EAJBS

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EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
BOTANY

ISSN 2090-3812

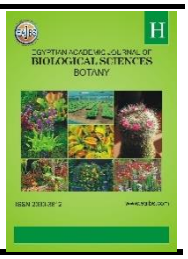
www.eajbs.com

Vol. 12 No.1 (2021)

Citation :*Egypt. Acad. J. Biolog. Sci.* (H. Botany) *Vol.12(1)pp21-77(2021)*
DOI: 10.21608/EAJBSH.2021.148134

Egypt. Acad. J. Biolog. Sci., 12(1):21- 27 (2021)

Egyptian Academic Journal of Biological Sciences
H. Botany
ISSN 2090-3812
www.eajbsh.journals.ekb.eg.



Ficus Species Genetic Diversity Based on Internal Transcribed Spacer (ITS) Region Analysis

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ARTICLE INFO
Article History
Received: 11/10/2020
Accepted: 12/1/2021

***Keywords*:**
Ficus, species, ITS, DNA, cluster, genetic, analysis

ABSTRACT

Ficus species (Moraceae) is a globally distributed species and considered one of the largest and most diverse genera of woody plants with growing genetic, environmental, economic, nutritional, and medicinal importance. This study was conducted to detect the genetic and evolutionary relationships among eight species of the genus Ficus (*Ficus microcarpa* var *nitida, F. retusa, F. benjamina* Vivian, *F. benjamina* Starlight, *F. cyathistipula, F. elastica Decora* and *F. binnendijkii,* and *F. religiosa*), by ITS1 and ITS4 primer, the DNA genetic analysis was performed. The eight *Ficus* species traits ITS phylogenetic analyses indicated that all species were separated into two main clusters containing four groups with identity ranging from 94-100%. The eight *Ficus* species relationships were estimated by a neighbor-joining (NJ) cluster analysis of genetic similarity matrices. The highest similarity was found between *F. benjamina* Vivian (Acc.no. MW301203), *F. binnendijkii* (Acc.no. MW301207), and between *Ficus microcarpa* var *nitida* (Acc.no. MW301202) and *F. retusa* (Acc.no. MW301205), gave the lower similarity index based on the analysis of the morphological features, and the lowest similarity was between *F. religiosa* (Acc.no. MW301209) and *F. cyathistipula* (Acc.no. MW301206).

INTRODUCTION

Ficus is a widely cultivated variety of plants, small trees that inhabit a diverse range of environmental zones; most of those are green (Halevy and Abraham 1989). *Ficus* is the common species in the Moraceae with about 850 species and is one of the biggest flowering plant taxa known today (Judd et al., 2008). The species now classified within *Ficus* have initially been split into several genera in the mid-1800s, providing the basis for a subgeneric classification when reunited into one genus in 1867. This classification put functionally dioecious species into four subgenera based on floral characters (Weiblen, 2000).

Since past decades, dozens of plant species are being used to cure various diseases. Many *Ficus* species had generally been documented for anticancer, anti-inflammatory, antihypertensive, and antiprotozoal (Ramadan *et al.*, 2009; Sirisha *et al.*, 2010). Peoples have used *Ficus* plants in folk medicine to cure diabetes, cough, and treat certain dermal diseases (Chopra *et al.*, 1950). In earlier studies, the medicinal use of fig leaf powder in hypoglycemia in type I people with diabetes was interested (Teixeira *et al.*, 2005). *Ficus benghalensis* wood ethanol extract has significant anti-inflammatory effects (Wanjari *et al.*,

2011). Within medicinal herbs, *Ficus religiosa* holds an important part. All plant parts are used to make herbal medicines. Its ingredients are positioned in the novel therapeutic sector as an essential component (Sandeep *et al*., 2018).

The primary source of phylogenetic rebuilding character was the genetic ribosomal DNA internal transcribed spacer (ITS) region. ITS sequencing has effectively addressed genetic trees at low taxonomic levels in organisms (Baldwin *et al*., 1995). *Ficus* morphological characters were analyzed individually and in a group, as an addition to ITS samples. The combination of morphometric data sources in a single analysis has been a matter of substantial discussion in several papers published (Bull *et al*., 1993; Donoghue and Kim, 1995; de Queiroz, 1996).

Phylogeny can also reveal details of the evolutionary process, leading to the development of hypotheses concerning morphological adaptation, physiological changes, or biogeography in the natural habitat (Alaklabi, 2014). This study aimed to detect the genetic and evolutionary relationships among *Ficus* species' eight genera through the ITS-DNA region.

MATERIALS AND METHODS

Plant Material:

Leaf samples from grown trees of *Ficus* species were gathered from local places in Saudi Arabia and identified initially by taxonomists. The voucher specimens are prepared and kept for molecular identification in the future at the University of Jeddah.

DNA Extraction:

DNA was isolated from 0.5 g fresh leaves according to Doyle and Doyle (1987) with modifications. The leaves milled in 2 ml of CTAB solution amended with 0.2% ß-mercaptoethanol and left in a water bath at 70°C for 30 min. The tubes were spined for 5 min at 4 °C, and 400 mL chloroform: isoamyl (24:1) was added to 600 mL of supernatant, repeated twice for 10 min at 4 °C. The pre-cooled isopropanol was added to pelt the DNA and left at 20°C for one hour, then centered at 15,000 rpm for 10 min. After washing the precipitated DNA with 70% ethanol, DNA was eluted in 100 µL of molecular grade water

Polymerase Chain Reaction (PCR):

The primer designated sequences for the ITS amplification region were imported from Macrogen Inc., Korea [ITS-1 (5' TCCGTA GGTGAACCTGCGG 3')] and [ITS-4 (5' TCCTCCGCTTATTGATATGC 3')] as reported by White *et al.* (1990). The amplicons were amplified in 0.2 tubes, and the reaction mixture consisting of 3 µL template DNA, 0.5 µL of each primer, DreamTaq mix 10 µL (Thermo Scientific), and the volume up to 25 µL. Amplifications were performed in a thermocycler (Techne- Progene, UK). The thermal cycler was programmed into three steps; the first step consisted of denaturation at 95.0 °C for 3 min, then the second step segmented into three stages for 34 cycles, each with 45 sec. at 94.0 °C to denature, 45 sec. at 55.0°C to anneal, and 1 min at 72.0 °C to elongate the amplicons. The last step was performed to finalize the elongation at 72.0°C for 7 min. PCR amplicons were electrophoresed on a 1.2% agarose gel, stained with Red Safe (iNtron, Korea). The DNA fragments were visualized by U.V. transilluminator. A 100bp DNA ladder (Thermo Scientific) was used as a molecular length standard.

DNA Sequencing and Analysis of The Amplified Regions:

The sequence was done with the same primers of amplification by HiSeq-2000 (Macrogen, Scientific Services Company, Korea). Similarities to known sequences were recorded using the BLAST nucleotide tool on NCBI site (http://blast.ncbi.nlm.nih.gov). Clustal W muscle alignment was done by MEGA 7 software, and the neighbor-joining phylogram was performed (Kumar *et al*., 2016). The obtained sequences were compared

with 16 accessions (MK472764, AB485849, JQ773840, HM368187, KP093097, HM368187, HM368206, HM368207, DQ455657, JX185793, JQ773846, JQ773843, JQ773866, HM368192, KJ845980, and JQ773964) obtained from GenBank. The obtained nucleotide sequences were accessioned with numbers reserved in GenBank

RESULTS AND DISCUSSION

Ficus plants belong to the Moraceae family; *Ficus* genus comprises approximately 850 species of big trees, shrubs, vines, and epiphytes (Woodland, 1997). In this study, eight *Ficus* species were morphologically identified, as showed in Figure (1)

An essential role of breeders is to develop selective breeding techniques, biological variation, relatedness, and parental genetic resources composition. Assessment of variability is vital for decoding evolutionary relatedness, including inheritance, or for the excellent implementation and the use of genome variability in propagating high yielding cultivars (Eldemery and Abdellatif 2014). The molecular identification and biodiversity were conducted by primers ITS-1 and ITS-4, amplifying the ITS gene and obtaining the predicted amplicons. A visible band of 600 bp has appeared in all tested *Ficus* species. The PCR products were purified and sequenced using ITS forward primer. The obtained nucleotide sequences were reserved in GenBank under accession numbers (Table 1). Different evaluations of ITS sequence data and phenotypic characteristics for *Ficus* species may demonstrate conflicting issues, but if the discrepancy is mostly uncontrollable flaws in phylogenetics analysis, a suitable combination will offer better the realistic assessment of taxonomy(Donoghue and Kim, 1995; de Queiroz 1996).

The results revealed the genetic and evolutionary relationships among eight genus *Ficus* species (*Ficus microcarpa* var *nitida*, *F. retusa, F. benjamina* Vivian, *F. benjamina* Starlight, *F. cyathistipula, F. elastica* Decora and *F. binnendijkii* and *F. religiosa*), based on the amplified ITS region reading sequences. The eight *Ficus* species traits' ITS region cluster analysis indicated that the eight species were separated into two main clusters containing four groups with identity ranging from 94-100%. The conditional approach supports integrated analyzes in the case of low contradiction while at the same time preferring independent analysis methods in profound inconsistencies. Concerns on independent vs. merged ITS evaluation, and phenotypic characters for *Ficus* have been discussed using computational analysis. Herre *et al*. (1996) Proposed that *Ficus* phenotype could offer inaccurate indications of phylogenetics due to integrating progression in genital characteristics; furthermore, published investigations have not explicitly tested this concept. Specificity approach was used to evaluate the development of the breeding program, including the marker genes in the rebuilding of phylogenetic analysis. (de Queiroz, 1996; Donoghue and Ackerly, 1996).

Fig.1. *Ficus* species selected for phylogenetic analysis based on ITS sequence analysis. A, *Ficus microcarpa* var *nitida*. B, *Ficus benjamina* (Vivian). C, *Ficus benjamina* (Starlight) . D, *Ficus retusa*. E, *Ficus cyathistipula* . F, *Ficus binnendijkii*. G, *Ficus elastica* (Decora). H, *Ficus religiosa.*

Table 1: Accession numbers in GenBank of all *Ficus* species used in this study

***Ficus* species**	**Accession number in GenBank**
Ficus microcarpa var *nitida*	MW301202
F. retusa	MW301205
F. benjamina Vivian	MW301203
F. benjamina Starlight	MW301204
F. cyathistipula	MW301206
F. elastica Decora	MW301208
F. binnendijkii	MW301207
F. religiosa	MW301209

The eight *Ficus* species relationships were estimated by neighbor-joining (NJ) analysis of genetic data. The maximum resemblance was found between *F. benjamina* Vivian (Acc.no. MW301203), *F. binnendijkii* (Acc. no. MW301207), and between *Ficus microcarpa* var *nitida* (Acc. no. MW301202) and *F. retusa* (Acc. no. MW301205), which have an extreme level of homogeneity, supported by the study of morphological features, the minimum resemblance has been reached between *F. religiosa* (Acc.no. MW301209) and *F. cyathistipula* (Acc.no. MW301206) Figure(2).

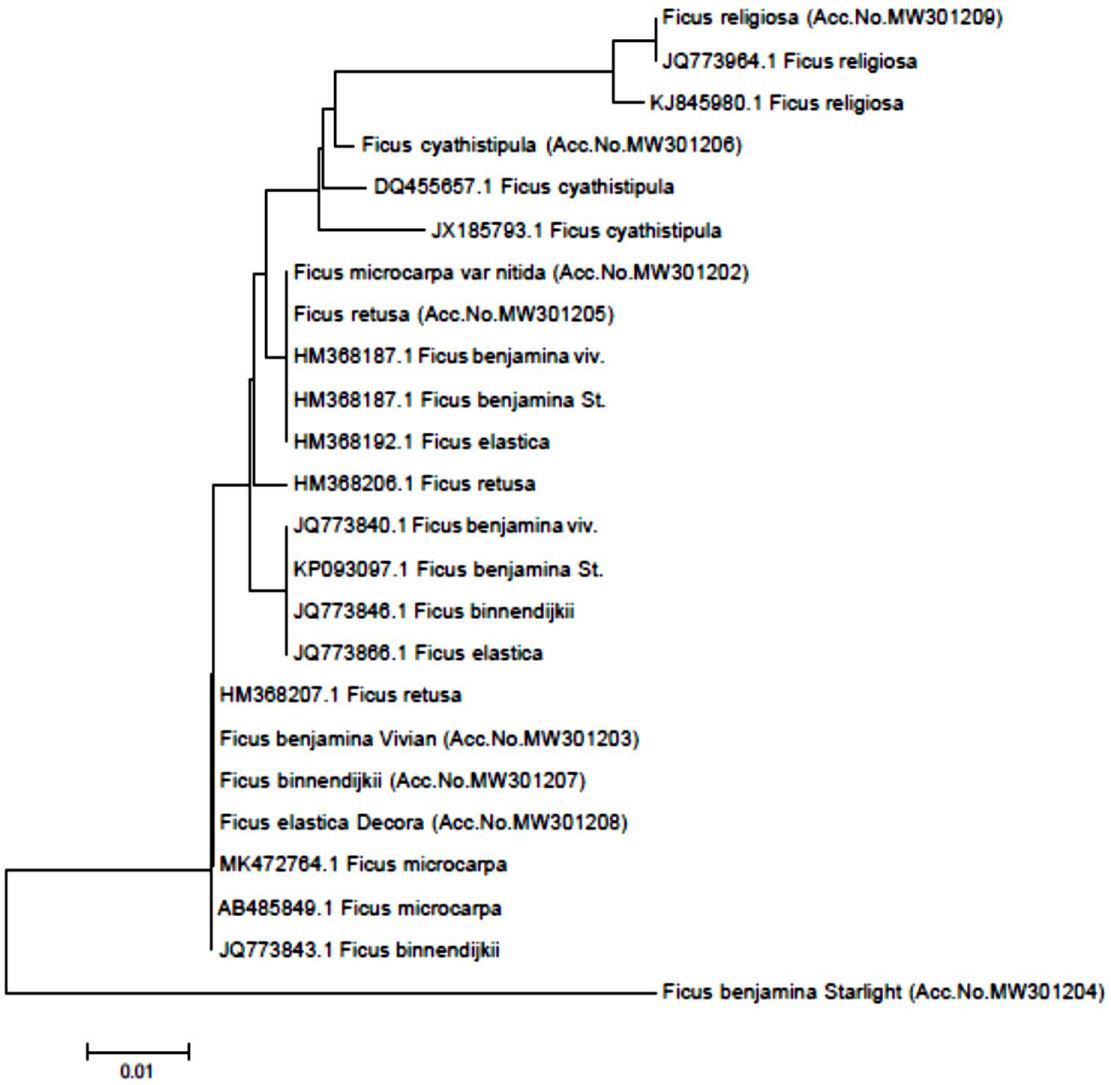

Fig.2. Neighbor-joining phylogram based on sequences of the eight *Ficus* species ITS gene degenerated with MEGA 7 software with 1000 replicates and bootstrap values (>50%). Accession numbers point to the sequences of *Ficus* species used in this study.

Conclusion

The morphology and molecular biodiversity in this study clarify the identification process of all *Ficus* species used. The ITS sequencing analysis draw a phylogenetic tree that compromised eight *Ficus* species in different groups with similarities ranging between 94 and 100%.

Conflict of Interest

There are no conflicts of interest to declare.

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