The Pharma Innovation Journal 2021; 10(12): 94-98

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; 10(12): 94-98
© 2021 TPI
www.thepharmajournal.com
Received: 06-09-2021
Accepted: 16-10-2021

Shubhada Tayade
Research Scholar, Department of Vegetable Science, Horticultural College and Research Institute, Periyakulam, Tamil Nadu, India

GJ Janavi
Professor and Head, Department of Vegetable Science, Horticultural College and Research Institute, Periyakulam, Tamil Nadu, India

B Rajagopal
Associate Professor (Agricultural Biotechnology), Department of Fruit Science, Horticultural College and Research Institute, Periyakulam, Tamil Nadu, India

Lakshmi Kashirajan
Senior Scientist, Department of Biotechnology, Division of Crop Improvement, Sugarcane Breeding Institute, Coimbatore, Tamil Nadu, India

Corresponding Author:
Shubhada Tayade
Research Scholar, Department of Vegetable Science, Horticultural College and Research Institute, Periyakulam, Tamil Nadu, India

Characterization of moringa (*Moringa oleifera* Lam.) genotypes using RAPD markers

Shubhada Tayade, GJ Janavi, B Rajagopal and Lakshmi Kashirajan

Abstract
Moringa is a member of the Moringaceae family, which includes 13 distinct species, the most widely farmed of which being *Moringa oleifera*. It is a tree of many varied virtues with large nutritional benefits and exceptional capacity to treat malnutrition, and it is generally regarded as a superfood across the world. Any genetic enhancement and production of superior cultivars requires an understanding of the genetic diversity among advanced breeding lines. The faster- breeding programme benefits from marker-assisted germplasm selection for the targeted character. In this work, a total of 25 accessions were screened using 24 Random Amplified Polymorphic DNA from different districts at Tamil Nadu that were collected and maintained in the Moringa genetic resource garden at Horticultural College and Research Institute, Periyakulam, Tamil Nadu (RAPD). It was discovered that RAPD markers can distinguish between various moringa accessions, which may help with high diversity studies and might also be used for further study based on their genetic and nutritional content, as well as the conservation of superior germplasm.

Keywords: Characterization, RAPD, moringa, *Moringa oleifera* Lam.

1. Introduction

Moringa oleifera Lam (*syn. M. ptreygosperma Gaertn.*) is a well-known, extensively spread, and naturalized member of the Moringaceae monogeneric family (Ramachandran *et al*., 1980) [1]. *M. oleifera* has diploid chromosomal constitution (2n=28) and is the most cultivated species among all. Moringa has proved to be an important food source containing proteins, vitamins, minerals and antioxidants, where it helps in overall wellness (Nautiyal and Venkataraman, 1987) [6]. The most malnourished parts of the world (Africa, Asia, Latin America, and the Caribbean) all have the potential to produce and use an edible plant called *Moringa oleifera*, sometimes known as "The Miracle Tree" (Palada, 1996; Fuglie, 1999) [5, 2]
Traditional healers have used various parts of *M. oleifera* to treat skin diseases, respiratory illnesses, ear and dental infections, hypertension, diabetes, cancer treatment, water purification, and have promoted its use as a nutrient dense food source for hundreds of years (Anwar *et al*., 2007; Castellón and González, 1996; Fahey, 2005; Fuglie, 1999) [3, 4, 7, 2]. In many third-world countries, it is also utilised in feeding programmes to combat malnutrition. Many compounds found in M. oleifera have anticancer and hypotensive properties, and have been used in Siddha therapy for centuries (Soller and Beckmann 1983) [8]. Traditional crop breeding operations for diverse qualities.
Might benefit from a better understanding of genetic diversity utilising targeted markers (Bretting and Widrelechner 1995) [9]. A faster-breeding programme will benefit from the use of marker- assisted germplasm selection for the targeted trait. Polymorphism detection at the genome level discloses the reality of variety and polymorphism, allowing for easy detection of genetic variation within a population (O'Neill *et al*. 2003; Wu *et al*. 2004) [10, 11]. As a result, in recent years, the use of genetic markers for crop enhancement has become increasingly popular (Garcia *et al*. 2004) [12].
Restriction fragment length polymorphism (RFLP) (Thomas *et al*. 2000) [14], amplified fragment length polymorphism (AFLP) (Kim *et al*. 2002) [15], microsatellites and mini satellites (Saini *et al*. 2013) [16], inter-simple sequence repeats (ISSR) (Zietkiewicz *et al*. 1994) [17], and RAPD are the most commonly used PCR-based molecular markers (Jacobson and Hedren 2007) [18]. Among them, RAPD is the most widely used, quick, and low-cost approach for determining plant genetic diversity (Yamanaka *et al*. 2003) [13]. Mgendi *et al*., 2010 [19] examined the genetic diversity of *Moringa oleifera Lam* throughout and between cultivated and non-cultivated provenances using 12 RAPD markers.

This present study is carried out to identifying variability between moringa genotypes at HC & RI, Periyakulam using RAPD markers. Identification of polymorphism at molecular level in moringa reveals diversity and polymorphism which will be helpful in detecting genetic diversity within the promising genotypes.

2. Materials and Methods

2.1 Plant materials

For this investigation, young leaves from 25 genotypes of *Moringa oleifera* L. were collected from the Moringa Genetic Resource Garden, Horticultural College and Research Institute, Periyakulam. Young leaves were often sampled in the morning and gathered in sterile zip lock bags before being immediately stored in an icebox to minimize exposure to sunlight, providing a high level of purity and avoiding any damage to the samples.

2.2 DNA extraction, purification and quantification

The CTAB technique (Doyle JJ, Doyle JL, 1990) was used to isolate genomic DNA, with some modifications to eliminate polysaccharides and increase purity. Fresh leaves (250 mg) were macerated in 1.5- 2 ml extraction buffer (1M Tris-HCl (pH 8.0), 0.5M EDTA (pH 8.0), 2 percent CTAB (w/v), 5M NaCl, 2 percent polyvinyl pyrrolidone (w/v)) in a water bath at 65 °C for 40- 45 minutes. After allowing the tubes to cool to room temperature, 1 mL of the supernatant was collected and transferred to fresh clean Eppendorf tubes. The tubes were filled with an equal volume of chloroform and isoamyl alcohol in a 24:1 ratio and well mixed. To achieve a residue-free solution, the tubes were centrifuged at 10,000 rpm for 10 minutes at 4°C. To get pure DNA, the supernatant was transferred to fresh sterile 1.5 ml centrifuge tubes and centrifuged at 10,000 rpm
for 10 minutes at 4°C with chloroform-isoamyl alcohol. The top layer containing DNA was transferred to a 1.5ml microfuge tube. The tubes were filled with an equal amount of ice-cold isopropanol and 50ul of 7.5M ammonium acetate and incubated at -20 °C for one hour. The DNA pellet was produced by centrifuging the tubes at 10000 rpm for 10 minutes at 4 °C, followed by two washes in 70% ethanol. The tubes were thoroughly air dried at room temperature, and the DNA pellet was dissolved in 100 µl 1X TE buffer before being kept at 4 °C. The DNA quality was tested on a 0.8 percent (w/v) agarose gel at 60V for 45 minutes to an hour.

2.3 RAPD marker analysis

Isolated DNA from the leaves of *M. oleifera* was used to study the genetic variation using RAPD markers (Eurofins Genomics India) as given in Table 1.

Table 1: Details of RAPD primers used for analysis

S. No.	Primers	Sequence
1.	OPA 03	AGTCAGCCAC
2.	OPA 04	AATCGGGCTG
3.	OPA 05	AGGGGTCTTG
4.	OPA 06	GGTCCCTGAC
5.	OPB 03	CATCCCCCTG
6.	OPB 04	GGACTGGAGT
7.	OPB 05	TGCGCCCTTC
8.	OPB 06	TGCTCTGCCC
9.	OPC 03	GGGGGTCTTT
10.	OPC 04	CCGCATCTAC
11.	OPC 05	GATGACCGCC
12.	OPC 06	GAACGGACTC
13.	OPD 03	GTCGCCGTCA
14.	OPD 04	TCTGGTGAGG
15.	OPD 05	TGAGCGGACA
16.	OPD 06	ACCTGAACGG
17.	OPE 02	GGTGCGGGAA
18.	OPE 03	CCAGATGCAC
19.	OPE 04	GTGACATGCC
20.	OPE 05	TCAGGGAGGT
21.	OPF 02	GAGGATCCCT
22.	OPF 03	CCTGATCACC
23.	OPF 04	GGTGATCAGG
24.	OPF 05	CCGAATTCCC

2.4. PCR amplification

With certain modifications, RAPD analysis was carried out using the approach reported by Mgendi *et al.*, 2010 [19]. Initially, the settings were tweaked by changing the annealing temperature (25-40 °C) and the number of cycles. The polymorphism was checked using 24 RAPD primers. 1.0 ul DNA template, 6.0 ul PCR Master mix (Synergy Scientifics), 0.5ul 2.5uM primer, and sterile distilled water were used to generate a final volume of 10ul for PCR amplification of RAPD primers. A thermocycler (MyGene L series) was used for PCR amplification. The amplification began with a 5-min denaturation at 94 °C, followed by 35 cycles of 30 seconds denaturation at 94 °C, 1 min primer annealing at 30 °C, 2 min extension at 72 °C, and 7 min final extension at 72 °C. Electrophoresis was done on a 1.5 percent agarose gel to examine the final PCR products for all the 25 genotypes.

3. Results

The DNA isolated from 25 selected genotypes of *Moringa oleifera* leaves were used to study genetic variations using RAPD technique. Initially, 24 RAPD primers were tested for polymorphism by performing PCR with DNA isolated from genotype PKM MO 50. The five best performing primers were identified and used for verifying polymorphism in 25 moringa genotypes.

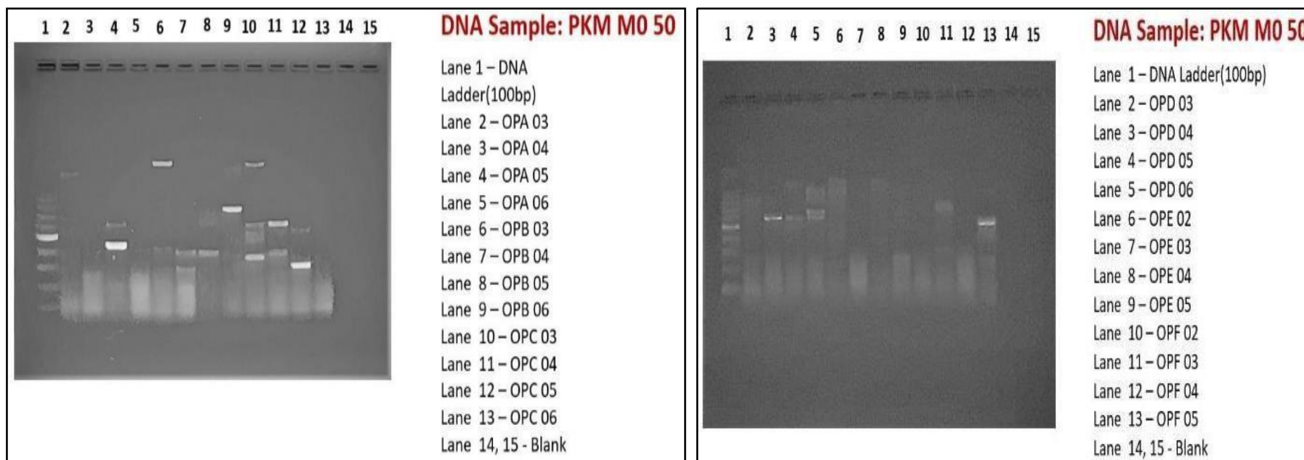

Fig 1: Screening of 24 RAPD primers using PKM MO 50 DNA

Fig.1 shows the testing of 24 RAPD primers for polymorphism with PKM MO 50. The five primers which polymorphism are OPE 03, OPD 04, OPB 05, OPB 06, OPC 05 and hence these primers were further used to characterize 25 *Moringa oleifera* genotypes

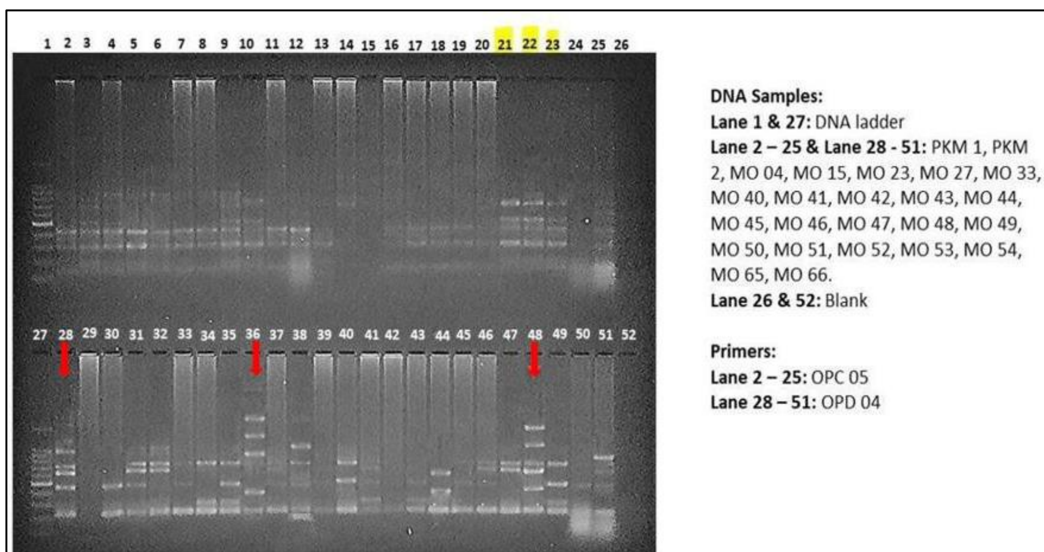

Fig 2: RAPD banding pattern in 25 genotypes of *Moringa oleifera*

Selected lane 21, 22, 23: PKM MO 52, PKM MO 53, PKM MO 54 showed similar bands with OPC 05 primer
Selected lane 28, 36, 48: PKM MO 53 shows unique bands with OPD 04 primer

Figure 2. Similar bands were observed in genotype PKM MO 52, PKM MO 53, PKM MO 54 by using OPC 05 primer, which indicates that these genotypes may be closely related. The Primer OPD 04 so far produced many distinct bands in all moringa genotypes. PKM 1, PKM MO 41 and PKM MO 54 showed most unique bands by using OPD 04 primer.

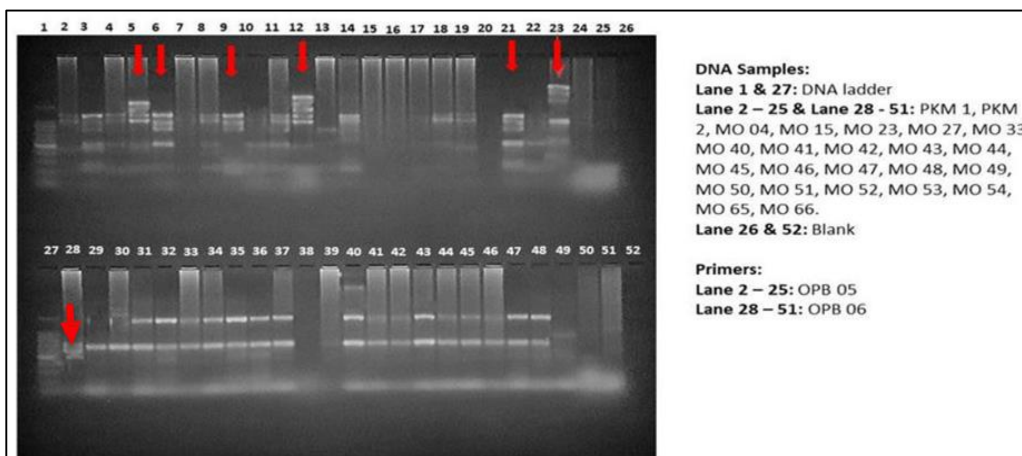

Fig 3: RAPD banding patterns in 24 genotypes of *M. oleifera*

Selected lane 5, 6, 9, 12, 21& 23: PKM MO 65 produced unique band with OPB 05 Selected lane 28: Primer OPB 06 produced distinct bands in PKM 1

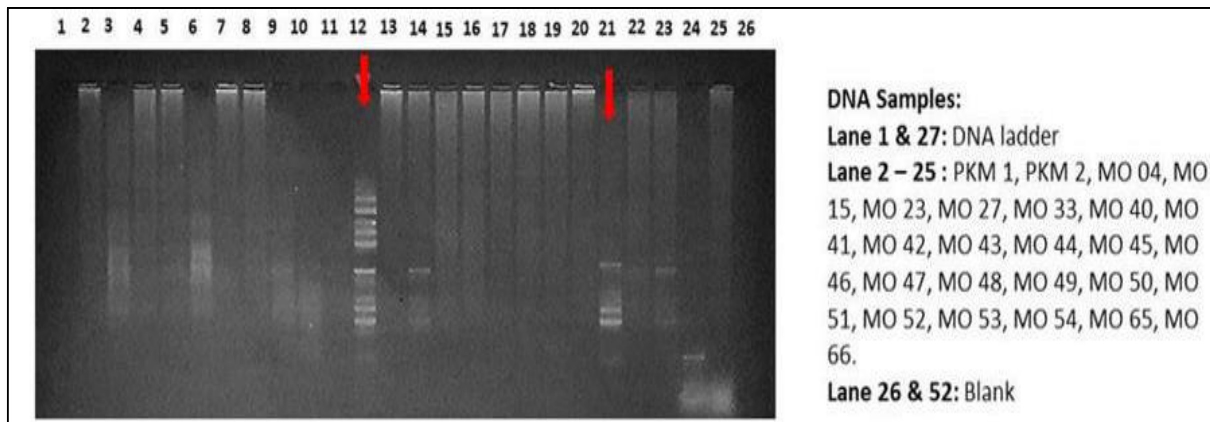

Fig 4: RAPD banding patterns in 25 genotypes of *M. oleifera*

Selected lane 12 & 21: PKM MO 44 PKM MO 52 produced unique band with OPE 03

Fig. 3 shows PKM MO 23, PKM MO 27, PKM MO 4, PKM MO 52 and PKM MO 54 producing distinct bands with OPB 05 and Fig. 4 shows primer OPE 03 produced different bands in PKM MO 44 and PKM MO 52 genotype.

3.2 Statistical analysis for construction of dendrogram using molecular data

The analysis of genetic diversity was relied on discrete variables of binary data matrix that comprise of the presence (1) and absence (0) of an allele per RAPD locus for each accession to determine the genetic link. In addition, a dendrogram based on Jaccard's similarity coefficients (Jaccard, 1908) was generated using the unweighted pair group approach with arithmetic averages to study the link between accessions (UPGMA). The NTSYS-pe 2.1 software program was used to estimate genetic diversity and perform cluster analysis.

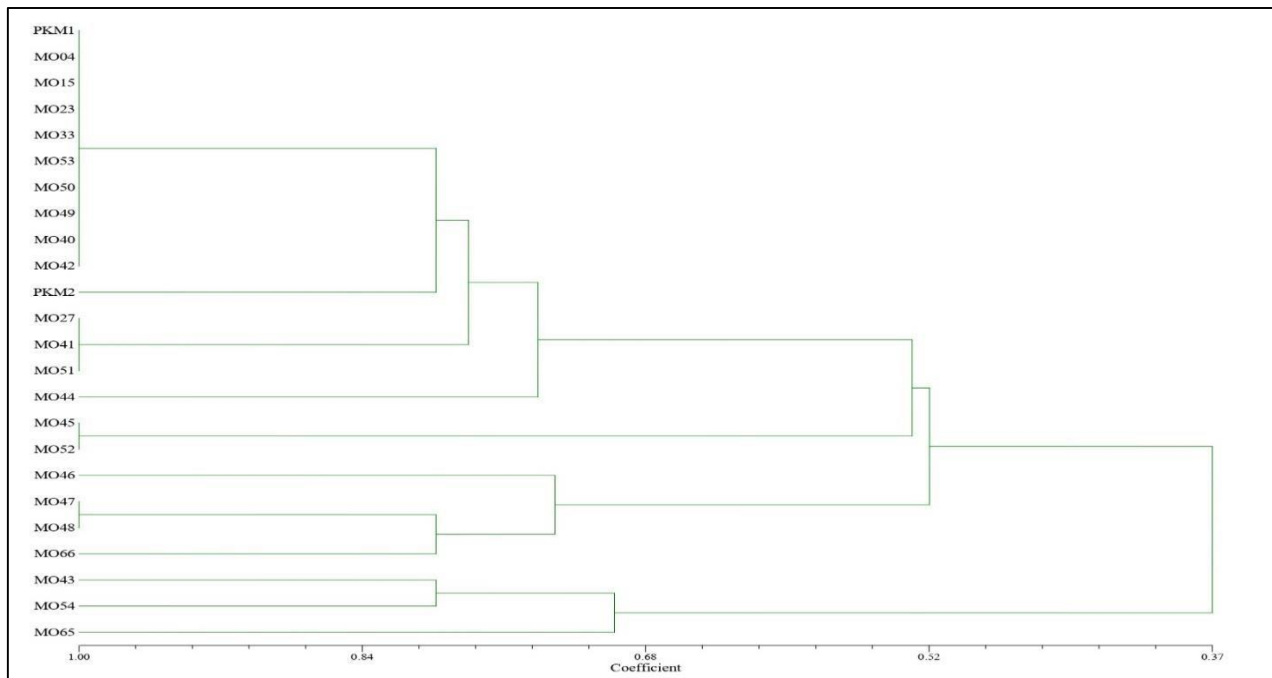

Fig 5: Dendrogram depicting clusters consisting 25 moringa genotypes

The entire population was divided into two Clusters formed at coefficient value 0.37, where the major Cluster I consisting 22 genotypes and minor Cluster II consisting 3 genotypes depicting that these genotypes are distinctly related to each other. PKM 1 which is at the top of the dendrogram is distinct from PKM MO 65, which is at the end of the dendrogram separated by different Clusters signaling towards high diversity. At coefficient value 0.52, the Cluster I divides itself into smaller Clusters showing relatedness and distinctness among the genotypes. Approximately at correlation coefficient value of 0.8, PKM 1 and PKM 2 appears distinct. The variety PKM 1, and the genotypes PKM MO 04, PKM MO 15, PKM MO 53, PKM MO 50, PKM MO 49, PKM MO 40 & PKM MO 42 are similar to each other with absolute correlation coefficient value of 1.0.

The genotypes PKM MO 27, PKM MO 41 & PKM MO 51 are closely related, PKM MO 45 & PKM MO 52 are related. Genotype PKM MO 47and genotype PKM MO 48 are closely related to each other and distinct from genotype PKM MO 46. The PKM MO 45 & PKM MO 52 are related with PKM MO

44 sub cluster with a correlation coefficient of 0.52.

3.3 Polymorphism information content
Each best-performing RAPD marker's polymorphism information content (PIC) was computed using the formula PIC = 2*f* *(1-*f*), where PIC is the marker's polymorphic information content, ***f*** is the frequency of present marker bands, and **(1-*f*)** is the frequency of missing marker bands (Roldan-Ruiz *et al*., 2000). Table 2. shows the results of the Polymorphism Information Content (PIC) value for the best five polymorphic primers. The primer OPB 05 (0.444) had the maximum PIC value, followed by primer OPC 05 (0.375), while the primer OPD 04 had the lowest PIC value (0.278). Both the primers OPB 06 and OPE 03 are on par. This demonstrates how effective RAPD primers are at differentiating the population.

Table 2: PIC value of RAPD markers

Sr. No.	Name of Primer	Sum of Bands	Frequency (Sum of Bands/ Total Genotypes)	1- Frequency	Pic Value (2*Frequency* (1-Frequency)
1	OPC 05	18	18/24	0.250	0.375
2	OPD 04	20	20/24	0.167	0.278
3	0113 05	16	16/24	0.334	0.444
4	0113 06	19	19/24	0.209	0.330
5	OPE 03	5	5/24	0.792	0.329

4. Discussion
Because RAPD is the most widely used approach for detecting numerous loci across the genome, it is strongly recommended for conducting research to identify genetic changes in plant species. The findings of the current study pave way for Moringa (*Moringa oleifera*) genotypes to be screened using molecular markers. Furthermore, improved polymorphic markers are needed for identifying diversity in moringa, since they will aid in the discovery of better genetic material for future breeding program and enhancement of this economically significant tree crop.

5. Reference

1. Ramachandran C, Peter KV, Gopalakrishnan PK. Drumstick (*Moringa oleifera*): a multipurpose Indian vegetable. *Econ Bot*. 1980.
2. Fuglie LJ. The Miracle Tree: Moringa oleifera: Natural Nutrition for the Tropics. Church World Service; Dakar, Senegal 1999.
3. Anwar F, Latif S, Ashraf M, Gilani AH. Moringa oleifera: A Food Plant with Multiple Medicinal Uses. Phytotherapy Research 2007;21:17-25.
4. Castellón CV, González CJR. Thesis Lic. Zootecnia Managua; Nicaragua, UCA: Utilización del marango (*Moringa oleifera*) en la alimentación de novillos en crecimiento bajo régimen de estabulación 1996, 44.
5. Palada MC. Moringa (*Moringa oleifera* Lam.): a versatile tree crop with horticultural potential in the subtropical United States. HortScience 1996;31:794-797.
6. Nautiyal BP, Venkataraman KG. Moringa (Drumstick)- An ideal tree for Social Forestry: Growing conditions and uses-Part 1. MYFOREST 1987;23(1):53-58.
7. Fahey JW. *Moringa oleifera*: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees Life J. 2005.
8. Soller M, Beckmann JS. Genetic polymorphism in varietal identification and genetic improvement Theoretical and Applied Genetics 1983;67:25-33.
9. Bretting PK, Widrelechner MP. Genetic markers and plant genetic resources management Plant Breeding Reviews 1995;13:11-86.
10. ’Neill RO, Snowdon RJ, Kohler W. Population genetics aspects of biodiversity Progress in Botany 2003;64:115-137
11. Wu CJ, Cheng ZQ, Huang XQ, Yin SH, Cao KM, Sun CR. Genetic diversity among and within populations of *Oryza granulata* from Yunnan of China revealed by RAPD and ISSR markers: implications for conservation of the endangered species Plant Science 2004;167:36-67.
12. GarciaI AAF, Benchimo LL, Barbosa AMM, Geraldi IO, Souza Jr CL, De Souza AP. Comparison of RAPD, RFLP, AFLP and SSR markers for diversity studies in tropical maize inbred lines Genetics and Molecular Biology 2004;27:579-588.
13. Yamanaka S, Nakamura I, Sato YI, Watanabe KN. Evaluation on genetic resources based on the diversity of functional regions genetic diversity of wild rice estimated by variations on xenobiotics-metabolizing cytochrome P450 gene-analogue Breeding Research 2003;5:24.
14. Thomas L, Albercht ME, Christina D, Marnik V. Martin K. Relationships among early European maize inbreds: IV. Genetic diversity revealed with AFLP markers and comparison with RFLP, RAPD, and pedigree data Crop Science 2000;40:783-791.
15. Kim MS, Moore PH, Zee F, Fitch MM, Steiger DL, Manshardt RE. Genetic diversity of *Carica papaya* as revealed by AFLP markers Genome 2002;45:503-512.
16. Saini RK, Saad KR, Ravishankar GA, Giridhar P, Shetty NP. Genetic diversity of commercially grown Moringa oleifera Lam. cultivars from India by RAPD, ISSR and cytochrome P450-based markers Plant Systematics and Evolution 2013;299:1205-1213
17. Zietkiewicz E, Rafalski A, Labuda D. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification Genomics 1994;20:176-183
18. Jacobson M. Hedren Phylogenetic relationships in Alisma (Alismataceae) based on RAPDs, and sequence data from ITS and turn L Plant Systematics and Evolution, 2007;265:27-44
19. Mgendi MG, Manoko MK, Nyomora AM. Genetic diversity between cultivated and non-cultivated *Moringa oleifera* Lam. provenances assessed by RAPD marker Journal of Cell and Molecular Biology 2010;8:95-102.
20. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus 1990;12:13-15.