

Molecular comparative study of the some cultivar from malvaceae family in Iraq

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ABSTRACT

This study is to investigate and compare the molecular relationships of eight cultivars and species belonging to the malvaceae family in Iraq for the period from 10/8/2022 to 1/2/2023. The plant; *Abelmoschus esculentus* var. bathrah, *Abelmoschus esculentus* var. lahlouba, *Abelmoschus esculentus* var. hussainawi, *Hibiscus sabdariffa* var. red, *Hibiscus sabdariffa* var. striped, *Malva parviflora*, *Althaea officinalis* var. red, *Althaea officinalis* var. white obtained from field trips and tours. Where the study was conducted using the polymerase chain reaction (PCR) polymerase chain reaction, through which it was reached to determine the final sequence of the region (ITS), and the genetic kinship tree showed the cluster distribution for it. The rest of the studied items were collected.

Keywords: Molecular, Comparative, Malvaceae

INTRODUCTION

Malvaceae is derived from the Latin word *Malva*, which means baker, and *Malva* was derived from the old term meaning plant [1]. According to academics that were interested in researching this family, the number of its genera was believed to be around 244 genera and 4,225 species, however opinions vary as to the count of its species [2]. And Elnaggar [3] mentioned that the family consists of approximately 110 genera and 2000 species that are dispersed throughout the tropics and subtropics. On the other hand, Thorne [4] mentioned that it is a large group that consists of approximately 2330 species of trees and herbs that are found in the tropics. According to Al-[5] Musawi's findings, the family is comprised of a total of 80 genera and 1000 species. Flora of Iraq [6] described eight genera and two families that belonged to the family, and that the genus *Abelmoschus* is one of the only species represented by the type *A. esculentus*, while the genus Baker recorded five species, and the same number is represented by the genus *Alcea*, while the genus *Hibiscus* is represented with six types. This information was taken from the Encyclopedia of Iraqi Plants. Such as okra *Abelmoschus esculentus* L., plants of the *Malvaceae* family are of significant commercial and medical value. Which may be used both fresh and dried. Due to its high protein and dietary fiber content, okra is one of the most significant plants in Central and West Africa [7] *Malva pariflora* L. leaves are also consumed after boiling due to their many health advantages, although certain species, such as *Althaea officinalis*, are used for ornamentation [8]. *Hibiscus sabdariffa*, a member of the *Hibiscus* family, is regarded as a medicinally significant plant [9]. The DNA Sequencing Technique is one of the modern techniques that is commonly used by taxonomists to solve taxonomic problems. This technique works by tracking the sequence of nitrogenous bases for the entire gene or for parts of the gene, as well as for the coding and non-coding regions, and this sequence can be obtained. DNA Sequencing Technique is one of the modern techniques that is commonly used by taxonomists to solve taxonomic problems. The results of the Polymerase Chain Reaction (PCR) were published in 2001. [10], which was discovered by Millis and Coworkers in 1983, it revolutionized molecular biology, and Karry Millis was awarded the Nobel Prize in Chemistry following this scientific discovery in 1983 [11]. Because of its potentially high tolerance to interrelationships, the ITS region, or a part of it called ITS2, is one of the DNA segments that is used the most in plant molecular systems in general and at the species level in particular among homogenous species and closely related genera. This is true both in general for plant molecular systems and at the species level [12, 13, 14]. The ITS region was initially suggested as a barcode for flowering plants [15], but it fell out of favor for some time because of concerns regarding incomplete concerted evolution of

different paternal and maternal poly-allelic copies, DNA contamination of different species, and some technical problems. It has been shown that these flaws do not result in major issues, and hence, they have been reinstated as a fundamental barcode for plants [16].

MATERIALS AND METHODS

Species sampling

The (ITS) region were sequenced from 8 accessions representing 8 cultivar of malvaceae. The (ITS) region were chose based on their documented utility in phylogenetics . Accessions for a cultivar with identical DNA sequences were represented by one accession in dataset to speed up phylogenetic analysis.

DNA Isolation, Amplification and sequencing.

For fresh material ,genomic DNA was isolated following Doyle&Doyle [17] In the case of herbarium sample the DNA isolation method was modified to optimize the procedure . In those cases ,the leaf material was extraction two to three times in CTAB+Beta mercaptoethanol and the supernatant solutions collected from each extraction were combined to increase the amount of DNA recovered .Additionally ,the solution was allowed to remain at-20 C overnight at the DNA precipitation step. the (ITS) region was amplifird using the universal primers ITS and the protocol from Tallberlet et al,[18] with a 50 c annealing Temperature and the polymerase chin Rection (PCR) method described in Wood et al., [19].Amplification products for ITS region were resolved on 0.8% TEB –agarose gels, excised, and cleaned QiA quick PCR purification . cycle sequencing was performed using performed using the ABI PRISM Big Dye terminator Cycle Sequencing Kit v.3.1(Applied Biosystem ,Foster city californaia, USA) and the products were resolved using Applied Biosystem 3730 automated sequencer at core sequencing Facility at the DNA Analysis Facility of Duke University.

RESULTS

Analysis of the area sequence (ITS) of species belonging to the *Malvaceae* family

The ribosomal nuclear region (ITS) was chosen to research the family's taxonomy, and its findings were processed using the aforementioned scientific tools to produce a genetic tree with strong support. In the Maximum Parsimony (MP) analysis approach, the *H. sabdariffa* STRIPED cultivar was put at the base of the genetic tree and was crossed with the other *H. sabdariffa* RED cultivar's clad.And with high support for their data, it reached (100%), which belonged to the main connective clade of *M. parviflora* and the two cultivars *Al.officinalis* WHITE and *Al. Officinalis* RED, in which the *type M. parviflora* approached the connective secondary clade of the varieties *Al.officinalis* WHITE and *Al. Officinalis* RED, and the reliability between them was extremely high (100%), whereas it was About the second main clade, three cultivars of okra were named after it, and it was subdivided into two more subclades. The first group consisted of *Ab.esculentus* BATHRAH and *Ab. esculentus* LAHLOUBA and to mate with the class *Ab. esculentus* HUSSAINAWI, with outstanding support, reaching (99%), as shown in Figure (1).In addition to that, another investigation was carried out using the approach of Maximum Likelihood. The resultant tree has the same topology as the one that came before it, as the two cultivars of Gujarat descended at the base of the tree, with an acceptable dependability of their data amounts to 67%. We find this to be the case. *officinalis* RED with outstanding backing (100%) The species *M. parviflora* and the cultivar *Ab. esculentus* HUSSAINAWI descended in the second main clade, togetherwith *Ab. esculentus* BATHRAH and *Ab. esculentus* LAHLOUBA, as shown in Figure (2). And the matter will become clear when completing the analysis by the third method, Bayesian analysis (BA), as the taxa did not differ in their distribution within the tree, as the taxa were distributed in the same way and with excellent support for all their data, as in Figure (3).

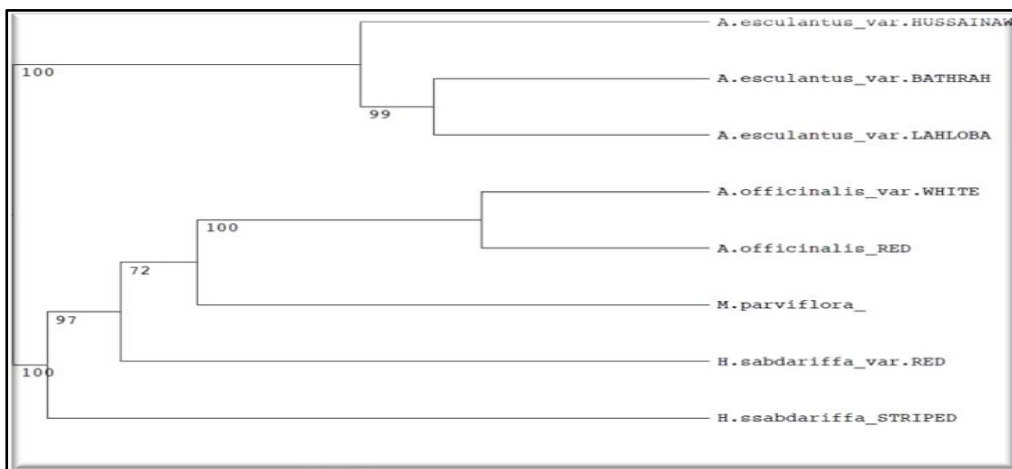


Figure (1): Using Maximum Parsimon, a genetic tree of species and cultivars of the *Malvaceae* family was constructed for the ITS region.

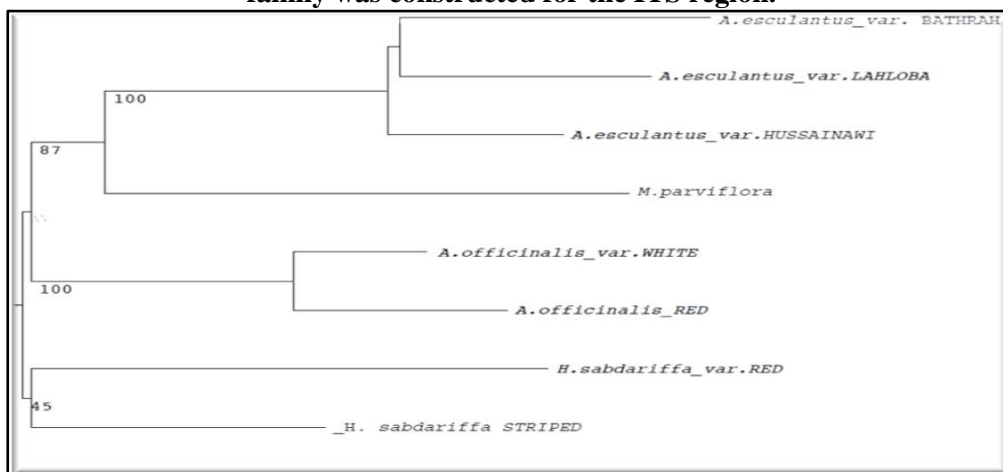


Figure (2): Using Maximum likelihood analysis, a genetic tree comprising species and cultivars of the *Malvaceae* family in the ITS region was constructed

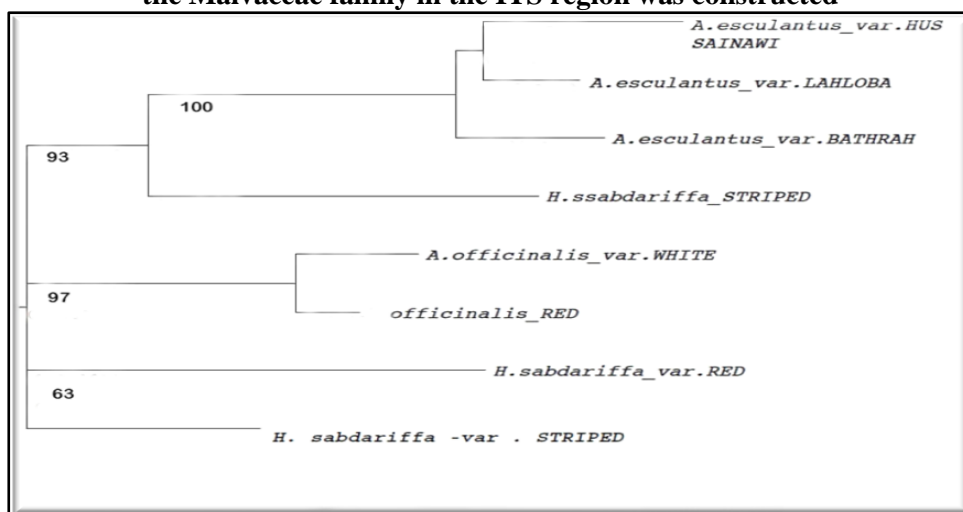


Figure (3): Genetic tree of *Malvaceae* species and cultivars in the ITS region using Bayesian analysis software.

DISCUSSION

Based on the prior DNA sequencing approach, the ribosomal nuclear region (ITS) was selected for the final sequences in the present investigation. In addition, the selection of the area was based on earlier research proving the significance of the region and the dependability of its significant taxonomic performance in distinguishing complicated taxonomic entities. And its significance in providing a view of the evolutionary and phylogenetic relationships between and within overlapping species, including plants of the *Malvaceae* family, in accordance with [20], when examining the family *Malvoiteae* of the *Malvaceae* family, based on the ribosomal nuclear region (ITS), thereby distinguishing between (65) and (65) alone. The taxonomy of Taxon belongs to the family, as does the research [21] that determined the evolutionary connections between the species of the genus *Abelmoschus* based on the (ITS) and (Trnc D) genes. As for analyzing the sequences and selecting the appropriate method to draw the evolutionary tree of the studied varieties, there are multiple methods for analysis, including Maximum parsimony, Likelihood Maximum, Bayesian analysis, upgma, and others, including what was used in our current study of the above methods, and this is consistent with what was used and confirmed by [22,23].

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