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Original Research Article

Genetic and Phenotypic Diversity of *Mentha (Mentha spp.)* Genotypes in Salah Al-Din Province, Iraq, Analyzed with RAPD Markers

Asmaa Adnan Marie[1]*, Ahmed Mohamed Salman[2], Ashwak I. AL-Obaedi[3]

[1]Depth of Biology College of Science University of Tikrit, Tikrit, Iraq
[2]Medical Laboratory Techniques, Al_Hadara University College, Iraq
[3]Researches and Islamic Studies Center, Al-Iraqia University, Iraq

***Corresponding Author:** Asmaa Adnan Marie
Depth of Biology College of Science University of Tikrit, Tikrit, Iraq

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Abstract: Samples of Mentha (*Mentha spp.*) were studied to assess the genetic and phenotypic variability among eight genotypes grown from ten locations in Salah Al-Din Governorate, Iraq. Genetic resemblance, genetic divergence and gene diversity based on RAPD technique: Analysis were estimated. A total of seventeen random primers were assayed, and only five gave reproducible PCR amplification profiles that are shown in the data analysis. Altogether 62 of the 86 DNA fragments were polymorphic by these primers and the percentage of polymorphism was 72.1%, which suggested that there was abundant genetic diversity within genotypes. The primer OPH-17 showed the highest level of discrimination (45%) and OPJ-01 was monomorphic. At the morphological level, the genotypes displayed clear differences in traits such as leaf shape, color, venation pattern, and growth density, reflecting underlying genetic variation. Cluster analysis using the UPGMA algorithm classified the genotype into two main genetic groups. The genetic distance between the genotype ranged from 0.1000 to 0.4010, which showed significant variability in genetic relation. These findings confirmed the effectiveness of RAPD markers in marking mentha genotypes and identifying primers which act as molecular markers in reproductive programs. In addition, the results emphasize, while supporting the value in improving this medical and culinary system, the usefulness of this technique for assessing the genetic relationship between mentha population in different places.

Keywords: *Mentha*, genetic variation, RAPD, morphological traits, molecular analysis, genetic fingerprinting, genetic distance, phylogenetic tree.

INTRODUCTION

The Mentha (*Mentha SPP*) family is an important genus in *Lamiaceae*, which is widely valuable for its economic, nutritional and medical roles due to its bioactive compounds, especially Menthol, which has various medicines, aromatic and food applications. (Saqib *et al.*, 2022; Yousefian *et al.,* 2023). The genus Mentha is widely distributed throughout the world and there are many hundreds of species. It is characterized by outstanding genetic diversity, which mainly leads to frequent natural hybridization and its wide ecological distribution (Lange *et al.,* 2024). Such variability has long justified its place in folk medicine and stressed the need for conservation and enhancement of Mentha genetic resources (Póvoa *et al.,* 2023).

Genetic diversity is one of the building blocks for crop improvement and conservation.

Among separate molecular techniques, randomly Amplified Polymorphic DNA (RAPD) is mostly used, as it is simple, cheap and effective to reveal polymorphism without the need for information before genome (Güler, 2023; Hromadová *et al.,* 2023; Bidyananda, 2024). These markers have been tested successfully in crops like Date Palm (Mahatma *et al.,* 2017), roses (Shehab, 2023), and sorghum (Zarea *et al.,* 2024). Recent studies also highlight their utility in medical and aromatic plants, including (Sutar *et al.,* 2023).

In Iraq, especially in Salah al-Din province, wild mentha populations are common and clear morphological variations. Nevertheless, information about their molecular diversity is still rare. For this reason, the current study was designed to analyze both genetic and morphological variation in the eight wild genotype collected from Salah al-Din using RAPD markers. The findings are expected to provide a valuable reference to breeding, improvement and conservation efforts.

Objectives of the Study

The study was conducted by implementing RAPD markers to investigate genetic differences between eight Wild Mentha (*Mentha SPP*) collected from the Salah al-Din province in Iraq. Furthermore, the purpose of this work is to check how these accesses are related to each other in terms of genetic equality and distance, and how these results correspond to their appealed functional symptoms. The results provide a useful reference point for future breeding, conservation and improvement programs that focus on local resources.

MATERIALS AND METHODS

Plant Material

In Salah al-Din province, Iraq, eight Wild Mentha (*Mentha SPP*) genotypes were identified and collected from ten different places. For each genotype, young and powerful leaves were selected, carefully preserved and later used for DNA -extraction -with morphological characterization.

Chemicals

All reagents used in this study were of analytical grade and suitable for molecular biology applications. The chemicals, their formulas, and main applications in DNA extraction and PCR procedures are summarized in Table 1.

Instruments and Equipment

All instruments were maintained and calibrated according to the manufacturers' instructions to ensure experimental accuracy. The main instruments employed for DNA extraction, amplification, and electrophoresis are listed in Table 2.

Laboratory Tools and Consumables

Routine laboratory tools, including micropipettes, microtubes, centrifuge tubes, and other consumables, were used throughout the experimental work. The principal items are summarized in Table 3.

DNA Extraction and Verification

Genomic DNA was extracted from fresh leaves using a modified CTAB protocol (Huang *et al*., 2013). The DNA quality and integrity were verified by agarose gel electrophoresis and quantified using a NanoDrop spectrophotometer.

Primers and RAPD Analysis

Seventeen RAPD primers (Operon Technologies, USA) were initially screened for their ability to generate clear and reproducible amplification patterns. Of these, five primers produced consistent and scorable bands and were selected for the final analysis. The sequences of the tested primers are presented in Table 4. The PCR reactions were performed in 20 ul versions according to the standard protocol (Green & Sambrook, 2019). The amplification products were separated by 2% agarose gel, which was fired with redsafe and conceived under UV light.

Data Analysis

The amplified bands were observed as being present (1) or absent (0) to construct a binary matrix. Genetic similarity coefficients and distances were estimated as Nei and Li (1979). Cluster analysis was carried out in NTSYS-pc by overlaying matrices using UPGMA method (Singh *et al*., 2020) and dendrogram were created in MEGA X software (Kumar *et al*., 2018). Morphologic characters as leaf shape, color, venation and growth density were also recorded to compare them with molecular information.

Table 1: Chemicals used in the study

Chemical (English)	Abbreviation / Formula	Application / Use
Agarose	—	Gel electrophoresis
Disodium EDTA	Na_2EDTA	DNA extraction buffer
Sodium acetate	CH_3COONa	DNA precipitation
Isopropanol	C_3H_7OH	DNA precipitation
Cetyltrimethylammonium bromide	CTAB	DNA extraction
Tris base	—	Buffer preparation
Tris-HCl	—	Buffer preparation

Chemical (English)	Abbreviation / Formula	Application / Use
Isoamyl alcohol	—	DNA purification
Boric acid	H_3BO_3	Buffer component
Hydrochloric acid	HCl	pH adjustment
Ammonium acetate	CH_3COONH_4	DNA precipitation
Bromophenol blue	—	Electrophoresis dye
Red Safe	—	DNA staining
Ethanol	C_2H_5OH	DNA precipitation / sterilization
Glycerol	$C_3H_8O_3$	DNA loading dye preparation
Sodium chloride	NaCl	Buffer preparation
Chloroform	$CHCl_3$	DNA purification
Liquid nitrogen	N_2 (liq.)	Sample preservation / grinding
Sodium hydroxide	NaOH	pH adjustment

Table 2: Instruments and equipment used in the study

Instrument	Application / Use
PCR Thermal Cycler	DNA amplification (RAPD)
Gel electrophoresis unit	DNA separation
UV transilluminator	DNA visualization
NanoDrop spectrophotometer	DNA purity and concentration
UV–visible spectrophotometer	DNA quantification
Centrifuge / Microcentrifuge	DNA precipitation / separation
Analytical balance	Precise weighing of chemicals
pH meter	Buffer pH adjustment
Water distiller	Distilled water preparation
Gel documentation system	Gel imaging and documentation

Table 3: Tools and consumables used in the study

Tool (English)	Specification
Micropipettes	2–20 µL, 10–100 µL, 100–1000 µL
Eppendorf tubes	0.5–2.0 mL
Micropipette tips	10 µL, 200 µL, 1000 µL
Glass centrifuge tubes	10 mL capacity
Pasteur pipettes	Plastic
Quartz cuvettes	For spectrophotometric measurements
Biohazard bags/containers	Waste disposal

Table 4 Primers used in RAPD reactions

Primer code	Sequence (5′ → 3′)
OPJ-01	CCCGGCATAA
OPJ-17	ACCCCCTATG
OPJ-05	CTCCATGGGG
OPH-19	CTGACCAGCC
OPM-15	GACCTACCAC

Table 5: Efficiency, discriminatory power, and unique DNA bands generated by RAPD primers in Mentha genotypes

Primer	Total bands	Polymorphic bands	% Polymorphism	Discriminatory Power (DP)	Unique bands	Size range (bp)
OPJ-01	30	0	0.0	0.00	0	400–1200
OPJ-17	45	33	73.3	0.45	2	500–900
OPJ-05	34	24	70.6	0.34	2	500–1600
OPH-19	28	20	71.4	0.30	1	400–1100
OPM-15	22	15	68.2	0.28	1	300–800
Total / Mean	159	92	72.1	0.35	6	—

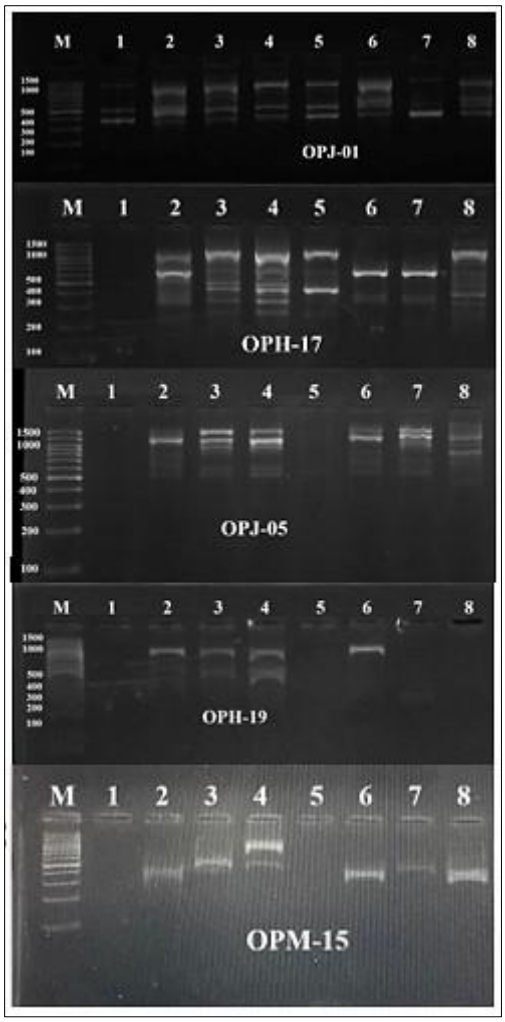

Figure 1: RAPD amplification profiles generated using primers OPH-17, OPJ-01, OPJ-05, OPH-19, and OPM-15 **with genomic DNA from eight *Mentha* genotypes collected from different locations in Salah Al-Din Province (Tikrit, Al-Alam, Baiji, Al-Shuwaish village, Hammad Shihab, and Balad). DNA fragments were separated on a** 2**% agarose gel**.

Table 6: Genetic distance values among eight Mentha genotypes based on RAPD markers

Genotype	M1	M2	M3	M4	M5	M6	M7	M8
M1	—	0.72	0.65	0.80	0.77	0.74	0.70	0.66
M2	0.72	—	0.68	0.83	0.79	0.76	0.73	0.69
M3	0.65	0.68	—	0.75	0.71	0.70	0.67	0.62
M4	0.80	0.83	0.75	—	0.85	0.82	0.78	

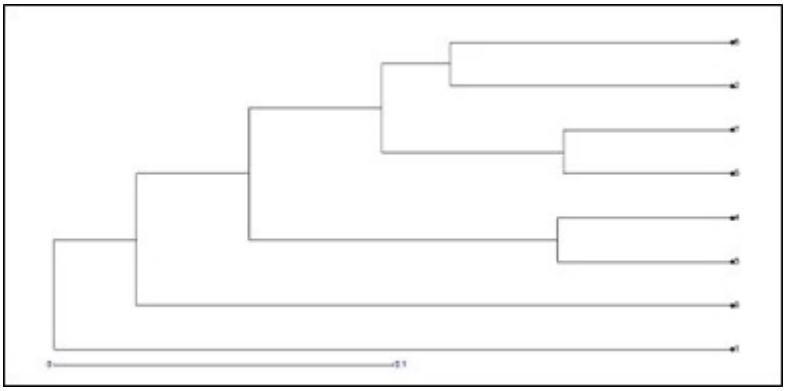

Figure 2: Represents the genetic relationship for eight varieties of mint based on the genetic distance of the RAPD

Figure 3: Photographs of Mentha plants collected from different locations in Salah Al-Din province

RESULTS AND DISCUSSION

Results

Genomic DNA Isolation

Good quality genomic DNA was isolated from young leaves of Mentha employing modified CTAB (Patel *et al*., 2021). The DNA showed good integrity on agarose gels and the purity values (A260/A280 = 1.65–1.80) met the requirement for PCR amplification.

RAPD Marker Analysis

Seventeen primers were initially screened, of which five generated clear and reproducible amplification patterns. These primers produced a total of 86 bands, 62 of which were polymorphic, corresponding to an average polymorphism rate of 72.1%.

Primer OPH-17 showed the highest discriminatory power (45%), whereas OPJ-01 did not produce polymorphic bands. The efficiency and discriminatory power of the primers were summarized in Table 5, and representative amplification profiles are shown in Figure 1.

Genetic Relationships and Dendrogram Analysis

Genetic similarity coefficients among the eight Mentha genotypes ranged from 0.62 to 0.89. The lowest similarity was observed between M3 and M8, while the highest was between M5 and M6. The genetic distance matrix is presented in Table 6. Cluster analysis grouped the genotypes into three major clusters, broadly reflecting their geographic distribution (Figure 2).

Morphological Characterization

The leaf forms, sizes, colors and vein structures as well as plant growth densities were distinct among the genotypes. The leaves of M1, M3 and M8 were wider with darker pigmentation, whereas the leaves of M4 and M6 were narrower with lighter pigmentation. Variation in stem diameter and branching was also reported. Representative images are presented in Figure 3.

DISCUSSION

The degree of polymorphism in RAPD analysis was high as reported previously among Mentha species (Kazemi *et al.,* 2012; Shinwari *et al*., 2011; Ibrahim *et al.,* 2017).

A number of studies have found a high degree of molecular polymorphism in Mentha Khanuja *et al.,* (2000) found 93.5% polymorphic RAPD bands in six Indian Mentha taxa, and Kazemi *et al.,* (2006) found 98.7% polymorphism among Iranian Mentha accessions. Shinwari *et al*., found predominant presence of S. entomophilum in Pakistani Mentha sp. (2011) identified 93.6–100% polymorphism per RAPD primer. Similarly, Hashem *et al.,* (2018) reported 69.23% polymorphism in M. spicata and M. piperita by certain RAPD primers from Egypt. Moreover, even the other studies have made up percentages of polymorphism 50%-75% in Mentha by marker system used (Attia *et al*., 2015; Afkar and Zand, 2020). We fall within this range, suggesting that Mentha spp. have a large amount of genetic variation within them around the world.

The supposed high discriminating power of OPH-17 in the present study was consistent with the results observed by Ona *et al.,* (2021), who found some primers to be more discriminant of closely related genotypes compared with others.

The lack of polymorphism for OPJ-01 indicates not all priming being informative, corresponding well with remarks by Al-Abdoulhadi *et al.,* (2012) in date palm.

The nine genotypes were grouped into three clusters according to their geographical background by the dendrograms. Analogous clustering is also reported in Mentha of Iran (Devi *et al.,* 2022) and other medicinal plants (Hussain *et al.,* 2024), showing that RAPD markers retain genetic as well as ecological discrimination.

The morphological diversity found here corroborated the molecular results. The variations in leaf morphology, and pigmentation showed correlation with genetic distances observed by RAPD in accordance with the previous findings on Mentha (Sharma and Patel, 2021; Javed *et al.,* 2022). These integrated approaches would facilitate the use of RAPD markers in germplasm resources assessment.

CONCLUSION

Farouk H. K and Asmaa N. A., 56 RAPD marker Iraq, was revealed to be informative study to evaluate the genetic differentiation degree among wild mentha genotypes in Salah al-Din. The dissimilarity in leaf symptom expression and growth traits resulted in high to moderate genetic variation as evidenced by GGE biplot. Once more these results illustrate the need for associating molecular and phenotypic data when analysing germplasm. The set can serve as valuable donor material for further breeding, enriching genetic diversity and conserving local Mentha genepool.

Recommendations

Perform more studies using different molecular marker systems such as ISSR, SSR and SNP-based methods that potentially could enhance the results presented here with RAPDs to provide a more of robust due in genetic relationships.

- Expand the sample size for more genotypes that have been collected from different ecological zones in Iraq that can sufficiently represent the natural diversity of Mentha spp.
- Integrate molecular findings with biochemical and phytochemical profiling in order to obtain a clearer view of genetic variation and its functional implications.
- Utilize the available information in breeding programm for improvement of mentha resources focusing medicinal and aromatic traits.
- Strengthen conservation efforts to conserve wild Mentha genotypes, especially vulnerable species of environmental stress and facing habitat destruction.
- Re-orientate future research towards global challenges rome and intensified pharma- cological utilizations of goodquality Mentha genetic resources.

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Conflict of Interest: The author declares no conflict of interest.

Key Findings: RAPD markers revealed a high level of genetic polymorphism (72.1%) among eight wild Mentha genotypes collected from different locations in Salah Al-Din Province, Iraq, while cluster analysis and genetic distance estimates demonstrated clear genetic differentiation that broadly reflected their geographic distribution. The integration of molecular and morphological data confirmed the effectiveness of RAPD markers for genetic diversity assessment and highlighted their potential application in Mentha breeding, conservation, and germplasm management programs.

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