Assessment of Genetic Diversity in Australian-grown Mangosteen (*Garcinia mangostana* L.) and its Wild Relatives

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Keywords: *Garcinia*, apomixis, RAF, crop improvement

Abstract

Mangosteen (*Garcinia mangostana* L.) is a popular dioecious tropical fruit tree cultivated throughout South East Asia and Northern Australia. Dioecious species are generally characterised by high levels of genetic diversity, however mangosteen is unusual in that phenotypic differences are uncommon. Almost all trees are female with only a few anecdotal reports of male trees reported. Furthermore, seeds from mangosteen are apomictic and consequently produce trees that are clones of the female parent. It has been suggested by some mangosteen growers in North Queensland that the occasional phenotypic variants observed in their plantations are a result of cross-pollination from wild related species. Whether these differences can be attributed to genetic variation or are purely a consequence of being cultivated at different geographical locations is unclear. To address these questions, we conducted a preliminary survey using a new high-efficiency DNA marker technique, Randomly Amplified DNA Fingerprinting (RAF), to examine the level of genetic diversity in a population of *G. mangostana* trees collected from orchards in Northern Queensland. Additionally, we examined the level of variation among a number of other *Garcinia* species including mangosteen's closest relative, *G. hombroniana*, as well as native Australian *Garcinia* species.

INTRODUCTION

Mangosteen, the "Queen of tropical fruits" (Fairchild, 1915), belongs to the large genus *Garcinia* (family *Guttiferae*), which contains about 400 species of evergreen trees or shrubs, distributed mainly in tropical Asia (Fairchild, 1930). Mangosteen is considered indigenous to Malaysia, and identified as a cultivated species (Verheij, 1991). Most of the 150,000 tons annual world production (Ross, 1997) is derived from backyard trees in Southeast Asian countries (Thailand, Malaysia, Indonesia) (Wieble et al., 1992). During the past few centuries, mangosteen has also been domesticated in other tropical areas including Northern Australia (Verheij, 1991). The species requires ultra-tropical climates (Alexander, 1984), has a juvenile phase of 10-15 years and is difficult to propagate by traditional vegetative means. However mangosteen seeds are considered homogenous due to apomictic reproduction and thus propagation occurs predominantly by seeds (Verheij, 1991).

Apparently, mangosteen developed by hybridization between the two Malaysian species *G. hombroniana* and *G. malaccensis* (Richards, 1990). Mangosteen is an unusual dioecious species in that it seems to be entirely female. Previously reported male mangosteen trees are now believed confused with closely related species (Richards, 1990). As males are absent in mangosteen, it has been suggested that all present day mangosteen trees are derived from one female, apomictic clone (Richards, 1990). This theory has been supported by a general lack of phenotypic variation in mangosteen within and between countries (Almeyda and Martin, 1976). However there have been occasional reports of trees with unusual characteristics, e.g. a race in the Sulu Islands (the Philippines) with thicker rind and more acid flesh (Burkill, 1966; Corner, 1988), and a

distinct, pear-shaped fruit occurring in Queensland (Australia). As for many other tropical fruit species, genetic research on mangosteen has been limited, and it is not known whether these differences are due to genetic or environmental factors (Almeyda and Martin, 1976). For genetic improvement of the species, e.g. reduction of the juvenile phase of the tree, there is a great need to attain knowledge regarding the genetic diversity in mangosteen. As an initial approach, we studied the genetic variability in a population of mangosteen and eight related *Garcinia* species collected in North Queensland, Australia, using the novel molecular marker technology Randomly Amplified DNA Fingerprinting (RAF) (Waldron et al., 2002). In this paper we also report on genetic analysis of *G. hombroniana* and its relatedness to mangosteen.

MATERIALS AND METHODS

DNA Extraction

During sample collection, young leaf material was chosen if available, as preliminary trials showed greatly enhanced DNA yields from young leaf material (~ 8 μg DNA per gram of tissue) compared to mature tissue (~ 0.5 μg DNA per gram of tissue) (Fig. 1). A modified CTAB protocol as described by Ramage et al. (2004) was used to extract DNA from leaf samples of mangosteen and various *Garcinia* species collected in North Queensland, plus a *G. hombroniana* specimen obtained from Malaysia (Table 1).

Randomly Amplified DNA Fingerprinting (RAF)

PCR and polyacrylamide gel electrophoresis was carried out as described by Waldron et al. (2002), using the primers listed in Table 2. Two experiments were conducted. I: The *G. mangostana* accessions recorded in Table 1 were examined using all the eight primers listed in Table 2 (Ramage et al., 2004). II: The other *Garcinia* species in Table 1 plus one representative from each of three *G. mangostana* varieties that were identified, were examined using primers AB-16 and AP-20 (Fig. 2).

Data Analysis

The presence or absence of all reproducible markers was recorded for each accession as binary data, which were subsequently subjected to UPGMA analysis (Unweighted Pair Group Method with Arithmetic Mean) (Sneath and Sokal, 1973) based on Nei & Li's genetic distance (Nei and Li, 1979), using Multi-Variate Statistical Package (MVSP) software, version 3.12d.

RESULTS

In experiment I, 650 RAF markers, of which 225 were polymorphic, were scored among the *G. mangostana* accessions. The analysis revealed three clusters among the investigated *G. mangostana* accessions: Variety 1; consisting of seven genotypes across 34 accessions, Variety 2; consisting of one accession and Variety 3; consisting of two genetically identical accessions (Fig. 3). These three clones were polymorphic at a large percentage (23-31%) of the loci screened. Within Variety 1, 26 mangosteen accessions had identical RAF profiles (represented by accession 21 in Fig. 3). The remaining eight accessions of Variety 1 showed genetic variations at very low levels (0.4-1.9% polymorphism among the markers present).

In experiment II, 588 RAF markers were scored across all the *Garcinia* species. Two of these markers were shared by all accessions. High levels of polymorphism were observed between the various species, with the exception of *G. griffithii* and *G. hombroniana*, which showed genetic relatedness in the same range as between the three mangosteen clones (Fig. 4).

Major phenotypic differences were observed between the mangosteen clones. Variety 2 and Variety 3 were known among some growers as the most distinctly different mangosteen trees growing in North Queensland. These accessions had similar tree shape, with branches pointing more upwards than those of normal mangosteen trees (represented

by most Variety 1 accessions). Variety 3 had not yet born fruit, however Variety 2 produced fruit with a more oblong shape and of larger size than normal rounded fruits (represented by most Variety 1 accessions).

Some of the accessions of Variety 1 that showed genetic variation (accessions 30, 32, and 50) had reportedly also different phenotypes (larger fruit, extremely poor growth, and different fruit season, respectively). The other accessions of Variety 1 showing different genotypes were all of normal phenotype. On the contrary, some accessions that reportedly had different phenotypes (accessions 18, 25, 26, 27, and 34) were genetically invariable.

DISCUSSION AND CONCLUSIONS

Traditional plant breeding and intensive farming systems have contributed to the narrowing of genetic resources in numerous crops, and extensive human impact in many areas is endangering a range of species. Conservation of biodiversity is therefore an essential issue in plant research, as maintenance of genetic diversity in plant populations is vital for adaptation to environmental changes. Studies to reveal the extent and distribution of genetic variation have thus become increasingly important (Palacios and González-Candelas, 1997). Moreover, genetic diversity and knowledge about the genetic diversity present in a species is necessary to aid plant breeding.

Our analyses have revealed low to moderate levels of genetic variation within *G. mangostana*, as well as high levels of genetic variation among the *Garcinia* species investigated. The genetic relationships determined among *Garcinia* species were not identical but consistent with results from the analysis of *Garcinia* species reported by Ramage et al. (2004) (a higher number of loci were screened in the latter study). Three clones were detected among the mangosteen accessions, and the degree of genetic variation observed between these clones was typical of variation between different cultivars. The genetic polymorphism observed among species and between clones of *G. mangostana* was associated with very specific morphological differences. The minor genetic differences observed within one of the mangosteen clones (Variety 1) were not associated with any major, distinct phenotypes. None of the mangosteen genotypes could be linked to a particular country of origin. However, the genetically varying mangosteen accessions were obtained from only four of 13 orchards, which could indicate an environment that promotes genetic instability at these plantations.

Considering the lack of genetic relatedness between mangosteen and the other wild *Garcinia* species investigated, it seems implausible that the variation among mangosteen trees in North Queensland can be due to cross-pollination from these species. *G. hombroniana* and *G. malaccensis* are regarded as the closest relatives and possible parents of mangosteen. We were unable to obtain *G. malaccensis*, however an accession of *G. hombroniana* was included in our study. Unexpectedly, this specimen appeared closely related to the *G. griffithii* accession, but not to *G. mangostana*. *G. hombroniana* and *G. griffithii* are not described as morphologically related; the former resembles mangosteen, producing a sour fruit with the flavour of peaches, while the latter yields an apple-like, very sour fruit (Burkill, 1966). We hold mislabelling of one of these accessions as an option, as we relied on others for identification of the original plant source, and we can therefore not exclude the possibility that both of these accessions may be *G. hombroniana* or *G. griffithii*. These two species appeared genetically closer to *G. mangostana* than did the other species, however the genetic distance to *G. mangostana* was larger than anticipated for a species that is regarded as the parent. There are possibly large genetic variations within *G. hombroniana*, which is a facultative apomict (Richards, 1990), and the accession we analysed could be genetically quite different to the *G. hombroniana* that may have been the original parent of mangosteen. Moreover, *G. hombroniana* germplasm may have been significantly altered during the centuries that have passed since mangosteen developed. The low relatedness between the investigated *G. mangostana* and *G. hombroniana* accessions may therefore not invalidate the theory of mangosteen's origin. Further characterisation of *G. hombroniana*, *G. mangostana* and *G.*

malaccensis germplasm may determine whether mangosteen is an interspecific hybrid between these two species, and could indicate whether the variation observed between the three mangosteen varieties is due to multiple hybridisation events between *G. hombroniana* and *G. malaccensis* during the development of mangosteen.

Chromosome counts reported for mangosteen have been very inconsistent (Richards, 1990), and may be indicative of aneuploidy. The less significant genetic variation detected within Variety 1 may be due to aneuploidy, and could be responsible for the minor morphological differences observed in this group. These differences might have performance implications, and thus cytological studies to reveal whether chromosomal instability may be a cause of variation in mangosteen would be valuable. However many of the differences in field performance reported by mangosteen growers are probably also caused by environmental factors. Environmental influence on gene expression in mangosteen could be further investigated by employment of a C-methylation sensitive DNA fingerprinting technique. Cultural practices such as shading of trees and pruning technique are also known to affect the performance and growing patterns of mangosteen trees, and would be worth further exploration.

Crop improvement in mangosteen is desired for more cost-effective and efficient production, and plant breeding strategies should be considered by mangosteen growers in Australia and other countries. Collaboration and exchange of plant material between countries may prove beneficial. The assumed parents of mangosteen, the Malaysian species *G. hombroniana* and *G. malaccensis*, represent desirable traits that are not present in mangosteen. For instance rapid growth and early heavy fruiting (5-8 years after planting) are qualities that are associated with *G. hombroniana*, but absent in mangosteen (Richards, 1990). Transfer of genes coding for such desirable traits to mangosteen would be very useful. However interspecific hybridisation in mangosteen has proved difficult (Richards, 1990), and could possibly be facilitated by in vitro techniques, e.g. protoplast fusion or embryo rescue techniques, as successfully carried out with *Carica papaya* and related species (Manshardt and Wenslaff, 1989; Drew et al., 1998). Protocols for regeneration of mangosteen plants in tissue culture have been reported by several authors (Goh et al., 1994; Normah et al., 1995; Te-chato and Lim, 1999, 2000). Establishment of a breeding program may take several generations and include an elevation of costs. However, DNA marker techniques are becoming more and more suitable for routine applications to tropical fruit crops (Henry, 1998), and may assist in plant breeding. The RAF technology has proven effective in research on mangosteen and could be used in further research, e.g. cultivar identification, and possibly as a tool for marker-assisted selection.

Our results disprove the theory put forward by some authors (Richards, 1990; Verheij, 1991), suggesting that all present day mangosteen trees may be derived from one original clone. The evidence that genetic diversity exists in mangosteen is promising for potential crop improvement. Further research to facilitate effective germplasm conservation of mangosteen and other *Garcinia* species is essential for exploration of the genetic resources in *Garcinia*.

ACKNOWLEDGEMENTS

We thank Rural Industries Research & Development Corporation (RIRDC), Australia, for supporting L. Sando through a travel/conference grant. J. McCormack, and other mangosteen growers in North Queensland, and Dr. M. Clyde at Universiti Kebangsaan Malaysia are gratefully acknowledged for supply of plant material. Dr. A. John Richards at University of Newcastle upon Tyne, UK, is appreciated for information about apomixis in Garcinia.

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Tables

Table 1. *Garcinia* samples examined.

Access-Ion nr.	Species	Field observations	Original seed source and year of planting (if known)	Current location
1[#]	G. mangostana	Grafted tree	Bogor, Java, Indonesia 1982[a]	Bellenden Ker
2[#]	*G .mangostana*	Normal	Bogor, Java, Indonesia 1982 [a]	Bellenden Ker
3*	*G. mangostana*	Normal	Bogor, Java, Indonesia 1985 [a]	Mirriwinni
4*	*G. mangostana*	Normal	Bogor or Madura, Java, Indonesia 1987 [a]	Mirriwinni
5*	*G. mangostana*	Normal	Java, Indonesia 1987	Mossman
6	*G. forbesii*	Monoecious form	Sarawak, Malaysia	Mossman
7	*G. griffithii*		Peninsular Malaysia	Mossman
8*	*G. mangostana*	Normal	Indonesia/Malaysia 1986	Mossman
10*	*G. mangostana*	Normal	Peninsular Malaysia	Mossman
11	*G. sp.* (unknown)		Kamerun	Mossman
13	*G. cambodia*		Borneo (Origin: Cambodia)	Mossman
14	*G. prainiana*		Northern Peninsular Malaysia	Mossman
15	*G. livingstonei*		East Africa	Mossman
16*	*G. mangostana*	Normal	Kuala Lumpur, Malaysia 1977	Daintree
17*	*G. mangostana*	Normal		Daintree
18*	*G. mangostana*	Narrower leaves, regular bearings, better crop		Daintree
19*	*G. mangostana*	Normal	1980-81	Daintree
20*	*G. mangostana*	Normal	1880	Mossman
21*	*G. mangostana*	Open tree, better crop (tree shaded)	Bogor, Java, Indonesia 1980 [a]	Mirriwinni
22*	*G. mangostana*	Dense tree, less crop (tree not shaded)	Bogor, Java, Indonesia 1980 [a]	Mirriwinni
23*	*G. mangostana*	Normal	Madura, Java, Indonesia 1986 [a]	Mirriwinni
24*	*G. mangostana*	Normal	Thailand 1990	Tully
25*	*G. mangostana*	Better crop	Bogor or Madura, Java, Indonesia 1987 [a]	Tully
26*	*G. mangostana*	Less crop	Bogor or Madura, Java, Indonesia 1987 [a]	Tully
27*	*G. mangostana*	Large fruit	Bogor or Madura, Java, Indonesia 1987 [a]	Tully
28*	*G. mangostana*	Normal	Thailand 1993-94	Kidner Bridge
29*	*G. mangostana*	Normal, but showed six months continuous flowering in 2000	Thailand 1975-77	Kidner Bridge
30	*G. mangostana*	Large fruit	[b]	Kidner Bridge
31	*G. mangostana*	Normal	Singapore 1992	Kidner Bridge
32	*G. mangostana*	Very poor growth	Bogor or Madura, Java, Indonesia 1997 [a]	Kidner Bridge
33*	*G. mangostana*	Good crop	Bogor or Madura, Java, Indonesia 1985 [a]	Silkwood
34*	*G. mangostana*	Early fruiting	1996	Silkwood
35*	*G. mangostana*	Normal	Bogor or Madura, Java, Indonesia 1982 [a]	Mena Creek
36*	*G. mangostana*	Normal	1975-81	Innisfail
37*	*G. mangostana*	Normal		Innisfail
39*	*G. mangostana*	Normal		Innisfail
41	*G. mangostana*	Distinctly different tree shape	Bogor or Madura, Java, Indonesia 1991 [a]	Innisfail
42	*G. mangostana*	Distinctly different tree shape	Bogor or Madura, Java, Indonesia 1991 [a]	Innisfail
43	*G. mangostana*	Normal	Bogor or Madura, Java, Indonesia 1991 [a]	Innisfail

44	*G. mangostana*	Distinctly different tree and fruit shape	Borneo 1994	Babinda
45	*G. mangostana*	Normal	Singapore 1991	Babinda
46	*G. warrenii*		Babinda, North Queensland, Australia	Bartle Frere
48	*G. dulcis*	Sour yellow mangosteen		Innisfail
49*	*G. mangostana*	Fruit season different from 50		Cairns
50[#]	*G. mangostana*	Fruit season different from 49		Cairns
51	*G. hombroniana*			Kebangsaan, Malaysia

[#] No genetic variations observed
* No genetic variations observed
[a] Tree obtained from the same nursery

Table 2. RAF primers (Operon Technologies).

Primer Name	**5'-3' Sequence**
AB-16	CCCGGATGGT
AE-11	AAGACCGGGA
AO-12	TCCCGGTCTC
AP-20	CCCGGATACA
AV-03	TTTCGGGGAG
BB-18	CAACCGGTCT
PO-5	CCCCGGTAAC
W-15	ACACCGGAAC

Figures

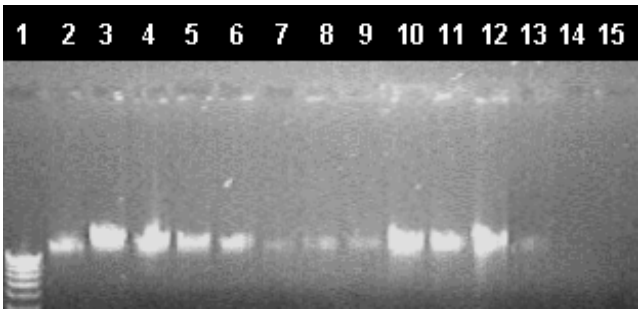

Fig. 1. Comparison of DNA yields extracted from young and mature mangosteen leaves.
Lane 1: DNA marker – greatest fragment 12,000 bp
Lane 2: 100 ng lambda DNA (weight standard)
Lane 3: 250 ng lambda DNA (weight standard)
Lanes 4-6: 1/33 of the total DNA extracted from ~1 gram of young mangosteen leaf tissue (~ 8 μg) (replicates)
Lanes 7-9: 1/10 of the total DNA extracted from ~1 gram of mature mangosteen leaf tissue (~ 0.5 μg) (replicates)
Lanes 10-12: 1/33 of the total DNA extracted from ~1 gram of young mangosteen leaf tissue (~ 8 μg) (replicates)
Lanes 13-15: 1/10 of the total DNA extracted from ~1 gram of mature mangosteen leaf tissue (< 0.5 μg) (replicates)

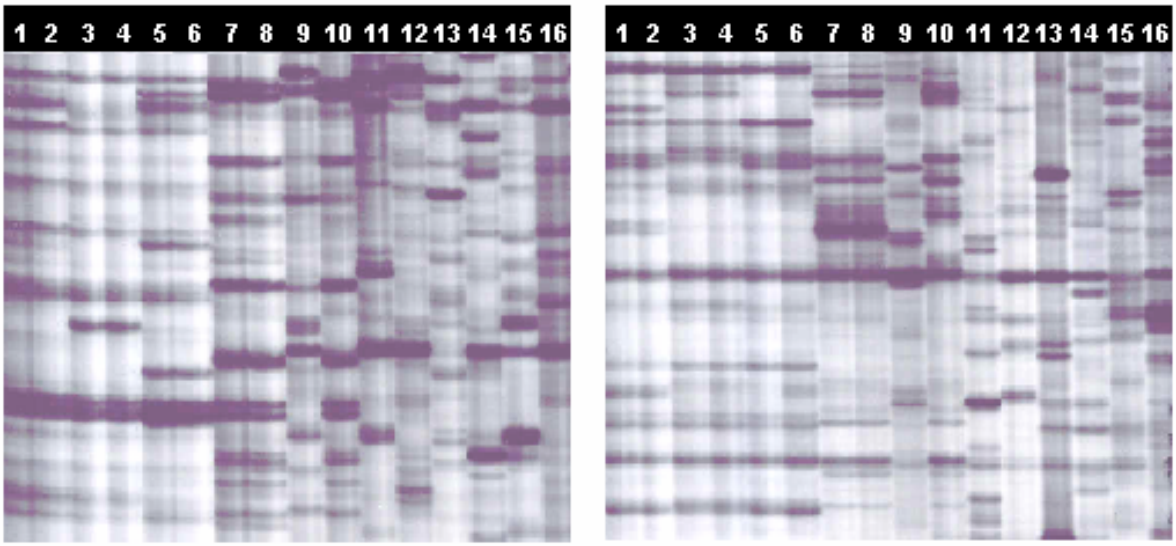

Fig. 2. Sections of RAF profiles for primers AB-16 (left) and AP-20 (right). Fragment range: 300-175 bp on 4% polyacrylamide gels.
Lanes 1-2: *G. mangostana* 1 Lane 11: *G. sp.* (unknown)
Lanes 3-4: *G. mangostana* 3 Lane 12: *G. cambodia*
Lanes 5-6: *G. mangostana* 2 Lane 13: *G. prainiana*
Lanes 7-8: *G. hombroniana* Lane 14: *G. livingstonei*
Lane 9: *G. forbesii* Lane 15: *G. warrenii*
Lane 10: *G. griffithii* Lane 16: *G. dulcis*
G. mangostana 1-3: refer to corresponding labels in Figure 4.

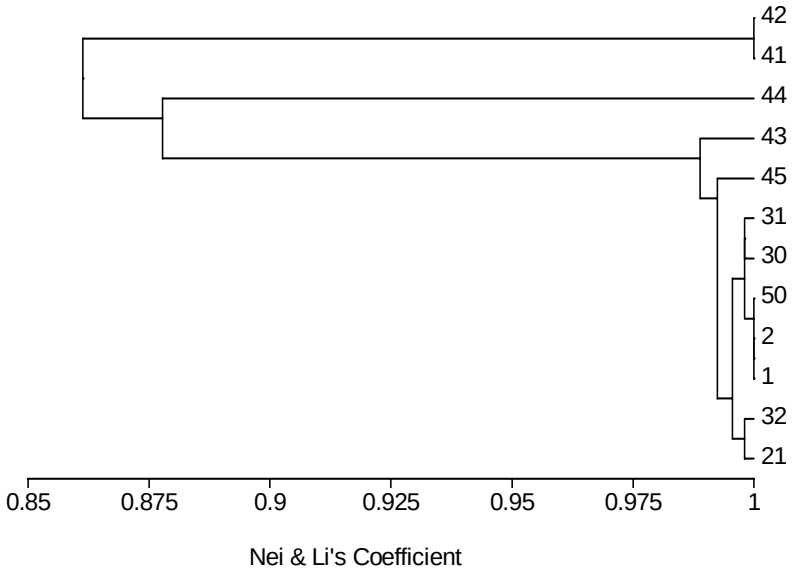

Fig. 3. Genetic relationships in mangosteen. UPGMA dendrogram obtained from RAF data, constructed by Nei and Li's coefficient. The numbers identifying each of the branches in the dendrogram refer to accession numbers. Accession 21 represents 26 identical accessions (identified by * in Table 1.). Three clusters identified among *G. mangostana* accessions: Variety 1; accessions 21-43, Variety 2; accession 44, Variety 3; accessions 41 and 42.

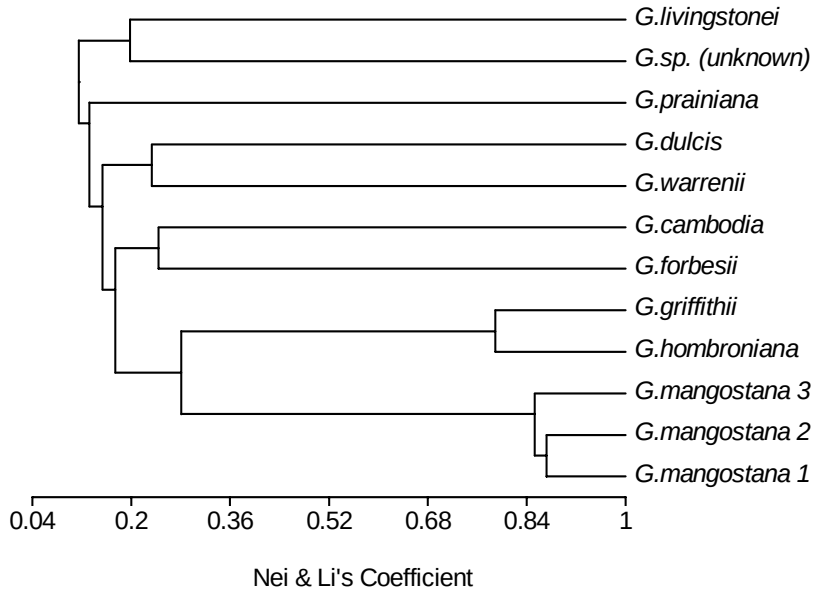

Fig. 4. Genetic relationships in *Garcinia*. UPGMA dendrogram obtained from RAF data, constructed by Nei and Li's coefficient. The numbers identifying each of the branches in the dendrogram refer to accession numbers. Each of the three mangosteen varieties (*G. mangostana 1-3*) is represented.