micropropagation of *Coffea arabica* L. and evaluation of genetic diversity in *Cocos nucifera* L. from Tanzania

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But you will not leave in haste or go in flight; for the Lord will go before you, the God of Israel will be your rear guard (Isaiah 52:12)

This thesis is dedicated to the memories of my loved ones Isaac, Tumaini and Martha Mayo (Isaiah 57: 1, Job 1: 21)

Abstract:

RAPD and ISSR markers were used to analyze diversity in *Coffe*a *arabica* L. and *Cocos nucifera* L. In coffee, both markers revealed within-provenance dissimilarity values that were lower than between-provenance values which accentuates the inbreeding nature of *C. arabica* and the effect of farmers' selection. ISSR analysis separated diploid coffee species from *C. arabica*. Overall provenances from the same location clustered together, provenances from Mbeya were clearly differentiated from the rest. In *Cocos nucifera* L. RAPD markers were used to analyse 120 accessions. Data were analysed by clustering based on Jaccard's (1908) coefficient and Nei genetic distances. Further analysis involved principal coordinate analysis (PCA) and bootstrap analysis. The results were able to discriminate between the different provenances and provide evidence of the different origins for the coconut palms in the northern and southern parts of Tanzania. The two major clusters also concur well with the history and distribution of coconuts in Tanzania. Development of a micropropagation protocol for new *Coffea arabica* L. varieties proceeded with testing five varieties on several media. Several benzyladenine (2.5, 5 and 10 μM) and triacontanol (2.85 and 11.38μM) combinations were tested.

Segments from different positions of the leaf were tested for their ability to produce embryos. Leaf segments did not differ in percent embryo-producing explants. BA had the most impact on the number of embryo-producing explants. The best combination of BA/TRIA was 10μM BA and 11.38μM TRIA. The presence of TRIA caused an increase in embryo-producing explants.

Contents

Appendix

Papers I – IV

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Masumbuko, L. I., Bryngelsson, T., Mneney, E. E. & Salomon, B. 2003. Genetic diversity in Tanzanian Arabica coffee using random amplified polymorphic DNA (RAPD) markers. Hereditas 139: 56 – 63.

II. Masumbuko, L. I. & Bryngelsson, T. 2005. Inter simple sequence repeat (ISSR) analysis of diploid coffee species and cultivated *Coffea arabica* L. from Tanzania. Genetic Resources and Crop Evolution (2005) 00: 1–10.

III. Masumbuko, L. I., Sinje, S. & Kullaya A. Genetic diversity and structure of East African Tall coconuts in Tanzania using RAPD markers. (Submitted).

IV. Masumbuko, L. I. & Welander, M. Micropropagation of new *Coffea arabica* L. varieties from Tanzania: The influence of benzyl adenine and triacontanol on somatic embryogenesis. (manuscript)

Study I: Genetic diversity of *Coffea arabica* L. from Tanzania. (Paper I and II)

Introduction

Bremer (1996) defines the *Rubiaceae* family as biologically and morphologically diverse with many different life forms, ranging from tiny herbs, epiphytes, lianas, and shrubs to tall trees. It also has various kinds of flowers with different pollination systems. The family has about 640 genera and about 10,000 species the majority of which are in the tropics.

The genus *Coffea* is one of the economically important members of the *Rubiaceae* family. The exact number of species within the genus *Coffea* is unknown, but it is assumed to be around 90 (Wilson, 1999). It is not uncommon to hear of new coffee species being discovered, for example in the Eastern Arc Mountains of Tanzania, Davis and Mvungi (2004) have described as new species *C. bridsoniae* and *C. kihansiensis*. The two most important species in this genus however are *Coffea canephora* Pierre and *Coffea arabica* L. The former, which is a diploid (2n = 2x = 22), is generally self-incompatible. It is found throughout the rain forest of the Congo River basin and on higher land to the northeast as far as the Ugandan and Tanzanian shores of Lake Victoria up to an altitude of 1500 m. It

also occurs in the coastal rain forest from the Congo area all the way to Côte d'Ivoire. There are several distinct types within this vast area but they are all considered to be *C. canephora* species (Wilson, 1999).

C. arabica is the most economically important and widely cultivated *Coffea* species. It is the only tetraploid (2n = 4x = 44) in the genus, and is self-fertile. Ethiopia is the centre of origin of *C. arabica* where it grows naturally at an altitude of 1300-1800 meters above sea level. From here it spread to Arabia, through trade caravans, where the discovery of brewing coffee was made in the 15th century (Purseglove, 1981).

History of world distribution of *C. arabica*

The Dutch were responsible for introducing *C. arabica var. arabica* into Java in 1699 and later on to Amsterdam Botanic Gardens in 1706. A progeny from Amsterdam tree was given to Louis XIV of France. Planting material from Amsterdam was sent to Surinam in 1718, where it spread into Brazil and Cayenne while from Louis XIV plant, planting material was sent to Martinique about 1720, and later spread to Jamaica in 1730, the Caribbean, Central and South America (Purseglove, 1981). Hence much of the *C. arabica var.*

arabica growing in the New World tropics came from a single tree. From the Amsterdam tree, progeny was sent to the Philippines in 1740 and Hawaii in 1825. In Africa, the French took *C arabica var. bourbon* from Ethiopia to Bourbon (now Réunion). In about 1718 they then spread *C. arabica* in their colonies, while Nyasaland got the same variety from the Edinburgh Botanic Gardens in 1878 whence it spread into Uganda in 1900 (Purseglove, 1981).

History of *C. arabica* introduction and distribution in Tanzania

The earliest documented introduction credits the Holy Ghost missionaries for introducing coffee into Tanzania (Table 1) (Fernie, unpublished report).

The importance of coffee to Tanzanian economy

Tanzania has 10 farming systems. One of them, the banana-coffee-horticulture system, found in Kagera, Kilimanjaro, Arusha, Kigoma and Mbeya regions is characterized by tree crops, high intensive land use, land scarcity and volcanic soils with high fertility. Coffee plays a leading role in this system both economically and environmentally. It is also the largest export crop; it contributes approximately

US$115 million to Tanzania's export earnings (Baffes, 2003). It is the largest export crop contributing about $115 million to export earnings and provides employment to some 400,000 families. About 95% of coffee cultivation is by smallholders with parcels of land from 1-2 hectares while 5% is grown on estates (Baffes, 2003). However, recent years have seen an upsurge in production of crops like cashew nuts and cotton (Table 2).

Constraints to coffee production in Tanzania

Tanzania's coffee industry experienced a decline from the early seventy's. Some of the factors that contributed to the decline are:

- Age of the trees which are up to 70 years old and low yields of about 250g per tree, make Tanzania's yields one of the lowest in the world.
- Lack of disease-resistant varieties.
- Removal of farm subsidies and the high cost of agricultural inputs.
- The quality of most Tanzanian coffee was poor, in the Class 8/9 range and therefore had to be sold to the most volatile and least profitable "blended" coffee market.

- Global coffee price fluctuations

Efforts to revitalise Tanzanian coffee industry.

Reforms have been undertaken to address the constraints. These are beyond the scope of this thesis but some will be mentioned. A number of farms which were previously nationalized have been privatized and replanted. Reforms in coffee marketing, which are favourable to the producers, have been undertaken. There has been an enormous increase in coffee processing capacity as a result of construction of new factories. Since 1993 twelve new factories have been built (Baffes, 2003). Coffee research has been restructured with the establishment of Tanzania Coffee Research Institute in 2001 which is financed by the industry. Non-governmental organisations have had very positive contributions in this revival. There have been improvements in coffee quality and the idea is to aim for "speciality coffee" markets.

Speciality coffees

The concept of "speciality coffee" simply means beans with special flavours as a result of interaction of the geographic microclimates

e.g. altitude, latitude, soil, rainfall, temperature etc. and the variety of coffee resulting in beans with unique flavour profiles, hence the term "specialty coffees." Good husbandry practices, processing and handling all are important parts in production of speciality coffees. Tanzania has the ideal environment, climate and altitude and coffee varieties to produce “specialty” *Arabica* coffee that commands premium prices in the world market. This seems to be the way forward in making Tanzanian coffee more competitive on global markets in order to benefit small-scale farmers.

Molecular genetic diversity analysis of *C. arabica*.

Characterization of genetic diversity within a crop plant is important in that it determines the extent to which the crop can be improved or otherwise changed by selection. Knowledge of the available variability will among other things lead to decision on plant breeding options and selection strategies. Traditionally breeders have used visible phenotypic features like fruit size, shape, colour etc. Such methods of selection of useful traits based on phenotypic features are however influenced by the environment. Molecular markers offer a more reliable approach. Data generated from molecular markers can

provide information on phylogenetic relationships, how divergent populations are and guidance on decisions concerning *in-situ* conservation strategies.

Molecular markers have been used by several workers to study genetic diversity in coffee. Use of six enzyme systems on *C. arabica* accessions from Kenya and Ethiopia failed to reveal polymorphism (Orozco-Castillo *et al.,* 1994), which contrasted to results of morphological variation detected in the same germplasm (Louarn, 1978), thus suggesting isozymes as being inappropriate for determining diversity in *C. arabica*.

Lashermes *et al.* (1996) used random amplified polymorphic DNA (RAPD) markers to successfully analyse the genetic diversity among cultivated and sub-spontaneous accessions of *C. arabica*. Anthony *et al.* (2001) conducted a study of genetic diversity of wild coffee (*C. arabica* L.) using RAPD markers. Orozco-Castillo *et al.* (1994) used RAPD markers generated by arbitrary primers to detect genetic diversity and selective gene introgression in *C. arabica*. The resulting dendrograms from RAPD profiles were consistent with the known history and evolution of *C. arabica*. From the results it was possible to separate the Ethiopian materials from the *Typica* and

Bourbon accessions which were included in the study, and classify the collected Ethiopian materials into four groups.

Anthony *et al.* (2002) were able to show a successive reduction of genetic diversity during the dissemination of coffee from its primary centre of diversity using amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSRs) markers. Both markers were used to assess polymorphism between and within cultivars from *Typica* and *Bourbon*, and relating these to four Yemen cultivars and eleven subspontaneous accessions from the primary centre of diversity. Ruas *et al*. (2003) successfully used inter simple sequence repeat (ISSR) markers for genetic differentiation of eight *Coffea* species and to identify the parentage of six coffee interspecific hybrids.

Analysis of genetic diversity within and among six *C. arabica* cultivars by AFLP markers showed cultivar Catimor to be more diverse while cultivar Caturra had the least diversity. Diversity between *C. arabica* and two other species, *C. canephora* and *C. liberica,* was also estimated. *C. canephora* was found to be closely related to *C. arabica* (Steiger *et al*., 2002). Lashermes *et al*. (2000) used AFLP markers to analyse introgressive breeding in coffee. Nineteen *Arabica* coffee introgression lines (BC_1F_4) and two Timor

hybrid accessions were analysed for introgression of *C. canephora* genetic material. These were compared to *C. arabica* and *C. canephora*. Genetic diversity in Timor hybrid-derived accessions appeared to be approximately double that found in *C. arabica*.

A combination of restriction fragment length polymorphism (RFLP) markers and genomic *in situ* hybridisation (GISH) were used to investigate the origin of the *C. arabica* species. Hybridisation between *C. eugenoides* and *C. canephora* was identified as the origin of *C. arabica* (Lashermes *et al*., 1999). Identification of DNA introgression fragments from *C. canephora* in four *C. arabica* lines and assessment of polymorphism among *C. arabica* and *C. canephora* accessions was done using microsatellites. Analysis managed to group *C. arabica* accessions from the *Typica* and *Bourbon* genetic bases separately according to their genetic origin. *C. canephora* from Central Africa was grouped with a canephora – derived hybrid, while *C. canephora* from West Africa was separated from the rest of the accessions. (Anthony *et al*., 2002).

Objectives of the study

The main objective of this study was to evaluate genetic diversity of Tanzanian cultivated *Arabica* coffee.

Specific objectives

- To use molecular markers to evaluate genetic diversity in Tanzanian cultivated *C. arabica* L.
- To provide an insight into the degree of genetic variability available in the Tanzanian cultivated *C. arabica*.
- Provide informed guidance on the need for introductions for overall improvement of coffee.

Materials and methods

Samples of coffee leaves from smallholders' farms were collected from five regions of Tanzania namely Kilimanjaro, Arusha, Tanga, Morogoro and Mbeya (Fig 1). DNA extraction was done at the Mikocheni Agricultural Research Institute (MARI) laboratory in Dar es Salaam, using a modified CTAB procedure as described by Aga *et al*. (2003). For random amplified polymorphic DNA (RAPD) (**Paper I**), and inter-simple sequence repeat (ISSR) analysis (**Paper II**), 10

base pair (bp) and 17 bp primers respectively were used for polymerase chain reaction (PCR).

Plant material

RAPD analysis involved 144 accessions representing sixteen locations while 100 accessions of *C. arabica* and 10 diploid coffee accessions were used in ISSR analysis (Table 3). The diploid coffee species comprised of two accessions from *C. eugenoides*, five from *C. zanguiberae* and three from *C. mufindiensis*.

Data analysis

ISSR data were analysed by NTSYS and MAPRF6-DDAT soft wares while additional software, DISPAN was employed for RAPD data.

RAPD analysis (Paper I)

Ten RAPD primers that detected polymorphism between accessions and gave reproducible banding patterns were chosen. They produced 86 fragments that had size ranging from 100-1400 bp. The average

dissimilarity values were 0.47 and 0.67, within and between-provenances respectively.

A dendrogram resulting from the between-provenance matrix showed two main clusters (Fig. 2). Kilimanjaro provenances were in the first two sub-clusters of the first cluster, while Arusha, Morogoro and Tanga provenances occupied the third and fourth sub-clusters. Maweni provenance stood alone then followed the second cluster, which had Mbeya provenances. One provenance from Kilimanjaro (Chombo) appeared at the lower end of the dendrogram.

Bootstrap analysis of the data showed values that were below fifty, except for two (Kibohehe- Kilema 71 and Kifumbu-Keiti 58). The resulting dendrogram based on Nei *et al*. (1983) genetic distances, resulted into two clusters with a more accurate grouping of the provenances according to their geographic origin (Fig. 3).

ISSR analysis (Paper II)

ISSR analysis generated 82 fragments ranging in size from 200 bp to 3500 bp. The overall mean dissimilarity value between-provenances (0.73), was higher than that of within-provenance (0.55). Cluster analysis of all accessions based on dissimilarity values generated a dendrogram with four clusters (not shown due to size of figure). The

first and second clusters had most accessions from Kilimanjaro and Arusha regions. The third cluster had accessions from Tanga and Morogoro while the fourth cluster had Mbeya and diploid coffee accessions. Similar grouping of the accessions was shown by principal coordinate analysis. The plot of the first three coordinates explained 43% of the observed variation. There were three distinct groupings. The first had Kilimanjaro and Arusha provenances; the second had Tanga and Morogoro provenances and the last group was comprised of Mbeya and diploid coffee species.

Cluster analysis based on Nei (1978) genetic distances (Fig. 4), gave a two-cluster dendrogram comprising of Kilimanjaro and Arusha accessions, Tanga and Morogoro accessions in two subclusters of the first cluster and Mbeya and diploid coffee accessions in the second. Cluster analysis of diploid coffee species using dissimilarity values gave a two cluster dendrogram, one with all *C. zanguiberae* accessions and the other with the *C. mufindiensis* accessions. The two *C. eugenoides* stood separate from the rest (Fig. 5). Cluster analysis based on Nei (1978) genetic distances (Fig. 6) separated C. eugenoides from the cluster of *C. zanguiberae* and *C. mufindiensis*. Principal coordinate analysis, the first three

coordinates of which explained 73% of the variation observed, showed the same pattern of grouping of the three species.

The mean dissimilarity values between provenances 0.73 were higher than within provenances 0.55 which underscores the low variability and the inbreeding nature and the effect of selection on cultivated Arabica coffee. Provenances from the same region clustered together.

Conclusions and recommendations

- RAPD and ISSR markers have shown their ability to identify variation in coffee. It was also evident that southern region, coffee accessions (Mbeya), were different from the rest of Tanzanian *Arabica* coffee; they could probably be of interest to the coffee improvement programme in Tanzania.
- The arabica coffee in Tanzania has low genetic diversity. This is a result of selection, its reproductive biology, and the narrow genetic base of the cultivated *Arabica* coffee. Similar trends have been observed in other studies (Anthony *et al*., 2001, 2002). They attributed the low genetic diversity in *C.*

arabica to its allotetraploid origin, reproductive biology and evolution (Lashermes *et al*., 1995, 1999).

- For rapid improvement in breeding work, we suggest widening of the existing genetic base by having more introductions especially from the centre of diversity (Anthony *et al.*, 2001), initiation of hybridisation programmes to create variability and use of diploid species as a source of desirable genes (Lashermes *et al*., 1995, 1999).

Study II: Diversity and structure of East African Tall coconuts (*Cocos nucifera* L.) in Tanzania (Paper III)

Introduction

The coconut palm, *Cocos nucifera* L. (2n = x = 32) is a monocot, that belongs to the family *Arecaceae* (*Palmae*). It is believed to have originated in the South West Pacific region (Purseglove, 1972; Child, 1974; Ohler, 1984). The two closest botanical relatives to the coconut have been found in Southern Africa (Uhl and Dransfield, 1987) and Madagascar (Dransfield, 1989) which makes it likely that the wild type coconut may have existed on the fringes of the Pacific and Indian oceans since the earliest time (Schuiling, 1991). Although

it is not possible to suggest when the coconuts were first introduced in Africa, it is certain that coconuts have existed on the coast of East Africa since time immemorial (Schuiling, 1991).

Botany

There are two major classifications of coconuts based on stature; *C. nucifera typica* (tall type) and *C. nucifera nana* (dwarf type). However, studies of Sri Lankan coconut germplasm identified an extra intermediate type, *C. nucifera aurantiaca* which is intermediate between *typica* and *nana* in (Liyanage, 1958). Tall types are cross-pollinated while dwarfs are self pollinated.

The inflorescence, a spadix, has fewer female flowers than male flowers which are enclosed together within a spathe which arises from the axil of each leaf; most of the male flowers are borne singly or in pairs towards the branch tips (Fig. 7). Pollination of the flowers is either by insects or wind. In tall types the male flowers mature and wither before the female flowers become receptive (a condition known as protandry) so that flowers in the same inflorescence cannot pollinate one another. This ensures cross-pollination. Generally in dwarfs, there is an overlap of the male and

female phases which promotes selfing. Hence, dwarfs are reasonably homozygous.

Coconuts in Tanzania

The East African Tall (EAT) coconut, a major tree-crop in Tanzania's coastal farming system, is cultivated mostly by smallholders. The coconut-producing area is a coastal strip of land which is approximately 50 km wide except in Coast region where it is 70 km running parallel to the coast. Other areas include the islands of Zanzibar, Pemba and Mafia which are off the coast of Tanzania. Further inland, there are other smaller areas around the shores of lakes Victoria, Nyasa and Tanganyika. However the area with potential for coconut production is estimated to range between 500,000 to 550,000 ha (Romney, 1984). Annual production is estimated at 90,000 tonnes of copra equivalent (Persley, 1992).

The coconut is an important source of vegetable oil in Tanzania. It is a smallholder crop that offers not only food, drink, income, shelter, job opportunities etc. to millions of people in Africa, but also plays an important role in the sustainability of the agricultural systems in the often fragile ecosystems of the tropical

coastal belts (Persley, 1992). One of the objectives of the coconut improvement program in Tanzania is to breed high yielding varieties that are resistant to prevailing biotic and abiotic stresses. To facilitate breeding, the Mikocheni Agricultural Research Institute (MARI) maintains a number of exotic and indigenous coconut accessions. More than 61 local and exotic accessions are maintained as active field collections. A large number of the accessions are EAT types, collected from different locations in Tanzania and neighbouring countries. Several factors make coconut genetic improvement difficult and these include its long generation time. It may take up to 7 years to reproduce (Sangare, 1992), it cannot be propagated vegetatively, and its maximum seed production peaks around 100 nuts per plant per year (Dhamodaran et al., 1991). Hence, coconut-breeding research is an expensive and a time-consuming undertaking. In view of this, it is extremely important that the different genetic materials are well characterized and evaluated. Morphological and agronomic traits have been used to characterize and evaluate coconut populations but they are time-consuming and labour intensive, and strongly influenced by environmental conditions (Akpan 1994, Ashburner et. al., 1997). Carotenoid differences (Fernando et al., 1997) and isozyme

variations (Carpio, 1982) have limited discriminatory capacity. With current developments in biotechnology, marker techniques have been employed to study genetic diversity in coconuts.

Coconut-specific microsatellite primers were used to evaluate the level and distribution of genetic diversity of Sri Lankan *C. nucifera* L. var *typica* to generate data that would help formulate future collection strategies and selection of parents for the breeding programme (Perera, 2001). The same workers used microsatellite primers to study genetic relationships among 94 coconut varieties/populations of tall and dwarf types representing the entire geographic range of the cultivation of coconut. Dwarf types were grouped in a single cluster within coconuts selected under cultivation, the "Niu Vai" type. These are predominant in Southeast Asia, Pacific islands and the west coast of Central America, implying that dwarf types evolved from "Niu Vai" types. Tall types also showed greater diversity compared to dwarfs (Perera *et al*., 2003). Amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) were employed in genetic diversity studies of coconut populations from various geographic regions of the world (Teulat *et al*., 2000, Lebrun *et al*., 1998). Other DNA markers that have been used in coconut genome analysis

include inverse sequence tagged repeats (ISTR) and random amplified polymorphic DNA (RAPD) markers. (Ashburner *et al*., 1997, Rohde *et al.*, 1995).

Objective of the study

The objective of this study of diversity using accessions from the coconut germplasm bank was to:

- Assist in formulating future prospection, collection and conservation strategies.
- Provide guidance on choice of populations for inclusion in the coconut breeding programme.

Materials and methods

Samples for DNA extraction were obtained from spear leaves of ten randomly selected accessions from each of twelve local provenances maintained at the Chambezi Research Station, Tanzania. Four provenances each represented the northern part of the coconut-growing belt (Livestock Breeding Station, Boza, Vuo and Mwambani in Tanga Region); the central part (Chambezi Green, Chambezi Brown, R.C. Bagamoyo and Tumaini) and the southern

part (Kilwa Singino, Mtoni, Ng'apa and Msanga Mkuu) (Table. 4). Five regions were covered in this study (Fig. 8). DNA extraction and RAPD analysis resulted in data that was analysed by MAPRF6-DDAT and NTSYS software.

Results and discussion

The accessions formed two major clusters, one with "central-south" provenances and the other with "northern" provenances (Fig. 9). Similar results were obtained from a bootstrap analysis after generating a Nei (1983) distance dendrogram (Fig. 10). However, only four of the bootstrap values were above fifty. The "north/central-south" divide is derived from an imaginary line cutting through the middle of Tanzania from Rukwa region in the west to Dar es Salaam in the east. The clustering is consistent with the history and distribution of coconut palms in Tanzania.

Schuilling (1991), points out "The Shirazi, who derive their name from the town of Shiraz on the Persian Gulf settled in East Africa from the 9th century AD onwards. Wild type coconuts may have grown spontaneously around their earliest settlements but there is no doubt that they imported coconuts as well". The Shiraz first settled along the coast of present-day Somalia and Kenya and later

from 11th century onwards moved southwards and settled in many towns along the coast as far south as Sofala in present-day Mozambique. At the beginning of the German colonial rule in Tanganyika, 75% of the coconut palms grew in the Tanga and Pangani districts "north", while only 4% grew in the "central-southern" Kilwa and Lindi districts (Schuiling, 1991). The German colonial authorities encouraged most of the plantings in the "central-south" with imports from or via Mafia and the Comoros; other possible sources were from German Pacific possessions. Thus in the south coconut palms have a more recent history. Coconut planting in the south has a more recent history and was promoted mostly from the beginning of the German colonial rule and some of the planting materials were from German colonies outside of the then Tanganyika.

Conclusions and recommendations

- With regard to prospection, collection and conservation strategies: Being a cross-pollinating species, EAT palms are likely to contain a large proportion of the total genetic variation. The total genetic variation of the species will be spread over populations depending on the impact and

direction of selection due to environmental variation and genetic drift. Therefore representative provenances of both “north” and “central-south” will be designated for prospection, collection and *in situ* conservation.

- Regarding guide on breeding populations and conservation, more emphasis should be directed to *in situ* conservation after identifying provenances with high variability. Selections can be made from such populations for the breeding programme.
- As for the breeding programme, *ex-situ* conservation should mainly be for facilitating breeding work or when the situation warrants and resources are not limiting. Data from fruit component analysis, individual palms and populations with phenotypes uncommon to the known diversity, palms naturally adapted to drought etc. should be collected for inclusion in the breeding programme and for *ex situ* conservation.

Study III: Micropropagation of new Coffee varieties from Tanzania (Paper IV)

Introduction

Coffee, the largest export crop, contributes approximately US$115 million to Tanzania's export earnings, and provides employment to some 400,000 families. Two- thirds of the coffee output is *Arabica* and one-third *Robusta*. Smallholders grow about 95 percent of the coffee while the remaining 5 percent is grown on estates (Baffes, 2003) Some years after embracing socialist policies, most Arabica coffee estates in Northern Tanzania, where most of it is grown, were nationalized. The Cooperatives and the Coffee Board which had authority over the coffee industry did not seriously address the needs of the coffee sector leading to serious deterioration of the coffee industry. In the early 1990's, political and economic reforms to revamp the agricultural sector were complemented by reforms in coffee research and development.

The succession of changes was followed by restructuring of the coffee research and development culminating in the formation of Tanzania Coffee Research Institute (TaCRI) in year 2000, which is supported by the coffee industry. Its goals are to contribute to the

revival of the coffee industry to sustainable prosperity and to improve the livelihoods of coffee producers as well as raising the country's profile as a reliable source of adequate volumes of high quality coffee. TaCRI has introduced new locally-bred, *Arabica* varieties that are high yielding and disease resistant. These were brought in to replace less productive, disease-susceptible old trees and varieties. Tanzania has an estimated 200 million old *Arabica* trees. Multiplying and distribution to farmers, of planting materials from these new varieties has started but the quantities are still marginal because of the limitation of the multiplication methods i.e. seed multiplication and vegetative cuttings. Plans are to distribute five million seedlings to farmers annually by 2006, hence the need for *in vitro* micro propagation.

Materials and methods

See manuscript for **Paper IV** for a detailed description.

Results and discussion

No significant differences were observed when comparing Medium A and B (Fig. 2 in **Paper IV**), however the former elicited a slightly higher average embryo production from the cultures than the latter.

Using leaf segments from in vitro cultured plants, Fuentes-Cerda et al. (2001) noted that nitrogen played a crucial role in embryo induction as elevated levels of nitrate and ammonium decreased culture embryogenicity. As the total nitrogen in the medium increased the response to somatic embryogenesis decreased.

The analysis of embryo-producing explants (EPEs) for the petiole end (PE) and distal end (DE) leaf segments showed no significant difference (Table 2 & 3 **Paper IV**). It is likely that data on number of embryos per explant might have been more informative (the present scores were taken at the early stages of embryo production). During data collection a few of the embryos had developed into plantlets (Fig 3 in Paper IV). A high concentration of ammonium and potassium nitrate in media B is the most probable explanation for the better performance of embryos and plantlets in Bioreactors with media B. (Fig. 4a & 4b in **Paper IV**)

Results (Table. 4 in **Paper IV**) showed that benzyladenine (BA) is the most influential factor in stimulating embryo production. BA at 10µM gave the highest EPEs percent of 48 and 30 in media A_V and A_{VI} respectively. A decrease in BA level caused a corresponding decrease in EPEs. Combining triacontanol (TRIA)

with BA resulted in more than two-fold increase in EPEs. This was observed by comparing media with and without TRIA i.e. A_{III}, A_{IV} against A. Presence of TRIA caused an increase in EPEs. Working with *C. arabica*-in vitro-regenerated leaf explants, Giridhar et al. (2004) had similar observations concerning the effect of BA\TRIA combination. Other effects of TRIA could not be ascertained at this stage of growth since it is also known to promote morphogenetic responses e.g. the number and length of roots, shoot growth, fresh weight, and chlorophyll content. With regard to the temporary immersion systems (RITA®), liquid media B is suitable for use in embryo development and plantlet growth.

Concluding remarks

- More trials with all varieties should be done on media $A_{V,}$ $A_{VI,}$ A_{III} and A_{IV} to find out if there are effects between variety and medium interaction. This will guide in the matching of media to variety.
- A repeat of the experiment on PE and DE leaf segments and collection of data on number of embryo per explant would shed more light on whether or not position of a segment on a leaf has influence on embryo production.

- It is possible to start micropropagation of the varieties with the available information and this should be considered.

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Table 1. Introduction and distribution of *Coffee arabica* in Tanzania

Year	Event
1877	The earliest known record shows that a Catholic missionary by the name of Father Horner made the first coffee introduction to Tanzania from Réunion (formerly known as Bourbon), (Kieran, 1966).
1879	It is possible that another introduction was made two years later from Bourbon to Mhonda in the Nguru Mountains in Tanganyika.
1880	A missionary of the Holy Ghost fathers, named Baur, introduced coffee seeds from Aden to Bagamoyo.
1883/4	Another introduction, whose origin was Ethiopia, was made this time to Morogoro (Cavalho, 1959).
1892	Missionary, Auguste Commenginger took coffee seed from Morogoro to Kilema mission in Kilimanjaro. About 115,000 coffee trees, whose origin is not given, were already planted in Usambara.
1893	Further introduction of coffee seed from Bagamoyo to Morogoro and Kilema.
Prior to 1914	"Menado" a strain from Java introduced and planted extensively in the Usambara mountains and at Mission stations in Iringa district.
1920's	"Kent" variety was introduced into Tanzania from South India. A "Nyasa" strain (from Malawi) was introduced into Tanzania shortly after the Bourbon; this was planted in the Nguru, and Uluguru mountains of Morogoro district. The origin of the Nyasa strain was the Blue Mountain region of Jamaica.

Source: Fernie, L. M. (undated)

Table 2. Agricultural production from mainland Tanzania ('000 tons)

Cash crops	1998/99	1999/00	2000/01	2001/02	2002/03[a]
Cashew nuts	103	121	122	67	88
Coffee	47	48	58	38	50
Cotton seed	106	101	123	149	189
Tea	22	25	26	25	28
Sisal	23	21	21	24	24
Tobacco	38	32	25	28	32

[a] Estimate.

Source: Bank of Tanzania

Table 3. Origins of *C. arabica* samples for ISSR analysis

Region	District	Location
Kilimanjaro (KIL)	Moshi Rural	Kilema RC Parish (Ki)
	Moshi Rural	Kibohehe (Kb)
	Moshi Rural	Keiti (Ke)
	Moshi Rural	Kifumbu (Kif)
	Moshi Rural	Chombo (Ch)
Arusha (AR)	Arumeru	Tengeru Farm (Te)
	Arumeru	Nkoanenkoli (Nk)
Tanga (TAN)	Lushoto	Gare (Ga)
	Lushoto	Bazo (Ba)
	Lushoto	Ziwai (Zi)
	Lushoto	Maweni (Ma)
Mbeya (MBY)	Tukuyu	Bugoba Masebe (Bg)
	Mbozi	Shiwanda (Sh)
	Ileje	Bwenda (Bw)
	Ileje	Ileje (Ij)
Morogoro (MOR)	Morogoro Rural	Luale (Lu)
Kilimanjaro (DpSp)		Lyamungu Gene Bank

Table 4. Names of coconut provenances and their regions of origin

Accession name	Abbrev-iation	Region of origin	Geographical location
Kilwa Singino	KS	Lindi	South
Mtoni	MT	Lindi	South
Ng'apa	NG	Lindi	South
Msangamkuu	MMK	Mtwara	South
Tumaini	TU	Dar es Salaam	Central
Chambezi green	CHG	Pwani	Central
Chambezi brown	CHB	Pwani	Central
Bagamoyo	BG	Pwani	Central
Livestock Breeding Station	LBS	Tanga	North
Boza	BOZ	Tanga	North
Vuo	VUO	Tanga	North
Mwambani	MWA	Tanga	North

Table. 5. BA\TRIA combination in the different media

Media	BA	TRIA
A	5	0
A_I	5	11.38
A_{II}	5	2.85
A_{III}	2.5	11.38
A_{IV}	2.5	2.85
A_V	10	11.38
A_{VI}	10	2.85

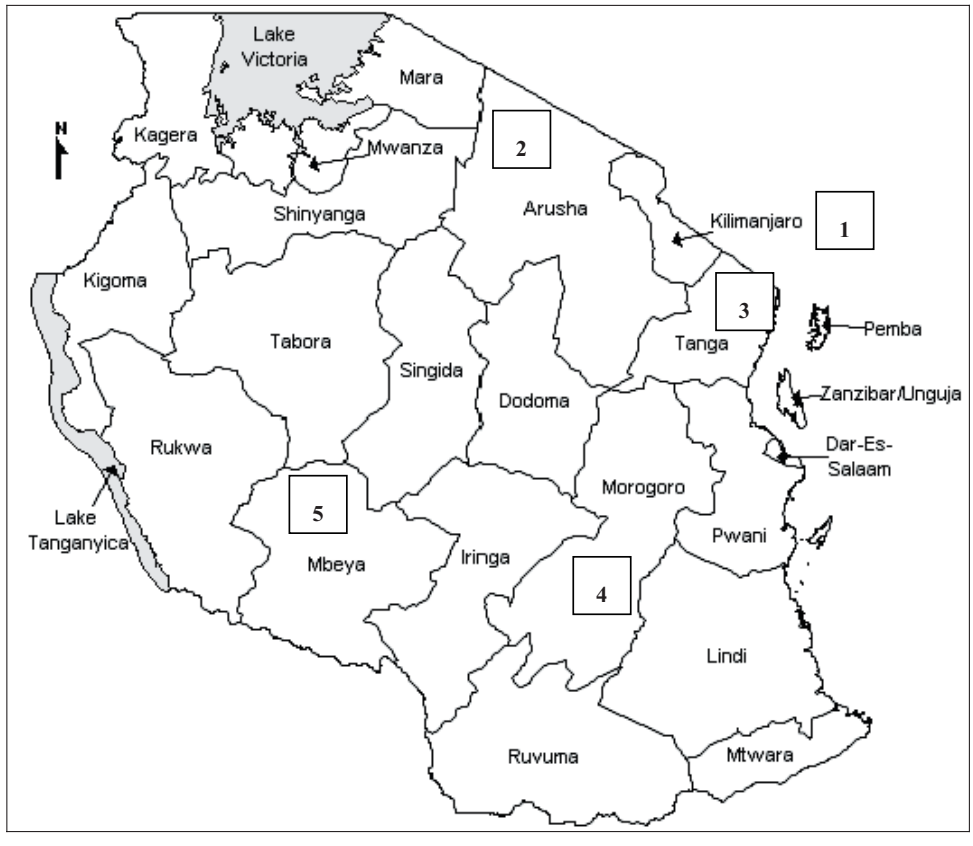

Fig. 1. Map of Tanzania showing regions were coffee samples were drawn for both RAPD and ISSR analysis.

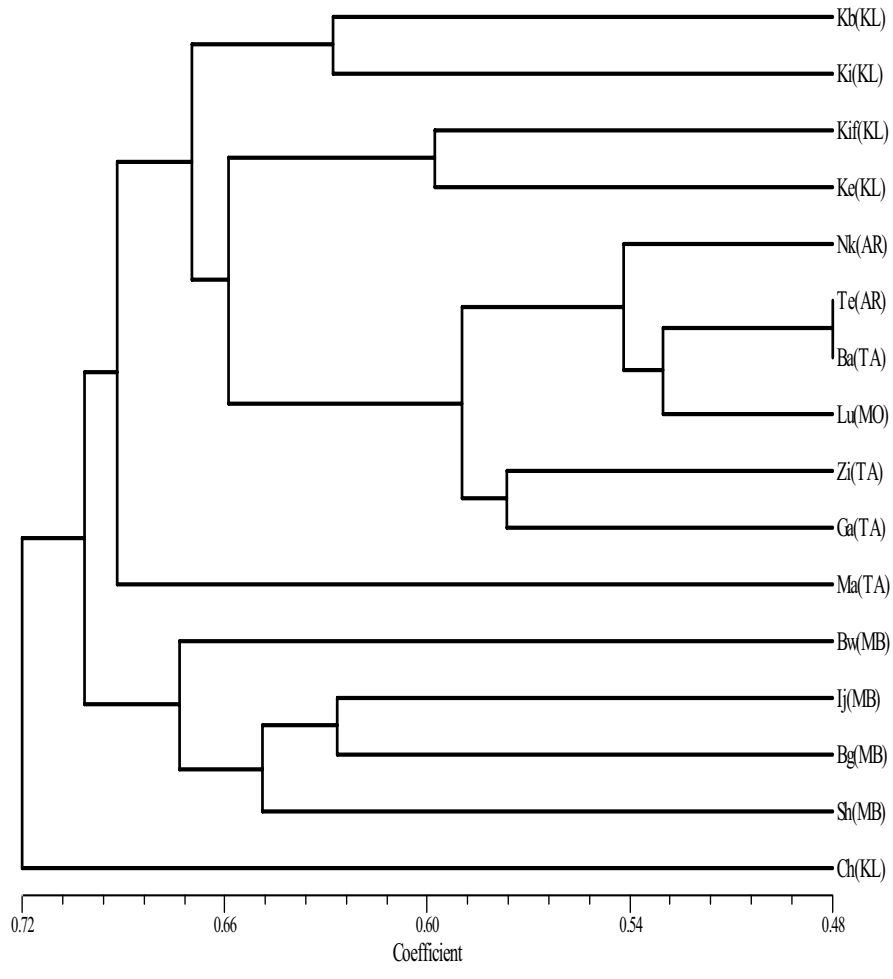

Fig. 2. Dendrogram of cultivated Arabica coffee based on between-provenance dissimilarity matrix from RAPD data using Jaccard's (1908) coefficient. KL – Kilimanjaro; AR – Arusha; TA – Tanga; Mo – Morogoro; MB – Mbeya.

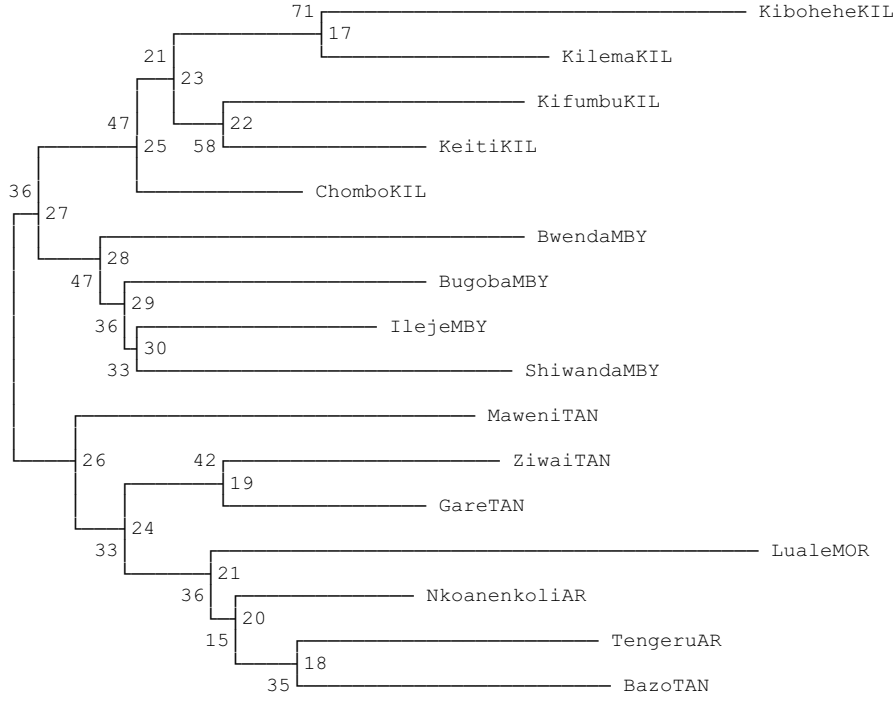

Fig. 3. Neighbour-joining dendrogram of RAPD data based on Nei (1983) distances.

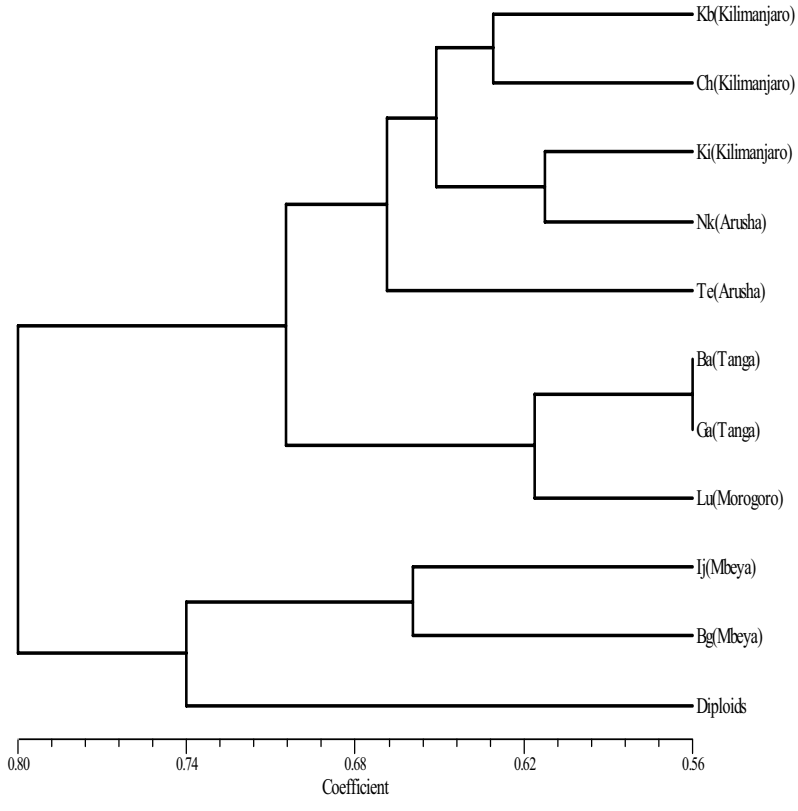

Fig. 4. A dendrogram based on ISSR data of ten *C. arabica* provenances and diploid coffee species using Nei (1978) genetic distances. (Location codes correspond to Table. 3).

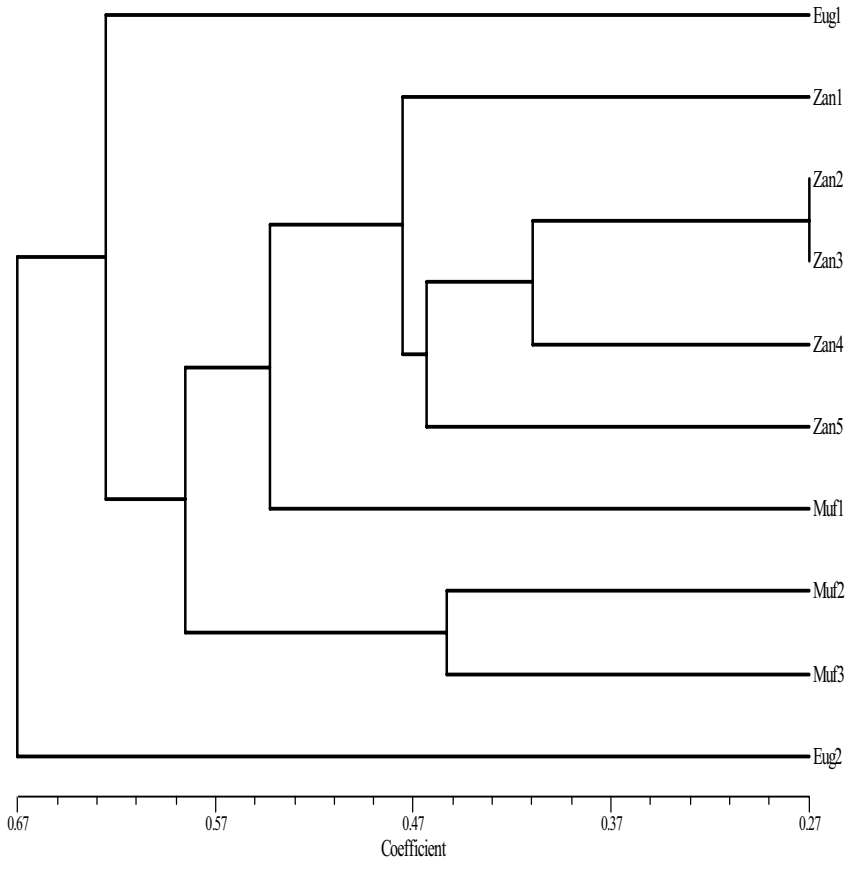

Fig. 5. ISSR data-dendrogram of diploid coffee species resulting from Jaccard's (1908) coefficient-dissimilarity matrix. *Eug, Eugenoides; Zan, zanguibariae; Muf, mufindiensis.*

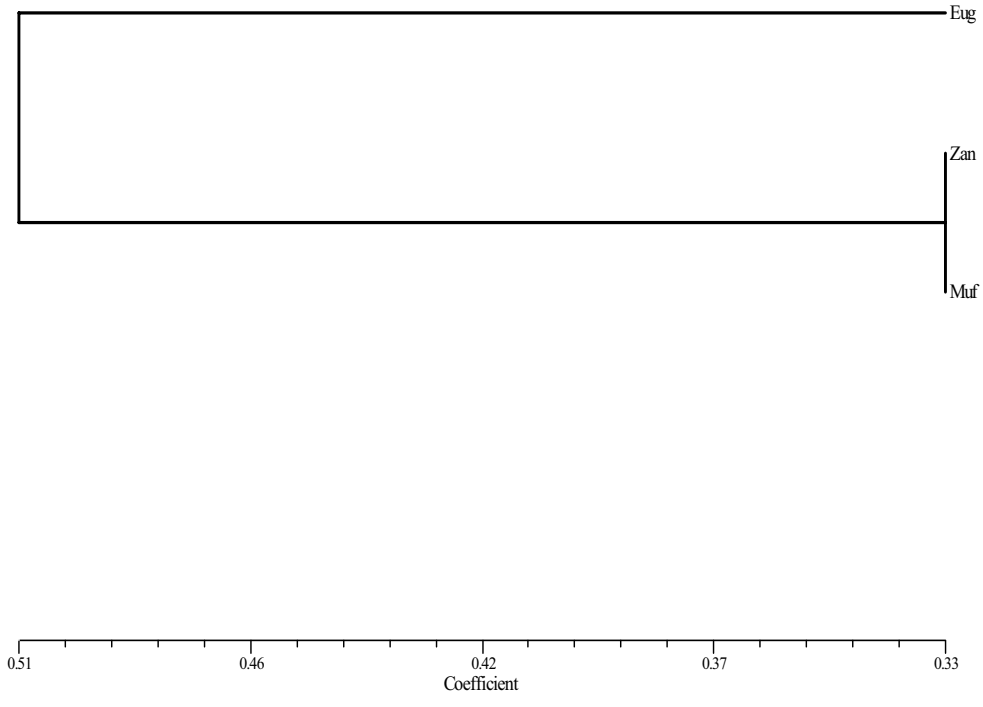

Fig. 6. ISSR-data dendrogram of three diploid coffee species using Nei (1978) genetic distances. *Eug, Eugenoides; Zan, zanguibariae; Muf, mufindiensis.*

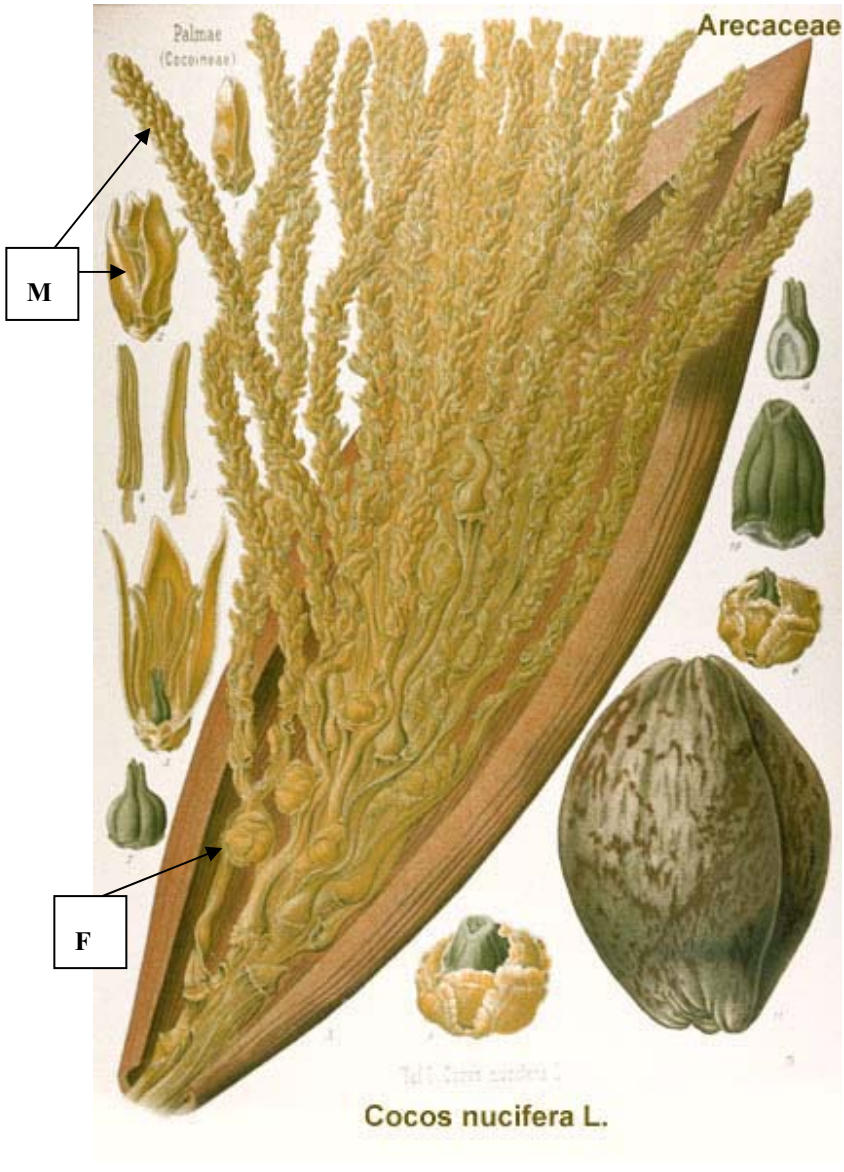

Fig. 7. The coconut palm inflorescence showing male and female flowers. M = male; F = female.

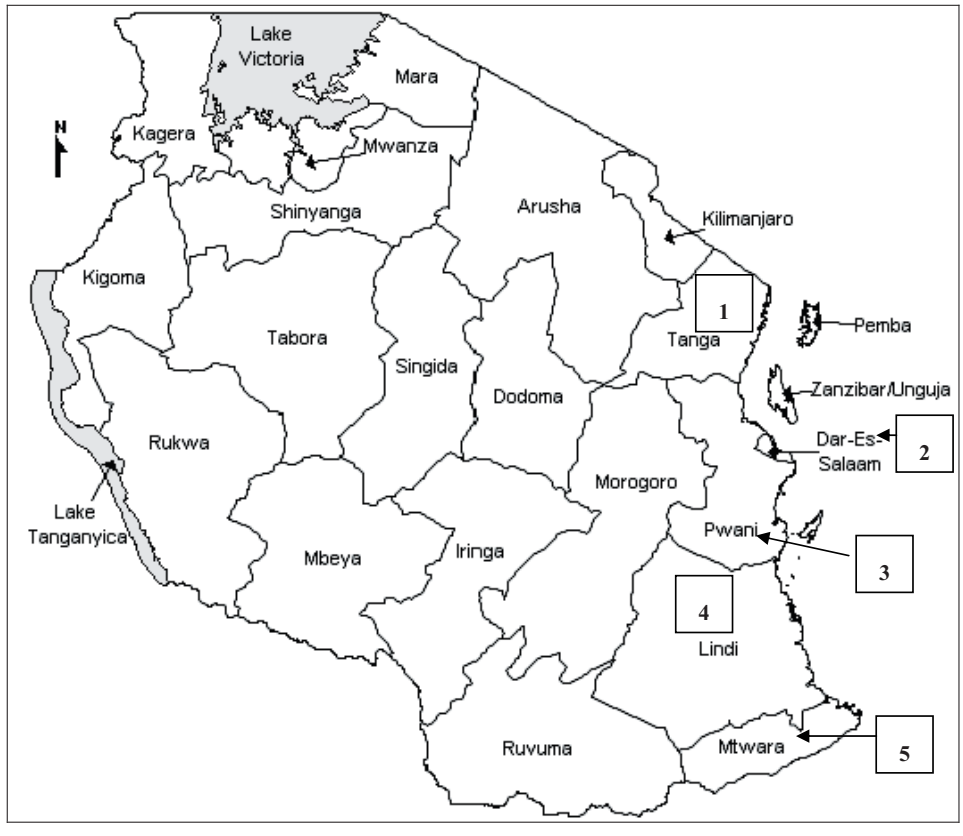

Fig. 8. A map of Tanzania showing the five regions where the EAT accessions originated.

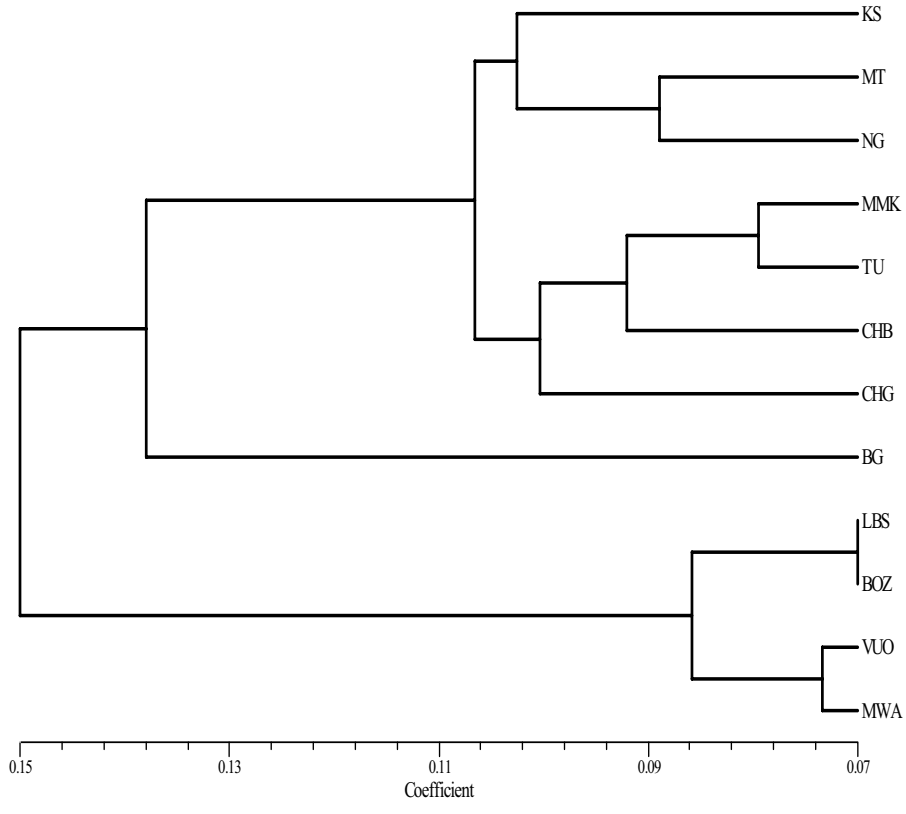

Fig. 9. UPGMA tree of twelve coconut provenances using Nei (1978) genetic distances. Abbreviations are from Table. 4.

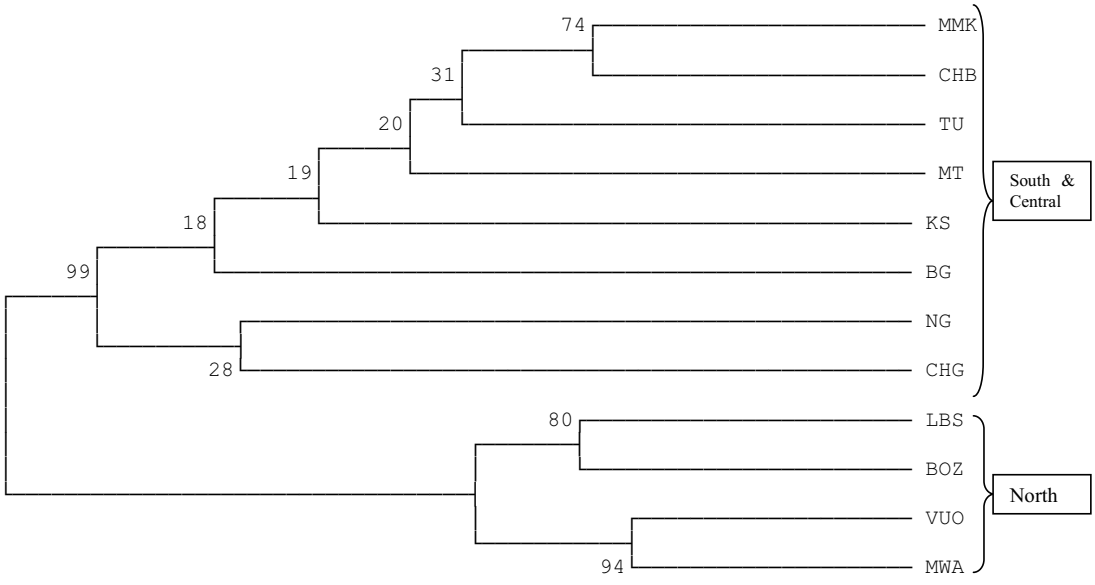

Fig. 10. UPGMA dendrogram using Nei *et al.,* (1983). Abbreviations are from Table. 4.