Chemical Science Review and Letters ISSN 2278-6783

www.chesci.com Research Article

# Molecular Characterization of Moringa (*Moringa Oleifera* Lam.) Germplasm Using DNA Barcoding

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**Abstract**

Moringa (*Moringa oleifera* Lam.) is an important tropical vegetable and medicinal plant belonging to the family Moringaceae that is used for its healing abilities, nutritional, industrial, agricultural, and socioeconomic values. Native to India, this genus contains 13 species that have been far and wild raised in Asia, Africa and worldwide for its multitudinal virtues. It occurs wild in the Sub-Himalayan regions of Northern India [1] and popularly known as moringa, drumstick, horseradish tree, ben oil tree or Murungai in Tamilnadu**.** Moringa is rich in nutrients and also has the largest genetic diversity. Hence the identification of ideal genotypes for commercial cultivation with prodigious potential for the future breeding programme is essential. Regardless of the vast genetic wealth of moringa India possess, its genetic diversity pattern and genetic makeup are yet to be investigated for successful breeding and crop improvement programme [2]. This investigation was carried out in moringa (*Moringa oleifera* Lam.) to study the extent of diversity among the selected 26 genotypes in the Germplasm Resource Garden of Department of Vegetable Science, HC&RI, Periyakulam. The analysis was based on molecular approach using DNA barcoding by employing *matK* and *ITS* markers, the DNA barcodes that were generated for all the twenty six moringa genotypes under study depicted the diversity among the species.

**Keywords:** Moringa, molecular characterization, DNA barcoding, *matK*, *ITS*, diversity

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## Introduction

*Moringa oleifera* is a plant native to India that thrives in tropical and subtropical parts of the world and is also called 'drumstick tree' or 'horseradish tree'. Drumstick (Sahjan or Sohanjana in India) has many vernacular names depending on the locale [3]. Each branch has its unique utilitarian, pharmacological, and bioceutical characteristics. Moringa is a treasured tree in India and other nations for its exquisite pods, which are eaten as a vegetable, and it is used medicinally in Guinea, Madagascar, and Burma. Various studies on biological features of this genus have been undertaken, particularly on *Moringa oleifera*, which has been studied since 1970s [4].

The assessment of genetic diversity present in the germplasm of various crop species is important, not only for the preservation and protection of agricultural genetic resources, also for other practical uses such as species diversification and heterosis exploitation. In any species where chronic inbreeding has resulted in the loss of genetic variety [5], a process that might be responsible for the failure of fresh recombination, increasing or expanding the genetic base is a serious problem, because sufficient amount of phenotypic variation are not present in some crop species.

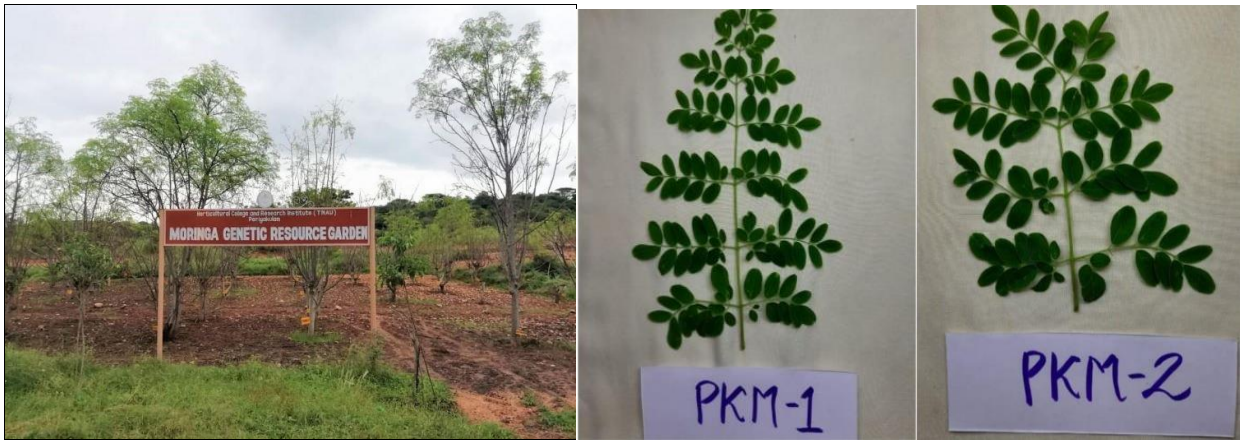
[6] Sheth *et al.* (2017) quoted, when used more precisely, "DNA barcoding" refers to the process of sequencing a short fragment of a gene, "DNA barcode," from a taxonomically unknown specimen and comparing it to a library of DNA barcodes from taxonomically known specimens in order to establish a taxonomic identification.

DNA barcoding is a good method for species identification and phylogenetic building, according to [7] Kang *et al.* (2017). However, recent research has come to a consistent conclusion about the universality of DNA barcoding. In three tropical cloud forests, the universality of tree species DNA barcodes such as *rbcL*, *matK*, *trnH-psbA*, and *ITS* was assessed, as well as their ability to identify species and construct phylogenies. Variations in DNA sequences are

extremely important for the construction of unique markers, which might be used to perform barcoding of DNA for various plants. Because it is characterised by a reliable, quick, and cost-effective method, DNA barcoding is utilized for identifying live creatures and offers additional vital assistance for identifying morpho- molecular differences in species as quoted by Rayan (2019) [8].

[8] used DNA barcodes to study the evolutionary connections between two species of the genus Moringa: *M. oleifera* and *M. peregrina*. A study carried out by Hausiku *et al*. (2020) [9], used nuclear Internal Transcribed Spacer (*ITS)* sequencing data analysis to determine the species boundaries of *Moringa ovalifolia* in Namibia. In account with the above mentioned literatures, this current investigation was framed to characterize the *Moringa oleifera* genotypes present at Germplasm Resource Garden of Department of Vegetable Science, HC&RI, Periyakulam, Tamilnadu, India.

## Materials and Methodology




Scheme 1. Moringa Genetic Resource Garden, HC& RI, Periyakulam, TNAU with varieties PKM 1 & PKM 2.

Twenty-six moringa genotypes (including two varieties, PKM 1 and PKM 2) that were collected from different regions of Tamil Nadu and India and maintained in Germplasm Resource Garden, HC &RI, Periyakulam were used for this investigation. Young leaves of selected genotypes were collected prior DNA isolation to avoid degradation and damage.

### *Genomic DNA isolation from Moringa genotypes*

The technique involves steps (**Figure 1**) to disrupt the cell and release the nuclear content and thereafter solubilizing the DNA. This was done by grinding 500 g of leaf sample in a pestle and mortar using liquid nitrogen. Disruption of cell membrane was achieved by using detergent SDS to the suspended pellet and kept in water bath for incubation.

After the incubation, 1.5 ml of 7.5M Ammonium acetate was added with quick mixing and placing it on ice for half an hour. Samples were later centrifuged at 14000 rpm for 10 minutes at 4°C. The supernatant was decanted without disturbing the pellet in 6.0 ml Isopropanol in a sterilized centrifuge tubes to precipitate DNA. Incubation was done by keeping the samples on ice for 30 minutes (or) overnight for complete precipitation of DNA. The pellet was again centrifuged at 15000 rpm for 15 minutes at 4°C then supernatant were discarded and the resultant pellet was re-suspended in 500µl of TE buffer and kept in water bath for 65°C for 15 minutes.

### *Agarose Gel Electrophoresis*

Electrophoresis of DNA **(Figure 2)** on 0.8 % agarose gel was followed by staining with the gel with ethidium bromide and viewing under UV revealed a high single molecular weight band. The gel was run at 50-70 V for 20 mins and DNA was visualized under Alpha Imager Gel Documentation Unit.

### *Polymerase Chain Reaction*

Polymerase Chain Reaction (PCR) is a technology for amplifying cloned or genomic DNA sequences using primers and specialized enzyme. For amplification of DNA barcode region, the most suitable set of primers for each target genes viz., *matK* and *ITS* were obtained from the literature and are described as below in **Table 1.**

On a single stranded DNA template, PCR employs the enzyme DNA polymerase to guide the synthesis of DNA from deoxynucleotide substrates. When a custom-designed oligonucleotide is annealed to a longer template DNA, the

*Taq* DNA polymerase inserts nucleotides to the 3' end. When a synthetic oligonucleotide is annealed to a single-stranded template with a complementary region, DNA polymerase can employ the oligonucleotide as a primer and elongate its 3' end to create an extended stretch of double-stranded DNA and the final PCR product was sent for sequencing **(Figure 3).**

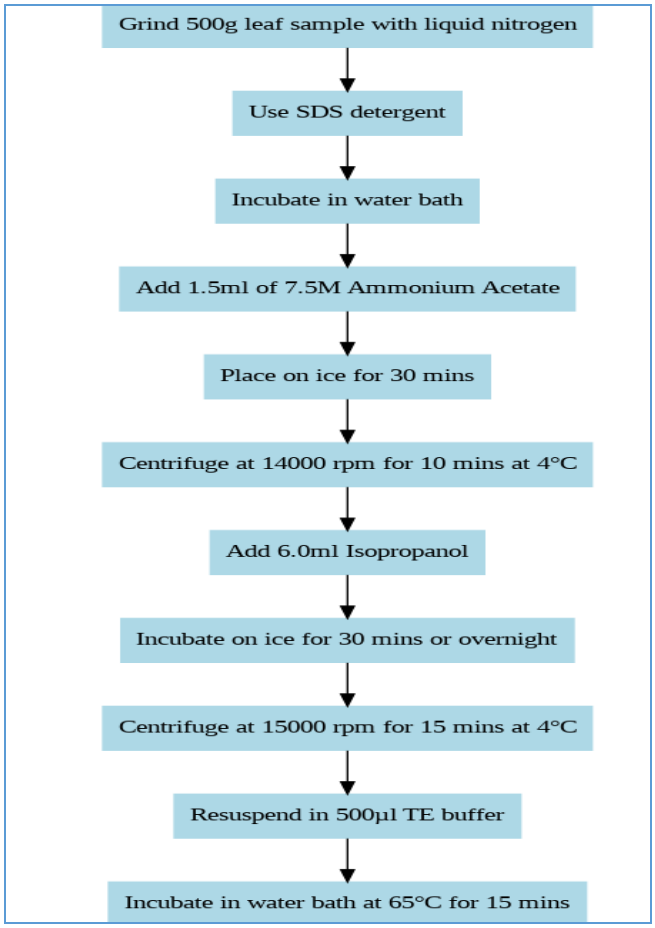



**Figure 1** Flowchart of methodology involving DNA Isolation

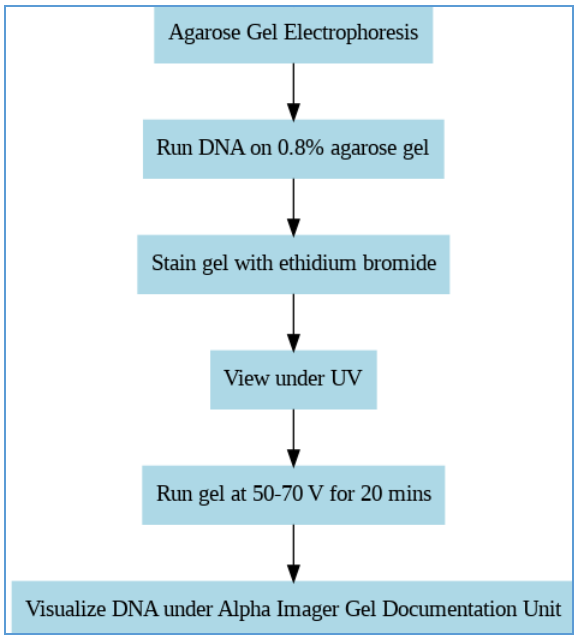



**Figure 2** Flowchart of methodology involved in Agarose gel Electrophoresis

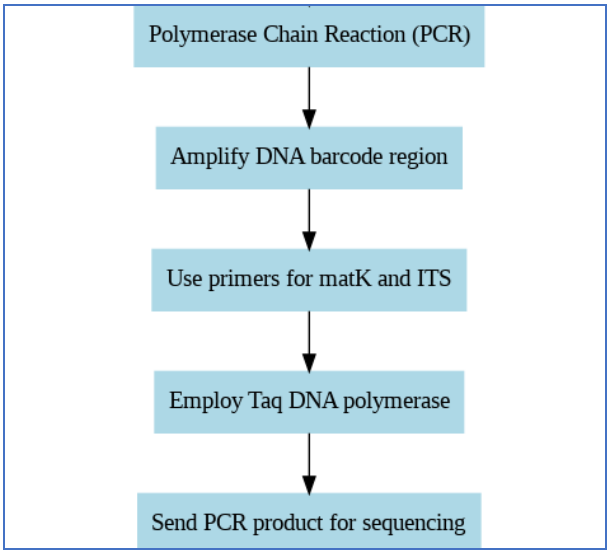



**Figure 3** Flowchart of methodology involved in PCR

**Table 1** Details of Primers used in DNA barcoding of Moringa germplasm.

| Name of the Gene | Name of the primer | Primer sequence (5' to 3' direction) | Reference |
|---|---|---|---|
| *matK 1* | Forward | CGATCTATTCATTCAATATTTC | Lahaye *et al.* (2008) |
| | Reverse | TCTAGCACACGAAAGTCGAAGT | |
| *ITS* | Forward | GGAAGGAGAAGTCGTAACAAGG | Selvaraj *et al.* (2012) |
| | Reverse | TCCTCCGCTTATTGATATGC | |

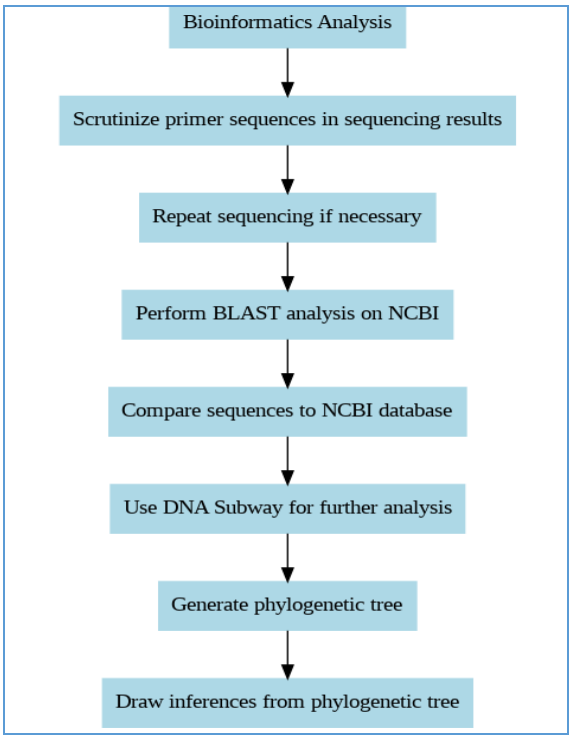



**Figure 4** Flowchart of methodology of Bioinformatics Analysis


**Figure 5** Results of DNA barcoding of investigated moringa germplasm using DNA subway by employing "MUSCLE" tool for sequences of the *matK* gene & *ITS* gene

***Bioinformatics analysis***

The primer sequences were initially scrutinized in the sequencing results (**Figure 4**) of all the 26 samples for required genes. The sequencing process was repeated if there were any ambiguities or fewer sequences than expected. Then, using the techniques provided in the Basic Local Alignment Search Tool (BLAST) accessible on the National Centre for Biotechnology Information's website, those affirmatively confirmed sequences were subjected to a simple nucleotide BLAST analysis (NCBI). The sample sequences were compared to moringa sequences deposited in the NCBI database to ensure whether they are > 90% related. The Blue Line of DNA Subway, available at http://dnasubway.iplantcollaborative.org/ was employed to analyse the above said moringa germplasm DNA sequences.

DNA Subway is an intuitive interface for analysing DNA barcodes. It analyzed the relationships between DNA sequences by comparing them to a reference sequence or sequence of our interest, the barcodes of twenty six genotypes with both *matK* and *ITS* gene has been presented in **Figure 5**. The phylogenetic tree generated from DNA sequences helped to draw inferences about how these genotype were related or different from each other on the basis of molecular characterization.

## Result and Discussion

Phylogenetic tree constructed *matK* gene sequences classified the sequenced genotypes into two groups (**Figure 6**) consisting of one out group and another containing all the moringa genotypes. The Group I consisted of two clusters which had genotype PKM MO 46 in Cluster I indicating that it is the most diverse moringa genotype. The Cluster II consist of the 24 moringa genotypes which further got subdivided into sub cluster consisting genotype PKM MO 54 on one node and PKM MO 23 genotypes on another node. This node was divided into two sub sub clusters. The sub sub cluster I showed that genotype PKM MO 42 is one of the diverse moringa genotype and the ruling varieties, PKM 1 and PKM 2 are diverse from each other. Moringa genotypes PKM MO 53 is located on a separate node which indicates high diversity and it also shares ancestry with PKM MO 44 and PKM MO 43. The visual interpretation is provided in **Figure 7**.

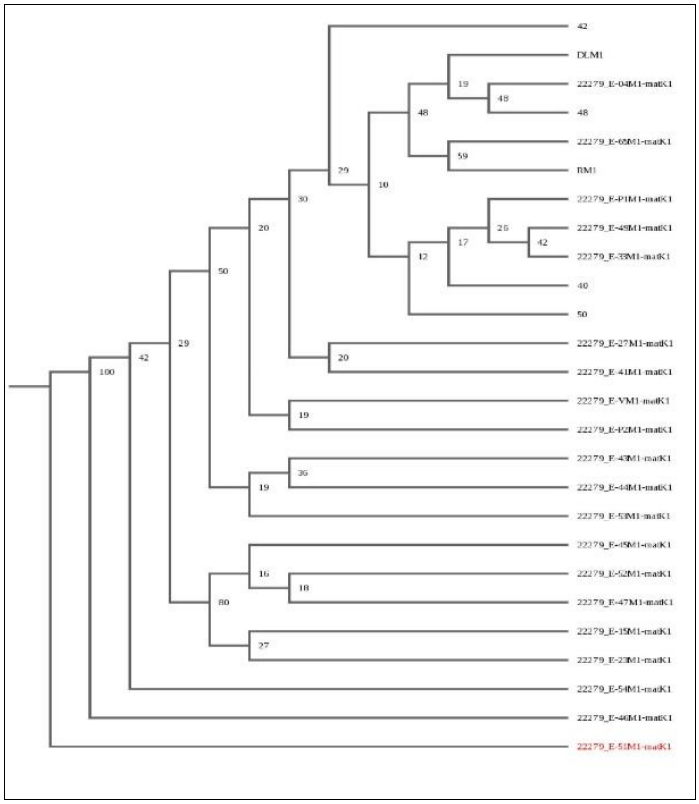



**Figure 6** Phylogenetic tree generated for *matK* gene

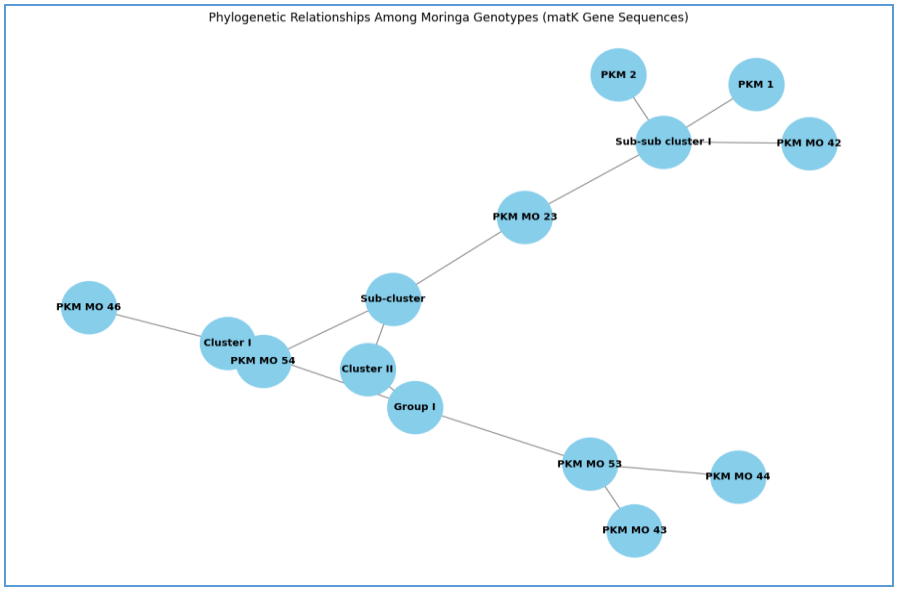



**Figure 7** Phylogenetic relationship between moringa genotypes determined by *matK* sequences

Similarly, the phylogenetic tree constructed by *ITS* gene sequences (**Figure 8**) classified all the investigated genotypes into one Group (I), which further divided itself into three major groups; Group (II), Group (III) and Group (IV). Group II comprised of moringa genotype PKM MO 47.

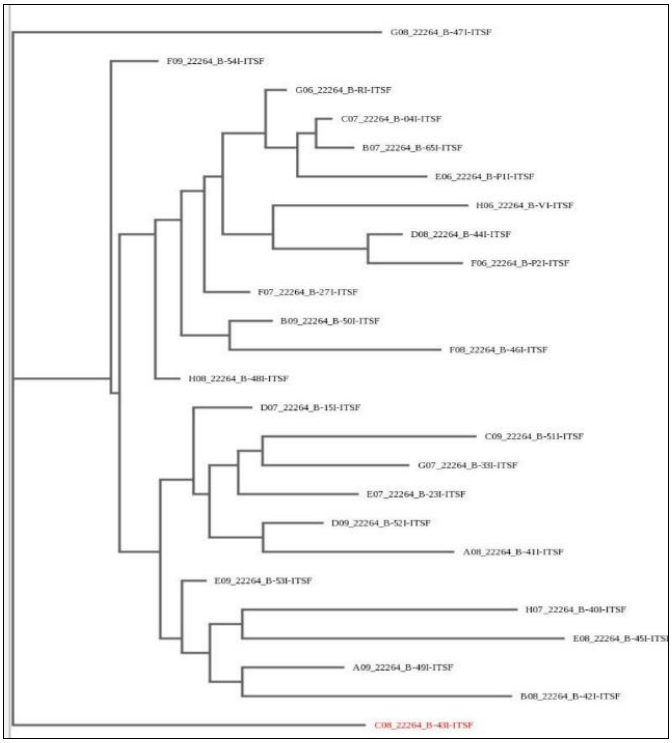



**Figure 8** Phylogenetic tree generated for *ITS* gene

Moringa genotype PKM MO 43 is the out- group in the phylogenetic tree. Group III included all the twenty-three genotypes, which divided itself into two sub groups; Group V and Group VI. The Group V consisted of the genotype PKM MO 54 present on a separate node, indicating higher rate of divergence.

The Clade I comprised of both moringa varieties PKM 1 and PKM 2, which are located on different clades indicating divergence among the two varieties, even though they share same ancestry. Moringa genotype PKM MO 48 is situated at the most distant clade which represents high divergence from the moringa varieties PKM 1 and PKM 2.

Moringa genotype PKM MO 15 is located at a distinct node, therefore confirming it's divergence from others. Genotype PKM MO 53 separates itself from other genotypes on different node suggesting it's divergence from the sub group. The visual interpretation is provided in **Figure 9**.

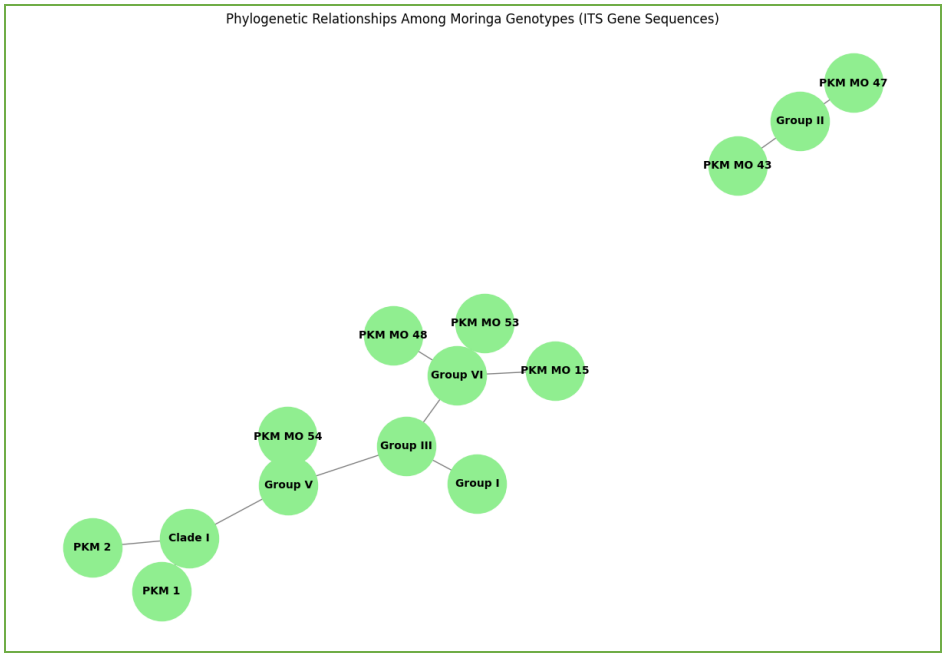



**Figure 9** Phylogenetic relationship between moringa genotypes determined by *ITS* sequences

DNA barcoding has been presented as a novel, quick, and practical technique of identifying changes in plants at the species or subspecies level by analyzing changes in DNA sequence in one or a few universal genomic areas [10]. The Consortium for the Barcode of Life Plant Working Group recognizes *matK* and *ITS* as the two key regions that generates DNA barcodes for classifying plant species (www.barcodeoflife.org). Because of the efficient species discriminating power, high quality sequence recovery, and simple experimental approach, matK gene is utilized as DNA barcoding gene [11].

Differential topology of *Moringa oleifera* genotypes could be used to determine superior moringa varieties or local types for next generation breeding venture. These researches offered support for choosing genetically varied moringa accessions for future breeding program through DNA barcoding [9, 12, 13].

The overall analysis of phylogenetic relationships constructed by both *matK* and *ITS* markers (**Figure 10**) leads to the conclusion that PKM MO 15, PKM MO 42 & PKM MO 54 are genetically closer genotypes to from check variety PKM 1 & PKM 2, whereas, significantly higher divergence was found in genotypes PKM MO 15, PKM MO 46, PKM MO 47 and PKM MO 53 from varieties PKM 1 & PKM 2 hence can be exploited as genetically diverse donor for moringa genetic improvement.

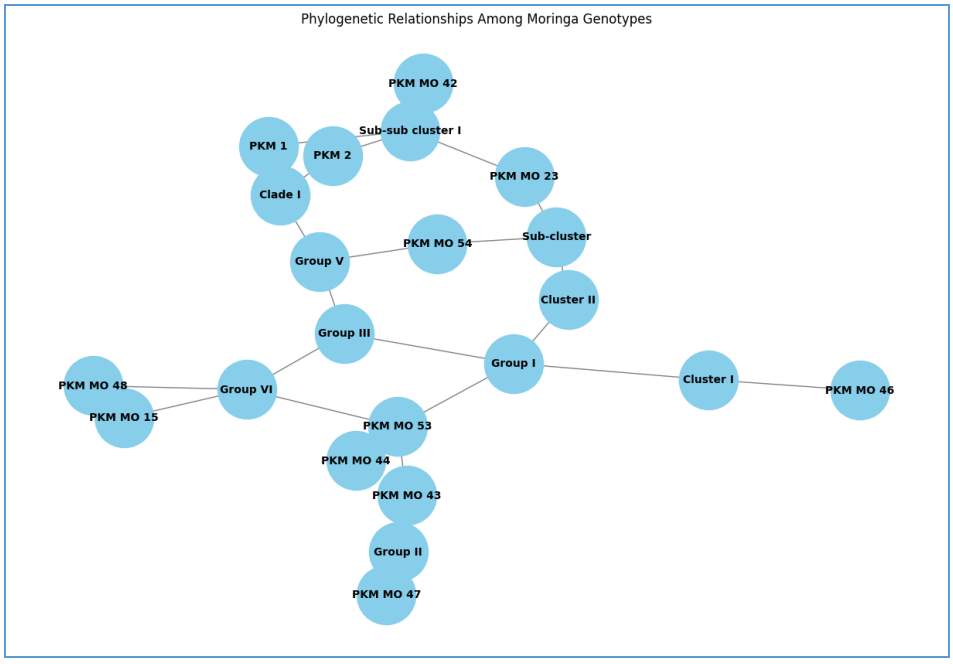



**Figure 10** Overall phylogenetic relationship between moringa genotypes

## Conclusion

Future prospects in Moringa cultivation and research are promising, focusing on developing customized cultivars with enhanced nutritional and medicinal properties, optimizing agricultural practices, and preserving genetic diversity. Advancements in sequencing technologies and bioinformatics are expected to significantly enhance the accuracy and utility of DNA barcoding, establishing it as a vital tool in global strategies for sustainable agriculture and biodiversity conservation. DNA barcoding also enables precise identification of Moringa species and their genetic variations, supporting the conservation of rare and endangered species. It helps ensure the traceability and authenticity of Moringa products in the global market, fostering quality control and consumer trust. As these technologies advance, they will play a crucial role in sustainable development, food security, and environmental stewardship, positioning Moringa as a key element in future agricultural and conservation initiatives.

## References

[1] Rajangam, J., R. Azahakia Manavalan, T. Thangaraj, A. Vijayakumar, and N. Muthukrishan. 2001. "Status of production and utilization of Moringa in southern India. Development potential for moringa product." Dar Es Salaam, Tanzania. http://www. moringanews. org/actes/rajangam_en. Doc

[2] Peter, D. 1989. "Monograph on Moringa oleifera" Indian Horticulture:33-34

[3] Tak, S., and I. Maurya. 2015. "Genetic variability in drumstick genotypes." Annals of Plant and Soil Research 17 (1):67-70.

[4] Mahmood, K.T., T. Mugal, and I.U. Haq. 2010. "Moringa oleifera: a natural gift-A review." Journal of Pharmaceutical Sciences and Research 2 (11):775.

[5] Graham, J., R. McNicol, and J. McNicol. 1996. "A comparison of methods for the estimation of genetic diversity in strawberry cultivars." Theoretical and Applied Genetics 93 (3):402-406.

[6] Sheth, B.P., and V.S. Thaker. 2017. "DNA barcoding and traditional taxonomy: an integrated approach for biodiversity conservation." Genome 60 (7):618-628.

[7] Kang, Y., Z. Deng, R. Zang, and W. Long. 2017. "DNA barcoding analysis and phylogenetic relationships of tree species in tropical cloud forests." Scientific reports 7 (1):1-9

[8] Rayan, W.A. 2019. "Evaluating the efficiency of DNA Barcode rbcL for detection of genetic relationships between four Moringa spp. genotypes." The egyptian journal of experimental biology (Botany) 15 (2):333-340.

[9] Hausiku, M., E. Kwembeya, P. Chimwamurombe, and A. Mbangu. 2020. "Assessment of species boundaries of the Moringa ovalifolia in Namibia using nuclear its DNA sequence data." South African Journal of Botany 131:335-341

[10] Vijayan, K., and C. Tsou. 2010. "DNA barcoding in plants: taxonomy in a new perspective." Current Science: 1530-1541.

[11] Ipek, M., A. Ipek, and P.W. Simon. 2014. "Testing the utility of matK and ITS DNA regions for discrimination of Allium species." Turkish journal of botany 38 (2):203-212.

[12] Wattoo, J.I., M.Z. Saleem, M.S. Shahzad, A. Arif, A. Hameed, and M.A. Saleem. 2016. "DNA Barcoding: Amplification and sequence analysis of rbcl and matK genome regions in three divergent plant species." Advancements in Life Sciences 4 (1):03-07.

[13] Ramesh, G.A., D. Mathew, K.J. John, and V. Ravisankar. 2022. "Chloroplast Gene matK holds the Barcodes for Identification of Momordica (Cucurbitaceae) Species from Indian Subcontinent." Horticultural Plant Journal 8:89-98.



Publication History
Received 08.03.2024
Revised 14.08.2024
Accepted 15.08.2024
Online 30.08.2024