*Asian Journal of Biology*

*6(2): 1-11, 2018; Article no.AJOB.41418*
*ISSN: 2456-7124*

# Analysis of Genetic Diversity among the Five Species of *Calamus* by RAPD Markers

**D. H. Tejavathi[1*], R. V. Madhusudhan[1], H. R. Raveesha[1], A. C. Lakshmana[2], R. Nijagunaiah[1] and B. S. Sumalatha[1]**

*[1]Department of Botany, Bangalore University, Bangalore 560 056, India.*
*[2]Forest and Environment, Govt. of Karnataka, Bangalore, 560 001, India.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author DHT designed the study, analyzed the data and wrote the draft of the manuscript. Author RVM performed the experiments. Authors HRR and BSS assisted in conducting the experiments and in literature survey. Author ACL assisted in collection and identification of taxa selected for the present study. Author RN performed the statistical analysis. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/AJOB/2018/41418
Editor(s):
(1) Vanessa da Silva Carrara, Department of Pharmacy, State University of Maringa, Brazil
Reviewers:
(1) Zeynel Dalkiliç, Adnan Menderes, Turkey.
(2) Ashwani Kumar, Chaudhary Charan Singh University, India.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/24847

***Original Research Article***

***Received 24th February 2018***
***Accepted 3rd May 2018***
***Published 28th May 2018***

## ABSTRACT

**Aims:** the aim of the present study was to determine the genetic diversity among the five commercially important species of *Calamus* using Random Amplified Polymorphic DNA (RAPD) markers.
**Study Design:** DNA extracted from mature leaves of selected species was used as template DNA for optimization of PCR amplification. Data was analysed with the help of software; numerical taxonomy and multivariate analysis version 2.02e (NTSYSpc) and unweighted pair group method with arithmetic mean (UPGMA).
**Place and Duration of Study:** The study was carried out at the Department of Botany, Bangalore University, Bangalore 560056, India during 2014.
**Methodology:** *Calamus huegelianus, C. nagabettai, C. prasinus, C. thwaitesii* and *C. vattayila*, known for their good quality canes, are selected for the present study to characterize them through RAPD analysis. Fifteen decamer primers were used for amplification. However, only five primers

**Corresponding author: E-mail: tejavathi_hanu@yahoo.com;*

have generated clear reproducible bands. Simple matching, similarity co-efficient, dendrogram and principal component analysis were composed by using NTSYSpc and UPGMA.

**Results:** Clear and reproducible 108 bands were scored with 64 polymorphic bands indicating 59.25 percent of polymorphism among the species selected. Higher percent of 76.00 was noted in *Calamus prasinus* and least of 43.67 in *C. huegelianus*. Similarity co-efficient value of 0.45 to 0.66 indicates less genetic divergence among the taxa selected. The cluster analysis with the help of dendrogram and principal component analysis has revealed that *C. vattayila* is distinct from others since it has 6 veined leaflets. The other selected four taxa are having three veined leaflets. Hence, clustering pattern was compared with distinguished phenotypic characters.

**Conclusion:** The previously mentioned data obtained through cluster, the principal component analysis clearly indicate that the taxa are clustered together based on sharing of common phenotypic characters. The present study is a step forward in understanding the genetic affinities among the selected taxa and their conservation strategies.

*Keywords: Calamus; polymorphism; RAPD; rattans.*

## 1. INTRODUCTION

*Calamus* is one of the important non-timber products (NTFP) of tropical forests of India categorised under Rattans along with other three genera; *Daemonorops, Korthalsia* and *Plectocomia* of the family Arecaceae. For years, rattans have played a major role in providing job opportunities and enhancing the economic situation of both tribal and other forest dwelling communities. It is estimated that more than half a million people are directly employed in harvesting and processing of rattans in the rural areas of South-East Asian countries [1,2]. Most of the raw materials for local processing and for supplying the rattan industry are still obtained by harvesting of unmanaged, wild rattan resources in natural tropical forests. Hence, both government and non-government organizations formulated several action programmes and strategies for the baseline information on conservation, sustainable management of genetic resources and utilization. Seed is the best source of propagation in these taxa, but its availability on a large scale is dependent on certain factors. Extraction of the plants before flowering and destruction of the natural habitats have led to the depletion of the germplasm of these taxa.

*Calamus* L. popularly known as cane is represented by 370 species distributed in three major geographical regions in India – Western Ghats of peninsular region, Sub-Himalayan hills and Andaman and Nicobar islands [3]. Species of *Calamus* are wind pollinated with their phenological behaviour being influenced by climatic, topographical, and edaphic factors. *Calamus* is dioecious and flowers only once in a year. The low frequency of mate plants and wastage of pollen during rains can lead to decreased pollination efficiency and low seed set [4,5].

*Calamus huegelianus*, *C. nagabettai, C. prasinus, C. thwaitesii* and *C. vattayila* are the taxa selected for the present study (Fig. 1). *C. huegelianus* is endangered, while *C. nagabettai* and *C. vattayila* are endemic to Western Ghats. *C. prasinus* and *C. thwaitesii* are comparatively having wide distribution. However, all the five species of *Calamus* selected for the present study are economically important and are extensively used in the furniture industry that led to their threatened status. *C. huegelianus, C. nagabettai and C. thwaitesii* are clump-forming canes, whereas *C. prasinus* and *C. vattayila* are solitary canes. Morphological analysis of all these five taxa selected revealed the existence of distinct variations both within and among the taxa. Taxonomically *Calamus* species are heterogeneous plants displaying considerable morphological variability [6,7,8]. The assessment of the genetic diversity of *Calamus* in Western Ghats is a prerequisite to overcome the threats to the genetic diversity present in wild populations and the need for preservation of these genetic resources. In addition, genetic diversity studies in biodiversity hotspots like Western Ghats, aids in identifying gene erosion, priority locations, and species for conservation [9].

Random Amplified Polymorphic DNA (RAPD) has been extensively used for the assessment of genetic diversity in a variety of plants, including *Calamus* species [2,10-14]. RAPD analysis is technically simple, less expensive, quick to perform and reliable, it requires very little plant material, and yields true genetic markers [15].

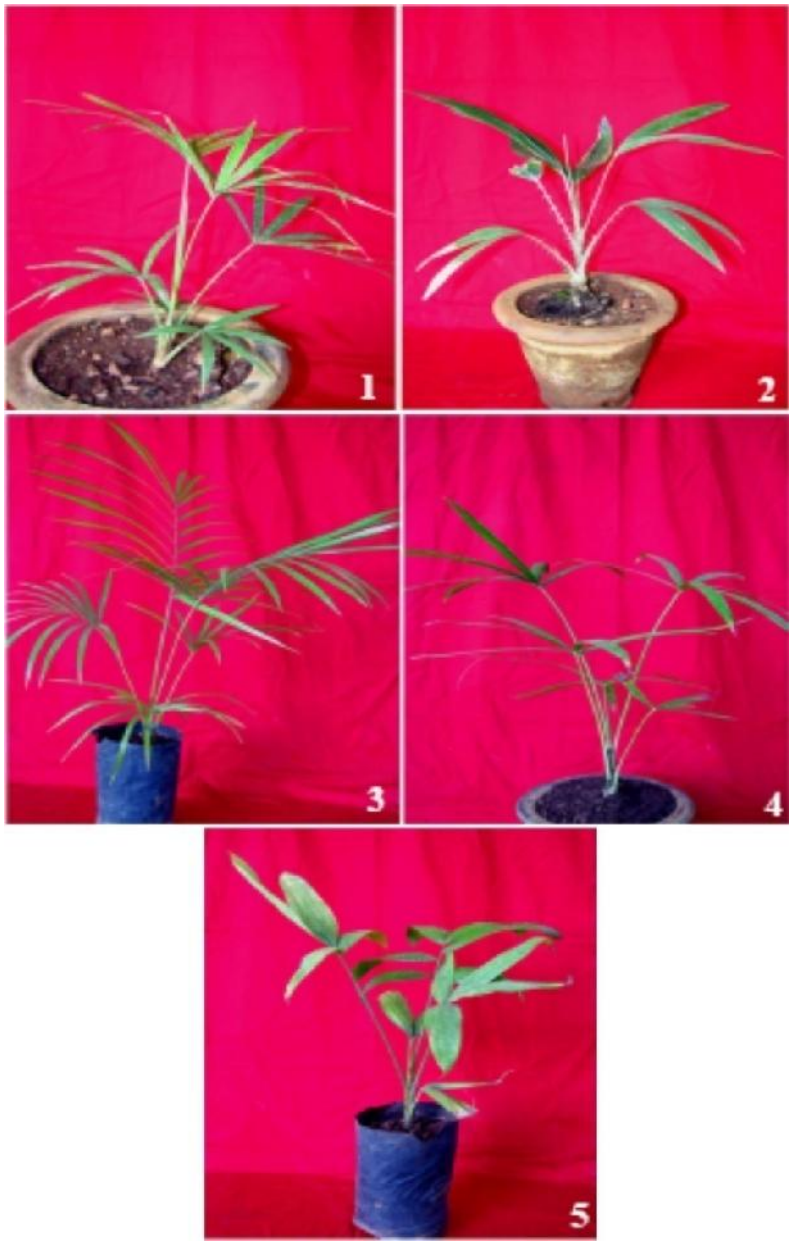


**Fig. 1. Selected species of *Calamus*: 1. *C. nagabettai* 2. *C. thwaitesii* 3. *C. huegelianus* 4. *C. prasinus* and 5. *C .vattayila***

Also, previous knowledge of the gene sequence of the sample is not required to perform the analysis. RAPD analysis provides information that can help to define the distinctiveness of species and phylogenetic relationships at the molecular level [13]. Reports on genetic analysis in *Calamus* species are limited [2,13,16-19].

The present investigation is an attempt to study the genetic relatedness/diversity among *C. huegelianus, C. nagabettai, C. prasinus, C. thwaitesii* and *C. vattayila,* and to correlate the phenotypic characters with the data obtained from genetic analysis by RAPD markers.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection for DNA Isolation

Seedlings were collected from a Subramanya range of Western Ghats and maintained in the polyhouse of the departmental garden of the

Department of Botany, Bangalore University (Fig. 1). Mature leaves from one-year-old seedlings of all the selected species of *Calamus* were used for DNA extraction. Total DNA was extracted from 500 mg of fresh leaves using the modified Laird method [20]. Further, the DNA sample was column purified to remove the inhibitors for PCR.

## 2.2 PCR-RAPD

The genomic DNA of five samples was used as template DNA for optimization of PCR amplification. Amplification was achieved by following the procedure outlined by Williams et al. [21] with slight modification. 2 µl of different template DNA was added to the 38 µl reaction mixtures which contained 2 x PCR master mixes of 20 µl, 1 µl of random primer, and 17 µl of deionised distilled water. PCR master mix is composed of 1.5 µl of Magnesium chloride ($MgCl_2$), 1.00 µl of dNTPS, one unit of *Taq* DNA polymerase. The master mix required for a set of 10 reactions was prepared fresh from the original stock, 40 µl of this mixture was aliquoted into 48 different labelled PCR vials. Amplification was achieved by Eppendorf Mastercycler programmed for initial denaturation at 94°C for 5 min followed by 40 cycles. Each cycle consisted of denaturation at 94°C for 30s, primer annealing at 45°C for 60s, primer extension at 72°C for 90s and a final extension for 7 min at 72°C. Five random primers were selected – OPB-10, OPD-02, OPD-08, OPC-06, and OPC-07 out of 15 primers tried for DNA fingerprinting. They yielded a maximum number of bands that are consistent and clear without any ambiguity.

Amplification products were resolved by electrophoresis on a 0.8% agarose gel containing ethidium bromide. Ethidium bromide stained DNA bands were viewed under UV transilluminator – Syngen G bio gel documentation system and photographed for documentation.

## 2.3 Data Analysis

The amplification products were scored for the present (1) and absent (0) of bands across the species to generate binary matrix. Percent polymorphism was calculated by the following formula,

$$\text{Percent polymorphism} = \frac{\text{Number polymorphic bands}}{\text{Total number of bands}} \times 100$$

The dendrogram and Principal component analysis (PCA) were generated by calculating similarity matrix (simple matching) and corresponding clustering by UPGMA using Numerical taxonomy and multivariate analysis version 2.02e software (NTSYSpc) [22].

## 3. RESULTS

In the present study, five primers were used to assess the morphological variation at the molecular level (Fig. 2). The size range of amplification products differed with selected primer as well as genotypes, which ranged from 100 bp – 3.5 kb. The RAPD bands produced by individual primer with different samples are tabulated (Table 1). Five primers used in the present study have generated 108 bands, 64 bands were found to be polymorphic, which represents 59.25% of polymorphism. The average number of bands and polymorphic markers per primer were found to be 21.6 and 12.8 respectively. *C. prasinus* has exhibited highest percent polymorphism with 11 polymorphic bands out of 16. The least percent of polymorphism was recorded in *C. huegelianus* with 9 polymorphic bands out of 20. Among the five primers, OPD-02 produced least percent polymorphism of 30.76 with at least a number of 13 bands. The highest percent of polymorphism of 82.60 was generated by OPC-07 (Table 2). The molecular weight of RAPD markers ranges from 1123 to 614bp in the profile generated by primer OPD-02. However, OPD-08 has generated 29 markers whose molecular weight ranges from 1322 to 342bp. Maximum number of 30 RAPD markers was generated by primer OPC-06 with 63.33% polymorphism, whose molecular weight ranges from 2564 to 329 bp, while the primer OPC-07 generated 23 markers with molecular weight ranging from 1310 to 388 bp. The primer OPB-10 generated least number of 13 bands with molecular weight ranging from 1432 to 615 bp.

## 3.1 Cluster and Principal Component Analysis

The RAPD bands were manually scored from the gel profile by assigning values '1' for the presence and '0' for the absence and binary matrix was generated. Thus, generated binary matrix from all five profiles generated by five primers was used for statistical analysis. The similarity co-efficient value in the distance matrix ranged from 0.45 to 0.66 indicating less genetic divergence among the taxa selected. Highest

similarity co-efficient of 0.66 is shown between *Calamus huegelianus* and *Calamus thwaitesii*, whereas the least similarity co-efficient of 0.45 is between *C. vattayila* and *C. nagabettai*. The dendrogram generated by overlapping the data obtained from five primers, generated two main clusters 'a' and 'b' (Fig. 3). Clusters 'a' is represented by *C. vattayila* that stood out from the rest of the species selected. Cluster 'b' is again divided into 'c' and 'd'; cluster 'd' is represented by *C. nagabettai* alone. Cluster 'c' is further splited into 'e' and 'f' sub clusters; "e" is composed of *C. prasinus* and 'f' cluster is composed of *C. huegelianus* and *C. thwaitesii.* PCA result corresponds with the grouping of taxa based on cluster analysis. *C. huegelianus* and *C. thwaitesii* are closely related, whereas *C. nagabettai* and *C. vattayila* have fallen apart in the 3-D plot (Fig. 4).

## 4. DISCUSSION

The depletion of genetic resources from natural habitats due to various anthropogenic activities in Southeast Asian countries has led to a serious threat to the rattan trade including India [23]. Hence, there is a need to conserve genetic resources of these species. *In situ* and *ex situ* conservation strategies are depended on the knowledge of the genetic structure, diversity of populations and mating system of the species. Information obtained from genetic marker screening is invaluable for identification of populations, which are desirable for forestry practices that inadvertently alter natural gene pool of domesticated species [17]. Genetic richness can be assessed by estimating the genetic diversity parameters like percent of polymeric loci and genetic diversity index.

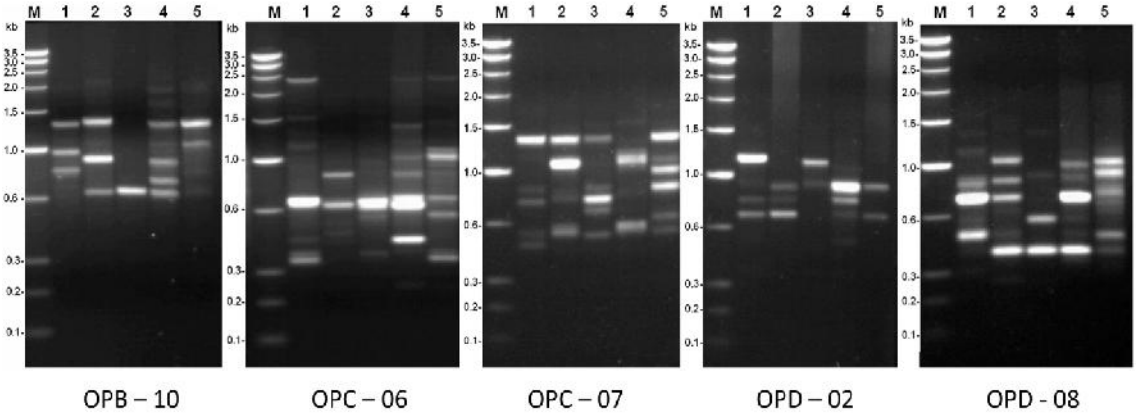


**Fig. 2. RAPD profiles generated by 5 primers**

*M Marker 1. C. nagabettai, 2. C. nuegelianus, 3. C. prasinus, 4. C. thwaitesii, 5. C. vattayila*

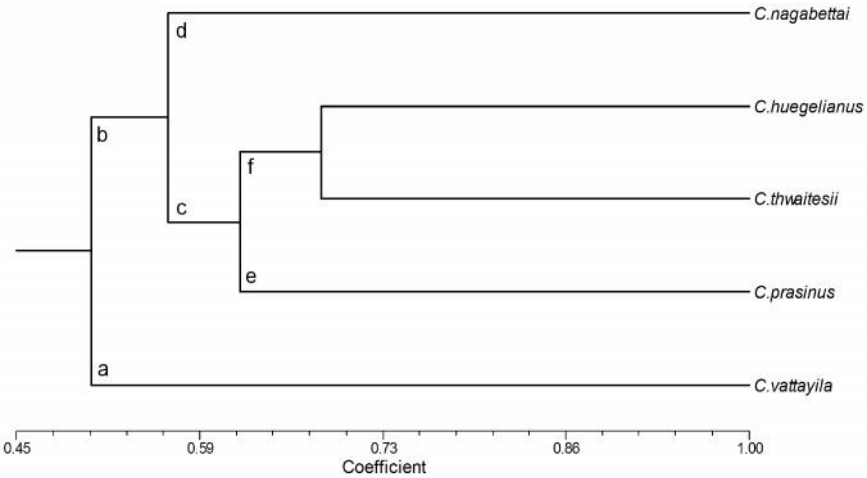


**Fig. 3. Dendrogram representing the simple matching, similarity co-efficient and UPGMA**

**Table 1. Total number of RAPD and polymorphic markers in RAPD profile**

| | Number of RAPD bands | | | | | Number of polymorphic bands | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Primers** | ***C. nagabettai*** | ***C. huegelianus*** | ***C. prasinus*** | ***C. thwaitesii*** | ***C. vattayila*** | ***C. nagabettai*** | ***C. huegelianus*** | ***C. prasinus*** | ***C. thwaitesii*** | ***C. vattayila*** |
| OPD-02 | 3 | 3 | 2 | 3 | 2 | 1 | 0 | 2 | 0 | 1 |
| OPD-08 | 6 | 6 | 4 | 6 | 7 | 4 | 2 | 2 | 2 | 4 |
| OPC-06 | 7 | 2 | 4 | 8 | 9 | 4 | 1 | 2 | 5 | 7 |
| OPC-07 | 5 | 5 | 5 | 2 | 6 | 4 | 3 | 4 | 2 | 6 |
| OPB-10 | 3 | 4 | 1 | 3 | 2 | 2 | 3 | 1 | 1 | 1 |
| Total bands | 24 | 20 | 16 | 22 | 26 | 15 | 9 | 11 | 10 | 19 |

**Table 2. Summary of RAPD analysis in five species of *Calamus***

| **Sl no.** | **Primers** | **Percent polymorphism** | | | | | **Total no. of RAPD bands** | **Total no. of polymorphic bands** | **Percent polymorphism** |
|---|---|---|---|---|---|---|---|---|---|
| | | ***C. nagabettai*** | ***C. huegelianus*** | ***C. prasinus*** | ***C. thwaitesii*** | ***C. vattayila*** | | | |
| 1 | OPD-02 | 33.33 | 00.00 | 100.00 | 00.00 | 50.00 | 13 | 04 | 30.76 |
| 2 | OPD-08 | 66.66 | 33.33 | 50.00 | 33.33 | 57.14 | 29 | 14 | 48.27 |
| 3 | OPC-06 | 57.14 | 50.00 | 50.00 | 62.50 | 77.77 | 30 | 19 | 63.33 |
| 4 | OPC-07 | 80.00 | 60.00 | 80.00 | 100.00 | 100.00 | 23 | 19 | 82.60 |
| 5 | OPB-10 | 66.66 | 75.00 | 100.00 | 33.33 | 50.00 | 13 | 08 | 61.15 |
| | Total | 60. 76 | 43.67 | 76.00 | 45.83 | 66.98 | 108 | 64 | 59.25 |

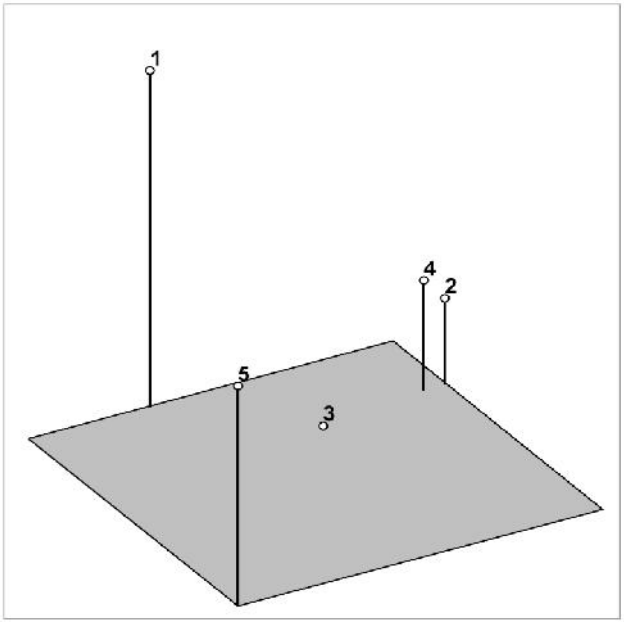


**Fig. 4. Principal component analysis of the five species of *Calamus***
*1. C. nagabettai 2. C. huegelianus 3. C. prasinus*
*4. C. thwaitesii 5. C. vattayila*

Various PCR-based marker techniques have been successfully introduced in the fingerprinting of plant genome [24] and in genetic diversity studies [25]. Among them Random amplified polymorphic DNA analysis (RAPD) is quick and well adopted for efficient non-radioactive DNA fingerprinting of genotypes [26]. RAPD has advantages over other markers, as the knowledge of the DNA sequence for the targeted genome is not required. This makes the method more popular for comparing the DNA of biological systems that have not had the attention of the scientific community or in a system in which relatively few DNA sequences are compared. In addition to this, RAPD is an inexpensive yet powerful method compared to the other marker system in analysing phylogeny of diverse plant and animal species.

The technical simplicity of the RAPD technique has facilitated its use in the analysis of genetic relationships in several genera [10,27]. The RAPD markers are well suited for genetic mapping, for plant and animal breeding applications and for DNA fingerprinting with particular utility for studies of population genetics [21]. Williams et al. [21] who for the first time described these markers have listed several advantages of this technique. Significant among them are as follows: 1) a universal set of primers can be used for genomic analysis in a wide variety of species, 2) no preliminary work, such as isolation of cloned DNA probes, preparation of fitters for hybridization or nucleotide sequence, is required 3) each RAPD marker is the equivalent of a sequence tagged site, which can greatly simplify information, transfer in collaboration research programme.

Five different species of *Calamus* were selected for the present study. The selected taxa are all economically important and are over exploited. Rattans are heterogeneous group of plants displaying considerable morphological variability [6,7,8]. Morphological analysis of the five taxa selected revealed the existence of variations both within and among the taxa [28].

Mature fresh leaves of all the five taxa were used for the DNA extraction. Stored samples were found to yield DNAs of reduced quality and quantity, which may be due to the formation of several irreversible complexes between polyphenols and DNA [29]. The isolation of genomic DNA and its purity play an important role in further processing. Removal of RNA and proteins is an important step in DNA purification, since they may hinder PCR amplification. The isolated DNA was column purified to remove any inhibitors that may come in the way of the PCR reaction.

DNA amplification was tried with different concentrations of genomic DNA. Out of these 2 µl of template DNA gave good and constant amplification and 1 µl of primer was found optimal. Higher primer concentration may promote mispriming and accumulation of non-septic products [30]. Both the magnesium ion and dNTPs concentrations are known to affect the relative intensity and the number of amplified bands. It was found that 1.5 µl of $MgCl_2$ and 1.00 µl of dNTPs were optimal. At higher Mg ion concentrations, *Taq* DNA polymerase activity is inhibited [31].

Selecting the right sequence for the primer is very important because different sequences will produce different band patterns and possibly allow for a more specific recognition of individual plants. For the present study, five primers were selected and they produced 108 bands. Similarity matrix data have shown that *Calamus thwaitesii* and *C. nagabettai* share 58% similarity, whereas *C. vattayila* and *C. prasinus* with 50% similarity have branched out separately, Both these species are solitary canes. *C. prasinus* has the highest percent of polymorphism (76.00%) which is a solitary cane and having three veined leaflets followed by *C. vattayila* (66.98%) which is also a solitary cane but having six veined leaflets. The least percent of polymorphism was seen in *C. huegelianus* (43.67%), an endangered species.

A few studies on phenetic and phylogenetic aspect in rattan species were carried out. Changtragoon, Szmidt & Wang [18] carried out a preliminary study on genetic analysis of selected taxa of *Calamus.* 20 primers were screened on the total DNA obtained from the leaf tissue. However, only 10 primers yielded reproducible fragments. Out of these, only 4 primers were selected for further studies. Different populations of *C. thwaitesii* were genetically analysed by Sreekumar & Renuka [2] and concluded that Goa population has a hot spot of genetic variation because of the presence of high genetic diversity. Subsequently, Sreekumar, Renuka, Suma & Balasundaran [19] reported 95% of polymorphism in *C. rivalis* and *C. metzianus* populations. Based on the RAPD and morphological data, they recommended the merger of these two species. Ramesha et al. [32] studied the genetic structure of the populations of *C. thwaitesii* in core, buffer, and peripheral regions of the three protected areas in Western Ghats. Their results indicated that the core and buffer regions maintain a better population's structure as well as higher genetic diversity than the peripheral regions. Sarmah et al. [13] characterized 15 rattan genotypes using 20 different random primers. Based on RAPD fingerprint, nearly 98.1% of polymorphism was detected among the genotypes. However, in the present study, percent of polymorphism in each taxa ranges from 43.67 to 76.00. Furthermore, sex identification of *Calamus* seedlings is a priority because *Calamus* takes 5 years or more to enter the reproductive phase. Molecular markers associated with sex in rattans are needed to identify the sex at an early stage. Li et al. [33] identified a male- specific SCAR marker (Cs Male 1) in *C. simplicifolius*. While Sarmah and Sarma [34] also identified a female-specific ISSR marker in *C. tenuis*. Nevertheless, in the present study, seedlings were selected randomly, sex identification studied were not carried out. Such studies are needed in all the five taxa that were selected since they are all economically important and are nearly threatened. This type of studies will definitely help in conservation efforts and breeding programmes. Hemanthakumar et al. [35] analysed the micropropagated plants of *C. thwaitesii* by ISSR markers and concluded that the microclones are genetically true to their parental origin.

UPGMA cluster analysis of the genetic similarity values has generated a dendrogram illustrating the overall genetic relationships among the taxa studied. The UPGMA cluster analysis assumes a constant evolutionary rate among the genotype and is typically most appropriate for diversity between and within the species. The clustering of the genotypes showed that *C. vattayila* has not clustered with other 4 taxa. Similarly *C. nagabettai* had stood out separately from other three taxa in the cluster 'd'. However, *C. huegelianus* and *C. thwaitesii* form a single cluster and the morphological characters of all the 5 taxa were analysed to study whether their morphological differences are reflected in the RAPD profile and dendrogram clustering. Hoey et al. [36] found a close correlation between morphological, isozyme and RAPD data, whereas Khanuja et al. [37], Singh et al. [11] and Joshi et al. [14] reported the lack of such similarity between morphological and RAPD data. Joshi et al. [14] are of the opinion that as all the loci responsible for the expression of morphological traits may not be covered by the molecular markers. This type of analysis in the present investigation of morphological characters versus clustering pattern has led to some

conclusion as follows; formation of main two clusters 'a' and 'b' may be based on leaflet vein characters, because the two sub groups that are formed under group 'b' which includes taxa that are having 3- veined leaflets, whereas the group 'a' which includes only one taxon, *C. vattayila* has 6 veins in the leaflets. This character has been used as a key character in the dichotomous key framed by Renuka [7]. However, *C. huegelianus* and *C. thwaitesii* are clustered with a similarity coefficient of 66. Morphologically both species are wide apart, each having their own distinguishing characters. The stem is thin about 1.5 cm in diameter in *C. huegelianus* and hence commonly called as 'Pencil cane'. Whereas the stem in *C. thwaitesii* is very stout and about 6 cm in diameter and commonly known as 'Handi betta'. Knee, a swelling of the leaf sheath below the petiole is present in *C. huegelianus*, whereas it is absent in *C. thwaitesii*. Involucre, the bracteole immediately surrounding the flower, is cup shaped in *C. thwaitesii* and concave in *C. huegelianus*. However, a few characters they share are; leaves are not clustered on the rachis, leaflets are 3 veined; cirrus is absent; both are clump forming. The other taxa which has fallen apart from this group is *C. prasinus*, which is a solitary cane. Though *C. vattayila* is also a solitary cane, it has not been grouped with *C. prasinus*. It is completely separated from other taxa, this may be due to the presence of 6 veined leaflets, the characteristic feature, not seen in any other taxa studied. The other taxa which again falls apart in the cluster 'b' is *C. nagabettai*. This taxon exhibits a distinguished morphological character that is the presence of cirrus- an extension of the leaf rachis, armed with reflexed thorns. Cirrus is an important key character in the dichotomous key that is present only in *C. nagabettai*, and not in other taxa [7]. The PCA also strengthens the inferences drawn from the cluster analysis of five species of *Calamus.* From the cluster and Principal Component Analysis (PCA) it can be concluded that the samples are clustered together based on the sharing of common phenotypic characters. However, a thorough morphological analysis is needed before coming to a conclusion. The highly allogamous nature of the rattan species creates heterogeneity among the species.

## 5. CONCLUSION

Random amplified polymorphic DNA markers were used to evaluate the genetic affinities among five economically important *Calamus* species. Based on the scoring of RAPD markers, percent of polymorphism among the selected taxa is recorded. Thus, obtained data was analyzed using the numerical taxonomy and multivariate analysis version 2.02e (NTSYSpc) and unweighted pair group method with arithmetic mean (UPGMA) softwares, which found that the taxa sharing the common phenotypic characters are clustered.

## ACKNOWLEDGEMENTS

Authors (DHT, RVM, HRR, ACL and RN) are thankful to Department of Science and Technology, New Delhi, India for funding this work.

## ETHICAL APPROVAL

This article does not contain any studies with human participants or animals performed by any of the authors.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Singh BH, Puni L, Jain A, Singh RS, Rao PG. Status, Utility, Threats and conservation options of rattans resources in Manipur. Curr Sci. 2004;87:90-94.
2. Sreekumar VB, Renuka C. Assessment of genetic diversity in *Calamus thwaitesii* Becc. (Arecaceae) using RAPD markers. Biochem Syst Ecol. 2006;47:397-405.
3. Uhl NW, Dransfield J. Genera palmarum: A classification of Palms based on the work of Harold E, Moore Jr. Lawrence, Kansas, USA: Allen Press; 1987.
4. Renuka C. A manual on the rattans of Andaman and Nicobar islands. Peechi, Kerala: Kerala Forest Research Institute; 1995.
5. Manohara TN, Ramaswamy SN, Shivamurthy GR. Calamus - dwindling resources? Curr Sci. 2007;92:290-92.
6. Basu SK. Rattans (canes) in India: A monographic revision, Rattan information centre, Kepong, Kuala Lumpur; 1992.
7. Renuka C. Rattans of Western Ghats: A taxonomic manual. Peechi, Kerala: Forest Research Institute. 1992;61-68.
8. Venkatesh CS. Problems and prospects of cane improvement. In: Proc. Rattan-India

seminar on rattan management and utilization. KFRI, Peechi and IRDC, Ottawa, Canada. 1992;135-39.
9. Arunachalam V. Genomics of cultivated Palms. Netherlands: Elsevier; 2012.
10. Nair NV, Nair S, Sreenivasan TV, Mohan M. Analysis of genetic diversity and phylogeny in *Saccharum* and related genera using RAPD markers. Genet Resour Crop Evol. 1999;46:73-79.
11. Singh R, Kumar J, Deshwal RP, Randhawa GJ, Walla S, Prasad R, Parmar BS. Morphological, chemical and genetic variability in neem accessions. Indian J. Plant. Genet Resour. 2002;15:237-47.
12. Lim W, Mudge KW, Weston LA. Utilization of RAPD markers to assess genetic diversity of wild populations of North American Ginseng (*Panax quinquefolium*). Planta Medica. 2007;73:21-24.
13. Sarmah P, Barua PK, Sarma RN, Sen P, Deka PC. Genetic diversity among rattan genotypes from India based on RAPD-marker analysis. Genet Resour Crop Evol. 2007;54:593-600.
14. Joshi N, Randhawa GJ, Firke PK, Purohit SD. RAPD analysis of diversity in 'Safed Musli' (*Chlorophytum borivilianum* Sant.et Fernand.), a rare Indian Medicinal herb. Med Aromat Plant Sci Biotechnol. 2008; 2: 86-89.
15. Rafalski JA, Vogel JM, Morgante M, Powell W, Andre C, Tingey SV. Generating and using DNA markers in plants. In: Birren B, Lai E, editors, Non-mammalian Genome Analysis. A practical guide. San Diego: Academic Press. 1996;75-134.
16. Changtragoon S, Szmidt AE. An integrated population genetic approach to conserve forest tree diversity in Thailand. In: Proc. ASEAN Seminar on Management and conservation of biodiversity, ASEAN Institute of Forest Management, Kuala Lumpur, Malaysia. 1993;217-22.
17. Changtragoon S, Szmidt AE. Evaluation of genetic diversity and resources of tropical trees in Thailand by using molecular markers. In: Proc. The 6th Annual International Workshop of BIO-REFOR. The University of Queensland, Brisbane. 1997;169-71.
18. Changtragoon S, Szmidt AE, Wang XR. Genetic diversity of rattans in Thailand by menas of RAPD analysis: Preliminary results. In: Proc. IUFRO' 96 conferences on diversity and adaptation in forest ecosystem in a changing world. University of British Columbia, Vancouver, Canada. 1996;1-9.
19. Sreekumar VB, Renuka C, Suma TB, Balasundaran M. Taxonomic reconsideration of *Calamus rivalis* Thu. Ex. Trim. and *C. metzianus* Schlecht (Arecaceae) through morphogenetic and molecular analysis. Botanical Studies. 2006;47:443-52.
20. Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A. Simplified mammalian DNA isolation procedure. Nucleic Acids Res. 1991;19:4293-294.
21. Williams JGK, Kubelik KJ, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful genetic markers. Nucleic Acids Res. 1990;18:6531-535.
22. Rohlf FJ. NTSYS-PC: Numerical taxanomy system ver 2.1. Setauket, New York: Exeter Publishing Ltd.; 2002.
23. Dransfield J, Uhl NW. Genera Palmarum 9. Kansas, USA: International Palm Society Press; 1987.
24. Welsh J, Mc Chelland M. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 1990;18:7213-218.
25. Tinker NA, Fortin MG, Mather DE. Random amplified polymorphic DNA and pedigree relationships in spring barley. Theor Appl Genet. 1993;85:976-84.
26. Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC. Comparison of RFLP and RAPD markers in estimating genetic relationships within and among cruciferous species. Theor Appl Genet. 1994;88:973-80.
27. Wilkie SE, Issac PG, Slater RJ. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. Theor Appl Genet.1993; 87: 7213-218.
28. Madhusudhan RV. *In vitro* conservation studies of selected species of *Calamus* L. PhD. Thesis. Bangalore University, Bangalore; 2016.
29. Wilfinger W. Isolation of DNA from plant tissue containing polyphenols and polysaccharides. MRC Technical Bulletin. 1999;7.
30. Innis MA, Gelfand DH. Optimization of PCRs, In: Innis MA, Gellf DH, Sninsky JJ, White TJ. editors. PCR protocols- A guide to methods and applications. New York: Academy Press. 1990;3-12.
31. Karihaloo JL, Brauner S, Gottlieb LD. Random amplification polymorphic DNA variation in the eggplant *Solanum*

*melongena* L. (Solanaceae). Theor Appl Genet. 1995;90:767-70.

32. Ramesha BT, Ravikanth G, Rao MN, Ganeshaiah KN, Shanker RU. Genetic structure of the rattan *Calamus thwaitesii* in core, buffer and peripheral regions of three protected areas in central Western Ghats, India: Do protected areas serve as refugia for genetic resources of economically important plants?. J Genet. 2007;86:9-18.
33. Li M, Yang H, Li F, Yang F, Yin G, Gan S. A male-specific SCAR marker in *Calamus simplicifolius* dioecious rattan species endemic to China. Mol Breeding. 2010;25: 549-51.
34. Sarmah P, Sarma RN. Identification of a DNA marker linked to sex determination in *Calamus tenuis* Roxb, an economically important rattan species in Northeast India. Mol Breeding. 2011;27:115-18.
35. Hemanthakumar AS, Preetha TS, Pillai PP, Krishnan PN, Seeni S. Micro-cloning of an economic rattan palm *Calamus thwaitesii* for eco-restoration programme. Biologia. 2014;69:618-24.
36. Hoey BK, Crowe KR, Jones VM, Polans NO. A phylogenetic analysis of Pisum based on morphological characters, and allozyme and RAPD markers. Theor Appl Genet.1996;92:92-100.
37. Khanuja SPS, Shasany AK, Srivastava A, Kumar S. Assessment of genetic relationships in Mentha species. Euphytica. 2000;111:121-25.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://prh.sdiarticle3.com/review-history/24847*