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Analysis of genetic diversity and sectional relationships in *Musa* using AFLP markers

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Abstract The AFLP technique was used to assess the genetic diversity and sectional relationships in 39 accessions representing the four main sections of the genus *Musa*. Eight AFLP + 3 primer pairs produced 260 polymorphic bands that were used in cluster and PCO analysis. A wide range of variability was observed among the species within the sections of the genus *Musa*. AFLP data was useful in separating the different sections of the genus as well as differentiating the different genomic groups of section *Eumusa*. Section *Rhodochlamys* ($x = 11$) appeared as a distinct entity and clustered closely with the *Musa acuminata* Colla complex of section *Eumusa* that has the same basic chromosome number. This relationship is congruent with previous studies. However, unlike previous proposals that questioned the identity of *Rhodochlamys* as a separate taxonomic unit, PCO analysis of the AFLP data showed that it is a distinct entity. *Musa laterita* Cheesman (*Rhodochlamys*) and *Musa schizocarpa* Simmonds clustered with the *M. acuminata* complex suggesting that they may be sources of useful genes for the improvement of the cultivated bananas. *Callimusa* formed a distinct unit and was closer to *Australimusa* than to the other sections. Although both sections share the same basic chromosome number of $x = 10$ these sections are genetically distinct

Keywords *Musa* · Sectional relationships · AFLP · Diversity · Genetic distance

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Introduction

The genus *Musa* is composed of 30–40 species and is usually divided into five sections: *Australimusa*, *Callimusa*, *Eumusa*, *Ingentimusa* and *Rhodochlamys* (Cheesman 1947; Simmonds 1966; Stover and Simmonds 1987) on the basis of the basic chromosome numbers and the orientation and arrangement of flowers. The sections *Australimusa* and *Callimusa* comprise about six species each with a basic chromosome number of $x = 10$, while *Eumusa* and *Rhodochlamys* contain 15 and 6 species, respectively, with $x = 11$ (Stover and Simmonds 1987; Karamura 1999). *Musa ingens* Simmonds is the only member in the section *Ingentimusa* with $x = 14$ (Argent 1976).

The section *Eumusa* is the largest, most diversified and most ancient group. It includes the diploid wild ancestors of modern bananas, *Musa acuminata* (AA) and *Musa balbisiana* (BB) Colla, which contributed the A and B genomes, respectively, to the edible bananas (Simmonds 1962). *M. acuminata* originated in Malaysia and evolved into a complex of diploid (AA) subspecies, which have been classified in several ways by taxonomists (Simmonds and Shepherd 1955; Shepherd 1988; Tezenas du Montcel 1988). *M. balbisiana* originated in India and, contrary to general opinion, recent reports on diversity within the species have indicated a wide morphological variation (Sotto and Rabara 2000). However, despite the existence of genetic variation (Shepherd 1988; Hari 1989) there is no record of subspecies classification in *M. balbisiana*.

Intra- and inter-specific hybridization of *M. acuminata* and *M. balbisiana*, in conjunction with chromosome restitution, gave rise to: (1) autoploids and homogenomic hybrids which comprise mainly the AAA dessert and East African highland and beer bananas, and (2) alloploids and heterogenomic hybrids comprising the plantains (AAB) and the cooking bananas (ABB). Many other genome groups also exist naturally or were produced by human intervention.

Rhodochlamys comprises several species that can hybridize with those of *Eumusa* due to weak reproductive

isolation between these sections. For example, *Musa flaviflora* Simmonds (*Eumusa*) and *Musa ornata* Roxb. (*Rhodochlamys*) hybridize easily to produce very vigorous plants (Shepherd 1988). It is thought that *M. ornata* itself may be a secondary species derived from a natural hybrid of *Musa velutina* Wendl. & Drude (*Rhodochlamys*) and *M. flaviflora* (Shepherd 1988). *Musa laterita* (*Rhodochlamys*) also hybridizes naturally with some subspecies of *M. acuminata* (Shepherd 1988).

The section *Australimusa* contains species that appear to be more geographic than genetic isolates in nature (Shepherd 1988). In contrast, species in *Callimusa* shows more differentiation and reproductive isolation than those of *Australimusa*.

The separation of species into sections and the delimitation of sectional boundaries in *Musa* have been done mainly on the basis of morphological differences, geographical distribution and hybridization studies (Simmonds 1966; Argent 1976; Swennen and Vuylsteke 1987; Ortiz 1997; Osuji et al. 1997; Karamura 1999). Morphological traits, however, are subject to genotype × environment interaction which reduces their discriminatory power for germplasm classification, particularly when accessions only display subtle differences, as commonly found within the *M. acuminata* complex (Simmonds 1962; Tezenas du Montcel 1988). Moreover, allopatric species that are distinguishable from each other because of geographical isolation, can hybridize under greenhouse conditions or when placed in the same geographical area. The success of hybridization within and between the sections of *Musa* raises questions about the genetic identity of the species and the reproductive isolating mechanisms between them.

Shepherd (1988) reported that *Australimusa* and *Callimusa* are morphologically and reproductively very different. However, he identified distinct morphological differences between *Eumusa* and *Rhodochlamys*, despite the absence of strong reproductive barriers between them. Simmonds and Weatherup (1990), rejected the section *Ingentimusa* indicating that *M. ingens* should not be placed in a separate section. They suggested that the genus *Musa* be divided into four sections (*Australimusa*, *Callimusa*, *Eumusa* and *Rhodochlamys*) and showed that there are two divergent groups within *Rhodochlamys*.

Recent developments in DNA marker technology provide alternative means of assessing genetic diversity and phylogenetic relationships (Staub and Serquen 1996; Saghai Maroof et al. 1997). DNA markers are more abundant than morphological markers and are largely unaffected by environmental influences, which makes them attractive for genetic analyses of plant populations. In particular, DNA marker systems based on the polymerase chain reaction (PCR) are well suited to applications in plant breeding (Rafalski and Tingey 1993) and are being used increasingly for genetic analyses in *Musa* (Jarret et al. 1993; Bhat and Jarret 1995; Kaemmer et al. 1997; Crouch et al. 1998a, b; Tenkouano et al. 1999; Pillay et al. 2001). Among these systems, the amplified fragment length polymorphism (AFLP) technique has the extra advantage of combining the speed of the PCR with the precision of the restriction fragment length polymorphisms (RFLPs) (Vos et al. 1995; Powell et al. 1996). Despite its potential, the AFLP technique has not been used for genetic analysis of the wild diploid accessions of the genus *Musa* (Engelborghs and Swennen 1999).

The objectives of the present study were to use AFLP analysis (1) to determine genetic relationships between a sample of *Musa* species, and (2) to assess the extent of genetic variation within the sections of *Musa*.

Materials and methods

Plant material

Forty accessions (Table 1) from the germplasm collection of the International Institute of Tropical Agriculture, Onne, Nigeria, were used in this study. The sample included 29 accessions of *Eumusa*, six *Australimusa*, one *Callimusa*, three *Rhodochlamys*, and one accession from the genus *Ensete* represented by *Ensete ventricosum* (Welw.) Cheesman.

DNA isolation

Approximately 10 g of leaf tissue from the cigar leaf (youngest unfurled leaf) was collected and transported on ice from the field to the laboratory and subsequently ground in liquid nitrogen with a mortar and pestle. Isolation of total DNA followed the protocol described by Gawel and Jarret (1991) with a few modifications. The ground tissue was added to an oak ridge tube containing 15 ml of pre-heated extraction buffer (4% of CTAB-hexadecyltrimethylammonium bromide, 100 mM of Tris-HCI, pH 8.0, 1.4 M of Nacl, 20 mM of EDTA, 4 µl/ml of β-mercaptoethanol) and incubated at 65 °C for 30 min. The samples were extracted with 15 ml of chloroform: isoamyl alcohol (24:1, v/v) and centrifuged at 6,000 rpm for 5 min. The upper aqueous phase was transferred to a new tube and extracted as before with chloroform: isoamyl alcohol. The DNA was precipitated by adding a two-thirds volume of ice-cold isopropanol and recovered by centrifugation at 6,000 rpm for 5 min. The DNA was dissolved in 600 µl of TE buffer (10 mM Tris, 1.0 mM EDTA, pH 8.0) and treated with 10 µg/ml of RNAse for 30 min at room temperature. The purified DNA was precipitated by adding a one-tenth volume of 3 M sodium acetate (NaOAc, pH 6.8), followed by two volumes of cold ethanol and centrifugation at 6,000 rpm for 5 min. The DNA pellet was washed with 70% ethanol, air-dried briefly and re-suspended in 200 µl of TE buffer. DNA concentrations were quantified with a Pharmacia Gene Quant II spectrophotometer (Pharmacia Biotech, England). To confirm the concentration and quality of the DNA samples, a 2-µl sample of the stock DNA solution was run in a 1% agarose gel stained in 1 µg/ml of ethidium bromide solution and compared visually with Lambda DNA standards of known concentration under UV illumination. An aliquot of the isolated DNA for each sample was diluted to 40 ng/µl in TE buffer and stored in a refrigerator (4 °C) for use in subsequent assays, while the stock DNA samples were stored at –20 °C.

AFLP procedure

The AFLP procedure was carried out as reported by Vos et al. (1995) with few modifications.

DNA digestion and adapter ligation

Approximately 40 ng of DNA was digested, simultaneously, with *Eco*RI and *Mse*I at 37 °C for 2 h. The restriction digestion was

Table 1 Plant materials of the genus *Musa* used in this study

S/No	Section	Species/hybrids	Subspecies	Genome	Name	Abbreviations
1	*Eumusa*	*M. acuminata* Colla	ssp. *microcarpa* Simmonds	AAw[b]	Borneo	Bor
2	*Eumusa*	*M. acuminata* Colla	ssp. *burmannicoides* De Langhe & Devreux	AAw	Calcutta 4	C4
3	*Eumusa*	*M. acuminata* Colla	ssp. *malaccensis* Simmonds	AAw	Selangor	Sel
4	*Eumusa*	*M. acuminata* Colla	ssp. *burmannica* Simmonds	AAw	Long Tavoy	LTav
5	*Eumusa*	*M. acuminata* Colla	ssp. *malaccensis*	AAw	Malaccensis Holotype	Mal
6	*Eumusa*	*M. acuminata* Colla	ssp. *microcarpa*	AAcv[b]	Tjau Lagada	TjL
7	*Eumusa*	*M. acuminata* Colla	ssp. *malaccensis*	AAcv	Pisang Lilin	Plin
8	*Eumusa*	*M. acuminata* Colla	ssp. *banksii* (F. Muell) Simmonds	AAcv	SF247	SF
9	*Eumusa*	*M. acuminata* Colla	ssp. *banksii* (F. Muell) Simmonds	AAcv	Madang	Mad
10	*Eumusa*	*M. acuminata* Colla	–	AAcv	Pisang Jari Buaya	PJB
11	*Eumusa*	*M. acuminata* Colla	ssp. *truncata* (Ridl.) Shepherd	AAw	Truncata	Tru
12	*Eumusa*	*M. acuminata* Colla	ssp. *zebrina* nom. nud.	AAw	Zebrina	Zeb
13	*Eumusa*	*M. balbisiana* Colla	–	BB	I-63	I-63
14	*Eumusa*	*M. balbisiana* Colla	–	BB	Honduras (HND)	HND
15	*Eumusa*	*M. balbisiana* Colla	–	BB	Montpellier (MPL)	MPL
16	*Eumusa*	*M. balbisiana* Colla	–	BB	Singapuri	Sing
17	*Eumusa*	*M. balbisiana* Colla	–	BB	Los Banos	Los
18	*Eumusa*	*M. balbisiana* Colla	–	BB	10852	10852
19	*Eumusa*	*M. balbisiana* Colla	–	BB	Butohan I	Butl
20	*Eumusa*	*M. balbisiana* Colla	–	BB	Etikehel	Etik
21	*Eumusa*	Desert Banana	–	AAA	Gros Michel	Gros
22	*Eumusa*	Desert Banana	–	AAA	Yangambi Km5	Km5
23	*Eumusa*	Plantains	–	AAB	Agbagba	Agb
24	*Eumusa*	Plantains	–	AAB	Batard	Bat
25	*Eumusa*	Plantains	–	AAB	Obino l'Ewai	Obino
26	*Eumusa*	Plantains	–	AAB	Asamiensa	Asa
27	*Eumusa*	Cooking banana	–	ABB	Bluggoe	Blug
28	*Eumusa*	Cooking banana	–	ABB	Fougamou	Foug
29	*Eumusa*	*M. schizocarpa* Simmonds	–	–	Schizocarpa	Schi
30	*Australimusa*	*M. peekeli* Lauterb	ssp. *peekeli* Argent	–	Peekeli	Peek
31	*Australimusa*	*M. fei* F. Muell.	–	–	Fei	Fei
32	*Australimusa*	*M. maclayi* F. Muell.	–	–	Maclayi	Mac
33	*Australimusa*	*M. lolodensis* Cheesman	–	–	Lolodensis	Lol
34	*Australimusa*	*M. textilis* Nee	–	–	Textilis	Txt
35	*Australimusa*	*M. peekeli* Lauterb.	ssp. *angustigemma* Argent	–	Angustigemma	Angust
36	*Rhodochlamys*	*M. laterita* Cheesman	–	–	Laterita	Lat
37	*Rhodochlamys*	*M. manni* Wendl.	–	–	Sanguinea	Sang
38	*Rhodochlamys*	*M. ornata* Roxb.	–	–	Ornata	Orn
39	*Callimusa*	*M. coccinea* Andrews	–	–	Coccinea	Coc
40	*Ensete*[a]	*E. Ventricosum* (W.) Ch.	–	–	Ensete	Ens

[a] *E. Ventricosum* is in the genus *Ensete*
[b] *M. acuminata* accession AAw = wild-type, AAcv = cultivar

stopped by transferring the reaction tubes to a 70 °C water bath and incubating for 10 min. The restricted DNA fragments were ligated to *Eco*RI and *Mse*I adapters by adding 0.25 µl of T_4 Ligase and 8 µl of adapter ligation solution to the reaction tubes and incubating at 20 °C for 2 h. The reaction was diluted 1:l0 by dissolving 1 µl of the ligation mix in 9 µl of 10 mM Tris EDTA.

Pre-amplification and selective amplification

Five microliters of the diluted DNA solution were pre-amplified using *Eco*RI + A and *Mse*I + C primers. Each reaction was composed of 10 µl of pre-amp primer mix, 1.8 µl of 10×reaction buffer, 1.8 µl of 25 mM magnesium chloride and 0.25 µl of *Taq* polymerase. PCR was done in a 9600 Thermal Cycler (Perkin Elmer) programmed to perform 20 cycles at: 94 °C for 30 s, 56 °C for 60 s, and 72 °C for 60 s. The pre-amplified DNA was diluted to 1:50 with TE buffer. Five microliters of the diluted product were used for selective amplification in a reaction tube containing 0.18 µl of *Eco*RI primer, 4.5 µl of *Mse*I primer, 5.72 µl of ddH_2O, 2.5 µl of 25 mM $MgCl_2$ 2.0 µl of 10×reaction buffer, and 0.1 µl of *Taq* polymerase. Eight primer pairs (E-AAC/M-CTC, E-AAG/M-CTA, E-ACA/M-CAA, E-ACT/M-CTG, E-AGC/M-CTT, E-AGG/M-CTA, E-ACA/M-CAG and E-ACG/M-CAA) from the GIBCO BRL commercial AFLP kit were used for the selective amplification. Selective amplification and associated PCR temperature cycles, stop reaction and polyacrylamide gel-electrophoresis were done according to Lin et al.(1996).

Silver nitrate staining

The silver staining process included fixing the gel in 10% (v/v) acetic acid for 20 min, rinsing three times in de-ionized water (5 min per rinse), staining for 15 min in a solution containing 0.1% (w/v) silver nitrate (BDH Analar grade) and 0.015% (v/v) formaldehyde. The stained plate was rinsed with de-ionized water for 10 s and developed in a cold (4–10 °C) developer solution containing 3% (w/v) sodium carbonate (BDH Analar grade), 0.015% (w/v) formaldehyde, and 0.002% (w/v) sodium thiosulphate until the DNA bands became visible. The gel was rinsed with distilled water and air-dried.

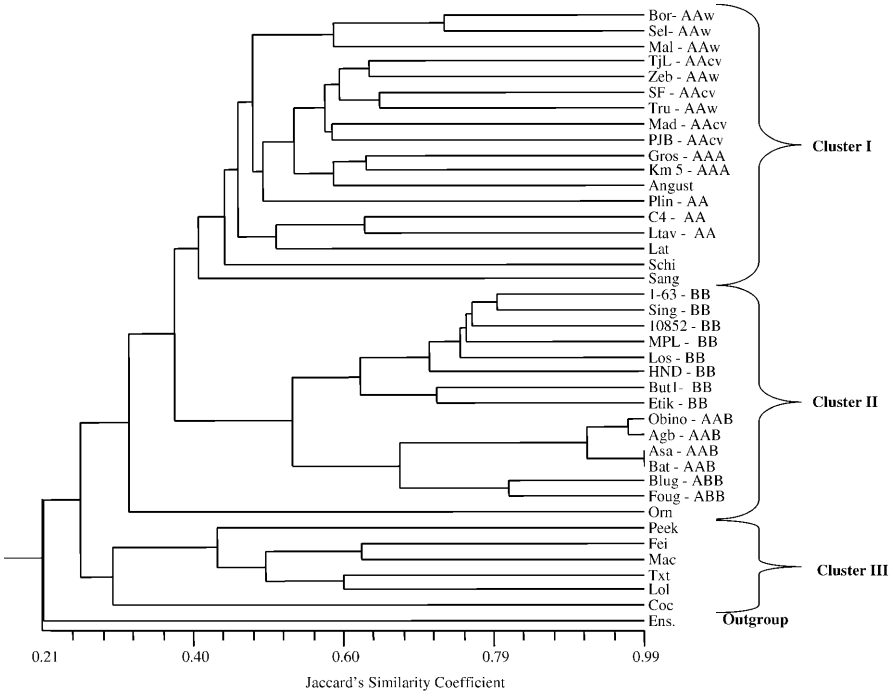

Fig. 1 Dendrogram of 39 *Musa* accessions evaluated using Jaccard's coefficient of similarity and UPGMA clustering. Three clusters were identified: (1) *M. acuminata* and *Rhodochlamys* accessions [including *M. ornata* (orn)]; (2) all accessions with at least a B-genome; (3) *Callimusa* and *Australimusa* accessions. *Ensete ventricosum* was distinguished from the other accessions. The accessions are labelled with codes listed in Table 1

Data analysis

Polymorphic bands were scored visually from the gels with the aid of a light box A band was considered polymorphic if it was present in at least one genotype and absent in the others. A matrix was generated in which each band was scored as a "1" if present and as "0" if absent.

The NTSYS-pc software package version 2.02f (Rohlf 1998) was used to calculate Jaccard's (1908) similarity coefficients among the genotypes as follows:

$$S_{ij} = N_{ij}/(N_{ii} + N_{ij} + N_{ij}) \quad (1)$$

where S_{ij} is the similarity index between the i^{th} and j^{th} genotype, N_{ij} is the number of bands present in both genotypes, N_{ii} is the number of bands present in the i^{th} genotype but absent in the j^{th} genotype, and N_{ij} is the number of bands absent in the i^{th} genotype but present in the j^{th} genotype. A dendrogram was constructed from the matrix of similarity coefficients, using the Unweighted Pair-Group Method of the Arithmetic average (UPGMA). Genetic distances (GD) were calculated as $[(1–S_{ij}) \times 100]$. Principal coordinate analysis (PCO) was also carried out to show multiple dimensions of the distribution of the cultivars in a scatter-plot (Keim et al. 1992).

Results

A total of 260 polymorphic bands ranging from 50 to 1,100 bp were scored. The average number of polymorphic bands per primer was 32.5 while the range for the eight primers was 23 to 45.

UPGMA clustering assigned the *Musa* accessions to three major clusters (Fig. 1). The first cluster included the diploid and triploid A-genome accessions of *Eumusa* but also included accessions of *Rhodochlamys* (*M. laterita* and *Musa manni* 'Sanguinea' Wendl.), *Australimusa* [*Musa peekeli* ssp. *angustigemma* (Lauterb.) Argent] and the S genome species, *Musa schizocarpa* Simmonds.

Table 2 Average genetic distances (AGD%) within and between taxonomic groups of *Musa*

Taxa	Average genetic distance %		
	Mean	Minimum	Maximum
Eumusa	53.4	1.3	77.2
Rhodochlamys	63.7	61.3	67.6
Australimusa	50.5	37.8	60.7
Rhodochlamys/*Callimusa*	75.6	69.6	74.5
Callimusa/*Australimusa*	70.1	66.7	76.7
M. acuminata (AA) Colla	50.84	27.2	66.2
M. balbisiana (BB) Colla	31.1	20.4	47.8
Plantains (ABB)	6.5	1.3	13.5
Cooking bananas (ABB)	18.7	–	–
AA/*Rhodochlamys*	61	44.2	73.1
AA/*Callimusa*	77.4	66.4	88.5
AA/*Australimusa*	75.5	66.9	84.5
AA/BB	67.4	59	77.2
AA/AAB	55.2	43.4	68.8
AA/ABB	58.2	51.5	65.5
BB/*Rhodochlamys*	68.3	61.7	73.2
BB/*Callimusa*	75.7	71.5	79.5
BB/*Australimusa*	72	64.9	78.4
BB/AAB	49.3	40.8	55.8
BB/ABB	41.8	37.5	49

Within the first cluster, three sub-groups of the seven subspecies of *M. acuminata* were observed (Fig. 1): (l) 'Borneo' (ssp. *microcarpa* Simmonds), 'Selangor' (ssp. *malaccensis* Simmonds), and 'Malaccensis holotype' (ssp. *malaccensis*); (2) 'Tjau Lagada' (ssp. *microcarpa*), 'Zebrina' (ssp. *zebrina* nom. nud.), 'SF247' [spp. *banksii* (F. Muell.) Simmonds], 'Truncata' [ssp. *truncata* (Ridl.) Shepherd], 'Madang' (ssp. *banksii*), and 'P. jari buaya' and (3) 'Calcutta 4' (ssp. *burmannicoides* De Langhe &

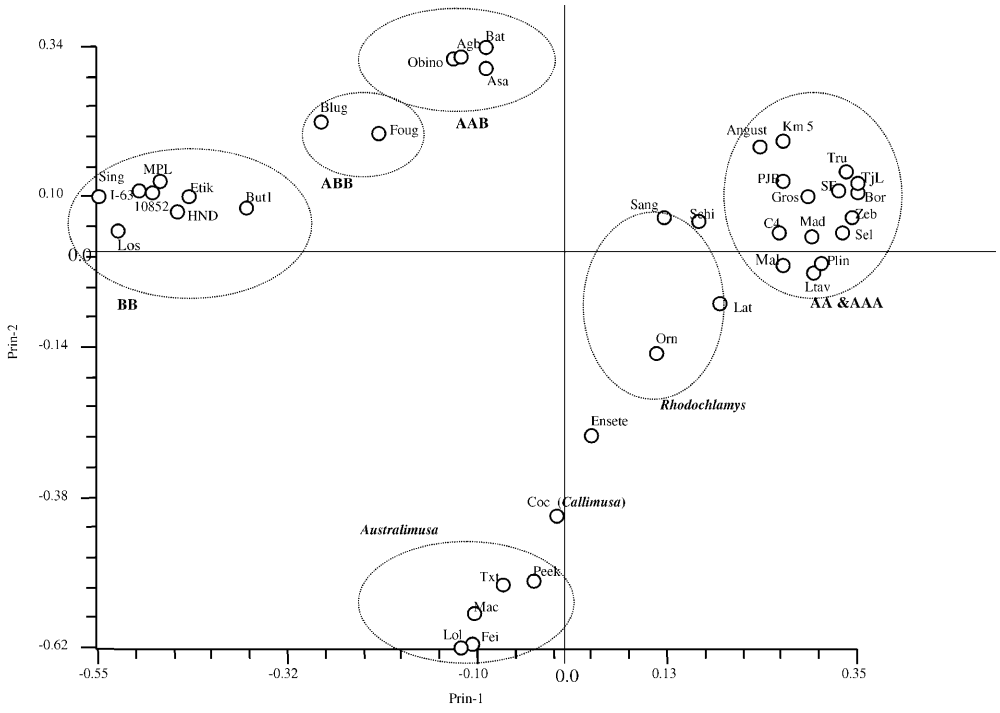

Fig. 2 Scatter plot of Principal Co-ordinate analysis of accessions of the genus *Musa*, using the AFLP data. Prin-1 distinguished *M. acuminata* (AA) from *M. balbisiana* (BB) accessions and Prin-2 distinguished *Eumusa* (AA, AAA, BB, AAB, and ABB) from *Australimusa* and *Callimusa*. The accessions are labelled with codes listed in Table 1

Devreux) and 'Long Tavoy' (ssp. *burmannica* Simmonds). The *Australimusa* accession, *M. peekeli* ssp. *angustigemma*, grouped closely with AAA dessert bananas 'Gros Michel' and 'Yangambi Km5'. 'Calcutta 4' and 'Long Tavoy' clustered with the *Rhodochlamys* accession, *M. laterita*. The 12 diploid *M. acuminata* (AA) accessions showed a wide genetic base with an average genetic distance (AGD) of 50.84% (Table 2).

The second cluster was composed of accessions with the B genome, including the homogenomic diploid *M. balbisiana*, and the heterogenomic allotriploid hybrids (Fig. 1). Three sub-groups were distinguished within this cluster: (1) the eight diploid BB accessions, which showed an AGD of 31%; (2) the triploid plantains (AAB); and (3) the cooking bananas (ABB) (Table 2). The ABB accessions were closer to the AAB accessions than to the BB accessions.

The third cluster consisted of species of section *Australimusa*: *M. peekeli* ssp. *Peekeli* (Lauterb). Argent, *Musa fei* F. Muell., *Musa maclayi* F. Muell., *Musa textilis* Nee and *Musa lolodensis* Cheesman. The single species of section *Callimusa* used in this study, *Musa coccinea* Andrews (section *Callimusa*), was isolated from the other sections and appeared closer to the section *Australimusa*. The AGD among the species of the sections *Eumusa* and *Australimusa* was 53.35% and 50.54%, respectively. The section *Rhodochlamys* showed the highest AGD of 63.71% (Table 2). *M. ornata* (*Rhodochlamys*) did not group with the other species of its section. The PCO scatter plot (Fig. 2) distinguished most of the species of section *Australimusa* with the exception of *Musa angustigemma* Simmonds that clustered with the *Eumusa*. The three species of *Rhodochlamys* formed a loose cluster. *Callimusa* was isolated from the other sections. The first principal coordinate axis (Fig. 2) separated *Eumusa* into its different genomic groups. Section *Rhodochlamys* appeared closer to *Eumusa* than the other sections (Fig. 2). The outgroup *E. ventricosum* (Welw.) Cheesman was well separated from the *Musa* accessions.

Discussion

The AFLP data demonstrated a wide range of variability among the species within the sections of the genus *Musa*. However, with the exception of the placement of *M. angustigemma* in the *Eumusa*, it was useful in separating the different sections of the genus as well as differentiating the different genomic groups of section *Eumusa* (Figs. 1, 2). The close relationship between the *M. acuminata* complex (AA and AAA) and section *Rhodochlamys* (Fig. 1) is congruent with the previous report of Shepherd (1988). This relationship is supported by the absence of reproductive barriers between the two sections (Simmonds 1954; Shepherd 1988). The existence of *Rhodochlamys* as a separate taxon was questionable in the past (Shepherd 1988). Our study showed a wide genetic variation among the species of the section *Rhodochlamys* (AGD = 63.7; range = 61.3–67.6%) suggesting that there are significant differences between their genomes. Simmonds and Weatherup (1990) identified two genetic groups in *Rhodochlamys* using morphological descriptors. The UPGMA clustering (Fig. 1) showed a very close relationship between species of *Rhodochlamys* and the *M. acuminata* complex. In contrast, the PCO analysis distinguished *Rhodochlamys* from the *M. acuminata* complex, showing that the section *Rhodochlamys* is a distinct taxonomic entity. It appears that fur-

ther work is necessary to determine the distinctiveness of *Rhodochlamys*.

Several authors have reported a close affinity between *M. acuminata* (*Eumusa*) and *M. laterita* (*Rhodochlamys*) (Simmonds 1954; Shepherd 1988). Shepherd (1988) observed that *M. acuminata*. ssp. *burmannica* ('Calcutta 4' and 'Long Tavoy') shares a homozygous segmental translocation with *M. laterita* and that their hybrids were fertile. Our study clustered *M. laterita* with 'Calcutta 4' and 'Long Tavoy' (Fig. 1) indicating that *M. laterita* is related to *M. acuminata* ssp. *burmannica*. This affinity between *M. laterita* and the diploid *M. acuminata* accessions suggests that *M. laterita* could be used for the improvement of the cultivated bananas. Similarly, our study showed a close affinity between *M. schizocarpa* (*Eumusa*) and the *M. acuminata* complex. It has been reported that hybrids between *M. acuminata* and *M. schizocarpa* do exist in nature (Argent 1976; Shepherd and Ferreira 1982; Tezenas du Montcel et al. 1995). *M. schizocarpa* is considered to have the S genome, which is different from the predominantly A and B genomes of cultivated bananas. The relationship between *M. schizocarpa* and the *M. acuminata* accessions suggests that the S genome could be exploited in breeding programs of the cultivated bananas. Our study also found that 'Selangor' (ssp. *malaccensis*) was well separated from *M. laterita*. This is contrary to the Simmonds (1954) report which indicated that *M. laterita* was more closely related to 'Selangor' than to 'Long Tavoy' (ssp. *burmannica*).

The single species of *Callimusa*, used in this study, formed a distinct unit and was closer to *Australimusa* than to the other sections. This relationship was not unexpected since both sections share the same basic chromosome number of $x = 10$. Shepherd (1988) showed that hybrids between some species of *Callimusa* and *Australimusa* had reduced chromosome pairing and much reduced fertility, implying that these sections are genetically distinct. With the exception of *M. peekeli* ssp. *angustigemma* that grouped with *Eumusa*, the other species of *Australimusa* (*M. lolodensis*, *M. fei*, *M. peekeli* ssp. *peekeli*, *M. textilis* and *M. maclayi*) clustered together. Crosses between these species show high levels of chromosome pairing suggesting that they are closely related. The AGD within the six species of section *Australimusa* was 50.5% while that within the *M. acuminata* complex was 50.84%. These values suggest that species of the section *Australimusa* are genetically similar. Shepherd (1988) suggested that species in the section *Australimusa* are 'geographic isolates' rather than 'genetic isolates' despite their morphological dissimilarities. He also suggested that they may be treated as subspecies as is the case in *M. acuminata*.

The grouping of *M. peekeli* ssp. *angustigemma* (*Australimusa*) with the *Eumusa* was unexpected and contrary to previous morphological data of Simmonds and Weatherup (1990) which showed *M. peekeli* ssp. *peekeli* was closely related to *M. peekeli* ssp. *angustigemma*. Argent (1976) proposed that *M. angustigemma* Simmonds should not be maintained as an independent species from *M. peekeli* on the basis of morphological characters and reduced it to subspecific rank under *M. peekeli* (*M. peekeli* ssp. *angustigemma*). Our study clearly distinguishes *M. peekeli* ssp. *angustigemma* from *M. peekeli* ssp. *peekeli* suggesting that the specific rank of *M. angustigemma* should be retained.

Although the AFLP data clearly distinguished the different sections of the genus *Musa* (*Australimusa*, *Eumusa*, *Rhodochlamys* and *Callimusa*) and the different genomic groups of *Eumusa*, it did not differentiate the subspecies of the *M. acuminata* complex. The level of polymorphism obtained with the eight primer pairs used in this study was inadequate to resolve subspecies relationships. To ascertain phylogenetic relationships at the infraspecific level in the genus *Musa*, we recommend that many AFLP primers and perhaps different enzyme combinations be used to screen for polymorphisms.

Prin I (see Fig. 2 legend) was able to distinguish the different genomic groups in the section *Eumusa*. It was interesting to find that the BB and AA accessions were placed at the extremes while the hybrid combination (AAB, ABB) fell in between. With further work, it would appear that the AFLP technique has the potential of uncovering genome-specific markers in *Musa*.

This study showed that AFLP is a very useful tool in determining taxonomic relationships in the genus *Musa*; it is also potentially useful to resolve some of the complicated taxonomic questions in the genus *Musa*.

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