Genetic diversity of Indonesian mangoes (*Mangifera indica* L.) using RAPD markers

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SUMMARY

Mango was introduced to the Caribbean in the XVIIIth century and became a popular garden tree. A regional programme for the inventory and conservation of fruit genetic resources undertaken in the French West Indies allowed the collection of 128 land races of mango in the Guadeloupe archipelago and in Martinique. Labelled accessions were selected according to local names, location and morphology. Microsatellite markers were developed for studying genetic diversity within this sample and within the germplasm bank maintained in Guadeloupe (Cirad) and for detecting duplicates. Nineteen microsatellite markers were selected and used to analyse a total of 307 accessions from India, South-East Asia, Florida, Africa and the Caribbean. Diversity was high within the sample with a total number of 140 alleles displayed. Results demonstrated the presence of duplicates in the germplasm bank and among the collected accessions, helping genetic resources management. Nevertheless, collecting efficiency was satisfactory with 73% of the material displaying distinct genetic profiles. Dissimilarities were calculated and a diversity representation was constructed using Neighbour Joining methodology. Accessions clustered in accordance with their geographical origin and their known history. The collected Caribbean accessions displayed a high variability, but shared some specific alleles and clustered together along with cultivars grown in Central (Mexico) and South America (Colombia) introduced from the South-East Asia, but also with cultivars from former French colonies in the Indian Ocean, indicating two introduction routes of mango to the French West Indies.

INTRODUCTION

Mangifera indica L. originated in a region including the north-eastern part of India (Assam), the western part of Myanmar, and Bangladesh. The mango was domesticated in this region and has been cultivated in India for 4000 years. Cultivation spread first to Malaysia and South-East Asia, supposedly expanded by Buddhist monks. Purseglove suggested that Phoenicians and Arabs spread the crop from India to East-Africa where it has been cultivated since the Xth century. According to Mukherjee (1997), the global spread of mango outside its original centres of domestication probably did not occur until the beginning of the European voyages of the XVth and XVIth centuries, when the Portuguese took the mango to West-Africa and from there to Brazil at the beginning of the XVIIIth century, and when the Spanish introduced polyembryonic mango types

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from The Philippines to Central America through the Pacific trading ports of Mexico and Panama.

In the West Indies, the first introduction reported was to Barbados during the XVIIIth century, then to Jamaica where it was obtained from a French ship plying between Mauritius and Haiti, highlighting the role of French traders in introducing the crop to the West Indies. During this period it is probable that all English, Spanish, French and Dutch, who were fighting over the Caribbean dominion, played an active part in spreading the mango throughout the region.

A regional inventory of fruit genetic resources has recently been undertaken in the French West Indies (FWI). The objective is to conserve the FWI fruit variability *in-situ* and *ex-situ*, as well as to try local varieties under standard conditions. The project includes a genetic diversity study of collected material in comparison with the diversity observed in the germplasm bank maintained locally in Guadeloupe.

Different markers have been used to study mango diversity: RAPD markers in Florida (Schnell 1993; Schnell *et al.* 1995), India (Kumar et al. 2001; Karihaloo et al. 2003) Venezuela (Lopez-Valenzuela et al. 1997) and Brazil (Barbosa de Souza et al. 2004); AFLP (Eiadthong et al. 2000; Hautea et al. 2001) and ISSR (Eiadthong et al. 1999; Gonzalez et al. 2002) Nevertheless, these molecular markers are dominant and they do not permit the differentiation of heterozygous from homozygous accessions.

The first co-dominant markers developed were isozymes (Degani et al. 1992; Pascua et al. 1996; Eiadthong et al. 1998). More recently three research groups chose to develop SSR markers (Honsho *et al.* 2005; Schnell *et al.* 2005) and Duval et al. (2005). These molecular markers are co-dominant, specific and highly variable. These qualities make them highly suitable to study diversity in supposedly related populations or cultivars.

MATERIAL AND METHODS

Plant material

One hundred and sixty five accessions were studied (Table 1). The germplasm bank maintained in Field experiment was extensively sampled with 86 accessions selected, including accessions of the related species *M. laurina* and *M.lalijiwa* and *M.indica* cultivars from India and indigenous Indonesia. Cukurgondang Pasuruan East Java.The accessions were selected according to their local names, location and morphology in order to cover the maximum of the observed variability.

DNA extraction

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DNA was extracted from fresh young leaves using a CTAB-based method (Doyle and Doyle, 1990). Samples weigthing 2 grams were mortared in liquid nitrogen and incubated for 30 minutes at 65 °C in extraction buffer (2% CTAB; 100 mM Tris HCl pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2% PVP; 0.2% mercaptoethanol). Nucleic acids were purified with chloroform/isoamyl alcohol (24:1) and phenol/chloroform/isoamyl alcohol (25:24:1), and then precipitated with isopropanol and dissolved in TE buffer. DNA concentrations were estimated on a 0.8% agarose gel stained with ETBr (ethidium bromide).

PCR Conditions

PCR was performed in a volume of 10 ul. The reaction mixture contained 10 x PCR buffer; 2.5 mM MgCl2; 0.1 mM of each nucleotide; 0.325 U *Taq* polymerase; 0.10 mM primer; and 10 ng template DNA. Amplification was performed in an Perkin Elmer Thermocycler (40 cycles: 95°C/1 mnt, 37°C/1 mnt, 72°C/1 mnt). In all, 7 primers were evaluated from the OPA 14, OPA 16, OPA 17, OPA 18, OPH 12, OPH 13, OPH 14 and OPH 19. PCR products were separated on 1.2% agarose gel, stained with ethidium bromide, and visualized under UV light. Only distinct, reproducible and informative bands were selected for further analysis.

Data analysis

For each sample assayed, RAPD bands were scored as 1 of (dominant band) or 0 (absent band). RAPD polymorphism was first analyzed with respect to primer and population. Polymorphic dominant bands were counted and their frequencies were calculated. A dissimilarity matrix was generated using an index derived from the Dice index:

$2n_{1,1}$

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SG = ------------------------

$$(2n_{1,1} + n_{1,0} + n_{0,1})$$

where $n_{1,1}$ is the number of alleles shared by individuals i and j, $n_{1,0}$ the number of alleles present in i and absent in j, and $n_{0,1}$ the number of alleles present in j and absent in i.

A Neighbour-Joining representation of the Dice dissimilarities between accessions was constructed using the NTSys software v. 2.1 (Rolf 2001).

RESULTS AND DISCUSSION

A total of 174 DNA bands, amplified by seven different were detected by the 7 different 10-mer oligonucleotide primers, were scored across the samples. An average of 12 DNA bands were amplified per sample/primer combination. Primer OPH-12 amplified as few as eight bands, whereas primer OPA 17 amplified as many as 14 bands. The approximate size of the amplified fragments ranged from 200 to 1500 bp (Table 2). Of the 81 DNA bands scored, 80 (99%) were polymorphic. Across all samples and primers, an average of 12 polymorphic bands were amplified per sample/primer combination. A wide range of genetic similarity was observed among the cultivars. The highest genetic similarity (0.97) was observed between cultivars Mangkok and Kidang kweni, while the lowest genetic similarity (0.76) was between the cultivars Kepodang and Madu
displayed RAPD profiles with polymorphic bands, but
many of these bands varied with thickness, molecular
weight, and intensity. This variation made the scoring
of RAPD bands difficult and less reliable. Thus, only
the robust RAPD bands were considered in this study.
Testing the reproducibility of some robustRAPD bands
revealed identical band patterns with respect to the individual
plants assessed and the primers tested, thus increasing
the confidence of using RAPD markers to detect
genetic variations in blue Twelve
their band frequencies in two selected populations (BMSC and UBC primers (Table 2) were selected to assay each
MSC) and their source populations (SPs). sample. A total of 69 polymorphic RAPD bands, consisting
of DNA fragments of molecular weights ranging
morphic dominant bands were counted and their frequencies from 330 to 1500 base pairs, were generated for each

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in each SP and selected population were calculated. sample. The number of polymorphic bands scored per

To assess the RAPD variation of blue grama in the 11 SPs, primer ranged from 3 to 10 with an average of 5 to 6

apvoeprualgaetiopno.lyMmeoarnphRicAbPaDndvfarreiaqtuioenncwy

iwthaisncaalpcouplautleadtifoonr weaecrhe bands per primer. Two primers (UBC389 and UBC570)

estimated following the method of Nienhuis et al. (1994) and had 10 bands scored, while UBC249 had only 3 bands.

from the sums of squares of Analysis of Molecular Variance No bands were found to be unique to plants from any

(AMOVA; Excoffier et al., 1992). For each population,RAPD SPs. The band frequencies detected in the SPs ranged

similarity between a pair of individual plants was first calcu- from 0 to 1, but most

The selected loci displayed 4 to 14 alleles with a mean value of 7.3 per locus and a total of 140 alleles of which 121 are specific to *M.indica*. Heterozygosity levels calculated using GDA software (Lewis 2001) ranged from 0.059 to 0.857 for H_O and for 0.146 to 0.853 for H_E.

Cultivar identification and Genetic Resources Management

Prior to the diversity study, a first dendrogram was constructed from the 304 accessions (three accessions were discarded for missing data) as an identity check, especially for some dubious varieties, and to detect duplicates

The 304 accessions displayed 207 different genotypes.

Some suspected identification errors in the germplasm bank were confirmed. They occurred generally during germplasm transfers and cover various mistakes (missing labels, mislabelled cuttings). Eight accession identities could be corrected according to morphological observations confirmed by molecular pattern comparisons with the original Floridian varieties.

Duplicates were detected in the germplasm bank. Their occurrence could be correlated in some cases with ID problems and are consequences of the same mistakes (i.e. a non-labelled cutting erroneously attributed and grafted as a new cultivar when in fact it is a second cutting of another cultivar). In other cases, expected synonymies were confirmed between accessions collected or exchanged in different regions or countries with different names. Last, some accessions issuing from clonal variation could not be differentiated from their parent (i.e. Valencia and Valencia Pride).

Ninety four genotypes were displayed among the 128 accessions collected in the French West Indies, which amounts to a minimum collecting efficiency of 73%.

While prospecting, various cultivars appeared to share morphological traits, but were collected separately, because they occurred in different locations and/or were known under different names. Some of these were found to share the same genotype based on the 19 SSR markers used and were probably plants of the same cultivar propagated vegetatively whether obtained from poly-embryonic

seeds or cuttings. Some of these could present significant morphological differences issuing from punctual mutations or epigenetic variation, vegetatively propagated.

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On the other hand, it is not unusual to find different genotypes grouped under the same cultivar name. Sometimes these genotypes share some morphological traits and are related to the original variety.

Morphological characterization is still in progress for all 128 accessions with the purpose of identity confirmation, and, if necessary, of selecting the most interesting phenotypes within the various accessions sharing the same genotype.

Diversity representation

A dissimilarity matrix was built with the 207 genotypes and a dendrogram was inferred using the Neighbour Joining method.

The genetic diversity tree (Figure 1) shows a loose assemblage of nearly equidistant branches with almost continuous variation. Nevertheless the accessions seem logically organized and six clusters could be delimited. The most distant node groups the three representatives of the parent species *M. applanata* and *M. laurina*. Nearest of these related Asiatic species, accessions originating from south-eastern Asia cluster with some poly-embryonic West-African varieties. Another group is constituted by Indian varieties together with East-African varieties. The Floridian varieties group with their Indian parents Mulgoba and Sandersha.

Caribbean accessions are the most numerous and group remarkably well, although they appear more or less separated into two groups. The largest group includes the majority of FWI accessions, the South American varieties Azucar and Chancleto, probably descendants of poly-embryonic accessions introduced by the Spanish, and some accessions exchanged with Trinidad. The smaller group groups the other FWI accessions, including the most famous, "Julie" and "Reine Amélie", with the majority of the accessions collected from the island of La Réunion (Indian Ocean).

Dispersion of mangoes from the centres of domestication

It is interesting to compare the structure observed in the diversity tree, although weak, with the history and hypotheses of mango dispersion.

African varieties

African varieties cluster separately: the poly-embryonic West African varieties, such as Améliorée du Cameroun and Alphonse de Goa, join the South-East Asian group, which is consistent with an early introduction by the Portuguese, probably from Macao or Timor (Mukherjee 1997; Rey *et al.* 2004). East African varieties (Mabrouk – Egypt A, B, C and D) cluster with the Indian varieties Mulgo Round, Bombay, Paheri and Alphonse, which indicates another introduction origin and supports an early dispersion by Phoenicians and Arabs. Rey (2004) reports the citation of Ibn Battuta who mentioned the presence of mango in Modagiscio as early as 1330. Last, some famous varieties grown in West Africa, such as Julie and Amélie (or Reine Amélie), were introduced later from the French West Indies.

Floridian varieties

The history of mango introductions to Florida during the XIXth century is well-known (Campbell 1992). The first successful introduction was made in 1861 from Cuba and was followed by the introduction of grafted varieties from India in 1889. These varieties did not fruit dependably under Floridian conditions, but produced well-adapted progeny such as Haden. Other accessions were introduced later from Asia, Africa, Central and South America, the Caribbean and Pacific Islands. Thus Florida became a secondary centre of diversity for mango and the origin of a great number of good quality commercial

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cultivars that are currently grown. Our results closely group the Floridian cultivars and display some sub-groups. Two of these cluster with Indian varieties, respectively Mulgoba (Haden, Springfels, Irwin, Lippens, Beverly) and Sandersha (Tommy Atkins, Valencia Pride). The majority of

these varieties are descendants of Haden, the first known Mulgoba progeny. The varieties found in the proximity of Sandersha could be hybrids between these two varieties, which were part of the early introductions, or their descendants.

Caribbean varieties

The accessions collected in the French West Indies display a high diversity. The dissimilarities observed are high which implies the intervention of sexual reproduction. Only one sub-group constituted by nine accessions, “Mangue fil”, is linked by consequently less high dissimilarities. These accessions are characterized by small fruits with fibrous texture. Heterozygosity calculated within this sub-group is very low (H_e=0.22 and H_o=0.25), even when compared with the heterozygosity calculated between related varieties such as Mulgoba and its descendants (H_e=0.51 and H_o=0.52). Investigations on the reproductive pattern of these “Mangue Fil” accessions are in progress.

Although well grouped, the Caribbean accessions segregate in two clusters. The observed grouping and sub-grouping did not match the geographic origin within the Caribbean islands that were sampled.

The most important cluster groups the majority of the Caribbean accessions with three Reunion island accessions, three varieties from Trinidad and three from South America, including the cultivar Manila, indigenous to The Philippines. These accessions could have been introduced from or via South America.

The second cluster groups the other Caribbean accessions, including Julie and Reine Amélie varieties with a sub-group of the majority of the accessions from La Réunion and one cultivar from Trinidad. This indicates that the introduction route from the former French possessions of the Indian Ocean and the French West Indies quoted by Kostermans and Bompard (1993) could have been the origin of some of the best Caribbean cultivars. The Floridian variety Carrie segregates in this group, which is consistent with its relationship with the Caribbean variety Julie (Morton 1987).

The marker set developed is suitable for investigating mango diversity and permitted a lot of information to be gathered. The data are still being studied to understand the dynamics of the creation of diversity. It would be interesting to introduce more accessions from the centres of origin and diversity to infer the relationships and to evaluate the genetic differentiation that has occurred in the Caribbean area.

REFERENCES

Barbosa de Souza, V. A., P. Sarmanho da Costa Lima, et al. (2004). "Genetic variability in mango genotypes detected by RAPD markers." Acta Horticulturae **645**: 303-310.

Billotte, N., P. J. L. Lagoda, et al. (1999). "Microsatellite-enriched libraries : applied methodology for the development of SSR markers in tropical crops." Fruits **54**: 277-288.

Campbell, R. J., Ed. (1992). A guide to mango in Florida. Miami, Fairchild Tropical Garden

Degani, C., M. Cohen, et al. (1992). "PGI isozyme diversity and its genetic control in mango." Hortscience **27**(3): 252-254.

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http://repository.unri.ac.id/

Duval, M. F., J. Bunel, et al. (2005). "Development of microsatellite markers for mango (*Mangifera indica* L.)." Molecular Ecology Notes.
Eiadthong, W., K. Yonemori, et al. (2000). "Amplified fragment length polymorphism analysis for studying genetic relationships among *Mangifera* species in Thailand." J-Am-Soc-Hortic-Sci **125**(2): 160-164.
Eiadthong, W., K. Yonemori, et al. (1998). "Isozyme polymorphisms of mango cultivars in Thailand." Thai Journal of Agricultural Science **31**(4): 555-568.

Eiadthong, W., K. Yonemori, et al. (1999). "Identification of mango cultivars of Thailand and evaluation of their genetic variation using the amplified fragments by simple sequence repeat (SSR) anchored primers." Scientia Horticulturae **82**(1/2): 57-66.
Gonzalez, A., M. Coulson, et al. (2002). "Development of DNA markers (ISSRs) in mango." Acta Horticultura **575**(1): 139-143.
Hautea, D. M., C. P. Padlan, et al. (2001). Molecular characterization of Philippine 'carabao' mango using RAPD and AFLP markers. Asian Agriculture Congress. 24-27 Apr 2001, Manila (Philippines), Society for the Advancement of Breeding Researches in Asia and Oceania, Tokyo (Japan); Asian Crop Science Association (Australia); Federation of Crop Science Societies of the Philippines, College, Laguna (Philippines) ; Food security and environment protection in the new millenium.Manila (Philippines).
Honsho, C., K. Nishiyama, et al. (2005). "Isolation and characterization of new microsatellites markers in mango (*Mangifera indica*)." Molecular Ecology Notes **5**: 152-154.
Karihaloo, J. L., Y. K. Dwivedi, et al. (2003). "Analysis of Indian mango cultivars using RAPD markers." Journal of Horticultural Science and Biotechnology. **78**(3): 285-289.
Kumar, N. V. H., P. Narayanaswamy, et al. (2001). "Estimation of genetic diversity of commercial mango (Mangifera indica L.) cultivars using RAPD markers." Journal of Horticultural Science and Biotechnology. **76**(5): 529-533.
Lewis, P. O. and D. Zaikin (2001). Computer Program for the Analysis of Genetic Data, Free program distributed by the authors over the Internet from http://lewis.eeb.uconn.edu/lewishome/software.html.
Lopez-Valenzuela, J. A., O. Martinez, et al. (1997). "Geographic differentiation and embryo type identification in Mangifera indica L. cultivars using RAPD markers." Hortscience **32**(6): 1105-1108.
Morton, J. (1987). Mango. Fruits of warm climates**:** 221-239.
Mukherjee, S. K. (1997). The Mango : Introduction : Botany and Importance. The Mango: botany, production and uses. R. E. Litz. Cambridge, University Press**:** 1-19.
Pascua, G. S., R. R. C. Espino, et al. (1996). "Diversity in mango accessions based on isozyme analysis and some morphological characters." Philippine Journal of Crop Science **21**(Supplement 1): 10.
Perrier, X., A. Flori, et al. (1999). Les méthodes d'analyse des données. Diversité génétique des plantes tropicales cultivées. S. M. Hamon P, Perrier X, Glasszmann J.C. Montpellier, France, Cirad**:** 43-76.
Rey, J.-Y., T. M. Diallo, et al. (2004). "La mangue en Afrique de l'Ouest francophone : variétés et composition variétale des vergers." Fruits **59**: 191-208.
Risterucci, A. M., L. Grivet, et al. (2000). "A high-density linkage map of *Theobroma cacao* L." Theor Appl Genet **101**: 948-955.

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Schnell, R. J. (1993). "Genetic relationships among *Mangifera* spp. based on RAPD markers." Acta Horticulturae(341): 86-92.
Schnell, R. J., C. T. Olano, et al. (2005). "Isolation and characterization of 15 microsatellite loci from mango (*Mangifera indica* L.) and cross-species amplification in closely related taxa." Molecular Ecology Notes.
Schnell, R. J., C. M. Ronning, et al. (1995). "Identification of cultivars and validation of genetic relationships in *Mangifera indica* L. using RAPD markers." Theoretical Applied Genetics **90**: 269-274.

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