Scientific Bulletin. Series F. Biotechnologies, Vol. XXIX, No. 2, 2025
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

POPULATION GENETIC STUDIES ON *CALAMUS NAGBETTAI* R.R. FERNALD & DEY. IN WESTERN GHATS REGION OF KARNATAKA

Umesh MADAR[1], Rajendra PUJAR[1], Devarajan THANGADURAI[1], Jeyabalan SANGEETHA[2], Pavitra CHIPPALAKATTI[1], Smita SHINDE[1]

[1]Department of Botany, Karnatak University, Pavate nagar Dharwad - 580003, Karnataka, India
[2]Department of Environmental Science, Central University of Kerala, Periye, Kasaragod, Kerala, India

Corresponding author email: umeshkmsep05@gmail.com

Abstract

Calamus nagbettai R.R.Fernald and Dey are spiny climbing palms belonging to the family Arecaceae which are mainly used for making furniture products and handcrafts. DNA markers have been employed to estimate the level of genetic diversity and distinguish the distribution patterns of genetic deviation within the populations. Studies on the genetic dissimilarity in twenty-six populations of Calamus nagbettai have been assisted by the development of molecular markers. Seven Random Amplified Polymorphic DNA (RAPD) markers were utilised to evaluate genetic diversity and genetic relationships between twenty-six accessions of Calamus nagbettai. The primers OPA-03 produced the maximum number of amplicons (260 amplicons), whereas OPA- 06 produced the least number of loci (75 amplicons). Genetic similarity coefficients among 26 wild populations of C. nagbettai ranged from 0.9 to 1.00 which was calculated using Jaccard's similarity coefficient. The dendrogram was constructed for RAPD data for all the 26 accessions of Calamus nagbettai which formed 2 major clusters A and B. The study concludes that RAPD is a potential molecular marker to evaluate the genetic diversity of C. nagbettai species.

Key words*: Calamus nagbettai, genomic DNA, genetic diversity, RAPD markers, Western Ghats.*

INTRODUCTION

Rattan is a unique plant that consists of climbing spiny palms with the diagnostic features of scaly fruits. Rattans are the main source of income for local people, especially those residing near forests (Dransfield and Manokaran, 1994). They are widely distributed throughout Southeast Asia, the Western Pacific, and the moist areas of Africa (Lalnuntluanga and Lalramnghinglova, 2010). They comprised more than 50% of the total palm taxa found in India. Rattans comprise approximately 13 genera with 568 species (Uhl and Dransfield, 1987). The highest number of genera and species is mainly found in Southeast Asia. *Calamus* tended to be the largest genus among the other 13 genera, comprising 375 species. In India, there are four rattan genera, *Calamus*, *Daemonorops, Korthalsia*, and *Plectocomia*, with 60 species that are widely distributed in three major regions: the Western Ghats of Peninsular India, Eastern and Northern India, and Andaman and Nicobar Islands. Tropical evergreen forests are said to be the ideal habitat for rattans, which harbor the majority of species in the Western Ghats region of Peninsular India. Peninsular India comprises one genus, *Calamus*, with 24 species.

Based on species adaptation, they tend to be widely distributed in evergreen, semi-evergreen, and moist deciduous forests. The utilization of rattans in various aspects has led to comprehensive work on canes in various parts of India, especially in the Western Ghats, Andaman, and Nicobar Islands. Furniture made out of rattan species is valued in many countries, and its export from producer countries has steadily increased over the years into a multibillion-dollar business. An increase in the demand for raw materials has resulted in the overexploitation of natural resources. This, along with the change in land-use patterns, has led to the erosion of rattan biodiversity. Approximately 15 *Calamus* species are endemic to the Western Ghats, and *C. nagbettai* is commonly found in the Karnataka and Kerala regions. The species is highly threatened because of its wide-ranging exploitation strategies for economic value. *C. nagbettai* is a spiny climbing cluster in

nature with huge thick rattans with long cirrus. Stems usually long up to 28 m or sometimes even more, if sheaths present measures up to 3.9-4.3 cm, without sheaths will be like 3 cm, black patches at the basal region. Fruits are ovoid with erect scales and are extremely channelled in the centre with a ruminate endosperm. The extensive demand for the furniture industry, which leads to indiscriminate harvesting, often blocks seed propagation and regeneration of the species. Consequently, most species are threatened. Hence, the need for the preservation of these genetic resources is essential for the evaluation of the genetic assortment of rattans. Preservation, fortification, and utilization of genetic resources are essential to guard the species. Several techniques are currently available for estimating genetic variation among wild populations of the species. Random Amplified Polymorphic DNA (RAPD), which is a typical molecular marker, is a powerful technique that can be used to categorize and complete plant genomes or to assess the phylogenetic relationship among genotypes of date palm (Cullis, 2011; Elshibli and Korpelainen, 2011). RAPD has been used for cultivar genotyping (Ben-Abdallah et al., 2000; Trifi et al., 2000) and for analyses of phylogenetic relationships and genetic diversity (Al-Khalifah and Askari, 2003; AI-Moshileh et al., 2004; El-Tarras et al., 2007). Genetic distance estimation assists in studying genetic diversity, a trait that is important for parent selection associated with genetic mapping and marker-assisted selection in breeding programs (Lapitan et al., 2007; Trethowan and Kazi, 2008). In this study, RAPD markers were used to determine the genetic relationships among 26 populations of *C. nagbettai* to evaluate the level of diversity present among species of commercial importance. The specific objective of this study was to characterize *C. nagbettai* genotypes using RAPD markers for future biodiversity conservation strategies and genetic improvement.

MATERIALS AND METHODS

Plant material and DNA isolation

Twenty-six distinct *Calamus nagbettai* populations' leaves were gathered from several sites in the Indian state of Karnataka, including Shivamogga, Subramanya, Agumbe, Dakshina Kannada, and Kodagu. Using the CTAB approach, 1 g of leaf material was used to isolate the whole genomic DNA, which was then ground into a fine powder using liquid nitrogen in a pre-cooled mortar and pestle (Doyle and Doyle, 1990). After that, it was put in Cetyltrimethylammonium Bromide (CTAB) extraction buffer that had been heated beforehand, incubated for 15 to 20 minutes at 65°C, and then allowed to cool to room temperature. Following a gentle inversion and thorough mixing, the 25:24:1 mixture of phenol, chloroform, and isoamyl alcohol was added and centrifuged. After the supernatant was moved to a new tube, 500 µl of cold isopropanol was added to precipitate the DNA. The pellets were centrifuged, then cleaned in 70% ethanol, dried, and dissolved in 1X TE buffer. RNase treatment eliminated the RNA. On a 1% agarose gel, the integrity and amount of isolated DNA were quantified visually and estimated using spectrophotometry (Figures 1 and 2).

Calamus nagbettai, a vulnerable species of Subramanya range mainly concerned with developing a conservation and utilization strategy. It is the prominent criteria to know the genetic variation and relatedness of *C. nagbettai*. The present study was carried out to analyze the genetic variation among *C. nagbettai* (26 accessions) collected from varied geographical regions of Western Ghats of Karnataka (Table 1).

Table 1. List of 26 populations of *Calamus nagbettai* Location, Accession name and GPS coordinates

Collection Area	Accession Name	Accessions	Latitude	Longitude
Agumbe Pond, Shivamogga	Agu	4	13.5027° N	75.0903° E
Subramanya, Dakshina Kannada	Sub	5	12.6695° N	75.6106° E
Subramanya-Dharmasthala, Dakshina Kannada	Sdh	3	12.9493° N	75.3791° E
Subramanya Road, Dakshina Kannada	Sro	3	12.8438° N	75.2479° E
Jogigundi, Agumbe	Jog	3	13.4854° N	75.1076° E
Sampaje, Kodagu	Sam	4	12.4973° N	75.5568° E
Karike, Kodagu	Kar	4	12.4397° N	75.4228° E

Figure 1. *Calamus nagbettai*: A - Habit.; B - Stem/Leaf sheath; C - Cirrus leaf modification; D - Fruit

DNA amplification conditions and gel electrophoresis

Ten primers were selected based on the number of polymorphic bands of the RAPD primers (Table 2) (GeNei^{TM}, Bangalore, India). DNA amplification was carried out in 20 µl reaction volume containing 50 ng genomic DNA, 10X Taqbuffer with 1.0 µl $MgCl_2$, 2.5 mM each dNTPs, 10 pM primer, and 3 units of TaqDNA polymerase (GeNei TM, Bangalore, India). PCR reaction was programmed to fulfil 38 cycles (for RAPD analysis) after an initial denaturation cycle for a 1 min at 95ºC which is followed by 1 min annealing temperature at 32-34ºC, for the extension factor again for 1 min at 72ºC which is further followed by final extension of 5 min at 72ºC to make certain completeness of the primer extension. Soon after completion of PCR, the end product was preserved at 4ºC until the gel electrophoresis was performed. The amplified products of each were size fractionated by electrophoresis on a 1.5% agarose gel with 0.5 µg/ml ethidium bromide 1X TBE buffer and visualized on UV transilluminator (120027GB Bangalore GeNei) and photographed. Each primer was tested three times, and the primers that produced repeatable fingerprints were taken into consideration for data analysis.

Table 2. List of 10 RAPD primers used for amplification with RAPD motifs and annealing temperature

Primers	Primer Sequence	Annealing Temperature (°C)
OPA-01	CAGGCCCTTC	34.0
OPA-02	TGCCGAGCTG	34.0
OPA-03	AGTCAGCCAC	32.0
OPA-04	AATCGGGCTG	32.0
OPA-05	AGGGGTCTTG	32.0
OPA-06	GGTCCCTGAC	34.0
OPA-07	GAAACGGGTG	32.0
OPA-08	GTGACGTAGG	32.0
OPA-09	GGGTAACGCC	34.0
OPA-10	GTGATCGCAG	32.0

RAPD data analysis

The banding patterns obtained from RAPD were represented as present (1) or absent (0), each of which was considered to be the independent characters in spite of its intensity. Jaccard's coefficient of similarity was used to create pair-wise similarity matrices using the NTSYS-pc SIMQUAL format. The similarity matrix was subjected to cluster analysis by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and a dendrogram was generated using the programme.

RESULTS AND DISCUSSIONS

A RET species' loss of genetic diversity can be mitigated by preserving high priority populations that were identified using molecular data. These populations can be chosen from a small number and should be protected. As a result, genetic markers have proven to be effective tools for defining species. Yet, each genetic marker has unique characteristics that must be taken into consideration. Nuclear DNA regions can be screened and analysed with great resolution using Random Amplified methods like RAPD. The RAPD markers' straightforward laboratory assay makes them a more widely used tool for obtaining intraspecific differences. Because they are quick and simple to use, and universal primers are widely available, random amplified polymorphic RAPD (a dominant DNA marker)

has become more popular in recent years as a technique for numerous sorts of genetic research on vegetative crops (Gillies et al., 1997). Several writers have already discussed the impact of various molecular marker reactions for varietal identification and species determination (Bellamy et al., 1996; Koch and Jung, 1997; Stallen et al., 2001; Van Cutsem et al., 2003). On the other hand, the preservation of genetic variety within the species is a fundamental goal of conservation because it is believed that loss of genetic variation reduces populations' capacity to adapt to environmental changes for survival (Hogbin and Peakall, 1999; Honjo et al., 2004; Yamagishi et al., 2010). Also, using these markers is very instructive and may be utilised to fix any issues that may arise from the debate over the gene and the species employed. As a result, the data produced by this study provide a clear picture of the genetic relationships between the aforementioned species, which suggests the significance of genetic diversity conservation within and among populations as well as management practises.

A total of 10 RAPD primers were screened out of which 7 primers were proved to be generating the amplicons. The primers OPA-01, OPA-02, OPA-03, OPA-04, OPA-06, OPA-07, OPA-08, were capable of generating bands in 26 populations of *C. nagbettai*. The primers OPA-03 produced the highest number of amplicons (260 amplicons), which was followed by OPA-04 (252 amplicons), OPA-02 (187 amplicons), OPA-01 (147 amplicons) (Figure 3), OPA-07 (130 amplicons) and OPA-08 is having (130 amplicons), whereas OPA-06 produced least number of loci (75 amplicons). With an average of 6-7 bands per primer, those primers produced 48 bands in total. The primer OPA-02 produces DNA bands with an average molecular weight of 200 to 2100 bp, while the primer OPA-01 can construct DNA bands with a molecular weight range of 400 to 1600 bp. The primer OPA-03 with 200 to 2200 bp, the primer OPA-04 creates 200 to 2100 bp, OPA-07 produces 200 to 2000 bp, OPA-08 has 100 to 1900 bp. The similarity matrix was calculated using Jaccard's similarity coefficient (Jaccard, 1908) for the pooled data of all RAPD primers. Genetic similarity coefficient among 26 wild populations of *C. nagbettai* ranged from 0.91 to 1.00 (Figure 3; Table 3). The dendrogram constructed using RAPD data from accessions of *Calamus nagbettai* was primarily formed of 2 major clusters of 26 accessions of *Calamus* species (Figure 4). The Cluster A consists of five accessions and in Cluster B remaining 18 accessions was found. The five accessions from Cluster A were basically collected from Sampage Forest Division and had genetic similarity of 0.93. The major Cluster B was further divided into two sub-clusters (Cluster C and Cluster D), one with Cluster C having 12 species which is again further divided into Cluster E and F. Cluster E comprises of eight populations which are collected from Agumbe Forest Division (AguA, AguB and AguC, AguD), Subramanya Forest Division (SubG, SubL), Subramanya-Dharmastala Forest Division (SdhJ, SdhK) the coefficient ranges at 0.978. The Cluster F comprises four populations all are collected from Subramanya region (SubE, SubF, SubH, SubI) at 0.972 coefficient value. The Cluster D was formed at level 0.976 with that of populations isolated from Sringeri and Karike two entirely different geographical regions, which shows more similarity due to some external factors which are equivalent to each other (SroM, SroO, SroN, KarW, KarZ, KarY). Subsequently, accessions of Jogi-gundi Forest were segregated from the rest of the accessions and forms a separate Cluster G at the level of genetic similarity of 0.93 (JogP, JogQ, JogR) as shown in the dendrogram constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). This shows that the *C. nagbettai* collected from varied regions shows more similarity to one another; generally, all accessions of a particular species formed compact clusters in the dendrogram.

Table 3. Similarity matrix of 26 populations of *Calamus nagbettai* for Jaccard's coefficient based on RAPD study

Accession	AguA	AguB	AguC	AguD	SubE	SubF	SubG	SubH	SubI	SdhJ	SubK	SubL	SroM	SroN	SroO	JogP	JogQ	JogR	SamS	SamT	SamU	SamV	SamW	KarX	KarY	KarZ
AguA	1.00																									
AguB	1.00	1.00																								
AguC	1.00	1.00	1.00																							
AguD	1.00	1.00	1.00	1.00																						
SubE	0.95	0.95	0.95	0.95	1.00																					
SubF	0.95	0.95	0.95	0.95	1.00	1.00																				
SubG	0.97	0.97	0.97	0.97	0.97	0.97	1.00																			
SubH	0.95	0.95	0.95	0.95	1.00	1.00	0.97	1.00																		
SubI	0.95	0.95	0.95	0.95	1.00	1.00	0.97	1.00	1.00																	
SdhJ	0.97	0.97	0.97	0.97	0.97	0.97	1.00	0.97	0.97	1.00																
SubK	0.97	0.97	0.97	0.97	0.97	0.97	1.00	0.97	0.97	1.00	1.00															
SubL	0.97	0.97	0.97	0.97	0.97	0.97	1.00	0.97	0.97	1.00	1.00	1.00														
SroM	0.93	0.93	0.93	0.93	0.93	0.93	0.95	0.93	0.93	0.95	0.95	0.95	1.00													
SroN	0.93	0.93	0.93	0.93	0.93	0.93	0.95	0.93	0.93	0.95	0.95	0.95	1.00	1.00												
SroO	0.93	0.93	0.93	0.93	0.93	0.93	0.95	0.93	0.93	0.95	0.95	0.95	1.00	1.00	1.00											
JogP	0.91	0.91	0.91	0.91	0.91	0.91	0.93	0.91	0.91	0.93	0.93	0.93	0.93	0.93	0.93	1.00										
JogQ	0.91	0.91	0.91	0.91	0.91	0.91	0.93	0.91	0.91	0.93	0.93	0.93	0.93	0.93	0.93	1.00	1.00									
JogR	0.91	0.91	0.91	0.91	0.91	0.91	0.93	0.91	0.91	0.93	0.93	0.93	0.93	0.93	0.93	1.00	1.00	1.00								
SamS	0.93	0.93	0.93	0.93	0.93	0.93	0.95	0.93	0.93	0.95	0.95	0.95	0.91	0.91	0.91	0.93	0.93	0.93	1.00							
SamT	0.93	0.93	0.93	0.93	0.93	0.93	0.95	0.93	0.93	0.95	0.95	0.95	0.91	0.91	0.91	0.93	0.93	0.93	1.00	1.00						
SamU	0.93	0.93	0.93	0.93	0.93	0.93	0.95	0.93	0.93	0.95	0.95	0.95	0.91	0.91	0.91	0.93	0.93	0.93	1.00	1.00	1.00					
SamV	0.93	0.93	0.93	0.93	0.93	0.93	0.95	0.93	0.93	0.95	0.95	0.95	0.91	0.91	0.91	0.93	0.93	0.93	1.00	1.00	1.00	1.00				
SamW	0.93	0.93	0.93	0.93	0.93	0.93	0.95	0.93	0.93	0.95	0.95	0.95	0.91	0.91	0.91	0.93	0.93	0.93	1.00	1.00	1.00	1.00	1.00			
KarX	0.95	0.95	0.95	0.95	0.95	0.95	0.97	0.95	0.95	0.97	0.97	0.97	0.97	0.97	0.97	0.95	0.95	0.95	0.93	0.93	0.93	0.93	0.93	1.00		
KarY	0.95	0.95	0.95	0.95	0.95	0.95	0.97	0.95	0.95	0.97	0.97	0.97	0.97	0.97	0.97	0.95	0.95	0.95	0.93	0.93	0.93	0.93	0.93	1.00	1.00	
KarZ	0.95	0.95	0.95	0.95	0.95	0.95	0.97	0.95	0.95	0.97	0.97	0.97	0.97	0.97	0.97	0.95	0.95	0.95	0.93	0.93	0.93	0.93	0.93	1.00	1.00	1.00

Due to the lack of valuable breeding systems in rattans the genetic mapping of the species is restricted and back-crossing is a difficult process. Hence, to our knowledge, nothing along these lines has been done. Many techniques are useful for at least attempting to identify genetic traits in rattans. The use of molecular markers was introduced to complement other well-developed techniques such as micropropagation and to develop objective tools for genotype diversity assessment (Gielis, 1995). RAPD markers are one of the efficient and inexpensive ways to provide molecular data. They have been successfully used in determining the genetic relationship of the species and used for DNA fingerprinting (Moreno et al., 1998). The first application of RAPD markers to characterize the genetic diversity was performed on *Dendrocalamus asper* in Indonesia which proved to be a phenomenal technique for analyzing genetic diversity of the species. The minimal genetic diversity of the species is explained by the fact that it has an asexual reproduction mechanism (Hamrick et al., 1992; Lee et al., 2000). The RAPD technique produced high polymorphism information content. Thus, the present investigation demonstrated the reliability of RAPD technique in determining the relationships in this plant group with high commercial importance and of 26 accessions of *Calamus nagbettai* which were obtained from different geographic locations i.e., Shivamogga, Subramanya, Agumbe, Dakshina Kannada and Kodagu. The present results warrant an extensive survey of genetic variation between the given populations of *Calamus nagbettai* using RAPD markers clarifies the phylogenetic relationships among the studied species for evaluating a relatively high level of polymorphism. However, our current study also showed that applicable molecular methods are useful in characterization at a biological level. This report will help us to find strategies for marker-assisted identification of *Calamus* genotypes in order to conserve *Calamus* genetic resources for different inbreeding approaches.

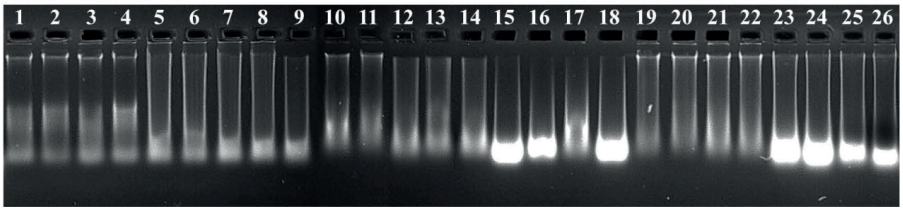

Figure 2. Genomic DNA of 26 populations of *Calamus nagbettai*:
1. AguA, 2. AguB, 3. AguC, 4. AguD, 5. SubE, 6. SubF, 7. SubG, 8. SubH, 9. Subl, 10. SdhJ, 11. SdhK, 12. SdhL, 13. SroM, 14. SroN, 15. SroO, 16. JogP, 17. JogQ, 18. JogR, 19. Sams, 20. SamT, 21. Samu, 22. SamV, 23. SamW, 24. KarX, 25. KarY, 26. KarZ

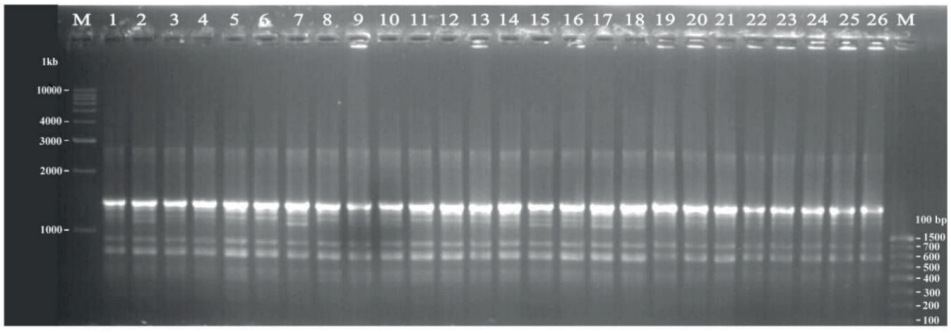

Figure 3. RAPD marker profile of 26 *Calamus nagbettai* populations produced by primer OPA-01 and ladder (Sigma USA):
1. AguA, 2. AguB, 3. AguC, 4. AguD, 5. SubE, 6. SubF, 7. SubG, 8. SubH, 9. SubI, 10. SdhJ, 11. SdhK, 12. SdhL, 13. SroM, 14. SroN, 15. SroO, 16. JogP, 17. JogQ, 18. JogR, 19. SamS, 20. SamT, 21. SamU, 22. SamV, 23. SamW, 24. KarX, 25. KarY, 26. KarZ

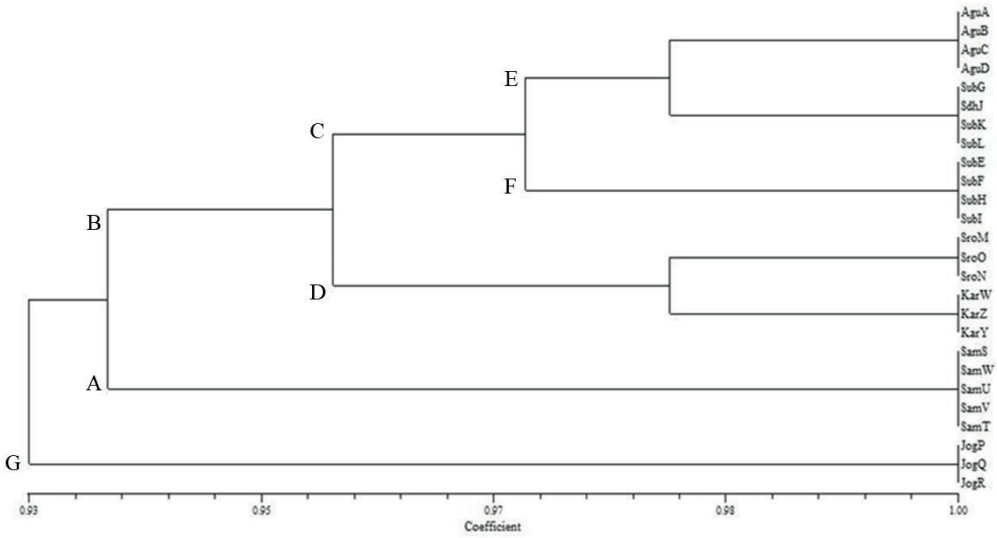

Figure 4. UPGMA dendrogram based on RAPD data for the studied populations of *Calamus nagbettai*

CONCLUSIONS

Accurate assessment of genetic diversity is an essential criterion for the establishment of appropriate conservation strategies, of a particular plant species.

The present study revealed the genetic variability among *C. nagbettai* in the natural population using RAPD markers which might be useful for further studies in the area of molecular characterization.

Over all, RAPD markers proved to be a fast, efficient technique for variability assessment that complements methods currently being used in genetic resources management and in plant systematics to confirm morphological differences among populations and/or to differentiate apparently similar populations.

The results of this study can be used as a baseline of relationships for future diversity assessment and genetic analysis of rattans in Southern India.

Using this marker is highly instructive as well as having the potential to alleviate any issues that may arise from the debate over the gene and the species employed.

Because of this, the data produced by this study provide a clear picture of the genetic relationship of the *C. nagbettai*, which implies significance for the preservation of genetic diversity within and among populations as well as management strategies.

ACKNOWLEDGEMENTS

First author thanks Dr. Pramod Gai, Director, and Dr. Suyaminder, Scientist, Karnataka Institute for DNA Research for permitting to utilize the institute's facilities and for their kind help and guidance during this research work. This work was supported by the Karnatak University in the form of UGC-UPE fellowship.

REFERENCES

Al-Khalifah, N.S., & Askari, E. (2003). Molecular phylogeny of date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia by DNA fingerprinting. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *107*(7), 1266–1270. https://doi.org/10.1007/s00122-003-1369-y

AI-Moshileh, A., Motawei, M., AI-Wasel, A., & Abdel-Latif, T. (2004). Identification of some date palm (*Phoenix dactylifera* L.) cultivars in Saudi Arabia using RAPD fingerprints. *Journal of Agricultural and Marine Sciences*, *9*(1), 1–3. https://journals.squ.edu.om/index.php/jams/article/view/617

Bellamy, A., Vedel, F., & Bannerot, H. (1996). Varietal identification in *Cichorium intybus* L. and determination of genetic purity of F1 hybrid seed samples, based on RAPD markers. *Plant Breeding*, *115*(2), 128–132.

Ben-Abdallah, A., Stiti, K., Leoivre, P., & Jardin P.D. (2000). Date palm (*Phoenix dactylifera* L.) cultivar identification using Random Amplified

Polymorphic DNA (RAPD). *Cahiers Agricultures*, *9*, 103–107

Cullis, C. (2011). Molecular markers in date palm. In: Jain, S.M., Al-Khayri, J.M. and Johnson, D.V., (Eds). *Date Palm Biotechnology.* pp. 361–370. Springer, Dordrecht.

Doyle, J.J., & Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*(1), 13–15.

Dransfield, J., & Manokaran, N. (1994). *Plant Resources of South-East Asia No 6: Rattans*. Prosea Foundation, Bogor, Indonesia.

Elshibli, S., & Korpelainen, H. (2011). Biodiversity in date palm: Molecular markers as indicators. In: Jain, S.M., Al-Khayri, J.M., & Johnson, D.V. (Eds). *Date Palm Biotechnology*. pp. 371–406. Springer, Dordrecht. doi:10.1007/978-94-007-1318-5_19

El-Tarras, A., Al-Tawatti, N., & Al-Malki, F. (2007). Genetic fingerprinting of some KSA cultivars using modern biotechnological techniques. *Biotechnology*, *6*, 263–267.

Gielis, J. (1995). Bamboo and biotechnology. *European Bamboo Society Journal*, *6*, 27–39.

Gillies, A.C.M., Cornelius, J.P., Newton, A.C., Navarro, C., Hernaandez, M., & Wilson, J. (1997). Genetic variation in Costa Rican populations of the tropical timber species *Cedrela odorata* L. assessed using RAPDs. *Molecular Ecology*, *6*, 1133–1146.

Hamrick, J.L., Godt, M.J.W., Sherman-Broyles, S.L. (1992). Factors influencing levels of genetic diversity in woody plant species. *New Forest*, *6*, 95–124.

Hogbin, P.M., & Peakall, R. (1999). Evaluation of the contribution of genetic research to the management of the endangered plant *Zieria prostrata*. *Conservation Biology*, *13*, 514–522.

Honjo, M., Ueno, S., Tsumura, Y., Washitani, I., Ohsawa, R. (2004). Phylogeographic study based on intraspecific sequence variation of chloroplast DNA for the conservation of genetic diversity in the Japanese endangered species *Primula sieboldii*. *Biological Conservation*, *120*, 211–220.

Koch, G., & Jung, C. (1997). Phylogenetic relationships of industrial chicory varieties revealed by RAPDs and AFLPs. *Agronomie*, *17*, 323–333.

Lalnuntluanga, L.K.J., & Lalramnghinglova, H. (2010). Status of research on rattans: A review. *Science Vision*, *10*(2), 51–56.

Lapitan, V.C., Brar, D.S., Abe, T., & Edilberto, D.R. (2007). Assessment of genetic diversity of Philippine rice cultivars carrying good quality traits using SSR markers. *Breeding Science*, *57*, 263–270.

Lee, S.L., Wickneswari, R., Mahani, M.C., & Zakri, A.H. (2000). Genetic diversity of *Shorea leprosula* Miq. (Dipterocarpaceae) in Malaysia: Implication for conservation of genetic resources and tree improvement. *Biotropica*, *32*, 213–224.

Moreno, S., Martin, J.P., & Ortiz, J.M. (1998). Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. *Euphytica*, *101*, 117–125.

Stallen, N.V., Noten, V., Neefs, V., Proft, M. D. (2001). The phylogenetic relationship between different *Cichorium intybus* cultivars and cultivar groups, as revealed by RAPDs. *Plant Breeding*. *120*(5), 425–428. https://doi.org/10.1046/j.1439-0523.2001.00633.x

Trethowan, R.M., & Kazi, A.M. (2008). Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat. *Crop Science*, *48*, 1255–1265.

Trifi, M., Rhouma, A. & Marrakchi, M. (2000). Phylogenetic relationships in Tunisian date palm (*Phoenix dactylifera* L.) germplasm collection using DNA amplification fingerprinting. *Agronomie*, *20*, 665–671.

Uhl, N.W., & Dransfield, J. (1987). Genera palmarum: A classification of palms based on the work of Harold E. Moore, Jr. *The L. H. Bailey Hortorium and the International Palm Society*. Allen Press, Lawrence, Kansas. pp. 610.

Van Cutsem, P., Du Jardin, P., Boutte, C., Beauwens, T., Jacqmin, S., & Vekemans, X. (2003). Distinction between cultivated and wild chicory gene pools using AFLP markers. *Theorotical and Applied Genetics*, *107*, 713–718.

Yamagishi, M., Nishioka, M., & Kondo, T. (2010). Phenetic diversity in the *Fritillaria camschatcensis* population grown on the Sapporo campus of Hokkaido University. *Landscape and Ecological Engineering*, *6*, 75–79. 10.1007/s11355-009-0084-5.