INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-5, Issue-3, July-Sept-2015 Coden: IJPAJX-CAS-USA, Copyrights@2015 ISSN-2231-4490
Received: 12th April-2015 Revised: 14th May -2015 Accepted: 25th May-2015
Research article

MOLECULAR IDENTIFICATION OF THE CULTIVATED *MORNINGA OLEIFERA IN SAUDI ARABIA*

Sanad M. AL-Sobeai[a], Khalid H. Alamer[b*] and Sayed A.M. Amer[b*]

[a]Shaqra University Sajir College of Arts & Science, Shaqra, Saudi Arabia
[b]Department of Biology, Faculty of Science, Taif University, Taif, Saudi Arabia
*Corresponding authors, e-mails: alamer_25@hotmail.com, yasser92us@yahoo.com

ABSTRACT: Seven hundred and nine nucleotides spanning the ITS region (ITS1 and ITS2 and the highly conserved 5.8S rDNA exon located in between) and its flanking regions of small and large ribosomal DNA subunits (18S and 26S genes) were amplified and sequenced for *Moringa oleifera* seedlings. The obtained data have been analyzed by maximum-parsimony and neighbor-joining methods in order to identify the cultivated plant molecularly. The parsimony could not be discriminated between the cultivated taxon from either *M. oleifera* or *M. peregrina*. The tree showed clustering of the three species with 100% bootstrap supports without clear identification of the seedlings sample and the neighbor-joining method showed similar tree topology. The genetic distance obtained from the sequenced data indicated that the smallest distance was found between cultivated and wild *M. oleifera* and between wild *M. oleifera* and *M. peregrina* while the distance between the cultivated *M. oleifera* and *M. peregrina* was higher. The current data therefore identified the seedlings sample to be affected by the ecological habitat of Saudi Arabia which is considered as an endemic habitat of *M. peregrina*. Further molecular study is necessary by collecting more wild and cultivated samples of both species in order to identify with clear resolution the molecular relationship between both wild and cultivated *Moringa*.
Key words: *Moringa*, Plant DNA, Saudi Arabia, plant nurseries

INTRODUCTION

Moringaceae is an angiosperm family consists of 13 [1] species which belong to only one genus called *Moringa* [2]. Morton [3] reported that the most common species are *Moringa peregrina* (forsk) fiori and *Moringa oleifera*. These species are the most widely cultivated of the Moringaceae family and was utilized by the ancient Egyptians, Romans and Greeks. *M. peregrina* has a wider range, growing from the Dead Sea area sporadically along the Red Sea coasts to northern Somalia and around the Arabian Peninsula to the mouth of the Persian Gulf [2]. In Saudi Arabia, *M. peregrina* is mainly distributed in South and North Hijaz [4,5,6]. *Moringa oleifera* is a small to medium sized tree native to the south central Asia from India to Nepal [7,8]. The species is widely cultivated in many parts of the world including Tanzania [9]. The studies on the genetic diversity of *M. peregrina* or *M. oleifera* are very limited. However, recent few investigations have tackled this point using RAPD-PCR [10] inter simple sequence repeat (ISSR) and cytochrome b [11,12] and internal transcribed spacer of the nuclear ribosomal DNA [13].
The present study therefore aimed to use the nuclear rDNA at the region spanning 18S gene, internal transcribed spacers (ITS1 and ITS2) and 5.8S gene to infer the molecular features of the cultivated *M. oleifera* in Saudi Arabia and such study could be a prerequisite for future crop improvement programme.

MATERIALS AND METHODS

Samples and DNA extraction

In this study, three samples of the cultivated *M. oleifera* species have been collected. The seeds of this cultivated plant were brought from Egypt and be cultivated in plant nurseries in Riyadh of Saudi Arabia. The samples were labeled, sealed in sterilized polythene bags, brought to the laboratory and were stored at -20 °C till their use for DNA isolation. DNA was isolated and purified by DNeasy Plant Mini Kit following the manufacture protocol.

PCR experiments

The following primers were used in this study; forward primer (ITS4) 5`- TCCTCCGCTTATTGATATGC-3` and the reverse primer (ITS5) 5`- GGAAGTAAAAGTCGTAACAAGG -3` [14] were designed for the amplification of *M. peregrina*.

A total volume of 25 µL PCR mixture (1 µL genomic DNA, 12.5 µL PCR Master Mix, 0.5 µL of each primer and 10.5 µL distilled sterilized H2O) was used. PCR amplification was carried out in a Techne thermocycler. The PCR conditions were 95 ˚C for 5 min as initial denaturation step followed by 35 cycles of 95 ˚C for 60 s denaturation, 56 ˚C for 60 s annealing and 72 ˚C for 60 s extension, the final extension was at 72.0 ˚C for 4 min. PCR products were run on 1% agarose gel containing ethidium bromide and visualized. The PCR products of the 3 samples were sent for sequencing using the same two primers used in the PCR amplification.

Data analyses

Seven hundred and nine nucleotides spanning 18S, ITS1, 5.8S and 28S genes from the nuclear DNA for the collected samples were sequenced in this study. Comparisons with sequences in the GenBank database were achieved in BLASTN searches at the National Center for Biotechnology Information site (http://www.ncbi.nlm.nih.gov).

The sequenced data were aligned with their counterparts found in the Genbank database for *M. peregrina* from Yemen (JX092071), *M. oleifera* (AY845130) from national botanical garden of Belgium, *M. ovalifolia* (AY461551) collected from national botanical garden of Belgium and *M. rivae* (JX092072) collected from Kenya. The aligned data were used for phylogenetic analyses and the sequence for *M. ovalifolia* was used for tree rooting. The gap-containing sites and unambiguous nucleotides were deleted so that 614 bp were left for phylogenetic analyses. The aligned nucleotides can be obtained from Sayed Amer upon request.

The phylogenetic analyses were conducted by maximum-parsimony and neighbor-joining methods packaged in PAUP* 4.0b10 [15]. Both methods were set to heuristic searches with the TBR branch swapping and 10 random taxon additions. Bootstrapping replicates were set to 10000 for both methods. The neighbor-joining was adjusted by Tamura-Nei distance option.

RESULTS

The amplified and sequenced ITS region, in this study, was shown in figure 1. The sequenced fragment was aligned with its counterparts for 3 *M. peregrina* and 4 *M. oleifera* samples that represented all published data for both species in the Genbank database. The length of ITS1, 5.8S and ITS2 regions, frequency of different bases and adenine-thymine and cytosine-guanine ratio (AT/CG and CG/AT) for the studied taxa are illustrated in Table 1. The entire ITS region was 629 bp in both cultivated *M. oleifera* and *M. peregrina* and was 631 bp in wild *M. oleifera*. The number of nucleotides in the ITS1 was 248 bp in the three studied taxa. The 5.8S region was 166 bp in both cultivated *M. oleifera* and *M. peregrina* and was 167 bp in wild *M. oleifera*. The ITS2 region was 215 bp in cultivated *M. oleifera* and *M. peregrina* and was 216 bp in wild *M. oleifera*. The AT/CG ratio was 37.67 / 62.33 % in cultivated *M. oleifera*, 73.36 / 62.64 % in *M. peregrina* and 37.34 / 62.66 % in *M. oleifera*.

The ITS2 region appeared more homogenous in all taxa followed by the 5.8S and the ITS1 regions. The aligned nucleotide sequence of the ITS1 region showed 4 base substitutions. The base at position 58 was cytosine in both wild species and it was guanine in the cultivated *M. oleifera* while bases number 59 and 84 were guanine in both wild species and were adenine in the cultivated taxon. For 5.8S region, only one substitution was found at the base number 283 where cytosine in both wild and cultivated *M. oleifera* was substituted with thymine in *M. peregrina*. The base number 124 was cytosine in the cultivated *M. oleifera* and was thymine in wild *M. oleifera*. Only two insertions were found one in the 5.8S at base number 278 and the other was in the ITS2 region at base number 621.

The data showed base frequencies of A = 19.9%, C = 31.9%, G = 30.7% and T = 17.5%. Of the 614 nucleotides used for tree analyses, 440 were constant and 174 were variables. From the variable sites, 157 were parsimony uninformative and 17 were informative under parsimony criterion. The consensus parsimony tree constructed showed consistency index (CI = 1.00), homology index (HI = 0.00), retention index (RI = 1.00) and rescaled consistency index (RC = 1.00).

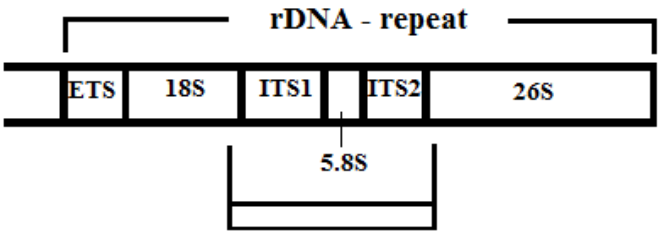

Figure 1. The gene arrangement of the nuclear DNA that spanning the rDNA genes. It consists of 18S, 5.8S, and 26S tracts and forms a tandem repetitive cluster. ETS is the external transcribed spacer while ITSs are the internal transcribed spacers 1 and 2 as numbered from 5' end.

A single parsimony tree (Fig. 2) was found in which the cultivated *M. oleifera* was grouped with both *M. oleifera* and *M. peregrina* in one polyphylic cluster (bootstrap= 100). Meanwhile, the sequenced fragments executed one neighbor-joining tree that showed a similar tree topology with the maximum-parsimony tree.

The pairwise genetic distances among the studied *Moringa* species are listed in Table 2. The genetic distance showed the smallest values between the cultivated *M. oleifera* and the wild *M. oleifera* and between *M. peregrina* and the wild *M. oleifera* (D= 0.005). The distances between either *M. peregrina* and *M. oleifera* and other species were high.

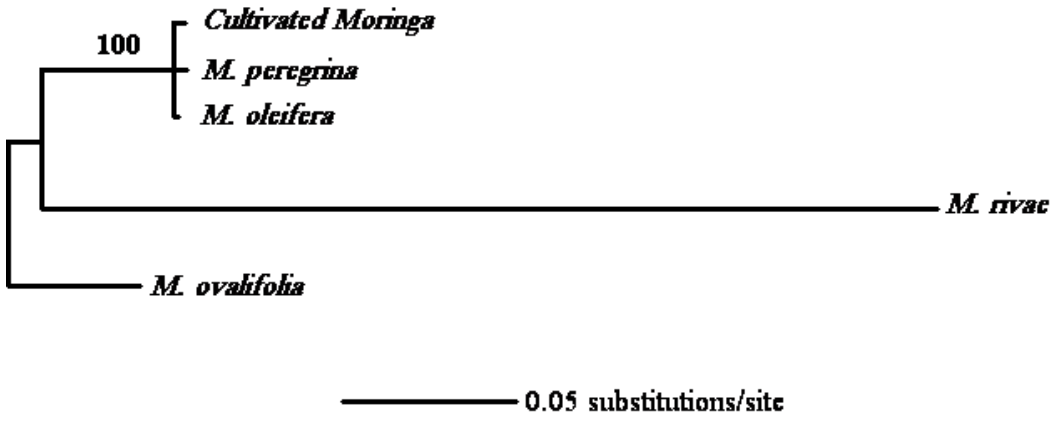

Figure 2. A neighbor-joining tree constructed from 614 bp sequenced fragments from ITS region of *Moringa* genome. Values at nodes refer to the bootstrapping of maximum-parsimony and neighbor-joining methods which were shown when they were over 50%.

Table 1. Base frequency and length of the sequenced region for the studied taxa.

Moringa taxa	ITS1					5.8S					ITS2					Total base frequency						
	A	T	C	G	Tot.	A	T	C	G	Tot	A	T	C	G	Tot.	A	T	C	G	AT%	CG%	Tot. length
M. oleifera (cult.)	54	35	85	74	248	44	34	43	45	166	31	39	72	73	215	129	108	200	192	37.67	62.33	629
M. peregrina	52	35	86	75	248	44	34	43	45	166	31	39	72	73	215	127	108	201	193	37.36	62.64	629
M. oleifera (wild)	52	36	85	75	248	44	34	43	46	167	31	39	73	73	216	127	109	201	194	37.34	62.66	631

Table 2. Pair wise genetic distances among the different *Moringa* species as calculated from the sequenced plant genome fragments in this study

	Cultivated *M. oleifera*	*M. peregrina*	*M. oleifera*	*M. ovalifolia*
Cultivated *M. oleifera*	--			
M. peregrina	0.007	--		
M. oleifera	**0.005**	**0.005**	--	
M. ovalifolia	0.088	0.088	0.086	--
M. rivae	0.297	0.298	0.296	0.303

DISCUSSION

ITS region is intervening spacers found between the 18S-5.8S-26S region separating the elements of the rDNA locus. It consists of ITS1 and ITS2 flanking from both sides the highly conserved 5.8S rDNA exon [16]. In angiosperms, the total length of this region varies between 500 and 750 bp [17] and in *Moringa*, it is approximately 630 bp. Unlike the coding regions, the ITS spacer exhibits more changes and therefore, is extensively used as a marker for systematic reconstruction at different taxonomic levels. After its first application in this regard [18], it has become widely used for phylogeny. This region is biparental inheritance, easy to be amplified and sequenced with several universal primers and suitable for evolutionary studies at the species or generic level [16,19, 20]. The spacer therefore, varies in its length from species to species. It length is 629 bp in the current sample and in *M. peregrina* while it is 631 bp in *M. oleifera*.

The biogeographic distribution of *M. peregrina* is North Africa and Arabia while that of *M. oleifera* is the Indian continent [1]. The genetic diversity within *M. peregrina* is very limited [13], while that within *M. oleifera* indicated a high level of population differentiation [21]. The genetic distance between the cultivated *M. oleifera* and *M. peregrina* was higher than that found between this taxon and both wild species. This result could indicate that the genetic structure of the cultivated *M. oleifera* did not altered by the new habitats of Saudi Arabia.

However, the small genetic variability could be induced by some genetic separation between cultivated and non-cultivated individuals. This possibly could be attributed to the genetic changes taking place in escapees' individuals as they adapt to the natural environment. Similar findings were revealed in Tanzanian *M. oleifera* [22]. These results may also be supported by recent findings [23] in which the authors revealed a large genetic diversity among the Indian populations of *M. oleifera* which can be used for crop improvement programme.

REFERENCES

[1] Olson M.E., 2002. Combining Data from DNA Sequences and Morphology for a Phylogeny of Moringaceae (Brassicales) Systematic Botany 27(1): 55–73.

[2] Sengupta A., Gupta M.P., 1970. Studies on seed fat composition of Moringaceae family. Fett. Wiss. Technol. (72): 6–10.

[3] Morton J.F., 1991. The horse radish tree, Moringa Rengosperma (Moringaceae) – a boon to arid lands? Econ. Bot. 45 (3), 318–333.

[4] Migahid A.M., (1978). Flora of Saudi Arabia Volume 1 Dicotyledon. Riyadh University Publication, p.101.

[5] Hall M., Miller A.G., Llewellyn O.A., Al-Abbasi T.M., Al-Harbi R.J. and Al-Shammari K.F. (2011). Important Plant Area in the Arabian Peninsula. Edinburgh Journal of Botany, 68(2): 183–197.

[6] Osman H.E., Abohassan A.A., 2012. Morphological and Analytical characterization of *Moringa peregrina* Populations In Western Saudi Arabia. College of Meteorology, Environmental Science and Arid land Agriculture, International Journal of Theoretical & Applied Sciences, 4(2): 174-184.

[7] Kantharajah A.S and Dodd W.A.1991. Rapid clonal propagation of *M. oleifera* Lam. Using tissue culture. South Ind Hort 39: 224-228.

[8] Parrota J.A., 2009. *Moringa oleifera* Lam. Wiley-VCH. Verlag GmbH and Co. KGaA, Weinheim.

[9] Murro J.K, Muhikambele V.R.M and Sarwat S.V.2003.*Moringa oleifera* leaf meal can replace cotton seedcake in the concentrate mix fedwith Rhodes grass hay for growing sheep. Livest Res Rur Dev 15 (11).

[10] Rufai S., Hanafi 1 M. M., Rafii 1. M., Ahmad 1.S., Arolu I. W. and Ferdous J., 2013, Genetic Dissection of New Genotypes of Drumstick Tree (*Moringa oleifera* Lam.) Using Random Amplified Polymorphic DNA Marker. Hindawi Publishing Corporation BioMed Research International Article ID 604598, 6 pages.

[11] Al Khateeb W., Bahar E., Lahham J., Schroeder D., Hussein E.,2013. Regeneration and assessment of genetic fidelity of the endangered tree *Moringa peregrina* (Forsk.) Fiori using inter simple sequence repeat (ISSR). Physiol. Mol. Biol. Plants (19): 157–164.

[12] Saini R.K., Saad K.R., Ravishankar G.A., Giridhar P., Shetty N.P., 2013. Geneticdiversity of commercially grown *Moringa oleifera* Lam. cultivars from Indiaby RAPD, ISSR and cytochrome P450-based markers. Plant Syst. Evol. (299):1205–1213.

[13] Alaklabi A., 2015. Genetic diversity of *Moringa peregrina* species in Saudi Arabia with ITS sequences. Saudi journal of biological sciences / v.22 no.2, pp.186-190.

[14] White T.J., Bruns T., Lee S. and Taylor J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis M. A., Gelfand D. H., Sninsky J. J. and White T. J. Academic Press, Inc., New York.

[15] Swofford D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

[16] Wheeler W.C., Honeycutt R.L. 1988. Paired sequence difference in ribosomal RNAs: evolutionary and phylogenetic implications. Mol Biol Evol 5:90–96.

[17] Baldwin B.G., Sanderson M.J., Porter J.M., Wojciechowski M.F., Campbell C.S., Donoghue M.J., 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. Ann Missouri Bot Gard 82:247–277.

[18] Porter C.H., Collins F.H 1991. Species-diagnostic differences in the ribosomal DNA internal transcribed spacer from the sibling species *Anopheles freeborni* and *Anopheles hermsi* (Diptera: Culicidae). Am J Trop Med Hyg (45):271–27.

[19] Liston A., Robinson W.A., Oliphant J.M, Alvarez-Buylla E.R., 1996. Length variation in the nuclear ribosomal DNA internal transcribed region of non-flowering seed plants. Syst Bot 21(2):109–120.

[20] Maggini F., Marrocco R., Gelati T.M., De Dominicis R.I 1998. Length and nucleotide sequences of the internal spacers of nuclear ribosomal DNA in gymnosperms and pteridophytes. Plant Syst Evol 213(3–4):199–205.

[21] Muluvi G. M., Sprent J. I., Soranzo N., Provan J., Odee D., Folkards G., Mcnico J. W. and Powell W. 1999. Amplified fragment Length Polymorphism (AFLP) analysis of genetic variation in *Moringa oleifera* Lam. *Molec Ecol*. **8**: 463-470.

[22] Mgendi M.G., Manoko M.K., Nyomora A.M.2010. Genetic diversity between cultivated and non-cultivated *Moringa oleifera* Lam. provenances assessed by RAPD markers. Journal of Cell and Molecular Biology 8(2): 95-102.

[23] Ganesan S.K., Singh R., Choudhury D.R., Bharadwaj J., Gupta V., Singode A.,2014.Genetic diversity and population structure study of drumstick(*Moringa oleifera* Lam.) using orphological and SSR markers Industrial Crops and Products (60): 316–325.

INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences