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# Assessment of the Validity of the Sections in *Musa* (Musaceae) using AFLP

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*Musa* L. (Musaceae) is currently separated into five sections (*Musa*, *Rhodochlamys*, *Callimusa*, *Australimusa* and *Ingentimusa*) based on chromosome numbers and morphological characters. However, the validation of this classification system is questioned due to the common occurrence of hybridizations across sections and the system not accommodating anomalous species. This study employed amplified fragment length polymorphism (AFLP) in a phenetic examination of the relationships among four sections (material of sect. *Ingentimusa* was not available) to evaluate whether their genetic differences justify distinction into separate groups. Using eight primer combinations, a total of 276 bands was scored, of which 275 were polymorphic. Among the monomorphic bands, 11 unique markers were identified that revealed the distinct separation of the 11-chromosome species from the 10-chromosome species. AFLP results suggest that species of sect. *Rhodochlamys* should be combined into a single section with species of sect. *Musa*, and likewise for species of sect. *Australimusa* to be merged with those of sect. *Callimusa.* 

**Key words:** Banana, *Musa*, Musaceae, section, *Rhodochlamys*, *Callimusa*, *Australimusa*, AFLP, DNA fingerprinting.

## INTRODUCTION

The first subgeneric classification of *Musa s.l.* began with three subgenera *Physocaulis*, *Eumusa* and *Rhodochlamys* (Sagot, 1887; Baker, 1893). Later, Cheesman (1947) laid the foundation for the grouping of banana species into four sections. He recognized subgenus *Physocaulis* as a distinct genus, *Ensete* with a chromosome number $n = x = 9$. Within *Musa s.s.*, he redefined subgenera *Eumusa* (now sect. *Musa*) and *Rhodochlamys* as two separate sections, and described an additional two sections, *Australimusa* and *Callimusa*. Cheesman (1947) also redistributed the species among the four sections to produce more homogenous groups.

Species of sections *Musa* and *Rhodochlamys* share common characteristics, possessing the same chromosome number ($n = x = 11$) and having bracts that are generally sulcate, glaucous and that become revolute on fading (Cheesman, 1947). This contrasts with species of sections *Australimusa* and *Callimusa*, which have chromosome number $n = x = 10$, and bracts that are smooth, polished on the outside and that do not become revolute on fading.

Species of sect. *Musa* are distinguished from those of sect. *Rhodochlamys* in being large plants, 3 m or more tall, with pendent inflorescences with dull coloured bracts, many flowers in two series per bract and reflexed fruits. In contrast, species of sect. *Rhodochlamys* are generally smaller in stature (less than 3 m), have erect inflorescences with brightly coloured bracts with a few flowers in a single series and the fruits are not reflexed. Species of sect. *Callimusa* are separated from those of sect. *Australimusa* by their unique seeds, which are cylindrical or barrel-shaped and possess a large apical chamber. In contrast, seeds of species of sect. *Australimusa* are similar to those of species in sect. *Musa* and *Rhodochlamys*, being subglobose or dorsiventrally compressed and possessing a small apical chamber.

Subsequent authors have followed these groupings, although Simmonds (1960) pointed out that three species (*Musa beccarii* N.W. Simmonds, *M. lasiocarpa* Franch. and *M. ingens* N.W. Simmonds) did not conform entirely to any of the existing sections. Since then, Wu (cited in Li, 1978) has placed *M. lasiocarpa* in its own monotypic genus, *Musella*, and Argent (1976) has created a new section, sect. *Ingentimusa* for *M. ingens*, which has a chromosome number of $n = x = 14$. Describing two new species from Borneo, *M. monticola* [Hotta ex] Argent and *M. suratii* Argent, Argent (2000) was unable to place them with any certainty into any section on morphological grounds. The placement of these two species and that of *M. beccarii* was discussed in Wong *et al.* (2001*a*).

There is a need to reassess the validity and usefulness of these sections in *Musa* because several authors have drawn attention to difficulties in placing species within existing sections (Simmonds, 1960; Argent, 1976), and the status of sect. *Rhodochlamys* as a valid section has been questioned by Cheesman (1947), Simmonds (1962), Shepherd (1990) and Jarret and Gawel (1995).

Taxonomic studies in *Musa* have been conducted using a wide array of techniques, such as morphological characters (Simmonds, 1962; Simmonds and Weatherup, 1990), isozymes (Bhat *et al.*, 1992), cytogenetics (Cheesman, 1947; Shepherd, 1959; Osuji *et al.*, 1997), molecular

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cytogenetics (Osuji *et al*., 1998), intergenic spacers (Lanaud *et al*., 1992), restriction fragment length polymorphisms (RFLPs) (Gawel and Jarret, 1991; Gawel *et al*., 1992), random amplified polymorphic DNA markers (RAPDs) (Howell *et al*., 1994), inter simple sequence repeats (ISSRs) (Godwin *et al*., 1997) and microsatellites (Grapin *et al*., 1998). Although these have provided a general understanding of *Musa* classification, the question of the validity of the sectional classification system is still unresolved. Amplified fragment length polymorphism (AFLP) (Vos *et al*., 1995) is a robust and reliable molecular technique recently employed in many plant systematic studies, involving, for instance, lettuce (Hill *et al*., 1996), soybean (Powell *et al*., 1996), rice (Aggarwal *et al*., 1999), *Caladium* (Loh *et al*., 1999, 2000*c*) and bamboo (Loh *et al*., 2000*a*). Levels of polymorphism in *Musa* were shown to be high when analysed using AFLP, and the technique was the most effective for genetic diversity analysis as shown in the studies of Crouch *et al*. (1999), Loh *et al*. (2000*b*) and Wong *et al*. (2001*a*, *b*).

The problems highlighted reveal the shortcomings of the current state of *Musa* classification. Hence, this study employs AFLPs in a phenetic examination of the relationships among sections *Musa*, *Rhodochlamys*, *Callimusa* and *Australimusa* of genus *Musa*, and evaluates whether genetic differences among the sections are sufficiently significant or distinct to justify maintaining the four sections as separate groups.

## MATERIALS AND METHODS

### *Plant material*

A total of 21 *Musa* species and subspecies was examined, with sample sizes ranging from three to five (Table 1). Two species of *Ensete*, *E. superbum* (Roxb.) Cheesm. and *E. glaucum* (Roxb.) Cheesm. were included as reference taxa, for comparison with *Musa*. The material included representatives from four sections of *Musa* (sect. *Ingentimusa* was excluded due to lack of available material) of both wild and cultivated origin and from a variety of introductions. Samples were collected from wild populations, the Singapore Botanic Gardens (Singapore), the Royal Botanic Garden Edinburgh (UK) and the Agricultural Park at Tenom (Sabah, Malaysia). Voucher specimens were deposited in the herbaria at Singapore Botanic Gardens and the Royal Botanic Garden Edinburgh.

Leaf tissue was used for AFLP analysis. Leaves were surface sterilized following the procedure described in Zhang *et al*. (1997). Briefly, leaves collected were swirled in 95 % ethanol for 1 min, 5 % bleach (NaOCl) for 5 min and then re-immersed in fresh 95 % ethanol for 30 s, after which they were blotted dry and stored in sealed plastic bags at –80 °C until required for DNA extraction.

### *DNA extraction*

DNA was extracted using the CTAB method according to Reichardt and Rogers (1993). Briefly, leaf tissue was pulverized using liquid nitrogen prior to the addition of 4 ml Solution I [2 % w/v CTAB (Sigma), 100 mM Tris-HCl, 20 mM EDTA, 1·4 M NaCl, pH 8·0] per gram of leaf tissue and incubated for 60 min at 65 °C. The homogenate was then extracted with an equal volume of chloroform/isoamyl alcohol (24 : 1) and centrifuged at 12 000 rpm for 5 min. The upper aqueous phase was recovered and incubated with 1/10 volume Solution II (10 % w/v CTAB, 0·7 M NaCl), pre-warmed to 65 °C. The aqueous phase was then extracted with one volume of chloroform/isoamyl alcohol (24 : 1) and recovered as before. To the recovered aqueous phase, one volume of Solution III (1 % w/v CTAB, 50 mM Tris-HCl, 10 mM EDTA, pH 8·0) was added and incubated overnight at 37 °C. The mixture was centrifuged for 5 min at 3500 rpm and the supernatant removed. The DNA pellet was re-dissolved in Solution IV (10 mM Tris-HCl, 0·1 mM EDTA, 1 M NaCl, pH 8·0) at 0·5–1 ml per gram starting material, followed by ethanol precipitation of the DNA. The pellet was washed with 70 % ethanol, dried and re-suspended in a minimal volume of TE buffer at 0·1–0·5 ml per gram starting material.

### *AFLP analysis*

AFLP analysis was carried out according to Vos *et al*. (1995) with minor modifications. Restriction digests of genomic DNA with *Eco*RI and *Mse*I were carried out at 37 °C for 1 h. Following heat inactivation of the restriction endonucleases, genomic DNA fragments were ligated to *Eco*RI and *Mse*I adapters overnight at 16 °C to generate template DNA for amplification. Polymerase chain reaction (PCR) was performed in two consecutive reactions. The template DNA generated was first pre-amplified using AFLP primers each having one selective nucleotide. The PCR products of the pre-amplification reaction were then used as template, after five-fold dilution in sterile water, for selective amplification using two AFLP primers, each containing three selective nucleotides. A total of eight primer combinations was used in this study (Table 2). The final PCR products were run on a 6 % denaturing polyacrylamide gel in 1 × TBE buffer. The *Eco*RI primers used were not radioactively labelled as in the original protocol. Instead, a modified silver staining method was used (Loh *et al*., 1999).

### *Data analysis*

For the diversity analysis, bands were scored as present (1) or absent (0) to form a raw data matrix. A square symmetric matrix of similarity was then obtained using Jaccard's Similarity Coefficient $[x/(y - z)]$, where $x$ is the number of fragments in common between two taxa, $y$ is the total number of fragments scored and $z$ is the number of fragments absent in both taxa, from the raw data matrix. Genetic diversity estimates (GDEs) were then calculated as 1 – Jaccard's Similarity Coefficient and used for cluster analysis using the UPGMA (unweighted pair group method with arithmetic mean) technique of the NEIGHBOR program in PHYLIP version 3·5c (Felsenstein, 1993). The dendrogram was drawn using TREEVIEW version 1·6·1 (Page, 1996).

TABLE 1. *Species of* Musa *and* Ensete *studied*

| Taxon | Accession No. | Source |
|---|---|---|
| *Ensete superbum* (Roxb.) Cheesm.*(8) | AR200/94-96-8474 | RBG/SBG |
| *Ensete glaucum* (Roxb.) Cheesm. (11) | AR215 | RBG |
| *Musa* sect. *Musa* | | |
| *M. acuminata* Colla ssp. *truncata* Ridl. (1) | RK4718/RK4889 | CH/FH |
| *M. acuminata* Colla ssp. *malaccensis* (Ridl.) Simmonds (2) | RK4890/CW1-5 | KKB/T |
| *M. acuminata* Colla ssp. *burmanica* Simmonds (12) | AR214 | RBG |
| *M. acuminata* Colla ssp. *siamea* Simmonds (9) | GA s.n. (siamea) | RBG |
| *M. balbisiana* Colla (3) | AR s.n./GA s.n. (balbisiana) | RBG/CI |
| *M. nagensium* Prain (14) | 19991679A | RBG |
| *M. sikkimensis* Kurz (15) | 19972089 | RBG |
| *M. itinerans* E. E. Cheesm. (7) | AR201 | RBG |
| *Musa* sect. *Rhodochlamys* | | |
| *M. laterita* E. E. Cheesm. (10) | GA s.n. (laterita) | RBG |
| *M. ornata* Roxb. (6) | 19961732/101-92-45/AL5 | RBG/SBG/AP |
| *M. velutina* H. Wendl & Drude (18) | 19702121 / 19980690 | RBG/SBG |
| *Musa* sect. *Callimusa* | | |
| *M. suratii* Argent (21) | AL6 | AP |
| *M. borneensis* Becc. (19) | GA s.n. (borneensis)/19992248/AL2 | RBG/SBG/AP |
| *M. campestris* Becc. (16) | 19773441/AL3 | RBG/AP |
| *M. coccinea* Andr. (13) | AR213 | RBG |
| *M. violascens* Ridl. (5) | RK4876 | FH |
| *M. gracilis* Holttum (23) | RK5088 | CR |
| *Musa* sect. *Australimusa* | | |
| *M. textilis* Née (4) | AL 7 | AP |
| *M. jackeyi* Hill (22) | 19990218 | SBG |
| *M. beccarii* Simmonds (20) | AL 1 | AP |
| *M. monticola* [Hotta ex] Argent (17) | 19891874/AL 4 | RBG/AP |

AP, Agricultural Park, Tenom, Sabah, Malaysia; CH, Cameron Highlands, Malaysia; CI, Camiguin Island, Philippines; CR, Chukai River, Trengganu, Malaysia; FH, Fraser's Hill, Malaysia; KKB, Kuala Kubu Baru, Selangor, Malaysia; RBG, Royal Botanic Garden, Edinburgh; SBG, Singapore Botanic Gardens, Singapore; T, Tapah, Perak, Malaysia.

* See Table 4.

TABLE 2. *Sequences of the primers and adapters used for AFLP analysis*

| Name/abbreviation | Enzyme | Type | Sequence (5′-3′) |
|---|---|---|---|
| GYY 101/EA+ | *Eco*RI | Adapter + | CTCGTAGACTGCGTACC |
| GYY 102/EA– | *Eco*RI | Adapter – | AATTGGTACGCAGTCTAC |
| GYY 103/MA+ | *Mse*I | Adapter + | GACGATGAGTCCTGAG |
| GYY 104/MA– | *Mse*I | Adapter – | TACTCAGGACTCAT |
| *GYY 105/E-A | *Eco*RI | Primer +1 | GACTGCGTACCAATTCA |
| GYY 107/E-AAC | *Eco*RI | Primer +3 | GACTGCGTACCAATTCAAC |
| GYY 108/E-AAG | *Eco*RI | Primer +3 | GACTGCGTACCAATTCAAG |
| GYY 109/E-ACA | *Eco*RI | Primer +3 | GACTGCGTACCAATTCACA |
| GYY 110/E-ACT | *Eco*RI | Primer +3 | GACTGCGTACCAATTCACT |
| GYY 111/E-ACC | *Eco*RI | Primer +3 | GACTGCGTACCAATTCACC |
| GYY 112/E-ACG | *Eco*RI | Primer +3 | GACTGCGTACCAATTCACG |
| GYY 113/E-AGC | *Eco*RI | Primer +3 | GACTGCGTACCAATTCAGC |
| GYY 114/E-AGG | *Eco*RI | Primer +3 | GACTGCGTACCAATTCAGG |
| *GYY 106/M-C | *Mse*I | Primer +1 | GATGAGTCCTGAGTAAC |
| GYY 115/M-CAA | *Mse*I | Primer +3 | GATGAGTCCTGAGTAACAA |
| GYY 116/M-CAC | *Mse*I | Primer +3 | GATGAGTCCTGAGTAACAC |
| GYY 117/M-CAG | *Mse*I | Primer +3 | GATGAGTCCTGAGTAACAG |
| GYY 118/M-CAT | *Mse*I | Primer +3 | GATGAGTCCTGAGTAACAT |
| GYY 119/M-CTA | *Mse*I | Primer +3 | GATGAGTCCTGAGTAACTA |
| GYY 120/M-CTC | *Mse*I | Primer +3 | GATGAGTCCTGAGTAACTC |
| GYY 121/M-CTG | *Mse*I | Primer +3 | GATGAGTCCTGAGTAACTG |
| GYY 122/M-CTT | *Mse*I | Primer +3 | GATGAGTCCTGAGTAACTT |

* Pre-selective primers

## RESULTS

### *AFLP profiles*

Figure 1 illustrates an AFLP profile generated using primer combination 1 (E-AAC, M-CAA). The eight primer combinations used in this study (Table 2) generated an average of 70 bands per primer pair. Only unambiguous bands were scored for analysis, giving a total of 276 unambiguous bands (35 bands per primer pair) of the size 50–500 bp. Of these, 275 bands (99 %) were polymorphic across the whole range of samples.

Eleven unique bands were observed for all the taxa examined (Table 3). *Musa* and *Ensete* were distinctly separated by the presence of two unique bands in each genus. *M. suratii*, *M. jackeyi* Hill and *M. itinerans* Cheesman were each characterized by two unique bands, and *M. sikkimensis* Kurz by one unique band, indicating that these species were distinct.

### *Genetic similarities*

Phenetic analysis based on genetic diversity estimates (GDEs) (Table 4) showed that the genus *Musa* was clearly separated from the genus *Ensete*, supporting their positions as distinct genera (Fig. 2). Within the genus *Musa*, species segregated into two main groups corresponding to the chromosome number: $n = x = 10$ in sect. *Callimusa* and sect. *Australimusa*; and $n = x = 11$ in sect. *Musa* and sect. *Rhodochlamys*. These molecular data supported the separation of *Musa* species into sections with chromosomes $n = x = 10$ and $n = x = 11$.

Within the *Rhodochlamys* and *Musa* clusters, *M. balbisiana* Colla formed a distinct branch, while the remaining species in the cluster were separated into two groups. The first cluster included *M. ornata* Roxb., the four subspecies of *M. acuminata* Colla, *M. laterita* Cheesman, *M. velutina* H. Wendl & Drude and *M. sikkimensis*, while the second cluster included *M. itinerans* and *M. nagensium* Prain. Species from sect. *Rhodochlamys*, *M. ornata*, *M. laterita* and *M. velutina* were embedded within sect. *Musa*, suggesting that the separation of sect. *Rhodochlamys* from sect. *Musa* was not clear-cut.

Within the *Callimusa* and *Australimusa* clusters, *M. coccinea* Andr. was distantly placed from the other species. The cluster divided into two subclusters. One subcluster included *M. jackeyi*, *M. campestris* Becc., *M. textilis* Née, *M. beccarii*, *M. monticola* and *M. borneensis* Becc.; while *M. suratii*, *M. gracilis* Holttum and *M. violascens* Ridl. formed the second subcluster, with *M. gracilis* clustering closer to *M. violascens* than to *M. suratii*. Species from sect. *Australimusa*, *M. beccarii*, *M. monticola*, *M. textilis* and *M. jackeyi* were nestled within species of sect. *Callimusa*, indicating a blurring of the distinction between sect. *Callimusa* and sect. *Australimusa.*

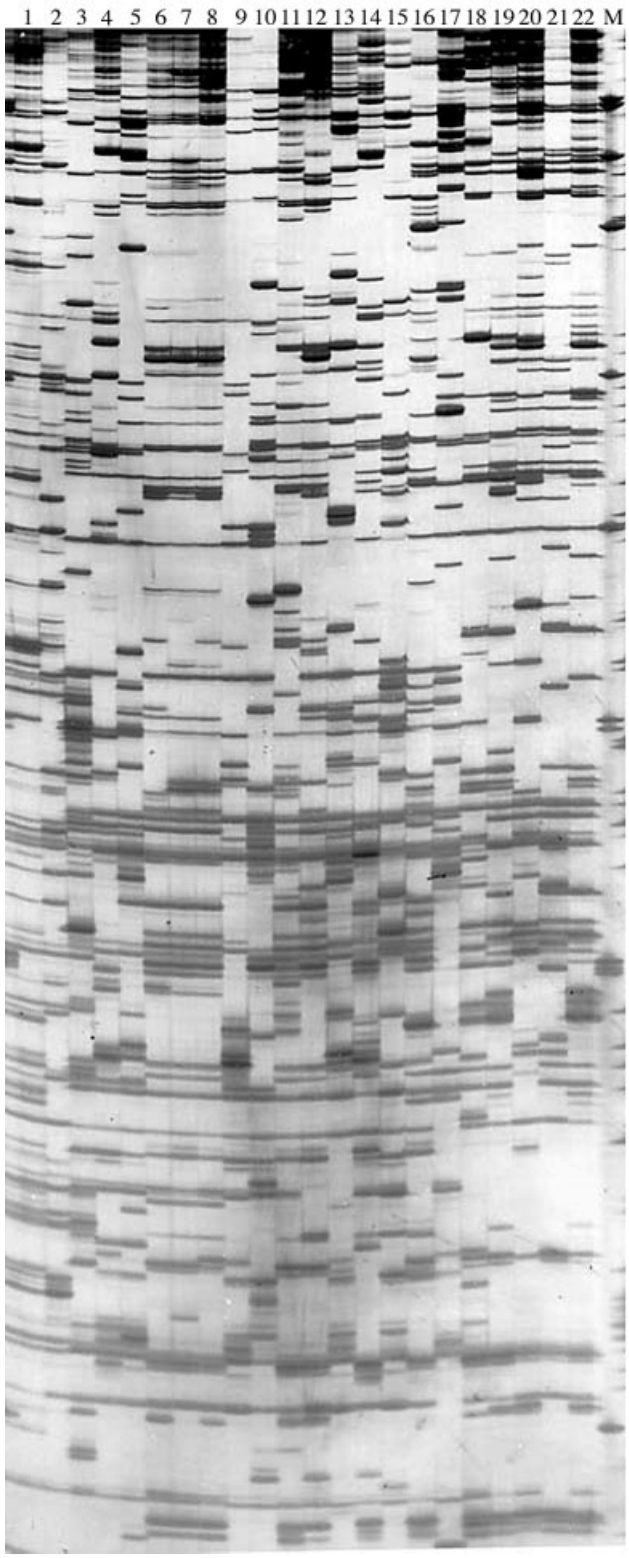


FIG. 1. AFLP profile generated by primer combination 1 (E-AAC, M-CAA). Lane 1, *Ensete superbum*; lane 2, *E. glaucum*; lane 3, *Musa itinerans*; lane 4, *M. laterita*; lane 5, *M. sikkimensis*; lanes 6–8, *M. gracilis*; lane 9, *M. acuminata* ssp. *malaccensis*; lane 10, *M. balbisiana*; lane 11, *M. textilis*; lane 12, *M. violascens*; lane 13, *M. ornata*; lane 14, *M. coccinea*; lane 15, *M. nagensium*; lane 16, *M. campestris*; lane 17, *M. velutina*; lane 18, *M. jackeyi*; lane 19, *M. beccarii*; lane 20, *M. suratii*; lane 21, *M. monticola*; lane 22, *M. borneensis*; lane M, pUC19/*Hpa*II molecular weight marker.

TABLE 3. *Taxonomic-specific genetic markers observed*

| Primer pair | *Eco*RI | *Mse*I | *Ensete* | *Musa* | *M. suratii* | *M. jackeyi* | *M. itinerans* | *M. sikkimensis* | Total number of unique markers per primer pair |
|---|---|---|---|---|---|---|---|---|---|
| 1 | AAC* | CAA** | – | 1 | – | – | 1 | – | 2 |
| 2 | AAG | CAC | – | – | – | – | – | 1 | 1 |
| 3 | ACA | CAG | – | – | – | – | – | – | 0 |
| 4 | ACC | CAT | – | – | – | – | – | – | 0 |
| 5 | ACG | CTA | | 1 | – | – | – | – | 1 |
| 6 | ACT | CTC | 1 | – | 1 | – | – | – | 2 |
| 7 | AGC | CTG | 1 | – | 1 | 2 | 1 | – | 5 |
| 8 | AGG | CTT | – | – | – | – | – | – | 0 |
| Total | | | 2 | 2 | 2 | 2 | 2 | 1 | 11 |

*Eco*RI*, *Eco*RI-adapter based primer; the selective nucleotides added at the 3′ end are indicated.
*Mse*I**, *Mse*I-adapter based primer; the selective nucleotides added at the 3′ end are indicated.

TABLE 4. *GDEs of eight primer combinations*

| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0·224 | 0·509 | 0·530 | 0·649 | 0·435 | 0·426 | 0·713 | 0·310 | 0·346 | 0·740 | 0·318 | 0·474 | 0·515 | 0·448 | 0·561 | 0·565 | 0·428 | 0·556 | 0·524 | 0·581 | 0·581 | 0·549 |
| 2 | | 0·487 | 0·512 | 0·644 | 0·351 | 0·424 | 0·704 | 0·242 | 0·278 | 0·731 | 0·251 | 0·452 | 0·478 | 0·396 | 0·553 | 0·512 | 0·338 | 0·506 | 0·530 | 0·540 | 0·588 | 0·525 |
| 3 | | | 0·546 | 0·565 | 0·494 | 0·503 | 0·751 | 0·469 | 0·490 | 0·749 | 0·534 | 0·490 | 0·563 | 0·482 | 0·540 | 0·521 | 0·455 | 0·544 | 0·545 | 0·599 | 0·588 | 0·547 |
| 4 | | | | 0·353 | 0·546 | 0·640 | 0·764 | 0·479 | 0·507 | 0·772 | 0·543 | 0·474 | 0·670 | 0·556 | 0·378 | 0·361 | 0·544 | 0·266 | 0·249 | 0·358 | 0·357 | 0·341 |
| 5 | | | | | 0·656 | 0·716 | 0·745 | 0·565 | 0·622 | 0·824 | 0·627 | 0·540 | 0·722 | 0·572 | 0·466 | 0·503 | 0·614 | 0·434 | 0·446 | 0·429 | 0·453 | 0·342 |
| 6 | | | | | | 0·413 | 0·688 | 0·397 | 0·377 | 0·725 | 0·401 | 0·495 | 0·468 | 0·445 | 0·532 | 0·468 | 0·422 | 0·549 | 0·594 | 0·581 | 0·594 | 0·558 |
| 7 | | | | | | | 0·718 | 0·379 | 0·435 | 0·772 | 0·439 | 0·589 | 0·419 | 0·474 | 0·599 | 0·547 | 0·407 | 0·656 | 0·604 | 0·637 | 0·646 | 0·617 |
| 8 | | | | | | | | 0·716 | 0·767 | 0·533 | 0·733 | 0·716 | 0·708 | 0·725 | 0·749 | 0·762 | 0·687 | 0·804 | 0·748 | 0·736 | 0·684 | 0·721 |
| 9 | | | | | | | | | 0·315 | 0·729 | 0·260 | 0·439 | 0·411 | 0·240 | 0·485 | 0·459 | 0·268 | 0·514 | 0·484 | 0·488 | 0·489 | 0·463 |
| 10 | | | | | | | | | | 0·742 | 0·186 | 0·474 | 0·457 | 0·452 | 0·525 | 0·561 | 0·423 | 0·522 | 0·542 | 0·545 | 0·560 | 0·512 |
| 11 | | | | | | | | | | | 0·726 | 0·725 | 0·792 | 0·741 | 0·795 | 0·798 | 0·724 | 0·792 | 0·787 | 0·788 | 0·728 | 0·768 |
| 12 | | | | | | | | | | | | 0·493 | 0·439 | 0·411 | 0·588 | 0·570 | 0·398 | 0·584 | 0·550 | 0·554 | 0·561 | 0·544 |
| 13 | | | | | | | | | | | | | 0·607 | 0·518 | 0·364 | 0·468 | 0·463 | 0·458 | 0·434 | 0·440 | 0·477 | 0·497 |
| 14 | | | | | | | | | | | | | | 0·472 | 0·695 | 0·629 | 0·466 | 0·664 | 0·689 | 0·677 | 0·641 | 0·661 |
| 15 | | | | | | | | | | | | | | | 0·564 | 0·514 | 0·328 | 0·601 | 0·590 | 0·585 | 0·547 | 0·585 |
| 16 | | | | | | | | | | | | | | | | 0·375 | 0·516 | 0·308 | 0·344 | 0·419 | 0·361 | 0·411 |
| 17 | | | | | | | | | | | | | | | | | 0·417 | 0·300 | 0·282 | 0·448 | 0·449 | 0·431 |
| 18 | | | | | | | | | | | | | | | | | | 0·539 | 0·494 | 0·505 | 0·558 | 0·545 |
| 19 | | | | | | | | | | | | | | | | | | | 0·343 | 0·425 | 0·480 | 0·377 |
| 20 | | | | | | | | | | | | | | | | | | | | 0·417 | 0·391 | 0·325 |
| 21 | | | | | | | | | | | | | | | | | | | | | 0·405 | 0·353 |
| 22 | | | | | | | | | | | | | | | | | | | | | | 0·411 |

Taxa 1–23 correspond to the list of species in Table 1

## DISCUSSION

AFLP has provided important information regarding the genetic relationships among taxa of sections of *Musa*. In addition, it has generated unique molecular markers for the identification of *Musa* species. The level of polymorphism in *Musa* and the number of loci generated per primer pair using AFLP compare favourably with other techniques. A study employing ISSRs in *Musa* (Godwin *et al*., 1997) generated 940 bands from ten primer pairs, but only 13·1 % were polymorphic, while RFLP analysis of *Musa* (Gawel *et al*., 1992) using 66 primers generated only 96 alleles, an average of two alleles per probe.

The distinct separation of the clusters comprising species with chromosome numbers $n = x = 11$ in sect. *Musa* and *Rhodochlamys*, and species with chromosome numbers $n = x = 10$ in sect. *Callimusa* and *Australimusa*, is in agreement with previous taxonomic alignment based on morphological data. Cheesman (1947) noted that chromosomal differences between taxa of sect. *Callimusa–Australimusa* and sect. *Musa–Rhodochlamys* were correlated with many small differences in their habits and physiology, and regarded chromosome number as the best and safest criterion of relationships within *Musa*. This study is in agreement with Cheesman's data and also the cytogenetic evidence of Simmonds (1962) and Shepherd (1990) and the more recent study on species in sections *Musa* and *Rhodochlamys* using RFLP by Jarret and Gawel (1995).

### *Relationships between sect.* Musa *and* Rhodochlamys

Based on phenetic analyses, no clear distinction was apparent between species of sect. *Rhodochlamys* and those

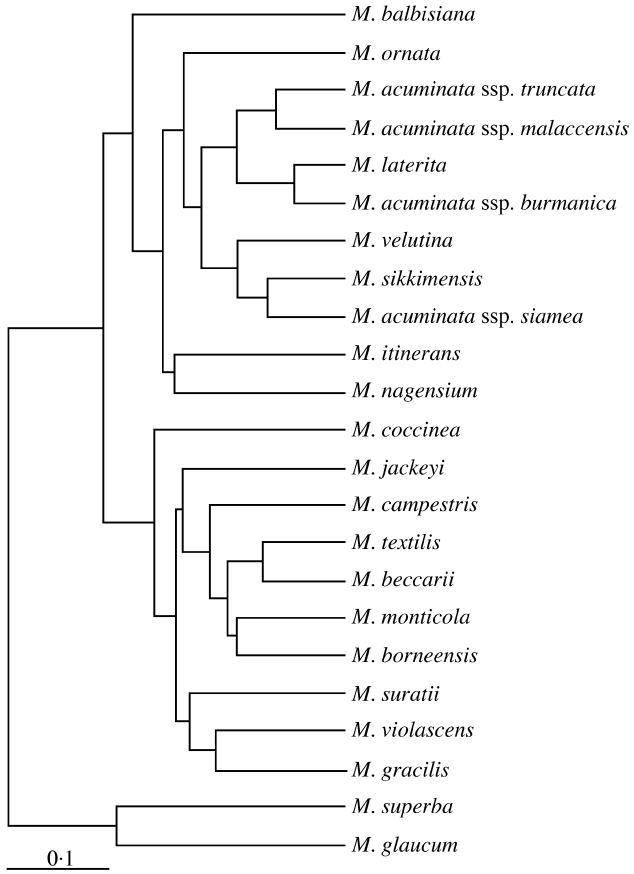


FIG. 2. Dendrogram showing genetic similarities between species of *Musa* and *Ensete* using UPGMA cluster analysis. Scale bar depicts GDEs.

of sect. *Musa*. *M. velutina* (sect. *Rhodochlamys*) was embedded within species of sect. *Musa*, and *M. laterita* (sect. *Rhodochlamys*) nestled within subspecies of *M. acuminata*. *Musa ornata* (sect. *Rhodochlamys*) also fell within the generally larger cluster of sect. *Musa*. These results suggested that sect. *Rhodochlamys* and sect. *Musa* are not sufficiently distinct genetically to warrant separation into two sections. This is in agreement with the conclusions of Simmonds (1962), Shepherd (1990) and Jarret and Gawel (1995).

*Musa balbisiana* was shown to be most distant in the present analysis. It is generally considered a distinct species (Cheesman, 1948; Simmonds, 1962) and other molecular studies have demonstrated its position as a species isolated within sect. *Musa* (Simmonds and Weatherup, 1990; Gawel and Jarret, 1991; Gawel *et al*., 1992; Jarret *et al*., 1992).

Genetic diversity estimates clearly showed that the three species of sect. *Rhodochlamys*, *M. ornata*, *M. laterita* and *M. velutina*, were genetically most closely related to *M. acuminata* in sect. *Musa*. Among the species in sect. *Rhodochlamys*, *M. laterita* clustered closely with *M. acuminata*. This is in agreement with the observation of Simmonds (1962) that *M. laterita* was closely related to *M. acuminata*, forming the nearest relationship between sections *Rhodochlamys* and *Musa*.

Hybridization is known to be common between species from sect. *Musa* and sect. *Rhodochlamys*, producing relatively vigorous offspring. According to Simmonds (1962), *M. acuminata* (sect. *Musa*) crosses effectively with *M. laterita*, *M. ornata* and *M. velutina* (all from sect. *Rhodochlamys*), while *M. balbisiana* (sect. *Musa*) hybridizes successfully with almost all species, including *M. laterita* and *M. velutina*. The weak reproductive barrier between the two sections supports the notion that they are not distinct.

*Musa acuminata* ssp. *siamea* did not cluster with the other subspecies of *M. acuminata* but clustered instead with *M. sikkimensis*. Lanaud *et al*. (1992) noted that ssp. *siamea* represented a highly diversified group. AFLP analysis suggests that it could be regarded as a separate species distinct from *M. acuminata*.

Cheesman (1947) noted that sect. *Musa* and sect. *Rhodochlamys*, although regarded as a close assemblage, were initially separated for convenience, sect. *Musa* including the edible bananas with dull bracts while sect. *Rhodochlamys* included the ornamental bananas with brightly coloured bracts. This view is no longer tenable in the face of genetic evidence and these two sections should be merged into a single section, sect. *Musa*.

### *Relationships between sect.* Callimusa *and sect.* Australimusa

These two sections were separated on the basis of conspicuous differences between their seeds (Cheesman, 1947). However, AFLP revealed no genetic justification for this separation, showing species of sect. *Australimusa*, *M. jackeyi*, *M. textilis*, *M. beccarii* and *M. monticola* (Wong *et al*., 2001*a*) clustering among species of sect. *Callimusa.*

Results obtained revealed that *M. textilis* (sect. *Australimusa*) clustered most closely with *M. beccarii* (sect. *Australimusa*; Wong *et al*., 2001*a*), with a GDE value of 0·249. However, *M. textilis* and *M. borneensis* of sect. *Callimusa* were also closely related, with a GDE value of 0·266, compared with genetic similarity between *M. textilis* and *M. jackeyi* in sect. *Australimusa* with a GDE of 0·357. Similarly, *M. jackeyi* was closely related to *M. campestris* of sect. *Callimusa* with a GDE value of 0·361, thus showing that species from sect. *Australimusa* were closely related to species from sect. *Callimusa,* and that the two sections were not distinct. Indeed, *M. suratii*, a new species described by Argent (2000), is not only intermediate between these two sections (Wong *et al*., 2001*a*) but has unique seed morphology that does not conform with that of either sect. *Callimusa* or sect. *Australimusa*.

Likewise, *M. coccinea* of sect. *Callimusa*, the species most distant from the other *Callimusa* species, was found to be genetically closely related to *M. textilis* and *M. jackeyi* of sect. *Australimusa* with GDEs of 0·474 and 0·477, respectively. This contrasted with the more distant relationship between *M. coccinea* and *M. violascens* (sect. *Callimusa*), with a GDE value of 0·540. This showed that species from two different sections were genetically more similar to one another than were two species from the same section (*M. coccinea* and *M. violascens*).

The distinction between sections *Callimusa* and *Australimusa* is based on a single character, that of seed structure. As mentioned above, *M. suratii* has unique seeds that do not conform to those of either sect. *Callimusa* or sect. *Australimusa*, thus breaking down the distinction between the two sections. In addition, hybridization is known to occur in the wild between species of both sections; for example, in Sabah, Borneo, hybridization occurs between *M. borneensis* in sect. *Callimusa* and *M. textilis* in sect. *Australimusa* (Kiew, 1998), showing that the sections are indeed not genetically distinct. The results of this AFLP analysis and those of Wong *et al*. (2001*a*) show that sections *Callimusa* and *Australimusa* are not genetically distinct and should be merged into a single section.

## CONCLUSIONS

Results of AFLP analysis showed that the 11-chromosome and 10-chromosome grouping are robust and justified and that the separation of *Musa* species into different groups based on their chromosome numbers provides a reliable means for classifying *Musa* species into sections. In contrast, the separations of sect. *Rhodochlamys* from sect. *Musa*, and sect. *Australimusa* from sect. *Callimusa* were not supported by the AFLP analysis. Indeed, there is more genetic variation within the two groupings, sect. *Musa*–*Rhodochlamys* and *Callimusa*–*Australimusa*, than there is between sect. *Musa* and sect. *Rhodochlamys* and between sect. *Callimusa* and sect. *Australimusa*, drawing attention to the fact that striking differences in morphological characters in *Musa* species are not always indicative of the same degree of genetic difference.

Results from the AFLP analysis provide evidence that sect. *Rhodochlamys* should be combined with sect. *Musa*, a view already mooted by Simmonds (1962), Shepherd (1990), and Jarret and Gawel (1995), and that sect. *Callimusa* and sect. *Australimusa* should also be combined into a single section.

In view of the importance of chromosome numbers in grouping species within the genus *Musa*, it will be of great interest to carry out a molecular study on the sole member of sect. *Ingentimusa* that has a chromosome number of $n = x = 14$.

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