Article

# Tamarindus Indica Tropical Populations Genetic Structure

**Nyadoi P[1,5], Jamnadass R[5]., Okori P[1], Okullo JBL[1], Obua J[1], Magogo Nasoro[2], Haji Saleh[3], Pushpakumara D.K.N.G[4], Roshetko J[5], Kalinganire A[5], Muchugi, A.[5], Temu A.B[5], Fluch S[6] and Burg K[6].**

[1]Makerere University P.O.BOX 7062 Kampala Uganda.
[2]Tanzania Forestry Research Institute P.O.Box 95 Luchoto, Tanzania
[3]Kizimbani Agricultural Research and Training Institute, P.O.Box 159, Zanzibar
[4]Faculty of Agriculture, University of Peradeniya, Peradeniya 20400 Sri Lanka
[5]World Agroforestry Centre (ICRAF), United Nations Avenue, Gigiri PO Box 30677-00100 GPO, Nairobi, Kenya and Austrian Institute of Technology Department of Environment and Health formally
[6]Austrian Research Centers GmbH–ARC, Department of Genetics and Bioresources, A-2444 Seibersdorf, Austria

Received April 5, 2010
Accepted May 2, 2010

**Abstract**

*Tamarindus indica* L. (tamarind) is a tropical tree species widely managed for fruit and other exported products in countries like India and Thailand. In Africa tamarind was earmarked for livelihood diversification however, conservation strategies, products/markets were not yet developed moreover, unsustainable utilisation and habitat losses has led to its populations and expectedly genetic resources erosion. Additionally, because tamarind population structure was not yet well defined even globally, knowledge on genetic structure requisite to classify the extent and nature of genetic erosion and thus conservation needs/strategies for its populations was lacking. The objective of our study was to generate knowledge on tamarind populations genetic structure which we hypothesised was influenced by habitats, latitudes and isolation by distance. We studied polymerase chain reaction–restriction fragment lengths polymorphisms (PCR-RFLP) in the slow evolving, organelle genomes of 311 tamarind sampled on-farm, woodland and riverbanks in island-mainland, higher-lower latitudes of nine geographic regions. Analysis revealed significant among geographic regions mitochondria genetic structure ($\Phi ST = 0.64$) but only one of its 6 haplotypes was global (overall frequency, 59.6%), one restricted to Kenya, Tanzania, Mexico (overall frequency, 12.8%) and the rest endemic to Kenya (2), Indonesia (1) and Thailand (1). The chloroplast was conserved ($\Phi ST \leq 0.02$), only one of its 16 haplotypes was global (overall frequency of 94%), 14 were rare endemics of East Africa and one restricted to Kenya-Indonesia (overall frequency<1%). Cytotypes genetic structure was significant ($\Phi ST = 0.49$) but with 51% variability within geographic populations and only one of the identified 20 was global (overall frequency, 51.89%), one was restricted to Burkinafaso, Indonesia, another one to Kenya, Tanzania, Mexico while the rest were endemic to East Africa and one in Thailand. Clearly, geographic population specific conservation strategies are needed for tamarind and the East African populations are worthy conservation priority as centre of diversity.

Key words: Conservation, chloroplast, mitochondria, genetic resources, *Tamarindus.*

## INTRODUCTION

Tamarind is a multipurpose, diploid (2n=24), hermaphrodite, out crossing tree of Fabaceae subfamily Caesalpiniaceae, Amherstiaeae tribe mono species genus Tamarindus (Nagarajan et al., 1998;Polhill and Raven 1981 in Diallo et al., 2007). Its fruit, wood and other products, shade and soil fertility enhancement services are utilized worldwide and exported in countries like India and Thailand (Yoneyama et al., 1993; Gunasena and Hughes, 2000; Nyadoi, 2005; Elsidig et al., 2006). In Africa, because of past low priority and research attention, no conservation strategies were yet in place for tamarind, its populations and expectedly genetic resources were declining under unsustainable utilisation and habitats loss (FAO, 2004; Nyadoi, 2005). Additionally because tamarind population structure was not yet fully defined; knowledge on genetic structure required to determine the nature and extent of genetic erosion in its populations and consequently their conservation needs and strategies was lacking.

Random amplified polymorphic DNA (RAPD) studies on tamarind in the recent past revealed high genetic diversity in East Africa populations with close relationship to Indian populations. However because of the shortcoming associated with RAPDs (Lowe et al., 2004) and also sampling limitations, specific conservation needs and management strategies required to enable sustainability of tamarind populations even within East Africa were not identified. In East Africa and in some other African countries, farmers prioritised tamarind conservation/product development to support livelihoods (FAO, 2004; Jama et al., 2005). Thus, there was need to generate detailed knowledge on tamarind populations genetic structure to guide identification of conservation needs and strategies required to enable sustainability of its populations for livelihood benefits. Therefore the objective of our study was to generate knowledge on tamarind populations' genetic structure. We hypothesized genetic structure in tamarind was influenced by habitats, latitudes and isolation by distance and investigated this using neutral genetic markers in the slow evolving recombination independent organelle (chloroplast and mitochondria) genome. The organelle genome based genetic markers instead of the highly evolving nuclear genome, in order to enable the long term evolutionary dynamics of tamarind genetic resources.

**MATERIALS AND METHODS**

**Study area**

To enable comparative genetic evolution assessment in postulated native, introduced and diverse environment populations; East -West Africa, South-South East Asia and Central America representing the geographic regions of postulated origin and introduced populations of tamarind worldwide were included as study sites (Figure 1). The samples were collected from islands (Sri Lanka, Indonesia, Lamu, Zanzibar) and mainland (Uganda, Tanzania, Kenya, Sahel Africa, Thailand and Mexico) on-farm, woodland and riverbank habitats in higher latitudes in the North above equator and lower latitudes in the South below equator. Within East Africa, diverse environments were included- climatic zones (areas with temperature range of 20 to 29 ºC, rainfall of less than 500 mm in semi arid–arid Kenya to about 2000 mm per annum in Tanzania and Uganda), vegetation types-Zanzibar Inhambane (Mombasa, Kilifi, Malindi, Lamu and Tanzania's Zanzibar), Somalia Masaai (Kitui, Tharaka, Baringo, Pokot and Samburu districts in Kenya), Lake Victoria regional, Sudanian and Guineo-Congolia (Gulu, Nebbi and Arua in Uganda) and the Zambesia Phytocoria in Tanzania (White, 1983) and position in the West and Eastern junctions of the Rift Valley. The diverse environments-sites from which tamarind were sampled n East Africa is shown elsewhere (Nyadoi et al., 2010). The sample size (number of tamarind trees) from which leaf and or seeds were collected from each of the studied sites are shown in table 1 below.

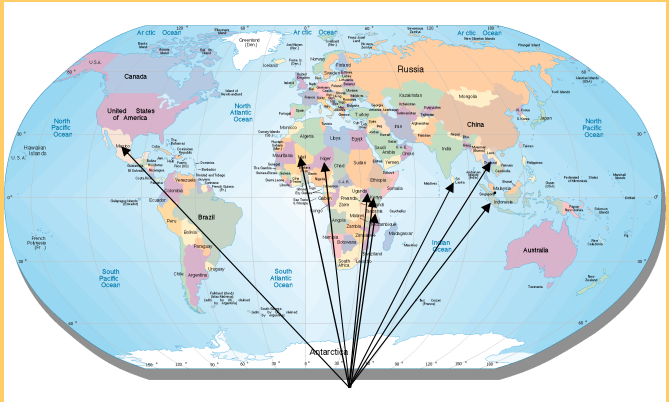



Figure 1. Global populations of tamarind sampled for genetic structure studies

Table 1. Number of tamarind trees from which leaf samples were collected in Africa, Asia and Central America

| Study site | Number of tamarind from which leaf were sampled | Seeds | Latitudes |
|---|---|---|---|
| Uganda | 61 | | 0-30ºC North |
| Tanzania | 58 | | 0-30ºC South |
| Kenya | 91 | 28 seeds from 2 trees | 0-30ºC South- 0-30ºC North |
| Niger/Burkina | 8 | | 0-30ºC North |

| | | | |
|---|---|---|---|
| Indonesia | 20 | | 0-30ºC North |
| Thailand | 11 | | 0-30ºC North |
| Sri Lanka | 40 | | 0-30ºC North |
| Mexico | | 20 seeds from fruits in supermarkets | 0-30ºC North |

Sampling strategy

Tamarind was sampled on-farm (on-farm here refers to farmlands, compounds of homes, administrative offices, community centres, schools, churches, mosques, trading centres and markets) and in wild habitats (riverbanks and woodlands). In each habitat, the first encountered tamarind was sampled and subsequent ones sampled at random systematic intervals ≥ 500 meters apart and ≤100 m apart where tamarind were found growing in pure stand. Where no tamarind was found at a given 500m interval, the next tamarind encountered would become the next sample regardless of distance. Environment and geographic information systems (GIS) data; latitudes, longitudes, altitudes coordinates were recorded using geographic position systems equipment (GPS model Garmin 3A, Garmin International, Inc. 1200 E. 151st Street, Olathe, KS 66062-3426, Kansas City metro area), the habitat, country, mainland or island status was recorded on site and the mean annual temperature and rainfall obtained from the GIS unit of World Agroforestry Centre-the International Centre for Research in Agroforestry (ICRAF), Nairobi Kenya.

In East Africa, healthy leaves were collected from sampled tamarind, the leaves were cleaned using clean cotton wool with distilled water and ethanol, and then packed in air tight plastic bags with silica gels. Mature tamarind fruit were also collected from two trees in Kenya and packed in airtight plastic bags but without silica gels. The leaf and fruit samples were transported to ICRAF and kept in storage for two months, in a refrigerator maintained at -20ºC. The leaf and fruit samples were then taken to Austria Research Centres Platform for Integrated Plant Clone Management Unit-PICME (ARC) where they were stored at 4ºC until DNA extraction and analysis. Leaf from the other countries were sampled by experts and sent to ARC lab. Niger/Burkinafaso samples were clones at ICRAF Sahel gene bank.

**DNA extraction and PCR**

Prior to DNA extraction, seeds were removed from fruits, pre-treated (washed with milique water and then incubated for some minutes in a water bath at 70ºC) to break dormancy. The seeds were then placed in moistened paper towels in Petri dishes and incubated in 25 ºC germination chamber to germinate. Seeds germinated within 2 weeks to 2 months. The seeds' embryos were extracted, placed in micro centrifuge tubes, frozen in liquid nitrogen for few seconds and stored at -20 ºC until DNA extraction. Extraction of DNA was carried out on 25-30 mg of leaf material and from each embryo in 2 ml microcentrifuge tubes following QIAGEN DNeasy plant minikit and extraction protocol (DNeasy Plant Kit Qiagen 2006) with slight modifications. Two ball bearings were added to each leaf/embryo in the centrifuge and frozen in liquid nitrogen for a few seconds before grinding in a Retsch mill (Retsch GmbH, Rheinischestraße 36, 42781 Haan, Germany) at 30 revolution cycles per second for 5 minutes. 400 µl buffer AP1 (lysis buffer) and 4 µl RNase were added to ground leaf/embryo material, vortexed to mix and incubated for 20-30 minutes at 65ºC (instead of 10 minutes as per Quiagen protocol) to enable cell wall break down. 100 µl of TRIS pH8–low salt buffer (instead of AE buffer from QIAGEN) was used to elute DNA (this modification was from experience of PICME Lab group that buffer AE interferes with PCR amplifications). The DNA elutes were stored at 4°C until quantity and quality tests. DNA were quantified using Nanodrop spectrophotometer according to manufacturers protocol (Nanodrop ND 1000, Rockland USA), verified for quality using 1% agarose gel electrophoresis in 1XTBE buffer run at 100V for 60 minutes and visualised using ethidium bromide stained gels on a UV light image documentation system (ASA, 3000). After genomic DNA quantity and quality verification, DNA quality for all samples were further tested for PCR amplification with 18s specific primer; The PCR master mix preparation included 20Nm dNTP, 4Mm primer, 50ng/µl DNA, 0.5 unit DNA polymerase (HOT START, QIAGEN), 1x PCR buffer (50 Mm KCL, 10 Mm Tris.CL and 1.5 mM MgCl2). The PCR amplification was carried out in a 25 µl reaction volume. The PCR involved hot start at 96ºC for 15', 96ºC for one min and 50-65 ºC (depending on the primer combination) for 1 min, and finally at 72 ºC for 1 min and a final extension at 72ºC for 10 min. All reactions were performed in PCR Biometra thermocyclers (Hoffmann-La Roche Ltd, USA). The reaction cycles were repeated 35 times. The amplicons were kept at 4 ºC or for longer time at -20ºC until further analysis. Verification of 18s amplicons was done using 1% agarose gel

electrophoresis. DNA extraction and quality tests were repeated for samples that did not amplify or those that had faint amplifications. SHAPE * MERGEFORMAT Mitochondrial and chloroplast specific primers were then tested for amplification and polymorphism in tamarind (appendix 1). The PCR master mix was prepared as for 18s ribosomal locus but with appropriate components' units as per each primer combination. Out of thirty five primers tested, only six; trnG, psbA and rplf2 genes found in the large single copy region of the chloroplast genome (McCoy et al., 2008) and Mitochondria Cox 11 loci amplified well.

**Restriction digestion of PCR products**

A pre-test of PCR amplicons was performed using 12 restriction enzymes; Alu1, BamH1, Cfo1, ECoR1, Hae111, Hind 111, Hinf1, HPA11, Mnl1, Sau3A1, Xho1 and Taq according to manufacturers' protocol (NEW ENGLAND Biolabs Inc, USA). Digestions were carried out in 20 µl reaction volumes at 37ºC and enzymes which generated polymorphic restriction products for each primer combination were selected (Table 2).

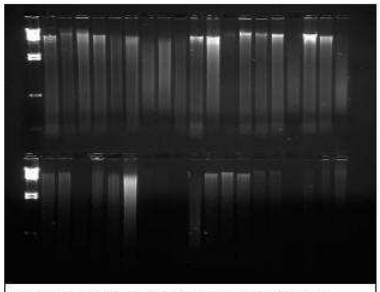
Table 2. Primers and restriction enzymes used for tamarind genetic diversity studies

| Primers | primer sequence | Enzyme | Plastid genome |
|---|---|---|---|
| Cox 11 F | 5'-TAGRAACAGCTTCTACGACG-3' | Alu1 | Mitochondria |
| Cox 11 R | 5'-GRGTTTACTATGGTCAGTGC-3' | Alu1 | Mitochondria |
| Nad9 F | 5'-GGTCATCTCAATTGGGYTCAG-3' | Mnl1 | Mitochondria |
| Nad9 R | 5'-TATAGTTGGGAGACTTTACC-3' | Mnl1 | Mitochondria |
| Rpl2F | 5'-ACCGATATGCCCTTAGGCACGGC-3' | BFCU1 | LSC chloroplast |
| TrnH-M5 R | 5'-GTGAATCCACCAYGCGCGGG-3' | BFCU1 | LSC chloroplast |
| Rpl2F | 5'-ACCGATATGCCCTTAGGCACGGC-3' | Hinf1 | LSC chloroplast |
| TrnH-M5 R | 5'-GTGAATCCACCAYGCGCGGG-3' | Hinf1 | LSC chloroplast |
| Trng2 III F | 5'-GTTTAGTGGTAAAAGTGTGATTCGTT-3' | Hinf1 | LSC chloroplast |
| Trng1 R (2) | 5'-CCGCATCGTTAGCTTGGAAGGC-3' | Hinf1 | LSC chloroplast |
| Trng2 III F | 5'-GTTTAGTGGTAAAAGTGTGATTCGTT-3' | BFCU1 | LSC chloroplast |
| Trng1 R (2) | 5'-CCGCATCGTTAGCTTGGAAGGC-3' | BFCU1 | LSC chloroplast |
| PSB A3 (7) F | 5'-TACGTTCRTGCATAACTTCC-3' | Hinf1 | LSC chloroplast |
| PSB5A (8) R | 5'-CTAGCACTGAAAACCGTCTT-3' | Hinf1 | LSC chloroplast |

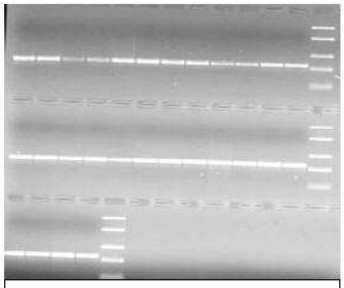
Following identification of polymorphic primers after restriction of their products, all 311 tamarind DNA were subjected to PCR and restriction digest, their products were prepared and subjected to electrophoresis in 8% non denaturing polyacrylamide gel (PAGE) and their visualisation was done on silver staining (Csaikl et al., 2002).

**PCR-RFLP data management**

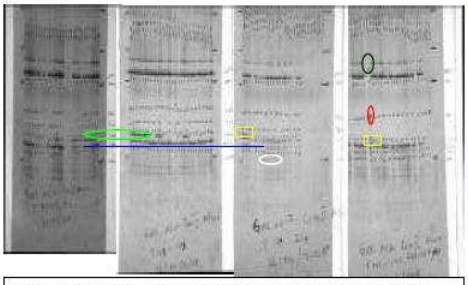
The restriction digest products were scored using a binary system of zero (0) for absence, one (1) for presence of restriction fragments. The figures below show some of the polymorphic restriction fragments obtained in the two investigated genomes for tamarind.



Genomic DNA of the tamarind from different populations



PCR products (NAD9) of the tamarind from different populations



Marked with oval, circles, rectangles and lines are the Cox 11-Alu 1 polymorphic restriction fragments scored for and used to generate haplotypes in the mitochondria genome of the tamarind

Marked with lines are some Rplf2 + trnHM-5 with Mnl1 polymorphic restriction fragments scored for/used to generate haplotypes in the chloroplast genome of tamarind from populations

Each tamarind restriction digest product was scored for the 29 and 7 restriction fragments generated from three chloroplast and one mitochondria loci respectively; (trnG digested with Hinf1, rplf2 digested with Hinf1, BCU and psbA digested with Mnl1, Hinf1) and Cox 11 digested with Alu1. The binary data from all restriction fragments were combined to generate haplotypes (a total of 6 for mitochondria and 16 for chloroplast) from 7 and 29 restriction fragments respectively. The mitochondria and chloroplast haplotype for each tamarind were combined to generate cytotypes and a total of 20 different were identified.

**Data analyses**

Frequencies and percentages overall and within populations were calculated for each of the identified six mitochondria haplotypes, 16 chloroplast haplotypes and 20 cytotypes. The calculations were performed in Microsoft Excel Computer Programme, tabulated and described. The haplotypes and cytotypes data matrix were then converted to Arlequin (Excoffier et al., 2005) and FAMD (Schlutter and Harris, 2006) Genetic diversity analysis soft ware programe compatible file format. Analysis of molecular variance (AMOVA) was then performed in both programmes to comparatively define genetic structure in tamarind within and among ; (i) Geographic regions, (ii) mainland and islands, (iii) lower and higher latitudes of Equator (iv) wild (woodland and riverbanks) and on-farm habitats. The two analytical soft ware programmes gave similar results however only FAMD results are presented in the results section here.

**RESULTS**

**Haplotypes and cytotypes found in tamarind mitochondria and chloroplast genomes**

From the 265 tamarind restriction digest products of Cox 11-Alu1, six haplotypes were identified and of these only one was widespread globally with an overall populations frequency of 59.62% and 100% within Niger, Uganda and Sri Lanka (Table 3). One haplotype was restricted to Kenya, Tanzania and Mexico (overall frequency of 12.83%). The most common haplotype in Kenya was endemic (overall frequency, 26.03% and 81% within Kenya), the second Kenyan endemic was rare (overall frequency<1%). Thailand and Indonesia had one rare (overall frequency <1%) haplotype each. From 212 tamarind that amplified well for the three chloroplast locus and were digested with appropriate restriction enzymes; 29 restriction fragments and from them 16 haplotypes were identified but only one of the 16 haplotypes was widespread globally (92.98% over all populations and 100% within Mexico, Burkinafaso, Niger, Sri Lanka and Thailand populations) (Table 4). One haplotype was restricted to Kenya and Indonesia (overall

frequency of <1%), four were endemic to Kenya, four in Uganda and six in Tanzania (Table 4).

Upon combination of the chloroplast and mitochondria haplotypes of 212 tamarind that amplified well in both genomes and were digested successfully with appropriate restriction enzymes, 20 different cytotypes were found. Only one of the twenty cytotypes was widespread globally (over all frequency of 51.89%) but rare in Kenya (Table 5). One cytotype was restricted to Burkinafaso and Indonesia with an overall frequency of 2.83% and the second restricted cytotype occurred in Kenya, Tanzania and Mexico (overall frequency of 13.68%). The most common cytotype in Kenya was endemic (overall frequency of 23.58% and 71% within Kenya (Table 5). Additional 5 cytotypes were endemic in Kenya, six in Tanzania, two in Uganda and two in Thailand. Uganda, Burkinafaso and Indonesia had one rare (overall frequency <1%) restricted but not well resolved cytotype. Because of incomplete resolution this cytotype was not included in population structure analysis.

Table 3. Mitochondria haplotypes of tamarind in populations from different geographic regions, habitats, mainlands, islands and latitudes in Africa, Asia and Central America

| | Haplotypes Frequencies | | | | | | |
|---|---|---|---|---|---|---|---|
| Populations | A | B | C | D | E | F | TOTAL |
| Combined populations | *158 | 34 | 69 | 1 | 2 | 1 | 265 |
| | ** | (12.83) | (26.04) | (0.38) | (0.75) | (0.38) | (100) |
| | (59.62) | | | | | | |
| Geographic origin | 16 | 0 | 0 | 0 | 2 | 0 | 18 |
| Indonesia | (88.89) | | | | (11.11) | | (100) |
| Kenya | 1 | 14 | 69 | 1 | 0 | 0 | 85 |
| | (1.18) | (16.47) | (81.176) | (1.18) | | | (100) |
| Mexico | 16 | 3 | 0 | 0 | 0 | 0 | 19 |
| | (84.21) | (15.79) | | | | | (100) |
| Niger | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| | (100) | | | | | | (100) |
| Sri Lanka | 28 | 0 | 0 | 0 | 0 | 0 | 28 |
| | (100) | | | | | | (100) |
| Tanzania | 38 | 17 | 0 | 0 | 0 | 0 | 55 |
| | (69.09) | (30.90) | | | | | (100) |
| Thailand | 10 | 0 | 0 | 0 | 0 | 1 | 11 |
| | (90.90) | | | | | (9.10) | (100) |
| Uganda | 48 | 0 | 0 | 0 | 0 | 0 | 48 |
| | (100) | | | | | | (100) |
| Habitats | 92 | 23 | 51 | 1 | 2 | 0 | 169 |
| On-farm | (54.44) | (13.61) | (30.18) | (0.59) | (1.18343) | | (100) |
| ICRAF | 3 | 0 | 0 | 0 | 0 | 1 | 4 |
| | (75) | | | | | (25) | (100) |
| River | 12 | 9 | 7(25) | 0 | 0 | 0 | 28 |
| | (42.86) | (32.14) | | | | | (100) |
| Wood | 51 | 2 | 11 | 0 | 0 | 0 | 64 |
| | (79.69) | (3.125) | (17.19) | | | | (100) |
| Mainland-island | 48 | 16 | 5 | 0 | 2 | 0 | 71 |
| Island | (67.61) | (22.54) | (7.04) | | (2.82) | | (100) |
| Mainland | 110 | 18 | 64 | 1 | 0 | 1 | 194 |
| | | | | (0.52) | | | |
| | (56.70) | (9.28) | (32.99) | | | (0.52) | (100) |
| Latitudes | 120 | 17 | 0 | 0 | 2 | 1 | 140 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| North | | (85.71) | (12.14) | | | (1.43) | (0.71) | (100) | | | |
| South | | 38 | 17 | 69 | 1(0.8) | 0 | 0 | 125 | | | |
| | | (30.4) | (13.6) | (55.2) | | | | (100) | NB; A – | | |

F, haplotypes generated using two approaches, manually based on visual inspection of restriction site polymorphisms and or based on excel computer programe soft ware analysis of binary data scored restriction fragments identified in the mitochondria Cox 11 Alu 1 restriction digest of tamarind samples from populations. *Frequency of haplotypes within or among populations, **() Percentage frequency of haplotypes within or among populations.

Table 4. Chloroplast haplotypes of tamarind in different populations from different geographic regions, habitats, mainlands, islands and latitudes in Africa, Asia and Central America

| | | Haplotypes frequencies | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Populations | A | B | C | D | E | F | G | H | 1 | J | K | L | M | N | O | P | TOTAL |
| Populations combined | *225 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 242 |
| | ** (92.98) | (0.41) | (0.83) | (0.41) | (0.41) | (0.41) | (0.41) | (0.41) | (0.83) | (0.41) | (0.41) | (0.41) | (0.41) | (0.41) | (0.41) | (0.41) | (100) |
| Geographic origin | 17 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 18 |
| | (94.44) | | (5.56) | | | | | | | | | | | | | | (100) |
| Indonesia | | | | | | | | | | | | | | | | | |
| Kenya | 76 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 81 |
| | (93.83) | (1..23) | (1.23) | | | | | | | (1.23) | (1.23) | (1.23) | | | | | (100) |
| Mexico | 19(100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 |
| | | | | | | | | | | | | | | | | | (100) |
| Burkinafaso | 5(100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| | | | | | | | | | | | | | | | | | (100) |
| Niger | 3(100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | | | | | | | | | | | | | | | | | (100) |
| Sri Lanka | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 27 |
| | (100) | | | | | | | | | | | | | | | | (100) |
| Tanzania | 38 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 45 |
| | (84.44) | | | (2.22) | (2.22) | (2.22) | (2.22) | (2.22) | (4.44) | | | | | | | | (100) |
| Thailand | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 |
| | (100) | | | | | | | | | | | | | | | | (100) |
| Uganda | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 33 |
| | (87.88) | | | | | | | | | | | | (3.03) | (3.03) | (3.03) | (3.03) | (100) |
| Habitat | 147 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 156 |
| On-farm | (94.23) | (0.64) | (1.28) | (0.64) | (0.64) | (0.64) | (0.64) | | | | | | | | (0.64) | (0.64) | (100) |
| ICRAF | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 |
| | (100) | | | | | | | | | | | | | | | | (100) |
| River | 21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 21 |
| | (100) | | | | | | | | | | | | | | | | (100) |
| Wood | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 54 |
| | (85.19) | | | | | | | (1.85) | (3.70) | (0.64) | (0.64) | (0.64) | (0.64) | (0.64) | | | (100) |
| Mainland-island | 64 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 69 |
| | (92.75362) | | (1.45) | (1.45) | (1.45) | (1.45) | | (1.45) | | | | | | | | | (100) |
| Island | | | | | | | | | | | | | | | | | |
| Mainland | 161 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 173 |
| | (93.06) | (0.58) | (0.58) | | | | (0.58) | | (1.16) | (0.58) | (0.58) | (0.58) | (0.58) | (0.58) | (0.58) | (0.58) | (100) |
| Latitude | 130 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 137 |
| North | (94.89) | (0.73) | (1.46) | | | | | | | | | | (0.73) | (0.73) | (0.73) | (0.73) | (100) |

| | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| South | 95 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 105 | | |
| | (90.48) | | | (0.95) | (0.95) | (0.95) | (0.95) | (0.95) | (1.90) | (0.95) | (0.95) | (0.95) | | | | | (100) | | |

NB: A – P, haplotypes generated using two approaches, manually based on visual inspection of restriction site polymorphisms and or based on excel computer programe soft ware analysis of binary data scored restriction fragments identified in the rplf2, trnG and psbA chloroplast loci and Hinf1, BFCU1, MnlI restriction digest of tamarind samples from populations. *Frequencies of haplotypes within and among populations and **() Percentage frequency of haplotypes within or among populations.

Table 5. Cytotypes of tamarind in different geographic, habitats, mainlands, islands and latitudinal populations in Africa, Asia and Central America

| | Cyto types frequencies | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AA | AX | AB | AC | BC | CC | CE | DB | EB | FB | GA | HB | 1A | JC | LC | MA | NA | AD | AE |
| Populations | | | | | | | | | | | | | | | | | | | |
| Combined populations | 110* | 6 | 29 | 50 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| | ( 51.89) ** | (2.83) | (13.68) | (23.58) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.47) | (0.4 |
| Geographic origin | 14 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | (82.35) | (5.88) | | | | | (5.88) | | | | | | | | | | | | (5.8 |
| Indonesia | | | | | | | | | | | | | | | | | | | |
| Kenya | 1 | 0 | 14 | 50 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| | (1.43) | | (20) | (71.43) | (1.43) | (1.43) | | | | | | | | (1.43) | (1.43) | | | (1.43) | |
| Mexico | 16 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | (84.21) | | (15.79) | | | | | | | | | | | | | | | | |
| Niger | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | (100) | | | | | | | | | | | | | | | | | | |
| Burkina | 1 | 5(100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | (100) | | | | | | | | | | | | | | | | | | |
| SriLanka | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tanzania | 20 | 0 | 12 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| | (100) | | (26.67) | | | | | (2.22) | (2.22) | (2.22) | (2.22) | (2.22) | (4.44) | | | | | | |
| Thailand | 26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | (57.78) | | | | | | | | | | | | | | | | | | |
| Uganda | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| | (90.91) | | | | | | | | | | | | | | | (4.17) | (4.12) | | |
| Mainland-island | 22 | 1(1.79) | 11 | 3 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| | (91.67) | | (19.64) | (5.36) | | | (1.79) | (1.79) | (1.79) | (1.79) | | | | | | | | | (1.7 |
| Island | | | | | | | | | | | | | | | | | | | |
| Mainland | 74 | 5 | 18 | 47 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 0 |
| | (47.44) | (3.21) | (11.54) | (30.13) | (0.64) | (0.64) | | | | | (0.64) | (0.64) | (1.28) | (0.64) | (0.64) | (0.64) | (0.64) | (0.64) | |
| Habitat | 36 | 1 | 19 | 39 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| On-farm | (64.29) | (0.73) | (13.87) | (28.47) | (0.73) | (0.73) | (0.73) | (0.73) | (0.73) | (0.73) | (0.73) | | | | | | | (0.73) | (0.7 |
| ICRAF | 74 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | (47.44) | (55.56) | | | | | | | | | | | | | | | | | |
| River | 9 | 0 | 9 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | (40.91) | | (40.91) | (18.18) | | | | | | | | | | | | | | | |
| Wood | 69 | 0 | 1(2.27) | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 0 | 0 |
| | (50.36) | | | (15.91) | | | | | | | | (2.27) | (4.55) | (2.27) | (2.27) | (2.27) | (2.273) | | |
| Latitude | 3 | 6(5.17) | 17 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 |

| | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| North | (33.33) | | (14.66) | (3.45) | | | (0.86) | | | | | | | | | | (0.86) | (0.86) | | (0.8 |
| South | 9 | 0 | 12 | 46 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | |
| | (40.91) | | (12.5) | (47.92) | (1.04) | (1.04) | | (1.04) | (1.04) | (1.04) | (1.04) | (1.04) | (2.08) | (1.04) | (1.04) | | | (1.042) | | |

NB; AA – AF, Cytotypes generated from the combination of tamarind individuals mitochondrial and chloroplast haplotypes generated using two approaches, manually based on visual inspection of restriction site polymorphisms and or based on excel computer programe soft ware analysis of binary data scored restriction fragments identified in the mitochondrial Cox 11 and Alu 1 digest and chloroplast rplf2, trnG and psbA loci digest with Hinf1, BFCU1, Mnl1, *Frequency of cyto types within and among populations and **() Percentage frequency of cyto types within or among populations.

**Tamarind populations genetic structure based on haplotypes and cytotypes**

Amount of genetic variation based on the Mitochondria genome of tamarind among geographic regions was 63.63% and 36.36% within populations and the genetic structure as revealed on AMOVA was strong (ΦST = 0.64). A test of population variation based on tamarind from latitudes higher North above equator and lower in the South below the equator revealed 57.60% variability within populations and 42.39% variation among populations with a differentiation index ΦST = 0.42 while, variability in different habitats and mainland-island populations were higher within populations (Table 6). There was no genetic structure in the chloroplast genome (Table 6). For cytotypes, within geographic populations variability was 51%, similar pattern was in higher-lower latitudes and no genetic structure occurred among habitats and mainland-islands populations (Table 6).

Table 6. Tamarind genetic structure in populations from different geographic regions, habitats, mainlands, islands and latitudes in Africa, Asia and Central America

| Genome of tamarind | Source of variation | Geographic regions | North-South | Mainland-Islands | Habitats |
|---|---|---|---|---|---|
| Mitochondria | Among populations % variation (Va) | 63.64 | 42.39 | 7.40 | 6.62 |
| | Within populations % variation (Vb) | 36.36 | 57.60 | 92.60 | 93.38 |
| | PhiST (ΦST) | *0.64 | *0.42 | 0.07 | 0.07 |
| Chloroplast | Among populations % variation (Va) | 0.56 | 1.73 | -0.52 | 1.58 |
| | Within populations % variation (Vb) | 99.44 | 98.27 | 100.52 | 98.42 |
| | PhiST (ΦST) | 0.01 | 0.02 | -0.01 | 0.002 |
| Cyto types | Among populations % variation (Va) | 48.97 | 26.22 | 6.08 | 7.88 |
| (Mitochondria and Chloroplast genome combined | Within populations % variation (Vb) | 51 | 73.78 | 93.92 | 92.12 |
| | PhiST (ΦST) | *0.49 | *0.26 | 0.08 | 0.08 |

The population differentiation index ΦST was computed as implemented in the AMOVA framework in FAMD (Schluter et al., 2006) based on the frequencies of the different mitochondria and chloroplast haplotypes and cyto types within and among the different populations of tamarind. The cyto types were generated from the combination of generated tamarind individuals' mitochondrial and chloroplast haplotypes. The haplotypes were generated using two approaches, manually based on visual inspection of restriction site polymorphisms and or based on excel computer programe soft ware analysis of binary data scored restriction fragments identified in the mitochondrial Cox 11 and Alu 1 digest and chloroplast rplf2, trnG and psbA loci digest with Hinf1, BFCU1, Mnl1. * refers to significant ΦST values for populations.

Below is a map (Figure 2) and notes summaries on the six mitochondria haplotypes, 16 chloroplast haplotypes and 20 cytotypes found in tamarind populations globally.

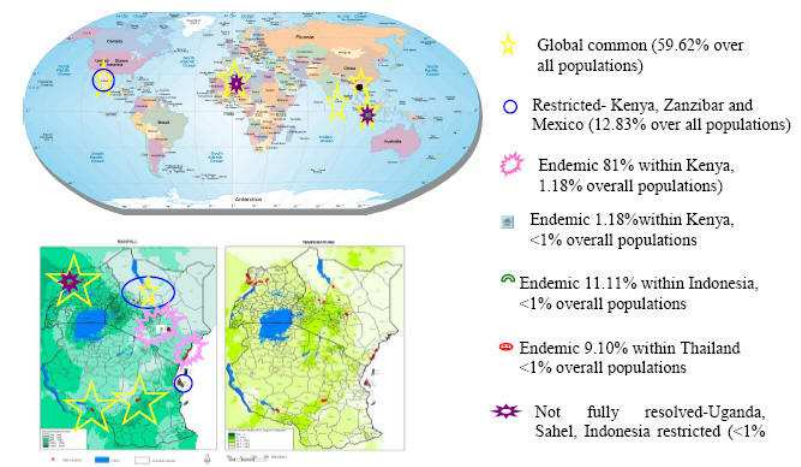



Figure 2. Mitochondria haplotypes found in global populations of tamarind

Note – Not shown in the figure;

- One global chloroplast haplotype (92.98% overall frequency), one Kenya, Indonesia restricted (1.23% overall), rare endemics (each<1% overall frequency)-Kenya (4), Tanzania (6), Uganda (4) found in the populations.

- Twenty cytotypes, of which one was global (51.89% overall populations and 100% in SriLanka, Niger), one restricted to Kenya, Tanzania, Mexico (13.68% overall) and the rest rare endemics (overall frequency <1% each) in Thailand (1), Uganda (2), Indonesia (3), Kenya (6, one of them 71.43% within Kenya) and 6 in Tanzania.

**DISCUSSION**

The goal of this study was to generate knowledge of genetic variation in tropical populations of tamarind using the slow evolving organelle (chloroplast and mitochondria) genome based neutral genetic markers. Analysis of molecular variance based on the mitochondria genome revealed strong genetic differentiation among geographic populations. Genetic structure for tamarind in higher latitudes North above equator and lower latitudes in the South below equator was strong but only within populations while, negligible genetic structuring is exhibited for island-mainland and different habitat populations. The presence of a population structure suggests absence of geneflow and potential for populations to drift apart and become distinct over a long time (Hartl and Clarke, 1997). Observed population structure in the mitochondria among geographic populations of tamarind therefore means potential for these populations to drift apart and even become distinct if left in the current state over a long time. Divergence among geographic regions has been found in many other angiosperms (Demesure et al., 1996; Latta et al., 1998), the current finding in tamarind thus provide additional evidence to entrench such diversity pattern in species.

Characteristically, the population structure causing mitochondria haplotypes were few, rare and restricted mainly to East Africa while the other geographic regions were composed largely of the single global haplotype or cytotype. In literature higher diversity is characteristics of founder population/centre of origin of species (Muratorio-Oddou et al., 2001). Therefore, the relatively higher diversity of tamarind genetic resources (haplotypes and cytotypes) within East Africa suggests origin from the region.

Among other factors, geographic barriers between islands and mainlands, North-South latitudes and on-farm and wild habitats are in literature known to cause genetic divergence, even speciation among species' populations (Hartl and Clarke, 1997; Erikson, 2001; Frankham, 2002; Grant and Grant, 2008). For tamarind, genetic differentiation among habitats, island-mainland populations were insignificant, and the population structure causing haplotypes and or cytotypes (genetic resources) were rare, endemic and or restricted to few geographic region(s), mainly East Africa. This diversity pattern hypothetically suggests two things; one a gene poor but stable ancestral tamarind genome (also evidenced by chloroplast genome conservation and absence of genetic structure among habitats, island–mainland and latitude populations) and two, an East Africa origin.

Taken alone, the observed high conservation of the chloroplast genome in tamarind is also in line with literature for other angiosperms, more recently in Diospyros kaki where PCR-RFLP revealed no polymorphism (Hu et al., 2008) and in the recent past, Sorbus

tormenalis in its France range (Muratorio-Oddou et al., 2001). Sorbus tormenalis is a hermaphrodite, pollinated by insects and dispersed by mammals and birds just like tamarind but for it, its chloroplast genome is known to be maternally inherited (Nagarajan et al., 1998; Muratorio-Oddou et al., 2001). Due to high homogeneity, it was not possible to determine tamarind organelle genomes inheritance mechanisms in the current study. But in many angiosperms, maternal and paternal inheritance is known for chloroplast and mitochondria respectively, although in some like Musa species maternal inheritance of mitochondria is reported (Curtis and Cleg, 1984; Faure et al., 1994; Demesure et al., 1995; Nwakanma et al., 2003). Based on literature from studies in other species, higher diversity in populations can be attributed to multiple maternal and paternal parents in founder populations (Muratorio-Oddou, 2001). Even though the inheritance mechanisms for tamarind organel genome was not identified, both its mitochondria and chloroplasts haplotypes and their cytotypes were more diverse (their rare, restricted and endemic nature not withstanding) within East Africa. This finding alludes to multiple maternal and paternal parents/centre of diversity/origin of tamarind being the East Africa populations.

Insightfully, the East Africa endemic and restricted genetic resources identified in the current study may have been responsible and or help explain the higher diversity reported for the region in the previous study involving RAPDS (Diallo et al., 2007). Similarly, the high homogeneity exhibited in tamarind organelle genome especially in populations outside East Africa (e.g. Niger, Sri Lanka, Mexico, Thailand) support findings of low genetic variability earlier reported in those regions (Shanthi, 2003; Pushpakumara and Gunasena in Elsidig et al., 2006). East African higher diversity and near homogeneity in the Asia (Sri Lanka, Thailand and to some extent Indonesia) and Central America (Mexico) observed in the current study also provides evidence of East African origin and confirms the introduced statuses of India and American populations. The rare-restricted-endemic nature of tamarind haplotypes/cytotypes responsible for genetic structure coupled with the global widespread status of only one genotype raise two hypotheses;

(1) These haplotypes or cytotypes may have been originally widespread but were reduced to small restricted populations by strong convergent selection or other population bottlenecks. In East Africa these bottlenecks may be habitat losses, destructive harvesting and poor regeneration reported for tamarind (Nyadoi, 2005) and known to cause genetic erosion in species (Frankham et al ., 2002 ; FAO, 2004; Lowe et al ., 2005). A gene poor ancestor moved from East Africa could have been the cause of observed near genetic homogeneity in Sri Lanka, Mexico and Thailand or, a convergent strong selection could have taken place despite diverse environment.

(2) Tamarind speciation from the Amherstiaeae may have involved genetically poor but a highly plastic, selection favoured ancestral gene pool. In fact evolution is known to start from a selection favoured ancestral genotype (Kauffmann, 1995). Similarly, genomic plasticity is the known force behind species' ability to colonise and survive in new environments with minimal or no evolution (Price et al., 2003; Grant and Grant, 2008). Indeed Tamarindus is the most derived among the twenty five Amherstiaeae genera (Polhill and Raven, 1981). A selection favoured, plastic gene poor ancestor coupled with little time since speciation is a plausible hypotheses for the observed current restricted and or endemic nature of its unique genetic resources visa-vie one global common one. The observed rare and unique genetic resources likely represent just emerging products of minimal drift and mutations or they may be remnants of genetic resources that were originally widespread. Weak populations' genetic structure despite diverse environments (different habitats, island-mainlands also support the hypothesis that tamarind ancestral genome may have been gene poor and stable or convergent selection occurred).

Based on the evidence of restricted genetic resources among geographic regions, the ancestral tamarind dispersal routes were most likely three; (i) East Africa→West Africa→South East Asia (Indonesia and Thailand) (ii) East Africa→South Asia (Sri Lanka/Indian region) and (iii) East Africa→Mexico/Americas. Interestingly, the first human migrants out of East Africa followed similarly routes (Sforzan and Feldman, 2003) and may have carried tamarind germplasm to geographic regions of their destiny. For example a Kenya ancestor is likely for the Kenya-Indonesia and Kenya, Zanzibar and Mexico restricted genetic resources. Tamarind restricted and the country endemic genetic resources may have been dispersed by tourists, traders, extension officers and or pollinators/bees. The Tharaka and Kitui based traders who sell tamarind fruits in Mombasa market could have aided the distribution of the Mombasa, Kilifi, Tharaka and Kitui (Kenyan endemic) haplotypes. Mexican tourists visiting the Samburu, Turkana and Pokot may have taken Kenya tamarind to Mexico, via Tanzania's Zanzibar Island giving rise to the Kenya, Zanzibar and Mexico restricted genetic resources. In Samburu, some farmers and extension officials reported planting tamarind seeds from Kenya

Forestry Research Institute (KEFRI) or from South Horr Forest. Similarly, in Zanzibar, agriculture station, it was reported that agricultural officials planted tamarind in the station but origin of seeds planted were not recorded. In literature (Gunasena and Hughes, 2000), tamarind fruits from Kenya are exported to Yemen and other destinations, genetic diversity studies involving such populations may reduce genetic resources endemism in East Africa. In terms of pollen dispersal, bees can move pollen from as near as 100 metres to several kilometres apart and are known to influence genetic variation in tropical trees (Di-Giovanni et al., 1991; Dow and Ashley, 1996; Young et al., 2000). Thus they could have facilitated dispersal especially of the locally widespread endemic Kenya haplotypes.

In general, whether the rare, endemic and restricted haplotypes and cytotypes of tamarind observed in the current study are remnants of once diverse genetic resources reduced under strong selection or they are just emerging products of limited drift and mutations; their rare-endemic and or restricted nature demonstrate they are endangered and need conservation interventions to enable sustainability (Kimura, 1983). To merit conservation investments however, the evolution and conservation value of these genetic resources need to be investigated. A phylogeny study of tamarind with its closest Amherstia relatives would for example shade light on the evolution of tamarind genetic resources while quantitative trait analyses would help elucidate their conservation value. But these further investigation needs not withstanding, it is clearly evident from findings of this study that specific/in-situ to geographic region population conservation strategies will be needed for the different tamarind genetic resources. Secondly, the relatively high genetic diversity in East Africa compared to near homogeneity in other geographic regions make the East African populations worthy of conservation priority as centre of diversity. Additionally, the need for conservation of the limited diversity found in Indonesia and Thailand withstanding, the single global widespread tamarind genetic resource conservation could be tied to its economic benefits if any and or other reasons.

**ACKNOWLEDGEMENT**

Funding for this study was received from the Third World Organisation of Women in Sciences, the International Foundation for Science, Austrian Development Agency and the Austrian Research Centres. The authors thank Professor Nagib Nasser and two anonymous reviewers for Gene Conserve Journal, for the review inputs they gave which helped us to improve the drafts of this manuscript. We thank Ratri B, and Alberto Lopez Luis for Thailand and Mexico tamarind leaf materials, Dr. Maria Berenyi, Karin Fohringer and Agnes Burg for technical assistance, Ben L, Phoebe Owino and Were Agnes for field assistance, Drs Sara Namirembe, Edward Kanju for collaboration assistance, Assoc. Prof J.R.S Kabogozza, Dr. Wilson Kasolo, Dr. Tony Simons, Dr. Denis P. Garrity for Institutional support and Rick Coe and Patricio Lopez for research methodological and analysis guidance.

**REFERENCES**

Excoffier L, Laval G and Schneider S (2005). Arlequin ver 3.11. An integrated Software Package for Population Genetics Data Analyses. Evolutionary Bioinformatics Online 1:47-50.

Csaikl UM, Burg K and Fineschi S (2002). Chloroplast DNA variation of white oaks in the alpine region Forest Ecology and Management 156:131-145.

Curtis SE, Clegg MT (1984). Molecular evolution of chloroplast DNA sequences. Molecular Biology Evolution. 1:191-301.

Demesure B, Sodzi N and Petit RJ (1995). A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molecular Ecology 4:129-131.

Demesure B, Comps B and Petit JR (1996). Chloroplast DNA phylogeography of the common beech (Fagus sylvatica L.) in Europe. Evolution, 50, 2215 – 2520.

Diallo OB, Joly LH, Mckey HM and Chevallier HM (2007). Genetic diversity of Tamarindus indica populations: Any clues on the origin from its current distribution? African Journal of Biotechnology Vol. 6 : 853-860.

Di-Giovanni F and Kevan PG (1991). Factors affecting pollen dynamics and its importance to pollen contamination: a review. Canadian Journal of Forest Research 21 : 1155-70.

Dow BD and Ashley MV (1996). Microsatellites analysis of seed dispersal and percentage of saplings in bur oak, Quercus macrocarpa. Molecular Ecology 5, 615-27.

Duminil J, Pemonge MH, Petit RJ (2002). A set of 35 consensus primer pairs amplifying genes and introns of plant mitochondrial DNA. Molecular Ecology Notes 2: 428-430

El-Siddig K, Gunasena HPM, Prasad BA, Pushpakumara DKNG, Ramana KVR, Viyayanand P and Williams JT (2006). Fruits for the Future 1-Revised edition-Tamarind (Tamarindus indica L). Monograph. 188p.

Eriksson G (2001). Conservation of noble hardwoods in Europe. Can. J. For. Res.

31:577-587.

Faure S, Noyer J, Careel F, Horry J, Bakry F and Lanund C (1994). Maternal inheritance of mitochondria genome in bananas (Musa acuminate). Curr Genet 25:265-269.

FAO (2004). Forest Genetic Resources No. 31. Rome, Italy.

Frankham RJ, Ballou JD and Briscoe DA (2002). Introduction to Conservation Genetics. Cambridge University Press.

Grant RB and Grant RP (2008). Fission and fusion of Darwin's finches populations. Philosophical Transactions of the Royal Society Biological Sciences 363:2821-2829.

Gunasena HPM and Hughes A (2000). Tamarind Tamarindus indica L. Fruits for the Future 1. (Eds. Hughes, A., N. Hag and R.W). Smith. International Centre for Underutilized Crops. UK. 169pp.

Hartl DL and Clark AG (1997). Principles of Population genetics. 3rd Edition, Sinuaer Associates. Inc. Sunderland, Massachusetts, USA. 542pp.

Hu D, Zhang Q and Luo Z (2008). Phylogenetic analysis in some Diospyros spp. (Ebenaceae) and Japanese parsimony using chloroplast DNA PCR-RFLP markers. Scienta Horticulture 117:32-38.

Jama B, Oginasako Z. and Simitu P (2005). Utilisation and Commercialization of dryland indigenous fruit tree species to improve livelihoods in East and Central Africa. Proceedings of a Regional Workshop, KEFRI Kitui Kenya June 20-24th 2005. ICRAF-ECA, ECA Working Paper No.7, 2005, World Agroforestry Center.

Jung, YH., Eun, Y.S and Seung, JC (2004). Phylogenetic analysis of plastid trnL-trnF sequences from Arisaema species (Araceae) in Korea. Euphytica 138:81- 88.

Kauffman SA (1993). The Origins of Order. Oxford Univ. Press, New York.

Kimura M (1983). The Neutral Theory of Molecular Evolution. Cambridge University Press: Cambridge.

Latta RG, Linhart BY, Fleck D and Elliot M (1998). Direct and indirect estimates of seed versus pollen movement within a population of ponderosa pine. Evolution 52: 61–67.

Lowe A, Harris S. and Ashton P (2004). Ecological Genetics. Design, Analysis and Applications. Blackwell Publishing, USA. 344pp.

Lowe A J, Boshier D, Ward M, Bacles CFE and Navarro C (2005). Genetic resources impacts of habitat loss and degradation; reconciling empirical evidence and predicted theory for neotropical trees. Heredity 95 : 255-273.

Mccoy RS, Kuehl VJ, Booke LJ and Raubeson AL (2008). The complete plastid genome sequence of Welwitschia mirabilis: an unusually compact plastome with accelerated divergence rates. BMC Evolutionary Biology 8:130

Muratorio-Oddou S, Petit JR, Le Guerroue B, Guesnet D, and Demesure B (2001). Pollen-versus Seed-Mediated Gene Flow in a Scattered Forest Tree Species. Evolution 55: 1123-1135.

Nagarajan A, Nicodemus AK, Mandal R, Verma K, Gireesan K and Mahadevan NP (1998). Phenology and Controlled Pollination Studies in Tamarind. Silvae Genetica 47:5-6.

Nishikawa T, Salomon B, Komatsuda T, Von BR, Kadowaki K (2002). Molecular phylogeny of the genus Hordeum using three chloroplast DNA sequences. Genome 45:1157–1166.

Nyadoi, P., Okori, P., Okullo, JBL., Obua, J., Burg, K., Fluch, S., Magogo, Nasoro., Haji, Saleh., Temu, A.B and Jamnadass, R. (2010). Variability of East Africa tamarind (Tamarindus indica L.) populations based on morphological markers. Gene Conserve 9:51-78.

Nyadoi P (2005). Population Structure and Socio-Economic Importance of Tamarindus indica in Tharaka District, Eastern Kenya. M.Sc. Thesis. Makerere University, Uganda.110 pp.

Nwakanma DC, Pillay M, Okoli BE and Tenkouano A (2003). Sectional relationships in the genus Musa L. inferred from the PCR-RFLP of organelle DNA sequences. Theo Appl Genet 107:850-856.

Polhill RM and Raven PH (1981). Biogeography of leguminoseae. In Advances in legume systematics, part 1.pp27-34. eds. Polhill R.M. and Raven P.H. Royal Botanic Gardens, Kew. 425 pp.

Price DT, Qvarnstro¨MA and Irwin ED (2003). The role of phenotypic plasticity in driving genetic evolution. Proc. R. Soc. Lond. B 270: 1433-1440.

Samuel R, Pinsker W and Kiehn M (1997). Phylogeny of some species of Cyrtandra (Cesneriaceae) inferred from the atpB/rbcL cpDNA intergene region. Botanica Acta 110: 503-510.

Schluter PM and Harris SA (2006). Analysis of multilocus finger printing datasets containing missing data. Molecular Ecology Notes 6: 569-572.

Shaw J, Lickey EB, Beck JT, Farmer SB, Liu W, Miller J, Siripun KC, Winder CT, Schilling EE and Small RL (2005). The tortoise and the hare II: relative utility of 21 non coding chloroplast DNA sequences for phylogenetic analysis. American Journal of Botany 92:142-166.

Shanthi A (2003). Studies on variations and associations in selected poulations, plantations and clones in tamarind (Tamarindus indica Linn.). Un published PhD Thesis. Barathiar University, Coimbatore, India.

Taberlet P, Gielly L, Pautou G and Bouvet J (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105-1109.

Tsumura Y, Kawahara T, Wiekneswari R and Yoshimura K (1996). Molecular phylogeny of Dipterocarpaceae in Southeast Asia using RFLP of PCR-amplified chloroplast genes. Theoretical and Applied Genetics 93: 22–29.

Tsumura, YK. Tomaru, N. Y and Ohba K. 1995. Molecular phylogeny of conifers using PCR-RFLP analysis of chloroplast genes. Theor. Appl. Genet. 91:1222–1236.

QIAGEN 2006. DNeasy® Plant Handbook. DNeasy Plant Mini Kit For miniprep purification of total cellular DNA from plant cells and tissues, or fungi.

White F (1983). The Vegetation Map of Africa. UNESCO, Paris.

Yoneyama, T., Muraokami, T. and Boonkerd, N. 1993. Natural abundance of 15N in tropical plants with emphasis on tree legumes, Planta.

Young A, Boshier D and Boyle T (eds.) (2000). Forest Conservation Genetics: Principles and Practice. CSIRO Publishing, Collingwood, Australia.

Appendix 1 Primers tested for tamarind amplification and diversity studies

| Primer | Foreword /reverse sequences | publisher | Pcr success with tamarinds remark |
|---|---|---|---|
| 1. tRNAleu (Intron 1 F) | 5'-CGAAATCGGTAGACGCTACG-3' | Taberlet et al 1991 | Amplified in some and failed in other individuals, Polymorphic with Hinf1 but poor resolution |
| tRNAleu (Intron1 R) | 5'-GGGGATAGAGGGACTTCAAC-3' | | Not used in final study |
| 2. MatkF<br>MatKR | | Nishikawa *et al*., 2002 | Amplified in some and failed in other individuals Not used in study |
| 3.trnL F<br>trnF | | Nishikawa *et al*., 2002 | Amplification failure Not used in study |
| 4. CCB 203 F<br>CCB 203 R | 5'-ASGTTCTACGGACCGATGCC-3'<br>5'-CACGGGGAGGGAGCRGGCGA-3' | Duminil *et al*., 2002 | Amplified in some and failed in other individuals Not used in study |
| 5. CR<br>RC | 5'-CACGGGTCGCCCTCGTTCCG-3'<br>5'-GTGTGGAGGATATAGGTTGT-3' | Demesure *et al.*, 1995 | Amplified in some and failed in others Not used in study |

| | | | |
|---|---|---|---|
| 6.CB<br>BC | 5'-GCATTACGATCTGCAGCTCA-3'<br>5'-GGGCTCGATTAGTTTCTGC-3' | Demesure *et al.*, 1995 | Amplified in some and failed in other individuals Not used in study |
| 7. NA41<br>NA14 | 5'-CAGTGGGTTGGTCTGGTATG-3'<br>5'-TCATATGGGCTACTGAGGAG-3' | Demesure *et al.*, 1995 | Amplification failure Not used in study |
| 8.NA42<br>NA24 | 5'-TGTTTCCCGAAGCGACACTT-3'<br>5'-GGAACACTTTGGGGTGAACA-3' | Demesure *et al.*, 1995 | Amplified in some and not in other individuals Not used in study |
| 10. ORF 25F<br>ORF25R | 5'-AAGACCRCCAAGCYYTCTCG-3'<br>5'-TTGCTGCTATTCTATCTATT-3' | Dumunil *et al.*, 2002 | Amplification failure Not used in study |
| 11.AS<br>SA | 5'-ACTTCTGGTTCCGGCGAACGA-3'<br>5'-AACCACTCGGCCATCTCTCCT-3' | Demesure *et al.*, 1995 | Amplification failure Not used in study |
| 12. CD<br>DC | 5'-CCAGTTCAAATCTGGGTGTC-3'<br>5'-GGGATTGTAGTTCAATTGGT-3' | Demesure *et al.*, 1995 | Amplification failure Not used in study |
| MatK F<br>trnK | 5'-AACCCGGAACTAGTCGGATG-3'<br>5'-TCAATGGTAGAGTACTCGGC-3' | Nishikawa *et al.*, 2002 | Amplification failure Not used in study |
| 13. trnlR<br>trnLFA | | Jung *et al.*, 2004 | Amplification failure Not used in study |
| 14..trnEDoyle 10<br>TrnTM 11 | GTCCTATCCATTAGACAATGG<br>CTACCACTGAGTTAAAAGGG | In PICME lab | Amplified in some and failed in others Not used in study |
| 15.TrnHF<br>PSB A3 (8) | | Tsumura *et al.*, 1995 | Amplification failure Not used in study |
| 16. atBSam 17<br>atBsam 20 | | Samuel *et al.*, 1997 | Amplification failure Not used in study |
| 17. rbcl F<br>rbcl R | | | Amplified in some and failed in others Not used in study |
| 20. ORF 62 P<br>trnf-M | CTTGCTTTCCAATTGGCTGT<br>CATAACCTTGAGGTCACGGG | Demesure *et al.*, 1995 | Amplification failure Not used in study |
| 21. trnG 2(III)<br>TrnG 1 R | GTTTAGTGGTAAAAGTGTGATTCG<br>CCGCATCGTTAGCTTGGAAGGC | Shaw *et al.*, 2005 | Amplified across populations Used in study |
| 22. rpl2f (2)<br>TrnH-M | ACCGATATGCCCTTAGGCACGGC<br>GTGAATCCACCAYGCGCGGG | | Amplified across populations Was used in study |
| 23. PSB A5 (7)<br>PSBA3 (8) | TACGTTCRTGCATAACTTCC<br>CTAGCACTGAAAACCGTCTT | Tsumura *et al.*, 1995 | Amplified across populations was used in study |
| 24. NAD9 F<br>NAD 9 R | GGTCATCTCAATTGGGYTCAG<br>TATAGTTGGGAGACTTTACC | | Amplified across populations Was used in study |
| 25. Cox 11 F<br>Cox 11 R | 5'-TAGRAACAGCTTCTACGACG-3'<br>5'-GRGTTTACTATGGTCAGTGC-3' | Duminil *et al.*, 2002 | Amplified across populations was used in study |
| 26.rps 14<br>Cob | | Demesure *et al.*, 1995 | Amplified in some and failed in others Not used in study |
| DT<br>TD | 5'-ACCAATTGAACTACAATCCC-3'<br>5'-CTACCACTGAGTTAAAAGGG-3' | Demesure *et al.*, 1995 | Amplification failure Not used in study |
| 27. rbcl samuel<br>Rbcl Samuel R | GAAGTAGTAGGATTGATTCTC<br>CCCTACAACTCATGAATTAAG | Samuel *et al.*, 1997 | Amplification failure Mot used in study |
| 28. Atp9 F<br>Atp 9 R | 5'-CCAAGTGAGATGTCCAAGAT-3'<br>5'-CTTCGGTTAGAGCAAAGCC-3' | Duminil *et al.*, 1995 | Amplification failure Not used in study |
| 29. NA12<br>NA21 | | | Amplified in some and failed in others Not used for study |
| 30. MatKF<br>trnlKR | | | Amplified in some and failed in others Not used for study |
| 31.ITS F<br>ITS R | | | Amplifies in all Not used in study |
| 32. FT<br>TF | | | Amplification failure Not used in study |
| 33. Rpoc F<br>Rpoc R | | | Amplification failure Not used in study |
| 34. Rps 4 F<br>Rps 4 R | 5'-CSTTTCYGCTCCGAAGAG-3'<br>5'-TCTCCGAAGATTGAGG-3' | | Amplification failure Not used in study |
| 35.IGR F<br>IGR R | | | Amplification failure Not used in study |
| 35. 18S F<br>18S R | | | Amplified in all Used to test DNA quality for PCR |
| 36. atB Samuel F<br>Rbcl Samuel R | GAAGTAGTAGGATTGATTCTC<br>CCCTACAACTCATGAATTAAG | Samuel *et al.*, 1997 | Amplification failed Not used in study |