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Diversity and Phylogenetic Analysis of the Genus *Musa*

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Abstract: The genus *Musa* of the family Musaceae has four sections namely Eumusa, Rhodochlamys, Australimusa and Callimusa. The section Rhodochlamys comprises of several ornamental bananas of commercial significance and they have the same basic chromosome number (2n = 22) like Eumusa. Though a lot of work has been carried out on Eumusa, reports on Rhodochlamys are very limited. In the present study efforts are made to standardize RAPD primers for *Rhodochlamys* section, to assay the amount of similarity and diversity in and between *Rhodochlamys* cultivars, to study the phylogenetic relationship within *Rhodochlamys* members and finally to assess the phylogenetic relationship of Rhodochlamys members with the Eumusa members using RAPD marker system.
Key words: Dendrogram, Diversity, Musa, RAPD, Rhodochlamys.

Introduction

India is the centre of origin with a great diversity for the family *Musaceae*. The family *Musaceae* is known not only for edible bananas but also for its members with other commercial utilities. There are two genera in this family i.e., *Musa* and *Ensete*. Though there is a proposal to include Musella as the third genus which is not yet confirmed[1]. Based on the basic chromosome numbers and morphological characters *Musa* species have been grouped into five sections namely *Eumusa, Rhodochlamys, Australimusa, Callimusa* and the fairly new addition, *Incertae sedis.* Of these, Eumusa and Rhodochlamys have basic chromosome number, 2n-22 while Australimusa and Callimusa have 2n-20 and *Incertae sedis* has 2n- 14 and18 (*M.ingens* and *M.beccari* respectively)[2].

Although the entire commercial banana industry is dependent on the section Eumusa, other sections also contribute to a greater extent for the creation of diversity and evolution of present day bananas. Of these, Rhodochlamys is the closely related section and has more genetic affinity with Eumusa. This is a section comprising valuable ornamental bananas of commercial importance.

General characteristics of section Rhodochlamys

They are slender and delicate plants found growing from mean sea level (MSL) to 1200m above MSL. Short to medium in stature (1.0-2.5 m), bear erect inflorescence with a short peduncle, uniseriate on which the hands are arranged. Fingers are short to medium, sometimes dehiscent upon maturity and usually female fertile producing a number of fertile seeds. Their wide range of bright coloured bracts characterizes the members. Interestingly they are highly adaptive to extremities of drought and temperature.

There are many reports available on the study of origin, distribution and taxonomy of *Musa* species under Eumusa[3,4,5,6]. Earlier reports on Rhodochlamys date back to early 1920s to 1950s. Of late, the taxonomy of the section Rhodochlamys is being worked out and many of the doubts on the earlier reports have been resolved. The purpose of this study is to standardize RAPD primers for *Rhodochlamys* members and assess the phylogenetic relationship not only in and between *Rhodochlamys* members but also in comparison with Eumusa members using RAPD marker system.

Materials and Methods

Test species

Five species from the section Rhodochlamys and two representative species from the section Eumusa were used. *Rhodochlamys* species were *M.ornata, M.laterita, M.velutina, M.aurantiaca* and *M.rosacea.* Among the Eumusa species used, one was *M.acuminata ssp burmannicoides*, var. Calcutta-4 and the other was *M.balbisiana*, type Attikol. Calcutta - 4 is a slender delicate diploid (AA) with duration of only six months and it carries resistant genes for many biotic and abiotic stresses. It is seen distributed in south and south east Asia. Attikol is a wild, hard seeded diploid (BB) with a duration of 15-16 months and is seen distributed in north east India.

DNA isolation

Young leaves of the test species were collected from the *Musa* germplasm from different locations. Genomic DNA from the fresh leaf samples (cigar leaf stage) was extracted with CTAB as described by[7] with minor modifications. The leaf tissue was frozen in liquid nitrogen, ground to a fine powder and mixed with 10 ml of pre-heated (65^{o}C) cetyl trimethyl ammonium bromide (CTAB) extraction buffer (1.5 % CTAB, 20 mM EDTA, 1.4 M NaCl, 10 mM Tris-HCl (pH 8.0), 1- 2% of 2-mercaptoethanol). The mixture was then shaken gently at 65^{o}C for 30 min. An equal volume of 24:1 chloroform/ iso-amyl alcohol was added gently mixed for 15 min. Then it was centrifuged at 8500 rpm for 20 min at 27^{o}C to remove the debris. The DNA was then precipitated by adding an equal volume of ice-cold iso-propanol to the aqueous phase. The precipitated DNA was dried and dissolved in 0.1X TE. The contaminant RNA was removed by digestion with 10 µg RNaseA for 1h at 37^{o}C. The DNA was further purified with an equal volume of phenol followed by equal volume of chloroform. It was pelleted using $^{1}/_{10}^{th}$ volume of 3M sodium acetate and double the volume of 100% ethanol. Finally, the dried pellet was dissolved in nuclease free water and stored at -20^{o}C. DNA concentration was determined spectrophotometrically and its integrity was checked using 0.8 % agarose gel electrophoresis.

Primers used and data analysis

In the present study a total of 75 random decamer primers were screened for polymorphism and reproducibility and best 56 primers were used for further work. Amplification was performed in a thermo cycler and amplified products were subjected to Agarose gel electrophoresis on 1.5% agarose. PCR amplified products of an individual genotype were treated as dominant markers (presence versus absence of an allele) as either present (1) or absent (0) and the allelic frequencies were not estimated. Only, well stained unambiguous polymeric bands were used for scoring. The binary matrix data file created was used as input file for data analysis. The Genetic Similarity (GS) matrix between the genotype was then estimated using the Jaccard's coefficient. Relationship among genotypes was evaluated using the unweighted pair-grouping method with arithmetic averages (UPGMA) and sequential agglomerative hierarchical and nested (SHAN) clustering methods were conducted using NTSYS program (version 2.02e, Exeter Software, Setauket, NY, USA)[8], to produce a dendrogram.

Results and Discussion

A total of seven species, five from the section Rhodochlamys and two representative species from the section Eumusa were used in the present study. After DNA isolation from these samples, 56 primers (Table-1) were used for DNA amplification and RAPD analysis. This study is an attempt to establish genetic diversity background in the section Rhodochlamys with RAPD markers.

From the 56 RAPD primers, that produced scorable discrete and reproducible amplicons, a total of 487 bands were identified with a mean of 9.36 bands per primer. Polymorphism was calculated based on the presence or absence of bands (**Table -2).** Out of 487 bands scored, only 8 were monomorphic (1.64%) and the

rest were polymorphic (98.36%). Maximum of 100% polymorphism was observed in 88.46% of the primers tested (46 primers). Minimum of 75% polymorphism was observed in the primer OPP-1 followed by OPE-16 (83.33%). The average polymorphism recorded in the present study was 98.66% indicating that there was substantial variation among the test accessions. Banding profile generated using primers OPE 17, 18 and OPV15,16 are indicated in Fig 1 and 2 respectively.

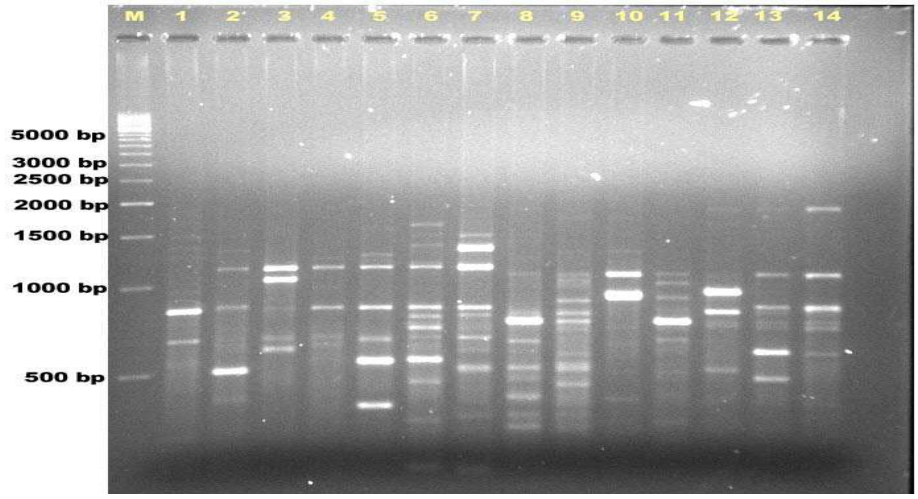

Fig.1: Banding profile generated using primers OPE 17 & 18

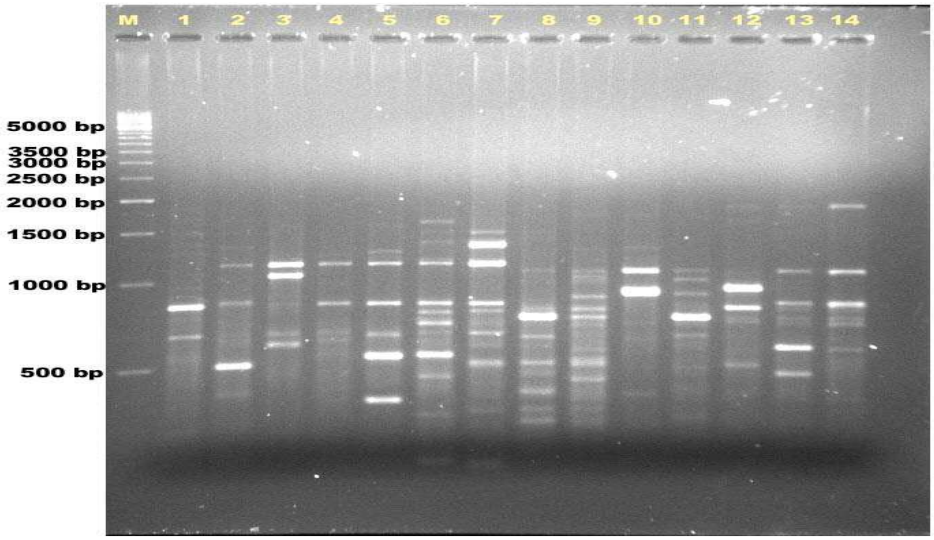

Fig. 2: Banding profile generated using primers OPV 15 & 16

Diversity analysis

In the present study, five members of the section *Rhodochlamys* were compared with the two representative members of the section *Eumusa. Rhodochlamys* members were *M.ornata, M.laterita, M.velutina, M.aurantiaca* and *M.rosacea. Eumusa* members used were *M.accuminata ssp. burmannicoides (AA)* and *M.balbisiana* (BB), the progenitors of the present bananas.

Cluster analysis was carried out on the basis of similarity co-efficient generated from RAPD profiles. Dendrogram drawn suggests that, the seven test species, clearly delineated into two major clusters that the similarity co-efficient between these two clusters was only 60%.

Clusters	Members
I	*M.acuminata ssp burmannicoides, M. laterita*
II	*M.ornata, M.velutina, M.rosaceae, M.aurantiaca, M.balbisiana*

Table 1: Analysis of Polymorphism obtained by 56 primers in different Musa species.

Primer Name	Number Of Bands	Number Of Monomorphic Bands	Number Of Polymorphic Bands	Polymorphism %
OPV 1	6	0	6	100
OPV 2	9	0	9	100
OPV 3	8	0	8	100
OPV4	11	0	11	100
OPV 5	5	0	5	100
OPV 6	8	0	8	100
OPV 7	16	1	15	93.75
OPV 8	8	0	8	100
OPV 9	15	1	14	93.33
OPV 10	13	0	13	100
OPV 11	0	0	0	0
OPV 12	15	1	14	93.33
OPV 13	6	0	6	100
OPV 14	11	0	11	100
OPV 15	12	0	12	100
OPV 16	15	0	15	100
OPV 17	4	0	4	100
OPV 18	9	1	8	88.88
OPV 19	7	0	7	100
OPV 20	7	0	7	100
OPA K 1	0	0	0	0
OPA K 2	12	0	12	100
OPA K 3	14	0	14	100
OPA K 4	11	0	11	100
OPA K 5	12	1	11	91.67
OPA K 6	8	0	8	100
OPA K 7	9	0	9	100
OPA K 8	0	0	0	0
OPA K 9	2	0	2	100
OPAK 10	6	0	6	100
OPAK 12	8	0	8	100
OPAK 13	9	0	9	100
OPAK 14	6	0	6	100
OPAK 15	7	0	7	100
OPAK 16	10	0	10	100
OPAK 17	10	0	10	100
OPK 7	10	0	10	100
OPK 8	9	0	9	100
OPK 9	6	0	6	100
OPK 10	5	0	5	100
OPE 15	9	0	9	100
OPE 16	6	1	5	83.33
OPE 17	7	0	0	100
OPE 18	11	0	11	100
OPE 19	15	0	15	100
OPE 20	9	0	9	100
OPP 1	8	2	6	75
OPP 2	9	0	9	100
OPP 3	7	0	7	100
OPP 4	9	0	9	100
OPP 5	10	0	10	100
OPP 6	9	0	9	100
OPP 7	5	0	5	100
OPP 8	8	0	8	100
OPP 9	8	0	8	100
OPP 10	9	0	9	100

Table 2: Details of RAPD in banana.

Details of polymorphism	**No.**
Total primers used	75
Primers which showed amplification	56
Total no of bands produced	487
No. of polymorphic bands	479
No. of monomorphic bands	8
Average no of bands per primer	9.36
Percentage of polymorphisim	98.36

Although it is believed that both *Eumusa* and *Rhodochlamys* sections share more than 90% homology, the present study on molecular characterization using RAPD system could only express 60% similarities **(**Fig-3).

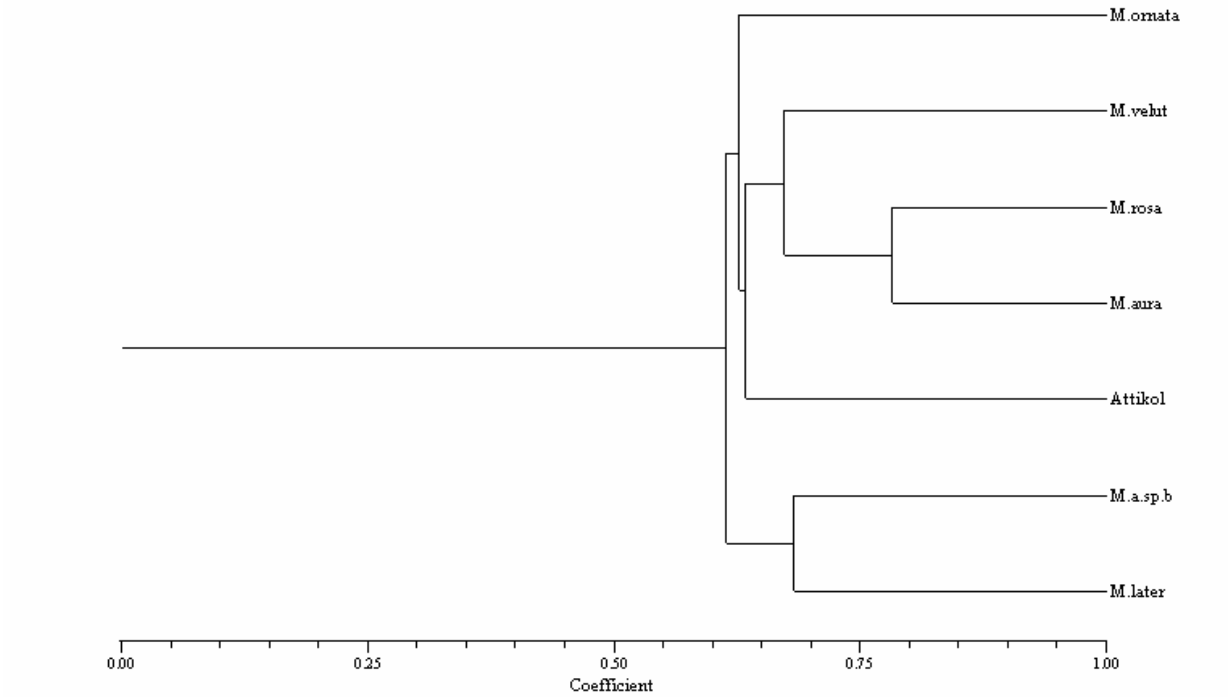

Fig 3: The dendrogram showing genetic relationship of seven *Musa* species.

First cluster comprised of only two accessions, *M.laterita* and *M.acuminata ssp. burmannicoides* both from different sections sharing a common homology up to 68%. *M.laterita*, clustering with *M.acuminata ssp.burmannicoides* while earlier reports also suggest that section Rhodochlamys is genetically closer to *M.acuminata*. This is probably the reason for prevalence of more natural hybrids of *Rhodochlamys* and *M.acuminata* in nature. Our results are also in conformity with earlier reports[6,9].

Second cluster had five of the test accessions, all the four *Rhodochlamys* members and *M.balbisiana* (Attikol). Attikol exhibited a similarity co-efficient upto 45% with other members of the 2nd cluster. Although it is believed that *Rhodochlamys* has more homology and affinity for *Eumusa,* especially *M.acuminata*, which is more closer than *M.balbisiana*[10]. In the present study, clustering of *Attikol* with *Rhodochlamys* members than *M.acuminata* needs further studies. This ambiguity needs to be rechecked and confirmed using more number of primers and marker systems.

M.ornata, a uni member subculture stood separately with almost 47% dissimilarity co-efficient from its other members. It has been disseminated widely through vegetative offshoots or as self pollinated seeds[11,12] and variability is not obvious. Its endemic nature and area of diversification is limited to Andhra Pradesh. Geographically, isolated individuals tend to accumulate genetic variations during the course of environmental adaptations[13]. This could be one of the reasons for its genetic uniqueness and separate clustering.

Similarly, *M.velutina* is also unique from other members exhibiting 44% dissimilarities. It is probably widespread but little visible in times of drought,to which it has scarcely any tolerance.It seeds readily and quickly.In favourable conditions, The furry red fruits may dehise at no more than seven to eight months from seed germination[14]. Morphological differences like dehiscent nature of fruits, medium tall nature of the plant and red pigmentation of the fruits could have contributed to this uniqueness. The phenotypic variation is expressed at genotypic levels as well.

M.rosaceae and *M.aurantiaca* delineated from others with 32% dissimilarity co-efficient. But among themselves they shared more than 78% genetic similarities. Phenotypically these two accessions are same except for their bract colour. It is orange in *M.aurantiaca* and pinkish red in *M.rosaceae*[10,15]. The same geographic area of origin and diversification in India could have contributed to its similarities and uniqueness.

In conclusion, high levels of polymorphism obtained in the study indicate RAPD markers as a suitable tool for genetic diversity studies. Common homology of 68% shows the genetic relatedness of Rhodochlamys members with present day banana cultivars.

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