Journal of Life Sciences 5 (2011) 387-392

Using Morphological Markers to Assess Variations between and within Cultivated and Non-cultivated Provenances of *Moringa Oleifera* Lam. in Tanzania

Mariam Godwin Mgendi[1], Agnes Morris Nyomora[2] and Mkabwa Katambo Manoko[2]

1. Department of Biological Sciences, College of Education, Dar es Salaam University, Dar es Salaam, P.O BOX 2329, Tanzania

2. Department of Botany, University of Dar es Salaam, Dar es Salaam, P.O BOX 35060, Tanzania

Received: September 28, 2010 / Accepted: November 26, 2010 / Published: May 30, 2011.

Abstract: The use of morphological markers to assess variations between and within cultivated and non-cultivated provenances of *Moringa oleifera* Lam. from selected coastal regions of Tanzania was studied. A descriptors list of 15 selected morphological characters was used to generate a character state matrix. Cluster analysis using NTSYS-pc and UPGMA program produced six clusters with similarity ranging from 66% to 100%. The majority cultivated and non-cultivated clusters with similarity ranging from 86-100% and 97-100% respectively reflected a clustering pattern based on the status of individuals whether cultivated or non-cultivated. Most individuals from same population clustered differently indicating high morphological variations within populations than between populations. The present results suggest that selection and conservation of elite germplasm could be achieved by considering individuals from both cultivated and non-cultivated plants.

Key words: Morphological variations, *Moringa oleifera*, cultivated and non-cultivated, NTSYS.

1. Introduction

Moringa oleifera is a multipurpose tree belonging to a monogeneric family of trees and shrubs, the Moringaceae [1]. Although the species is indigenous to northwest India, it is now cultivated throughout the Middle East, and in almost the whole tropical belt [2]. In Tanzania, *M. oleifera* was introduced by Indian and Arab traders in the 1900s as a source of vegetable and medicine in the restricted Indian community [3]. It used to be planted along the Coast and main railway line and around homes for demarcation of individual plots. Most individuals of *M. oleifera* introduced during this time were left to grow naturally and some escaped to the wild through seed dispersal. Currently, *M. oleifera* is cultivated mainly for seed and leaf production at a commercial scale in most regions of Tanzania including Shinyanga, Dodoma, Singida, Arusha, Moshi Mbeya, Iringa and along the Coastal strip which runs from Tanga in the north to Mtwara in the south [3].

M. oleifera is one of the world's most useful trees, as almost every part of the tree can be used for food, or has some other beneficial properties [4-6]. Various parts of *M. oleifera* such as leaves, seeds pods, barks and roots are used for various purposes including human consumption, medicinal and industrial uses [7]. *M. oleifera* is also the most nutritionally food crop as it is rich in proteins, vitamins and minerals thus a good source of nutritious vegetable [8-10]. However, the diverse morphological variability of this species probably due to high out-crossing nature and/ or a wide range of its geographical occurrence provide a limitation towards improving cultivated *M. oleifera* products. A wide range of variability of its

Corresponding author: Mariam Godwin Mgendi, M.Sc., assistant lecturer, research field: plants ecology and evolution. E-mail: mamge2006@yahoo.co.uk.

characteristics and character states such as pod length, size and number of seeds per pod, leaf size and shape and flower parts has been documented [4, 7, 11]. A study had revealed considerable morphological variability among *M. oleifera* accessions from different locations [12]. However, no study has been conducted so far to investigate morphological variability of *M. oleifera* provenances found in Tanzania. It is also not known whether there is morphological variability between non-cultivated (escapees) individuals and currently cultivated *M. oleifera* in Tanzania. Such information would facilitate improvement of *M. oleifera* products by selecting seed materials from individuals with superior qualities. It is on the basis of phenotype that certain groups of plants are considered to be of good quality and therefore preferentially selected. Therefore, selection based on the qualities of products is essentially important not only for improvement of cultivated individuals but also for conservation purposes. This study was therefore undertaken to assess morphological variations between and within cultivated and non-cultivated provenances of *M. oleifera* from selected coastal regions of Tanzania.

2. Materials and Methods

This study was conducted from six selected sites that were randomly selected from three selected coastal regions namely Tanga, Coast and Lindi. Three sites among the six consisted of only cultivated populations and the other three were sites consisting of non-cultivated populations (Table 1). Morphological data were collected from 20 individual plants randomly selected from each cultivated population and 10 individual plants from non-cultivated populations. Morphological data were collected using a descriptors list of 15 characters (Table 2). Selection of these characteristics was based on the description of the genus

Table 1 Location and population status where morphological data were collected.

Site	Geographical coordinates	Region	Status	No. of individuals
1. Pajaroya	0429011/9415992	Tanga	Cultivated	20
2. Hale	0461934/9420190	Tanga	Cultivated	20
3.Ubena-Chalinze	0411502/9278086	Coast	Cultivated	20
4. Horohoro	0517044/9470182	Tanga	Non-cultivated	10
5. Bagamoyo	0488904/9287160	Coast	Non-cultivated	10
6. Kilwa	0556543/9013414	Lindi	Non-cultivated	10

Table 2 Descriptors list of morphological characteristics used in the study.

Characters	Character states		
1. Habit	(1) Tree	(2) Shrub	(3) Sub-shrub
2. Flower colour	(1) White	(2) Yellow	(3) Red
3. Flower symmetry	(1) Regular	(2) Nearly regular	(3) Irregular
4. Petals	(1) Equal	(2) Unequal	
5. Anthers	(1) Yellow	(2) Orange	
6. Sepals	(1) Equal	(2) Unequal	
7. Sepals fusion	(1) Fused	(2) Unfused	
8. Leaves	(1) 2 pinnate	(2) 3 pinnate	(3) 4 pinnate
9. Seeds	(1) Winged	(2) Nearly- unwinged	(3) Unwinged
10. Seed cover	(1) Hard	(2) Membranous	
11. Bark	(1) Smooth	(2) Rugose	(3) Corky
12. Bark colour	(1) White	(2) Grey	(3) Pale buff
13. Pod length (cm)	(1) 0-30	(2) 30-60	(3) 60-90
14. Receptacle	(1) Long-tubular	(2) Intermediate	(3) Short-cuplike
15. Leaflets shape	(1) Elliptic	(2) Obovate	

Moringa in Flora of Tropical East Africa [11]. Each character state from each individual was observed and recorded as present or absent.

3. Data Analysis

All morphological data collected were coded as binary whereby (1) indicated the presence of a character, (0) indicated the absence of a character and (999) indicated missing data [13]. A character state matrix created was used to generate similarity matrix using Dice similarity coefficient [14]. Cluster analysis was performed from similarity matrix using Unweighted Pair Group Method with Arithmetic Averages (UPGMA) program. A dendrogram was generated using the SAHN clustering program. All analyses were done using Numerical Taxonomy System (NTSYS-pc) version 2.10 software [15].

4. Results and Discussion

Cluster analysis produced a dendrogram with a total of 6 clusters (I-VI) with similarity ranging from 66-100% (Fig. 1). Similarities within clusters ranged from 90% to 100% with majority individuals within provenances being morphologically similar at 100%. Clustering pattern mainly reflected the status of individuals whether cultivated or non-cultivated regardless of their geographic origin. As depicted in the dendrogram, cluster I comprised majority of individuals (31) from cultivated populations (MCI) from Pajaroya, Hale and Ubena-Chalinze and seven (7) non-cultivated individuals from Horohoro and Kilwa were the exception. Cluster II was made up of the majority non-cultivated individuals (MNCI) (22) regardless of their geographical sources i.e. Kilwa, Bagamoyo and Horohoro and 3 individuals from the cultivated population of Ubena-Chalinze were the exception. The majority cultivated (MCI) and the majority non-cultivated (MNCI) clusters i.e. cluster I and II were 80% morphologically similar while the distance within the majority cultivated ranged from 86% to 100% and within majority non-cultivated ranged from 97% to 100%. Clusters III, IV and V grouped all individuals from cultivated populations (CI) i.e. Pajaroya and Hale and Ubena-Chalinze. Cluster VI was made up of individuals from both cultivated and non-cultivated populations (BCNI). Three outliers labeled a, b and c did not fall in any of the clusters. The results also showed that, individuals from closer locations like those from Pajaroya, Hale and Horohoro clustered together first before they clustered with individuals from distant locations e.g. Kilwa and Bagamoyo.

Generally, morphological data recorded for all individuals showed that some characteristics such as flower colour and symmetry, colour of the anther, seed cover, seed wings, sepal fusion and leaf characteristics exhibited similar character states. Flowers in all populations were white in color, irregular with five unequal numbers of petals. Leaf shape was 3-pinnate with elliptic or obovate terminal leaflets and all seeds were winged and membranous. However, some characteristics such as growth form/habit, bark texture, bark color and pod length varied between individuals and between provenances. Bark colour exhibited 3 states i.e. white, grey or pale buff. Similarly, while some individuals had corky texture others had rugose or smooth texture. Pod length ranged from 30 to 60 cm.

In the present study morphological characters have been used to assess diversity within and between cultivated and non-cultivated populations of *M. oleifera* from six selected sites from Coastal regions of Tanzania. Total separation between cultivated and non-cultivated escapees from cultivation would have indicated a considerable level of variation between cultivated and non-cultivated individuals which would have been attributed to different selection pressure experienced in different habitats i.e. in cultivation and populations in the wild. Since natural selection is expected to act more in the natural habitat rather than on cultivation, these variations would have shown a change on phenotype. Similarly, it was expected that individuals from different populations would have

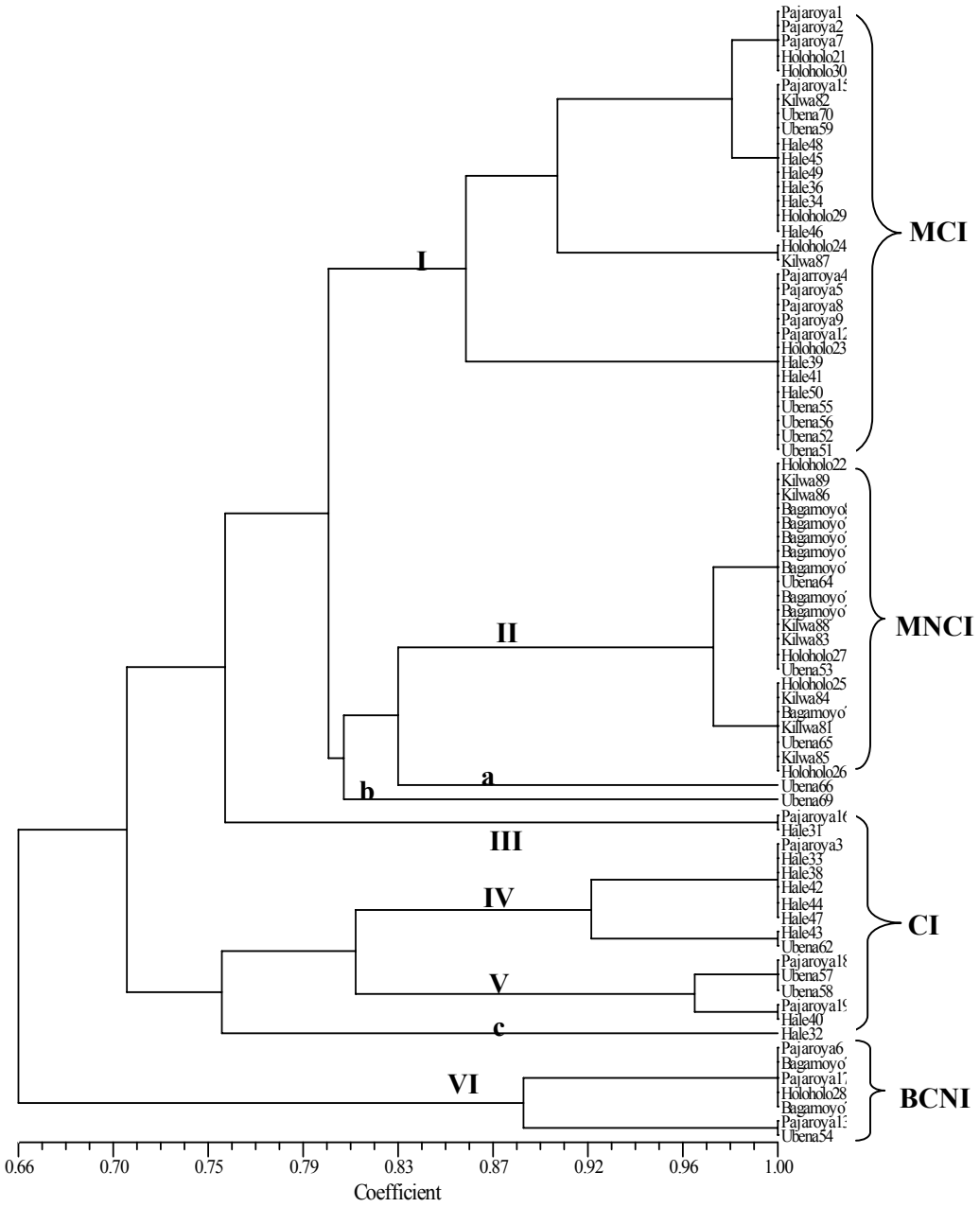


Fig. 1 Phenetic dendrogram based on morphological variation of individuals from cultivated and non-cultivated populations of *M. oleifera*.

MCI = Majority Cultivated Individuals; MNCI = Majority Non-cultivated Individuals; CI = Cultivated Individuals; BCNI = Both Cultivated and Non-cultivated individual; a, b and c = outliers.

clustered differently or in sub-clusters to indicate differences in phenotypes due to habitat or environmental variations [16]. It was also expected that individuals from the same population would cluster together first before they clustered with individuals from different populations but this was not the case.

Most individuals neither fell into clusters to reflect the separation between cultivated and non-cultivated individuals nor clustered according to their geographic origin. As depicted in the dendrogram, three (3) clusters with the majority individuals were comprised of a mixture of cultivated and non-cultivated

individuals while the other three (3) clusters with fewer individuals were made up of only cultivated individuals indicating that some non-cultivated individuals were no longer similar to cultivated individuals. This view is in line with the fact that two of the mixed clusters had more non-cultivated individuals than cultivated ones and/or more cultivated individuals than non-cultivated ones. In the majority cultivated cluster (MCI) with 31 individuals only seven (7) individuals were from non-cultivated populations while in the majority non-cultivated cluster (MNCI) with 22 individuals only three (3) were from cultivated populations. Although cluster VI (BCNI) had comparative number of cultivated and non-cultivated individuals of *M. oleifera*, the two groups with majority individuals described above suggest that escapees from cultivation can be separated from cultivated individuals based on morphology. The three clusters made of only cultivated individuals CI (III, IV and V) support this observation. However, it is not clear whether the morphological characteristics that differentiate the two groups are genetically fixed.

The study also revealed that most individuals from the same population clustered differently. For instance, individuals from Pajaroya were distributed in almost five different clusters. The observed trend suggests considerable levels of morphological variations within populations which may be caused by environmental heterogeneity as experienced by naturally occurring populations and/or genotypic plasticity of the species [17]. Different micro-environments generate different selection pressures that may lead to genetic heterogeneity, thus producing the observed morphological differences. Similarly, environmental heterogeneity such as elevation and moisture availability often generate significant barriers to gene flow and thus enhances genetic differentiation among semi-isolated or isolated populations [18]. Nevertheless, the interactions between environmental conditions and genotypes may also have a significant impact on the observed morphological variations [17]. The morphological variations within population observed under this study could also be due to differences in genotypes which may be caused by the use of different seed sources and/or breeding systems of the species. Different genotypes are known to have different degrees of responses to a given environment and some may produce a wider range of phenotypes [17]. However, high levels of cross-pollination (74%) in *M. oleifera* [19] increases heterogeneity of the genotype, thus may have contributed to phenotype variability within populations. On the other hand, lack of separation based on their geographical origin probably suggests that these individuals originated from a narrow genetic pool which is common in most cultivated crops. Narrow genetic pool could also be attributed to the use of cuttings for vegetative propagation. Furthermore, clustering of individuals from close locations like Hale and Pajaroya may be attributed to the close proximity of the sites which may suggests the use of common seed source.

5. Conclusions

Based on the clustering pattern observed, it can be concluded that some cultivated individuals are morphologically different from non-cultivated individuals. The two groups with majority individuals suggest that escapees from cultivation can be separated from cultivated individuals based on morphology. From this information, one can easily select and conserve from both cultivated and non-cultivated populations. Considerable morphological differences between cultivated and non-cultivated populations of *M. oleifera* observed in this study indicate the potential and usefulness of morphological data as a tool for selection and conservation of elite germplam as revealed by other similar studies [12, 20-22]. However, since morphological characteristics are more easily influenced by environmental factors often resulting in genetic plasticity, they may fail to provide the correct information to base selection or conservation as they do not reveal whether such morphological characters are

genetically fixed or not. Morphological variability revealed under this study provides baseline information for further studies. The study was limited to the use of only morphological data, so there remains a need for further studies to assess genetic diversity using molecular markers in order to determine whether the characters used to cluster individuals based on morphology are genetically fixed or not. Genetic based markers are highly recommended as they provide an efficient and unbiased estimate, free of environment effects.

Acknowledgment

The authors would like to thank the University of Dar es Salaam Sida-Sarec for the generous financial support.

References

[1] C. Ramachandran, K.V. Peter, P.K. Gopalakrishnana, Drumstick *Moringa oleifera*: A multipurpose Indian vegetable, Economic Botany 34 (1980) 276-283.

[2] S.A. Jahn, H.A. Mussad, H. Burgastaller, The tree that purifies water, cultivating multipurpose Moringaceae in the Sudan, Unasylva 152 (1986) 23-28.

[3] W. Creighton, *M. oleifera* Seed Production in Tanzania: Development Potential for *Moringa* Products, Optima of Africa Ltd., Dar es Salaam, Tanzania, 2001.

[4] F.J. Morton, The horseradish tree, *Moringa pterygosperma* (Moringaceae) a boon to arid lands, Economic Botany 45 (3) (1991) 318-333.

[5] D.W. Odee, G.M. Muluvi, J. Machua, M.E. Olson, M. Changway, Domestication of Moringa Species in Kenya, Development Potential for Moringa Products, Dar es Salaam, 2001.

[6] R. Hsu, M. Sharon, A. de Witte Lucienne, *Moringa oleifera,* Medicinal and Socio-economic Uses, National Herbarium Leiden, The Netherlands, 2006.

[7] N. Foidl, H.P.S. Makkar, K. Becker, The Potential of *Moringa Oleifera* for Agricultural and Industrial Uses, Moringa Workshop, Dar es Salaam, 2001.

[8] M. Price, The *Moringa* Tree, ECHO, 17391 Durrance Rd., North Ft. Myers FL 33917 USA, 1985.

[9] S. Litherland, Large scale water purification article, 1995, available online at: http://www.treesforlife.org. (accessed on May 20, 2007)

[10] J.W. Fahey, *Moringa oleifera*: A review of the medical evidence for its nutritional, theurapeutic, and prophylactic properties Part 1, Trees for Life Journal 1 (5) (2005), available online at: http://www.tfljournal.org/article.php/20051201124931586. (accessed on September, 2008)

[11] B. Verdcourt, R.M. Polhill, Flora of Tropical East Africa-Moringaceae, CRC Press, 1986.

[12] D.S. Resmi, V.A Celine, L. Ramony, Variability among drumstic (*M. oleifera.* Lam.) accessions from central Kelara, Journal of Tropical Agriculture 43 (1-2) (2005) 83-85.

[13] S. Robert, R.P. Toby, Homology and Systematics, Coding Characters for Phylogenetic Analysis, Systematics Association, U.K., 1999.

[14] L.R. Dice, Measures of the amount of ecological association between species, Ecology 26 (1945) 297-302.

[15] F.J Rohlf, NTYSY-pc Numerical Taxonomy and Multivariate Analysis System, Exeter Publishing Co, New York, 2000.

[16] B.R Murray, A.M. Gill, A comperative study of interspecific variation in fruit size among Australian *Eucalypts*, Ecography 24 (2001) 651-658.

[17] D. Briggs, S.M. Walters, Plant Variation and Evolution, 3rd ed., Cambridge University Press, 1997.

[18] Y.B. Linhart, C.G. Michael, Evolutionary significance of local differentiation in plants, Annual Review of Ecology and Systematics 27 (1996) 237-277.

[19] G.M. Muluvi, J.I. Sprent, D. Odee, W. Powell, Estimates of out crossing rates in *Moringa* using amplified fragment length polymorphism (AFLP), African Journal of Biotechnology 3 (2) (2004) 146-151.

[20] L. Rongbai, M.P. Pandey, S.K. Pandey, D.K. Dwivedi, Agro-morphological characterization of ovary culture derived plants of rice (*Oryza sativa* L.), Euphytica 106 (1999) 197-203.

[21] R. Vashistha, B.P. Nautiyal, M.C. Nautiyal, Conservation status and morphological variations between populations of *Angelica glauca* Edgew and *Angelica archangelica* Linn in Garhwal Himalaya, Current Science 91(11) (2006) 1537-1542.

[22] M.G. Figuiredo, J.L.F Pires, M.B. Lovato, Variability in fruit and seed morphology among and within populations of *Platymenia* (Leguminoceae-Mimosoideae) in area of the Cerrado, the Atlantic Forest and Transitional sites, Plant Biology 8 (2006) 112-119.