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Research Article

Genetic diversity between cultivated and non-cultivated *Moringa oleifera* Lam. provenances assessed by RAPD markers

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Abstract

Knowledge of population genetic diversity is one of the prerequisites for development of plant species conservation strategies. In the case of wild species that represent genetic resources for cultivated plants, an understanding of their genetic diversity is essential as they are the source of genetic material that are used to improve elite varieties. A total of 12 RAPD primers were used to investigate the genetic diversity between and within cultivated and non-cultivated provenances of *Moringa oleifera* Lam. from costal regions of Tanzania. Cluster analysis using NTSYS-pc and UPGMA program produced five clusters with similarity ranging from 54% to 96%. Clustering pattern revealed a trend of genetic separation between cultivated and non-cultivated individuals which could be attributed to genetic changes taking place in escapees' individuals as they adapt to the natural environment. Based on the results, selection of elite germplasm and conservation of *M. oleifera* genetic resources could be achieved by considering individuals both from wild and cultivated plants.

Keywords: Genetic diversity, RAPD Markers, *Moringa oleifera*, NTSYS, UPGMA

Moringa oleifera'nın ekili olan ve olmayan kökenlerinin genetik çeşitliliğinin RAPD markörleri ile değerlendirilmesi

Özet

Populasyon genetik çeşitliliği bilgisi bitki türlerini koruma stratejileri geliştirmek için ön koşullardan bir tanesidir. Ekili bitkiler için genetik kaynak teşkil eden yabanıl bitkiler durumunda, elit çeşitleri geliştirmek için genetik material kaynağı olduklarından onların genetik çeşitliliğini anlamak gereklidir. Tanzanya'nın kıyı bölgelerinden *M. oleifera* Lam'ın ekili ve ekili olmayan kökenlerinin aralarında ve kendi içlerinde genetik çeşitliliği araştırmak için toplamda 12 RAPD primeri kullanıldı. NTSYS-pc ve UPGMA programı kullanarak yapılan grup analizi %54 ile %96 arasında değişen benzerlikle beş grup oluşturdu. Gruplama şekli ekili ve ekili olmayan bireyler arasında, doğal çevreye uyum sırasında firari bireyler arasında oluşan genetik değişikliklere ilişkilendirilebilecek genetik ayrışma akımı olduğunu ortaya çıkardı. Bu sonuçlara dayanarak, elit gametlerin seçimi ve *M. oleifera* genetik kaynaklarının korunumu, rüzgardan ve ekimden gelen bireyleri dikkate alarak başarılabilir.

Anahtar sözcükler: Genetik çeşitlilik, RAPD markörleri, *M. oleifera*, NTSYS, UPGMA

Introduction

Moringa oleifera is a small to medium sized, evergreen or deciduous tree native to Northen India, Pakistan and Nepal (Kantharajah and Dodd, 1991; Parrota, 2009). The species is widely cultivated and has become naturalized in many parts of the world including Tanzania (Murro et al., 2003). M. oleifera was introduced in Tanzania by Indian and Arab traders in the 1900s as a source of vegetable and medicine in restricted Indian community (Creighton, 2001). It also used to be planted all along the Coast and main railway line and around homes for demarcation of individual plots. Currently, *M. oleifera* is found in the wild and it is mainly cultivated for seed and leaf production at large scale in most regions of Tanzania including Morogoro, Dodoma, Shinyanga, Tabora, Mbeya and Iringa (Creighton, 2001). However, it grows best along the Coastal strip which runs from Tanga in the north to Mtwara in the south.

Non-cultivated individuals of *M. oleifera* found in most historical towns such as Bagamoyo, Kilwa and Tanga they include individuals that escaped from cultivation to the wild through seed dispersal. It is however expected that since adaptation to the wild conditions for escaped plants is necessary, changes that take place in such plants will be genetically controlled. Plants growing under wild conditions must adapt to uncontrolled environmental conditions and stresses in order for them to survive and reproduce (Manoko et al., 2007). To maintain the quality and quantity of products from cultivated individuals, different groups of farmers and plant breeders would like to select and improve their product from genetically heterogenous populations. Highest level of genetic diversity revealed by provenances of *M. oleifera* from Kenya and India indicates the genetic potential of the species (Muluvi et al., 1999). However, there is lack of information for *M. oleifera* found in Tanzania. It is also not known whether there is a genetic distance between cultivated and non-cultivated *M. oleifera* provenances. Such information would facilitate the improvement of *M. olifera* products from cultivated individuals and the conservation of *M. oleifera* genetic resources. This study was therefore designed in order to assess whether or not there is a genetic diversity within and between cultivated and non-cultivated *M. oleifera* provenances found in selected coastal regions of Tanzania using RAPD markers.

Materials and methods

Study Sites and Collection of Leaf Materials

This study was conducted from three non-cultivated populations from Horohoro (Tanga), Kilwa (Lindi) and Bagamoyo (Coast) and four cultivated populations from Hale (Tanga), Ubena-Chalinze (Coast), Pajaroya (Tanga), and UDSM (Dar es Salaam) (Figure 1). 4 to 20 individuals from each site were randomly selected depending on the size of the population. 20 individuals were selected from each cultivated population and 10 from non-cultivated populations. 6 individuals were sampled from UDSM site which included four individuals of *M. oleifera* and two individuals of *M. stenopetala* thus making a total of 96 individuals. From each individual 20-30 young leaves were collected and were kept in polythene bags containing silica gel and stored in ambient temperature until DNA extraction.

Extraction and Purification of DNA

DNA was extracted according to Dellaporta et al (1983) and Mneney et al (2001) with minor modifications. To easy grinding 2-3gm of silica gel dried leaves of Moringa were mixed with acid washed sand and were ground to powder using a pestle. DNA extraction buffer containing 3% (w/v) CTAB, 1.4M NaCl, 20mM EDTA pH 8.0, 100Mm Tris-HCl pH 8.0, 1% (w/v) PVP-40T (soluble), 0.2% β- Mercaptoethanol preheated to 65°C was added during the process of grinding and the resulting slurry was transferred to 50ml eppendorf tubes. The dried DNA pellet was dissolved in 1000µl $T_{10}E_1$ (10Mm Tris-HCl, 1Mm EDTA) and stored at -4°C. DNA was purified by adding 2µl of RNAse (10mg/ml) and left overnight at 4°C followed by addition 500µl of phenol:chloroform:isoamyl (25:24:1) to dissolve organic matter. Precipitation of DNA was achieved

by addition of 2/3 volume of Sodium acetate and 1/3 cold absolute ethanol. DNA quality was checked by electrophoresis of 3µl of DNA from each sample in 1% agarose gel in 1 X TAE buffer stained with 0.5µl ethidium bromide (EtBr) for 30 minutes at 100V. DNA concentration in each sample was measured using eppendorf Biophotometer and was diluted at the ratio of 1:9 i.e. 1µl DNA and 9µl PCR grade water to a final concentration of 25ng/ µl.

RAPD DNA amplification

A total of 12 suitable primers were selected from 30 primers after screening (Table 1). PCR under the following settings were performed using 25 µl total volume containing 1.0µl (25ng/25µl) of genomic DNA, 2.0µl of primer, and 22.0µl of illustra^{TM} puReTaq Ready-To-Go PCR^{TM} Beads consisting of 200µM dNTP, 1.5mM $MgCl_2$, 50mM KCl, 10mM Tris-HCl, Bovine Serum Albumin ≈2.5 units of pure Taq DNA polymerase and PCR grade water. Amplifications were performed using thermocycler at the initial denaturation temperature of 94.5 ºC for 3s (1 cycle), denaturation at 94.5 ºC for 1 minute, anneling at 37ºC for 1.5 minutes and extension at 72 º C for 1.5 minutes all in 35 cycles followed by final extension at 72ºC for 5 minutes. Amplification products were analyzed by electrophoresis through 1.4% agarose gels (Invitrogen life technologies) in 0.5 X TBE running buffer (Sambrook et al., 1989) followed by ethidium bromide staining. The molecular fragments were estimated using 100bp PCR marker (Biolabs). The reactions were repeated at least twice and only reproducible, relatively intense bands were scored.

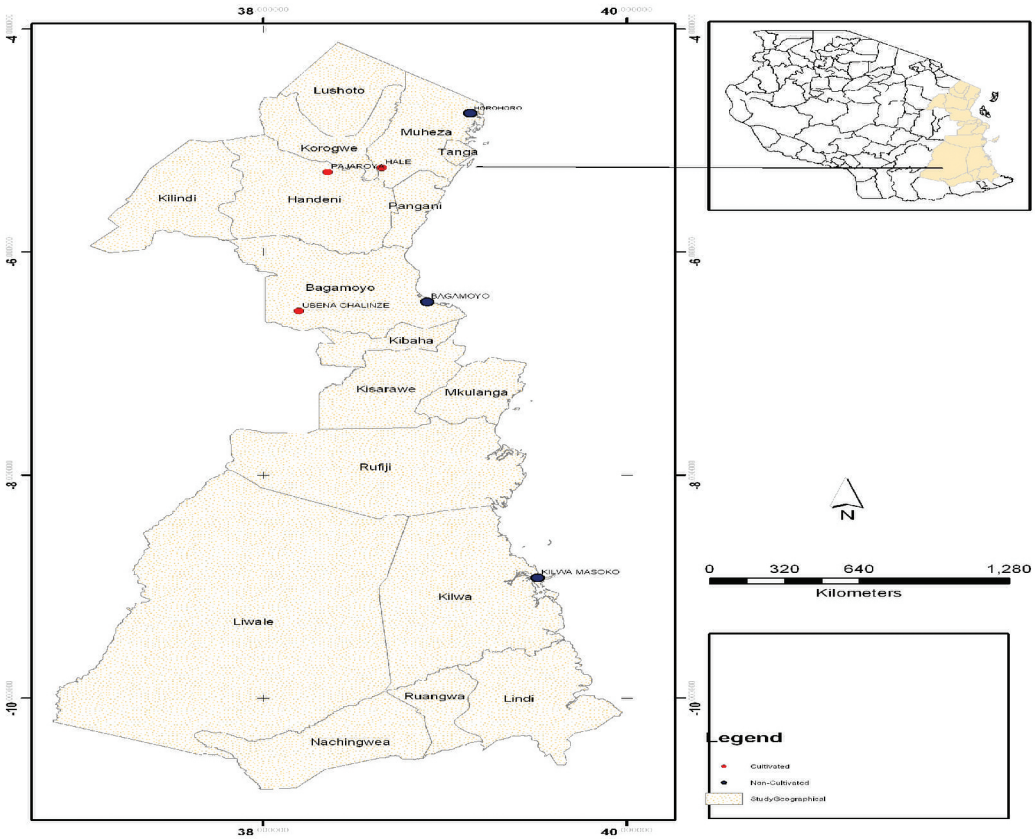


Figure 1. Map showing study sites

Table 1. Sequences of RAPD primers used in this study

RAPD Primer	Primer Sequence
KFP-1	5'-GGC TCG TAC C-3'
KFP-3	5'-GTT AGC GGC G-3'
KFP-4	5'-CGG AGA GTA C-3'
KFP-5	5'-CCT GGC GAG C-3'
KFP-6	5'-TCC CGA CCT C-3'
KFP-7	5'-CCA GGC GCA A-3'
KFP-8	5'-ACG CGC TGG T-3'
KFP-9	5'-GAC TGG AGC T-3'
KFP-10	5'-ACG GTC CGC C-3'
KFP-11	5'-CGC GAC GTG A-3'
KFP-13	5'-CAG TCG GGT C-3'
KFP-21	5'-GTA GGC GTC G-3'

Cluster Analysis

Data matrix of RAPD profiles for fragments of similar molecular weight from each individual were scored as present (1) or absent (0). Genetic similarities were estimated using Dice coefficient (Dice, 1945) as recommended when using RAPD data (Lamboy, 1994). Cluster analysis was performed using Un-weighted Pair Group Method with Arithmetic Averages (UPGMA) (Sneath and Sokal, 1973) using NTSYS-pc version 2.1 (Rohlf, 2000) software. The dendrogram was generated using the SAHN clustering program.

Results

A total of 98 bands of 100 to 1500bp were scored and 86 of them (89.6%) were polymorphic. On average each primer produced 8.1 RAPD bands, the largest number (10) obtained for primers KFP-8 and KFP-13 and the lowest number (6) obtained from KFP 6 primer. An example of the part of the variation (RAPD profile) detected using KFP 13 is shown in Figure 2.

Clustering Pattern

Cluster analysis produced five clusters that can be split into two groups A and B and. One outlier individual (h1) did not fall in any of the five clusters (Figure 3). Group A which was made up of all *M. oleifera* individuals, had four clusters with the genetic distance ranging from 65% (for most divergent individuals) to 96% (for most related individuals). Cluster I of group A had two sub-clusters Ia and Ib which were similar by 74%. Sub-cluster Ia had majority individuals (50) from cultivated populations (MCI) except 3 individuals from the non-cultivated population from Horohoro. Sub-cluster Ib contained majority individuals (20) from non-cultivated populations (MNCI) from Kilwa and Bagamoyo except one cultivated individual from Pajaroya. The genetic distance within the majority cultivated cluster ranged from 76% to 93% and the genetic distance within the majority non-cultivated ranged from 80% to 96%. Cluster II consisted of cultivated individuals from Hale and Ubena and two non-cultivated individuals from Horohoro. Cluster III was made up of the remaining cultivated individuals from Pajaroya. Cluster IV had two individuals i.e. Ubena 64 (cultivated) and Bagamoyo 80 (non-cultivated). The two most related non-cultivated and cultivated individuals are Ubena-Chalinze (69) and Horohoro

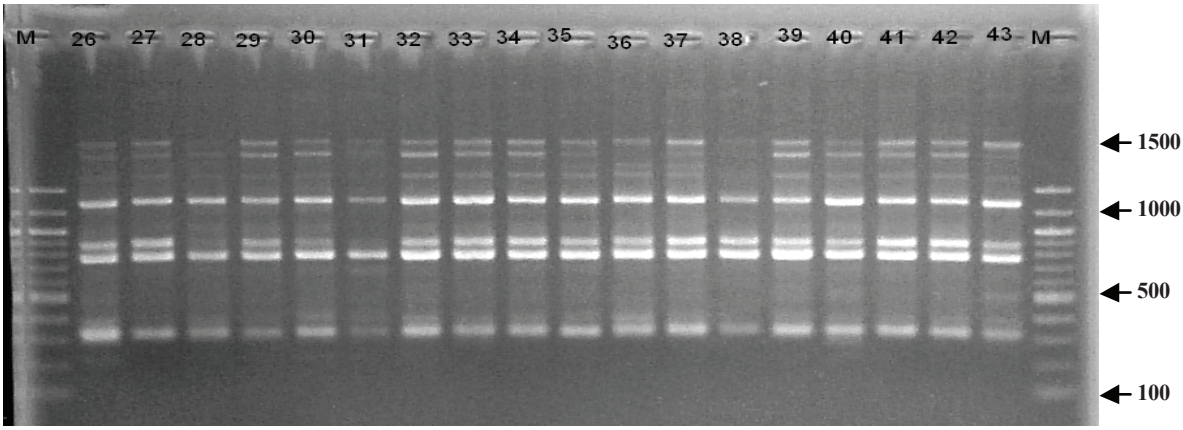


Figure 2. RAPD profile for *M. oleifera* produced using primer KFP-13. The numbers above are the individual serial numbers; M is a 100bp Molecular marker

(30) which were 90% similar. The shortest genetic distance among cultivated individuals from one population were samples number 92 and 93 from UDSM and samples number 14 and 17 from Pajaroya which were 94.5% similar while for non-cultivated individuals were samples number 74 and 75 from Bagamoyo which were 96% similar. Group B was made up of only two individuals of *M. stenopetala* which were genetically separated from group A at 0.54.

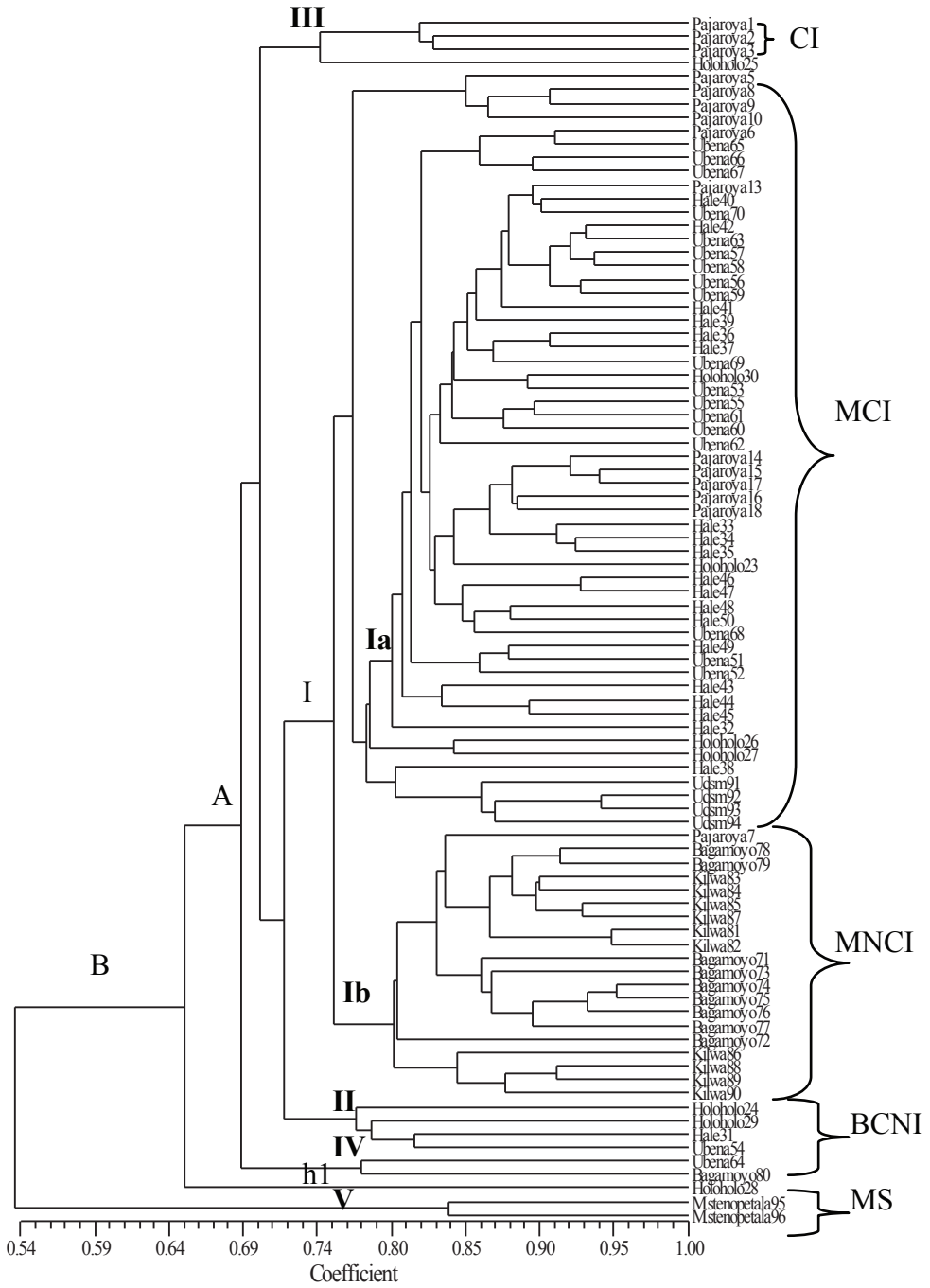


Figure 3. Phenetic dendrogram of genetic analysis computed from similarity matrix of genetic distance based on Dice coefficient

MCI= Majority Cultivated Individuals, MNCI= Majority Non-cultivated Individuals,
BCNI= Both Cultivated and Non-cultivated Individuals, CI= Cultivated Individuals, MS= M. stenopetala

Discussion

In the present study, we have used RAPDs to find out genetic diversity between cultivated and non-cultivated *M. oleifera* from selected costal regions of Tanzania. The obtained results indicated a trend of genetic separation between cultivated and non-cultivated individuals suggesting that the two groups are evolving differently. Under cultivation, genetic changes are slowed down because both competition and natural selection are reduced, since environmental conditions are kept at optimum and pests are artificially controlled (Manoko et al., 2007). Under the wild environment, individuals are exposed to competition, predation, herbivores and diseases. Since individuals in cultivation change less due to their uniformity of their environment, therefore the genetic distance observed between the cultivated and non-cultivated individuals could be attributed to genetic changes that take place in escapee's individuals as they adapt to the natural environment. These results are similar to many other studies that reached similar conclusion that wild individuals evolve differently genetically from individuals in cultivation (Lashermes et al., 1996; Desplanque et al., 1999; Virginie et al., 2002; Zenglu and Randall, 2002; Baldoni et al., 2006).

The clustering of a few individuals from non-cultivated populations with individuals from cultivated populations suggests the possibility that, non-cultivated individuals have recently escaped from cultivation and therefore have had not enough time to be genetically separate. As a result, they still share their genetic make up with those in cultivation. According to Schaal et al (1998), historical relationships contribute to the genetic structure of most plant populations with those having recent common ancestry being genetically more similar than those having more distant common ancestry. Since evolution is a slow process, it is therefore only possible to get total genetic separation between the two groups after a very long period of time.

On the other hand, clustering of individuals from same population in different clusters indicate high genetic variation within population which may be attributed to the use of different seed sources, mutation and/or breeding system. This conform to what is expected for out-crossing species and it also agrees with other similar studies which had similar observations (Huff et al., 1993; Rosseto et al., 1995). Since *M. oleifera* is a predominantly out-crossed species, it was expected to maintain most variations within populations than between populations. However, such results were contrary to that of Muluvi et al (1999), where high levels of between-population differentiation were reported.

Lack of clustering according to geographical areas indicates that individuals from different geographical areas are not significantly different genetically. This association may be the result of similar agroclimatic conditions of the study area, spread of planting materials from cuttings, seed movement and/or high rates of gene flow between adjacent populations. Genotypes from different geographical areas may be genetically similar to genotypes with immediate spatial relationships. Several studies have reported similar observations in other plant species (Padmesh et al., 1998; Virginie et al., 2002) although some other studies were able to find separation of genotypes based on geographic origin (Keskitalo et al., 1998; Desplanque et al., 1999; Zenglu and Randall, 2002). Separation between the distant located genotypes e.g. Kilwa and Pajaroya may explain the fact that evolutionary forces are not evenly distributed amongst populations. Individuals from different geographical locations are expected to be genetically different due to varying selection pressure that allow fixation of a particular character. The total genetic variation of the species is likely to be distributed among populations as the impact and direction of natural selection varies from one population to another due to environmental variation and genetic drift (Lawrence and Rajanaidu, 1985). Based on the results it can therefore be concluded that, selection of materials for improvement of cultivated individuals and conservation of *M. oleifera* genetic resource can be achieved by considering individuals from both the wild and from cultivation. However, further research are needed to identify elite genetically fixed morphological characters from wild individuals that can be used for improving productivity of cultivated *M. oleifera*.

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