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AFLP characterization and genetic diversity analysis of Indian banana and plantain cultivars (*Musa* spp.)

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Abstract

DNA profiles of 92 banana and plantain cultivars were generated with 12 amplified fragment length polymorphism (AFLP) primer pairs. The average polymorphism information content (PIC) values of the polymorphic AFLP markers varied from 0.270 to 0.341, and the profiles could distinguish all the 92 cultivars analysed. It was possible to identify differences between two accessions of the AAB genome cultivar Rasthali, indicating the presence of intra-cultivar genetic variation. A UPGMA (unweighted pair group method of arithmetical means) dendrogram generated with 1581 AFLP products identified four major clusters, each comprising cultivars of similar genomic constitution. All the ABB cultivars grouped in a single cluster, which was well separated from the rest. The within-group similarity coefficients indicated that the most diverse group was AAB, followed by AAA. However, significant genetic diversity was also present among AA, AB and ABB Indian banana cultivars, supporting the notion that India, along with other neighbouring south-eastern Asian countries, is the centre of diversity for cultivars of banana and plantain. The presence of substantial diversity among the banana and plantain landrace cultivars could be exploited in varietal improvement programmes.

Keywords: AFLP profiling; cultivar identification; Indian banana and plantain landraces; germplasm classification

Introduction

The genus *Musa* contains 40 species, divided into the four sections *Eumusa* (haploid chromosome number, $n = 11$), *Rhodochlamys* ($n = 11$), *Callimusa* (mostly $n = 10$) and *Australimusa* ($n = 10$). The edible species are found in *Eumusa* and *Australimusa*, and the ornamental types are in *Rhodochlamys* and *Callimusa* (Simmonds, 1962). The edible *Musa* germplasm includes *M. acuminata* Colla. (AA), *M. balbisiana* Colla. (BB), and their hybrids and polyploids, AA, AAA, AAAA, AB, AAB, ABB and ABBB genomic groups (Simmonds, 1962). The AA, AB, AAA and AAB groups contain the dessert bananas, which are consumed as fruits, while the ABB group comprises the cooking bananas. Plantains, which are cooked before consumption due to their high starch content, belong to the AAB genomic group.

India is considered to be one of the centres of origin and diversity for the AB, AAB and ABB groups, as these species are cultivated in varied agroclimatic regions of the country and a wide spectrum of variability is reported to occur (Simmonds and Shepherd, 1955). Most of the Indian cultivars belong to *Eumusa*. Approximately 12 species occur in the north-eastern and western Ghats regions of India (Hore *et al.*, 1992). In addition, considerable diversity for edible cultivars also occurs in these two regions of the country (Jain, 1963). Concerns about genetic erosion in its primary and secondary centres have generated interest in germplasm collection, identification,

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classification and conservation. Over 600 accessions of *Musa* germplasm have been collected, but these have not been systematically characterized and classified. The practice of assigning local names to cultivars on the basis of their fruit and plant characteristics has resulted in numerous synonyms and homonyms which need to be sorted out for effective and economical germplasm conservation, management and utilization (Shanmugavelu *et al.*, 1992).

A numerical scoring system based on a range of morphological features of stem, leaf, inflorescence and fruit has been used to classify individuals into various genomic categories. This system of cultivar identification, based entirely on morphological descriptors (Simmonds and Shepherd, 1955; IPGRI, 1991), is time consuming, subjective in nature and prone to errors in the hands of an inexperienced curator because environmental conditions greatly influence the expression of the morphological markers. Further, no proper 'keys' exist for identification of closely related cultivars. Hence, biochemical markers (isozymes) have been employed for cultivar identification, diversity analysis and establishment of genetic relationships among banana and plantain germplasm (Jarret and Litz, 1986; Bhat *et al.*, 1992a, b), but these markers are not ideal for distinguishing closely related cultivars. Furthermore, tissue specificity and a paucity of polymorphic marker loci limit the application of isozyme markers in phenetic studies.

DNA marker techniques such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSR) analyses have been shown to be appropriate tools for cultivar identification and assessment of genetic diversity. RFLPs and RAPDs have been employed for establishing phylogenetic relationships, diversity analysis and cultivar identification in *Musa* (Gawel *et al.*, 1992; Jarret *et al.*, 1992, 1993; Lanaud *et al.*, 1992; Bhat *et al.*, 1994, 1995). However, AFLPs combine the advantages of both RFLPs and RAPDs (Powell *et al.*, 1996), and no prior sequence knowledge is required for generating fingerprints and markers distributed throughout the genome.

This study was conducted with the aim of (i) characterizing the Indian banana and plantain cultivars to assess the genetic diversity present and (ii) classifying the *Musa* cultivars to specific genomic groups.

Materials and methods

Plant materials

The *Musa* clones analysed were collected from various parts of India. The *M. acuminata* ssp. *banksii* Colla., *M. balbisiana* var. tani, Boodles Altafort, Bluggoe and Pisang Seribu were procured from the International Network for the Improvement of Banana and Plantain, Germplasm Transit Centre, Leuven, Belgium. A total of 92 genotypes of *Musa* (Table 1) were included in this study. These collections were first characterized for morphological traits and maintained in the field genebank at the National Bureau of Plant Genetic Resources, Regional Station at Thrissur, Kerala State, India (Amalraj *et al.*, 1993). Based on the criteria defined by Simmonds and Shepherd (1955) and Simmonds (1962), the scores recorded for the morphological traits were used tentatively to classify the accessions into AA, BB, AB, AAA, AAAA, AAB and ABB genomic groups. The genomic constitution of some of the cultivars could not be determined unambiguously, so their genomic group was not designated (Table 1). The accessions *M. acuminata* ssp. *banksii* (AA), *M. balbisiana* var. tani (BB), Njalipoovan (AB), Rasthali, Poovan (AAB) and Bluggoe (ABB), which were classified into specific genomic groups based on a previous RAPD (Bhat and Jarret, 1995) and RFLP study (Bhat *et al.*, 1994), were included to test the validity of composition of the clusters and genomic constitutions deduced from the morphological studies.

DNA extraction and AFLP analysis

Total genomic DNA was extracted from 5 g of young leaf material and purified following the protocol of Saghai-Maroof *et al.* (1984). AFLP electropherograms (Vos *et al.*, 1995) were generated for each cultivar using ABI Prism fluorescent dye labelling and detection technology (Perkin Elmer) protocols. The AFLP kit from Perkin Elmer Applied Biosystems (USA), optimized for genome size of 500–6000 Mb was used following the manufacturer's instructions. Genomic DNA and enzyme concentrations, and reaction volume, were first optimized. Genomic DNA (100 ng) was digested with *Eco* RI and *Mse* I, and the DNA fragments were ligated with *Eco* RI and *Mse* I adaptors in a single reaction.

Pre-selective amplification was carried out with non-selective primers. The pre-amplified DNA was diluted 1:20 with 10 mM Tris–0.1 mM EDTA buffer, pH 8.0 ($TE_{10:0.1}$) and was used as a template for selective amplification. The selective amplifications were carried out with primers having three selective nucleotides. The *Eco* RI primers were labelled with three different fluorescent dyes (6FAM, NED and JOE; Table 2). The thermal cycling conditions for pre-selective and selective amplification were as described by the manufacturer. The selective amplification product (3 μl) was mixed with 0.25 μl of Gene Scan ROX-500 internal size standard and 19.5 μl of deionized formamide in a 0.5 ml sample tube.

Table 1. List of banana and plantain cultivars and their genomic constitution

S. No.	Abbreviation	Name of cultivar	Genomic constitution[a]
AA group cultivars (*M. acuminata* Colla.)[b]			
1	Kadl 432	Kadali	AA
2	Matt 487	Matti	AA
3	Mabk 2c	*M. acuminata* ssp. *banksii* Colla.	AA
4	Snsk 489	Sannasenkadali	?
5	Skzn 436	Sikuzani	?
6	Tngt 450	Tongat	AA
M. balbisiana Colla. accessions[b]			
7	Mblb 3c	*M. balbisiana* Colla.	BB
8	Mbtn 12c	*M. balbisiana* var. tani	BB
AB group cultivars (*M. acuminata* Colla. × *M. balbisiana* Colla.)[b]			
9	Chkb 400	Chikkabale	AB
10	Nypn 496	Neypoovan	AB
11	Njpn 485	Njalipoovan	AB
12	Pdmg 422	Padati Moongil	?
13	Pngl 423	Poongalli	AB
14	Rsgl 441	Rasgally	?
15	Vykn 406	Veliyakunnan	AB
AAA group cultivars (*M. acuminata* Colla.)[b]			
16	Ankn 462	Anaikomban	AAA
17	Chnd 492	Chandrabale	?
18	Gujrb 409	Gujarbale	AAA
19	Jahj 501	Jehaji	AAA
20	Krvz 473	Karivazhai	?
21	Kola 393	Kola	?
22	Mnrj 482	Manoranjitham	?
23	Nttu 398	Nattu	AAA
24	Pchk 458	Pachakkappa	AAA
25	Snzb 428	Sanzibar	?
26	Snvz 413	Senvazhai	AAA
27	Tkkd 395	Thenkadali	AAA
AAAA group cultivar (*M. acuminata* Colla.)[b]			
28	Bdla 508	Boodles Altafort	AAAA
AAB group cultivars (*M. acuminata* Colla. × *M. balbisiana* Colla.)[b]			
29	Adkn 380	Adakkakunnan	?
30	Amsr 14c	Amrit Sagar	AAB
31	Ayrk 391	Ayiramkkai	AAB
32	Brdm 293	Bardhaman	AAB
33	Brgb 379	Bergibale	?
34	Bthb 301	Boothibale	AAB
35	Chak 347	Chakkia	?
36	Chmp 321	Champa	?
37	Chnb 435	Cheenabale	AAB
38	Chnl 367	Chenali	?
39	Dsmb 319	Desimalbhog	AAB
40	Ernb 365	Ernabanian	?
41	Jafr 299	Jafarikela	?
42	Kali 338	Kali	AAB
43	Kalb 366	Kalibale	AAB
44	Krmp 344	Karimpoovan	AAB
45	Kmdn 456	Koombdyn	AAB
46	Kmbk 360	Koombillakkai	AAB
47	Krsv 334	Krishnavazhai	AAB
48	Lalk 469	Lalkela	?
49	Mdhb 314	Madhubas	?
50	Malk 357	Malakali	?
51	Mlvz 313	Malavazha	?
52	Mlbg 326	Malbhog	AAB
53	Mann 364	Mannan	AAB
54	Mrth 316	Marthaman	AAB

Table 1. *Continued*

S. No.	Abbreviation	Name of cultivar	Genomic constitution[a]
55	Mas 359	Mas	?
56	Mnth 336	Monthan	AAB
57	Mors 445	Morris	AAB
58	Musa 308	*Musa* cv. 308	?
59	Musa 309	*Musa* cv. 309	?
60	Musa 312	*Musa* cv. 312	?
61	Namr 351	Namrai	AAB
62	Nndn 303	Nendran	AAB
63	Ndpd 286	Nendrapadathi	AAB
64	Pchd 292	Pachanadan	AAB
65	Pdth 291	Padathi	AAB
66	Plyk 296	Palayankondan	AAB
67	Prmp 331	Permapadathi	AAB
68	Psgm 375	Pisang Man	?
69	Psgs 353	Pisang Seribu	AAB
70	Povn 284	Poovan	AAB
71	Rjsr 342	Rajasiree	?
72	Rsth 288	Rasthali 288	AAB
73	Rsth 290	Rasthali 290	AAB
74	Srml 343	Sirumalai	AAB
75	Tkld 352	Thekkan Thalladn	AAB
76	Tmlb 325	Thulasimalbhog	?
77	Vann 318	Vannan	AAB
78	Vlpd 348	Velipadathi	AAB
79	Vnmn 346	Veneethamannan	?
ABB group cultivars (*M. acuminata* Colla. × *M. balbisiana* Colla.)[b]			
80	Blgg 191	Bluggoe	ABB
81	Bbbt 189	Booditha Bontha Bathees	ABB
82	Guri 195	Gauria	?
83	Klmn 193	Kallu Monthan	?
84	Knbs 16c	Kanai Bansi	ABB
85	Mdvz 211	Madavazha	?
86	Mlmn 207	Malai Monthan	ABB
87	Matn 184	Mathura Annan	?
88	Ntvz 194	Nattu Vazhai	?
89	Peyn 5c	Peyan	ABB
90	Pdmn 212	Pidimonthan	ABB
91	Pdmn 190	Podhamanua	?
92	Sbmn 192	Sambrani Monthan	ABB

[a] Genomic constitution designated based on study of morphological variation (Amalraj *et al.*, 1993); ? denotes genomic constitution assigned from morphological studies not conclusive.
[b] Genomic groupings based on results of present study.

The samples were denatured at 95°C for 5 min in a thermal cycler and snap cooled over ice. The denatured samples were analysed in an automated DNA sequencer by capillary electrophoresis (ABI 310, Perkin Elmer Applied Biosystems).

Primer screening

Sixty-four primer combinations from Perkin Elmer Applied Biosystems were tested for selective amplification with three diverse cultivars: Amrit Sagar (AAB), Thiruvananthapuram (AAB) and Kanai Bansi (ABB). Twelve of the primer pairs producing consistently good and polymorphic amplification products were selected for further analysis (Table 2).

Data analysis

Accuracy of estimates of the size of DNA fragments in an automated analyser is restricted to the range of the internal size standard used. Hence, only the amplification products in the size range of 50–450 bp were considered as these are within the range of the internal DNA size standard, Gene Scan ROX-500 (Table 2). Each amplification product was

Table 2. Characteristics of the AFLP amplification products obtained with 12 selected primer pairs used to analyse genetic diversity among 92 banana and plantain cultivars

S. No.	Primer combinations (*Eco* RI/*Mse* I)	Total number of amplification products	Number of polymorphic products	% Polymorphism	Range of amplification products per cultivar	Average number of amplification products per cultivar	Average PIC per primer
1	E-ACA/M-CTC	135	135	100	10–115	55.53	0.291
2	E-ACG/M-CTC	128	128	100	10–96	43.67	0.270
3	E-AGC/M-CTC	118	118	100	8–77	41.27	0.276
4	E-ACT/M-CAA	131	131	100	16–86	61.25	0.319
5	E-AAG/M-CAA	133	133	100	12–101	65.16	0.329
6	E-ACC/M-CAA	129	129	100	17–93	61.98	0.316
7	E-ACA/M-CAC	137	137	100	7–100	53.97	0.297
8	E-AGG/M-CAC	140	140	100	14–105	65.02	0.341
9	E-ACC/M-CAC	136	136	100	11–87	49.91	0.292
10	E-ACT/M-CAG	130	130	100	9–75	50.26	0.291
11	E-AGG/M-CAG	137	137	100	18–92	59.51	0.313
12	E-ACC/M-CAG	127	127	100	13–81	54.35	0.311
Total		1581	1581	100	7–115	50.91	0.280

treated as a separate trait and scored as present (1) or absent (0) across the *Musa* genotypes analysed. The 'Genescan' and 'Genotyper' software packages (Perkin Elmer Applied Biosystems) were used for processing, scoring and conversion of the results into a '1/0 matrix'. This matrix of cultivars × AFLP markers was used to calculate pair-wise Jaccard similarity coefficients (Jaccard, 1908). The similarity coefficients matrix was then subjected to cluster analysis using UPGMA, and a dendrogram depicting the relationships among the cultivars was constructed. The goodness of fit of the dendrogram was analysed by the Mantel's test of correlations between similarity indices and co-phenetic values (Mantel, 1967). All computations used NTSYS-pc version 1.70 software (Exeter Software, New York, USA). The genomic designation of the cultivars analysed was decided on the basis of their placement in specific clusters.

Informativeness of markers

The frequency, p_i, of each marker (amplification product) was calculated from the 1/0 data matrix using the formula:

p_i = (Number of genotypes possessing the ith marker)/(Total number of genotypes analysed).

The frequency of the recessive allele, q_i, was calculated as $q_i = 1 - p_i$ following the Hardy-Weinberg population equilibrium equation. The polymorphism information content (PIC) for each marker was then computed as $\text{PIC} = 1 - (p_i^2 + q_i^2)$. The average PIC values for each primer pair, which has a range of 0–0.50, was derived by averaging the PIC values over the markers scored for each primer pair.

Results

Selection of primers and level of polymorphism

Of the 64 primer pairs screened, 16 generated polymorphic amplification products. However, only 12 were used in the final analysis, as these generated a sufficient number of polymorphic amplicons across the cultivars screened (Table 2). A total of 1581 polymorphic products in the size range 50–450 bp were generated among the 92 genotypes analysed. The number of products scored ranged from 118 (E-AGC/M-CTC) to 140 (E-AGG/M-CAC) with a mean of 131 per primer combination (Table 2). The average number of products per genotype per primer pair ranged from 41.27 (E-AGC/M-CTC) to 65.16 (E-AAG/M-CAA). PIC values ranged from 0.270 (E-ACG/M-CTC) to 0.341 (E-AGG/M-CAC). The number of products per accession varied widely; for example, E-AGC/M-CTC generated 8–77 products and E-ACA/M-CAC generated 7–100. In order to confirm the validity of this wide range observed, the experiment was repeated for the extreme accessions. No significant variation in the number of products generated was observed. Profiles for five of the *Musa* cultivars, Adakkakunnan (AAB), Anaikomban (AAA?), Bergibale (AAB), Jahaji (AAA) and Malbhog (AAB)), obtained with the primer E-AGG/M-CAC are presented in Fig. 1.

Genetic diversity and cluster analysis

The binary data matrix comprising 1581 polymorphic AFLP markers was used to calculate similarity coefficients between the cultivars which ranged from 0.150 (Mas and

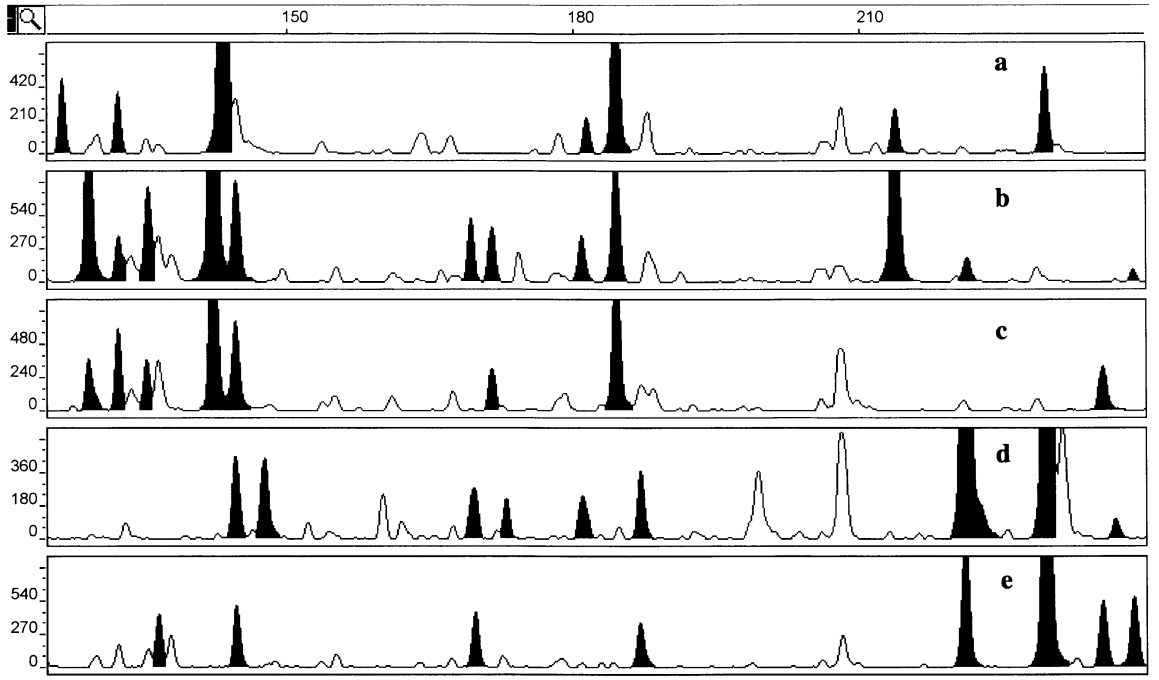

Fig. 1. AFLP profiles for five banana cultivars: (a) Adakkakunnan (AAB), (b) Anaikomban (AAA), (c) Bergibale (AAB), (d) Jahaji (AAA) and (e) Malbhog (AAB). These profiles were obtained by selective amplifications of total genomic DNA fragments from the above cultivars with the primer combination E-AGG/M-CAC. Some of the polymorphic amplicons have been highlighted with dark filled-in peaks.

Jahaji, AAB and AAA cultivars, respectively) to 0.802 (Chandrabale and Vannan, AAA and AAB types, respectively). The Jaccard similarity coefficients (JSC) within the AA, AB, AAA, AAB and ABB cultivar groups revealed the presence of much genetic diversity within the genomic groups. The AAB banana and plantains were the most diverse as the JSC varied from 0.155 to 0.752, followed by AAA types (range: 0.222–0.726) and AB cultivars (range: 0.258–0.752). The ABB group (range: 0.333–0.779) and AA (range: 0.357–0.714) were the least diverse. A cluster analysis was carried out to study genetic similarity between genotypes. Mantel's test indicated very good fit ($r = 0.92$) for the clusters in the dendrogram, indicating a high degree of association between the cultivars and the patterns of clustering. At a Jaccard's similarity coefficient average of 0.50, the *Musa* genotypes could be grouped into four distinct clusters with 22 genotypes not included in any of the clusters (Fig. 2). Each of the four major clusters identified comprised at least one previously identified reference cultivar and this, along with the morphological scoring, formed the basis for designation of the genomic constitutions for the rest of the cultivars. The genomic constitution of cluster I was inferred to be AAB, as it comprised the two previously identified AAB group cultivars, namely Nendran and Malbhog. Cluster II comprised the types BB or ABB, and cluster III comprised the types AA, AAA, AAAA, AB and AAB. Cluster IV contained only the ABB type. Cluster I with 27 genotypes was resolved into two sub-clusters, IA and IB, with 19 and nine cultivars, respectively. Cluster II was the smallest with only four genotypes, including two *M. balbisiana* (BB) and two ABB cultivars, namely Peyan (ABB) and Kanai Bansi (ABB). Cluster III was the largest group comprising 28 genotypes in two subclusters, A and B. Cluster IV with 11 genotypes included 10 ABB cultivars. In addition, there were 22 genotypes not included in any of the major clusters and these comprised 15 AABs, five AAAs and one cultivar each of the AA and AB groups (genomic constitution determined by morphological analysis). Diversity within the genomic groups was high (Table 3). However, the maximum diversity observed was among AAB genotypes as JSC values in this group ranged from 0.155 to 0.781, followed by AAA and ABB genotypes.

Discussion

The assessment of genetic diversity and management of genetic resources are of prime importance in plant breeding for the introgression of exotic genes and characteristics into established cultivars. In addition, molecular characterization of genotypes offers an opportunity for the identification of duplicates and effective germplasm management. The aim of the present study was AFLP

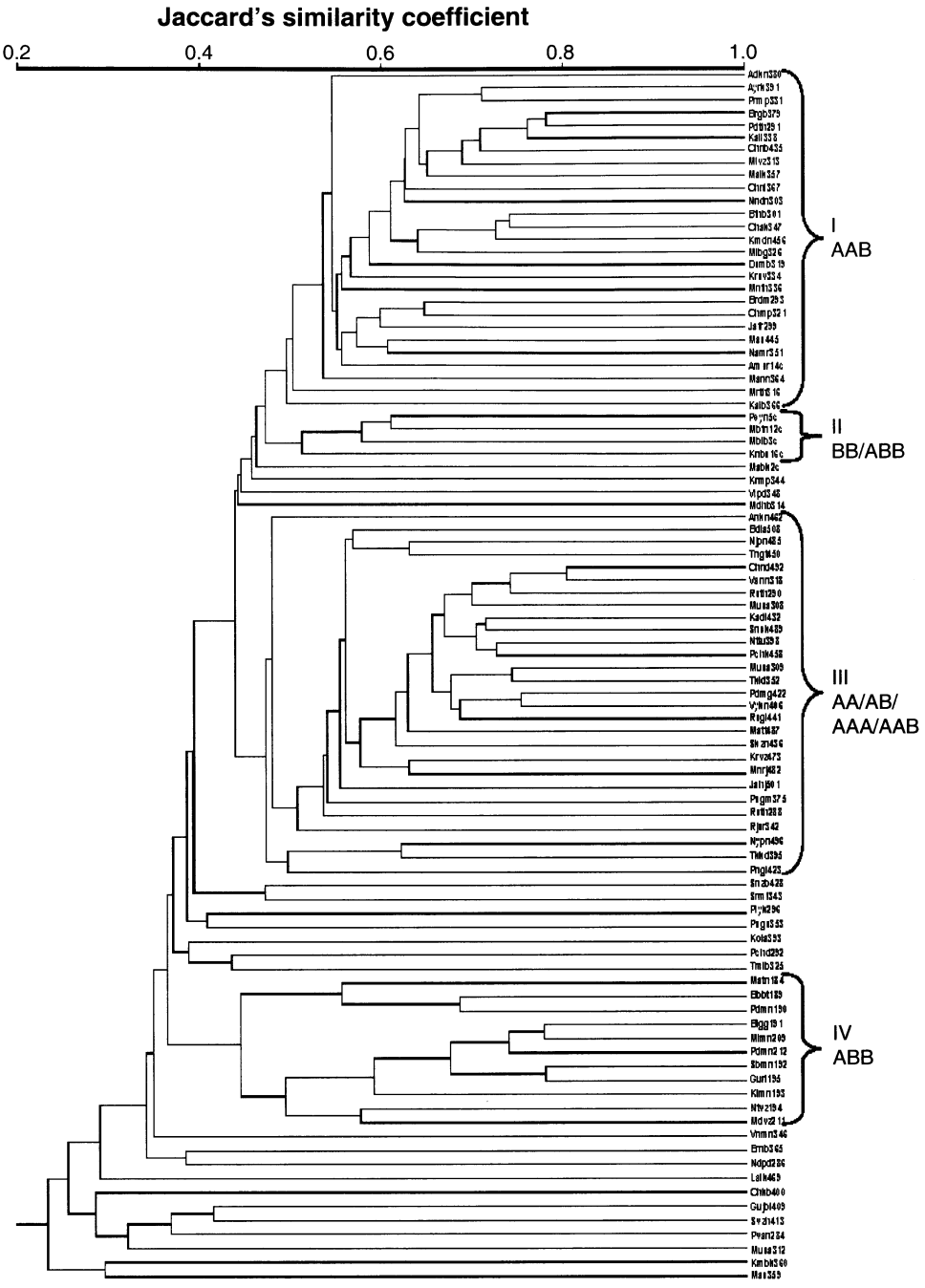

Fig. 2. UPGMA dendrogram depicting the diversity and relationships among Indian banana and plantain cultivars.

profiling for the unambiguous identification of *Musa* accessions, and the study of genetic diversity present among Indian materials. The technique employed here, namely fluorescent labelling, automated fragment detection and scoring technology, offers significant advantages over radioactive detection due to its increased sensitivity, scoring accuracy and genotyping efficiency by elimination of human errors. Multiplexing of primers in PCR amplification and electrophoresis reduces the cost incurred in the AFLP analysis (Zhang *et al.*, 2000).

Cultivar identification

The ability of AFLPs to distinguish closely related cultivars of plantains and somaclonal variants had been demonstrated earlier (Engelborghs *et al.*, 1998). But the 12 most informative primer combinations identified in the present study for use in cultivar identification generated highly polymorphic amplicons and are different from those used previously (Engelborghs *et al.*, 1998). More conserved markers like isozymes were used earlier

Table 3. Indicators of within-group genetic diversity among banana and plantain accessions of various genomic constitutions

Genomic group	Number of cultivars in each group	Jaccard's similarity coefficient	
		Average	Range
AA	6	0.523	0.357–0.714
AB	7	0.531	0.258–0.752
BB	2	0.64	–
AAA	12	0.423	0.222–0.726
AAB	51	0.439	0.155–0.752
ABB	13	0.524	0.333–0.779
AAAA	1	–	–
Total	92		

to characterize *Musa* cultivars (Jarret and Litz, 1986; Bhat *et al.*, 1992a, b). The limited polymorphism encountered in isozymes was not sufficient for unambiguous identification of all the cultivars. Therefore, DNA-based markers such as RAPDs (Bhat *et al.*, 1994) and RFLPs (Bhat and Jarret, 1995) were employed. Although these studies demonstrated the utility of RAPD and RFLP techniques in cultivar identification, they suffer from problems such as inconsistency (RAPDs) or smaller sample throughput (RFLPs). The AFLP technique in comparison (Vos *et al.*, 1995) has better consistency and applicability in cultivar profiling, as the genomic coverage per assay is high. The feasibility for multiplexing three to four primer pairs in a PCR reaction and electrophoresis increases the sample throughput enormously, although fragment detection does require an automated DNA sequencer.

The number of markers available in an AFLP analysis is much higher in comparison to RFLPs or even RAPDs. Bhat *et al.* (1994) reported that as many as 20 RAPD primers were required to distinguish the 57 *Musa* genotypes analysed. Further, it was shown that 107 polymorphic RFLP products from 12 genomic probe hybridizations were needed to obtain consistent groupings of these varieties (Bhat and Jarret, 1995). In comparison, the AFLP profiles obtained with the 12 primer pairs in this study effectively differentiated even two of the most similar cultivars (Chandrabale and Vannan), as they differed for 20% of the 1581 AFLP products compared. Koombillakkai and Mas (both AAB), two of the most diverse cultivars, differed from the rest for more than 70% of the products studied. AFLP profiling was also useful in resolving homonyms in banana. For example, two accessions collected as Rasthali (accession numbers 288 and 290) differed in their profiles with all the primer pairs, and clustered in different groups in the dendrogram. This showed that these two accessions, although they bear the same name in two different regions, are genetically distinct from one another.

Morphological classification versus AFLP grouping

Earlier studies had demonstrated clear agreements between classifications of *Musa* germplasm based on morphological variations (Simmonds and Shepherd, 1955) and molecular markers (Bhat *et al.*, 1995). Unlike RAPDs (Munthali *et al.*, 1992; Kaemmer *et al.*, 1993; Newbury and Ford-Lloyd, 1993; Bhat and Jarret, 1995) and RFLPs (Gawel and Jarret, 1991a, b; Gawel *et al.*, 1992; Bhat *et al.*, 1994), AFLPs are more robust and technically less demanding. The present study has demonstrated that the AFLPs identified can be used to classify *Musa* germplasm into specific genomic groups. Demarcation of AAB, BB and ABB types appears less ambiguous in the resulting dendrogram although some ambiguity still exists between AA, AB and AAA types as each of these groups did not segregate effectively. The clusters identified groups of accessions with similar genomic composition. The grouping in clusters I–IV was broadly in agreement with morphology-based classification.

This analysis has also given putative genomic designations to unclassified cultivars. For example, the cultivar Adakkakunnan grouped along with AAB genotypes in cluster IA. This indicated an AAB genomic constitution for Adakkakunnan. Similarly, Pisang Man and Gauria grouped with identified AAB and ABB genotypes in clusters III and IV, respectively. The results also indicated the presence of highly diverse genotypes in the AAB group, as 18 of such cultivars did not group in any of the clusters. This agrees with earlier reports regarding the presence of highly diverse genotypes in the AAB group based on analysis of variation at isozyme loci (Lebot *et al.*, 1993). These conclusions support the study by Ude *et al.* (2002a, b) and Wong *et al.* (2002) which demonstrated genomic specificity of AFLP markers in *Musa* and their utility in solving problems related to identification of the sections under this genus.

Non-grouping of some of the cultivars such as Karimpoovan, Madhubas, Koombillakkai, Mas, Gujarbale and Chikkabale with their purported genotypic groups may reflect problems associated with the expression of morphological characteristics used in genomic identifications. As a result, wrong classifications cannot be ruled out as scoring for morphological characteristics requires considerable experience and skill, and is highly subjective in nature.

Genetic diversity among cultivars

Very high genetic diversity was observed among the cultivars studied, and this supports the view that India is one of the secondary centres of diversity for bananas and plantains (Simmonds, 1962). Such a high genetic

diversity may be due to the essentially vegetative mode of propagation as somatic mutations will accumulate, and novel types emerge through bud sports. High genetic diversity observed was for the five major *Musa* genomic groups, while the Indian AAB accessions have a similarity index in the range of 0.155–0.781 (average 0.439) compared to highly narrow genetic diversity reported in the 76 African plantains (genetic distance in the range 0–0.220; Crouch *et al.*, 2000). Among the genomic groups, AAB appears to be the most diverse, as these accessions are distributed in two distinct clusters in the dendrogram. Variation in consumer preferences with respect to flavour, colour, culinary requirements and other fruit characteristics may have contributed to this result.

Eleven cultivars mapping to the lower extreme of the dendrogram (Fig. 2) were previously designated as AAA, AAB and AB, but they did not group with any of the major four clusters. This may reflect the presence of highly diverse genotypes in these three genomic groups. Of the 22 ungrouped genotypes, 15 were AAB and five were AAA. This is consistent with the suggestion of Lebot *et al.* (1993) that a high level of genetic diversity within AAB groups occurs in Asia. However, the extent of genetic diversity reported in the present study on the basis of AFLP markers is much higher in comparison to that based on RAPD (Bhat *et al.*, 1994) and RFLP (Bhat and Jarret, 1995) analysis. This can be attributed to the inclusion of genotypes from a wider range of environments covering a wider spectrum of morphological variation in AAB genome cultivars.

In conclusion, we have demonstrated the usefulness of AFLP markers for *Musa* cultivar identification, classification and diversity analysis. The Indian banana and plantain cultivars appear to be highly diverse. The range of diversity occurring in India for the five groups of banana and plantains surpasses the reported diversity for these groups elsewhere (Swennen *et al.*, 1995; Ortiz, 1997; Crouch *et al.*, 2000). The increased resolution associated with the large number of AFLP markers generated per primer pair provides sufficient polymorphism to fingerprint *Musa* cultivars, identify distinct types, and resolve the problems of homonyms and synonyms, thereby aiding in more efficient germplasm management.

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