Genetical and morphological studies on *Ficus* trees

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ABSTRACT: In this current study, DNA barcoding and fifteen plant species of *Ficus* genus belongs to family moracea were used. The plant species were collected from the botanical garden of Antoniadis Alexandria government, Egypt. To authenticate the morphological identification and measure the evolutionary rate among them, *rbcL* and *matK* genes were used as genes universal DNA barcoding. The obtained results for *rbcL* gene amplification success were 93% and the *matK* gene amplification 80%. The DNA sequencing was got for 14 species of *rbcL* gene and 11 species for *matK* gene in addition, the *matK* and *rbcL* sequences for all samples were checked by GenBank databases and the accession numbers were detected for all the studied species. Finally, the *rbcL* was suggested as a plant barcode for its discriminatory power at high taxonomic levels than *matK* barcode.

Keywords: DNA barcoding, *Ficus* genus, *rbcL*, *matK*, Morphological, Genetic, GenBank

INTRODUCTION

Egypt is rich in biodiversity because it contains significant differences in its ecosystem from dry, salty, drought, desert, water bodies, flat lands, and mountainous environments, which are differed in temperature and humidity, besides it has distinct geographic location between three continents of Africa, Europe, and Asia. The diversity of the Egyptian plants includes various life forms such as trees, shrubs, bushes herbs, water and parasitic plants. So, the discriminatory and identification of plant cover in Egypt are very important for conservation and improvement of such plant biodiversity. Usually, plant description dependents on morphological character such identification is not always reliable and effective **(Ali et al., 2014).** In these cases plant species can be identified by using DNA chloroplast barcode marker which is a beneficial tool for plant species description or identification and phylogenetic construction **(Kang et al., 2017).**

The barcode consortium of life in 2009 considered the two genes *rbcL* and *matK* can be considered as molecular markers which used to explain the diversity and determine affiliation the plant samples to their species, in which the morphological diagnostic characters are not accurate enough **(O. Elansary et al., 2017).** Besides has various applications and used for ecological surveys to unknown taxon **(Dick & Kress, 2009)**.

The chloroplast genes (*rbcL* and *matK*) are widely used for standard barcodes and defined regions of the chloroplast DNA (maturasek or *matK* and ribulose- 1.5 biphosphates carboxylase oxygenase large subunit or *rbcL*). The selected *rbcL* and *matK* as a barcode region was dependent on the accurate recovery of the *rbcL* district and the *matK* has discriminatory power (**Hollingsworth et al., 2011).**

The *Ficus* genus has multiple different species of woody plants and shrubs, it consists of approximately 850 species spread in tropical and semitropical regions. Most species are diploid including the chromosome number (2n= 26) (**Condit, 1964)**. Some species are used as fuel and animal feed. Other species: *F. elastica* produces crude rubber as a natural extraction from its stem in its original habit. Another species like *F. benjamina*, *F. lyrata warb*., and *F. pumila* are grown for their ornamental value both as landscape plants and foliage ornamental ones used for inside decorative (**Fang et al., 2007**). There are

famous species worldwide for their fruits that contain important nutrients like *F. sycomorous*, it was a sacred tree for the ancient Egyptians and *F. carica* where spread along the northwest coast from Alexandria to Matrouh. Some species of *Ficus* are used as interior and outdoor ornamental plants. Some *Ficus* species are used as traditional medicine (**Nawaz et al., 2019**).

In Egypt, the *Ficus* trees are widespread for ornamentation the gardens and roads inside and outside cities and some species are used as plant fence for farms beside the two-fruit production species; *F. carica* and *F. sycomorous*. To develop a program for conserve this biological diversity of such species, we must survey the different species planted in different regions, by making a complete morphological description of them, and applying molecular genetic barcoding which gives the accurate differences and similarity among these biological variants in addition to give a phylogenetic tree to describe the evolutionary relation among them.

MATERIAL AND METHOD:

Plant material:

Leaves of 15 different trees of *Ficus* species were collected, in Zip lock plastic bags, from Antoniadis - garden – Alexandria - Egypt. The species name is, *F. retusa, F. benjamina, F. afzelii, F. microcarpa, hawii, F. lyrata, F. elastica Roxb ex Hornem, F. benjamina var golden, F. sycomorus, F. elastica decora, F. religiosa, F. altissima, F. benghalensis, F. aspera, F. tinctoria and F. platyphlla*.

Morphological description:

To clarify the specification of the plant species, Leaf morphology of the different species as structure, shape, color, surface texture and dimension was taken under consideration as recommended by those **(Mostafa et al., 2020, IPGRI, C. (2003) and Fatihah et al., 2014**) (see Table 2 and 3).

DNA barcoding analysis:

DNA extraction and PCR amplification of *rbcl* and *matk* genes:

Total genomic DNA was extracted from leaves from different plant species by using i- genomic plant DNA Extraction mini kit (lot No: 13110251) company of (intron biotechnology, Inc. South Korea). The PCR was performed for the extracted DNA of each studded species using the primers of the two candidate genes **(Maloukh et al., 2017).**as found in Table 1

Table (1): Sequence of primers used in the current study.

Locus	Sequence	Tm
rbcL a	(F) (5`ATGTCACCACAAACAGAGACTAAAGC3`)	57.2°C
	(R)(5`GTAAAATCAAGTCCACCRCG3`)	52.8°C
matK-KIM	(F) (5`CGTACAGTACTTTTGTGTTTACGAG3`)	53.9°C
	(R)(5` ACCCAGTCCATCTGGAAATCTTGGTTC3`)	60.4°C

To determine the optimum annealing temperatures of the primers used, the reaction volume was 25µl containing 12.5 µl Taq Red Mix, 2x Master mix (bioline) (Master mix with dye), 1.25 µl (12.5 µmole) forward primer, 1.25 µl (12.5 µmole) reverse primer,3 µl DNA template and complete the reaction with 7 µl d H_2O (distilled water). Reaction PCR condition were performed as follows: Initial DNA denaturation at 94°C for 5 minutes, followed by 35-40 cycles of final DNA denaturation at 94°C for 45 second, primer annealing temperature at 50°C for 45 second, DNA strand extension at 72°C for 1 minute, and final extension at 72°C for 7 minutes and 12 °C for ∞. The PCR products were verified by electrophoresis in 1.6 % agarose gel stained with ethidium bromide. The PCR products were sent to colors medical laboratories. Eltehad Square, Maadi –Cairo - Egypt for DNA sequencing and the sequences were obtained. All the obtained sequences were submitted to GenBank.

Sequencing and phylogenetic analysis

Results obtained based on comparative (*rbcL* and *matK*) chloroplast genes analysis have allowed the elucidation of some disputable questions of systematics and phylogeny of the fifteen *Ficus* species studied and the resultant of interesting new data. PCR product of 600 - 900 bp size amplification was observed using *rbcL* and *matK* primers respectively **(Fig. 1)** in fifteen *Ficus* taxa. These sequences are visualized by Chromas 2.4.4. The gained forward and reverse sequences were assembling and aligned using BioEdite software

version 7. 0.5,3 **(Hall, 1999**) the *matK* and *rbcL* sequences for all samples were checked by GenBank databases. The Phylogenetic tree analysis relationships among the different species samples, were conducted through Neighbor Joining (NJ) trees using MEGAX version 10.0.1 (**Kumar et al., 2018).**

RESULT:

Morphological study:

Fourteen morphological parameter of the fifteen *Ficus* species are presented in Tables (2 and 3) .The obtained results showed the smallest length and width of leaf were recorded for *F. microcarpa Hawaii* (4.72 and 0.92cm respectively) (Fig 2 and 3), while the tallest length observed in *F. lyrata, F. afzelii* and *F. aspera* (24. 46 and 23.2 and 22.41 cm, respectively) (see Fig .4) and the largest width in two species were pointed to *F. lyrata ,F. platyphylla* (14.76 and 14.56 cm, respectively)**.** The leaf petioles length ranged from 0.52 cm for *F. tinctoria* to 11.8 cm for *F. religiosa* (Fig. 4). The leaf texture of the *Ficus* species was recorded as smooth in all species except for *F. aspera parcelii* and *F. sycomorus*, which was recorded as rough. *F. aspera parcelii* has serrated leaf margin trait while, the *F. platyphylla, F. lyrata* and *F. bengamina* have waved leaf edge, while the other species have entire leaf edge.

The shape of leaf in the most species were elipetic ovate except for both *F. afzelii, F. religiosa* and *F. tinctoria* which showed obovate, cordate and lencolate shape respectively. Simple type of leaves was arranged in alternate shape on stem in all species.

The base of leaf was differed from cordate, cuneate, trunceate and rounded in (*F. lyrata, F. platyphlla, F. aspera* and *F. religiosa),* (*F. retusa , F. afzelii, F. microcarpa, F. tinctoria and F. altissima), .*(*F.benghalensis* and *F. sycomuros) and* (*F. elastica decora, F. elastica .Roxb ex .Horenm, F. benjamina golden* and *F. benjamina) respectively.*

The apex acute of leaf was observed in *F. retusa, F. microcarpa, F. altissima* and *F. afzelii*, while the apex acuminates was observed in the most species except for *F.platyphlla* was cuspidate (Table 2).

To determine the leaf colour as an optical trait by eye as green, shiny green and variegated colour. According to this categorical classification species *F. benjamina* has shiny green colour. While the species *F. microcarpa hawaii, F. benjamina var gold* and *F. aspera parcelii* have variegated colour. The most of Ficus species have green colour. The venation of leaf was pinnate in *F. retusa, F. benjamina, F. microcarpa hawaii, F. tinctoria, F. elastica,* F. elastica Roxb ex Hornem and *F. benjamina golden.* However, the leaf venation were prominent noticed in the other *Ficus* species (Table 3). The milky latex observed in the most species except *F. tinctoria* (Table. 3).

Figure (1) : Leaf shapes of *Ficus sp*: *(1- Ficus retusa, 2- Ficus benjamina, 3- Ficus afzelii, 4- Ficus microcarpa. hawii, 5- Ficus lyrata, 6- Ficus elastica Roxb, 7- Ficus benjamina var golden, 8- Ficus sycomorus, 9- Ficus elastica. 10- Ficus religiosa, 11- Ficus alttisima, 12- Ficus benghalensis. 13- Ficus aspera, 14- Ficus tinctoria, 15- Ficus platyphlla).*

Table (2): Quantitative and qualitative morphological data were measure from mature plants). Using 10 randomly replication for studied the leaf, shape, length (cm), and width (cm),, apex. base , petiole length (cm), surface texture (determined as 0= rough and 1=smooth), margin, (recorded as Waved Entire and Serrated).

Species	Length (cm)	Width (cm)	Petiole length (cm)	Apex	Base	Shape	Surfac texture	Arrange ment	Margin
F. retusa	9	3.228	1.32	Acute	Cuneate	Eliptic	smooth	1	Entire
F. Benjamina	7.6	3.46	1.08	Acuminate	Rounded	Eliptic (oval)	smooth	1	Slightly wave
F.afzelii	23.2	6.8	3.24	acute	Cuneate	obovate	smooth	1	Entire
F. microcarpa-hawii	4.72	2.22	0.92	Acute	Cuneate	Eliptic	smooth	1	Entire
F.lyrara	24.46	14.76	1.74	truncate	Cordate	Fiddle leaf	smooth	1	wave
F. elastica Roxb.ex Hornem	12.65	6.32	3.94	Shortly Acuminate	Rounded	Eliptic (oval)	smooth	1	Entire
F. benjamina gold	7.64	4.8	1.26	Acuminate	Rounded	Eliptic (oval)	smooth	1	Entire
F. sycumorus	8.75	5.16	2.72	subacute	Truncate	Eliptic	Rough	1	Entire
F. elastic	21.62	12.08	4.42	Acuminate	Rounded	Eliptic (oval)	smooth	1	Entire
F. religiosa	17.496	10.44	11. 8	Long Acuminate	Cordate	cordate	smooth	1	Entire
F. altissima	16.8	7.9	4.38	Acute	Cuneate	ovate	smooth	1	Entire
F. benghalensis	10.84	6.6	2.6	Rounded	Trunceat	ovate	smooth	1	Entire
F. aspera	22.41	12.46	0.88	accuminate	Cordate	oval	rough	1	Serrated
F. tinctoria	8.66	3.14	0.52	accuminate	Cuneat	lanceolate	smooth	1	Entire
F. platyphlla	16.26	14.56	10.3	cuspidate	Cordate	elliptic	smooth	1	wave

Table (3): Leaf color (obtained as variegated and Green), type leaves (record as compound and simple), , arrangement venation (record as 0= pinnate and 1= prominent), latex (record as 0=absence , 1= present) , aerial roots (record as 0=absence , 1= present).

Species	Leaf color	Leaf venation	Aerial root	Milky latex	Leaf type
F. retusa	green	0	1	1	simple
F. Benjamina	Shiny green	0	0	1	simple
F. afzelii	green	1	0	1	simple
F. microcarpa-hawii	Variegated	0	1	1	simple
F. lyrara	green	1	0	1	simple
F. elasica Roxb.ex Hornem	green	0	0	1	simple
F. benjamina gold	variegated	0	0	1	simple
F. sycumorus	green	1	0	1	simple
F. elastic	green	0	0	1	simple
F. religiosa	green	1	0	1	simple
F. altissima	green	1	0	1	simple
F. benghalensis	green	1	1	1	simple
F. aspera	variegated	1	0	1	simple
F. tinctoria	green	1	0	0	simple
F. platyphlla	green	1	1	1	simple

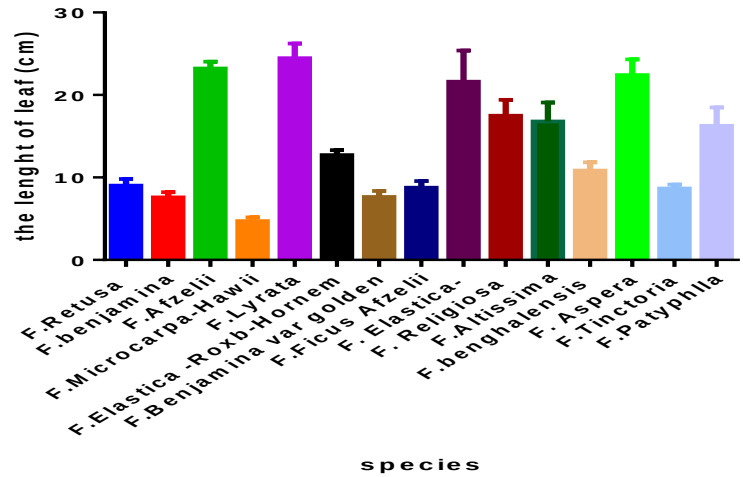

Figure (2): Histogram of 15 species from *Ficus* genus determined length of leaf (cm)

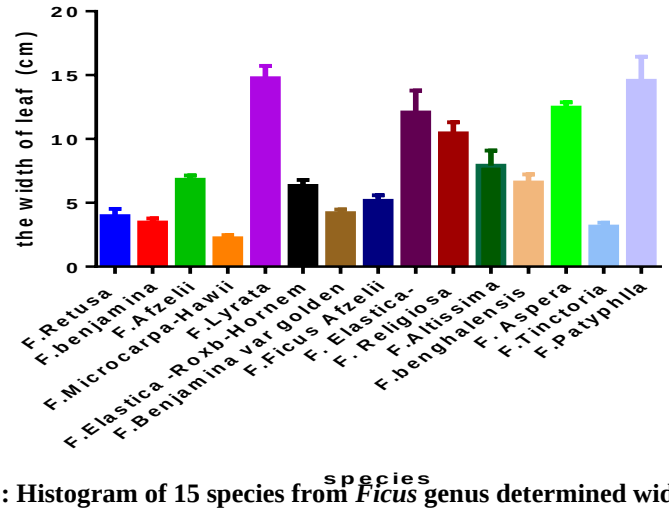

Figure (3): Histogram of 15 species from *Ficus* genus determined width of leaf (cm)

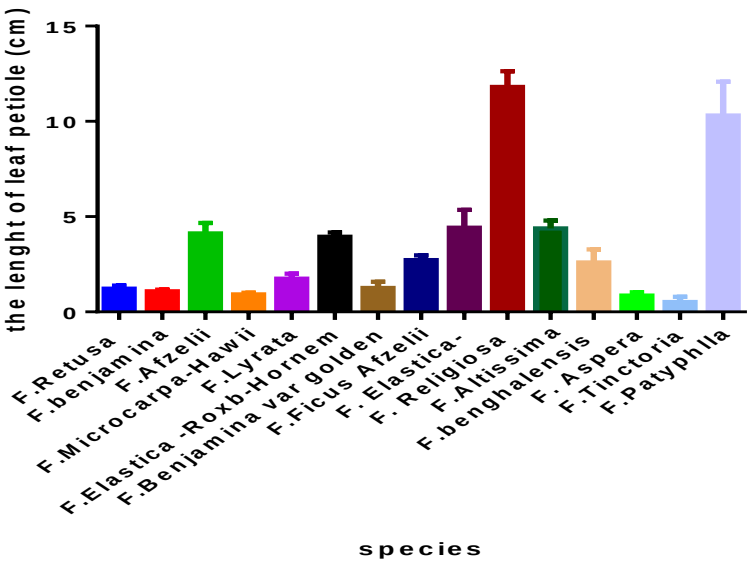

Figure (4): Histogram of 15 species from Ficus genus determined length of petiole (cm)

***RbcL* gene: PCR amplification, and sequencing.**

The amplification of *rbcL* yielded PCR products about 93% (14/15) of species. The query sequences identified on species level of 14 plants were 97 to 100% in either of the algorithms. The generated query sequences of 14 plants were matched with the reference sequences in BLAST /NCBI, (Table.4). The identification success was equally great for 12 species using the *rbcL* locus. Accession numbers are obtained for the respective plant species: *F. retusa* (MN102667.1), same accession number (KT718118.1) for *F. benjamina* and *F. benjamina var gold* was determines. All of *F. lyrata* (JQ773728.1), *F. microcarpa* (MN099002.1), *F. elastica Roxb. ex Hornem* and *F. elastica decora* were distinguished by accession number (MN098997.1). Accession number for *F. religiosa, F. altissima, F. benghalensis, F. tinctoria and F. platyphlla* were (KF381142.1), (GU135133.1), (MG946836.1), (JQ773784.1) and (KX783880.1) respectively. On the other hand two different morphological species were identified as the same species through sequence alignment with *F. hirta* (MN364796.1) this can be, explained on the basis that these two species are hybrids of both species.

***MatK* gene PCR amplification and sequencing:**

The gene of *matK* amplified only 80% (12/15) of the tested plant taxa and rate 73.33 %(11/15) for sequences . When the *matK* sequences were aligned with the reference sequences in BLAST/ NCBI (Table.4), only 60 %(9/15) resulted in correct species identification. Accession numbers were (GU935043.1) for *F. retusa,* (JQ773506.1) for *F. benjamina* and *F. benjamina var Gold,* (AB925064.1) for *F. microcarpa,* (JX495717.1) for *F. sycumorus,* (JQ773471.1) for *F. elastica decora,* (KR530802.1) for *F. altissima,* (MG946963.1) for *F. benghalensis* and (JQ773602.1) for *F. tinctoria.* Two of the query sequences mis-matched, the *F. elastica Roxb. ex Hornem* with *Ficus sp moore* 315 (EU002177.1) and *F. aspera* with *F.* h*irta* chloroplast complete genome (MN364706.1). The *matK* algorithm is not able to identify the species due to the absence of species specific unique regions.

Phylogenetic tree Analysis for *rbcL gene*:

The relationships among 14 *Ficus* species estimated the sequences were alignment using Bioedit software and the construction for the phylogenetic tree using the NJ method by MEGA x software. The phylogenetic tree divided into two main clusters, the first cluster was further separated into two subgroups, the first subgroup contains (*F. afzelii G.Don*) as out group and the second sub*group contains (F. elastica decora, F. benjamina var gold prestigious, F. elastica Roxb ex Hornem and F. platyphlla).* The most closely related species (*F. benjamina var gold and F. elastica decora*) as sister group. The second cluster cont ained nine species *(F.lyrata , F.benjamina, F.tinctoria, F. altissima, F. aspera, F. benghalensis, F. retusa, F. religiosa and F. microcarpa. hawii*). The highest similarity was found between (*F. aspera* and *F. benghalensis*) and between (*F. retusa and F. microcarpa hawii*) (**Fig. 6)**

Phylogenetic tree Analysis for *matK* gene:

The relation among 11 *Ficus* species measured by the sequences were alignment using Bioedit software and the phylogenetic tree was constructed using the NJ method by MEGA x software this alignment. The phylogenetic tree was divided into two main clusters. The first cluster involved 10 species *(F. elastica decora, F. benjamina var gold prestigious, F. elastica Roxb ex Hornem, F.benjamina, F.tinctoria, F. altissima, F. benghalensis, F. retusa, F.sycomorus and F. microcarpa. hawaii*).The highest similarity was found between (*F. benjamina var gold, F. microcarpa hawaii, F. elastica decora, F. benghalensis and F. altissima*).The second cluster contained only one species *F. aspera*. **(fig. 7).**

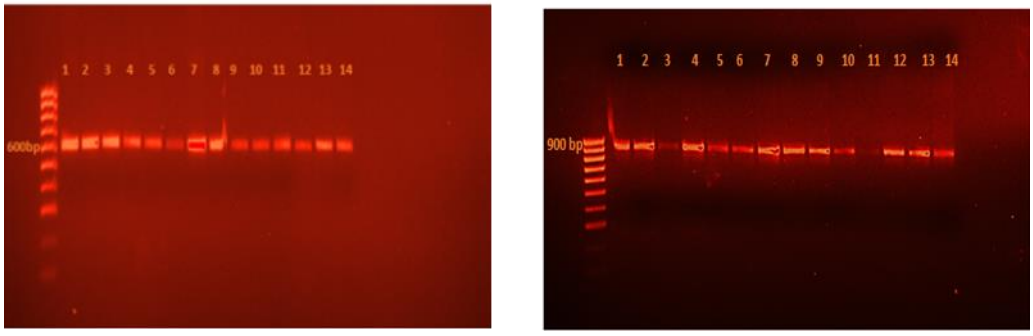

Figure (5): Gel electrophoresis for 14 species of *Ficus* tree after purification of *rbcL* and *matK*

Table (4): Result of Matching with reference sequence on Blast Ncbi for *rbcL* and *matK.*

Names	Matching with reference sequence on Blast Ncbi for *rbcL*	rbcL Blast GenBank Accession No.	Matching with reference sequence on Blast Ncbi for *matK*	*matK* Blast GenBank Accession No.
F. retusa	***F. retusa***	***MN102667.1***	***F. retusa***	***GU935043.1***
F. benjamina	***F. benjamina***	***KT718118.1***	***F. benjamina***	***JQ773506.1***
F. afzelii	***F. hirta***	***MN364706.1***	***……...***	***……...***
F. microcarpa-hawii	***F. microcarpa***	***MN099002.1***	***F. microcarpa***	***AB925064.1***
F. lyrara	***F. pandorata***	***JQ773728.1***	***……...***	***……...***
F. elastica Roxb.ex Hornem	***F.elastica***	***MN098997.1***	***F. sp moore 315***	***EU002177.1***
F. benjamina gold	***F.benjamina***	***KT718118.1***	***F. benjamina***	***JQ773506.1***
F. sycumorus	***.....***	***.....***	***F. sycumorus***	***JX495717.1***
F. elastica	***F. elastica***	***MN098997.1***	***F.elastica***	***JQ773471.1***
F. religiosa	***F. religiosa***	***KF381142.1***	***……***	***……..***
F. altissima	***F. altissima***	***GU135133.1***	***F. altissima***	***KR530802.1***
F. benghalensis	***F. benghalensis***	***MG946836.1***	***F. benghalensis***	***MG946963.1***
F. aspera	***F. hirta***	***MN364706.1***	***F.hirta***	***MN364706.1***
F. tinctoria	***F. tinctoria***	***JQ773784.1***	***F.tinctoria***	***JQ773602.1***

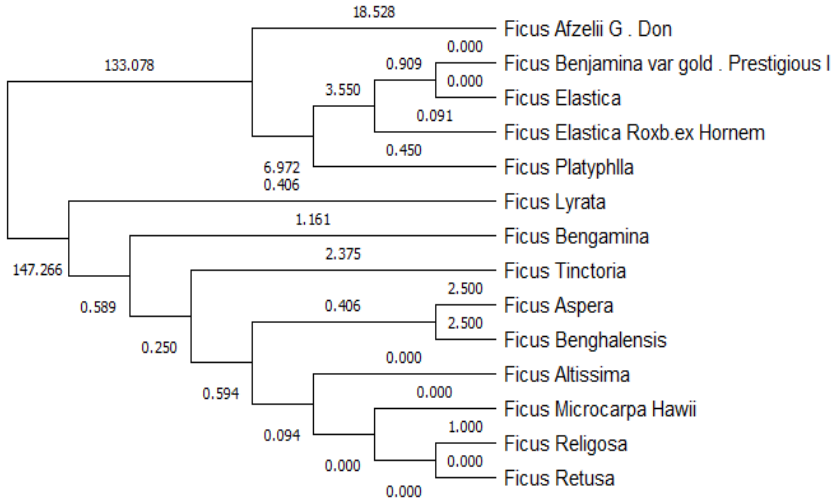

Figure (6): Molecular Phylogenetic tree analysis using *rbcL* gene for14 Species by Neighbor-Joining method (Saitou and Nei, 1987).The optimal tree with the sum of branch length = 322.71875000 is shown. The evolutionary distances were computed using the number of differences method **(Nei and Kumar, 2000)**. There were a total of 582 positions in the final dataset. Evolutionary analyses were conducted in MEGA X **(Kumar *et al.*, 2018).**

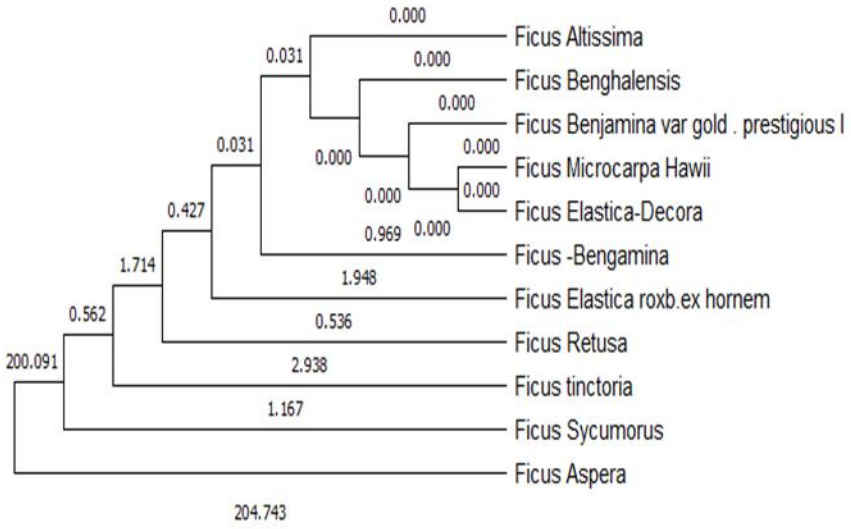

Figure (7): Molecular Phylogenetic tree analysis using *matK* gene for11 Species by Neighbor-Joining method (Saitou and Nei, 1987).The optimal tree with the sum of branch length = 415.15625000 is shown. The evolutionary distances were computed using the number of differences method (**Nei and Kumar, 2000**) and are in the units of the number of base differences per sequence. There were a total of 751 positions in the final dataset. Evolutionary analyses were conducted in MEGA X **(Kumar *et al.*, 2018)**.

The evaluation of variation among species with three parameters:

With different parameters such as nucleotide frequencies, measure of Tajima D value, and the measured values of transition/transversion bias (R) indicated the existence of a wide divergence pattern of *rbcL* and *matK* in fourteen *Ficus* specie. We further computed the Maximum Composite Likelihood (MCL) measure of the pattern of nucleotide substitution according to (**Tamura et al., 2004)** and Compute nucleotide frequencies .

The *rbcL* sequence divergence among taxa:

The *rbcL* was used as a DNA barcode region for the identification of most plants at the species level. *rbcL* is used for phylogenetic studies due to the facility of amplification, alignment, and sequencing. The sequence was (600 bp) in each of the *Ficus* species and the averages of nucleotide frequencies were A (28.82%), T/U (29.50%), C (20.42%), and G (21.26%). Nucleotide frequency of *rbcL* were variable in the fourteen of the *Ficus* species (Table 5). The average of GC was (**41.75%**) and AT (**58. 3%**)**.** This experiment analysis showed that transversions were more than transitions. Moreover. result of transition/transversion bias were (*R*= 0.956) in Table (7) and the evaluated nucleotide diversity value(π) using the Tajima Neutrality test, as shown in table (9). A total of 312 segregating sites (S) from a total 582 position demonstrating a nucleotide diversity rate of (0.254768) among species of *Ficus* genus were recorded. A positive value of the D test of Tajima was obtained for the *rbcL* region.

The *matK* sequence divergence among taxa:

The *matK* sequence length was (900 bp) in each *Ficus* species and the averages of nucleotide frequencies were A (36.8%), T/U (31.8%), C (15.7%), and G (15.7%). which were so far identical in the eleven species of *Ficus* (Table 6), the average GC was (31.4%) and AT was (68.63%).In the present investigation we found that transversions less than transitions. Moreover, *matK* indicated evolutionary rate for *Ficus* species, because the results of transition/transversion bias (*R*= 1.172) in Table (8) and the evaluated nucleotide diversity value (π), using the Tajima Neutrality test, as shown in Table (9). There were a total of 751 positions with a total of 411 segregating sites (S) demonstrating a lower nucleotide diversity rate (0.101416) among species of *Ficus* genus. A negative value of the test D of Tajima was obtained for the *matK* region.

Table (5): The evolutionary analyses of nucleotide frequencies among 14 species of *Ficus* genus for *rbcL* gene.

The names of species	T(U)	C	A	G	Total	AT	GC
Ficus afzelii g . don	28.0	22.5	30.4	19.1	582	58.4	41.6
Ficus altissima	30.4	19.4	28.0	22.3	583	58.4	41.7
Ficus aspera	30.2	19.6	28.0	22.3	583	58.2	41.9
Ficus benjamina	30.2	19.4	28.1	22.3	583	58.3	41.7
Ficus benghalensis	30.0	19.7	28.0	22.3	583	58	42
Ficus benjamina var Gold Prestigious I	28.1	22.5	30.0	19.4	583	58.1	41.9
Ficus microcarpa hawii	30.4	19.4	28.0	22.3	583	58.4	41.7
Ficus elastica Roxb.ex Hornem	28.1	22.3	30.0	19.6	583	58.1	41.9
Ficus elastica	28.1	22.5	30.0	19.4	583	58.1	41.9
Ficus lyrata	30.4	19.6	28.1	22.0	583	58.5	41.6
Ficus platyphlla	28.0	22.1	30.4	19.6	583	58.4	41.7
Ficus religosa	30.5	19.2	28.0	22.3	583	58.5	41.5
Ficus retusa	30.4	19.4	28.0	22.3	583	58.4	41.7
Ficus tinctoria	30.4	19.6	28.0	22.1	583	58.4	41.7
Avg.	29.5	20.5	28.8	21.2	582.9	58.3	41.75

Table (6): The evolutionary analyses of nucleotide frequencies among 11 species of *Ficus* genus for *matK* gene.

The names of species	T(U)	C	A	G	Total	AT	GC
Ficus altissima	**31.2**	**15.6**	**37.5**	**15.7**	**751**	**68.7**	**31.3**
Ficus aspera	**37.3**	**16.1**	**30.9**	**15.7**	**751**	**68.2**	**31.8**
Ficus bengamina	**31.2**	**15.6**	**37.4**	**15.8**	**751**	**68.6**	**31.4**
Ficus benghalensis	**31.2**	**15.6**	**37.5**	**15.7**	**751**	**68.7**	**31.3**
Ficus benjamina var gold prestigious I	**31.2**	**15.6**	**37.5**	**15.7**	**751**	**68.7**	**31.3**
Ficus microcarpa hawii	**31.2**	**15.6**	**37.5**	**15.7**	**751**	**68.7**	**31.3**
Ficus elastic	**31.2**	**15.6**	**37.5**	**15.7**	**751**	**68.7**	**31.3**
Ficus elastica Roxb. ex Hornem	**31.4**	**15.6**	**37.3**	**15.7**	**751**	**68.7**	**31.3**
Ficus retusa	**31.3**	**15.6**	**37.5**	**15.6**	**751**	**68.8**	**31.2**
Ficus sycomorus	**31.4**	**15.6**	**37.3**	**15.7**	**751**	**68.7**	**31.3**
Ficus tinctoria	**31.2**	**15.8**	**37.2**	**15.8**	**751**	**68.4**	**31.6**
Avg.	**31.8**	**15.7**	**36.8**	**15.7**	**751.0**	**68.6**	**31.37**

Table (7): Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution (Transition /Transversion) for *rbcL*

	A	T	C	G
A	-	*7.44*	*5.15*	**11.36**
T	*7.27*	-	**9.34**	*5.36*
C	*7.27*	**13.49**	-	*5.36*
G	**15.39**	*7.44*	*5.15*	-

Each entry shows the probability of substitution (r) from one base (row) to another base (column) (**Tamura et al., 2004).** The overall transition/transversion bias is $R = 0.956$, where $R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)]$.

Table (8): Maximum Composite Likelihood Estimate of the Pattern of Nucleotide Substitution for *matK*

Each entry shows the probability of substitution (r) from one base (row) to another base (column)

	A	**T**	**C**	**G**
A	-	*6.75*	*3.32*	**9.52**
T	*7.82*	-	**8.49**	*3.34*
C	*7.82*	**17.23**	-	*3.34*
G	**22.31**	*6.75*	*3.32*	-

(Tamura et al., 2004). The overall transition/transversion bias is R = 1.172, where R = [A*G*k_1 + T*C*k_2]/[(A+G)*(T+C)].

Table (9). Result from Tajima's Neutrality Test (Tajima D, 1989).

Locus	***m***	***S***	p_s	Θ	π	***D***
rbcL	14	312	0.536082	0.168572	0.254768	2.308605
matK	11	411	0.547270	0.186847	0.101416	-2.216143

The analysis involved 14 and 11 nucleotide sequences for *rbcL* and *matK* genes respectively .There were a total of 582 and 751 positions respectively. in the final dataset. Evolutionary analyses were conducted in MEGA X **(Kumar et al., 2018**).

DISSCUSION:

Ficus trees belonged to the *Moraceae* family are well known in the field of classical medicine. It is rich source of flavonoids and phenolic acid which make them able to protect against disorders of oxidative stress like as **F. exasperate (Akanni et al., 2014).** Some species such as the *F. benjamina* and *F. retusa* are considered as an important ornamental plant. There are other species produce fruits for nutrition such as *F. sycomorus* and *F. carica* in addition, *F. elastica* is a source of natural rubber because its contain on milky latex (**Augustus and Seiler, 2011).**

In this study, leaves were collected from *Ficus* trees for the determination of the potentiality of *rbcL* and *matK* regions as a barcode for 15 species. Identification using *rbcL* and *matK* regions matched with morphological identification 86% and 72% to species level respectively .The amplification rate of rbcl was 93% for species; while the amplification rate of *matK* was 80%. The low rate of *matK* amplification in plant taxa could be due to the great size for product amplification that is susceptible to degradation **(Fazekas et al., 2012)**. DNA barcode should be unique identifiers, short sequence and universality **(Stoeckle, 2003).**

The generated query *rbcl* sequences of 14 plants were matched with the reference sequences in GenBank database and obtained accession number for most species. Although there were shape and texture leaf surface difference between *F. afzeli*, and *F. aspera* they showed similarity result with *F. hirta* this results were in agreements with **(Nio et al., 2018)** *Codiaeum variegatum (L.) Blume*.

The average AT nucleotide composition for *rbcL* of 14 species of *Ficus* was found to be (58. 3%) higher than GC content (41.75%) Similarly, in *matK*, average AT contents (59.46%) were higher as compared to the GC contents (31.4%). Similar findings have been reported by **(Ismail et al., 2020)** in *Acacia sp*.

The nucleotide transition substitution for *rbcL* gene was lower than transversion (R = 0.956 bp) and observed nucleotide diversity was higher than expected (л= 0.254768) indicated moderate of evolution rate among species **(Ahmed and Fadl, 2019)**. However, The *matK* gene showed a high rate of nucleotide substitutions (R = 1.172) that inhibited PCR amplification.

Tajima D test Neutrality for *rbcL* revealed positive value, determined balancing selection to increase of moderate frequency alleles. On contrast The Tajima D for *matK* was negative value this indicated that this gene under purify selection were removing variation between individuals (**Biswas and Akey, 2006).**

Finally these results strengthen that *rbcL* is suitable gene for identification at the species level, similar results were in accordance with **(Maloukh et al., 2017)** and contrary to **(chen et al., 2010**)

CONCLUSION

The two barcode regions of *rbcL* and *matK* have a similar low identification at the cultivar level. Plastid *matK* region has few of GC content and more nucleotide substitutions, which evolves faster than rbcL region between the tested plants. Based on the estimation of recoverability, goodness of sequence and level of species discrimination the *rbcL* was higher than the *matK* gene in amplification, sequencing and identification. The *rbcL* could resolved various species belonged to *Ficus* genus region so, the *rbcL* region consider as a favorable barcode locus for different plant species.

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الملخص العربى

دراسات وراثية ومورفولوجية على أشجار الفيكس

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تهدف هذه الدراسة الى توثيق النباتات وأعادة تصنيف وتعريف الانواع عن طريق الفروق المورفولوجية التقسيمية التقليدية وأيضا بأستخدام تقنية الترميز الوراثى DNA barcoding وذلك بمقارنة تتابعات *rbcL* و *matK* المستخدمة فى الدراسة كجينات ترميز الحمض النووى وذلك بين الانواع الخمسة عشر المختلفة التى تم جمعها من حديقة انطونيادس –الاسكندرية وهي := *F. retusa, F. benjamina, F. afzelii, F. microcarpa hawii, F. lyrata, F. elastica Roxb ex Horenm, F. benjamina var golden, F. sycomorus, F. elastica, F. religiosa, F. altissima, F. benghalensis, F. aspera, F. tinctoria and F.platyphlla* لتوضيح حالة الشجرة الوراثية وكشف درجة التشابه والتماثل بينهم وتحديد ما إذا كانت الشفرة الوراثية الخيطية قادرة على ملاحظة الأختلافات وحل الغموض ما بين هذه الانواع وكانت النتائج كالتالى: نسبة نجاح تضخيم الجين *rbcL* كانت 93 ٪ ونسبة نجاح تضخيم الجين *matK* كانت 80 ٪. تم فحص تسلسل *matK* و *rbcL* لجميع العينات بواسطة قواعد بيانات GenBank حيث تم تحديدaccession numbers لـ 14نوع لتتابعات جين *rbcL* و ل11 نوع لتتابعات *matK* وبناءا على ذلك تعتبر منطقة *rbcL* مكانًا مناسبًا للرموز الشريطية لجميع أنواع النباتات في العمل الحالى نظرا لقوته التمييزية على تعريف الانواع بالمقارنة بجين ال *matK* . ولقد أظهر تحليل الشجرة الوراثية phylogenetic tree ان اكثر الانواع تماثل كان بين *F. , F.retusa benjamina, F. microcarpa hawii,* واكثرهم تباعد *F. afzelii*.