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Assessment of phylogenetic lineage of landraces (AA) and wild *Musa acuminata* Colla. through morphotaxonomic traits and microsatellite markers

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ABSTRACT

Phylogenetic lineage of *Musa acuminata* Colla. and its landraces was assessed through morphotaxonomic traits and microsatellite markers. Test accessions were morphotaxonomically characterized for 107 traits using *Musa* descriptor and documented. An average PIC of 0.55 was observed using microsatellite markers and a total of 139 alleles were identified with a mean of 8.1 alleles per primer pair. Matti, a landrace could be a parthenocarpy form of *M. acuminata* ssp. *burmannica* or might have originated through natural crosses involving *M. acuminata* ssp. *burmannica* as one of its parents. Four wild *acuminata* forms, *viz.*, Pagalaphad wild, Chengdawt, Lairawk and Meghalaya wild, which have been newly identified, proved their uniqueness. Anaikomban and Pisang Lilin had grouped in the same cluster and have proved their genetic proximity.

Key words: *Musa acuminata* Colla., microsatellite markers, phylogeny, morphotaxonomy, molecular characterization.

INTRODUCTION

India is one of the recognized centers of origin for *Musa* along with other South East Asian countries and Pacific Islands. Explorations in Indian subcontinent have proved the occurrence of variability for *M. acuminata* Colla. (Simmonds, 11, 12), which has the advantage of parthenocarpy that is not observed in *M. balbisiana* Colla. Most of the present day bananas have been derived from two major progenitors, *viz., M. acuminata* Colla. and *M. balbisiana* Colla. Prolonged cultivation and natural introgression between them have led to a vast diversity in *Musa* ranging from delicate edible diploid *acuminata* (AA) types to hardy hybrid triploid (ABB) types.

The commercial parthenocarpic triploids, which dominate the banana industry of the world, are Grand Naine, Williams etc. (AAA), others like Plantain, Silk, Pome (AAB) and cooking bananas (ABB). Their commercial production is hurdled by various biotic and abiotic stresses and thrust is on genetic improvement of these traits, either through conventional or non-conventional approaches. Their improvement relies on the desired resistant genes that are present in the wild species or their parthenocarpic diploid landraces (AA or BB). The diploids needed for improvement has to be either selected from the existing gene pool or developed synthetically through inter-diploid crosses (Faure *et al*., 6). Both the selection of diploids and development of synthetic diploids require the basic knowledge on phylogenetic relationships which is needed for targeted breeding. Phylogenetic relationships could be studied either through morphotaxonomic or molecular characterization, which are complementary to each other. Though a lot of phylogenetic and diversity analyses have been reported in *Musa* (Creste *et al*., 3; Uma *et al*., 15), studies on diversity and phylogenetic relationship exclusively among the *M. acuminata* Colla., and their potential landraces is very meagre. In the present study, wild gene sources involving wild species and landraces belonging to AA genome of Indian origin have been included along with few exotic diploids of Asian origin (especially Indonesia and Philippines) for better understanding of the phylogeny.

MATERIALS AND METHODS

Six Indian states were explored for *Musa* genetic diversity and a total of 50 accessions were collected including wild species, landraces and commercial cultivars, which were planted in NRCB, Trichy field gene bank located at 10°50 N´ latitude, 74°50 E´ longitude and 90 m above mean sea level. Among them, nine *acuminata* wild species and parthenocarpic landraces were chosen for the study and the remaining accessions were introduced from ITC (International Transit Center), Belgium, which were basically from Asia, *i.e.* either from Malaysia or the Philippines. The test accessions were morphotaxonomically characterized for 107 traits using IPGRI/INIBAP *Musa* Descriptor (Anon, 2) and documented. The collections were classified as wild species, varieties/cultivars and landraces based on the definitions by Daniells

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et al. (5). The morphotaxonomic data for 107 traits of 14 accessions including four exotic potential diploids were subjected to Multivariate Hierarchical Cluster Analysis using NTSYS Pc version 2.01e (Rohlf, 10). Distance among the accessions was computed using the SIMINT with DIST option. A phenetic tree was constructed using TREE PLOT of NTSYS.

All the 14 test accessions were included for diversity analysis using microsatellite markers to confirm the results obtained through morphotaxonomic characterization. The fresh cigar leaf samples required for this study were collected from the field gene bank, NRCB, Tiruchirappalli. The genomic DNA was isolated with CTAB as described by Gawel and Jarret (7) with minor modifications. A total of 21 pairs of microsatellite primers previously identified in *M. acuminata* and *M. balbisiana* were used to assess polymorphism. The above primers were synthesized by Genei, Bengaluru, India. The details of the primers used in the current study are furnished in Table 1. The PCR protocol of Lagoda *et al.* (9) was adopted with minor modifications. Both normal and Touch down PCRs (TD – PCR) were used depending on the availability of primer information. However, all the PCR reactions were performed on an Eppendorf gradient master cycler (Eppendorf, Hamburg, Germany) in 25 µl reaction containing 15 ng of genomic DNA, 100 µM of each of the dNTPs, 0.2 µM of each of the primer, 1X PCR buffer (100 mM Tris-HCl, pH 9.0, 500 mM KCl, 10 mM $MgCl_2$, 0.1% gelatin) and 1U of *Taq* DNA polymerase. Those primers pairs whose specific annealing temperatures were known, PCR programme consisted of an initial denaturation at 94°C for 4 min. followed by 35 cycles at 94°C for 30 sec and extension at 72°C for 1 min. terminated by a final extension at 72°C for 8 min. followed by incubation at 4°C. For those primer pairs whose specific annealing temperatures were not known or in cases where specific annealing temperatures did not give sufficient amplification, touch down PCR programme was adopted.

Allele separations were performed on 38 x 30 cm sequencing unit (Sequi- Gen- GI, Bio- Rad, Australia.) using Urea-polyacrlamide gel electrophoresis. The gel mixture containing urea – polyacrylamide 5 percent along with APS (prepared fresh) and TEMED was poured immediately on a preset template. Electrophoresis was performed at 2.3 kV, 75 mA and 60 W for 2 h. After the completion of electrophoresis, the amplified bands were visualized by silver nitrate staining as reported by Kaemmer *et al.* (8). The gel was dried under ambient conditions, and then was scanned (Fig. 2). Each accession was evaluated for the presence (1) or absence (0) of an allele. Molecular sizes of the amplified fragments were estimated using a 100 bp DNA Ladder Plus (Fermentas). The NTSYS Version 2.01e (Rohlf, 10) was used to calculate genetic similarities based on simple matching (SM) coefficients. The relationship among the germplasm accessions were calculated with a phenotypic cluster analysis using Unweighted Pair Grouping Method with Arithmetic averages (UPGMA) and Sequential Agglomerative Hierarchical and Nested (SAHN) clustering method (Sneath and Sokal, 13) and plotted in a phenogram using the same software. The relative discriminatory value of the microsatellite marker was estimated by its Polymorphic Information Content (PIC), which measures the information content as a function of a markers ability to distinguish between genotypes (Anderson *et al*., 1).

RESULTS AND DISCUSSION

The dendrogram derived through morphotaxonomic characterization showed high degree of dissimilarities varying from 52-100%, however molecular characterization exhibited only 0-25% dissimilarities (Fig. 1). In both the dendrograms, the total 14 accessions have been grouped into two major clusters. These clusters were further classified into small sub-clusters (Table 2). Cluster Ia subgroup of morphological characterization brings in five accessions together, where *M. acuminata* ssp. *burmannica* and *M. acuminata* ssp. *burmannicoides* shared about 70% similarities, which could be attributed to their common phenotypic characteristics. These two wild accessions clustered with Pisang Jajee with 55% similarity and they have clustered with Matti with 50% similarity, which has common characteristics like maroon coloured male bud and whitish male flowers, which might have contributed to its clustering with these wild forms. Anaikomban is morphologically very different from the aforesaid accessions except maroon colored male bud which has led to its grouping with other wild forms with just 30% similarities. In molecular characterization, *M. acuminata* ssp. *burmannica* had grouped with Matti sharing 82% similarity. Similarly, *M. acuminata* ssp. *burmannicoides* and Pisang Jajee had grouped in another cluster with 85% similarities. *M. acuminata* ssp. *burmannica* had grouped with Matti in both morphotaxonomic and molecular characterization and proved its genetic propinquity. The results suggested that *M. acuminata* ssp. *burmannica* might have contributed for the development of parthenocarpic diploid Matti.

Cluster Ib sub-cluster had Pagalaphad wild, Lairawk and Chengdawt having 50-60% dissimilarities as expressed by their morphological characteristics. Despite their differences in molecular characterization, they have grouped in one cluster and proved their

Table 1. Details of polymorphic primer pairs.

Name of primer	Sequence	Mean alleles generated	PIC value
AGMI 24	TTTGATGTCACAATGGTGTTCC	12	0.52
AGMI 25	TTAAAGGTGGGTTAGCATTAGG		
AGMI 33	AGTTTCACCGATTGGTTCAT	7	0.55
AGMI 34	TAACAAGGACTAATCATGGGT		
AGMI 67	ATACCTTCTCCCGTTCTTCTTC	9	0.54
AGMI 68	TGGAAACCCAATCATTGATC		
AGMI 93	AACAACTAGGATGGTAATGTGTGGAA	10	0.58
AGMI 94	GATCTGAGGATGGTTCTGTTGGAGTG		
AGMI 125	TCCCATAAGTGTAATCCTCAGTT	5	0.52
AGMI 126	CTCCATCCCCCAAGTCATAAAG		
AGMI 133	GTGGTTTGGCAGTGGAATGGAA	11	0.40
AGMI 134	TGACCCTCCGACACCTATTTGG		
AGMI 161	TGAGGCGGGGAATCGGTA	11	0.52
AGMI 162	GGCGGGAGACAGATGGAGTT		
Ma SSR 07a	AAGAAGGCACGAGGGTAG	6	0.51
MaSSR 07b	CGAACCAAGTGAAATAGCG		
Ma SSR 08a	GGAAAACGCGAATGTGTG	7	0.52
Ma SSR08b	AGCCATATACCGAGCACTTG		
Ma SSR 18a	CGTCACAGAAGAAAGCACTTG	9	0.61
Ma SSR18b	CCTCTCCATCGTCATCAATC		
Ma SSR 24a	GAGCCCSTTAAGCTGAACA	8	0.55
Ma SSR 24b	CCGACAGTCAACATACAATACA		
Ma SSR 2-10 F	GGGTTCCGTGAAGATTGATT	7	0.64
Ma SSR 2-10 R	TGGACAACTGACGACCATAAT		
Ma SSR 3-132 F	AACGCGAATGTGTGTTTTCA	7	0.51
MaSSR3-132 R	TCCCTCTTCAACCAAAGCAC		
Mb SSR 1-12 F	CCTCTTCTCTCCTTCACTTTCTCA	5	0.70
Mb SSR 1-12 R	AGGATGGCGGAGATCTGGTCA		
Mb SSR 1-69 F	CTGCCTCTCCTTCTCCTTGGAA	9	0.54
Mb SSR 1-69 R	TCGGTGATGGCTCTGACTCA		
Mb SSR 1-100 F	TCGGCTGGCTAATAGAGGAA	8	0.65
Mb SSR 1-100 R	TCTCGAGGGATGGTGAAAGA		
Mb SSR 1-146 F	CCGTTGGATTTCTCCCCCACA	-	-
Mb SSR 1-146 R	GAAGAACTGGGCTTACCCAGGA		
Mb SSR 1-149 F	CCGAAACGAAGGTTACAACAA	8	0.62
Mb SSR 1-149 R	GCGCCACCTGTATCACTGT		
AGMI 101	TGCAGTTGACAAACCCCACACA	-	
AGMI 102	TTGGGAAGGAAAATAAGAAGATAGA		
AGMI 95	ACTTATTCCCCCGCACTCAA	-	
AGMI 96	ACTCTCGCCCATCTTCATCC		
AGMI 123	TTCATAATTGCAAGAAAGATAA	-	
AGMI 124	GGAGGTACAGGGGATGAGGACT		

A = Morphotaxonomic characterization, B = Molecular characterization

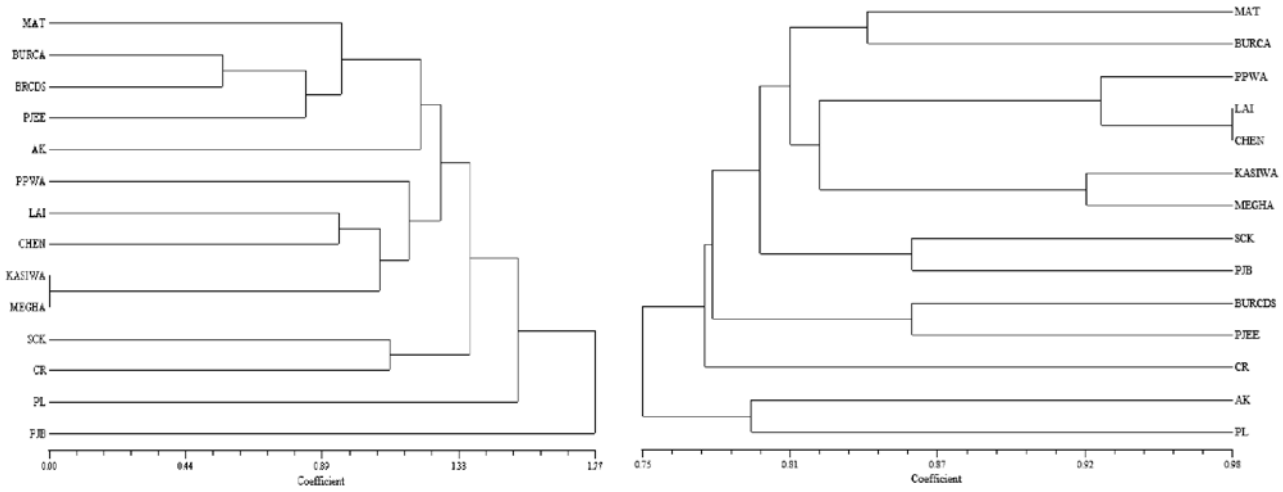
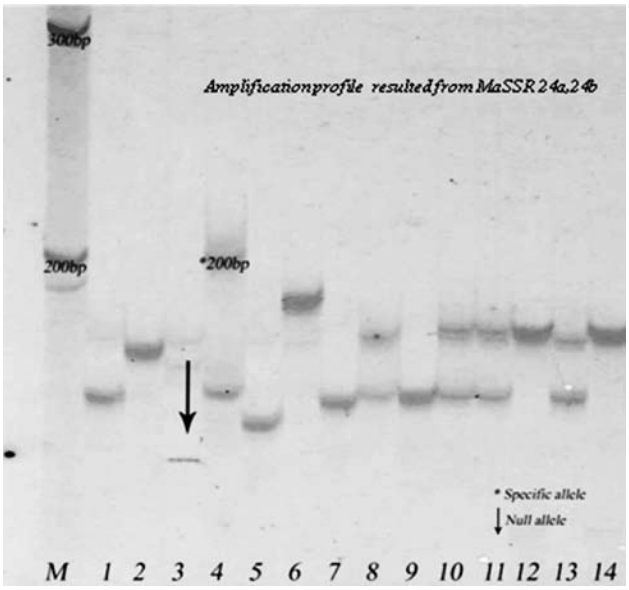


Fig. 1. Dendrogram showing the genetic affinities among the test accessions.



1. Matti, 2. Anaikomban, 3. Sanna Chenkadali, 4. cv Rose, 5. Pisang Jari Buaya, 6. Pisang Lilin, 7. *M.ac.*ssp.*burmannica*, 8. *M.ac.*ssp. *burmannicoides*, 9. Pagalaphad wild, 10. Lairawk, 11. Chengdawt, 12. Khasi wild, 13. Pisang Jajee, 14. Meghalaya wild

Fig. 2. Bending profile produced by the SSR marker Ma SSR 24a / 2b

common geographical origin North eastern India. Khasi and Meghalaya wild forms grouped with 100% similarities and suggested that they might be synonyms of each other and they have joined with aforesaid wild forms by 60% dissimilarities and proved their distinctness. In molecular characterization, all these five wild forms have grouped together with more than 90% similarities. Khasi Wild and Meghalaya Wild have been identified as synonyms in morphotaxonomic characterization and this has been confirmed through molecular characterization. Lairawk and Chengdawt clustered together sharing 92% similarities and joined with the aforesaid wild forms at 81% similarity level. The results derived using microsatellite markers proved their genetic proximity by grouping in one cluster and suggested that their genetic proximity will facilitate the development of improved synthetic diploids in gene pyramiding programmes and these distinct wild forms can be elevated to their systematic position as subspecies of *M. acuminata* Colla., and strengthen the family *Musaceae.*

Cluster Ic had Sanna Chenkadali and cv. Rose with 40% similarities. This similarity might have been caused by their common morphological characteristics, *viz.*, reddish coloured pseudostem. But in molecular analysis, they have grouped in separate clusters. Sanna Chenkadali, has grouped with an exotic introduction (Malaysia), *viz.*, Pisang Jari Buaya with 82% similarities. This clustering could be due to common origin of South and South East Asia. Cultivar Rose stood uniquely and formed an uni-member cluster, although cvs. Rose and Sanna Chenkadali grouped together in morphotaxonomic characterization, lot of differences have been recorded for various phenotypic traits like fruit peel colour, bunch orientation etc. and the same has been confirmed at molecular level.

Cluster Id had Pisang Lilin, an exotic introduction stood as uni-member and proved its exotic origin. In microsatellite analysis, Pisang Lilin and Anaikomban have grouped with 80% similarities and exhibited their genetic proximity. However, in morphotaxonomic characterization, Anaikomban grouped with Matti and Pisang Jajee. The results of molecular characterization suggested that though their phenotypic expressions

Table 2. Clustering pattern of *Musa acuminata* wild forms and landraces.

Cluster	Name of the accession	
	Morphotaxonomic analysis	Microsatellite analysis
Cluster Ia	Matti, *M. ac.* ssp. *burmannica, M. ac.* ssp. *burmannicoides*, Pisang Jajee and Anaikomban.	Matti and *M. ac.* ssp. *burmannica.*
Cluster Ib	Pagalaphad Wild, Lairawk, Chengdawt, Khasi Wild and Meghalaya Wild.	Pagalaphad Wild, Khasi Wild, Meghalaya Wild, Lairawk, and Chengdawt
Cluster Ic	Sanna Chenkadali and cv. Rose	Sanna Chenkadali and Pisang Jari Buaya
Custer Id	Pisang Lilin	*M. ac.* ssp.*burmaniccoides* and Pisang Jajee
Cluster Ie	-	cv. Rose
Cluster II	Pisang Jari Buaya	Anaikomban and Pisang Lilin

are different, they are genetically closer. Similar results have also been reported in diversity analysis using RAPD and AFLP techniques and concluded that there is no strong correlation between genetic diversity and phenotypic character (Crouch *et al*., 4; Ude *et al*., 14). Their genetic closeness could be successfully exploited in gene pyramiding programmes for the development of improved synthetic diploids with good quality fruits and disease resistance.

Cluster II Pisang Jari Buaya exhibits merely 100% dissimilarities with the cluster I. Persistent male flowers are one of its unique morphological features; which might have contributed for its separate clustering. However, in molecular characterization this has grouped with an indigenous landrace Sanna Chenkadali. This clustering might be due to common origin of South and South East Asia. Twenty one primers pairs were used to assess the microsatellite polymorphism, in which 17 primer pairs (81.01%) produced discrete, reproducible amplicons and they were considered for the genetic diversity analysis. Rest of them produced non-specific products even after changing amplification conditions.

A total of 139 alleles were identified from the 17 primers pairs with a mean of 8.1 alleles per primer pair. The primer pairs tested exhibited 100% polymorphism. AGMI 24/25 exhibited the highest polymorphism with 12 alleles followed by AGMI 133/134 and AGMI 161/162 with 11 alleles each. The lowest polymorphism was observed in Mb SSR 1-12 and AGMI 125/126 with 5 alleles each. PIC values ranged from 0.40 (AGMI 133/134) to 0.70 (Mb SSR 1-12) with an average of 0.55. PIC value above 0.5 was registered in 60% of the primer pairs tested (Table 1). All the seventeen primer pairs, which were considered for the genetic analysis, produced specific alleles. Among them, AGMI 133/134 produced the maximum number of specific alleles (6) followed by AGMI 24/25 (5) and they also produced highest number of alleles, *viz.,* 11 and 12, respectively. Mb SSR 1-12 and Ma SSR 8a, 8b produced only one specific allele.

Musa ac. ssp. *burmannica* might have contributed for the development of parthenocarpic diploid Matti, both having Indian Peninsula as their origin. Newly identified *M. acuminata* wild forms, *viz.,* Pagalaphad Wild, Lairawk, Khasi Wild and Chengdawt can be elevated to sub species of *M. acuminata* Colla. and strengthen the family Musaceae. Pseudostem colour, which is one of the important traits for morphotaxonomic characterization, has not contributed to any changes at genetic level. Genetic improvement of banana is quite difficult owing to the crop inherent problems like sterility, parthenocarpy and polyploidy. Under such circumstances, the basic information obtained in the present study is expected to facilitate the selection of genetically closer parents with desired trait combination in banana breeding programmes.

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