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# GEOGRAPHIC CHARACTERISATION OF AFRICAN PROVENANCES OF *FAIDHERBIA ALBIDA*

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## ABSTRACT

Polymerase Chain Reaction (PCR) analysis based on Internal Franscribed Spacers (ITS) and Random Amplified Polymorphic DNA (RAPD) were carried out on sixteen provenances of *Faidherbia albida* currently growing in a provenance trial in semi-arid Baringo district in Kenya. The objectives of the study were to: (i) determine the phylogenetic relationship among the 16 provenances in order to establish the species centre of origin, and (ii) determine the extent of genetic diversity in *F. albida* using PCR markers. ITS data did not produce any consistent regional or geographic pattern. RAPD data produced a dendrogram clearly grouping the provenances into Western, Southern and Eastern African regions. The study revealed the utility of RAPD markers in understanding geographic variation and phylogenetic relationships among *F. albida* populations in Africa.

*Key Words*: DNA, Genetic diversity

## RÉSUMÉ

L'Analyse de Réaction par Polymérase en Chaine (PCR) basée sur Espaceurs Internes Franscrits (ITS) et l'Amplification Aléatoire à ADN Polymorphique (RAPD) étaient entrepris sur 16 provenances de *Faidherba albida*, croissant actuellement en test de provenance dans le District semi-aride de Baringo au Kenya. Les objectifs de l'étude étaient : (i) déterminer les relations phylogénétiques parmi les 16 provenances en vue d'établir le centre d'origine de l'espèce et (ii) déterminer l'ampleur de la diversité génétique au sein de *F. Albida* en utilisant les marqueurs PCR. Les données ITS n'int fourni aucun motif géographique ou régional constant. Les données RAPD ont produit un dendrogramme groupant clairement les provenances en régions africaines occidentales, australes et orientales. L'étude a révélé l'utilité des marqueurs RAPD dans la compréhension de variations géographique et les relations phylogénétiques parmi les populations de *F. Albida* en Afrique.

*Mots Clés*: ADN, diversité génétique

## INTRODUCTION

*Faidherbia albida* is an important agro-silvo-pastoral species in arid and semi-arid lands throughout Africa. Its unique reverse phenology of retaining and shedding leaves during the dry and wet seasons, respectively, provides a favourable microclimate for crops and green fodder, which is rich in protein and carbohydrates during the dry season. It enriches the soil and is also used for timber, medicines and food (Wickens, 1969).

In Africa, drought and human interference have endangered the genetic resources of this species, prompting its inclusion among the endangered plant species (Cook and Grut, 1989). It has not been domesticated and is little used outside its natural range (Saka and Bunderson, 1989).

Plantation projects in Chad and Niger failed (Kirmse and Norton, 1984), possibly due to lack of basic knowledge on the amount of genetic diversity as well as the breeding systems (Joly, 1991).

Investigations of genetic variation of 16 African provenances of' *Faidherbia albida* based on morphological characteristics in Kenya, showed consistency across Africa, but could not separate Eastern from Southern African provenances, probably due to environmental influence (Dangasuk *et al.*, 1997; Dangasuk, 1999). This shows one of the weaknesses of morphological characterisation in determining genetic diversity as well as the phylogenetic relationship among the provenances (Dangasuk *et al.*, 2002).

Molecular and biochemical techniques provide a powerful set of tools for the study of plant population genetics (Dangasuk *et al.*, 2002). Isozyme analysis in *F. albida* showed a significant deviation from the Hardy-Weinberg equilibrium and deficiency in heterozygotes (Joly, 1991; Dangasuk, 1999; Dangasuk and Gudu, 2000), which could be due to inbreeding. However, a high rate of inbreeding or selfing would be expected to generate a considerable genetic differentiation between provenances, which was not detected in the isozyme data. In addition, the isozyme results could not show significant variation in genetic structure within provenances of *F. albida,* hence, firm conclusions on phylogenetic relationships among populations could not be drawn (Dangasuk, 1999; Dangasuk, 2001). Recent reviews on the levels of variation detected in a range of plant species based on isozyme data revealed that tropical tree species maintain most of their variation within populations (Hamrick, 1990). This suggests that the classical forestry approach, which considers provenance or geographic variation as an accurate predictor of the diversity spectrum within species, may be inappropriate.

For a better understanding of population genetics of *F. albida,* there is need to use markers able to show more variations, which would allow the identification of individuals within populations that are genetically different. Restriction fragment length polymorphism (RFLP) is the commonly used DNA marker for many species to measure genetic diversity and construct genetic linkage maps (Bardakci, 2001). However because RFLP requires large quantities of species-specific pure DNA, it is time consuming, labour intensive and the need for radioactive isotopes makes it too expensive for many populations-based studies. Polymerase Chain Reaction (PCR) based DNA markers are becoming more popular and widespread research technique (Bardakci, 2001). PCR based DNA markers are gaining popularity because of their simplicity, high probability of success and because they provide flexibility in detecting genetic variation since a variety of primers can be used which are designed to reveal particular types of polymorphism based on selective amplification of DNA (Rafalski and Tingey, 1993; Bardakci, 2001).

One of the PCR based DNA marker that has found wide application in phylogentic analysis is the specific amplification of the nuclear ribosomal DNA especially the ribosomal RNA (rRNA) genes and the more variable Internal Transcribed Spacers (ITS) mainly because they are ubiquitous and well conserved DNA sequences (Raue *et al.*, 1988). According to Som *et al.* (2000), the extent of conservation of the rRNA genes and the ITS sequences vary depending on the organism, however the variation may be small hence cases of simple amplification of the ITS yields insufficient information (Bailey and Doyle, 1999). Currently, the amplification of the ITS is routinely followed by either direct sequencing of the fragments or restriction of the fragment using restriction enzymes to show the details of the ITS fragment polymorphism (Lanfranco *et al.*, 1999).

Random Amplified Polymorphic DNA (RAPD) is another (PCR) based DNA markers that is gaining popularity. Its simplicity, wide applicability and the fact that it requires only a small amount of DNA, makes it an ideal technique for gene mapping, population genetics and molecular evolutionary genetics in both plants and animals (Bardakci, 2001). RAPD analysis requires no prior DNA sequence information and it relies on short, random oligonucleotide primers for amplification of unspecified target DNAs. RAPDs, therefore, represent a PCR based technology that is immediately applicable to organisms from diverse taxa and, because of the large number of primers available for analysis, it provides good overall genome coverage. It is

therefore the simplest and cheapest method for analysis of genetic variation. The nuclear ribosomal DNA is well suited for evolutionary and phylogenetic study and hence was used for this study.

This study aimed at (i) determining the phylogenetic relationship among the 16 provenances from different geographic regions of Africa in order to establish the species centre of origin, and, (ii) determining the extent of the genetic diversity in *F. albida* using DNA markers.

## MATERIALS AND METHODS

**Plant materials.** Bulk seeds of sixteen *F. albida* provenances from different geographical regions of Africa were used in this study (Table 1). The samples were representative of the entire natural distribution range of the species in Africa (Fig. 1). A field trial was conducted at Noiweit, located at 0°10'N and 36°00'E and at an altitude of about 1,500 meters above sea level (masl), in Mogotio division of Baringo district. The district is classified as semi-arid (Teel, 1984), with a mean annual rainfall range between 500 and 800 mm. It has mean annual temperature of 21°C.

Four months old potted seedlings were transplanted into the field in April 1997, in a randomised complete block design (RCBD) with repeated observations. There were five replications and in each replication, there were 16 experimental units representing each of the provenances of *F. albida*. In each unit, there were two seedlings from the same provenance. In total, there were ten seedlings per provenance in the whole design at a spacing of 3 m x 3 m.

In June 2002, at the onset of the rainy season, young succulent leaves were collected from one tree per provenance in each of the blocks, making a total sample size of five trees per provenance. The leaves from each tree were immediately put into separate polythene bags, sealed and kept in liquid nitrogen to be retrieved later for DNA extraction.

**Extraction of total DNA.** DNA extraction was carried out on 0.5 g of liquid nitrogen-frozen leaves from each tree according to the protocol of Dellaporta *et al.* (1983), modified as follows: 0.5 g of leaf tissue was weighed and placed in a pre-chilled mortar. The leaves were then ground to a fine powder in liquid nitrogen and the powder transferred into a 1.5 ml eppendorf tube containing 700 µl of extraction buffer (100 mM Tris Hcl pH 8.0, 50 mM EDTA pH 8.0, 500 mM Nacl and 10 mM mercaphethanol added last). The mixture

TABLE 1. Collection site data for 16 provenances of *F. albida* used in this study

| CI | Code | Provenance | Country | Zone | Latitude | Longitude | Altitude (m) | Rain (mm) | Temp. °C |
|---|---|---|---|---|---|---|---|---|---|
| OFI | 1 | Bignona | Senegal | WA | 12°45'N | 16°25'W | 10 | 1408 | 26.5 |
| OFI | 2 | Mana Pools | Zimbabwe | SA | 15°45'S | 29°20'E | 360 | 628 | 25.1 |
| OFI | 3 | Chawanje | Malawi | SA | 14°36'S | 34°48'E | 600 | 900 | 24.2 |
| OFI | 4 | Bolgatanga | Ghana | WA | 10°46'N | 01°00'W | 201 | 1057 | 28.2 |
| OFI | 5 | Mwembe | Tanzania | EA | 04°08'S | 37°51'E | 860 | 569 | 23.5 |
| OFI | 6 | Dumisa | Zimbabwe | SA | 22°13'S | 31°24'E | 280 | 438 | 24.7 |
| OFI | 7 | Debre zeit | Ethiopia | EA | 08°43'N | 38°59'E | 1850 | 730 | - |
| OFI | 8 | Pongola river | South Africa | SA | 22°20'S | 30°03'E | 540 | 340 | - |
| OFI | 9 | Rama | Ethiopia | EA | 14°23'N | 38°48'E | 1350 | 742 | 19.1 |
| OFI | 10 | Lake Awassa | Ethiopia | EA | 07°03'N | 38°25'E | 1650 | 961 | - |
| OFI | 11 | Hoanib river | Namibia | SA | 19°15'S | 13°23'E | 350 | 98 | 24.0 |
| CDF | 12 | Moulvouday | Cameroun | WA | 10°23'N | 14°50'E | 330 | 815 | - |
| CDF | 13 | Tera | Niger | WA | 14°00'N | 00°45'E | 240 | 458 | - |
| CDF | 14 | Banbora | Burkina Faso | WA | 10°34'N | 04°46'W | 280 | 800 | - |
| KFI | 15 | Kainuk | Kenya | EA | 01°14'N | 35°09'E | 500 | 230 | 25.0 |
| KFI | 16 | Tot | Kenya | EA | 01°30'N | 35°45'E | 750 | 800 | 21.0 |

CI = Collector's Identity; OFI = Oxford Forestry Institute; CDF = Cirad-Foret; KFI = Kenya Forestry Research Institute; WA = West Africa; SA = Southern Africa; EA = East Africa; — = missing data

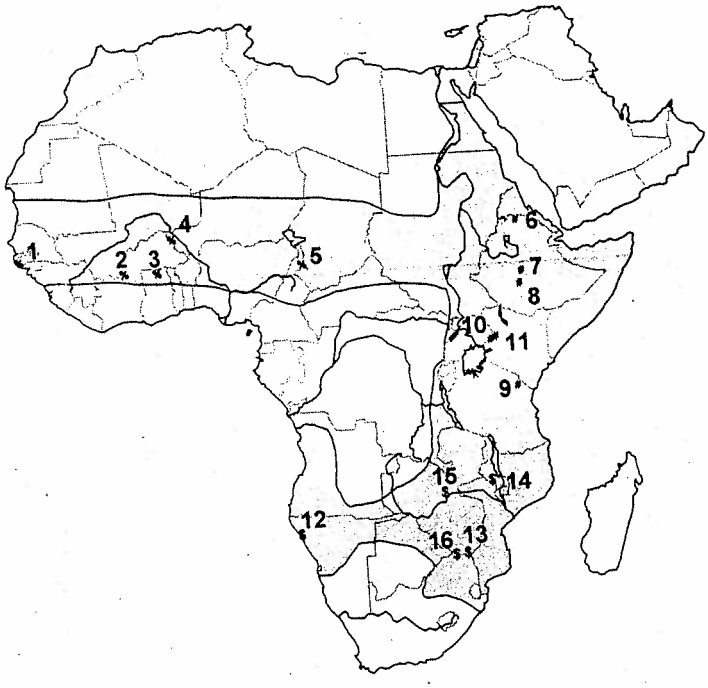


Figure 1. Natural distribution range (redrawn from Wood, 1992) and location of *Faidherbia albida* provenances used in this study (sites details in Table 1).

was homogenized by vortexing for 1 min then 70 µl of sodium dodecyl sulfate (SDS) added and mixed by gentle shaking. The mixture was incubated at 65° C for 1 hour in a water bath. Then 350 µl of phenol and 350 µl of chloroform were added and mixed thoroughly after which the mixture was centrifuged at 13000 rpm for 15 min. The supernatant was transferred into a clean 1.5 ml eppendorf tube and 700 µl of chloroform added, then centrifuged at 10,000 rpm for 15 min and the supernatant again transferred into a clean 1.5 ml tube. To this, 0.8 ml of ice cold isopropanol was added and mixed by gently shaking before adding 0.2 ml of 3 M sodium acetate. It was again mixed by gentle shaking of the tube followed by overnight incubation at -20 °C. The solution was then centrifuged at 10,000 rpm for 15 min to pellet the DNA after which the pellet was washed in 70% ethanol before re-suspension in 100-200 µl of Tris EDTA (TE) pH 8.0. To this was added RNase to make a final concentration of 20 µg/ml and the solution was incubated at 37 °C for 1 hour. The solution was then made up to 500 µl by adding TE pH 8.0 followed by addition of an equal volume (1:1) of phenol: chloroform solution. The resultant DNA pellet was re-suspended in 100-200 µl Tris EDTA (pH 8.0). The DNA was quantified by taking optical density (OD) readings on spectrophotometer at wavelength of 260 nm. The DNA concentration was then determined following the method by Sambrook *et al.*, (1989). Aliquots of 50 µl were then made and diluted to a concentration of about 60 ng/µl and stored at 4°C, ready for PCR reactions.

**PCR Conditions for ITS Amplification.** The PCR was carried out in 20 µl volumes made up of the following: 2 µl Reaction Buffer (Thermoperfect Pol), 1.2 µl of $MgCl_2$ (25 mM), 1 µl of each primer (10 pmol Sigma Genosys), 4 µl of deoxyribonucleotides (dNTPs) 0.5 mM, 1 µl of Template (60 ng $\mu l^{-1}$) and 1.5 U of Taq polymerase (Thermoperfect Pol). The mixture was then made up to 20 µl with sterile double distilled water. The

reactions were then overlain with 10 µl of mineral oil (Sigma). The reaction was carried out in a Crocodile III, Appligene oncor, thermal cycler programmed as follows: an initial denaturation of 4 min at 94 °C followed by 45 cycles of denaturation (4 °C for 30 sec); annealing (60 °C for 60 sec) and extension at 72 °C for 2 min. This was followed by a final extension of 5 min at 72 °C.

The ITS-1 site specific primer sequences used were adapted from Hillis and Dixon (1991) and synthesized by Sigma Genosys. For ITS-1 the primers used were forward 18d-5' (GCCTGCGGCTAATTTGACTCAACACGG) and reverse 5.8c-3' (AGGGTTCGATTCCGGAGAGGGAGCCTGAGAAA. While for ITS-2 the primers used were: forward 28y-5' (CTATCCGGATTCCCCTAGTAACGGCGAGT) and reverse 28z-3' (AGACTCCTTGGTCCGTGTTTCAAGAC).

**PCR Conditions for RAPD Amplification.** Amplification of the total DNA was carried out according to the procedures described by Dellaporta *et al.* (1983). Amplification was conducted in a 10 µl reaction volume comprising: 2 µl of 10x reaction buffer, 50 ng Template, 1.5 U Taq DNA polymerase (Thermoperfect pol), 10 pmol primer (GIBCO BL Custom primers), 1.5 mM $MgCl_2$, 5.25 µl sterile $ddH_2O$, 0.1mM dNTPs and overlaid with mineral oil (Sigma). The reaction was performed in a PCR thermal cycler (Crocodile III, Appligene oncor) programmed as follows: an initial denaturation of 6 min at 94 °C followed by 45 cycles of 60 sec denaturation at 94 °C; 60 sec annealing at 34 °C; extension at 72 °C for 2 min. This was then followed by a final extension of 10 minutes at 72 °C. Finally the reaction was held at 4°C till ready for separation of the PCR products by gel electrophoresis. Eleven primers were tested in total and six were selected for amplification based on the consistency, strength of amplification products and number of bands generated (Table 3). The six primers were Primer 1 (GGCTCGTACC); Primer 2 (CGTCCGTCAG); Primer 11 (CGCGACGTGA); Primer 4 (CGGAGAGTAC); Primer 5 (CCTGGCGAGC); and Primer 7 (CCAGGCGCAA). Of the six primers, only three (primers 1, 2 and 4) gave consistently reproducible banding patterns, showing a clear variation among the 16 provenances of *F. albida*. Therefore, primers 1, 2 and 4 were selected for screening each of the five representatives of the 16 *F. albida* provenances. The presence and absence of bands generated by these three primers was used to assay genetic differences among the provenances.

**Electrophoresis.** The PCR products were separated by electrophoresis on a 1.2% agarose gel and stained in ethidium bromide in a Tris Acetate EDTA (TAE buffer). The fragments were viewed under ultra violet (UV) light and photographed using a Polaroid camera.

**Statistical analysis.** ITS results were obtained directly from the bands and not subjected to any statistical analysis. The RAPD data were analysed using Numerical Taxonomy and Multivariate Analysis System (NTSYS pc 2.1) statistical package (Rolf, 1993). Using Jaccard's (1908) coefficients, a dendrogram based on between-provenance dissimilarity matrix was generated (Fig. 6). The coefficients were calculated as:

$$J_{ij} = a/(a+b+c)$$

Where:

a = the number of fragments present in both provenance i and j;
b = the number of fragments present in i and absent in j; and
c = the number of fragments present in j and absent in i

## RESULTS

**ITS-1 and ITS-2 Amplification.** There was strong amplification of both ITS-1 and ITS-2. For ITS-1, a single fragment of 900bp long amplified consistently for all the five representative of each of the 16 provenances of *F. albida* (Fig. 2). Amplification of ITS-2, (Fig. 3) showed two fragments 560 and 750bp long. The two fragments were not consistently amplified in all the 16 provenances as shown in Figure 3. The 560bp long fragment was present in all the provenances; the 750bp fragment was polymorphic between provenances. The amplification of the ITS did not

TABLE 2. Summary of profiles of ITS-1 and ITS-2 bands in sixteen *F. albida* provenances

| Population code | Origin | Region | ITS-1 | ITS-2 |
|---|---|---|---|---|
| 5 | Mwembe (Tanzania) | East Africa | 900bp | 560bp |
| 7 | Debre zeit (Ethiopia) | East Africa | 900bp | 560, 750bp |
| 9 | Rama (Ethiopia) | East Africa | 900bp | 560, 750bp |
| 10 | Lake Awassa (Ethiopia) | East Africa | 900bp | 560, 750bp |
| 15 | Kainuk (Kenya) | East Africa | 900bp | 560bp |
| 16 | Tot (Kenya) | East Africa | 900bp | 560, 750bp |
| 2 | Mana Pools (Zimbabwe) | South Africa | 900bp | 560, 750bp |
| 6 | Dumisa (Zimbabwe) | South Africa | 900bp | 560, 750bp |
| 8 | Pongola River (S. Africa) | South Africa | 900bp | 560, 750bp |
| 3 | Chwanje (Malawi) | South Africa | 900bp | 560bp |
| 11 | Hoanib river (Namibia) | South Africa | 900bp | 560bp |
| 1 | Bignoma (Senegal) | West Africa | 900bp | 560, 750bp |
| 4 | Bolgatanga (Ghana) | West Africa | 900bp | 560, 750bp |
| 12 | Moulvouday (Cameroun) | West Africa | 900bp | 560, 750bp |
| 13 | Tera (Niger) | West Africa | 900bp | 560bp |
| 14 | Banora (Burkina faso) | West Africa | 900bp | 560bp |

bp = base pair

TABLE 3. The eleven RAPDs primers used for the *Faidherbia albida* studies

| Primer | Code | Sequence | Mwt(µg/µmole) | TM (1M Na+) | % GC |
|---|---|---|---|---|---|
| 1 | C02 | GGCTCGTACC | 3157 | 34 | 70 |
| 2 | C03 | CGTCCGTCAG | 3157 | 34 | 70 |
| 3 | C04 | GTTAGCGGCG | 3237 | 34 | 70 |
| 4 | C05 | CGGAGAGTAC | 3237 | 32 | 60 |
| 5 | C06 | CCTGGCGAGC | 3182 | 36 | 80 |
| 6 | C07 | TCCCGACCTC | 3077 | 34 | 70 |
| 7 | C08 | CCAGGCGCAA | 3175 | 34 | 70 |
| 8 | IC10 | ACGCGCTGGT | 3197 | 34 | 70 |
| 9 | IC11 | GCATGGAGCT | 3221 | 32 | 60 |
| 10 | IC16 | ACGGTGCGCC | 3182 | 36 | 80 |
| 11 | IC24 | CGCGACGTGA | 3206 | 34 | 80 |

Mwt = molecular weight, TM = total molarity, and %GC = percent guanine-cytosine content.

clearly distinguish the 16 provenances according to the geographic origin since a single fragment was common for all the ITS-1. The polymorhic fragment generated in ITS-2 did not show any particular trend appearing in some individuals and was absent in other individuals of the same provenance (Table 2).

**RAPD Amplification.** Banding pattern obtained from primers 1 and 2 from a representative of the 16 provenances of *F. albida* are shown in Figures 4 and 5. In Figure 4, the upper row represent samples from the East African region, while the lower row represents samples from Southern and West Africa. From this figure, provenance 1 is the least polymorphic, followed by 13 and 14 all of West African origin. Among the East African samples, provenance 16 was the least polymorphic, followed by provenance 15 both of Kenyan origin.

Generally, using primer 2 RAPD profiles (Fig. 4), apart from provenance one, the rest had a common fragment (C03-600). The Southern African provenances had fragment C03-1400 which was absent in the other regions apart from provenance 12 from West Africa. The East African provenances apart from provenance 16, had fragment C03-1300, this fragment was absent in the other regions. The two Kenyan provenances

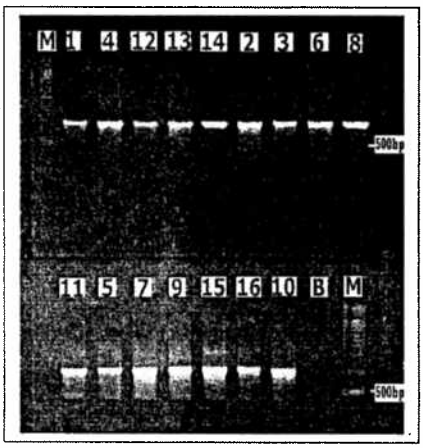


Figure 2. PCR amplification of ITS-1 region. Lane M = size marker, lane B = blank space and lanes 1 - 16 = *F. albida* provenances as given in Table 1.

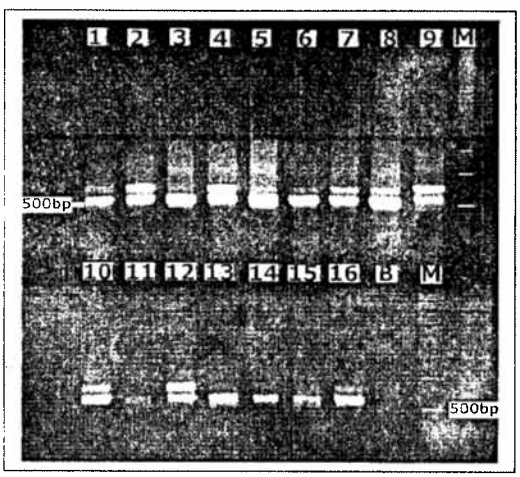


Figure 3. PCR amplification of the ITS-2 region. Lane M = size marker, lane B = blank space and lanes 1 - 16 = *F. albida* provenances as given in Table 1.

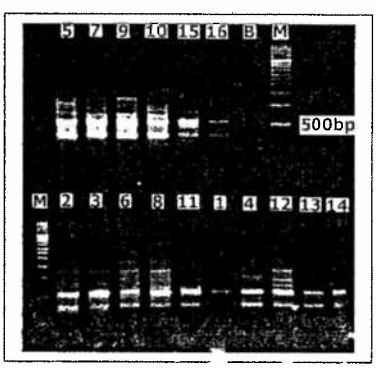


Figure 4. RAPD profiles of the 16 provenances of *F. albida* as shown in Table 1 generated by primer 2.[(5,7,9,10,15,16) East African; (2,3,6,8,11) South African; (1,4,12,13,14) West African]. Lane M = size marker and lane B = blank space

15 and 16 had a unique fragment C03-270 which, was absent in all the other provenances. Based on banding patterns, provenance pairs 13 and 14; 7 and 10; 5 and 9 were similar.

For Primer 1 (Fig. 5), two provenances, 13 and 9 failed to amplify for unknown reasons. Fragment (CO2-520) was common in all the provenances. Fragments CO2-490, and CO2-470 were weakly polymorphic among the 16 provenances. Fragment CO2-470 was common in all the regions, but absent only in provenance 3 and 14 from East and West Africa, respectively. Fragment CO2-490 was common in all Southern African provenances, but was absent in provenance 1 and 16 among the West and East African provenances, respectively.

The dendrogram generated by NTSYS p.c. 2.1 based on Jaccard's coefficient (Jaccard, 1908), distinct clusters were evident between three groups (Fig. 6). The first group comprising of fa1, fa4, fa12, fa14 and fa13 were all from West African

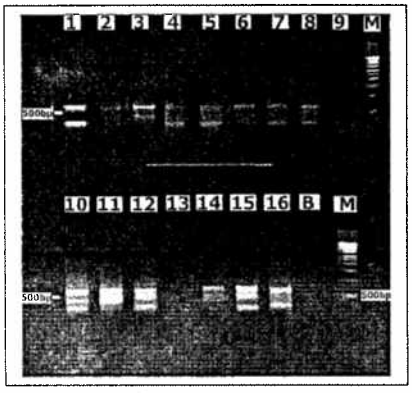


Figure 5. RAPD profiles of the 16 provenances of *F. albida* as shown in Table 1 generated by primer 1. [(5,7,9,10,15,16) East African; (2,3,6,8,11) South African; (1,4,12,13,14) West African]. Lane M = size marker and lane B = blank space.

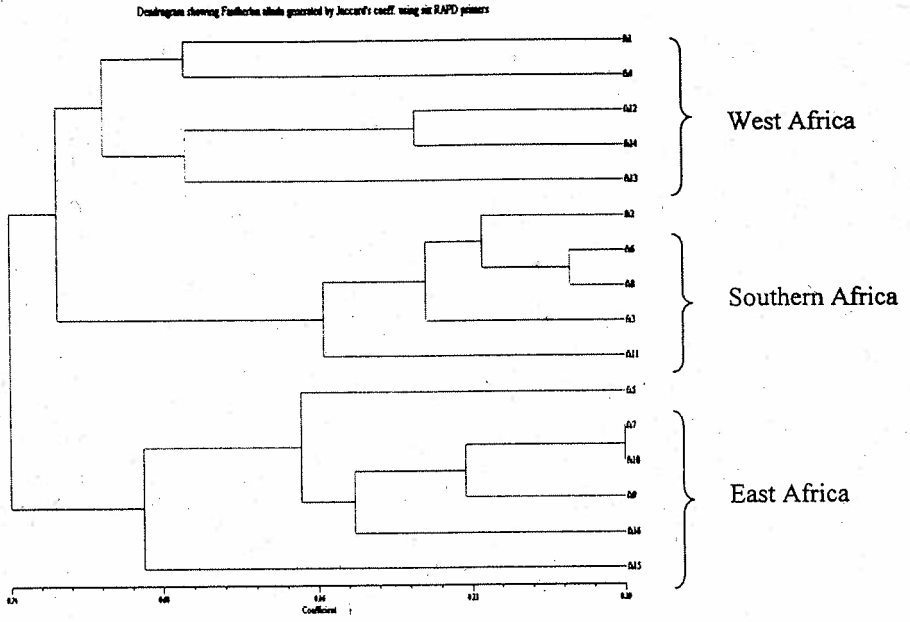


Figure 6. Dendrogram showing clusters of the 16 provenances of *F.albida* generated by NTSYS pc 2.1 (Rolf, 1993) based on Jaccard's (1908) coefficients.

provenances; the second group comprising of fa2, fa6, fa8, fa3 and fa11 was exclusively Southern African provenances of *F. albida*. Similarly, group three comprising of fa5, fa7, fa10, fa9, fa16 and fa15 was made up of East African provenances. It is evident from the dendrogram that the Southern African group although located midway between the West and East African groups, had the least genetic diversity (0.46). Followed by the East African (0.67) and finally the West African (0.70) groups based on Jaccard's coefficients. From the dendrogram, provenances 7 and 10 from Debre zeit and Lake Awassa both from Ethiopia had the smallest variation (0.19). Provenances 15 and 16 both from Kenya had large variation over 0.6. Provenance 6 from Zimbabwe was found to be closer to a South African provenance 8 (0.25) than to provenance 2 (0.30) another Zimbabwean provenance.

## DISCUSSIONS

There was no variation revealed by the amplification of the ITS-1, but some little polymorphism was revealed by amplification of the ITS-2 region. However, there was no particular trend in the banding patterns of this polymorphic fragment. This may be expected since according to Palumbi (1996), the ribosomal RNA genes evolve slowly and, hence, may not produce much variation at lower taxonomic levels. However, since the non-coding DNA sequences evolve more rapidly than the coding regions, they are a potential source for genetic variation useful in resolving relationships at lower taxonomic levels (Bailey and Doyle, 1999).

The RAPD analysis proved to be an effective tool for the detection of genetic variability in *Fadherbia albida* as it showed high degree of polymorphism among the 16 provenances. The banding pattern for primer 2 showed that the East Africa provenances of Mwembe (5), Debre zeit (7), Rama (9) and Lake Awassa (10) were the most polymorphic, while Tot (16) and Kainuk (15) all from Kenya had the least polymorphism in the region (Fig. 4). This is in agreement with the findings of Harris *et al.*, (1997) and Dangasuk and Gudu (2000) based on allozyme markers.

The dendrogram (Fig. 6) based on six primers categorised the provenances into three distinct regional clusters representing the East African, Southern African and West African regions, contrary to earlier reports that one needs at least 9-15 primers to correctly group outcrossing species into their corresponding geographical origins (Stiles *et al.*, 1993; Sabatti *et al.*, 2001). The genetic distance based on Jaccard's (1908) coefficient showed that the West African provenances had the greatest distance and thus by implication the greatest diversity. This could be due to the large area covered by the provenances representing the West African region given that there were no two provenances from the same country. Second in genetic diversity were the East African provenances and least were the Southern African, which tends to agree with Ibrahim (1996), Harris *et al.* (1997) and Dangasuk and Gudu (2000).

Based on the RAPD results the West African provenances were placed closer to the South African provenaces, which is contrary to the earlier theories based on allozyme and morphological studies that *F. albida* originated from Ethiopia and spread first southwards and later westwards (Joly, 1991; Ibrahim 1996; Dangasuk *et al.*, 1997). A major drawback of RAPD markers in population genetic studies of outbreeding organisms such as *F. albida* is that they are dominant, thus gene frequency estimates for such loci are necessarily less accurate than those obtained with codominant markers such as allozyme (Bardakci, 2001). According to Lynch and Milligan (1994) from two to ten times more individuals need to be sampled for dominant markers to achieve the same degree of statistical power as codominant markers such as allozymes. In addition, William et al. (1993) and Smith and Williams (1994) reported that RAPD analysis gives more accurate estimates between closely related populations and less accurate estimates for distantly related populations. More extensive work needs to be done with more experimental units per provenance and involving more primers so as to map out the evolution and spread of *F. albida* in the African continent.

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## REFERENCES

Bardakci, F. 2001. Random Amplified Polymorphic DNA markers. *Turk J. Biol* 25:185-196

Bailey, C. D. and Doyle, J. J. 1999. Potential phylogenetic utility of low-copy nuclear gene pistillata in Dicotyledonous Plants: comparison to nrDNA ITS and trnL Intron in Sphaerocardamum and other Brassicaceae. *Mol. Phylog. and Evol.* 13:20-30.

Cook, C. C. and Grut, M.1989. *Acacia albida* in east Niger. In: *Agroforestry in sub-Saharan African.* World Bank Technical Paper, 0253-7494; No. 112. pp. 32-34.

Dangasuk, O. G., Gudu, S., Aman, R. A. and Brandl, T. 2002. Isozyme Variation and Growth Performance in 16 Provenances of African *Faidherbia albida* (Del.) A. Chev. in Kenya. *Discovery and Innovation* (Special Edition, April 2002): 26-36

Dangasuk, O. G. 2001. Molecular Genetics of *Faidherbia albida. European Tropical Forestry Research Network NEWS*, Biotechnology Special Issue, 34:15-17

Dangasuk, O. G. and Gudu, S. 2000. Allozyme variation in 16 natural populations of *Faidherbia albida* (Del.) A Chev. *Hereditas* 133:133-145.

Dangasuk, O. G. 1999. Genetic diversity in *Faidherbia albida* (Del.) A. Chev. Ph.D. Thesis, Moi University, Eldoret, Kenya. 155pp.

Dangasuk, O. G., Seurei, P. and Gudu, S. 1997. Genetic Variation in Seed and Seedlings Traits in 12 African Provenances of *Faidherbia albida* (Del.) A.Chev. at Lodwar, Kenya. *Agroforestry Systems* 37:133-141.

Dellaporta S. L., Woods, J. and Kick, J. B. 1983. A plant DNA mini-preparation: Version II. *Plant. Mol. Biol.* Reporter 1:19-21.

Hamrick, J. L. 1990. Isozymes and the analysis of genetic structure in plant population. In: *Isozymes in Plant Biology.* Soltis, E.D. and Soltis P.S. (Eds.), pp. 87-105. Chapman and Hall, London, UK.

Harris, S. A., Fagg, C. W. and Barnes, R. D. 1997. Isoenzyme variation in *Faidherbia albida* (Leguminosae, Mimosoideae). *Pl. Sys. Evol.* 207:119-132.

Ibrahim, A. M.1996. Conservation and utilization of *Faidherbia albida* (Del.) A.Chev. (Syn. *Acacia albida*) gene resources. In: *Tree improvement for sustainable tropical forestry.* Dieter, M. J., Matheson, A. C.; Nickels, D. C.; Hardwood, C. E. and WALKER, S. M. (Eds.), pp. 404-408. Proceedings of QRFI-IUFRO Conference, Caloundra, Queensland, Australia.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.* 44:223-270

Joly, H. I. 1991. Population genetics of Acacia albida (syn. *Faidherbia albida*). *Bull. Int. Group study of Mimosoideae.* 19:86-95.

Kirmse R. D. and Norton, B. E. 1984. The potentials of *Acacia albida* for desertification control and increased productivity in Chad. *Biol. Conserv.* 29:121 – 141.

Lanfranco, L., Delpero, M. and Bonfante, P. 1999. Inter-sporal variability of ribosomal sequences in endomycorrhizal fungus *Gigaspora margarita. Mol. Ecol.* 8:37-45.

Lynch, M. and Milligan, B. G. 1994. Analysis of population genetic structure with RAPD markers. *Mol. Ecol.* 3:91-99.

Palumbi, S. R. 1996. Nucleic acids II: the polymerase chain reaction. In: Molecular Systematics 2nd edition. Hillis, D. M; Moritz, C. and Mable, B. K. (Eds.), pp. 205-247. Sinauer Associates, Inc. USA,

Rafalski, J. A. and Tingey, S. V. 1993. Genetic diagnosis in plant breeding: RAPDs, microsatellites and machines. *Trends Genet.* 9:275-280.

Raue, H. A., Klootwijk, J. and Musters, W. 1988. Evolutionary conservation of structure and function of high molecular weight ribosomal RNA. *Prog. Biophys. Mol. Bio.* 51:77-129

Rolf, F. 1993. NTSYS-pc (Numerical Taxonomy and Multivariate Analysis System). Applied Biostatistics Inc., Setander, NY.

Sabbati, M., D'Ovidio, R., Tanzarella, O. A. and Mugnozza, G. S. 2001. Assessment of

geographical variation by RAPD markers among Italian open-pollinated progenies of *Populus alba* L. *Gen. Res. and Crop Evol.* 00:1-6.

Saka, A. R. and Bunderson, W. T. 1989. The potential of *Acacia albida* and *Leucaena leucocephala* for small holder crop production system in Malawi. In: *Trees for development in sub-Saharan Africa.* Proc. IFS Reg. Sem. 20-25 February 1989, Nairobi, Kenya. pp. 150-161.

Som, I., Azam, A., Bhattacharya, A. and Battacharya, S. 2000. Inter- and intra-strain variation in the 5.8s ribosomal RNA and internal transcribed spacerssequences of Entamoebahistolyca and comparison with Entamoeba dispar, Entamoeba moshkovski and Entamoeba invadens. *Inter. Jour. Parasit.* 30:723-728.

Sambrook, J., Fritch, E. F. and Maniatis, T. 1989. Molecular cloning. A laboratory Manual 2nd Ed III. Cold Spring Harbour Laboratory Press. Cold Spring NY. pp. 395.

Smith, J. S. C. and Williams, J. G. K. 1994. Arbitrary primer mediated fingerprinting in plants: case studies in plant breeding, taxonomy and phylogeny. In: *Molecular Ecology and Evolution: Approaches and Applications.* Schierwater, B.; Streit, B.; Wagner, G. P. and DeSalle, R. (Eds.), pp 5-15. Birkhauser Verlag Basel, Switzerland.

Stiles, J. L., Lemme, C., Sondur, S., Morshidi, M. B. and Manshardt, R. 1993. Using randomly amplified polymorphic DNA for evaluating genetic relationships among papaya cultivars. *Theor. Appl. Genet.* 85:697-701.

Teel, W. 1984. A pocket dictionary of trees and seeds in Kenya. KENGO, Nairobi, Kenya.

Wickens, G. E. 1969. A study of *Acacia albida* Del. (Mimosoideae). *Kew Bull.* 23:181-202.

Williams, J. C. K., Hanafey, M. K., Rafalski, J. A. and Tingey, S. V. 1993. Genetic analysis using random amplified polymorphic DNA markers. *Meth. Enzym.* 218:704-740.

Wood, P. J. 1992. The botany and distribution of *Faidherbia albida.* In: *Faidherbia albida* in the West African Semi-Arid Tropics. Vandenbeldt, R. J. (Ed.), pp. 9-18. Proc. Workshop, Niamey, Niger.