Euphytica **67**: 163–175, 1993.
© 1993 *Kluwer Academic Publishers. Printed in the Netherlands.*

Genetic relationships among cultivated bananas and plantains from Asia and the Pacific[1]

V. Lebot[1], K.M. Aradhya[2], R. Manshardt[2] & B. Meilleur[3]
[1] CIRAD, BP 745, Antananarivo, Madagascar; [2] Department of Horticulture, University of Hawaii, Honolulu, Hawaii 96822, USA; [3] Amy Greenwell Ethnobotanical Garden, Bishop Museum, Captain Cook, Hawaii 96704, USA

Received 12 November 1992; accepted 16 March 1993

Key words: banana, isozymes, multivariate analysis, *Musa* spp., plantain, zymotype

Summary

Isozyme variation was studied to determine genetic relationships among 563 accessions of *Musa,* including diploid (AA and BB), triploid (AAA, AAB, and ABB), and a few tetraploid (ABBB) clones from Asia and the Pacific. Several open-pollinated seedling progenies of wild, diploid *M. acuminata* and *M. balbisiana* were also studied. Cryogenic preservation of leaf tissue in liquid nitrogen allowed sampling of a wide array of germplasm from Papua New Guinea and several Pacific Islands without transporting propagules which are subjected to quarantine regulations. Electrophoretic variation was recorded in three enzyme systems, MDH, PGI and PGM. In total, 52 distinct electromorphs were identified among 192 different isozyme phenotypes (zymotypes). Multivariate analyses of the data clearly differentiated the major genome groups and revealed patterns of association within groups. The isozyme data suggest that the genes contributed by the *M. acuminata* genome to the triploid Pacific plantain AAB subgroup are similar to those of the *acuminata/banksii* complex of Papua New Guinea. It is likely that the Pacific plantain subgroup, including the Hawaiian Maoli, Pōpō'ulu and Iholena cultivars, originated in Papua New Guinea/Melanesia, rather than in Asia or the Malay Archipelago.

Introduction

Genetic relationships among domesticated banana and plantain clones involve the variation in two different genomes (A = *M. acuminata,* B = *M. balbisiana*) and different ploidy levels. Triploids (AAA, AAB, and ABB) are by far the most important and numerous edible types, diploids (AA and AB) are less common and tetraploids (AAAA, AAAB, AABB, ABBB) are rare. The wild progenitor species are all native to tropical Asia and Australasia, and consequently the origins of the cultivated derivatives must lie somewhere in that region (Stover & Simmonds, 1989).

The conventional method of classifying domesticated banana and plantain clones is based on fifteen diagnostic morphological traits for which the two wild progenitor species, *M. acuminata* and *M. balbisiana,* differ (Simmonds & Shepherd, 1955). Chemosystematic studies of bananas and plantains, employing isozyme and flavonoid analyses, have provided impressive confirmation of the conven-

Journal Series no. 3783 of the Hawaii Institute of Tropical Agriculture and Human Resources.

tional classification scheme. Jarret & Litz (1986a, b) used 18 enzyme systems to study variation in 58 clones of different ploidy level, including eight clones of subspecies of *M. acuminata* and several *M. balbisiana* accessions. They identified species- and subspecies-specific alleles and concluded that isozyme markers can be used to identify cultivars, subspecies and species. Bhat et al. (1992) employed three enzyme systems to identify clones and did not observe any genome specific isozyme patterns, but was able to distinguish 29 clones out of 44 included in the study. Horry (1989) assayed eight isozyme loci in 113 wild and cultivated diploids from diverse geographic origins. He observed that *M. acuminata* and *M. balbisiana* displayed highly divergent allelic distributions. The greater diversity within *M. acuminata* was attributed to its proliferation and differentiation into a number of subspecies as a consequence of geographic isolation. He concluded that isozymes are particularly useful for evolutionary studies and classification of bananas. Banana classifications based on isozyme analyses and anthocyanin variation corroborated genomic classifications obtained using morphological characters.

The above studies indicated a center of domestication for *M. acuminata* in the Malay Archipelago. Interspecific hybrids must have originated when semisterile, parthenocarpic, and highly variable diploid *M. acuminata* was brought in contact with less variable *M. balbisiana* in the geographic fringe areas from India through south China and the Philippines to New Guinea (Stover & Simmonds, 1989).

A distinctive set of cooking bananas (AAB), which are referred to collectively as the Pacific plantain subgroup, has remained anomalous. This subgroup was first described in Hawaii (Pope, 1926), where the Maoli, Pōpō'ulu, and Iholena types are thought to have been introduced in prehistoric times by Polynesians. Apart from Hawaii, they are found throughout the islands of the south, central and eastern Pacific (Pope, 1926; Daniells, 1990; Lebot et al., 1994). The Pacific plantains are not to be confused with the *fe'i* bananas of the section Australimusa, although they share much the same geographic range.

Horry & Jay (1988) analyzed anthocyanin composition of bracts in 59 wild and cultivated forms of *Musa*. Their results suggested two independent centers of domestication for *M. acuminata,* one in Southeast Asia and the other in Papua New Guinea. Their analysis was based on a small sample of clones, but it suggested that the 'A' genome in AAB plantains is more closely allied to Papuan *M. acuminata* ssp. *banksii* than to Asian *M. acuminata.*

Despite these recent studies, knowledge of biochemical variation in bananas and plantains is based on only a small portion of the existing germplasm. The inaccessibility of germplasm is often cited as 'the principal obstacle hindering research' (Jarret & Litz, 1986b).

In the present paper, we have employed isozyme analysis in a more comprehensive survey of banana and plantain germplasm, focussing particularly on elucidating the genetic relationships between the Pacific plantains and other *Musa* groups. We present evidence that the Pacific plantain subgroup and other closely related plantains originated in New Guinea or Western Melanesia as a result of hybridization between members of the *M. acuminata/ banksii* complex and *M. balbisiana.*

Materials and methods

Plant materials

A total of 563 *Musa* accessions from Asia and the Pacific were included in the survey (Table 1). All but a few accessions have been previously described morphologically and classified into genomic groups by various investigators (Daniells, 1990; Sharrock 1990; Simmonds, 1954; Stover & Simmonds, 1989; Tezenas du Montcel, 1990, 1991). The following research organizations have contributed germplasm from their collections:

French Polynesia: Station de Recherches Agronomiques de Papara, Tahiti, Service de l'Economie Rurale. Hawaii: College of Tropical Agriculture and Human Resources, University of Hawaii; Kanewai Cultural Garden, Center for Hawaiian Studies, University of Hawaii; Amy B.H. Greenwell Ethnobotanical Garden, Bishop Museum; Waimea Arboretum and Botanical Garden; Dr. A. Brash (private collection). New Caledonia: Station de Re-

cherche Fruitière de La Foa-Pocquereux, Institut de Recherches sur les Fruits et Agrumes. Papua New Guinea: Laloki Research Station, Department of Primary Industries. Vanuatu: Tagabé Agricultural Station, Agriculture Department. Western Samoa: Alafua Campus, University of the South Pacific.

For accessions from French Polynesia, New Caledonia, Papua New Guinea, and Vanuatu, small portions of young leaves were collected in 1.5-ml microcentrifuge tubes in the field, preserved in liquid nitrogen and transported in a cryogenic shipping container to the University of Hawaii, Honolulu, Hawaii, for electrophoresis. Leaf samples preserved in liquid nitrogen revealed banding patterns identical to those obtained from fresh materials, even after four months of storage. This simple preservation technique permitted sampling of a wide range of germplasm. Banana seeds were also obtained from Malaysia (*M. acuminata*), Western Samoa (*M. acuminata* ssp. *banksii*), and Hawaii (*M. acuminata* ssp. *zebrina* and *M. balbisiana*) to study the progenies for isozymic variation.

Enzyme electrophoresis

Eighteen enzyme systems were assayed using a variety of buffer systems (Table 2). Among them, histidine-citrate pH 6.5 was found to be the best for resolving *Musa* isozymes. About 2.0 mg of fresh or frozen leaf tissue was homogenized in five drops of chilled extraction buffer (Lebot et al., 1991). In the case of frozen samples, the leaf tissue was dropped into extraction buffer before thawing. The homogenate was absorbed on 0.2- × 1.0-cm chromatographic paper (Whatman 3MM CHR) wicks and loaded into 12.5% starch gels (Sigma Chemical Co.) previously prepared in histidine-citrate gel buffer and cooled to 4° C. The gel buffer consisted of 0.016 M histidine (free base) and 0.002 M citric acid, and the tray buffer contained 0.065 M histidine and 0.007 M citric acid (Cardy et al., 1983).

Samples were electrophoresed in a refrigerator for 6 to 7 hours at a constant 200 V. At the end of electrophoresis, the gels were sliced horizontally into six slabs and stained for different enzyme systems following Shaw & Prasad (1970). Each accession was electrophoresed at least twice to confirm the zymogram. Accessions with the same cultivar name or identification, but originating from different sources, were electrophoresed in adjacent lanes to confirm cultivar identity.

Isozyme genotypes of cultivated diploid clones and open-pollinated seedlings of wild diploids were inferred from isozyme phenotypes observed among different individuals and from reports of earlier workers (Jarret & Litz, 1986a, 1986b; Horry, 1989). However, zymograms of triploids and tetraploids were complex, and hence could not be given a genetic interpretation. Consequently, analysis of isozyme data was based on phenotypes, rather than ge-

Table 1. Origin of *Musa* accessions studied for isozyme variation

Origin	AA	AAA	AAB	ABB	ABBB	BB	Total
Seedling progenies[1]	(240)	–	–	–	–	(120)	(360)
Asian clones[2]	6	32	13	4	–	4	59
Hawaii	1	0	20	–	–	–	21
French Polynesia	1	2	28	11	2	–	44
Western Samoa	0	0	4	1	1	–	6
New Caledonia	0	0	21	5	–	–	26
Vanuatu	11	5	23	14	–	–	53
Papua New Guinea	133	70	86	57	8	–	354
Total accessions	152	109	195	92	11	4	563
Zymotypes identified[3]	51	65	49	22	3	2	192

[1] Open pollinated seedling progenies. [2] Clones existing in germplasm collections known to be Asian in origin (from Daniells, 1990; Stover & Simmonds, 1987). [3] Identified based on composition of electromorphs in zymograms.

notypes. Zymograms of MDH, PGI, and PGM from different accessions were scored for the presence or absence of each electromorph (band).

Data analysis

A total of 52 distinct electromorphs were used as isozyme descriptors. If two accessions differed by at least one electropmorph they were considered as two different zymotypes. Multivariate relationships among zymotypes were appraised separately for each genome group, using the principal components analysis (PCA) on the respective variance-covariance matrices among the 52 electromorphs. The genetic relationships among different zymotypes were visualized by plotting them along the first two principal axes. The zymotype clusters were identified based on the results of UPGMA cluster analysis performed on the Jaccard's similarity coefficient matrices. The similarity matrices and the UPGMA dendrograms are available upon request. All computations were made using the computer program NTSYS-pc version 1.6 (Exeter Publishing Ltd., Setauket, N.Y. 1987).

Results

Among the 18 enzyme systems assayed, six enzyme systems, ACO, IDH, MDH, PGI, PGM and 6-PGD exhibited polymorphisms. However, only MDH, PGI and PGM could be scored accurately and consistently, and the zymograms observed are illustrated schematically in Figs 1, 2 and 3, respectively.

Isozyme variation

Malate dehydrogenase (MDH). This dimeric enzyme with two zones of activity was less variable than PGI or PGM. A third zone of activity, the most cathodal, was found to be highly polymorphic, but inconsistent banding patterns did not permit accurate scoring. Twelve different zymograms with 13 electromorphs were identified (Fig. 1). The most anodal zone, *Mdh-1,* was highly polymorphic, as re-

Table 2. Enzyme systems and buffers investigated

Enzyme system	Abbreviation	Buffer System[1]
Acid phosphatase	ACP	HC
Aconitase[2]	ACO	HC, TC
Alcohol dehydrogenase	ADH	HC, TC
Aldolase	ALD	HC
Diaphorase[2]	DIA	HC, TC
Esterase	EST	HC
Glutamate oxaloacetate transaminase	GOT	HC
Glutamate dehydrogenase	GDH	HC, TC, TEB
Glucose-6-phosphate dehydrogenase	G6PDH	HC
Isocitrate dehydrogenase[2]	IDH	HC, TC
Leucine aminopeptidase	LAP	HC, MC
Malate dehydrogenase[3]	MDH	HC, TC, TEB
Malic enzyme	ME	HC, TC, TEB
Peroxidase	PER	HC, MC
Phosphoglucomutase[3]	PGM	HC
Phosphoglucose isomerase[3]	PGI	HC, TC
Shikimate dehydrogenase	SKDH	HC, MC
6-Phosphogluconate dehydrogenase[2]	6-PGD	HC, TC

[1] Buffer systems: histidine-citrate (HC), pH 6.5; Tris-citrate (TC), pH 6.1; morpholine-citrate (MC), pH 8.1; and Tris-EDTA-borate (TEB), pH 8.6. [2] Enzyme systems producing unclear banding patterns but not used in analysis. [3] Well resolved on HC buffer and used in the analyses.

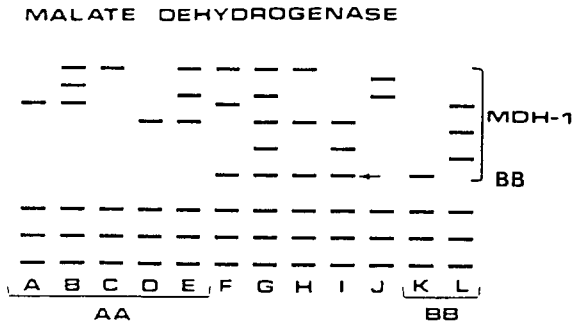

Fig. 1. Zymograms observed at MDH for each genomic group.

ported in previous studies (Jarret & Litz, 1986a, 1986b; Horry, 1989). Five alleles were identified in the diploid accessions, two being unique to *M. balbisiana* and two being specific to *M. acuminata.* The three cathodal bands were common to all accessions.

Diploid *M. acuminata* clones of Southeast Asian origin ('Lakatan', 'Berangan', 'Tuu Gia' and seedlings of wild *M. acuminata* from Malaysia) and an AB hybrid ('Ney Poovan'), also of Asian origin, exhibited zymograms A, B or C. Wild and cultivated diploid bananas from Oceania (*M. acuminata* from Papua New Guinea, *M. acuminata* ssp. *banksii* from New Guinea and Western Samoa, *M. schizocarpa* and their hybrids), exhibited zymograms C, D, or E.

Triploid AAA cultivars also exhibited zymograms C, D, or E, while AAB hybrids presented zymograms E, F, or G. All clones of the Pacific plantain AAB subgroup (Iholena, Pōpō'ulu and Maoli) exhibited zymogram G, while Asian AAB clones ('Brazilian', 'Dwarf Apple' and 'Go Sai Heong') displayed the zymogram E. Triploid ABB hybrids presented zymograms F, H, I, or J ('Ice Cream'), while tetraploid ABBB or ABBS (S = *M. schizocarpa*) hybrids presented zymograms F, H and I.

Phosphoglucose isomerase (PGI). Altogether, 56 different PGI zymograms were observed, and these contained a total of 19 scorable electromorphs.

Three loci were identified among the diploid *M. acuminata* clones, including *Pgi-1* (most anodal) with two alleles, *Pgi-2* (middle) with five alleles, and *Pgi-3* (most cathodal) with three alleles. In total, diploid *M. acuminata* accessions exhibited 11 different zymograms (A–K) (Fig. 2a), while the two *M. balbisiana* accessions exhibited the same zymogram (L). Also among the diploids, hybrid AB cultivar 'Ney Poovan' and 13 accessions from Papua New Guinea exhibited a unique zymogram (A) which contains alleles from both species. The zones of enzyme activity were not clearly resolved among triploids. The Cavendish cultivars, 'Cocos' and 'Golden Aromatic' were distinct from other AAA cultivars in presenting the zymogram B (Fig. 2b). Altogether, 18 zymograms were recorded among AAA accessions.

All triploid interspecific hybrids exhibited the fast moving *M. balbisiana* electromorph (Fig. 2c and 2d). Among the Pacific plantain AAB subgroup, Maoli and Iholena cultivars presented zymogram A, while Pōpō'ulu cultivars were distinct in possessing zymogram B (Fig. 2c). Asian AAB banana clones displayed zymogram B ('Go Sai Heong'), zymogram C ('Brazilian' and 'Walha') or zymogram M ('Mysore' and 'Poovan'). Altogether, the AAB banana and plantain clones exhibited 13 different zymograms, while ABB triploids and ABBB and ABBS tetraploids also produced a total of 13 zymograms (Fig. 2d), all but two differing from those of the AAAB group.

Phosphoglucomutase (PGM). This was the most variable of the three enzyme systems assayed. Altogether, 73 different zymograms with 20 distinct electromorphs were observed. Three regions exhibiting good enzyme activity, including *Pgm-1* (most anodic) with three alleles, *Pgm-2* (middle) with four alleles, and *Pgm-3* (most cathodic) with three alleles, were identified among diploids (Fig. 3). Wild and cultivated diploids of *M. acuminata, M. acuminata ssp. banksii, M. schizocarpa* and their hybrids exhibited zymograms A to M (Fig. 3a). Diploids of Asian origin exhibited zymograms N ('Lakatan' and 'Berangan'), P ('Tuu Gia'), R (*M. acuminata ssp. zebrina*) and Q, B, and S for seedling progenies of *M. acuminata* from Malaysia. Three species-specific alleles were observed in *M. balbisiana* (zymogram T).

The Cavendish cultivars were differentiated from other triploid AAA clones by the presence of a unique slow moving allele at *Pgm-1* (Fig. 3b, zymogram A). However, 'Valery', the only giant Ca-

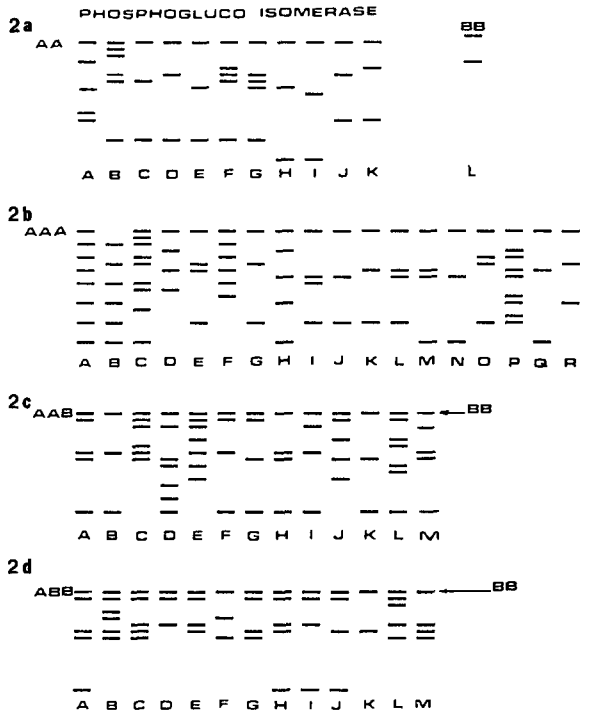

Fig. 2. Zymograms observed at PGI for each genomic group. (Alleles specific to *M. balbisiana* are indicated with arrows.)

vendish assayed in our study, differed from other Cavendish cultivars by the absence of this electromorph (zymogram B). Altogether, 13 different zymograms were observed among AAA accessions.

Most of the cultivars that are hybrids of *M. acuminata* and *M. balbisiana* presented combinations of species-specific PGM electromorphs (Fig. 3c, 3d). In the Pacific plantain AAB subgroup, Iholena clones exhibited zymogram A, distinct from closely related Maoli and Pōpō'ulu clones, which produced zymogram B. The Asian AAB clones lacked PGM alleles specific to *M. balbisiana*; 'Brazilian', 'Walha', 'Poovan' and 'Mysore' had zymogram J, while 'Go Sai Heong' had zymogram K. A total of 20 different zymograms were observed among AAB accessions. The ABB genomic group also contained 20 different zymograms (Fig. 3d).

Multivariate analyses of zymotypes

Diploids (AA). Fifty-one different zymotypes obtained from among 152 wild and cultivated diploids were subjected to PCA. The first two principal axes accounted for 31.8% of the total variability. Projection of zymotypes on the plane of these two axes revealed two major clusters (Fig. 4). The Asian *M. acuminata*, consisting of 'Berangan' (1), 'Tuu Gia' (3), and wild forms of Malaysian *M. acuminata* (49, 50, and 51) belonging to cluster I, exhibited divergence from Papua New Guinea and Vanuatu diploids in cluster II. *Musa acuminata* ssp. *banksii* accessions (6, 7, and 31) from Papua New Guinea and Western Samoa were closely related to Papuan *M. acuminata* AA cultivars in cluster II, while Papuan *M. schizocarpa* (39 and 42) were somewhat intermediate between the two clusters. The two Asian subspecies of *M. acuminata*, ssp. *zebrina* ('Mai'a 'Oa', 47) and ssp. *sumatrana* (48), were closely related. The diploid clone 'Lady Finger' (2) found in Hawaii is included as an outlier in Fig. 4 to emphasize our contention that its genomic constitution is AB (Fig. 2a, zymogram A). We suspect that it is 'Ney Poovan', rather than the diploid AA 'Sucrier'.

Triploids (AAA). Sixty-five zymotypes identified

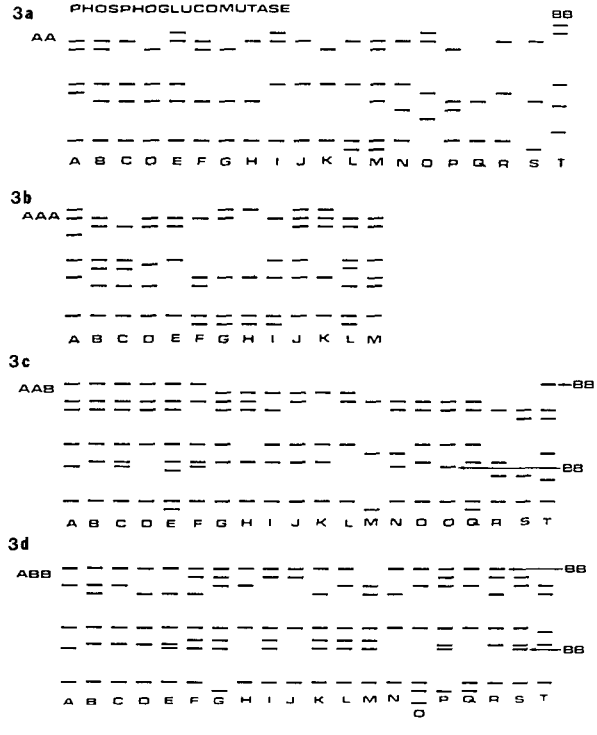

Fig. 3. Zymograms observed at PGM for each genomic group. (Alleles specific to *M. balbisiana* are indicated with arrows.)

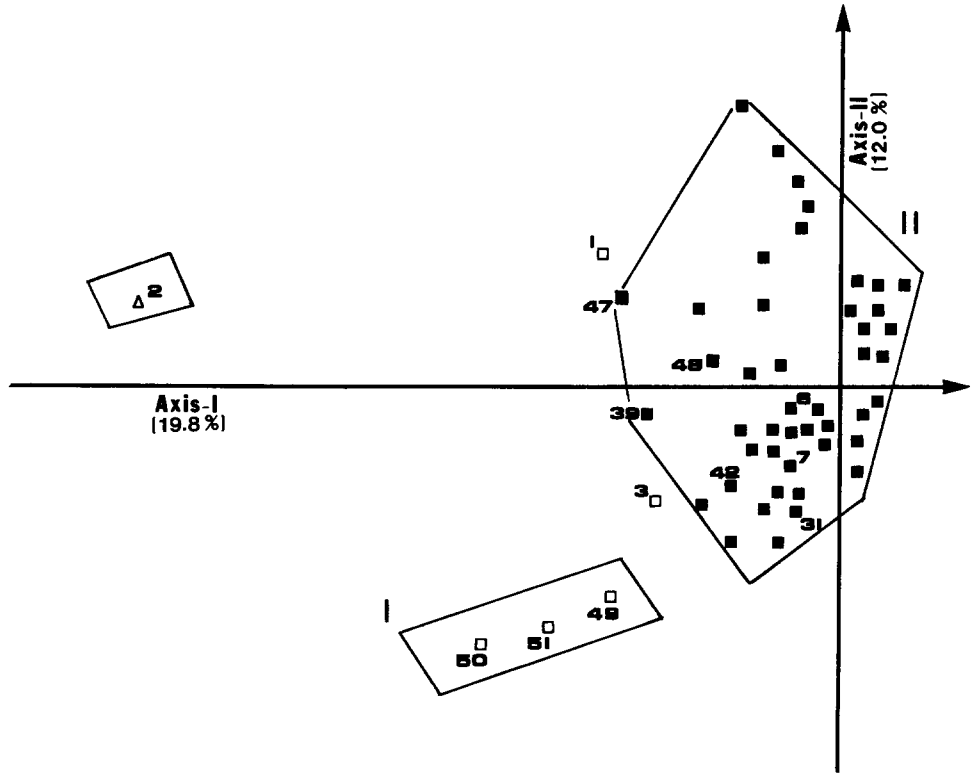

Fig. 4. PCA of diploid *M. acuminata* (AA) zymotypes. (Each dot represents a zymotype which includes several accessions.)

from a total of 109 accessions were subjected to PCA. The first two principal vectors, accounting for 32% of the total variation, revealed three distinct clusters (Fig. 5). Cluster I included exclusively Papuan triploids. It is not known whether these triploids are endemic to Papua New Guinea or are early introductions through human migration. Several triploid AAA cultivars from Papua New Guinea exhibited zymotypes identical to Papuan diploid AA cultivars.

Cluster II included the Asian cultivars 'Valery' (2), nine other Cavendish cultivars (3), and 'Cocos' and 'Golden Aromatic' (1). Cultivars present in this cluster have significantly diverged from other AAA cultivars by possessing a number of unique PGI and PGM electromorphs (Fig. 2b and 3b). Three cultivars from Papua New Guinea ('Keemerey' from Madang, 'Fako Fako' from Goodenough and 'Bumbu' from Morobe), which may represent recent introductions from Asia, were also found in cluster II.

Cluster III consisted of 14 Asian cultivars, including 'Colorado Blanco' and 'Cuban Red' (4), 'Green Red' (21), and others collected in Papua New Guinea, but presumably of Asian origin.

Triploids (AAB). A total of 195 AAB accessions were electrophoresed and 49 different zymotypes were identified. Four clusters were resolved by PCA (Fig. 6), in which the first two principal axes accounted for 27.5% of the total variation in this group.

The zymotypes representing the Maoli (1), Pōpō'ulu (2) and Iholena (4) cultivars of the Pacific plantain AAB subgroup aggregated with 19 other related zymotypes, including 'Dwarf Horn Plantain' (3) and 'Laknau' (15), to constitute cluster I. Cultivars 'Aumalie' and 'Lapoa', from Western Samoa, exhibited zymotypes identical to Maoli (1). Among the other Samoan clones, 'Fai Ause' was found to be identical to Hawaiian Pōpō'ulu (2) and 'Mamae' to Hawaiian Iholena (4). Cultivars 'Hopa Green' and 'Hopa Red' from Tonga were identical in zymotype to Pōpō'ulu from Hawaii. In Papua New Guinea, ten local cultivars, all from New Bri-

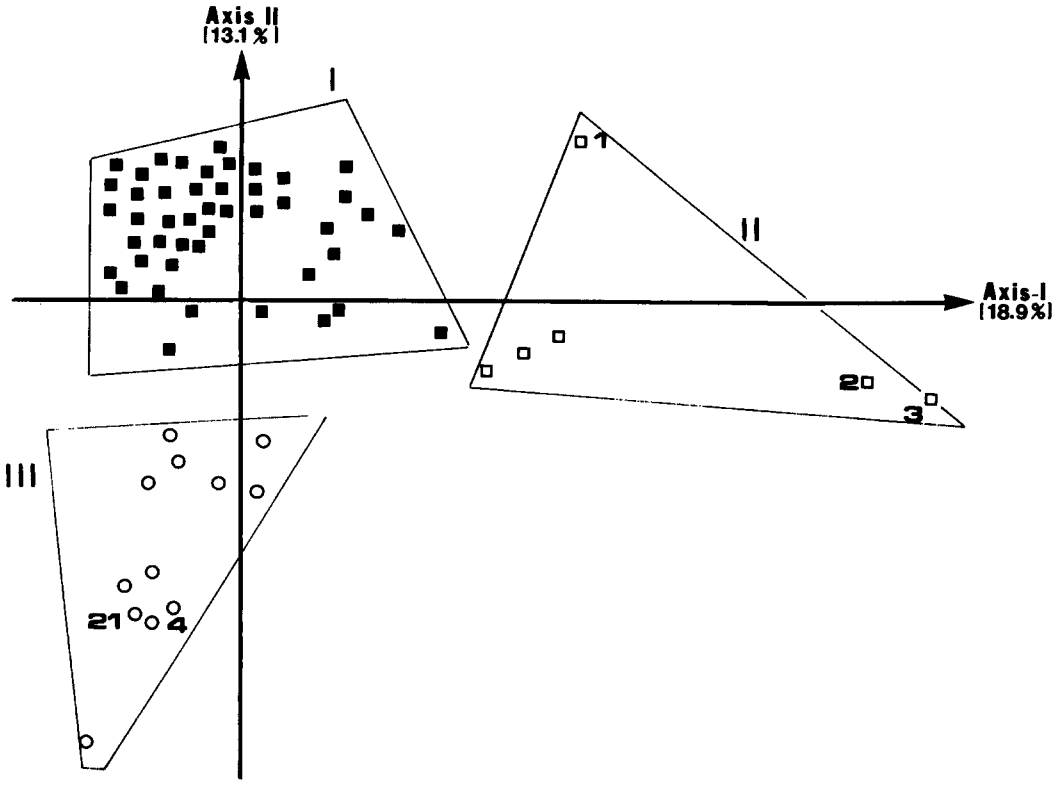

Fig. 5. PCA of triploid (AAA) zymotypes.

tain and Madang ('Morga Marvee', 'Mailay', 'Kabai', 'Abinakenau', etc.), produced zymotypes identical to Hawaiian Maoli, and six cultivars (including cultivar 'Morpa' from Mt. Hagen) were found to be identical to Iholena of Hawaii. The zymotype of the Hawaiian Pōpō'ulu was not observed within the Papua New Guinea germplasm collection. However, in Vanuatu, 12 local cultivars presented zymotypes identical to Pōpō'ulu, six to Maoli and seven to Iholena. The latter was not identified in New Caledonia, where 16 accessions produced zymotypes identical to Maoli and four to Pōpō'ulu. 'Pisang Rajah' (38) and cultivar 'Yourh' (33) from Wamu in Papua New Guinea were found to be closely related to each other, although they are rather distinct from other members of the Pacific plantain AAB subgroup comprising cluster I (Fig. 6).

Cluster II included seven cultivars possessing PGM zymograms J or K (Fig. 3c). This cluster includes cultivars of the Asian AAB dessert banana subgroup, such as 'Mysore' (9), 'Brazilian' (6), 'Go Sai Heong' (7) and 'Silk' (28).

Cultivars in cluster III originated from Papua New Guinea and were characterized by MDH zymogram F.

Cluster IV consisted of 14 cultivars originating in New Britain, with the exception of 'Morpa' from Madang.

'Eslesno' (10), an introduction to Hawaii from Puerto Rico, differed from other AAB accessions with a number of unique bands at PGI (Fig. 2c, zymogram E).

Several accessions classified as AAB, based on their morphology, lacked *M. balbisiana* alleles at MDH and PGM. For example, cultivars 'Mysore', 'Silk', 'Brazilian', and 'Walha' presented MDH zymogram E, which does not possess alleles from *M. balbisiana.* Several other AAB accessions from Papua New Guinea presented PGM zymograms M, O, Q, R, S, and T which also lack *M. balbisiana* alleles. However, the classification of these accessions as

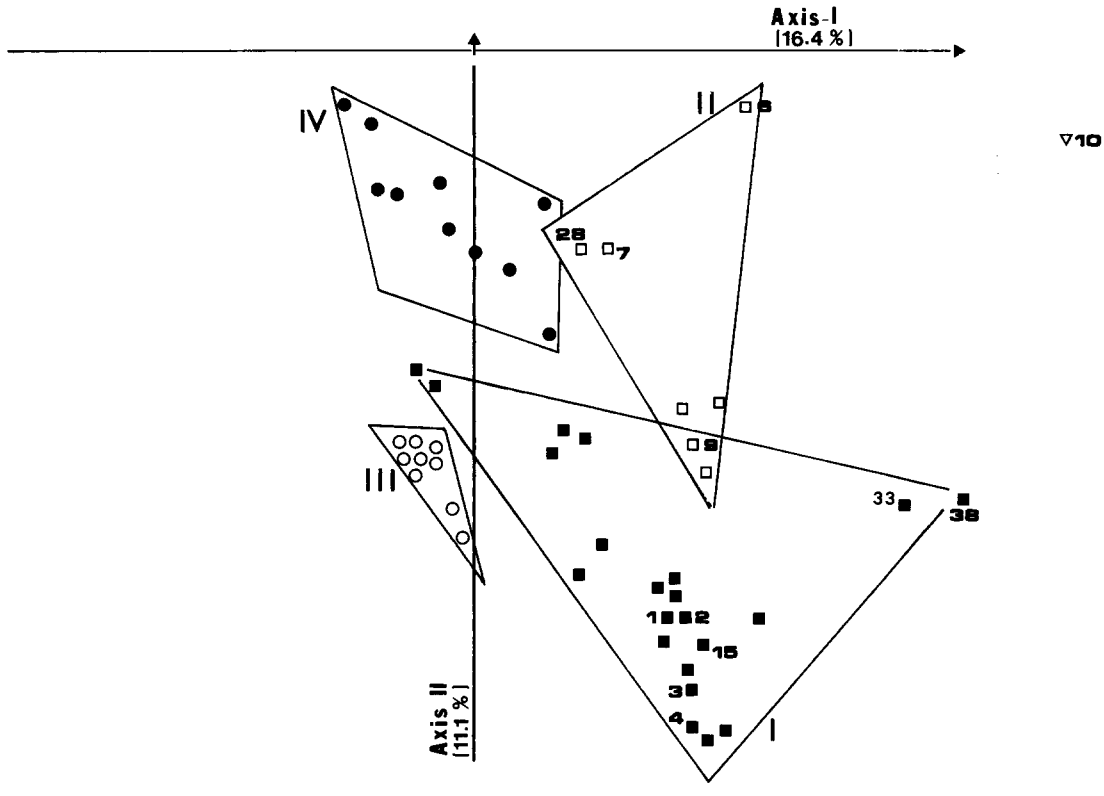

Fig. 6. PCA of triploid hybrid (AAB) zymotypes.

members of the AAB genomic group is supported by the fact that they possess *M. balbisiana* alleles at PGI.

Triploids (AAB) and Tetraploid (ABBB). A total of 103 cultivars were electrophoresed. The PCA conducted on the resulting 25 zymotypes identified four clusters (Fig. 7). The first and second principal vectors accounted for 37% of the total variation in this group. Cluster I includes cultivars 'Ice Cream' (synonym 'Blue Java') (1) and 'Paka' (synonym 'Monthan') (2) from Hawaii, and cultivars 'Bluggoe' (2), 'Pata' (Western Samoa) (2), 'Poro'ini' (French Polynesia) (2), 'Katiene' (New Caledonia) (2) and eight related zymotypes from Papua New Guinea and Vanuatu.

Cluster II includes cultivars 'Largo' (3) and 'Daru' (23) from the Western Province of Papua New Guinea.

Cluster III includes 'Sugaroo – Yawa' (4) and 27 other Papuan cultivars with identical zymotypes.

Cluster IV includes cultivars 'Kalapua Large' (17) and 'Kalapua Small' (17), which are classified as tetraploid ABBB and are the most popular cultivars in Papua New Guinea. 'Kalapua Large' is known under the local names of 'Fa'i pata Tonga' in Western Samoa and 'Poro'ini fe'i' in French Polynesia. 'Pisang awak' (10) appears to be distantly related to other ABB/ABBB cultivars.

Genetic differentiation among different genome groups. Isozyme variation within and among the five main genomic groups was summarized by analyzing together all 192 zymotypes by PCA. The first two principal axes accounted for 22% of the total variation. The zymotypes projected on the plane of the first two principal axes indicate that *M. acuminata* and *M. balbisiana* accessions have diverged significantly (Fig. 8). All *M. acuminata* zymotypes, including diploids and triploids, clustered together on the left side of the second axis, while all diploid, triploid and tetraploid accessions containing *M.*

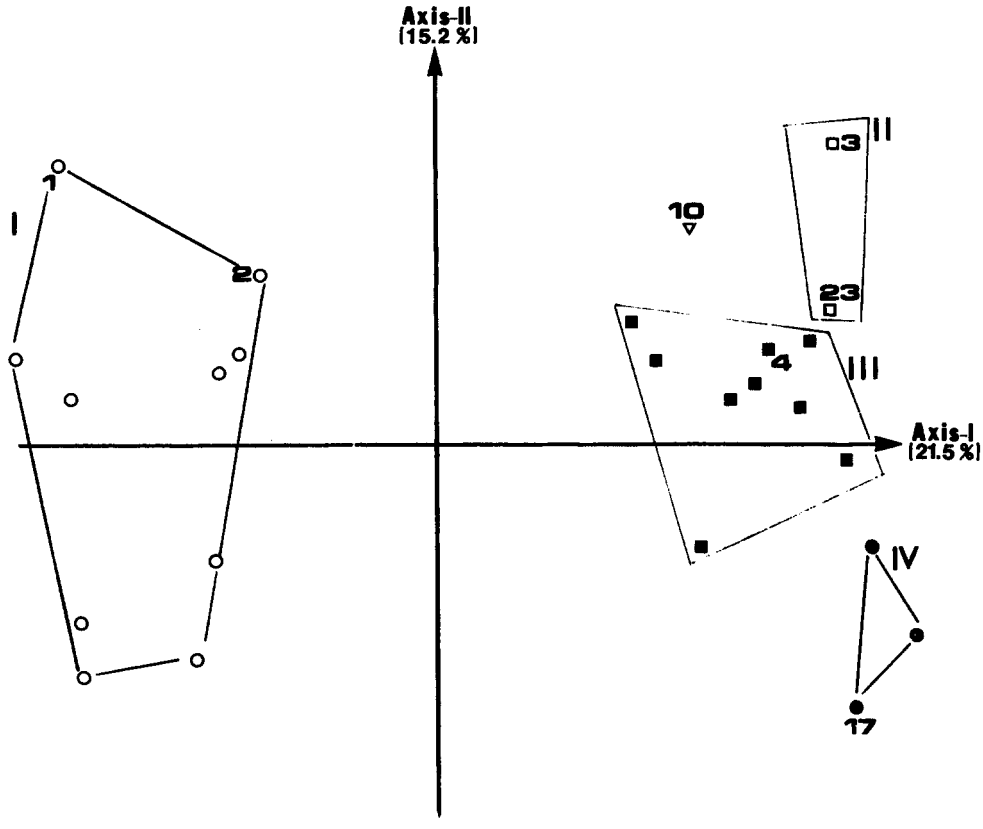

Fig. 7. PCA of triploid and tetraploid hybrid (ABB and ABBB) zymotypes.

balbisiana alleles clustered to the right side of the second axis.

The Cavendish cultivars (50 and 51) were distinctly separated from other *M. acuminata* zymotypes. The zymotypes of triploid interspecific hybrids are continuously distributed between the divergent progenitor species. The affinity of interspecific hybrids towards either of the two progenitor species was roughly proportional to the ratio of A and B genomes they possessed. The AAB genomic group appears to encompass the greatest amount of zymotype variation.

Discussion and conclusions

The effectiveness of isozymes for the classification of bananas and plantains has been previously reported (Jarret & Litz, 1986a, 1986b; Horry & Jay, 1988; Horry, 1989; Bhat et al., 1992). In this study, we used liquid nitrogen to preserve and transport leaf samples of germplasm maintained at various research centers in the Pacific. This technique allowed us to sample a wider array of the banana and plantain germplasm available in collections, overcoming restrictions imposed by quarantine regulations on movement of propagules. Hence, this survey was more comprehensive than any previous investigations of *Musa* germplasm from the Pacific.

Multivariate analyses, such as PCA, which reduce multidimensional variation to two or three simply explainable and meaningful dimensions, can contribute greatly to understanding of complex genetic relationships. Previous applications of this technique in the genus *Musa* have generally yielded consistent results, whether based on morphological data (Simmonds & Weatherup, 1990a, 1990b), isozyme data (Horry, 1989), or flavonoid data (Horry & Jay, 1988). Likewise, the multivariate analysis of isozyme variation in the present study clearly dif-

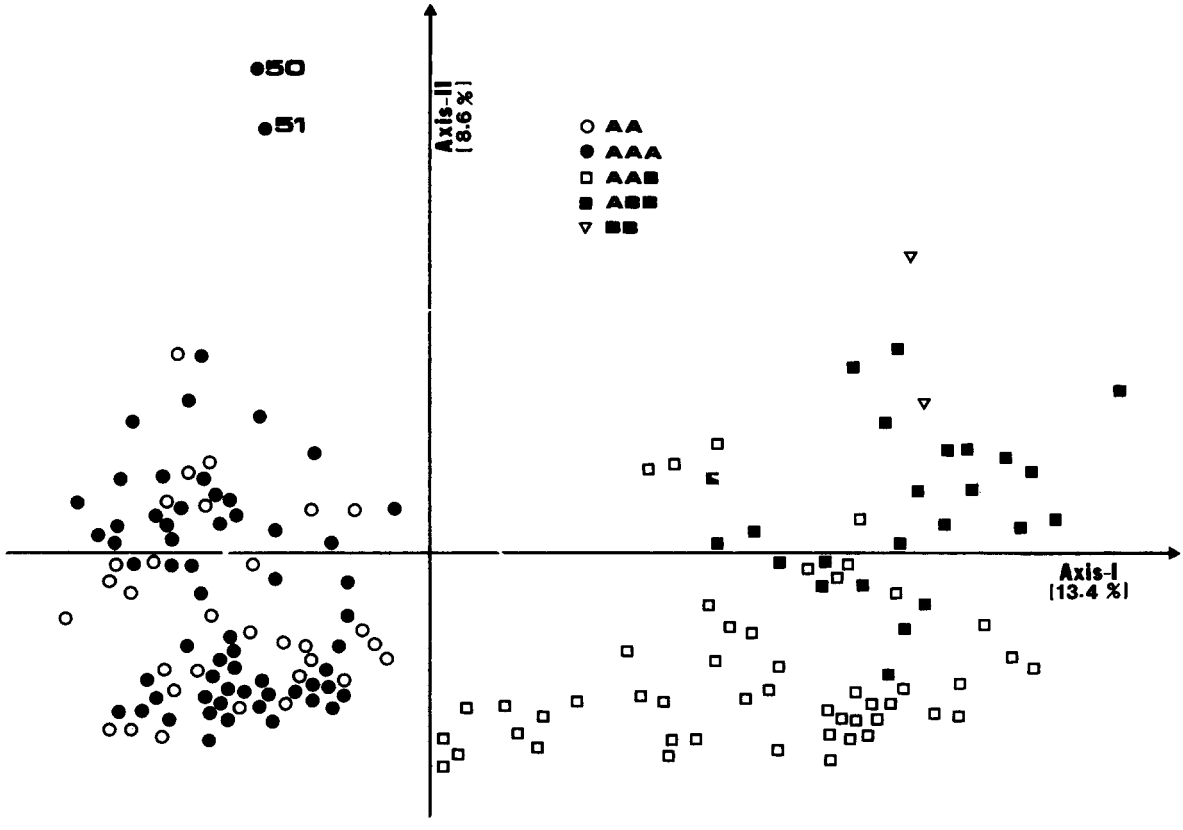

Fig. 8. Combined PCA of *Musa* zymotypes.

ferentiated banana and plantain groups into different genome groups, as well as indicated the pattern of relationships between genotypes within genome groups. However, in the present study, the first two principal axes used to resolve the clusters accounted for only about 30% of the total variation on the average. This emphasizes the multidimensional nature of isozymic variation in the genus *Musa.*

The genetic divergence between *M. acuminata* and *M. balbisiana* was reflected in the existence of unique alleles in both species for all three enzyme systems. The diploid *M. acuminata* complex includes many subspecies distributed throughout South East Asia and New Guinea. The germplasm included in the present study may represent only a part of the range of variation present in the species complex. Zymotypes of wild and cultivated diploid *M. acuminata* from Asia ('Berangan' 'Tuu Gia', *M. acuminata* ssp. *zebrina, M. acuminata* ssp. *sumatrana,* and seedlings of wild Malaysian *M. acuminata* ssp. *malaccensis*?) differed slightly from Papuan *M. acuminata* (*M. acuminata* ssp. and *M. acuminata* ssp. *banksii*) by being more variable, and expressing at low frequency a few additional alleles at *Mdh-1* and *Pgm-2*. However, unlike Jarret & Litz (1986b), we did not find any isozyme alleles that differentiated the subspecies of *M. acuminata* in Papua New Guinea (ssp. *banksii,* ssp. *sumatrana* and ssp. *zebrina*). Furthermore, the study indicated that the wild *M. schizocarpa* from Papua New Guinea has only narrowly diverged from diploid *M. acuminata* ssp. This supports the observations of Argent (1976) that hybridization, genetic recombination, and introgression between these groups is frequent. The isozyme data do not support separate species status for any members of the *M. accuminata* complex studied.

Several triploid AAA cultivars from Papua New Guinea exhibited zymotypes identical to Papuan diploid AA cultivars. This suggests the involvement

of Papuan diploid *M. acuminata* in the origin of some of these triploids, as opposed to the view that they were imported from Asia as triploid domesticates.

The three zymotypes identified in the Pacific plantain AAB subgroup corresponded to the three well-defined and morphologically distinguishable sets of related cultivars called Iholena, Maoli and Pōpō'ulu in the Hawaiian vernacular (Lebot et al., 1994). Most probably, they represent three original clones that were widely introduced throughout the Pacific and which later became diversified on individual archipelagos through human selection of naturally occurring somatic mutants. The Hawaiian Maoli, Pōpō'ulu and Iholena cultivars exhibited identical zymograms at MDH (zymogram G), but were differentiated at PGM, where Iholena cultivars exhibited zymogram A, while Maoli and Pōpō'ulu cultivars exhibited zymogram B. Although the Maoli and Pōpō'ulu cultivars have been grouped together in previous studies (Daniells, 1990; Horry, 1989; Tezenas du Montcel, 1990), our work shows that they are distinguishable by differences in their PGI zymograms, as well as by morphology. At PGI, Iholena and Maoli cultivars exhibited zymogram A, while Pōpō'ulu cultivars had zymogram B.

Isozyme fingerprinting has allowed us to discover members of the Pacific plantain subgroup from Hawaii to French Polynesia, Western Samoa, Tonga, New Caledonia, Vanuatu and Papua New Guinea. Accessions originating in New Britain exhibited zymotypes identical to Maoli. Others had zymotypes identical to Iholena, but Pōpō'ulu zymotypes were absent from Papua New Guinea.

The isozyme data indicate that the members of the Pacific plantain subgroup are closely related, and their geographic distribution suggests a link with Papua New Guinea. Furthermore, our isozyme studies suggest that the *acuminata/banksii* complex of Papua New Guinea is the most likely source of the A genome in the Pacific plantains. This is mainly due to the fact that we identified all the *acuminata* allozyme types that are present in the Pacific plantains from among diploid AA clones collected in Papua New Guinea. Our findings support the contention of Horry & Jay (1988) that the plantains 'would thus not have originated from a classical Malayan *M. acuminata,* but instead from a Papuan *M. acuminata* ssp. *banksii*'.

Particularly, in this study, the slow-migrating electromorph at *Mdh-1*, which is present in Pacific plantains and Papuan diploid *M. acuminata,* was absent among the Asian diploids. However, our result may have been biased by the large sample (n = 133) of Papuan material included in this survey, as opposed to a much smaller sample (n = 6) from Asia. It is possible that larger samples might reveal the missing allele in other populations from Asia. Nevertheless, the fact that the Pacific plantain subgroup has never been reported in Asia or the Philippines, and that zymotypes identical to those of Maoli and Iholena cultivars occur in Papua New Guinea, are strong support for their origin in New Guinea. Although the Pōpō'ulu zymotype was not identified from New Guinea, these cultivars are widely represented in Vanuatu and New Caledonia. Pōpō'ulu may have originated in Melanesia as a mutation from Maoli, which was further selected by local farmers.

Parthenocarpic diploid AA clones with plantain-like characteristics are well represented in Papua New Guinea and Vanuatu, but are absent from New Caledonia and Polynesia. These may represent the ancestral *M. acuminata* stock from which the AAB plantains might have originated by hybridization with *M. balbisiana.* Diploid cultivars present in Vanuatu are most likely ancient introductions from Papua New Guinea, since wild *M. acuminata* are absent in this archipelago.

The isozyme analysis of banana and plantain germplasm identified a large amount of variation in the AAB group. This is apparently due to the genetic heterogeneity of the progenitor species over the wide geographic range in which hybridization occurred. For example, the AAB banana cultivars 'Silk', 'Mysore', 'Go Sai Heong', 'Brazilian' and 'Walha' formed a cluster of related cultivars that was separate from the cluster containing the AAB plantains, including 'Dwarf Horn Plantain', 'Laknau', and the Maoli, Pōpō'ulu, and Iholena cultivars. The former subgroup originated from hybrids involving Asian subspecies of *M. acuminata,* whereas the latter subgroup seems to be derived from Pa-

puan subspecies of *M. acuminata*. Interestingly, our findings agree with the observation of Tezenas du Montcel (1990) that some *M. acuminata* diploids in Papua New Guinea are similar in appearance to the False Horn or True Horn plantain. These conclusions, based on analyses of morphology, isozyme variation and geographic distribution, lend credibility to the suggestion that New Guinea should be considered a secondary center of *Musa* domestication (Horry & Jay, 1988).

Based on the limited collection of *M. balbisiana* studied (n = 4) it appears that the species is polymorphic. Further study of *M. balbisiana* on a broader sampling basis should improve our understanding of the geographic origin and genetic organization of triploid hybrids.

Acknowledgements

Support for this research was provided by the Hawaii-Bishop Research Institute, INIBAP, and the Hermès Small Grants Program. We gratefully acknowledge H. Tezenas du Montcel, INIBAP, France; R. Kambuou, DPI, Papua New Guinea; E. Lavigne, IRFA, New Caledonia; V. Tiollier, Dept. of Agric., Vanuatu; C. Garnier, Economie rurale, Tahiti; R. Hamilton, UH; R. Fenstermacher and A. Brash for providing germplasm.

References

Argent, G.C.G., 1976. The wild bananas of Papua New Guinea. Notes from the Royal Botanic Garden, Edinburgh 77–114.

Bhat, K.V., S.R. Bhat & K.P.S. Chandel, 1992. Survey of isozyme polymorphism for clonal identification in *Musa*. I Esterase, acid phosphatase and catalase. J. Hort. Sci. 67: 501–507.

Cardy, B.J., C.W. Stuber, J.F. Wendel & M.M. Goodman, 1983. Techniques for starch gel electrophoresis from maize (*Zea mays* L.). Mimeograph Series No. 1317, Institute of Statistics, North Carolina State University, Raleigh.

Daniells, J., 1990. The banana varieties of Tonga, Western Samoa and the Cook Islands. Musarama 1: 6–10.

Horry, J.P., 1989. Chimiotaxonomie et organisation génétique dans le genre *Musa*. Fruits 9: 455–474.

Horry, J.P. & M. Jay, 1988. Distribution of anthocyanins in wild and cultivated banana varieties. Phytochemistry 8: 2667–2672.

Jarret, R.L. & R.E. Litz, 1986a. Isozymes as genetic markers in bananas and plantains. Euphytica 35: 539–549.

Jarret, R.L. & R.E. Litz, 1986b. Enzyme polymorphism in *Musa acuminata* Colla. Journal of Heredity 77: 183–186.

Lebot, V., K.M. Aradhya & R.M. Manshardt, 1991. Geographical survey of genetic variation in kava (*Piper methysticum* Forst. f. and *P. wichmannii* C. DC.). Pacific Science 45: 169–185.

Lebot, V., B.A. Meilleur & R.M. Manshardt, 1994. Genetic diversity in Eastern Polynesien cultivated bananas. Pacific Science 48 (in press).

Pope, W.T., 1926. Banana culture in Hawaii. Washington, Government Printing Office, 48 p.

Sharrock, S., 1990. Collecting *Musa* in Papua New Guinea. p. 140–157. In: R.L. Jarret (Ed). Identification of Genetic Diversity in the Genus *Musa*: Proceedings of an international workshop held at Los Baños, Philippines, p. 140–157.

Shaw, C.R. & R. Prasad, 1970. Starch gel electrophoresis of enzymes – a compilation of recipes. Biochem. Genet. 4: 297–320.

Simmonds, N.W., 1954. Notes on banana varieties in Hawaii. Pacific Science 8: 226–229.

Simmonds, N.W. & K. Shepherd, 1955. The taxonomy and origins of the cultivated bananas. Journal of the Linnean Society, London 55: 302–312.

Simmonds, N.W. & S.T.C. Weatherup, 1990a. Numerical taxonomy of the cultivated bananas. Tropical Agriculture 67: 90–92.

Simmonds, N.W. & S.T.C. Weatherup, 1990b. Numerical taxonomy of the wild bananas (*Musa*). New Phytologist 115: 567–571.

Stover, R.H. & N.W. Simmonds, 1989. Bananas, 3rd edn. Longman, London.

Tezenas du Montcel, H., 1990. *Musa acuminata* ssp. *banksii*: status and diversity. p. 211–218. In: R.L. Jarret (Ed). Identification of Genetic Diversity in the Genus *Musa*: Proceedings of an international workshop held at Los Baños, Philippines.

Tezenas du Montcel, H., 1991. Rapport de Mission en Nouvelle Caledonie. INIBAP fm/HTM/A95R91, France, 12 p.