International Journal of Botany

ISSN: 1811-9700

science alert

ANSInet
an open access publisher
http://ansinet.com

International Journal of Botany 7 (3): 237-242, 2011
ISSN 1811-9700 / DOI: 10.3923/ijb.2011.237.242
© 2011 Asian Network for Scientific Information

Detection of Genetic Variability using Random Amplified Polymorphic DNA Markers in Some Accessions of *Moringa oleifera* Lam. from Northern Nigeria

[1]B.Y. Abubakar, [2]R. Wusirika, [1]S. MuA'zu, [1]A.U. Khan and [1]A.K. Adamu
[1]Department of Biological Sciences, Ahmadu Bello University,
Funtua Road, Samaru-Zaria 810261, Nigeria
[2]Department of Biological Sciences, Michigan Technological University, Dow 740, 1400 Townsend Dr,
Houghton, MI 49931, USA

Abstract: To investigate genetic variability in *Moringa oleifera* Lam., 75 accessions from the Sudan and Guinea savanna zones in Nigeria were taxonomically analysed using Random Amplified polymorphic DNA (RAPD) markers. The electrophoresis bands were analysed using NTSYSpc software and the result of their matrices indicated different variability in the accessions. High degrees of polymorphism (74%) among the accessions were observed in terms of genetic relationship and were grouped into five clusters. This high variability can be utilised for mapping out breeding strategies in the production of cultivars with better yield of *M. oleifera* to meet the pressing needs of these multi-purpose crop to our growing populations.

Key words: *Moringa oleifera*, RAPD, variation, northern Nigeria

INTRODUCTION

Moringa oleifera Lam. commonly named horse-radish tree, drumstick tree, mothers best friend, Indian ben etc., is one of the thirteen known species belonging to the family Moringaceae with only one genus *Moringa*. It is a fast growing drought resistant tree or shrub. It originated from sub-Himalayan tracts of Northern India, distributed worldwide in the tropics and sub-tropic (Olson, 2002). The Moringaceae is one of the 15 families that produce mustard oil (glucosinolates) and related to other mustard oil plants like Brassicaceae, Caricaceae etc (Chase *et al*., 1998). In West Africa, the family is represented by 10 species whereas in Nigeria the plant is represented by the only species of *M. oleifera* (Keay, 1989).

The plant is multi-purpose with a tremendous variety of potential uses and recently attracted the attention of several authors (Tsaknis *et al*., 1999; Olson, 2002; Muluvi *et al*., 2004). In recent years, the leaf of this plant with its rich micronutrient contents like zinc, iron and vitamin A has been used in treating malnutrition in children and improving the diets of lactating mothers in some African countries. Being a local resource, its use in checking child undernutrition would mean saving the meagre African financial resources in counter funding which depend on imported solutions. In water purification, Foidl *et al*. (2001) reported that up to 99% of colloids can be removed from dirty water and works more effectively than the imported alum (alluminium sulphate).

At an International conference (Anonymous, 2001) entitled "what potential for moringa? participants express the need among other things to collect and assess genetic diversity, select best provenances based on product (leaves, seeds, green pods etc.) and genetic and propagation improvement of the crop. Bosch (2004) observed that apart from Indian breeding programmes, very little breeding has been achieved so far. He further observed that no breeding work has been undertaken in Africa. In Nigeria with wide range of uses of moringa products, most research efforts are focused mainly on medicine (Ezeamuzie *et al*., 1996), amino acid composition, water treatment (person. communication) but, not targeted towards the improvement of this important plant. A prerequisite of any genetic improvement programme is a focus in genetic variability in the local germplasm so that breeding strategies can be mapped out. Many authors (Waycott, 1995; Singh *et al*., 2004; Chakrabarty *et al*., 2007; Lattoo *et al*., 2008; Wang *et al*., 2010) have used the Random Amplified Polymorphic DNA (RAPD) molecular markers developed by Williams *et al*. (1990) in plant genetic variability studies. There is no record of any study that estimates the genetic variability in *M. oleifera* in Nigeria. Therefore, we have conducted this study to analyze genetic diversity present in some accessions of this valuable vegetable from northern Nigeria using RAPD marker.

Corresponding Author: B.Y. Abubakar, Department of Biological Sciences, Ahmadu Bello University, Funtua Road, Samaru-Zaria 810261, Nigeria Tel: +234 80615 70 509

Table 1: *Moringa oleifera* accessions used and their origin

S. No.	Accession	Place of collection (acquisition)	GPS
1	1DTJG	Dutse, Jigawa State, Nigeria (1)	11°44'N 9°17'E
2	2JHJG	Jahun, Jigawa State, Nigeria (5)	12°06'N 9°25'E
3	7ARKB	Argungun, Kebbi State, Nigeria (2)	12°26'N 4°40'E
4	9GWKB	Gwandu, Kebbi State, Nigeria (3)	12°16'N 6°33'E
5	12KFKD	Kafanchan, Kaduna State, Nigeria (4)	11°34'N 8°18'E
6	16GZKN	Gezawa, Kano State, Nigeria (2)	12°14'N 8°04'E
7	18TWKN	Tudunwada, Kano State, Nigeria (5)	12°01'N 8°34'E
8	23DMKT	Dutsin-ma, Katsina State, Nigeria (4)	12°27'N 7°29'E
9	27KFKT	Kafur, Katsina State, Nigeria (3)	11°39'N 7°42'E
10	30DDSK	Dogondaji, Sokoto State, Nigeria (2)	12°06'N 6°19'E
11	32GRSK	Goronyo, Sokoto State, Nigeria (3)	13°27'N 5°40'E
12	35BDZM	Bodinga, Zamfara State, Nigeria (4)	11°58'N 9°97'E
13	39WNZM	Wanzamai, Zamfara State, Nigeria (3)	15°18'N 6°92'E
14	42NMAD	Numan, Adamawa State, Nigeria (9)	9°26'N 12°18'E
15	51JMBA	Jamaare, Bauchi State, Nigeria (3)	11°38'N 9°52'E
16	54BAU	Bauchi, Bauchi State, Nigeria (5)	10°30'N 10°0'E
17	59GMB	Gombe, Gombe State, Nigeria (4)	10°17'N 11°10'E
18	63KGGM	Kaltingo, Gombe State, Nigeria (2)	9°38'N 11°05'E
19	65MLTR	Malobe, Taraba State, Nigeria (4)	7°47'N 10°13'E
20	69MMYB	Mamado, Yobe State, Nigeria (4)	11°42'N 11°05'E
21	73DMT	Damaturu, Yobe State, Nigeria (2)	12°53'N 11°13'E
22	75ZRKD	Zaria, Kaduna State, Nigeria (1)	11°09'N 7°39'E

MATERIALS AND METHODS

Plant material: A total of 75 seed accessions of *Moringa oleifera* (Table 1) were collected from two sites 70-100 km apart in each 12 states of NE and NW regions of northern Nigeria. Two populations in each sites separated by 50-100 m were used for the collection.

DNA extraction: ZR plant/seed DNA kit-D6020 from Zymo Research (www.zymoresearch.com) was used and its protocols for extraction adopted. All extracted DNA were stored at -20°C before use. DNA quality was checked using NanoDrop® ND-1000 spectrophotometer at 260 and 280 nm absorbance.

Primers: A total of 24 arbitrary primers were used in this study as shown in the Table 2.

Randomly amplified polymorphic DNA assay was carried out in 15 μL amplification, sample was prepared containing 1 μL genomic DNA, 7.5 μL Mix Gotaq (with $MgCl_2$, 2.5 L of 1 mM dNTP, 0.6 of Taq DNA polymerase, 10 mM Tris HCl pH 9.0, 50 mM KCl and 0.01% gelatin), 3 μL primer and 3.5 μL dd water.

PCR amplification: BIORAD thermal cycler™ (My Cycler™) was used for PCR amplification with the following conditions: Pre-denaturation at 94°C for 4 mins, 45 cycles of denaturation at 94°C for 1 min, annealing at 34°C for 1 min, elongation step at 72°C for 2 mins, Final cycle at 72°C for 5 mins and final products of amplification was then cooled at 4°C. RAPD-PCR products were then analysed by electrophoresis (using GCMGU-602T from C.B.S Scientific Coy) with 1 kb DNA ladder-N3232 (from New Englang BioLabs-www.neb.com) on a 1.2% agarose gel with 1XTAE buffer and stained with 5 μL ethidium bromide. Power was supplied at 100 V h^{-1}. A negative control was also prepared containing all the reaction mixtures minus the genomic DNA. DNA banding patterns were then visualized using FOTO/UV® Transilluminator from FOTODYNE Incorporated.

Table 2: The arbitrary primers were obtained from IDT Integrated DNA Technologies, Coralville, IA, USA (www.idtdna.com)

RAPD primer	Sequence (5'-3')
OPA-7	GGTGACGCAG
OPA-8	GTGACGTAGG
OPA-10	GTGATCGCAG
OPA-13	CAGCACCCAC
OPA-17	GACCGCTTGT
OPA-18	AGGTGACCGT
OPA-19	CAAACGTCGG
OPA-20	GTTGCGATCC
OPB-17	AGGGAACGAG
OPB-20	GGACCCTTAC
OPF-20	GAGGATCCCT
OPH-19	CTGACCAGCC
OPM-6	CTGGGCAACT
OPM-8	TCTGTTCCCC
OPO-2	ACGTAGCGTC
OPO-3	CTGTTGCTAC
OPO-13	GACAGGAGGT
OPQ-2	TCTGTCGGTC
OPBB-7	GAAGGCTGGG
OPBC-2	ACAGTAGCGG
OPBC-10	AACGTCGAGG
OPBD-18	ACGCACACTC
OPBD-19	GGTTCCTCTC
OPU-17	ACCTGGGGAG

Data analysis: Amplification products were scored for presence (1) or absence (0) of bands in a binary matrix at same base molecular weights. This data was then used to generate genetic matrix using Jaccard's similarity

coefficients of NYSYSpc (Rohlf, 2009). This similarity matrix was then used for clustering of the genotypes by SAHN of the software by UPGMA (unweighted pair group with arithmetic mean.

RESULTS AND DISCUSSION

A total of 24 primers consisting of 10 mer nucleotide repeats were used to carry out the RAPD analysis screening of the 75 accessions of *M. oleifera.* Out of these primers, six were found to give clear polymorphic bands (Table 3). Average polymorphic bands per primer is 5.16 from a total of 42 amplified bands in the size range of 150-400 bp. Primer OPA 19 gave the highest polymorphism of 100% while OPBC 10 gave the lowest of 55.56%. PIC ranged from 0.46 to 0.95 indicating that the least informative primer was OPO_3 but, has the highest Resolving Power (Rp) of 0.92 as shown in Table 3.

The gel electrophoresis pattern as shown in Fig. 1 and the rest gave a clear variation in terms of scorable and unscorable bands. The primer OPA-19 gave a total of 44 scorable bands with unique bands to accessions 1DTJG, 5JHJG, 10GWKB, 29KFKT and GMB61 at a band size of 400 bp, likewise at 380 and 150 bp; cultivars 14KFKD, 26DMKT, 30DDSK and 20TWKN, 38BDZM, 57BAU, GMB60, 68MLTR gave unique bands respectively. At base sizes of 510 and 240 bp of primer OPF-20, conspicuous bands were noted in 25DMKT, 27KFKT, 36BDZM, 44NMAD, 52JMBA, 57BAU, 66MLTR, 70MMYB, 75ZRKD and 7ARKB, 18TWKN, 19TWKN, 25DMKT, 36BDZM, 38BDZM, 52JMBA, respectively. Common scorable bands at 200 and 175 bps were found in all the OTUs but, 7ARKB, 15KFKD, 16GZKN, 18TWKN, 25DMKT, 37BDZM, 64KGGM, 68MLTR, 72MMYB, 74DMT and 10BGKB, 19TWKN, 20TWKN, 22TEKN, 28KFKT, 37,BDZM 55BAU, 58BAU, 69MMYB, respectively using the primer OPO-3. Primer OPB-17 has revealed common bands at 300 bp in all accessions but, 7ARKB, 15KFKD, 30DDSK, 37BDZM, 43NMAD, 65MLTR and 68MLTR while unique bands at 160 bp are common to OTUs 10GWKB, 25DMKT, 37BDZM and 43NMAD. Similar amplification patterns were observed at 398 bp of the primer OPBC-10 in all but, samples 13KFKD and 39WNZM. The most unique bandings of RAPD amplification patterns were observed in the primer OPB-20 in accessions 2JHJG, 4JHJG, 6JHJG, 7ARKB, 15KFKD, 18TWKN, 19TWKN, 22TWKN, 26DMKT, 30DDSK, 32GRSK, 41WNZM, 56BAU, 60GMB, 61GMB, 65MLTR, 68MLTR, 71MMYB-ZRKD75.

This dissimilarity matrix was then calculated for all the genotypes (or accessions) by SAHN of the software via UPGMA (unweighted pair group with arithmetic mean) as shown in Table 3. The dendrogram generated by UPGMA cluster analysis (Fig. 2) revealed at about 74% dissimilarity index, the accessions were grouped into five clusters. The first cluster grouped 22 accessions, followed by second and third cluster with 27 and 10 accessions each respectively. While the third and fourth clusters contained 10 and 6 accessions, respectively.

Polymerase Chain Reaction (PCR) amplification have revealed an average polymorphic bands per primer of 5.16 from a total of 42 amplified bands in the size range of 150-400 bp, with primers having 50-50% GC ratio showing higher polymorphism. This suggest that the higher polymorphism in these primers may be due to stability of base complementation when G pairs with C by 3 hydrogen bonds than A with T (Ragunathachari *et al*., 2000). According to Prevost and Wilkinson (1999), the resolving power was a characteristic of a primer which reflects overall suitability of a marker system for the purpose of identification and it was related to the number of accessions distinguished by that primer. The dendrogram generated by UPGMA cluster analysis revealed at about 74% dissimilarity index, the accessions were grouped into five clusters, indicating a high genetic variation of between the accessions. In the same vein, different accessions of *M. oleifera* from northern Nigeria indicated low similarity coefficient (26%) thus, crossing between the genotypes with low similarity coefficient will manifest high heterosis. Likewise the work of Fu *et al*. (2003) indicated 51.2% genetic differentiation coefficient in *Changnium smyrnioides* using RAPD marker suggesting that the species were of a higher genetic diversity among the populations of these plants. From this result, it was observed that there was the need to take individuals from more different populations if we need to construct an artificial conservation area so as to preserve their diversity in the future.

Table 3: Polymorphism exhibited by the RAPD primers in *M oleifera* accessions

Primer	Sequence	No of amplified bands	No. of polymorphic bands	% Polymorphism	PIC	Rp
OPA 19	CAAACGTCGG	8	8	100.00	0.66	0.68
OPB 17	AGGGAACGAG	8	5	62.50	0.72	0.56
OPB 20	GGACCCTTAC	8	7	87.50	0.64	0.72
OPBC 10	AACGTCGAGG	9	5	55.56	0.95	0.10
OPF 20	GAGGATCCCT	6	4	66.67	0.67	0.66
OPO 3	CTGTTGCTAC	3	2	66.66	0.46	0.92
Total	42	31				

PIC: Polymorphic Information Content, Rp: Resolution power

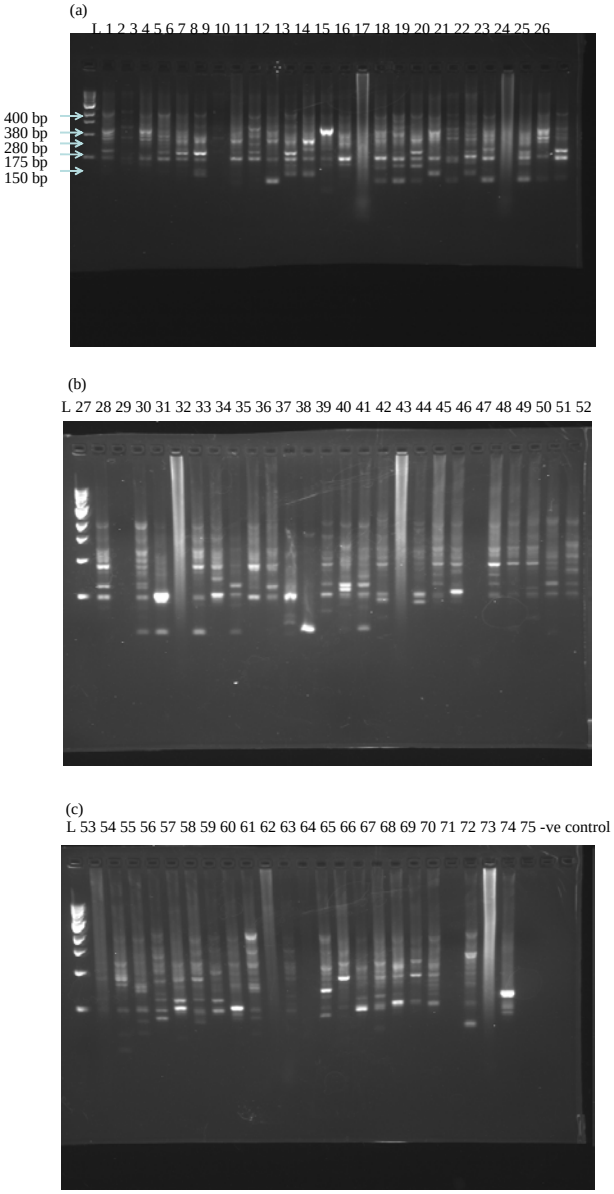

Fig. 1(a-c): RAPD amplification pattern of *M. oleifera* accessions from northwest and northeast Nigeria using primer OPA-19; (a) 1DTJG, 2JHJG, 3JHJG, 4JHJG, 5JHJG, 6JHJG, 7ARKB, 8ARKB, 9GWKB, 10GWKB, 11GWKB, 12KFKD, 13KFKD, 14KFKD, 15KFKD, 16GZKN, 17GZKN, 18TWKN, 18TWKN, 19TWKN, 20TWKN, 21TWKN, 22TWKN, 23DMKT, 24DMKT, 25DMKT, 26DMKT, (b) 27KFKT, 28KFKT, 29KFKT, 30DDSK, 31DDSK, 32GRSK, 33GRSK, 34GRSK, 35BBDZM, 36BDZM, 37BDZM, 38BDZM, 39WNZM, 40WNZM, 41WNZM, 42NMAD, 43NMAD, 44NMAD, 45NMAD, 46NMAD, 47NMAD, 48NMAD, 49NMAD, 50NMAD, 51JMBA, 52JMBA and (c) 53JMBA, 54BAU, 55BAU, 56BAU, 57BAU, 58BAU, 59GMB, 60GMB, 61GMB, 62GMB, 63KGGM, 64KGGM, 65MLTR, 66MLTR, 67MLTR, 68MLTR, 69MMYB, 70MMYB, 71MMYB, 72MMYB, 73DMT, 74DMT, 75ZRKD

Generally, the observed genetic variability of 74% with the six RAPD primers among the 75 accessions of *M. oleifera* analysed, confirms the ability of this marker to detect variation in the samples. The accessions from

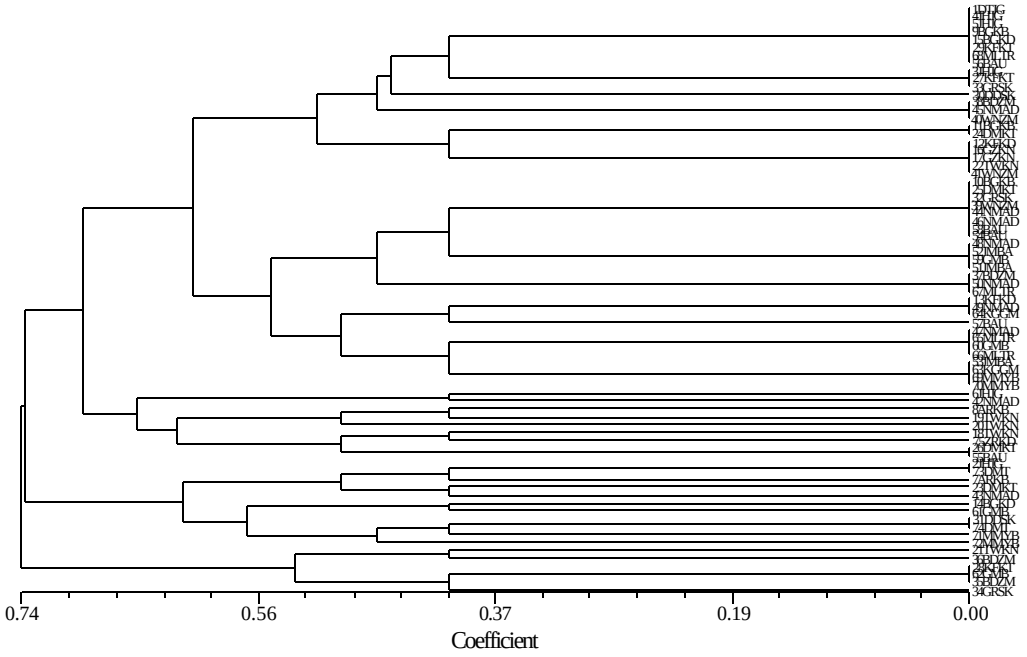

Fig. 2: Cluster analysis of *Moringa oleifera* accessions on Jaccard similarity index in a dendrogram by UPGMA using RAPD data. Key: I= Cluster 1, II= Cluster 2, III= Cluster 3, IV= Cluster 4 and V= Cluster 5

different population were clearly grouped into 5 genetic clusters using the RAPD primers irrespective of area of collection suggesting closer parentage ramets and genetic variability among the *M. oleifera* accession from northwest and north east states of Nigeria.

ACKNOWLEDGMENT

The authors are grateful to the Management of Ahmadu Bello University, Zaria for financial assistance to BYA through the UBR (University Board of Research) and STEPB grants.

REFERENCES

Anonymous, 2001. Developmental potential for moringa products. Workshop on October 29-November 2, 2001, Dar es Salam, Tanzania. http://www.moringanews.org/seminaire_en.html

Bosch, C.H., 2004. *Moringa oliefera* Lam. In: Plant Resources of Tropical Africa. 2. Vegetables, Grubben, G.J.H and O.A. Denton (Eds.). PROTA Foundation, Wegeningen, Netherlands, pp: 392-395.

Chakrabarty, D., V.N. Gupta and S.K. Datta, 2007. Varietal identification and assessment of genetic relationships in hippeastrum using RAPD makers. Plant Biotechnol. Rep., 1: 211-217.

Chase, M.W., K. Bremer, P.F. Stevens, A.A. Anderberg and A. Backlund *et al.*, 1998. An ordinal classification for the families of flowering plants. Ann. Missouri Bot. Gard, 85: 531-553.

Ezeamuzie, I.C., A.W. Ambadederomo, F.O. Shode and S.C. Ekwebelem, 1996. Anti-inflammatory effects of *M. oleifera* root extract. Int. J. Pharmacognosy, 34: 207-212.

Foidl, N., H.P.S. Makkar and K. Becker, 2001. The Potential of *M. oleifera* for Agricultural and Industrial Uses. In: The Miracle Tree: Multiple Attributes of Moringa, Fuglie, L.J. (Ed.). CTA/CWS, Dakar, Senegal, pp: 45-76.

Fu, C., Y. Qiu and H. Kong, 2003. RAPD analysis for genetic diversity in *Changium smyrnioides* (Apiaceae), an endangered plant. Bot. Bull. Acad. Sin., 44: 13-18.

Keay, R.W.J., 1989. Trees of Nigeria. 2nd Edn., Oxford Science Publication, New York, ISBN: 0-19-854560-6, pp: 476.

Lattoo, S.K., R.S. Dhar, S. Khan, S. Bamotra, M.K. Bhan, A.K. Dhar and K.K. Gupta, 2008. Comparative analysis of genetic diversity using molecular and morphometric markers in *Andrographios paniculata* (Burm. f.) Nees. Genet. Resour. Crop Evol., 55: 34-43.

Muluvi, G.M., J.I. Sprent, D. Odee and W. Powell, 2004. Estimates of outcrossing rates in *Moringa oleifera* using amplified fragment length polymorphism (AFLP). Afr. J. Biotechnol., 3: 146-151.

Olson, M.E., 2002. Combining data from DNA sequences and morphology for a phylogeny of Moringaceae (Brassicales). Syst. Bot., 27: 55-73.

Prevost, A. and M.J. Wilkinson, 1999. A new system of comparising PCR primers applied to ISSR fingerprinting of potato cultivars. Tag Theor. Appied Genet., 98: 107-112.

Ragunathachari, P., V.K. Khanna, U.S. Singh and N.K. Singh, 2000. RAPD analysis of genetic variability in Indian scented rice germplasm (*Oryza sativa* L.). Curr. Sci., 79: 994-998.

Rohlf, F.J., 2009. NTSYSpc: Numerical Taxonomy and Multivariate Analysis Systems. Version 2.2E. Exeter Publishing Ltd., Setauket, New York.

Singh, A.P., S. Dwevedi, S. Bharti, A. Srivastava, V. Singh and S.P.S. Khanuja, 2004. Phylogenetic relationships as in *Ocimum* revealed by RAPD markers. Euphytica: Nwtherlands J. Plant Breedings, 136: 11-20.

Tsaknis, J., S. Lalas, V. Gergis, V. Dourtoglou and V. Spiliotis, 1999. Characterization of *Moringa oleifera* variety mbololo seed oil of Kenya. J. Agric. Food Chem., 47: 4495-4499.

Wang, X.F., W.H. Zheng, H.X. Zheng, Q.Q. Xie, H.Y. Zheng, H. Tang and Y.L. Tao, 2010. Optimization of RAPD-PCR reaction system for genetic relationships analysis of 15 camellia cultivars. Afr. J. Biotechnol., 9: 798-804.

Waycott, M., 1995. Assessment of genetic variation and clonality in the seagrass (*Posidonia australis*) using RAPD and allozyme analysis. Mar. Ecol. Prog. Ser., 116: 289-295.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey, 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18: 6531-6535.