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Molecular Characterization of Genetic Diversity and Structure in South Indian *Musa* Cultivars

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Abstract: Ten STMS loci were used to genotype thirty eight *Musa* cultivars representing six genome groups (AA, AB, BB, AAA, AAB and ABB). Number of alleles per locus ranged from 1 to 4 with a total of 27 alleles with an average of 2.7 alleles per primer pair. Moderate levels of average genetic diversity was observed (HE=0.42). Observed number of alleles per locus was high (2.5) in AA group followed by an average of 2.1 in AAB group. Average genetic diversity and Shannon's information index was highest in AA group with mean values 0.42 and 0.69, respectively. Average Fst value of 0.33 and an average Fis value of -0.11 were observed over all loci. The cluster analysis using the Unweighted Pair Group Method using Arithmetic mean (UPGMA) method displayed five clusters within two main groups that can be described by ploidy and genome constitution.

Key words: Banana, microsatellites, molecular characterization, *Musa*, STMS, south India

INTRODUCTION

Bananas and plantains are monocotyledonous plants in the genus *Musa* (Musaceae, Zingiberales). The centre of origin of the group is in South-East Asia, where they occur from India to Polynesia. The centre of diversity has been placed in Malaysia or Indonesia, although considerable diversity is known throughout the range. The plants are distributed mainly on margins of tropical rainforests (Heslop-Harrison and Schwarzacher, 2007). The genus *Musa* is divided into four sections on the basis of morphological characters and differences in basic chromosome numbers viz., *Eumusa* (x = 11), *Rhodochlamys* (x = 11), *Australimusa* (x = 10) and *Callimusa* (x = 10 or x = 9) (Dolezel and Bartos, 2005). Section *Eumusa* consists of 11 wild species (Horry *et al*., 1997) and is geographically wide spread with considerable variability. The species are found throughout South East Asia from India to Pacific Islands. Sections *Callimusa* and *Rhodochlamys* consist of non parthenocarpic species that have no nutritionally valuable fruits and are important only as ornamentals (Pillay and Tripathi, 2007). *Callimusa* is chiefly represented in and around the Isle of Borneo. The section *Australimusa* is a poorly understood group of parthenocarpic edible types, collectively known as Fe'i bananas, characterized by their erect fruit bunches and generally red sap. This section also includes *M. textilis* which is of commercial importance for its fibre. The distribution of *Australimusa* extends from the Philippines, New Guinea and Australia in the west to Hawaiin islands and Samoa in the East (De Langhe, 1969).

Modern cultivated bananas evolved from intra-and inter-specific hybridization between two wild diploid species of section *Eumusa*, *M. acuminata* Colla. and *M. balbisiana* Colla., that contributed the A and B genomes, respectively (Simmonds, 1995). Commercially cultivated bananas are seed sterile diploid, triploid or tetraploid clones containing various combinations of the A and B genomes coming from these two diploid wild progenitors (Simmonds and Shepherd, 1955). Presence of S and T genomes in the cultivated and wild types as represented by *M. schizocarpa* and *M. textilis* has also been reported (Uma and Sathiamoorthy, 2007). Further evolution occurred through polyploidization and accumulation of somatic mutations (Stover and Simmonds, 1987). Inter-crossing among species and sub species has resulted in the appearance of sterility, a trait that was selected during domestication, together with parthenocarpy and vegetative propagation (Simmonds, 1995). The first cultivated bananas were diploids and triploid forms originated from the crossing between partially sterile diploids with fertile-male forms. Since triploids were more productive and vigorous, with larger fruits, they were selected over the diploid clones (Sharrock, 1998).

The evolutionary pathway subtending the emergence of cultivated varieties include the appearance of vegetative parthenocarpy and female sterility in *M. acuminata*, allowing the production of pulp without seeds as evidenced by the occurrence of parthenocarpic and seedless diploid *M. acuminata* (Simmonds, 1962). Edibility of mature fruits of diploid *M. acuminata* (AA) is

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due to female sterility and parthenocarpy, which have been selected and maintained by humans. Triploid AAA cultivars arose from these diploids, following crosses between edible diploids and wild *M. acuminata* subspecies, giving rise to a wide range of AAA genotypes. Humans took the diploid and triploid *M. acuminata* cultivars to areas where *M. balbisiana* is native and natural hybridizations resulted in the formation of hybrid progeny with the genomes AB, AAB and ABB. Tetraploids were also resulted from the natural hybridizations (Uma *et al*., 2001). Genome composition has played an important role in the classification of bananas. Edible bananas are classified into different genomic groups based on a system created by Simmonds and Shepherd (1955). This system assigns a score of 1 to 5 for 15 selected morphological features that differentiate *M. acuminata* from *M. balbisiana*. The total score determines the relative contribution of these wild species to the constitution of the clone. The major genomic groups include AA, BB, AB (diploids), AAA, AAB, ABB (triploids), AAAA, AAAB, AABB and ABBB (tetraploids).

Generally crop landraces and wild relatives are important sources of new genetic variation for the future improvement of highly bred cultivars. Many of these genetic resources are, however, in danger of being lost, as old cultivars are replaced because of modernization in farming techniques, changes in socio-economic conditions and by introduction of new high yielding cultivars. Furthermore the growers of landraces are often aging and the younger members of the families might not be interested in maintaining the landraces for the future (Christensen *et al*., 2011). Knowledge about genetic diversity in available germplasm is very useful for plant breeders. It supports their decision on the selection of cross combinations from large sets of parental genotypes and is always helpful when widening the genetic basis of a breeding program (Ganapathy *et al*., 2011). Morphological diversity measures often underestimate or overestimate the actual amount of genetic diversity, as they are based on observable morphological and phenological characteristics rather than actual population genetic structure. Population structures along with genetic relationship and diversity are useful for developing strategy of conservation and utilization of crop genetic resources. Molecular markers have become useful tools for the evaluation of genetic diversity in germplasm collections, as well as for genetic mapping and marker-assisted breeding (Phumichai *et al*., 2008) because of their highly polymorphic nature, frequent occurrence in genome, selective neutral behaviour, easy access, easy and fast assay, high reproducibility and easy exchange of data between laboratories (Joshi *et al*., 1999). Microsatellite markers have become the markers of choice because they are locus-specific, codominant, PCR-based, highly polymorphic and useful at a range of scales from individual identification to fine-scale phylogenies. The only disadvantage in using microsatellites is that prior information about the sequence for developing primer combinations is needed.

Bananas and plantains are believed to be one of earliest plant species to be domesticated (Denham *et al*., 2003). The long history of domestication and cultivation with widespread dispersal of cultivars with many synonyms has resulted in a great deal of confusion in the identification and classification of cultivars in *Musa*. Since banana is native to Indian sub continent, a high level of genetic diversity may be expected among individual plants within and between genomic groups. Although a wide range of morphometric variability exists in banana populations native to the South India (Amalraj, 1992), no extensive study has been undertaken on harnessing molecular markers to assess the diversity of these populations. Thus, the objectives of the present study were to (a) identify polymorphic STMS markers with high informative value for the South Indian banana in terms of population differentiation and identification, (b) estimate the genetic population structure and (c) compare the genetic diversity between different genomic groups.

MATERIALS AND METHODS

Plant material collection and DNA extraction: Leaf samples of thirty eight banana cultivars representing AA, AB, BB, AAA, AAB and ABB genomic groups were collected from Banana Nursery, Peringammala, Thiruvananthapuram, Kerala, India and Banana Research Station, Kannara Thrissur, Kerala (Table 1). Fresh, young and healthy cigar leaves were used for DNA isolation. Homogenization was accomplished by freezing fresh leaf tissue in liquid nitrogen and then either grinding with a mortar and pestle. DNA was extracted from homogenized leaf tissue using CTAB-based protocol modified from Saghai-Maroof *et al*. (1984). DNA was suspended in TE buffer (pH = 8) and digested with RNase A (QIAGEN) at 37°C for 1 h. DNA concentration was estimated with a DNA fluorimeter (Hoeffer Scientific, San Fransisco, USA) using Hoechst 33258 (Bisbenzimide) as the DNA intercalating dye (Brunk *et al*., 1979). DNA samples were diluted to y 20 ng μL^{-1} before conducting PCR.

Amplification of STMS loci and fragment separation: Fifteen primer pairs of Ma series, specific to *Musa* spp. (Jarret *et al*., 1994) were screened for usefulness on a

Table 1: List of banana cultivars used for the present study

Name of the cultivar	Genome constitution	Place of collection
Pisanglilin	AA	Banana nursery Peringammala, TVM
Matti	"	"
Kadali	"	"
Sannachenkadali	"	"
Chingan	"	Botanical garden, Dept. of Botany
Calcutta 4	"	BRS, Kannara, Thrissur
Sikuzani	"	"
Adukkan	AB	Banana Nursery, Peingammala, TVM
Poomkalli	"	"
Njalipoovan	"	"
Valiyakunnan	"	BRS, Kannara, Thrissur
Adakkakunnan	"	"
Padalimoongil	"	"
Velipputtubale	"	"
Red Banana	AAA	Banana Nursery, Peingammala, TVM
Robusta	"	"
Dwarf Cavendish	"	"
Grandnaine	"	"
Green red	"	"
Monsmarie	"	"
Grosmichel	"	"
Poovan	AAB	"
Perumpadali	"	"
Dudhsagar	"	"
Mysore ethan	"	"
Palayankodan	"	"
Krishnavazhai	"	"
Charapadathi	"	"
Nendran	"	"
Quintal nendran	"	"
Padathi	"	"
Velipadathi	"	"
Kosthabontha	ABB	"
Karpooravalli	"	"
Boothibale	"	"
Kanchikela	"	"
Peyan	"	"
Elavazhai	BB	BRS, Kannara, Thrissur

diverse subset of cultivars from the collection. Based on criteria of PCR amplification and allele scoring consistency ten best markers were chosen for further amplification reactions (Table 2). Each PCR reaction mixture (25 μL) consisted of 1X PCR buffer (10 mM Tris pH 8.3 and 50 mM KCl), 25 mM $MgCl_2$, 1U Taq DNA polymerase (AmpliTaq, Applied Biosystems), 200 μM each dNTPs, 1.0 μM each of forward and reverse primers and 20 ng of genomic DNA. PCR was performed under the following conditions: denaturation at 93°C for 1 min, primer annealing (for 2 min, temperature specified in Table 2 and 45 sec extension at 72°C for 30 cycles). Amplified STMS fragments were mixed with 2.5 μL gel loading dye (6X dye: 0.25% bromophenol blue, 0.25% xylene cyanol FF, 30% glycerol, w/v) and centrifuged briefly in a microfuge before loading. The amplification

Table 2: Forward and reverse primer sequence details used for STMS analysis

Primer	Sequence 5'-3'	Repeat unit	Annealing temp
Ma1-2	GATGATGGTGAGAGGCTGATGA GGTCGGTATGGGAAGCACC	$(GA)_{11}$	62
Ma1-16	TTTGCCTGGTTGGGCTGA CCCCCCTTTCCTCTTTTGC	$(GA)_{10}$	63
Ma1-17	AGGCGGGGAATCGGTAGA GGCGGGAGACAGATGGAGT	$(GA)_{14}$	63
Ma1-27	GAGCCCATTAAGCTGAACA CCGACAGTCAACATACAATACA	$(GA)_9$	60
Ma2-4	CTCCTTTGTGAGCTCGGCATAT AGGGTCCAAGAAACTCCTCC	$(CT)_{13}$	63
Ma2-7	TGAATCCCAAGTTTGGTCAAGA CCAACTCTTGTCCCTCACTTCA	$(GA)_{11}$	62
Ma3-41	GAAGCATCCAATGGACCTA GCGAACTCACAATAGCGA	$(GA)_{12}$	58
Ma3-48	CCCGTCCCATTTCTCA TTCGTTGTTCATGGAATCA	$(GA)_{15}$	58
Ma3-50	GGTGGATGGGCTGGGTA GGATCCAAGCTTATCGAGTT	$(GA)_{12}$	58
Ma3-55	GGTGCTCTTCGGAGGA CGTTTATATCCATCCCA	$(GA)_{10}$	58
Ma3-59	GCTTGTCTCTCACCCACTC ACCGACTCCCCAATAGG	$(AT)_{12}$	58
Ma3-60	TGGCTGACAATTACATGACA GCGCACTGTGGTGTGT	$(GA)_{14}$	58
Ma3-79	CTGGTCCTTTTCAGTTCACTC TAGGCAGCTCCCAATCA	$(AT)_6$	58
Ma3-81	CTAGGCTTCCTGCTGCTC TGAGCGAATTTGATCAGAAC	$(CT)_{10}$	58
Ma3-90	GCACGAAGAGGCATCAC GGCCAAATTTGATGGACT	$(CT)_{11}$	58

products were size separated electrophoretically on 3.5% Metaphor (FMC Bioproducts, USA) + 0.7% agarose gels in 1X TAE buffer at 70 V for one hour 30 min or till the bromophenol blue dye traveled less than 2/3rd the length of gel. A 100 bp DNA ladder was used as molecular size standard. Gels were stained with ethidium bromide (1 mg mL^{-1}) and viewed under UV light.

Data analysis: Each amplification product was considered as a DNA marker and patterns were scored for presence of each allele in a cultivar. Genetic variation at each locus was characterized in terms of the number of alleles, observed heterozygosity (H_O), expected heterozygosity (H_E), Shannon's diversity index (I), gene flow (Nm) and gene differentiation coefficient (F_{ST}) using the genetic analysis packages POPGENE Version 1.32 (Yeh *et al*., 2000). Polymorphism Information Content (PIC) for each STMS primer set was determined as described by Botstein *et al*. (1980). The UPGMA algorithm was used to construct a dendrogram from a distance matrix based on Nei's (1972) genetic distances. MVSP 3.0 was used to perform principal coordinate analysis that plots the relationship between distance matrix elements based on their first two principal coordinates.

RESULTS

Efficiency of STMS primers to detect polymorphism among *Musa* cultivars: A total of fifteen primer pairs were tested to assess the microsatellite polymorphism in 38 *Musa* cultivars. Five primers (33%) produced null alleles in several individuals or produced a non-defined amplification profile, with several alleles or very faint bands. A cultivar was assigned null allele for a microsatellite locus whenever an amplification product could not be detected for a particular genotype-marker combination. The remaining ten primers produced well defined discrete banding pattern which revealed 27 alleles based upon the presence (1) and absence (0) of alleles. The absence of an amplification product with these ten primers in an individual was considered as missing data. PIC values for the STMS primers were quite high and ranged from 0.29 to 0.77 with an average of 0.53 (Table 3).

High genetic polymorphism was observed among the cultivars in the present study. Of the ten loci analysed, 90% was polymorphic among the selected cultivars. The highest polymorphism was observed with the primers Ma1-17 and Ma 3-60 with 4 alleles and the mean number of alleles observed per primer was 2.7. The primer Ma3-55 was monomorphic. The over all size of amplified products ranged from 50 to 290 bp. The size differences between the smallest and largest alleles for a given STMS locus varied from 20 to 60 bp. The mean value of Shannon's information index, which is a measure of gene diversity, was 0.70±0.38. Average genetic diversity computed was 0.42±0.22 in terms of Nei's expected heterozygosity (Table 4).

Genetic differentiation among different *Musa* genomic groups: The percentage of polymorphism for the ten STMS loci ranged from 60 to 80% among the five major *Musa* genomic groups (AA, AAA, AB, AAB and ABB). Observed number of alleles per locus was high (2.5) in AA group of cultivars followed by an average of 2.1 in AAB group (Table 5). Estimates of observed and expected heterozygosity showed variation between different *Musa* genomic groups. Observed heterozygosity was higher than expected heterozygosity values in all genomic groups, except AA. AAA group showed highest observed heterozygosity with a mean value of 0.50 followed by ABB group. Average genetic diversity in terms of Nei's expected heterozygosity ranged from 0.2 to 0.42 among the five major *Musa* genomic groups. Nei's expected heterozygosity and Shannon's information index was highest in AA group with mean values 0.42 and 0.69, respectively. F-statistics for the 38 banana cultivars for each STMS locus as well as their averages over all loci were also computed. Average Fst (Fixation index) value over all loci was found to be 0.33. The average Fis (average inbreeding coefficient within the cultivars) value was -0.11, which indicated the absence of inbreeding.

Table 3: Data on number of alleles, Polymorphism information content (PIC), number of genotypes with multiple alleles, product size range obtained using 10 STMS markers in 38 banana cultivars

				Product size (bp)	
Primer	No. of alleles	PIC	No. of genotypes with multiple alleles	Range	Difference
Ma1-2	2	0.42	14	120-140	20
Ma1-16	3	0.63	25	150-180	30
Ma1-17	4	0.69	22	110-150	40
Ma1-27	3	0.74	12	110-130	20
Ma2-7	3	0.53	15	50-100	50
Ma3-48	3	0.66	17	160-180	20
Ma3-55	1	-	-	290	-
Ma3-60	4	0.77	6	90-150	60
Ma3-79	2	0.29	7	80-120	40
Ma3-90	2	0.52	17	120-140	20

Table 4: Summary of STMS analysis for 38 *Musa* cultivars

Analysis	Values
No. of loci	10
% of polymorphic loci	90%
Total no. of alleles	27
	2.7/locus
No. of alleles specific to each loci	1-4
Highly polymorphic loci	Ma 1-17 and Ma 3-60
PIC	0.29 -0.77
Average	0.53
Shannon's information index	0.7
Average genetic diversity (Nei expected heterozygosity)	0.42
H_O	0.34
H_E	0.42
Fst	0.33
Nm	0.50

Table 5: Genetic diversity within different *Musa* genomic groups

	Group				
Parameters	AA	AB	AAA	AAB	ABB
na*	2.5	1.7	1.8	2.1	1.9
I**	0.69	0.33	0.45	0.46	0.5
Nei***	0.42	0.21	0.3	0.29	0.33
H_O	0.25	0.24	0.5	0.36	0.38
H_E	0.45	0.22	0.32	0.3	0.37

***Nei (1973) expected heterozygosity, *na: Observed number of alleles, **I: Shannon's Information index

Genetic similarity estimation and cluster analysis: Nei's (1972) genetic identity measures calculated for 38 *Musa* cultivars ranged from 0.43 to 0.98 with an average of 0.71. Highest similarity was observed between AAA cultivars Grandnaine and Dwarf Cavendish. Maximum genetic distance was observed between Boothibale (ABB) and Red banana (AAA). Nei's genetic identity measures showed variation among the cultivars of same genomic group (Table 6).

UPGMA derived dendrogram on the basis of STMS data indicated two major clusters (Fig. 1). All the AA and

Table 6: Nei's genetic identity measures within different *Musa* genome groups

Group	Range	Average
AA	0.49-0.92	0.68
AB	0.78-0.97	0.89
AAA	0.68-0.98	0.89
AAB	0.63-0.97	0.85
ABB	0.69-0.90	0.86

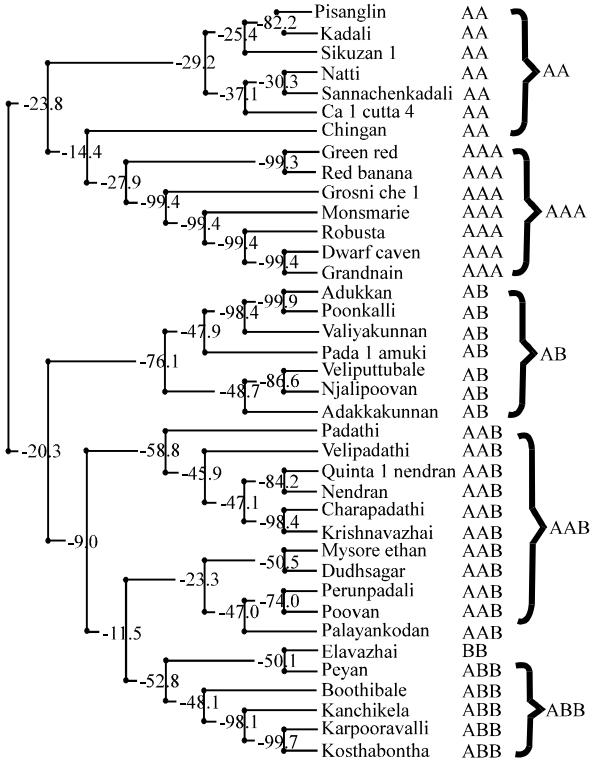


Fig. 1: UPGMA derived dendrogram based on STMS data

AAA cultivars were grouped together in cluster 1 and all the other bispecific (AB, AAB and ABB) cultivars were grouped together in cluster II. Cluster 1 included two sub clusters 1 A and 1 B. All diploid AA cultivars were grouped in cluster 1 A and triploid AAA cultivars were grouped in cluster 1 B. However, Chingan (AA) showed an association with cluster 1 B. Cluster II included two sub clusters (II A and II B). Cluster II A grouped all the AB diploid cultivars together. Cluster II B was again divided into two sub clusters B1 and B2. Cluster B1 grouped six AAB cultivars together. Cluster B2 grouped five AAB cultivars and ABB cultivars separately. UPGMA derived dendrogram on the basis of STMS data grouped AAB cultivars in two clusters while the cooking bananas having ABB genome constitution were grouped together. Elavazhai (BB) was found to be linked with the ABB cluster. Bispecific diploid cultivars with AB genomic

Table 7: Genetic similarity between different *Musa* genomic groups

Group	AA	AB	AAA	AAB	ABB	BB
AA	****	0.8268	0.911	0.9059	0.7792	0.7177
AB		****	0.8313	0.7762	0.7673	0.594
AAA			****	0.8165	0.6215	0.5826
AAB				****	0.8518	0.8414
ABB					****	0.8719
BB						****

***Nei's (1973) expected heterozygosity

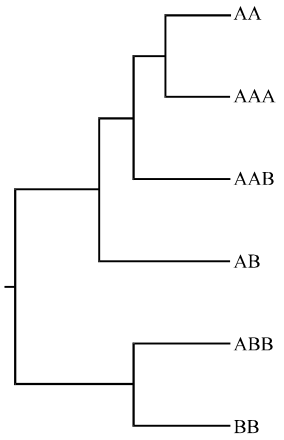
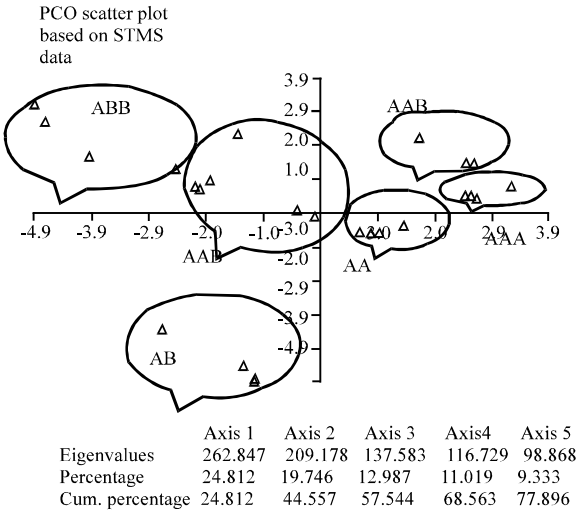


Fig. 2: Genetic relationship between *Musa* genomic groups

constitution were found to be sandwiched between the triploid AAA cultivars and AAB cultivars.

Genetic relationships between *Musa* genomic groups: Similarity matrices were constructed based on Nei (1978) unbiased measures of genetic distance for different *Musa* genomic groups (Table 7). Genetic similarity was lowest between AAA and BB genomic groups with a value of 0.5826. Highest genetic similarity value (0.9110) was observed between AA and AAA groups. Average value of genetic similarity among the six genomic groups was observed to be 0.78. Dendrogram constructed on the basis of Nei's genetic distance method revealed two major clusters (Fig. 2). First cluster included AA, AAA, AAB and AB genomic groups. Of which, AA and AAA groups were clustered together showing close association. ABB and BB groups were grouped separately as a different cluster.

Principal co-ordinate analysis: The results of the PCO analysis were comparable to the cluster analysis (Fig. 3). The first five most informative principal components explained more than 77% of the total variation. PCO analysis based on STMS markers showed clear grouping of banana cultivars based on their ploidy and genomic constitution. AA cultivars showed affinity to AAA cultivars. AB and ABB formed distinct clusters and were



	Axis 1	Axis 2	Axis 3	Axis4	Axis 5
Eigenvalues	262.847	209.178	137.583	116.729	98.868
Percentage	24.812	19.746	12.987	11.019	9.333
Cum. percentage	24.812	44.557	57.544	68.563	77.896

Fig. 3: PCO scatter plot

separated from rest of the cultivars. However, AAB cultivars formed two groups.

DISCUSSION

The molecular characterization of banana cultivars using STMS markers indicated enough polymorphism to differentiate different genomic groups. In the present study, 90% of the loci were polymorphic among the selected cultivars. Polymorphism in a given population is often due to the existence of genetic variants represented by the number of alleles at a locus and their frequency of distribution in a population. The higher level of polymorphism associated with STMS markers may be a function of the unique replication slippage mechanism responsible for generating simple sequence repeat allelic diversity (Pejic *et al*., 1998).

In the present study, PIC values for the tested primer pairs ranged from 0.29 to 0.77 with an average of 0.53. Seven primers in banana showed an average PIC value greater than 0.5 suggesting that the STMS markers used in this study were highly informative, because PIC values higher than 0.5 indicate high polymorphism. DeWoody *et al*. (1995) suggested that markers with PIC values of 0.5 or higher are highly informative for genetic studies and are extremely useful in distinguishing the polymorphic rate of a marker at a specific locus. Khar *et al*. (2011) also reported similar observations in onion. PIC value is a reflection of allele diversity and frequency among the cultivars and an average PIC of 0.578 has been reported in rice using 38 SSR markers (Ravi *et al*., 2003). PIC values ranging from 0.23 to 0.81 with an average of 0.62 have been reported in Sorghum using 28 SSR markers (Agrama and Tuinstra, 2003). In red clover, PIC values for seven SSR markers ranged from 0.64 to 0.85 with a mean value of 0.75 (Dias *et al*., 2008). Smith *et al*. (1997) also reported highest PIC values for SSR markers containing dinucleotide repeats. This difference may be associated with the use of acrylamide gels for allele detection in their studies. Acrylamide gels have greater resolving power than agarose gels. The increased resolution of acrylamide over agarose gel separation could result in the detection of larger number of allele per locus. This may be particularly important for SSR loci containing dinucleotide repeats whose amplification products are in the 130 to 200 bp range. PCR products differing by two base pairs cannot be resolved with agarose gels (Agrama and Tuinstra, 2003). However, Cregan *et al*. (1994) suggested that metaphor agarose (2.5%) gels separate alleles that differ by only 6-8 base pairs. Liu *et al*. (1995) also reported that it is possible to map most microsatellite loci using metaphor agarose. Crouch *et al*. (1999) used metaphor agarose in the VNTR based diversity analysis of 2x and 4x full-sib *Musa* hybrids.

The genetic composition of a population is usually described in terms of allele frequencies, number of alleles and heterozygosity. In the present study the mean number of alleles observed per primer was 2.7 and among the *Musa* genomic groups it varied from 1.7 (AB group) to 2.5 (AA group). Oriero *et al*. (2006) reported a total of 23 alleles with an average of 2.56 alleles per primer in 40 *Musa* accessions using nine B-genome derived SSR markers. Using 9 primers Creste *et al*. (2004) reported a total of 115 alleles with an average of 12.8 alleles per primer from 58 *Musa* genotypes including 49 diploid AA genotypes of which 17 were wild. Grapin *et al*. (1998) analysed 59 diploid AA genotypes (35 wild and 21 cultivars) with a different set of nine primers and identified 72 alleles with a mean of 8 alleles per primer. Difference in the results might be due to the use of agarose gels instead of polyacrylamide gels and the samples (2 wild and 36 cultivars) selected for the present study. Ge *et al*. (2005) reported a total of 29 alleles with an average of 2.32 alleles per primer from 15 *Musa balbisiana* populations using five SSR loci.

Heterozygosity of a population is measured by determining the proportion of genes that are heterozygous and the number of individuals that are heterozygous for each particular gene. Average expected heterozygosity is an indicator of genetic diversity in a population (Nassiry *et al*., 2009). Heterozygosity corresponds to a probability that two alleles taken at random from a population can be distinguished using the marker in question. Thus a convenient quantitative

estimate of marker utility and the polymorphism detected can be given in terms of Nei's genetic diversity, Shannon's information index and estimate of gene flow (Zhao *et al*., 2006). Average genetic diversity computed in terms of Nei's expected heterozygosity and Shannon's Information index was 0.42 and 0.70, respectively for the 38 cultivars. The generally high heterozygosity per locus found within the banana collection is typical in naturally out-crossing, perennial and clonally propagated species that are highly selected for greater adaptability, vigor and productivity under cultivation and harbor recessive somatic mutations as heterozygotes (Aradhya *et al*., 2003). AAA group showed highest observed heterozygosity followed by ABB group. The reason could be that in the selection process higher heterozygosity levels were favoured and intensified by improvements in horticultural performance, as evidenced in domesticated *Vitis* cultivars (Sefc *et al*., 2000).

Average genetic diversity among five major *Musa* genomic groups ranged from 0.20 to 0.42 and Shannon's Information index ranged from 0.45 to 0.69. In the present study diploid AA group showed highest genetic diversity in terms of Nei's expected heterozygosity and Shannon's index while diploid AB group was least diverse. Observed number of alleles per locus was also highest in AA group with a mean value of 2.5 while it was lowest in AB group. The high level of genetic diversity detected in AA diploid cultivars might derived from their putative inter-sub specific hybrid origin, with limited fertility due to heterozygosity for chromosome structural abnormalities, maintained by vegetative propagation (Creste *et al*., 2003). Grapin *et al*. (1998) concluded that most cultivated AA diploids were heterozygous for most of the microsatellite loci. Somatic mutation that accumulates genetic variation within clonally persisting clumps may account for some of the heterozygosity especially given rapid mutation of SSR fingerprints (Ge *et al*., 2005). Oriero *et al*. (2006) reported an average genetic diversity of 0.411 and average proportion of observed heterozygous individuals as 0.630 in 40 *Musa* accessions using nine SSR primers.

Genetic structure of the populations was analyzed through Wright's F-statistics. The F_{ST} varied among the loci from 0.07 (Ma 1-2) to 0.59 (Ma 3-60) with a mean value of 0.33, indicating a high level of population differentiation among the cultivars. F statistics (Wright, 1951) quantify the extent of between-within population differentiation. An F_{ST} value of 0.13 was reported among 40 *Musa* accessions including 23 banana and 17 plantain accessions (Oriero *et al*., 2006). Verma *et al*. (2010) used F statistics to determine the population differentiation of Indian *Perilla* landraces based on STMS markers and revealed that Uttarakhand population having relatively low level of heterozygosity consequently resulted in greater population differentiation. Kumar *et al*. (2010) reported higher population differentiation among common landraces of rice with an average F_{ST} value of 0.72. The mean number of migrants per generation among populations (Nm), based on F_{ST} calculations was 0.50. When Nm>1, gene flow is able to offset the differentiation among populations caused by isolation and genetic drift (Wang, 1996). Low levels of gene flow among the cultivars suggest that genetic differentiation might have occurred through genetic drift or random sampling of gametes.

The primers used in the present study were able to separate cultivars containing the *M. acuminata* genome alone, from interspecific hybrids of *M. acuminata* and *M. balbisiana* and to arrange cultivars into groups based on genomic constitution. Cultivars of AA, AB, AAB and ABB formed separate groups. Cultivars of AAB group formed two clusters and ABB group was more closely related to the BB group. Bhat *et al*. (1994) also observed distribution of cultivars in the same genomic groups into separate clusters based on cpDNA RFLPs. Sub divisions within the AAB group may reflect differing roles for specific *M. acuminata* sub species in the phylogeny of individual cultivars.

The microsatellite polymorphisms are good indicators of genomic micro differentiations, which may not reflect in morphological or physiological traits but germplasms with higher microsatellite polymorphisms are assumed to have higher allelic variation in functional genes. The results of this study revealed high levels of diversity in South Indian *Musa* germplasm. Therefore, we would expect that there is value in greater sampling of *Musa* cultivars in Kerala to search for new useful alleles and for germplasm collection and conservation. The notable polymorphism and genetic diversity detected within populations suggest that the future sampling and analyses should be focused at individual level.

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REFERENCES

Agrama, H.A. and M.R. Tuinstra, 2003. Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. Afr. J. Biotechnol., 2: 3334-340.

Amalraj, V.A., 1992. Collecting banana germplasm in South India. India. Plant Genet. Resour. Newsletter, 88: 64-66.

Aradhya, M.K., G.S. Dangl, B.H. Prins, J.M. Boursiquot, M.A. Walker, C.P. Meredith and C.J. Simon, 2003. Genetic structure and differentiation in cultivated grape, *Vitis vinifera* L. Genet. Res., 81: 179-192.

Bhat, K.V., R.L. Jarret and Z.W. Liu, 1994. RFLP characterization of Indian *Musa* germplasm for clonal identification and classification. Euphytica, 80: 95-103.

Botstein, D., R.L. White, M. Skolnick and R.W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet., 32: 314-331.

Brunk ,C.F., K.C. Jones and T.W. James, 1979. Assay for nanogram quantities of DNA in cellular homogenates. Anal. Biochem., 92: 497-500.

Christensen, S., R.V. Bothmer, G. Poulsen, L. Maggioni, M. Phillip, B.A. Andersen and R.B. Jorgensen, 2011. AFLP analysis of genetic diversity in leafy kale (*Brassica oleracea* L. convar. acephala (DC.) Alef.) landraces, cultivars and wild populations in Europe. Genet. Resour. Crop Evol., 58: 657-666.

Cregan, P.B., A.A. Bhagwat, M.S. Akkaya and J. Rongwen, 1994. Microsatellite fingerprinting and mapping of soybean. Meth. Mol. Cell Biol., 5: 49-61.

Creste, S., A.T. Neto, S.O. Silva and A. Figueira, 2003. Genetic characterization of banana cultivars (*Musa* spp.) from Brazil using microsatellite markers. Euphytica, 132: 259-268.

Creste, S., A.T. Neto, R. Vencovsky, S.O. Silva and A. Figueira, 2004. Genetic diversity of *Musa* diploid and triploid accessions from the Brazilian banana breeding program estimated by microsatellite markers. Genet. Resour. Crop Evol., 51: 723-733.

Crouch, J.H., H.K. Crouch, A.Tenkouano asnd R. Ortiz, 1999. VNTR-based diversity analysis of 2x and 4x full-sib *Musa* hybrids. Electron. J. Biotechnol., 2: 99-108.

De Langhe, E.A., 1969. Bananas (*Musa* Spp.). In: Outlines of Perennial Crop Breeding in the Tropics, Ferwerda, P.P. and F. Wit, (eds.). H. Veenman and Zonen, Wageningen, Netherlands.

DeWoody, J.A., R.L. Honeycutt and L.C. Skow, 1995. Microsatellite markers in white tailed deer. J. Hered., 86: 317-319.

Denham, T.P., S.G. Haberle and C. Lentfer, 2003. Origins of agriculture at Kuk Swamp in the Highlands of New Guinea. Science, 301: 189-193.

Dias, P.M.B., B. Julier, J.P. Sampoux, P. Barre and M.D. Agnol, 2008. Genetic diversity in red clover (*Trifolium pretense* L.) revealed by morphological and microsatellite (SSR) markers. Euphytica, 160: 189-205.

Dolezel, J. and J. Bartos, 2005. Plant DNA flow cytometry and estimation of nuclear genome size. Ann. Bot., 95: 99-110.

Ganapathy, K.N., B.N. Gnanesh, M.B. Gowda, S.C. Venkatesha, S.S. Gomashe and V. Channamallikarjuna, 2011. AFLP analysis in pigeonpea (*Cajanus cajan* (L.) Millsp.) revealed close relationship of cultivated genotypes with some of its wild relatives. Genet. Resour. Crop Evol., 58: 837-847.

Ge X.J., M.H. Liu, W.K. Wang, B.A. Schaal and T.Y. Chiang, 2005. Population structure of wild bananas, *Musa balbisiana*, in China determined by SSR fingerprinting and cpDNA PCR-RFLP. Mol. Ecol., 14: 933-944.

Grapin, A., J.L. Noyer, F. Carreel D. Dambier, F.C. Baurens, C. Lanaud and P.J.L. Lagoda, 1998. Diploid *Musa acuminata* genetic diversity with sequence tagged microsatellite sites. Electrophoresis, 19: 1374-1380.

Heslop-Harrison, J.S. and T. Schwarzacher, 2007. Domestication, genomics and the future for banana. Ann. Bot., 100: 1073-1084.

Horry, J.P., R. Ortiz, E. Arnaud, J.H. Crouch and R.S.B. Ferris *et al*., 1997. Banana and Plantain. In: Biodiversity, Conservation and use of Plant Genetic Resources in CGIAR Centres, Fuccillo, D., L. Sears and P. Stapleton (Eds.) University Press, Cambridge. Pp: 67-81.

Jarret, R.L., K.V. Bhat, P. Cregan, R. Ortiz and D. Vuylsteke, 1994. Isolation of microsatellite DNA markers in *Musa*. InfoMusa, 3: 3-4.

Joshi, S., P. Ranjekar and V. Gupta, 1999. Molecular markers in plant genome analysis. Curr. Sci., 77: 230-240.

Khar, A., K.E. Lawande and K.S. Negi, 2011. Microsatellite marker based analysis of genetic diversity in short day tropical Indian onion and cross amplification in related *Allium* spp. Genet. Resour. Crop. Evol., 58: 741-752.

Kumar, S., I.S. Bisht and K.V. Bhat, 2010. Population structure of rice (*Oryza sativa*) landraces under farmer management. Ann. Appl. Biol., 156: 137-146.

Liu, Z.W., R.L. Jarret, S. Kresovich and R.R. Duncan, 1995. Characterization and analysis of simple sequence repeat (SSR) loci in seashore paspalum (*Paspalum vaginatum* Swartz.). Theor. Appl. Genet., 91: 47-52.

Nassiry, M.R., A. Javanmard and R. Tohidi, 2009. Application of statistical procedures for analysis of genetic diversity in domestic animal populations. Am. J. Anim. Vet. Sci., 4: 136-141.

Nei, M., 1972. Genetic distance between populations. Am. Naturalist, 106: 283-292.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA., 70: 3321-3323.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89: 583-590.

Oriero, C.E., O.A. Odunola, Y. Lokko and I. Ingelbrecht, 2006. Analysis of B-genome derived simple sequence repeat (SSR) markers in Musa spp. Afr. J. Biotechnol., 5: 126-128.

Pejic, I., P. Ajmone-Marsan, M. Morgante, V. Kozumplick, P. Castiglioni, G. Taramino and M. Motto, 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. Theor. Applied Genet., 97: 1248-1255.

Phumichai, C., W. Doungchan, P. Puddhanon, S. Jampatong and P. Grudloyma, 2008. SSR-based and grain yield-based diversity of hybrid maize in Thailand. Field Crops Res., 108: 157-162.

Pillay, M. and L. Tripathi, 2007. Banana. In: Genome Mapping and Molecular Breeding in Plants, Chittaranjan, K. (Ed.) Springer, Berlin, Heidelberg. Pp. 281-301.

Ravi, M., S. Geethanjali, F. Sameeyafarheen and M. Maheswaran, 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa* L.) using RAPD and SSR markers. Euphytica, 133: 243-252.

Saghai-Maroof, M.A., K.M. Soliman, R.A. Jorgensen and R.W. Allard, 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal locations and population dynamics. Proc. Natl. Acad. Sci., 81: 8014-8018.

Sefc, K.M., M.S. Lopes, D. Mendonca M.R. dos Santos, L.M. da C. Machado and A. Da C. Machado, 2000. Identification of microsatellite loci in olive (*Olea europaea*) and their characterization in Italian and Iberian olive trees. Mol. Ecol., 9: 1171-1173.

Sharrock, S., 1998. Collecting the *Musa* Genepool in Papua New Guinea. In: Collecting Plant Genetic Diversity, Guarino, L., V.R. Rao and R. Reid (Eds.). CAB International, Wallingford, pp: 647-658.

Simmonds, N.W. and K. Shepherd, 1955. Taxonomy and origins of cultivated bananas. J. Linn. Soc. London Bot., 55: 302-312.

Simmonds, N.W., 1962. The Evolution of Bananas. Longman, London, UK., Pages: 170.

Simmonds, N.W., 1995. Bananas. In: Evolution of Crop Plants, Smart, J. and N.W. Simmonds (Eds.). Longman, UK., pp: 370-375.

Smith, J.S.C., E.C.L. Chin, H. Shu, O.S. Smith and S.J. Wall*et al*., 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.) comparisons with data from RFLPs and pedigree. Theor. Appl. Genet., 95: 163-173.

Stover, R.H. and N.W. Simmonds, 1987. Bananas. 3rd Edn., Longman, London UK.

Uma, S. and S. Sathiamoorthy, 2007. Status of Genetic Diversity in Banana in India. In: Banana: Technological Advancements, Singh, H.P. and S. Uma (Eds.). AIPUB, Tiruchirappalli, India.

Uma, S., S. Sathiamoorthy and R. Nicolas, 2001. Confirmation of occurrence of a natural tetraploid banana in India. Indian J. Plant Genet. Resour., 14: 350-353.

Verma, N., M.K. Rana, K.S. Negi, G. Kumar, K.V. Bhat, Y.J. Park and I.S. Bisht, 2010. Assessment of genetic diversity in Indian Perilla [*Perilla frutescens* (L.) Britton] landraces using STMS markers. Indian J. Biotechnol., 9: 43-49.

Wang, J., 1996. Inbreeding and variance effective sizes for nonrandom mating populations. Evolution, 50: 1786-1794.

Wright, S., 1951. The genetical structure of populations. Ann. Eugen., 15: 323-354.

Yeh, F.C., R.C. Yang, T.B.J. Boyle, Z.H. Ye and J.X. Mao, 2000. POPGENE 32, Microsoft Windows based Software for Population Genetic Analysis. Version 1.32, Molecular Biology and Biotechnology Centre, University of Alberta, Alberta, Canada.

Zhao, W.G., J.Q. Zhang, Y.J. Wang, T.T. Chen and Y. Yin *et al*., 2006. Analysis of genetic diversity in wild populations of mulberry from western part of north east China determined by ISSR markers. Genet. Mol. Biol., 17: 196-203.