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RFLP-based phylogeny of *Musa* species in Papua New Guinea

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Summary. Random genomic probes were used to detect restriction fragment length polymorphisms (RFLPs) in 26 accessions of *Musa* representing eight species from Papua New Guinea (PNG), *M. textilis, M. jackeyi* and one accession of *Ensete*. Ninety-eight phylogenetically informative characters were scored and analyzed cladistically and phenetically. Results generally agreed with previous morphology-based phylogenetic analyses. However, the closest wild relative of the edible *M. fehi* (fe'i banana) appears to be *M. lolodensis*. *Musa angustigemma* is sister species with *M. boman* and *M. jackeyi* and is distinct from *M. peekelii*, with which it is often united. *Musa boman* is unambiguously placed in section *Australimusa*. The diploid parthenocarpic landraces of section *Musa* unique to PNG are closely related to, but apparently distinct from, *M. acuminata* ssp. *banksii*. The evolution of the fe'i bananas and the *M. acuminata*-derived diploid landraces of PNG are discussed.

Key words: Fe'i bananas – Taxonomy – Evolution – *M. acuminata* – *M. fehi* – *M. banksii* – RFLP – Papua New Guinea

Introduction

The bananas (genus *Musa*) are one of the most important tropical fruit crops in the world. Modern cultivated races of banana (usually triploid, parthenocarpic and clonally propagated plants) are grown throughout the tropics; the world production of bananas, including plantains, exceeds 5.7×10^4 metric tons annually (FAO 1983). Successful breeding of improved cultivars depends upon the availability of *Musa* germ plasm (Rowe 1981). However, the genetic base of *Musa* continues to be diminished due to the ongoing eradication of primitive, vegetatively propagated and sexually reproducing landraces from most of the areas where they were once the predominant vegetation (MacDaniels 1947).

New Guinea (PNG), and the surrounding islands, including New Ireland and New Britain, are the greatest remaining reservoir of edible diploid banana germ plasm in the world today. These islands also support a variety of native species of *Musa* that could be valuable for banana or abaca breeding. The native flora of the islands includes eight species belonging to three sections. One of the three sections, sect. *Ingentimusa* (containing only *Musa ingens*), has apparently never produced edible types. The remaining two sections, sects. *Musa* and *Australimusa*, both include parthenocarpic edible landraces in addition to related wild species.

The most important cultivated bananas belong to *Musa* sect. *Musa* (sometimes referred to as sect. *Eumusa*). There are three species of this section native to New Guinea: *M. schizocarpa, M. balbisiana* and *M. acuminata* ssp. *banksii* (sometimes treated as a distinct species, *M. banksii*; Argent 1976). Modern triploid cultivars, derived from *M. acuminata* or from *M. acuminata* × *M. balbisiana* hybrids, are widely grown in New Guinea, as they are throughout the tropics. However, fruit of the many diploid *M. acuminata* parthenocarpic landraces remains a food of local importance (Sharrock 1990), grown in backyard gardens. The origin of these parthenocarpic diploids is obscure. They may have been bred locally from the indigenous *M. acuminata* ssp. *banksii*, or introduced from western Indonesia or the Malay Peninsula, where diploid races derived from the indigenous *M. acuminata* ssp. *malaccensis* were once common but have

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since been displaced by higher-yielding triploid cultivars introduced from India (Simmonds 1962).

The third section found in PNG is *Musa* sect. *Australimusa*. Five native species, *M. boman, M. bukensis, M. lolodensis, M. maclayi* and *M. peekelii*, are included in this section; a sixth taxon is sometimes treated as a distinct species, *M. angustigemma* (Simmonds 1948), and sometimes as *M. peekelii* ssp. *angustigemma* (Argent 1976). Two additional species of sect. *Australimusa, M. textilis* (Philippines and Borneo) and *M. jackeyi* (Queensland), are not found in PNG. All species in section *Australimusa* are highly interfertile, although they may differ from one another by single inversions or translocations (Richardson 1957; Shepherd, 1990).

Section *Australimusa* also contains a poorly understood array of parthenocarpic edible forms, collectively known as *M. fehi* Bert. These edible types, often referred to as "fe'i bananas", were originally distributed from the Moluccas to Hawaii and Tahiti. Historical records often describe the fe'i bananas as being gathered from feral plants growing in remote and/or inhospitable sites (especially talus slopes at the bases of cliffs), rather than from around settlements. As fe'i bananas are highly seed and pollen sterile, such feral plants are probably the asexually propagated remnants of old plantings, the plants having originally been transported from island to island as corms (Dodds 1946; MacDaniels 1947; Simmonds 1962). As noted by Simmonds (1962), early reports of highly seed-fertile bananas from the fe'i area probably refer to other species of *Musa*. Since some individual clones are believed to have been distributed from the Moluccas to Tahiti (MacDaniels 1947), the transport of corms must have once been extensive. In addition to the use of the fruit as food, the leaves of *M. fehi* were widely used as plates for eating, and also for thatching, bedding and packing and as a source of fibers for lashings and weaving; the pseudostems were a source of a dark red dye, and were sometimes used as floats for small rafts. The fruit has also been used medicinally (MacDaniels 1947).

Although the fe'i bananas were an important food source to the inhabitants of the Society and Marquesas Islands and of Fiji, they must have originated farther west, since wild species of *Musa* sect. *Australimusa* are not found east of the Solomon Islands. However, very little is known about the origins of this crop. Simmonds (1956a) suggests that *M. maclayi* is the most likely wild ancestor of the fe'i bananas, but suggests that hybridization with other species may also have been important in their development. Tezenas du Montcel (1990) states that the fe'i bananas originated from *M. maclayi*. Alternatively, Cheesman (1947) notes similarities between the fe'i bananas and *M. lolodensis* and states these two species "probably have fairly close affinities".

The genetic diversity of *Musa* in PNG is threatened by the replacement in cultivation of the endemic diploid populations with introduced triploid clones, and the loss of the fe'i bananas through rural development programs. In response to this threat, the junior author ($S_\alpha S_\alpha$) directed extensive collecting activities within Papua New Guinea from 1986 to 1989. All but one of the native species, *M. bukensis*, were collected for maintenance and study. Earlier taxonomic and biodiversity studies of *Musa* from this area used a series of morphological descriptors (MacDaniels 1947; Simmonds 1948, 1956b; Argent 1976). We have elected to utilize restriction fragment length polymorphisms (RFLPs) in our examination of this material. RFLPs have been used effectively to establish and refine systematic relationships and to examine genetic diversity in several crop genera including *Lycopersicon* (Miller and Tanksley 1990), *Brassica* (Song et al. 1988), *Solanum* (Debener et al. 1990), *Lens* (Havey and Muehlbauer 1989) and *Glycine* (Menancio et al. 1990).

The study reported here was undertaken to investigate systematic relationships within and between representatives of sects. *Musa* and *Australimusa* from PNG using RFLPs as genetic markers.

Materials and methods

Plant material

Unless noted otherwise, the plant material utilized in this study (Table 1) was collected in PNG from 1986 to 1989 (Sharrock 1990). Accessions of *M. balbisiana* (I-63), *Ensete* and *M. acuminata* ssp. *malaccensis* (II-357) were obtained from the Fundacion Hondurena de Investigacion Agricola (FHIA) and were included for comparison. *Musa textilis* was originally collected in the Philippines and was obtained through the International Network for the Improvement of Bananas and Plantains (INIBAP) germ plasm transit center at the Katholieke Universiteit Leuven (KUL). *Musa jackeyi* was collected in Queensland, Australia and obtained from the Maroochy Horticultural Research Station, Nambour. All plant materials, with the exception of the *Ensete* sp., *M. acuminata* ssp. *malaccensis* and *M. balbisiana*, were received for analysis as tissue cultures. Upon receipt, in vitro plantlets were recultured onto Murashige and Skoog (1962) medium containing 3% (w/v) sucrose and 0.7% agar and allowed to grow to 5–7 cm in height. Prior to being transferred to soil, plants were acclimated in vitro by removal of the tube enclosures and left for a period of 7 days at room temperature. Young leaf tissue was harvested when plants were about 1 m in height. Leaf tissue of *Ensete* was harvested in the field at FHIA, placed on wet ice and transported to Griffin. Leaf material of *M. balbisiana* (I-63) was harvested in Griffin from plants grown from seed obtained from FHIA. Corms of *Musa acuminata* ssp. *malaccensis* were obtained from FHIA and transported to Griffin.

DNA isolation

All leaf tissue was freeze dried, ground to a fine powder in liquid N and stored at −135 °C. Lyophilized, powdered leaf tissue (500 mg) was extracted with 15 ml of CTAB extraction buffer as described previously (Gawel et al. 1992). DNA was precipitated with isopropanol, removed with a glass hook, blotted on a paper towel and resuspended in TRIS-EDTA (TE) pH 8.0 by heating at 65 °C for 1 h. DNA isolated from *M. jackeyi* could not be collected with a glass hook. In this instance, isopropanol-precipitated DNA was collected by centrifugation at 5,000 *g* for 5 min

Table 1. Plant materials analyzed for RFLPs

ID no.	Name or species	Origin or collection site
PNG 228	*M. acuminata* (Inori)	Koipa, Oro Province
PNG 257	*M. acuminata* (Mala)	Mendi, Southern Highlands
PNG 249	*M. acuminata* (Buka)	Siriseta, Oro Province
PNG 181	*M. a.* ssp. *banksii*	Ono, Madang
PNG 276	*M. a.* ssp. *banksii*	OK Mart, Western Province
PNG 292	*M. a.* ssp. *banksii*	Manus Island
II-357	*M. a.* ssp. *malaccensis*	[a]
PNG 147	*M. balbisiana*	Kokopo, East New Britain
I-63	*M. balbisiana*	[a]
PNG 158	*M. peekelii* ssp. *angustigemma*	Yelso, Madang
PNG 315	*M. peekelii* ssp. *peekelii*	Rasarik, New Ireland
PNG 316	*M. peekelii* ssp. *peekelii*	Rasarik, New Ireland
PNG 172	*M. schizocarpa*	Malpine, Madang
PNG 223	*M. schizocarpa*	Pusa Hambo, Oro Province
PNG 282	*M. schizocarpa*	Epo Estate, Gulf Province
PNG 329	*M. schizocarpa*	Pwaepwae, Normandy Island
PNG 339	*M. maclayi* ssp. *maclayi*	Sebutuia Bay, Fergusson Island
PNG 340	*M. maclayi* ssp. *aluluia*	Sebutuia Bay, Fergusson Island
PNG 274	*M. fehi* (Skai)	Nigerum, Western Province
PNG 294	*M. fehi* (Sar)	Manus Island
PNG 331	*M. fehi* (Kwaputa)	Sebuluyu, Normanby Island
PNG 341	*M. boman*	Sosi, West Sepik
PNG 364	*M. lolodensis*	Yawreng, East Sepik
	M. jackeyi	Queensland, Australia [a]
BS 079	*M. textilis*	[a]
	Ensete sp.	[a]

[a] Fundacion Hondurena de Investigacion Agricola, La Lima, Honduras
[b] Maroochy Horticultural Research Station, Nambour, Queensland, Australia
[c] Katholieke Universiteit Leuven, Belgium

at 20 °C, and the pellet resuspended in TE. All extracts were centrifuged at 10,000 *g* for 5 min to remove undissolved DNA and the supernatant transferred to clean tubes. DNA extracts were stored at 4 °C.

Restriction enzyme digestion, electrophoresis and blotting

Five micrograms of DNA was digested with 15 U of either *Eco*RI, *Hin*dIII, *Bam*HI or *Msp*I for 8 h at 37 °C according to the manufacturers' (IBI) recommendations. Fragments were separated on 0.8% agarose gels at 2 V/cm for 20 h in TEA buffer (Sambrook et al. 1989). Gels were stained with ethidium bromide, photographed, denatured in 1.5 *M* NaCl, 0.5 *M* NaOH for 30 min, neutralized in 3 *M* Na acetate pH 5.5 for 30 min and blotted according to Southern (1975) onto nylon membrane (BioTrans-ICN).

Library construction, probe selection, labeling and hybridization

Probes were selected at random from genomic libraries of *M. acuminata* ssp. *malaccensis* and *M. balbisiana* as described by Gawel et al. (1992). Recombinant *E. coli* colonies were selected on X-gal and the plasmids isolated following an alkaline extraction procedure (Sambrook et al. 1987). Whole plasmids were random-primer labeled (BRL) with ^{32}P and used to probe genomic digests.

Data analysis

Cladistic analyses were performed using PHYLIP (Felsenstein 1985). Bootstrap samples were generated with the BOOT subprogram (global option) of PHYLIP. Cluster analyses were calculated with Biosys-1 (Swofford and Selander 1989) using the genetic distance algorithm of Nei (1972) and the unweighted pair-group method with arithmetic averaging (UPGMA) algorithm. Principal component analyses were performed using the PRINCOMP procedure of SAS (1988).

Results and discussion

Ninety-eight phylogenetically informative characters were detected and scored (Table 2) using 40 (25 *M. acuminata* and 15 *M. balbisiana*) genomic probes. Probes isolated from the *M. acuminata* ssp. *malaccensis* and *M. balbisiana* libraries were equally effective in detecting polymorphisms. The resulting cladogram (Fig. 1) was 136 steps, with an overall homoplasy of 28%.

Our results confirmed the close relationship of *M. acuminata* and *M. schizocarpa*. However, as in other recent studies of relationships in *Musa* (Simmonds and Weatherup 1990; Gawel et al. 1992), our data suggest only a distant relationship between *M. balbisiana* and the other species of sect. *Musa*. Our analysis did not place *M. balbisiana* on the same clade as the other species of this section. However, this separation was not well supported in the bootstrap analysis (only 42 of 100 bootstrap samples) and must be considered tentative. The diploid Papuan landraces from this section all cluster with *M. acuminata*; neither *M. schizocarpa* nor *M. balbisiana* seems to have been involved in the development of these races.

All accessions of *M. acuminata* shared a large number of polymorphisms. For this reason, our analysis did not separate the two subspecies of *M. acuminata*, and the parthenocarpic diploids did not cluster clearly with either subspecies. The similarity of ssp. *banksii* and ssp. *malaccensis* supports the subspecific treatment of these two taxa by Cheesman (1950), Simmonds (1962) and Simmonds and Weatherup (1990), rather than the treatment of Argent (1976), who considers *banksii* to be a distinct species. However, it is not possible to tell from this data whether the parthenocarpic cultivated races arose locally from ssp. *banksii*, from ssp. *malaccensis* in western Indonesia or the Malay Peninsula, or if they are derived from imported races that subsequently crossed with local wild bananas.

Within sect. *Australimusa*, the relationships between the wild species generally support the findings of Gawel

et al. (1992), especially in the clear inclusion of *M. boman* in this section. *M. peekelii* ssp. *angustigemma* clusters with *M. boman* rather than with *M. peekelii* ssp. *peekelii*. Its exclusion from the latter branch is supported by a high bootstrap statistic (96% of bootstrap samples).

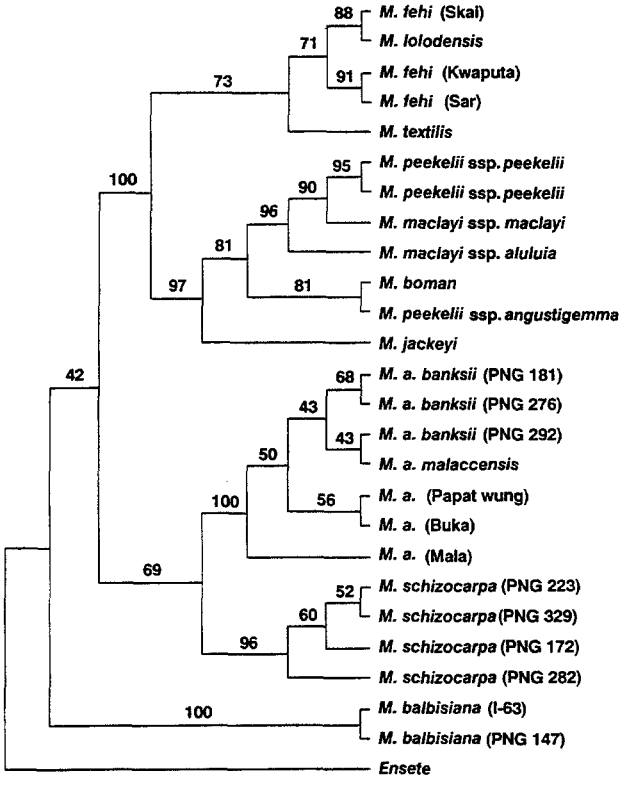

Fig. 1. Cladogram of RFLP-based character states. *Numbers* on branches represent the number of times a clade was present in 100 bootstrap analyses. *Ensete* is the outgroup

These data suggest that this taxon should be treated as a distinct species, *M. angustigemma*, as in the original treatment by Simmonds (1948).

To further examine relationships within section *Australimusa* a principal component analysis (PCA) was conducted on the RFLP-derived characters. The first two principal components accounted for 73% of the variation among the species included (Fig. 2). The PCA confirms the clear separation of *M. peekelii* ssp. *angustigemma* from *M. peekelii* ssp. *peekelii* and illustrates the close relationship of *M. peekelii* ssp. *angustigemma* to *M. boman*. The PCA also emphasizes other divisions within sect. *Australimusa*, including the overlapping coordinate distributions of *M. fehi* cvs 'Kwaputa', 'Skai' and 'Sar' and *M. lolodensis*, all of which are well removed from *M. textilis*, *M. jackeyi* and *M. maclayi*. The affinities of *M. jackeyi*, now an endangered species, had not previously been investigated.

The fe'i bananas are excluded from the branch leading to five of the seven sect. *Australimusa* species, including *M. maclayi*, which was considered by Simmonds (1956) and Tezenas du Montcel (1990) to be the likely wild ancestor of *M. fehi*. The bootstrap statistic supporting this branch is sufficiently high (96% of bootstrap samples) that the five species on it can be confidently excluded from the ancestry of the fe'i cultivars. Our analysis strongly suggests that *M. lolodensis* is the closest wild relative of the fe'i bananas. The bootstrap statistic for the branch uniting the fe'i accessions and *M. lolodensis* falls short of statistical significance (71%), providing insufficient evidence to rule out the hypotheses that the fe'i bananas were derived from *M. textilis* or from *M. lolodensis* × *M. textilis* hybrids. However, in the principal component analysis (Fig. 2) *M. fehi* clusters with *M. lolodensis* far from *M. textilis*; and geographically *M. textilis* is found only in the Philippines and Borneo, outside the

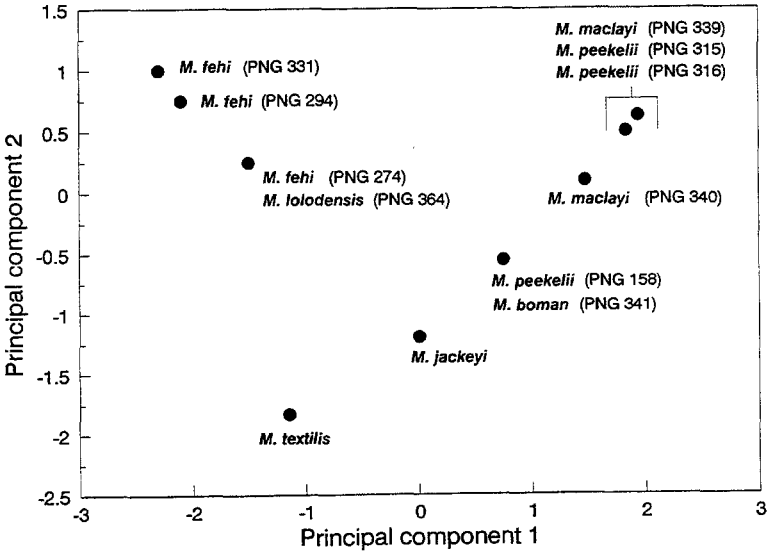

Fig. 2. Principal component analysis of RFLP-based character states of *Musa* sect. *Australimusa* species

range of *M. fehi*. It thus seems unlikely that *M. textilis* was involved in the development of the fe'i cultivars.

It has been recognized for decades that the fe'i cultivars represent a parthenocarpic group derived independently from all other edible bananas. Although they have been a widespread and locally important food plant and grow well in marginal habitats (such as talus slopes) in the wet tropics, little is known about their origin and biological characteristics. Our data indicates that *M. fehi* is derived from *M. lolodensis* (native to New Guinea and the Moluccas). Although the high level of racial variation within *M. fehi* has been taken as evidence that the development of the fe'i bananas may have involved interspecific hybridization (Simmonds 1962), we found no evidence that any of the three fe'i races we examined were intermediate between *M. lolodensis* and any other species. Further work on the relationships of different fe'i cultivars and the level of genetic variability within *M. lolodensis* should yield a much clearer picture of the evolution, ethnobotany and future potential of this plant. Unfortunately, indigenous populations of these plants are rapidly being lost due to habitat disturbance and displacement by high-yielding triploid bananas, and collections and reliable field observations of fe'i bananas and their relatives are few. The latter is especially true of western New Guinea and the Moluccas, an area that may contain a significant number of unique fe'i bananas and other species of sect. *Australimusa*. For instance, Rhumphius (1750) described a plant from the Moluccas (later named *M. troglodytarum* by Linnaeas in 1762) which, as far as can be determined from the brief description provided, seems to be intermediate in form between *M. lolodensis* and *M. fehi*. The fruit is edible, although it is diuretic, but it is seedy, unlike the parthenocarpic *M. fehi*. The plant is described as being rare, but occasionally cultivated in gardens and eaten by indigenous peoples. Unfortunately, there are no modern reports of such bananas from eastern Indonesia, but this is a very promising area for future field work.

In the past, study of the genus *Musa* has been severely limited by the unsuitability of plant material for traditional herbarium and greenhouse techniques. Since herbarium specimens are usually fragmentary and poorly preserved and greenhouse material seldom flowers reliably in temperate areas, previous studies (Baker 1893; Cheesman 1947; Simmonds 1962) have relied upon verbal descriptions, drawings and photographs (Brewbaker and Umali 1956). In addition, until recently there existed no centralized genebank through which propagules could be obtained, so that the identity of material used by earlier workers cannot be independently confirmed.

The use of RFLPs shows great promise for characterizing *Musa* accessions and examining the taxonomic relationships of *Musa* species and cultivated forms. Suitable material can be obtained from juvenile, nonflowering greenhouse material, so that studies may be carried out with limited resources in cold or dry climates. The establishment of an in vitro genebank (Jarret 1990) should facilitate the acquisition of plant material for future analysis. This will allow the preservation and study of species and races that are currently available only as poorly preserved herbarium material. An in vitro genebank will also aid the study of wild species and cultivated races that are currently disused and endangered, but may be potentially useful for breeding, or as crops in their own right.

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