig. S1, Fig. 4, Fig. 5, Fig. 6, Table S2, Table 4, Table 5).

The comparative analysis of RAPD, ISSR based marker systems revealed ISSR to be the best marker as it generated the highest percentage of polymorphisms, marker index (MI), average heterogeneity (Hav) and multiplex ratio (MR) (Table 5).

4. Discussion

Despite the medicinal importance of the genus *Memecylon*, the information on phylogenetic relationships and genetic diversity is sparse. Identification of *Memecylon* species based on morphology is dynamic due to their close morphological similarities and their broad geographic distribution [31]. While making species determinations in Madagascan *Memecylon* species, both morphological and eco-geographical factors were taken into account because, in several cases, different species have converged on similar vegetative morphologies, leading to taxonomic confusion [32,33].

Memecylon species exhibit simplesiomorphy and synapomorphy. Morphological parameters used in traditional plant systematics caused difficulty in classification when plant such as some *Memecylon* species overlaps with geographical distributions and is similar in gross morphology except certain differences in floral structure [4]. Hence, DNA barcoding such as 5s, *psbA-trnH*, *rpoc1*, *ndh* and *atpF-atpH* regions, RAPD and ISSR genotyping techniques was attempted for measuring genetic variation and determination of genetic relationships among five *Memecylon* species.

So far, authentication and genetic diversity analysis have not been carried out for the selected Indian *Memecylon* species. However, few studies have been carried out on phylogenetic relationship of Melastomataceae family based on combined exon and intron sequences of nuclear glyceraldehyde-3-phosphate dehydrogenase gene and also ITS and ETS marker has been developed for 167 samples of African *Memecylon* species which acts as a key for the authentication of African *Memecylon* species [14].

Although the aligned sequence of marker *psbA-trnH* is the shortest compared to 5s and *atpF-atpH* sequence used in this analysis, *psbA-trnH* has the highest parsimony informative sites. In the *psbA-trnH* it is observed that *Memecylon* species is sister to *Mouriri crassifolia* and closely related to *Lijndenia jasmonoides* and *Warneckea pulcherrima*. Also in *atpF-atpH*, *Memecylon species* is sister to *Mouriri crassifolia*. This observation is in concordance to the observation made by Stone [14]. Therefore, *psbA-trnH* exhibits better discrimination ability of the *Memecylon* species than other markers used in this study.

Memecylon accessions, inferred by both sequence and diversity analysis were in accordance with their morphological characters. *M. malabaricum* is similar to *M. wightii* [4,7] with the only difference in the cylindrical and winged stem. *M. umbellatum* and *M. edule* have common attributes and grouped into same clade indicating that it could be synapomorphic [10,11]. The markers 5s, *psbA-trnH*, *rpoC1* and *ndh* have phylogenetically resolved isolates both within and among *Memecylon* species according to their morphological characters. However, *atpF-atpH* phylogeny did not correlate with morphological traits.

The comparative analysis of RAPD, ISSR based marker systems revealed ISSR to be the best marker as it generated the highest percentage of polymorphisms, MI, Hav and MR. Similar results were observed in *Moringa oleifera* [34]. The dendrogram generated from the binary data matrices of the two marker systems was found highly concordant with each other.

5. Conclusions

The nuclear and chloroplast DNA sequence is useful in examining the genetic variation of complex species at the inter-specific level, and the proposed phylogenetic relationship among *Memecylon* species based on nuclear, chloroplast DNA sequence and Jaccard dissimilarity coefficient values is convincing. Therefore, further work has to be carried out using *ITS*, *matK* and *rbcL* markers in combination including the *Memecylon* species of Western Ghats and compare them with rest of the deposited sequences of other *Memecylon* species from different parts of the world.

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Conflict of interest

The authors declare that there is no conflict of interest.

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