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# Genetic Diversity of Etamba in Sri Lanka

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**ABSTRACT**

***M. zeylanica* is endemic to Sri Lanka as a wild mango species. RAPD analysis was used to assess the genetic diversity of *M. Zeylanica*. Primer OPA -12 was the most useful in genetic diversity studies of Etamba. Intra- specific variation was found in the population of *M. zeylanica* even within the same climatic zones.**

**Keywords :** Endemic species, Genetic diversity, *M. Zeylanica,* RAPD

## INTRODUCTION

*Mangifera zeylanica* (Blume) Hook is endemic to Sri Lanka as a wild species commonly known as 'Etamba` (Dassanayake and Fosberg, 1983). It is a slow growing, large plant and bears edible fruit in abundance, but it is not a cultivated species. Etamba is mainly found in Intermediate Zone and Wet Zone forests and conserved *in situ* under the management of the Forest Department. However, the natural population of Etamba is declining in unreserved areas due to destruction of forests and also threatened by habitat loss (Toby, 2002). In these areas, Etamba occurs as scattered plants so that many people do not recognize their inherent value apart from the utility value (timber, firewood, medicine) and thus are not interested in their maintenance and propagation (Kostermans and Bompard, 1993). Mukherjee (1985) has listed *Mangifera zeylanica* as one of the threatened species in the world.

It has been reported that the *M. zeylanic*a species also has some several variations in relation to the fruit morphology and quality. Some have larger fruits than others, some are sweet and others sour when ripe, some have very little and others have more of the edible pulp. The loss of genetic diversity of Etamba has also been accompanied by loss of their genes that may be useful in the future emphasizing the need for conservation.

Identification of genetic diversity of Etamba is important for a successful breeding program. Assessment of diversity traditionally has been through morphological characters, which has often found to be less effective. This problem is further compounded by the perennial nature of the mango crop. Therefore, the use of molecular methods could be an efficient way to identify genetic diversity of Etamba. Among the molecular markers, Random Amplified Polymorphic DNA (RAPD) markers are simple, versatile, relatively inexpensive and only nanogram quantities of DNA required (Williums *et al.,* 1990).

RAPD has been used for both genetic characterization and estimation of genetic diversity in mango (Bally *et al.,* 1996; Hemanth kumar *et al.,* 2001; Lopex *et al.,*

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1997; Ravishanker *et al.,* 2000; Schnell and knight 1993; Weeraratne *et al.,* 2005). However, little is known about the genetic diversity of Etamba. Therefore, the objective of this paper is to assess the genetic diversity of Etamba by using RAPD markers.

## MATERIALS AND METHODS

Leaf samples of *M. zeylanica* were collected from six plants in five locations to cover the Wet Zone (Ratnapura, Kandy, Peradeniya and Gampola) and Intermediate Zone (Bibile) during the period of January to February 2004. Two samples were able to collect from Gampola and one of them (Gampola 2) known as 'Rathamba' suspected as another Etamba also included in this study. Fruits were collected whenever available and possible morphological characters were recorded using IPGRI descriptor list for Mango.

Total genomic DNA was extracted from both tender and mature leaves of each sample using CTAB method described by Doyle and Doyle (1987) with modifications. Small scale DNA extraction was done using 200 mg of fresh tissue of each variety. DNA concentration and purity were determined by both spectrophotometrically and by visualization of DNA resolved by electrophoresis on agarose gels stained with ethidium bromide. The DNA of each sample was quantified based on UV absorbency measurements.

RAPD analysis was carried out using five arbitrary oligonucleotide primers (OPA-8, OPA-12, OPA-15, OPA-18, and OPA-19) obtained from Operon Technologies Inc. USA. DNA amplification was achieved by the protocol outlined by Williams *et al*., (1990) with slight modifications. The RAPD reactions were carried out in 25 µl volume consisting 25 ng of template DNA, 1x1.5 mM *Taq* reaction buffer (pH 8.3), 1.2 mM dNTPs and 1.2 mM primer, 2.5 U *Taq* polymerase (Takara Schuzo Co; Ltd, Japan). Amplification was performed in a thermal cycler for 40 cycles after an initial denaturation at 94 $^0$C for 3 min. In each cycle, denaturation for 1 min at 93 $^0$C, annealing for 3 min at 35 $^0$C and extension for 2 min at 72 $^0$C was programmed with a final extension step at 72 $^0$C for 10 min after the 40 cycle. Negative control was used initially to check the fidelity of PCR reaction. Amplified DNA fragments were separated out on 1.4% agarose gel stained with ethidium bromide (0.5 µg ml$^{-1}$).

RAPD bands were scored for presence (1) or absence (0) of by visual inspection of gel photographs. The sizes of RAPD products were estimated by comparison with 1 kb ladder. The cluster analysis and genetic distances performed by computer software POPGENE version 1.31 (Francis and Boyle, 1999) using unweighted pair-group methods with arithmetic averages (UPGMA) and Nei's genetic distance was used for the RAPD analysis.

## RESULTS AND DISCUSSION

All leaf samples of Etamba had matured except for the sample collected from Peradeniya. The OD value of DNA obtained from these samples was 1.1 to 1.6 (260 nm per 280 nm). The quality of these DNA was also confirmed by gel electrophoresis. According to Sambrook *et al.,* (1989), the OD valve of these samples found to be below 1.8 denoting that the DNA could have been contaminated with protein or phenol. Hence, this protocol adopted in this study should be improved to avoid polyphenol contamination for further studies.

Six individuals collected from Wet zone and Intermediate zones were screened with five random primers. Three out of five primers that revealed clear polymorphic amplification pattern were selected for genetic analysis. The

gel pattern for the primer OPA12 is shown in Figure1. The number of bands for each primer varied from 2 to18 and the size of bands ranged from 450 to 2000bp. Out of 25 bands 15 were polymorphic and shared among at least two individuals; 9 bands were polymorphic and unique and one band was monomorphic (Table 1). Figure 1 shows eight unique bands against primer OPA-12, of which bands 2.8 kb, 2.1 kb and 1.7 kb specific to individual of Peradeniya; bands 2.7 kb and 800bp specific to Ratnapura; band 400bp specific to Gampola II (Rathamba) and 200bp specific to Kandy.

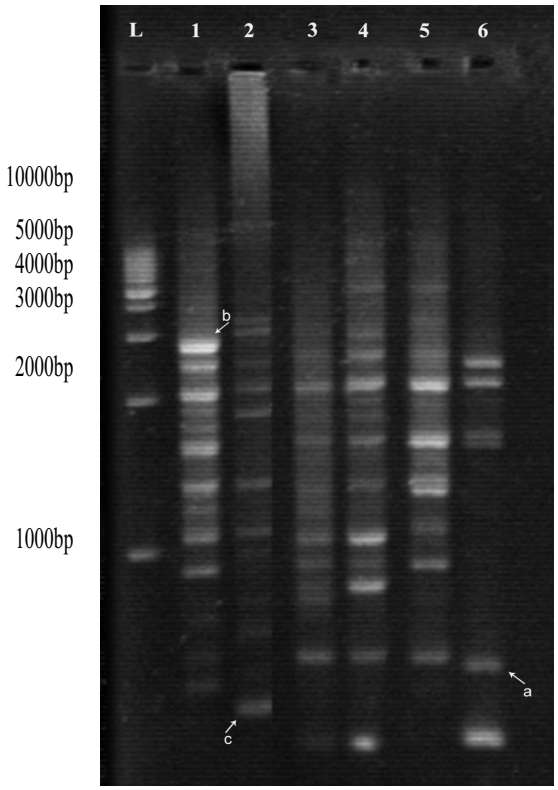


**Fig. 1.: RAPD profile for *M. zeylanica* obtained with OPA-12 Primer**

**L- 1 kb Ladder, 1- Peradeniya, 2- Kandy, 3- Bibile, 4- Rathnapura, 5- Gampola 1, 6- Gampola 2**

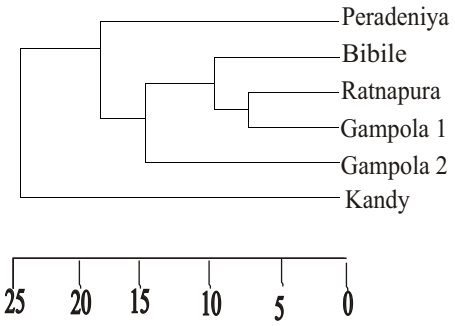


**Fig. 2:** Dendogram of six individuals of M.zeylanica Using RAPD

All 25 RAPD markers were analyzed to assess the intraspecific variation of *M. zeylanica* species. A dendrogram based on Nei's (1978) genetic distance (Figure 2) revealed that the six individuals were grouped into one cluster, with two distinct individuals of Kandy and Peradeniya. The Etamba collected from Bibila (IZ) appeared to be mixed with Etamba collected from Wet zone in the cluster analysis and was closely connected with Ratnapura and Gampola I. Etamba from Ratnapura and Gampola 1 were more closely related when compared to other individuals of Wet zone. Rathamba collected from Gampola was similar to Etamba. This is the first report of Etamba which is bearing purplish red fruit with prominent beak. According to RAPD analysis, Etamba collected from Bibila (IZ) and those collected from Gampola 1 and Ratnapura (WZ) were closely related to each other even they are different from some morphological characters. However, variation found in Wet zone population could not be compared with Intermediate zone due to limited number of sample collected from IZ.

The genetic distance of individuals of *M. zeylanica* was estimated based on RAPD data using Nei`s genetic distance. Genetic distance between individuals of Wet zones and

**Table 1: RAPD markers produced by selected primers for *M. zeylanica***

| Primer | Primer sequence | Polymorphic bands | | Monomorphic bands | Total |
|---|---|---|---|---|---|
| | | Shared | Unique | | |
| OPA-12 | TCGGCGATAG | 10 | 8 | 0 | 18 |
| OPA-15 | TTCCGAACCC | 4 | 1 | 0 | 05 |
| OPA-18 | AGGTGACCGT | 1 | 0 | 1 | 02 |
| Total | | 15 | 9 | 1 | 25 |

Intermediate zone ranged from 0.223 to 0.916. The lowest genetic distance (0.223) was observed between individuals of Ratnapura and Gampola I which belongs to Wet Zone. The highest genetic distance (0.916) was also observed from Wetzone in between Etamba of Ratnapura and Kandy. A genetic variation exhibited within the population of *M. zeylanica* in same climatic zones (Wet zone) even within the small scale distance eg. Kandy, Peradeniya and Gampola m ay emphasize the relatively wider diversity in the gene pool of *M. zeylanica*.

Leaf characters (leaf texture, leaf margin and leaf shapes) are almost same in all collected samples except leaf tips. All the leaves were thickly coriaceous in texture, lanceolate in leaf shape with flat margin. Individuals collected from Peradeniya, Bibile and Gampola 1 had obtuse leaf tips while others acuminate (1mm- 3 mm) from a rounded apex of the leaves (Figure 3). Fruit bearing period of the tree is October to January in Intermediate zone while those from Wet zone vary from January to March. Fruits could be collected from all the individuals except those from Kandy and Peradeniya. However, the matured fruits could be found only from Bibile indicating that early fruiting observed in IZ. The fruits of Bibile are round in shape about 4.5 cm in length and about 3.5 in width. The skin colour of immature fruits is green and becomes red at maturity. Fruit is fully ripe when it is dropped. However, skin of immature fruit shown in Figure 4 is infected with scab like fungal disease. Unripe fruits collected from Bibile are slightly acidic while ripe fruits are very juicy and fruit can be suck out. The skin colour of Rathama is purplish red even at immature stage, 4-5 cm in length and having a prominent beak. Immature fruits collected from Ratnapura and Gampola 1 are green (Figure 4). It was reported that Etamba in Jaffna and Batticaloa districts of Dry zone are green, small elongated fruits each about 3.8 cm long and 2 cm in wide (Paul and Gunerathnam , 1938). Similar characters reported from Knuckles range belongs to IZ. The value of their inherent characters still not discovered, there may be one or more useful traits that can be used to enrich the cultivated mangoes. Hence, Diversity and distribution of *M. zeylanica* need to be studied in details incorporating more collections represent in three climatic zones and mapped their diversity for future conservation or research purposes.

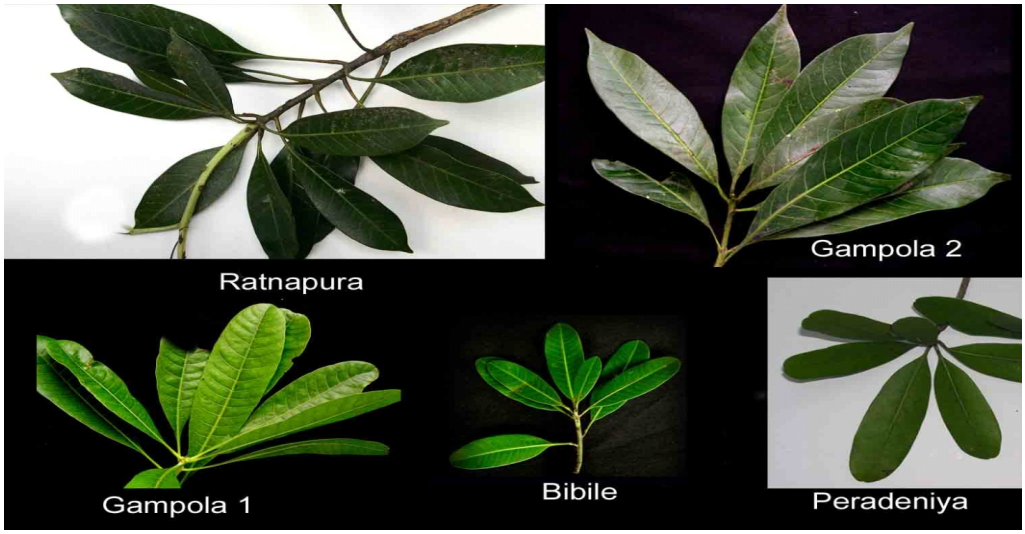


**Fig. 3 : Leaf characters of *M. zeylanica* collected from different location**

**Flat margin, lanceolate, Obtuse ends - Peradeniya, Bibile and Gampola**
**Flat margin, lanceolate, acuminate ends - Kandy, Ratnapura and Gampola 2**

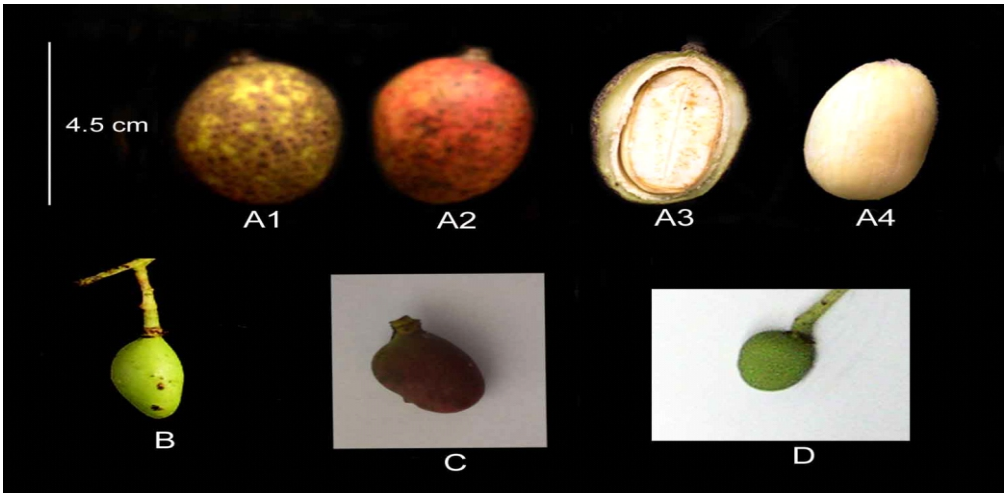


**Fig. 4 : Fruit characters of *M. zeylanica* collected from different location**

**A1- Immature fruit in Bibile, A2- Mature fruit, A3- Cross section of immature fruit,**

**A4- Stone of fruit, B- Immature fruit in Gampola 1, C- Immature fruit of Rathamba**

**D- Immature fruit of Ratnapura**

## CONCLUSIONS

RAPD markers could be used as an efficient tool for estimating genetic diversity in *M. zeylanica* than morphological markers. Primer OPA-12 was the most useful in genetic diversity studies of Etamba. Rathamba collected from Gampola

with purplish red fruit and prominent beak was identified as another Etamba tree. Intra-specific variation was found in the population of *M. zeylanica* even within the same climatic zones. This strongly implies the existing rich diversity and the importance of conserving endemic species for future utilization.

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