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Molecular Investigation Comparative between *Dombeya* and *Bombax* Plant Species of Exotic Malvaceae

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Abstract: The study focuses on the Malvaceae plant family, highlighting its significance in understanding plant diversity in Iraq. The research involves classifying these plants, examining their properties and physical characteristics, and focusing on their molecular evolution and potential threats. The exotic plants are species that are not naturally present in the local environment . their intestines during their long journey back and forth between their original habitats and wintering areas.the plant Dombeya wallichii and Bombax ceiba are the exotic plants cultivated for ornamental purposes, this plants grew adapting to the environmental conditions in Iraq. Both plants perennial trees, so the study Chose to compare with Marva parviflora because that represents the family Malvaceae and it phant grew and distributed very well in Iraq, native throughout the temperate, subtropical, and tropical regions of Europe, Africa, and Asia . Molecular studies are emphasized for their accuracy and comprehensiveness in identifying species.. Molecular information is crucial for taxonomic determination, with evolutionary theory being an important tool in plant taxonomy. Individual molecular evolutionary data for Alcea are limited in determining interspecies relationships and identifying species. Chloroplasts are play a crucial role in sustaining life on earth, intracellular gene transfer and conservation, diversity, this genetic basis by chloroplast transgenes was engineered to enhance plant agronomic traits or produce high-value agricultural (biomedical products. chloroplast genome sequences impact understanding the origins of economically important cultivated species. DNA sequencing technology, especially PCR, is widely used for solving classification problems by tracing the sequence of nitrogenous bases in genes. The study involves DNA isolation from plant samples using the Genomic DNA GENEzol™ DNA Reagent Plant Kit at the Macrogen Center in South Korea and analyzing the samples through agarose gel electrophoresis. Genetic variations within the trnH-psbA intergenic spacer sequences were analyzed to study the biodiversity patterns of three plant isolates from the family Malvaceae (designated O1, W1 and W2) collected from Babylon. The results show successful PCR amplification of the TrnH-psbA region in some genera of the Malvaceae family, with sequence similarities to reference sequences. Sequencing reactions indicated that the identity of samples O1, W1 and W2 belonged to Marva parviflora, Dombeya wallichii, and Bommax ceiba, respectively. Variations were detected in some samples, and the alignment results showed the exact identity of the samples after NCBI BLASTn analysis. The study provides valuable insights into the genetic diversity and evolutionary relationships within the Malvaceae family in Iraq.

Keywords: *Malvaceae, Iraqi environment, Plant diversity, Molecular systematics, Genetic polymorphisms, Phylogenetic tree, DNA sequencing.*

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Introduction

Malvaceae family species are under study for their taxonomic system, phytochemistry, ethnomedicine, molecular study, and conservation approaches. DNA-based studies are one of the best tools that offer a wide range of knowledge from basic biology to

advanced biological studies. However, the Malvaceae family, is considered a relatively small group in plant taxonomy. The systematic position of the family in order to support a close relationship with related families is still controversial (1-3). The current information on the genetic content of the Malvaceae family has shown sufficient support to confirm the family as monophyletic and sisterly to the remaining families included in the order of the Malvales Iraq has a unique and special floristic value in Western Asia because of its diverse ecological conditions, topography, various altitudinal gradients, and diverse plant habitats. Despite the high floristic value of Iraq, it is not widely studied in terms of ecological and biological diversity(4, 5). These a large and economically important family of flowering plants and inhabit most parts of the world due to their notable capacity to adapt to harsh environmental conditions such as extreme temperatures, low nutrient status, and high soil salinity. The family plays a fundamental role as a source of medicine, fiber, ornamentals, foresters, dyes, food, fodder, nectar, shelter, and traditional uses. However, the number of other impacts is highly increasing(6, 7).

Unrootedphylogenetictrees were constructed based on UPGMA, NJ, ML, and MR algorithms using the hypervariable data of the population structure of the 7 Excel SSR marker alleles, and the results showed that a high degree of genetic diversity was present in the population of the seven Malvaceae plant groups(8, 9). This study of varying levels of genetic differentiation provided valuable diversity information on a population of Malvaceae plants in Iran, which was significant for further germplasm conservation, integration, and breeding studies(10, 11). This conclusion provides important insights into the population structure of the plant through an in-depth investigation of genetic biodiversity, clarifying the information of the plant status of the population on an Iranian scale (12). Seeds such as gum, gum arabic, okra, niger seed, cotton, borage, and sesame were imported as crop seeds from different foreign countries. These varieties have different morphological descriptions, and we cannot distinguish them without the result of molecular differentiation. DNA barcoding or DNA matrix is a commonly used genetic marker in biological research, and the most commonly used one is part of the conservation of the gene nucleus, chloroplast, and organism DNA. Malvaceae family species have great economic importance like cotton. This tribe contains valuable grain resources widely grown in the western Mediterranean region (13, 14). At the molecular level, we use the simple sequence repeat (SSR) hypervariable DNA fingerprinting marker for plants such as seeds. Residents of Iraq see exotic The migratory birds in the fall provide a good opportunity for the seeds to move with these birds outside the borders. The genus Hibiscus includes more than 300 deciduous or evergreen shrubs, and small trees native to warm and tropical regions around the world.The first reports of exotic Malvaceae species in Iraq were in the middle of the last century, such as Hibiscus syriacus, Pavonia foetida, and Urena lobate(15). In the beginning of the present century, in the environs of Kirkuk, and after that in the other Iraqi's phytogeographical regions, other exotic Malvaceae species appeared, such as Abutilon theophrasti, A. grandifolium,

Hibiscus sabdariffa, H. cannabinus, and Malva parviflora. These naturalized as a result of the expanded agriculture in the last hundred years in the areas where they were cultivated as crops or ornamental plants, and they are now weed plants. Due to its great ecological, medicinal, and nutritional importance, some Malvaceae naturalized or cultivated species were the subject of diversity and enzyme studies. Not all the exotic Malvaceae species in Iraq have been molecularly analyzed, which is a deficiency in molecular investigation in the genus Hibiscus and the family Malvaceae. This study used molecular markers, e.g., the internal transcribed spacer (ITS) of 45S and the intergenic spacer trnH-psbA of the cpDNA, to investigate seven exotic Malvaceae species in Iraq by comparing them to other global Malvaceae species.

Plant molecular biology focuses on understanding molecular interactions within plant cells, elucidating the structure and function of plant components, and their roles in growth, development, and environmental responses. Malvaceae family have crucial due to relevance of Iraq's plant diversity The studies Molecular, advanced technologies(SEM, TEM) and evolutionary relationships (16, 17,18).the sequencing of DNA is doing revolutionizes taxonomy and evolutionary analysis into plant taxonomy. Malvaceae family is very importance significant of the context understanding plant diversity in Iraq.the aim of study to classification, properties, characteristics physical and molecular evolution and the potent(19, 20). Molecular studies in species has significantly enhanced of karyotype studies. advancements have crucial in solvie complex biological and plant taxonomy. The problem of the research to identification for plant species and comprehensive classification of the Malvaceae in Iraq. This was tested in the Seoul; South Korea and the results are send to Iraq. also uses comprehensive phylogenetic analysis to understand the evolutionary relationships within the family(21,22).

Their taxonomic system, photochemistry, ethno medicine , molecular study, are study for species Malvaceae. DNA-based studies are one of the best tools that offer a wide range of knowledge from basic biology to advanced biological studies. The Malvaceae family, with about 244 genera and 4,225 species, is considered a relatively small group in plant taxonomy. Iraq has a unique and special floristic value in Western Asia because of its diverse ecological conditions, topography, various altitudinal gradients, and diverse plant habitats(1).

A study found high genetic diversity in the population of seven Malvaceae plant groups in Iran, providing valuable information for germplasm conservation, integration, and breeding studies. Seeds such as gum, gum arabic, okra, niger seed, cotton, borage, and sesame were imported as crop seeds from different foreign countries. The study recommends involving native and sensitive weed species in sustainable agriculture systems that aim to conserve diversity. The phylogeny investigation of a genus, tribe, or family is a front in botany to study diversity, diagnosis, historical relations, evolution, and many other subjects. appeared in the environs of Kirkuk and other Iraqi's phytogeographical regions. The species have great economic importance like cotton, This research contributes to the field of plant taxonomy by:

- Utilizing advanced molecular

techniques to enhance the accuracy of species identification.

- Constructing a comprehensive phylogenetic tree to understand the evolutionary relationships within the Malvaceae family.
- Identifying specific genetic variations that could help in conservation efforts and understanding environmental adaptability.

Material and methods

DNA Isolation: Using the Genomic DNA GENEzol™ DNA Reagent Plant Kit, DNA (genomic, mitochondrial, and chloroplast) was purified from plant tissues. PCR Amplification: The TrnH-psbA spacer gene was amplified using PCR. The reaction mixture was prepared with specific primers and reagents, followed by thermal cycling. Gel Electrophoresis: DNA samples were analyzed using agarose gel electrophoresis. Sequencing: PCR amplicons were sequenced commercially by Macrogen Inc., Seoul, South Korea. Sequence alignment and comparison with reference sequences from GenBank were performed to identify genetic variations and evolutionary relationships. here we will detailing the methods used in my study, particularly focusing on PCR amplification of the TrnH-psbA spacer gene and subsequent Sanger sequencing for genetic polymorphism analysis in Malvaceae samples. described methods:

DNA Isolation from Plant Samples:

Kit Used: Genomic DNA GENEzol™ DNA Reagent Plant Kit (Geneaid Biotech Ltd.) Purpose: Extraction and Purification of total DNA (genomic, mitochondrial, and chloroplast DNA) for plant tissues. The Polymerase Chain Reaction technique (PCR): Nucleic acid sequencing of PCR amplicons that allows of determination exact nucleotides sequence in DNA a specific fragment in the genetic research and various medical applications and used in the research such genetics, forensics, and diagnostics medical, DNA sequences and genetic variations. Nucleic acid sequencing of PCR amplicons is a crucial technique used to determine the order of nucleotides in a DNA or RNA molecule. Target of the Region: TrnH-psbA spacer region.

Procedure: Preparation of the PCR reaction mixture. Dissolve primers of this per specifications. Mixing concentrated stock with a practical solution (1:9 ratio). Addition of PCR components (antibody, hot medium, DNA polymerase start, Mgc12.Dntps, booster, stabilizer). Adjusting volume with RNAse-free water to 20 ml. Setting up thermal cycler (PCR) with specific conditions for each primer.

Primers: The required concentration was prepared according to the leaflet provided by the American supplier (IDT). To dilute the primers, 20 µl of the primer solution was taken and the volume was adjusted to 100 µl by adding double-distilled water (ddH2O). The primers were then stored in the freezer. For diagnostics, 20 µl of the primers were taken and dissolved in 80 µl of double-distilled.

Table (1): Primers sequences used for genes amplification.

Primer	Sequences (5---- 3)	Base
Trn f	CGCGCATGGTGGATTCACAATCC	-23MER
Trn r	GTTATGCATGAACGTAATGCTC	-22MER
Rbcl f	ATGTCACCACAAACAGAGACTAAAGC	-26MER
Rbcl f	CTTCTGCTACAAATAAGAATCGATCTC	– 27MER

Table (2): Program for qPCR amplification

Steps	Temperature	Time	Cycles
Reverse transcription	37℃	15 MIN.	1
Reverse transcriptase inactivation and GoTaq® DNA Polymerase activation	95 ℃	10 MIN.	1
Denaturation	95 ℃	10 SEC.	40
Annealing and data collection	60 ℃	30 SEC.	

Sequencing Methods

1-Nucleic acids sequencing of PCR amplicons: Positive cases indicate that the pathogen's specific genetic sequence is present. Very close sequence matches (false or extraneous targets present in the sample) or targeted sequence errors (poorly designed primers) can also lead to positive results. It is essential to verify the specificity of the primer sequence and to confirm that the positive result is truly revealing the presence of the targeted sequence, and not the presence of an extraneous sequence in the sample. Without confirming the specificity, it can be very easy to jump to a conclusion that results in a costly management decision based on information that is simply not accurate. Good laboratory practices underscore the need for getting the right answer the first time, perhaps especially when the decision is complex and difficult. In certain cases, there may also be value in the negative results. For example, a seed company may be able to save money by treating seeds without detectable endophytes differently from seed with detectable endophytes and avoid overtreating some seeds. Sequence analysis can reveal many features of amplified sequences, such as variation in sequence, single nucleotide polymorphisms, heterozygosity, sequence of targeted genes, and more. Most importantly, sequencing the amplicons identifies the specific amplified sequence. While its most common application is to take advantage of the amplification process (as a primer set binds specifically to certain sequences), it is critical to remember that in most PCRs, the sequence obtained is derived from the primer binding sites. If the purpose of the reaction is to amplify a unique sequence of interest, great care is needed to ensure that the sequence does not include the primer binding site or a non-unique internal target. Even when the purpose of the reaction is to simply amplify a full-length template, the use of an appropriate primer is a critical step. Furthermore, sequencing the amplicons to determine the target's status is equally important. The resolved PCR amplicons were commercially sequenced from both directions, forward and reverse directions, following the instruction manual of the sequencing company (Macrogen Inc. Geumchen, Seoul, South Korea). Only clear chromatographs obtained from ABI (AppliedBiosystem)

2-The sequencing data: The results of PCR sequencing technique for the studied samples were based on the protocol for both (15). The nucleic acid sequencing of PCR amplicons is a crucial step in molecular research, which allows identification of the exact order for nucleotides in a sample DNA.

3-Comprehensive construction of the evolutionary phylogenetic tree: The comprehensive evolutionary tree of the studied species was constructed according to the neighbour-joining protocol as described by (26). The observed changes were compared with

the neighboring homologous reference sequences.

Result and discussion

PCR amplification of the TrnH-psbA region: The results showed that the DNA extracted from some genera of the Malvaceae family was amplified in the TrnH-psbA region, as the amplification of the polymerase product was found to have a length of between 500-750 pairs for the genus, compared with the similar genotype as in panel (1).

Five samples were used in the target site for the study. Samples were examined to obtain serial amplification of the trnH-psbA gene spacer for the species that were partially examined. This is due to the fact that the variation of trnH-psbA intergenic spacer sequences can be used for genotyping, and due to the potential ability in adapting to changing genetic diversity. Sequencing reactions indicated the exact identity after NCBI blasting of these PCR amplifiers (22,23)). Regarding the trnH-psbA intergenic spacer amplicons of O1, the NCBI BLASTn the engine found that the similarity rate through studying the samples reached 99%, as in reference trnH-psbA intergenic spacer sequences of Malva parviflora (GenBank acc. KR735623.1) (Fig. 1a). Concerning the trnH-psbA intergenic spacer amplicons of W1, the NCBI BLASTn engine showed 100% sequence similarities between the sequenced samples and the reference trnH-psbA intergenic spacer sequences of Hibiscus rosa-sinensis (GenBank acc. NC_042239.1) (Fig. 1b). Concerning the trnH-psbA intergenic spacer amplicons of W2, the NCBI BLASTn engine showed up to 99% sequence similarities between the sequenced samples and the reference trnH-psbA intergenic spacer sequences of Brachychiton discolor (GenBank acc. KM895402.1) (Fig. 1c). Concerning the trnH-psbA intergenic spacer amplicons of W1, t was found through the NCBI BLASTn engine that the sequence similarity was 100% between the sequenced samples and the reference trnH-psbA intergenic spacer sequences of Dombeya wallichii (GenBank acc. OL312183.1) (Fig. 1d). Concerning the trnH-psbA intergenic spacer amplicons of W1, the NCBI BLASTn engine about 100% sequence similarities between each of sequenced samples ,and reference trnH-psbA intergenic spacer sequences of Bombax ceiba (GenBank acc. NC_037494.1).

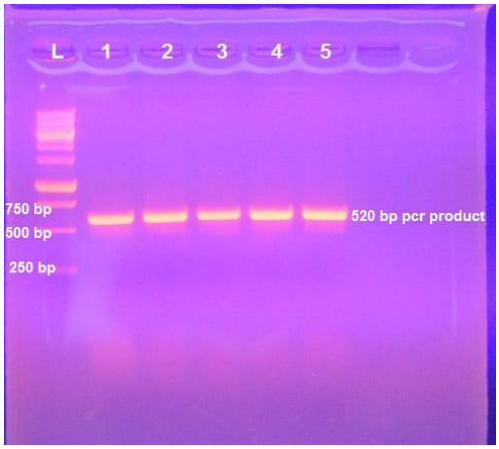

Figure (1): The Polymerization products through the trnH-psbA of Malvaceae family.

A) *Marva parviflora*

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                10        20        30        40        50        60        70        80        90
100
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
ATAACTTCCCTCTAGACCTAGCTGCTATTGAAGCTCCATCTACAAATGGGTAAGACTTTGGTTTTAGTGTATACGAGTTTTTGAAAATAAAGGAGCAATA
O1
..........................................................................................................
              110       120       130       140       150       160       170       180       190
200
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
ACCAATTTCTTGTTCTATCAAGAGTGTTGGTATTGCTCCTTTATTTAGATTTAGTAGTCTTTTTTTGCGTTACACCTTTTTTTTCTTTACATTTACATAA
O1
..........................................................................................................
              210       220       230       240       250       260       270       280       290
300
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
GTTTGTTTTTTCTTTACTTTAACTTTTTTATTTTACCTTATTTATTTCTGATTATCATAAGAAAAAAAAAGATTTTTTAATGAGTTTGATTTCATTTTAT
O1
..........................................................................................................
              310       320       330       340       350       360       370       380       390
400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
TGGATTTGACTTAGTATTATACGTTCGTTATGTAATAATATATTATATATTAACGAATTTTTTTTATTAAAAAAAAAGTAAAAAAGTTCTTACCAATCTT
O1
..........................................................................................................
              410       420       430       440       450       460       470       480       490
500
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
TTGTTTGGAATTTTCTATATATCTTATCTCAGAAATACGAGAAAAAAAAAAACGAATGATGAATGATAGAAATTGAGATATTTGAAATATTTAAACAATA
O1     ..........................................-
.....................................
              510       520       530       540
      ....|....|....|....|....|....|....|....|.
ref.  AAAAAAATACTATAGTATTATAGTAGAGGGGCGGATGTAGCCAAGT
O1    ...........................................
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B) *Dombeya wallichii*

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                10        20        30        40        50        60        70        80        90
100
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
GAGCTCCATCTACAAATGGATAAGACTTTGGTTTTAGTGTATACGAGTTTTTGAAAATAAAGGAGCAATAACCAATTTCTTGTTCTATCAAGAATGTTGG
W3
..........................................................................................................
              110       120       130       140       150       160       170       180       190
200
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
TATTGCTCCTTTATTTAGATTTAGTAGTCTTTCTTTTCTTTACATAAGTTTGTTTTTTCTTTACTTTAACTTTACTCTTTTTATTGGATTTTACTTGGTA
W3
..........................................................................................................
              210       220       230       240       250       260       270       280       290
300
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
TTATACGTTCGTTATGTACTAATTTCGTTATGTACTAATATTATGTATATGTACTAAATATCTAATTCTAATATATTGTATAATTTATATACAATTAAAA
W3
..........................................................................................................

              310       320       330       340       350       360       370       380       390
400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
AATATAAAAATAAAAAAGGTCTTACCAATCTTTTGTAAAGTTTTTGTTTAAAAAAAAAAAAATAAATATAAAGATGGGAATTTTCTATATTTCAAWTCTC
W3
..........................................................................................................
              410       420       430       440       450       460       470       480       490
500
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
ref.
AAAAAAAAAAAAAAAATTAAATAATTAAAAAATAATGATGAATGATAGAAATTTAAATCTTTTACATTTTCAATTTTTATTTGAAATAAAATATTTAAAC
W3
..........................................................................................................
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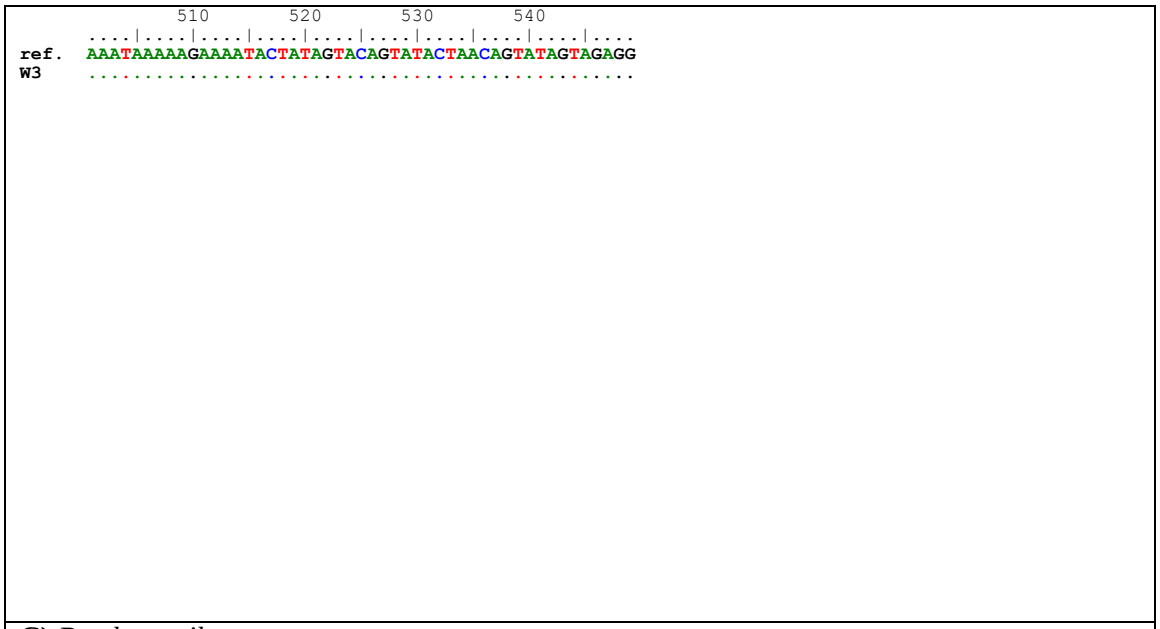


C) *Bombax ceiba*

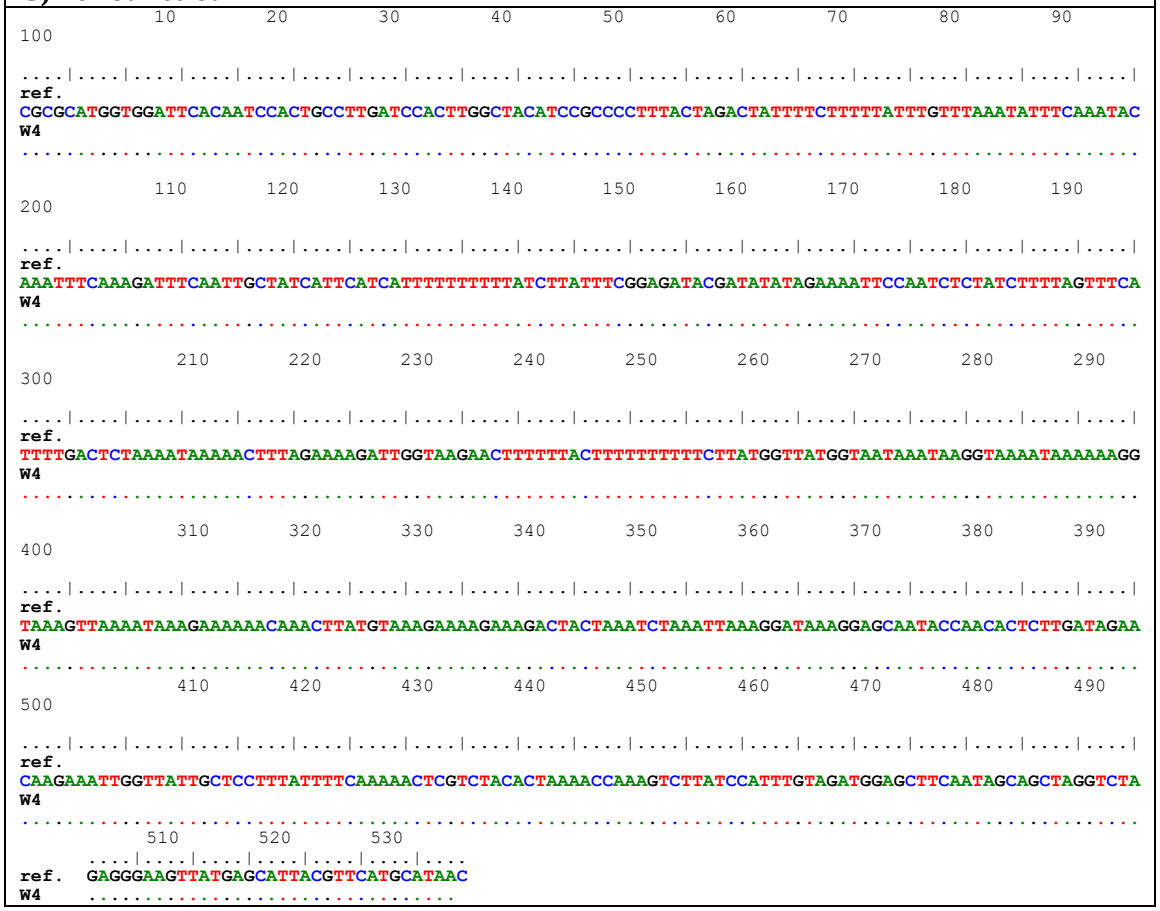
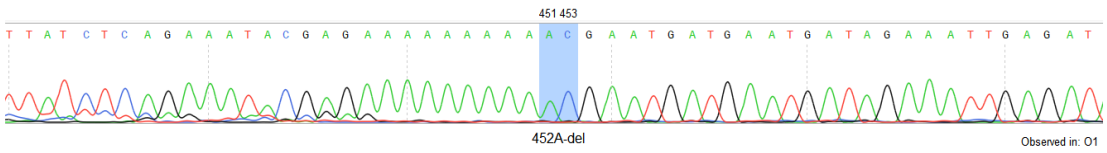


Figure (2): Nucleic acid sequences alignment of Malvaceae family samples with their corresponding reference sequences of the trnH-psbA intergenic spacer genomic sequences. The symbol "ref" refers to the NCBI referring sequence of *Malva parviflora* (GenBank acc. no. KR735623.1), *Dombeya wallichii* (GenBank acc. no. OL312183.1), and *Bombax ceiba* (GenBank acc. no. NC_037494.1) in branches A, B, C, respectively.

Marva parviflora



B) *Dombeya wallichii*

----------NO VARIATION DETECTED----------

C) *Bombax ceiba*

----------NO VARIATION DETECTED----------

Figure (3): The chromatogram of the investigated sequences in the amplified PCR products sequence of *Malva parviflora* (GenBank acc. no. KR735623.1), *Dombeya wallichii* (GenBank acc. no. OL312183.1), and *Bombax ceiba* (GenBank acc. no. NC_037494.1) in branches A, B, C, respectively. The symbols "del" and ">" refer to the identified deletion and substitution mutations, respectively.

The conclusion here pertains to the presence or absence of nucleic acid variations in the trnH-psbA intergenic spacer regions of different samples from the Malvaceae family compared to their most similar reference sequences. The findings for each species are as follows:

*Malva parviflora: One variation was detected in the DNA of the sample when compared to the reference sequence (GenBank acc. no. KR735623.1).

*Dombeya wallichii: No nucleic acid variations were found in the sample when compared to the reference sequence (GenBank acc. no. OL312183.1).

*Bombax ceiba: No nucleic acid variations were found in the sample when compared to the reference sequence (GenBank acc. no. NC_037494.1)(24, 25).

1- The figure referenced (Fig. 2) would provide visual evidence of these alignment results, showing the comparison between the samples' sequences and their corresponding reference sequences. The overall conclusion indicates that some species within the Malvaceae family exhibit genetic variation in the trnH-psbA intergenic spacer region, while others do not, highlighting potential genetic stability or variability within the family. Our results showed the absence of any nucleic acid variation observed in the investigated samples of Dombeya wallichii, and Bombax ceiba as compared with the most homologous reference sequences. Whereas, the results A single deletion of DNA was found in the samples examined of Marva parviflora, which was represented by the deletion of Adenine in the 452th position of the amplicon (Fig. 3a). These differences were confirmed by serial chromatography of the samples and explained in detail.

2-The results of the molecular study a major role in proving molecular evolutionary relationships, as the genetic kinship tree showed distribution and the occurrence of the studied species and varieties within two main clades.

3-Expanding the molecular study to include genes and other genetic regions using (NGS) Next Generation Sequence technology for complete genes in mitochondria, plastids, or the nucleus. Highlighting its importance in showing genetic relationships in the plant genome.

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Conclusion

This research demonstrates that Expanding the molecular study to include genes and other genetic regions using (NGS) Next Generation Sequence technology for complete genes in mitochondria, plastids, or the nucleus. Highlighting its importance in showing genetic relationships in the plant genome.

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