Theor Appl Genet (2003) 107:850–856
DOI 10.1007/s00122-003-1340-y

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Sectional relationships in the genus *Musa* L. inferred from the PCR-RFLP of organelle DNA sequences

Received: 10 March 2003 / Accepted: 30 April 2003 / Published online: 25 June 2003
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Abstract The objective of this study was to construct a molecular phylogeny of the genus *Musa* using restriction-site polymorphisms of the chloroplast (cpDNA) and mitochondrial DNA (mtDNA). Six cpDNA and two mtDNA sequences were amplified individually in polymerase chain reaction (PCR) experiments in 13 species representing the four sections of *Musa*. *Ensete ventricosum* (W.) Ch. was used as the outgroup. The amplified products were digested with ten restriction endonucleases. A total of 79 restriction-site changes were scored in the sample. Wagner parsimony using the branch and bound option defined two lines of evolution in *Musa*. One lineage comprised species of the sections *Australimusa* and *Callimusa* which have a basic number of x = 10 chromosomes, while most species of sections *Eumusa* and *Rhodochlamys* (x = 11) formed the other lineage. *Musa laterita* Cheesman (*Rhodochlamys*) had identical organellar genome patterns as some subspecies of the *Musa acuminata* Colla complex. The progenitors of the cultivated bananas, *M. acuminata* and *Musa balbisiana* Colla, were evolutionarily distinct from each other. *Musa balbisiana* occupied a basal position in the cladogram indicating an evolutionarily primitive status. The close phylogenetic relationship between *M. laterita* and *M. acuminata* suggests that species of the section *Rhodochlamys* may constitute a secondary genepool for the improvement of cultivated bananas.

Keywords Organelle DNA · Molecular phylogeny · *Musa* · PCR-RFLP · Sectional relationships

Communicated by H.F. Linskens

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Introduction

The genus *Musa* L. comprises about 30 to 40 species of perennial rhizomatous herbs that are native to the wet tropical lowlands of Southeast Asia, the Pacific islands and up to the northern most tip of Australia (Simmonds 1962). The wild species of the genus are diploid with basic chromosome numbers of x = 11, 10, and rarely 9 and 7 (Purseglove 1988). The genus is divided into the sections *Eumusa, Rhodochlamys, Australimusa* and *Callimusa* on the basis of morphological characters and differences in basic chromosome numbers. Information on the evolutionary relationships in the genus would improve our understanding of taxonomic delimitations in *Musa* and assist in the selection of additional genotypes for the improvement of cultivated bananas. The economic importance of the genus lies primarily in the cultivated bananas of the section *Eumusa* that are major sources of dietary carbohydrates for over 100-million people worldwide (Rowe 1981).

The first formal classification of the genus *Musa* was established by Baker (1893) whereby three subgenera, *Physocaulis, Eumusa* and *Rhodochlamys*, were recognized. The subgenus *Physocaulis* included the wild bananas with seeded inedible fruits while the subgenus *Eumusa* consisted of species with edible fruits. The remaining species with inedible fruits and brightly

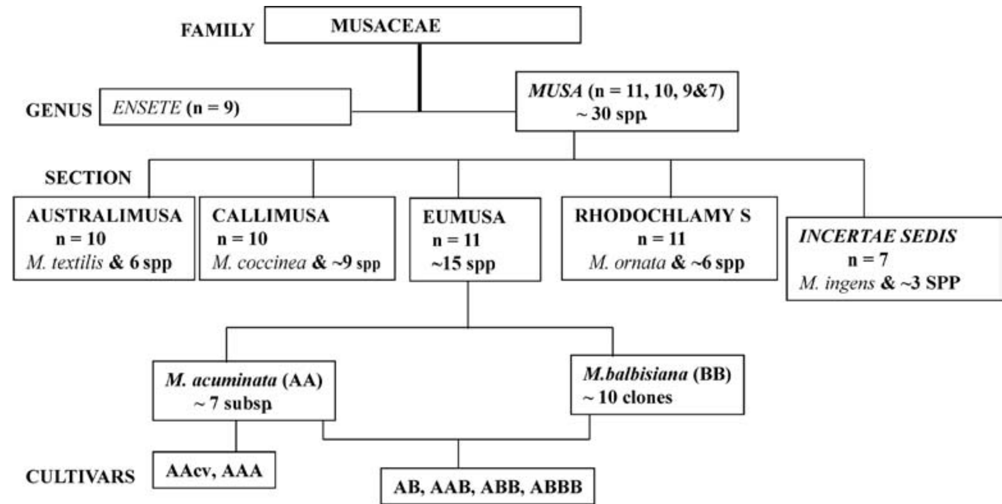

Fig. 1 Classification of the family Musaceae showing sectional treatment of the genus *Musa*. Based on Cheesman (1947), Simmonds (1962), Simmonds and Shepherd (1955)

coloured bracts formed the subgenus *Rhodochlamys*. Baker's (1893) classification was revised by Cheesman (1947) whose detailed taxonomic treatment of the genus *Musa* (Fig. 1) has been widely adopted. Cheesman's (1947) classification was based primarily on chromosome number, pseudostem stature, inflorescence characters and seed morphology. The genus was divided into four sections: *Eumusa, Rhodochlamys, Australimusa* and *Callimusa*. Sections *Eumusa* and *Rhodochlamys* contain species with a basic chromosome number of $x = 11$, while those of sections *Australimusa* and *Callimusa* have $x = 10$. Subsequently, Shepherd (1959) reported basic numbers of 7 for *M. ingens* Simmonds and 9 for *M. beccarrii* Simmonds. Thereafter, section *Ingentimusa* was created to accommodate *M. ingens* (Argent 1976), although other taxonomists prefer to assign accessions with uncertain affiliation to the species *Incertae sedis* Simmonds (Simmonds 1962; De Langhe 1969).

Cheesman's classification provided a general framework of *Musa* taxonomy which was used for almost half a century without any significant modifications (De Langhe 2000). However, several issues such as: (1) the evolutionary relationships among the four sections of the genus *Musa*, (2) the separation of sections *Eumusa* and *Rhodochlamys* with apparently little taxonomic support, and (3) the relationship between the wild progenitor species/subspecies and the cultivated clones have not been resolved. Simmonds (1954) found that *M. laterita* (*Rhodochlamys*) crossed more easily with the *M. acuminata* (*Eumusa*) subspecies than with species from its own section. Natural hybridization between the two species occurs in northern Thailand where *M. laterita* is sympatric with *M. acuminata* ssp. *siamea* (Simmonds 1960). Extensive intersectional crosses between *Rhodochlamys* and *Eumusa* (Shepherd 1999) suggested that sectional isolation is virtually non-existent. Thus, although unique and irreplaceable, morpho-taxonomy must be supplemented with additional techniques in order to guarantee the level of sensitivity needed to correctly identify the full-range of genetic diversity in the genus *Musa* (De Langhe 1990).

Recently, considerable attention has been given to the use of organellar genomes in the evolutionary studies of plants. The availability of universal primers capable of amplifying specific regions of the mitochondrial (Demesure et al. 1995) and chloroplast (Badenes and Parfitt 1995; Tsumura et al. 1996, Fofana et al. 1999) genomes using the polymerase chain reaction (PCR) has made it possible to explore differences in organelle DNA sequences for taxonomic and phylogenetic purposes. Because of its uniparental mode of inheritance and its low mutation rate relative to the nuclear genome, the chloroplast genome is considered an ideal system for the study of genetic variation and phylogenetic relationships in plants (Curtis and Clegg 1984; Palmer 1987). Although the mitochondrial genome evolves at a much faster rate than the cpDNA, it has also been used to determine genetic relationships in plants (Palmer 1988; Carreel et al. 2002). In *Musa*, the mtDNA and cpDNA are paternally and maternally inherited, respectively (Faure et al. 1994).

The objective of this study was to construct a molecular phylogeny of the genus *Musa* using restriction-site polymorphisms of the cpDNA and mtDNA, and to compare the derived phylogenetic relationships with the present morpho-taxonomic classification of the genus.

Materials and methods

Plant material

The plant materials used in this study (Table 1) were chosen to represent the four sections of the genus *Musa* and to reflect the geographical distribution and morphological diversity within species. *Ensete ventricosum*, a species from the only other genus in the family *Musaceae*, was included as an outgroup taxon. Leaf samples for DNA extraction were collected from plants in the field genebank of the International Institute of Tropical Agriculture (IITA) at Onne (4°43′N, 7°01′E) in Southeast Nigeria. Leaf samples for three accessions (Table 1) were kindly supplied by Dr.

Table 1 List of *Musa* accessions, their genome composition, chromosome numbers and origin of plants used in this study

Serial no.	Species/Cultivar/Hybrid	Genome	Chromosome number	Source/Origin
	Section Australimusa			
1	*M. peekeli* Lauterb.	TT	20	IITA[a]
2	*M. textiles* Nee.	TT	20	CARBAP[b]
	Section Callimusa			
3	*M. coccinea* Andrews		20	CARBAP
	Section Rhodochlamys			
4	*M. laterita* Cheesman		22	IITA
5	*M. ornata* Roxb.		22	CARBAP
6	*M. velutina* Wendl. & Drude		22	IITA
	Section Eumusa			
7	*M. basjoo* Sieb.		22	IITA
8	*M. schizocarpa* Simmonds	SS	22	IITA
	M. balbisiana Colla			
9	'Los Banos'	BB	22	IITA
10	'Singapuri'	BB	22	IITA
	M. acuminata Colla			
11	ssp. *zebrina* nom. nud.	AA	22	IITA
12	ssp. *microcarpa* Simmonds	AA	22	IITA
13	ssp. *burmannicoides* De Langhe & Devreux	AA	22	IITA

[a] IITA = International Institute of Tropical Agriculture, Onne, Nigeria
[b] CARBAP = Centre Africain de Recherches Régionales sur Bananiers et Plantains, Njombé, Cameroon

Table 2 Nucleotide sequence of primers used in PCR-RFLP analysis of chloroplast and mitochondria DNA regions and sizes of the corresponding PCR products in some plants

Primer pair	Primer sequence (5′ to 3′) (forward and reverse)	Tm (°C)	Size (base-pairs)	Source
Chloroplast genome:				
tRNA thr-tRNAleu *(IGR 1)	CAT TAC AAA TGC GAT GCT CT TCT ACC GAT TTC GCC ATA TC	55 °C	577	Taberlet et al. 1991
tRNAleu-tRNAleu (tRNAleu intron 1)	CGA AAT CGG TAG ACG CTA CG GGG GAT AGA GGG ACT TCA AC	58 °C	389	Taberlet et al. 1991
tRNAleu-tRNAphe (IGR 2)	GGT TCA AGT CCC TCT ATC CC ATG TCA CCA CAA ACA GAA ACT AAA	55 °C	2,569	Taberlet et al. 1991
tRNAlys (*trn*K gene)	AAC CCG GAA CTA GTC GGA TG TCA ATG GTA GAG TAC TCG GC	58 °C	3,603	Tsumura et al. 1996
*rpo*C (RNA polymerase)	GCA GTT TCT TGA AAA CTC GC TGT ACA CGC GGT AGA AAA AT	58 °C	3,200	Tsumura et al. 1996
tRNAthr-tRNAphe (IGR 3)	CAT TAC AAA TGC GAT GCT CT ATT TGA AAC TGG TGA CAC GAG	55 °C	1,550	Fofana et al. 1999
Mitochondrial genome:				
nad^{exonB}-nad^{exonC} ($Exon^{B–C}$ intron 2)	GCA TTA CGA TCT GCA GCT CA GGA GCT CGA TTA GTT TCT GC	58 °C	1,640	Demesure et al. 1995
rsp14-cob (Apocytochrome b protein, IGR 4)	CAC GGG TCG CCC TCG TTC CG GTG TGG AGG ATT AGG TTG T	58 °C	656	Demesure et al. 1995

* IGR, Inter-Genic Region

K. Tomekpe from the Centre Africain de Recherches Régionales sur Bananiers et Plantains (CARPAB) station in the Cameroon.

DNA Isolation

Approximately 5 g of tissue sample from young unfurled leaves was collected, immediately submerged in liquid nitrogen and subsequently ground in liquid nitrogen with a mortar and pestle. Genomic DNA was extracted according to the protocol of Gawel and Jarret (1991a). Six cpDNA and two mtDNA sequences were amplified in each of the accessions by PCR using universal primers synthesised by MWG-Biotech laboratories, Ebersberg, Germany. Table 2 lists the nucleotide sequence, annealing temperature (Tm), and the genomic regions amplified by the primers used in this study.

PCR-amplification

The amplification reaction was done in a 50-μl volume consisting of 100 ng of sample DNA, 0.5 μM of each forward and reverse primer, 1.5 mM of $MgCl_2$, 200 μM each of dTTP, dCTP, dGTP and dATP, 1 unit of *Taq* polymerase (Advanced Biotechnologies, Surrey, UK) in a reaction-buffer containing 75 mM of Tris-HCl (pH 8.9) and 20 mM of $(NH_4)_2SO_4$. The thermal cycling profile consisted of a single denaturation step of 4 min at 94 °C, followed by 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 50–58 °C (depending on primer Tm), 1–3 min extension (depending on

fragment size) at 72 °C, with a final 7-min incubation at 72 °C using a Perkin Elmer model 9600 thermal cycler. The sizes of the amplified fragments were determined by separating 2 μl of the PCR products in a 1% agarose gel, staining in 1 μg ml^{-1} of ethidium bromide solution and visualising under UV light. Estimation of fragment sizes was done by comparison with a 1-kilobase ladder (Life Technologies, Paisley, UK).

Restriction endonuclease analysis

Restriction-site differences among the accessions were surveyed by digesting each of the amplified fragments sequentially with ten restriction enzymes having four or five base-recognition sequences. The following restriction enzymes were used: *Sau*3A1, *Taq*1, *Hae*lll, *Hin*fl, *Dde*l, *Rsa*l, *Alu*l, *Msp*l, *Cfo*l and *Hpa*ll. The digested DNA fragments were separated by electrophoresis on 2–3% agarose gels in TBE buffer at 5 Vcm^{-1} for 1 to 3 h depending on the size of the initial amplified fragment. Gels were stained and photographed as explained earlier. Fragment sizes were estimated by comparison with a 100-base pair ladder (Life Technologies, Paisley, UK).

Data analysis

Restriction-site differences among the accessions were determined by comparing the sizes of restriction fragments. An apparent gain in restriction site was indicated when a larger fragment was replaced by two smaller ones and this was scored as '1', while absence of the restriction site was scored as '0'. Variation in the sizes of the undigested PCR product was scored as insertion/deletion mutations. Such size variants were coded from 0 to 4 (smallest to largest) and scored as multi-state characters. The combined restriction-site data for cpDNA and mtDNA sequences were used to reconstruct phylogenetic relationships employing the Phylogenetic Analysis Using Parsimony (PAUP) version 2.4.1 computer software (Swofford 1990). The Wagner parsimony method with the branch and bound option was used to find the most-parsimonious trees. The neighbour-joining (NJ) method of Saitou and Nei (1987) was also used to analyze the same data using the NEIGHBOR algorithm in PHYLIP (Phylogeny Inference Packages) version 3.5c (Felsenstein 1993). Bootstrap analysis was performed with PHYLIP to evaluate the level of support for the branch nodes. One hundred replications of the original data were generated with the programme SEQBOOT, and analysed with the MIX programme using the multiple data-set option and a majority-rule consensus tree constructed using the CONSENSE programme. The estimated phylogenetic trees were plotted using the programme DRAWGRAM.

Results

Table 3 shows the sizes of the amplified products, the number of fragments generated by restriction analysis and the number of restriction site changes scored for each sequence. Fragment sizes ranged from 400 to 2600 bp for the tRNA leu intron (intron 1) and the tRNA lys gene (*trn*K) respectively.

RFLP analysis of the six cpDNA regions and the one mtDNA region with ten restriction endonucleases yielded a total of 79 restriction-site changes and 12 size mutations (indels). The *rps*14-cob region was not included in restriction analysis due to the consistently low PCR-product yield. Figure 2 illustrates representative restriction fragment patterns generated by digesting the chloroplast gene, *trn*K, with *Taq*1 (A) and *Hinf*1 (B), while Fig. 3 is a *Cfo*1 restriction fragment pattern for the *nad*1 exon$^{b–c}$ intron.

Twelve distinct organellar DNA patterns or haplotypes were identified in the sample. Wagner parsimony with the branch and bound algorithm yielded five equally parsimonious trees. All the trees were 149 steps long with a consistency index of 0.624. The trees had similar

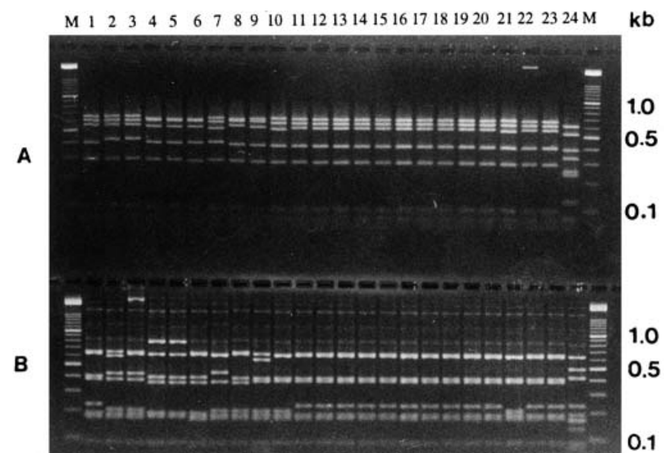

Fig. 2 Restriction fragment patterns generated by digesting the chloroplast gene, *Trn*K, with *Taq*1 (**A**) and *Hinf*1 (**B**)

Table 3 Sizes of PCR-amplified sequences, number of restriction fragments and restriction-site changes generated using ten restriction enzymes

Primers	Size (base pairs)	No. of size variants	No. restriction fragments	No. restriction-site changes
Chloroplast DNA				
IGR 1	1,400–1,600	2	40	7
IGR 2	600		36	5
IGR 3	1,600–2,100	2	58	18
trnK	2,600		74	10
rpoC	700		28	2
Intron 1	400–420	3	20	5
Mitochondrial DNA				
Intron 2	1,350–1,700	5	118	32
IGR 4	1,400	–	–	–
Total	11,120	12	374	79

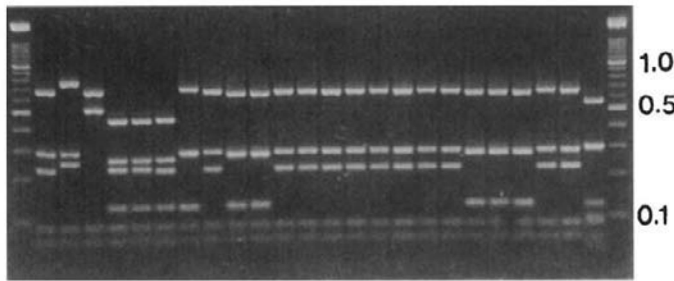

Fig. 3 *Cfo*I restriction fragment pattern for the *nad*1 exon^{b-c} intron

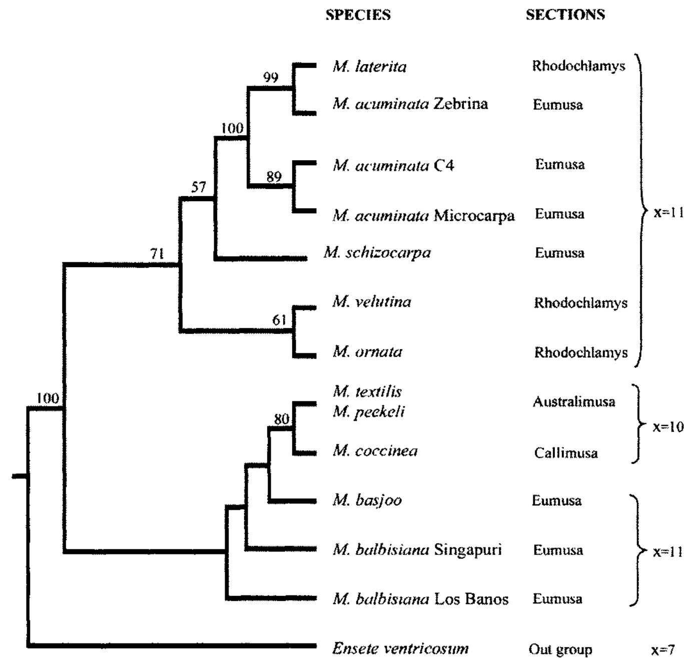

Fig. 4 One of the five most-parsimonious trees of organelle DNA phylogeny obtained by Wagner parsimony analysis of restriction-site variation in PCR-amplified chloroplast and mitochondria DNA sequences in *Musa*. Bootstrap support values greater than 50% are indicated at the nodes. Haploid chromosome numbers are denoted as *x*

topologies with only minor differences. Bootstrap analysis with 100 replications gave good support values for the major clades (Fig. 4). The NJ method resulted in a tree with the same basic features as the most-parsimonious tree (Fig. 5).

Two major clades were identified. The basal clade included *M. textilis* Nee. and *M. peekeli* Lauterb (*Australimusa*), *M. coccinea* Andrews (*Callimusa*), *M. basjoo* Sieb, and the *M. balbisiana* clones 'Los Banos' and 'Singapuri' (*Eumusa*). Species of the sections *Australimusa* and *Callimusa* shared a sister-group relationship. Two cpDNA haplotypes represented by the 'Singapuri' and 'Los Banos' patterns were detected in *M. balbisiana*.

In the second clade, *M. velutina* Wendl. & Drude, *M. ornata* Roxb. and *M. laterita* (*Rhodochlamys*) grouped with the *Eumusa* species *M. acuminata* and *M. schizocarpa* Simmonds. *M. laterita* was separated from the other *Rhodochlamys* species and grouped closely with the *M. acuminata* ssp. *zebrina* in a well-supported group.

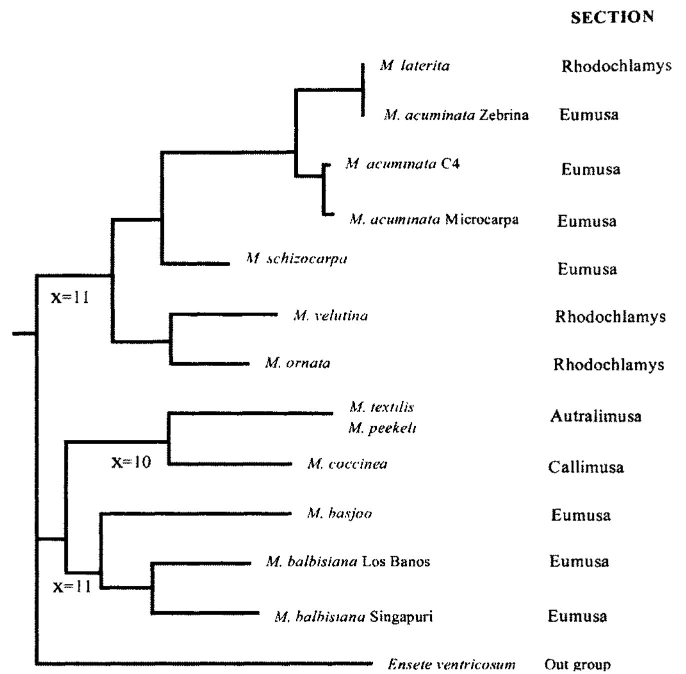

Fig. 5 Neighbour-Joining tree of organelle DNA phylogeny reconstructed using data from restriction-site variations in PCR-amplified chloroplast and mitochondria DNA sequences in the genus *Musa*. Haploid chromosome numbers are denoted as *x*

Discussion

This study used PCR-RFLP of the organelle genomes to determine sectional relationships in the genus *Musa*. The data produced phylogenetic trees with similar topologies (Figs. 4, 5) with both the Wagner parsimony and neighbour-joining methods. When phylogenetic trees constructed by different methods agree, as was the case in this study, confidence in the trees and the data used for their construction is increased (Tsumura et al. 1996).

The basal position of *M. balbisiana*, close to the outgroup, suggests that it has the more primitive organellar genomes amongst the species examined. On the contrary, the *M. acuminata* subspecies were terminally located in the cladogram suggesting a more-recent origin of these subspecies. This interpretation agrees with the conclusions from anthocyanin diversity (Horry and Jay 1990). The latter study reported that *M. balbisiana* was evolutionarily primitive having fairly unique and unmodified anthocyanin forms. *M. balbisiana* was also found to be most distant and isolated within the section *Eumusa* in a study using AFLP (amplified fragment length polymorphic) markers (Wang et al. 2002). However, in the latter study *M. balbisiana* was more closely linked to the *Eumusa-Rhodochlamys* complex, while in

this study it was placed more closely with the *Australimusa-Callimusa* complex.

The cladogram (Figs. 4, 5) showed two major lines of evolution in the genus *Musa*. One line included species of sections *Australimusa, Callimusa* and two species of section *Eumusa*, namely, *M. basjoo* and *M. balbisiana*. The sister-group relationship of *Australimusa* and *Callimusa* was expected since both sections have species with $x = 10$ chromosomes. Both sections also display distinctive cylindrical or barrel-shaped seeds with a marked external transverse groove (Cheesman 1947). The two sections are morphologically similar with species of the section *Callimusa* being distinguished by their smaller pseudostem. Despite their similarity, the high bootstrap value (80%) suggests that sections *Autralimusa* and *Callimusa* are distinct taxa with a close genetic relationship. Similar results were obtained in an AFLP study of these taxa (Ude et al. 2002; Wang et al. 2002). The relatively close genetic relationship between *M. balbisiana* (*Eumusa*) and *M. textilis* (*Australimusa*) is also supported by previous analysis of cpDNA in *Musa* (Gawel and Jarret 1991b). Similarly, a numerical taxonomic analysis of morphological data placed section *Australimusa* close to *M. balbisiana* (Simmonds and Weatherup 1990). The existence of the cultivar 'Canton', a natural hybrid of *M. textilis* and *M. balbisiana* (Simmonds 1962), is additional evidence of the close genetic affinity of *M. balbisiana* with *Australimusa*.

The second line of evolution contained species of sections *Rhodochlamys* and *Eumusa* both with $x = 11$. The initial subdivision of the $x = 11$ species of the genus *Musa* into sections *Rhodochlamys* and *Eumusa* relied on the presence of small pseudostem size (<3 M), erect inflorescence with few hands and brightly coloured bracts in *Rhodochlamys* species (Cheesman 1947). However, these characters appear to be unstable in the genus *Musa* and have evolved independently and repeatedly in different taxa. For example, the short pseudostem and erect inflorescence with brightly coloured bracts have evolved in the ornamental species *M. coccinea* of the section *Callimusa*. Similarly, the erect inflorescence is a distinctive feature of the Fe'i bananas that evolved from species of the section *Australimusa*. The tenuous basis for initially separating the two sections was tacitly acknowledged by Cheesman (1947) in the statement that he "was inclined to regard the division between *Eumusa* and *Rhodochlamys* as unessential, though it is convenient to maintain it as long as it remains as well marked in the field as it is at present".

Another feature of the organelle DNA phylogeny was the close genetic relationship between the species of section *Rhodochlamys* and the subspecies of *M. acuminata*. This is also in agreement with previous studies using AFLP markers (Ude et al. 2002; Wang et al. 2002). In this study, *M. laterita* (section *Rhodochlamys*) shared identical chloroplast genome restriction patterns with *M. acuminata* subsp. *zebrina*. It would appear that little genetic differentiation exists between species of section *Eumusa* and those of *Rhodochlamys*.

The species of section *Eumusa* appeared to be very diverse. The range of morphological variation and geographic distribution of this section encompasses that of the genus *Musa* as a whole (Simmonds 1962). This was reflected in the distribution of the *Eumusa* species in both clades suggesting that section *Eumusa* is a heterogeneous assemblage of species. Previous studies using chloroplast DNA polymorphisms (Gawel and Jarret 1991b), and RFLPs of the nuclear genome (Gawel et al. 1992), found a much-wider variation in the section *Eumusa* compared to that found in the other three sections of the genus *Musa*. A numerical taxonomic study of 23 morphological characters showed such a wide diversity in section *Eumusa* that it was subdivided into two informal groups, *Eumusa*-1 and *Eumusa*-2 (Simmonds and Weatherup 1990).

Phylogenetic analysis of organelle DNA variation provided new insights in sectional relationships in *Musa*. Species belonging to section *Eumusa* were widely dispersed in different clades suggesting that the *Eumusa*, as presently defined, is a heterogeneous group. Our result supports the splitting of section *Eumusa* into two groups, *Eumusa*-1 and *Eumusa*-2, as suggested by Simmonds and Weatherup (1990). Our study suggests that the progenitors of modern bananas, *M. balbisiana* and *M. acuminata*, should be in different groups. The organelle DNA phylogeny did not distinguish species belonging to the section *Rhodochlamys* from those of the *M. acuminata* complex of section *Eumusa*. This is in agreement with morphological data, cross-hybridization experiments (Simmonds 1954; Shepherd 1999), RFLP of the nuclear genome (Gawel et al. 1992) and AFLP data (Ude et al. 2002; Wang et al. 2002). On this basis, Wang et al. (2002) suggested that section *Rhodochlamys* should be combined with section *Eumusa*. Our study supports such an idea.

This study provides additional support for the joint treatment of section *Rhodochlamys* and the *M. acuminata* species complex of section *Eumusa*. This will ensure that sectional classification in *Musa* is in line with genetic relationships in the genus. Our study suggests that species of section *Rhodochlamys* could form a secondary genepool for the improvement of *M. acuminata* cultivars. Finally our study is supportive of the statement of De Langhe (2000) that sectional classification in the genus *Musa* ought to be reviewed to accommodate the present state of knowledge in *Musa* systematics.

Acknowledgements Funding for this research was provided, in part, by the Directorate General for International Cooperation (DGIC), Belgium. D.C. Nwakanma was funded by the IITA Graduate Research Fellowship Program. This is IITA manuscript number 03/003/JA.

References

Argent CGC (1976) The wild bananas of Papua New Guinea. Notes Royal Bot Gard Edinburgh 35:77–114

Badenes ML, Parfitt DE (1995) Phylogenetic relationships of the cultivated *Prunus* species from an analysis of chloroplast DNA variation. Theor Appl Genet 90:1035–1041

Baker JG (1893) A synopsis of the genera and species of Museae. Ann Bot 7:189–229

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331

Carreel F, Gongalez de Leon G, Lagoda P, Lanaud C, Jenny C, Horry JP, Tezenas du Montcel H (2002) Ascertaining maternal and paternal lineage within *Musa* by chloroplast and mitochondrial DNA RFLP analyses. Genome 45:679–692

Cheesman EE (1947) Classification of the bananas. Kew Bull 2:106–117

Curtis SE, Clegg MT (1984) Molecular evolution of chloroplast DNA sequences. Mol Biol Evol 1:291–301

De Langhe E (1969) Bananas (*Musa* spp.). In: Ferwarda FP, Wit F (eds) Outlines of perennial crop breeding in the Tropics. Misc Papers, No. 4, Agricultural University, Wageningen, The Netherlands, pp 53–78

De Langhe EA (1990) Identification of genetic diversity in the genus *Musa*: a general introduction. In: Jarret RL (ed) Identification of genetic diversity in the genus *Musa*. INIBAP, Montpellier, France, pp 8–16

De Langhe EA (2000) Diversity in the genus *Musa*: its significance and its potential. Acta Hort 540:81–88

Demesure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Mol Ecol 4:129–131

Faure S, Noyer J, Carreel F, Horry J, Bakry F, Lanaud C (1994) Maternal inheritance of chloroplast genome and paternal inheritance of mitochondrial genome in bananas (*Musa acuminata*). Curr Genet 25:265–269

Felsenstein J (1993) PHYLIP (Phylogeny Inference Package) version 3.2. Cladistics 5:164–166

Fofana B, Baudoin JP, Vekemans X, Debouck DG, du Jardin P (1999) Molecular evidence for an Andean origin and a secondary gene pool for the Lima bean (*Phaseolus lunatus* L.) using chloroplast DNA. Theor Appl Genet 98:202–212

Gawel NJ, Jarret RL (1991a) A modified CTAB DNA extraction procedure for *Musa* and *Ipomoea*. Plant Mol Biol Rep 9:262–266

Gawel NJ, Jarret RL (1991b) Chloroplast DNA restriction fragment length polymorphisms (RFLPs) in *Musa* species. Theor Appl Genet 81:783–786

Gawel NJ, Jarret RL, Whittemore AP (1992) Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of *Musa*. Theor Appl Genet 84:286–290

Horry JP, Jay M (1990) An evolutionary background of bananas as deduced from flavonoid diversification. In: Jarret RL (ed) Identification of genetic diversity in the genus *Musa*. INIBAP, Montpellier, France, pp 41–45

Palmer JD (1987) Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. Am Natl 130:6–29

Palmer JD (1988) Intraspecific variation and multicircularity in *Brassica* mitochondrial DNAs. Genetics 118:341–351

Purseglove JW (1988) Tropical crops: monocotyledons. Longman Scientific and Technical/John Wiley and Sons, New York, USA

Rowe PR (1981) Breeding an intractable crop. Bananas. In: Rachie KO, Lyman JM (eds) Genetic engineering for crop improvement. Working papers. The Rockefeller Foundation, pp 66–84

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Shepherd K (1959) Two new basic chromosome numbers in *Musaceae*. Nature 183:1539

Shepherd K (1999) Cytogenetics of the genus *Musa*. INIBAP, Montpellier, France

Simmonds NW (1954) Isolation in *Musa*, sections *Eumusa* and *Rhodochlamys*. Evolution 8:65–74

Simmonds NW (1960) Notes on banana taxonomy. Kew Bull 14:198–212

Simmonds NW (1962) The evolution of the bananas. Longman, London

Simmonds NW (1966) Bananas. 2nd edn. Longman, London

Simmonds NW, Shepherd K (1955) The taxonomy and origins of the cultivated bananas. J Linn Soc (Bot) 55:302–312

Simmonds NW, Weatherup STC (1990) Numerical taxonomy of the wild bananas (*Musa*). News Phytol 115:567–571

Swofford DL (1990) PAUP: phylogenetic analysis using parsimony, version 2.4. Illinois, Natural History Survey, Champaign, Illinois

Taberlet P, Gielly L, Patou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Mol Biol 17:1105–1109

Tsumura Y, Kawahara T, Wickneswari R, Yoshimura K (1996) Molecular phylogeny of *Dipterocarpaceae* in Southeast Asia using RFLP of PCR-amplified chloroplast genes. Theor Appl Genet 93:22–29

Ude G, Pillay M, Nwakanma D, Tenkouano A (2002) Analysis of genetic diversity and sectional relationships in *Musa* using AFLP markers. Theor Appl Genet 104:1239–1245

Wang C, Kiew R, Argent G, Set O, Lee SK, Gan YY (2002) Assessement of the validity of the Sections in *Musa* (*Musaceae*) using AFLP. Ann Bot 90:231–238