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Genetic Diversity and Biochemical Activity of Leaves and Fruits of Main *Ficus* Sp. Grown in Egypt

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Abstract: *Ficus* sp. is a globally distributed species with growing genetic, economic, environmental, nutritional and medicinal importance. Egypt is one of the countries which the *Ficus* sp. dominate its cities and northern and eastern costs resorts on the Mediterranean and Red seas. In this study we investigated the genetic relations among nine *Ficus* sp. in Egypt (*Ficus retusa* L., *F*. Dejentle, *F*. Golden, *F. religiosa*, *F. trigonata, F. carica, F. sycamorus, F. elastica* Decora and *F. benjamina*) using 15 RAPD primers. Leaves and fruits extracts were investigated for their antioxidant activity by 1, 1-diphenyl, 2-picrylhydrazyl (DPPH) and determination of total phenolic content using Folin-Ciocalteu's method. The results showed high polymorphism among all primers. Data analysis using genetic distance and UPGMA showed the close genetic relatedness between *F*. Golden and *F*. Dejentle, twining of species and that the furthest species was *F*. Decora, furthermore, the highest antioxidant activity in both of leaves and fruits methanolic extracts was found in *F*. Decora. Top three species in leaves phenolic contents were *F. religiousa, F. sycamorus* and *F. retusa*, while the highest leaves and fruit phenolic content was found in *F. religiousa*. Our data represent the first specialized genetic study of Egyptian Ficus trees, which will facilitate future breeding and selection programs in this field. The analyses of biochemical activities showed the Egyptian grown *Ficus* sp. is a rich source of antioxidants for human health and pharmaceutical industry.

Key words: *Ficus* sp. · DPPH · Phenolic · Diversity · RAPD

INTRODUCTION

Ficus sp. Linn (family Moraceae) represents one of the largest genera of flowering plants with about 800 species [1]. The genus Ficus is well-known for its high diversity and the large number of varieties/accessions scattered across the tropical and semitropical areas of the planet. Such diversity is facing genetic erosion and hampered accession/cultivar morphological identification. Protection of genetic resources and further plant breeding programs urged genetic identification of *Ficus* sp. with simple genetic methods like ISSR [2], therefore the need for an accurate method of phylogenetic relationships and genetic identification is crucial. Egypt is known for its trees diversity including *Ficus* which is found in Egyptian main gardens like Orman garden in Cairo and Antoniades garden in Alexandria (over 50 species in Egypt). These gardens contain many *Ficus* species including *Ficus retusa* L., *F*. Dejentle, *F. golden*, *F. religiosa, F. trigonata, F. carica, F. sycamorus, F. elastica* Decora and *F. benjamina. Ficus* sp. includes several street trees "used in street decoration and fighting pollution" like *F. retusa*, *F*. Decora and *F. sycamorus* which are main street trees in Egypt. *F. retusa* is a fast growing evergreen tree with leathery green leaves reaches the height of 15 meters or more in some cases and tolerates trimming and formation process.

The genus usually tolerate pruning, trimming and formation especially *F. retusa*. Thousands of tons of leaves, branches and fruits of *F. retusa* and other species annually remain from trimming and formation process of these trees are disposed improperly as either garbage or

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in the best cases as compost. The annual production of fruits of these street trees was not studied in Egypt although fruits contain a antioxidants and many medical compounds. The genus produces fruits, globally known as figs. *F. carica* and *F. sycamorus* are considered important fruit trees in Egypt. The *F. carica* is genetically diverse, contains hundreds of accessions, the world production of its fruits is about one million tons and it is mostly concentrated in the Mediterranean [3]. The medicinal value of leaves and fruits of local grown *Ficus* sp. as optimal resources of antioxidants and phenolic compounds are not well studied for our knowledge. Fruits of several species like *F. sycomorous, F. carica* and others are freshly eaten in Egypt also they were used in ancient and medieval medicament for the treatment of several diseases as cancer, tumors and as anti-inflammatory [4]. Other species like *F. religiosa*, *F. benjamina* and *F. bengalensis* in India are either consumed fresh or their extracts are used in folk medicine and had anticancer and antibacterial activities [5, 6, 7].

In India and China, plant parts differ in their uses were leaves of *F. racemosa* are used for the treatment of gastrointestinal problems [8], bark of *F. arnottiana* and *F. hispida* shows hypoglycaemic activity [9, 10], Roots of *F. bengalensis* inhibit insulinase and fruit extracts exhibits anti-tumour activity [11]. Ethanolic wood extracts of *F. glomerata* has Anti-HIV-1 integrase activity [12]. *F. religiosa* leaves extracts used for sexual disorders asthma, cough and others [7].

In this study we used RAPD markers to elucidate the genetic relationships among main *Ficus* sp. in Egypt, in addition to biochemical assays to study natural Egyptian resources of antioxidants and phenolic contents and to find possible relation with genetic characters.

MATERIALS AND METHODS

Chemicals: Methanol, ethanol, Gallic acid and 2, 2-diphenypicrylhydrazyl (DPPH) from Sigma Aldrich Egypt. Sodium carbonate and Folin-Ciocalteu's reagent from Merck Chemical Co.

Plant Material: Leaf samples from grown trees of *Ficus retusa* L., *Ficus* Dejentle, *Ficus* Golden, *F. religiosa, F. trigonata, F. carica, F. sycamorus, F. elastica* Decora and *F. benjamina* located in Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt were obtained on 11 July 2012. Fruit samples were obtained during the period from 15 April to 16 September 2012 for each of *F. retusa, F. religiousa, F. carica*, *F. sycamorus* and *F. lyrata*. Trees were identified by Dr. Nader Elshanhory and Dr. Hesham Ali, Antoniadis Research Centre and obtained voucher numbers in Egypt barcode of life project (www.egyptbol.org), Faculty of Agriculture, Alexandria University and their voucher numbers were Hosam 00034, Hosam 00100, Hosam 00099, Hosam 00035, Hosam 00029, Hosam 00382, Hosam 00383, Hosam 00033, Hosam 00028, Hosam 00021 for *Ficus retusa* L., *Ficus* Dejentle, *Ficus* Golden, *F. religiosa, F. trigonata, F. carica, F. sycamorus, F. elastica* Decora, *F. benjamina* and *F. lyrata*, respectively.

DNA Extraction: Young leaves were taken from each plant (except *F. lyrata*) and thoroughly washed with water then ethanol to remove dust and other contaminants. The DNA was extracted using QIAGEN DNA extraction kit, USA. The DNA was quantified spectrophotometrically at 260 nm and by electrophoresis on 0.8% agarose gels.

RAPD Analysis: PCR reactions were performed using 15 RAPD primers showed in Table 1. Total Genomic DNA of each accession was diluted in sterile double distilled water to a concentration of 10 ng/ml for RAPD analysis. PCR was performed in PEQLAB thermocycler, Germany in a 25 µl reaction volume containing 200 µM of each dNTP (MBI Fermentas), 3.0 mM $MgCl_2$, 0.48 µM primer, magnesium-free reaction buffer and 1U *Taq*DNA polymerase (Promega, USA). After initial heating for 5 min at 94°C, samples were PCR amplified using 40 cycles (94°C, 20 S; 42°C, 20 S, 72°C, 1 min.) followed by a final extension of the PCR products for 4 min at 72°C. The products of amplification were analyzed by electrophoresis in 2.0% agarose gels with 1xTAE running buffer, visualized by ethidium bromide staining and photographed under UV light with a digital Canon power shot G7 camera. Each reaction was repeated twice and negative controls accompanied the reactions without adding DNA for increasing the fidelity of the data. Fifteen RAPD primers (Table 1) were used; all of them yielded polymorphic markers. DNA extraction and PCR were performed in the Department of Floriculture and Ornamental Horticulture, Faculty of Agriculture, Alexandria University in July 15th 2012.

Data Analysis: All visible and unambiguously scorable fragments amplified by the primers were scored by visual observation. The amplified bands were scored as (1) for presence and (0) for the absence of all samples. The scores obtained using primers in the RAPD analysis were then joined and used to estimate genetic distances using Jaccard coefficient [17] in a computer program,

Table 1: Code and sequence of RAPD primers used in this study.

Primer	Sequence5`-3`
OPA-7	GAAACG GGT G
OPA-11	CAATCG CCG T
OPC-13	AAGCCT CGT C
RAPDA1	GGTGCGGGAA
RAPDA2	GTTTCGCTCC
RAPDA3	GTAGACCCGT
RAPDA4	AAGAGCCCGT
RAPDA5	AACGCGCAAC
RAPDA6	CCCGTCAGCA
OPF-08	GGGATATCGG
OPF-07	CCGATATCCC
OPH-14	ACCAGGTTGG
OPH-20	GGGAGACATC
OPD-05	TGAGCGGACA
UBC-211	GAAGCGCGAT

RAP Distance [18]. UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram was created using genetic distances in PAUP4 [19].

Preparation of Methanolic Extracts: Preparation of methanolic extract followed the protocol of Salem *et al*. [13] with some modifications. The fresh leaves were washed, cut into small pieces (30g) then extracted by soaking with 300 ml of 80% aqueous methanol. After filtration the residue was processed similarly with the same amount of solvent. The methanol extract was concentrated to dryness under reduced pressure at 45°C with a rotary evaporator, lyophilized and were stored at 4°C until further use.

Antioxidant Capacity: Free radical scavenging activity of the samples was determined using the 2, 2-diphenypicrylhydrazyl (DPPH) method following Elansary *et al.* [14] with some modifications. The reaction mixture was mixed for 10 s and left to stand in fibber box at room temperature in the dark for 30 min. The absorbance was measured at 517 nm, using a UV scanning spectrophotometer (Unico® 1200). Total antioxidant activity (TAA) was expressed as the percentage inhibition of the DPPH radical and was determined by the following equation: Where TAA is the total antioxidant activity and Abs is the absorbance, Abs control: The absorbance of control reaction and Abs sample: The absorbance of the sample.

$$\%TAA = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Tests were carried out in triplicate. The concentration of sample required to scavenge 50% of DPPH (SC50) was determined. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity.

Total Phenolics Content Determination: Determination of total phenolics content in methanolic extracts of leaves and fruits was carried out according to Amerine and Ough [15] and Singleton and Rossi [16] using a Folin-Ciocalteau colorimetric method, calibrating against Gallic acid as the reference standard and expressing the results as Gallic acid equivalents (GAE).

RESULTS AND DISCUSSION

RAPD: RAPD analysis of nine species of *Ficus* revealed that all primers were polymorphic. Polymorphic primers produced a total of 163 bands (Fig. 1). The number of bands per sample ranged from 6-15, corresponding to an average of 10.86 bands per primer. In this study, genetic distances were developed on the basis of the scorable banding patterns of the 9 genotypes using 15 RAPD primers. Distances among the nine accessions ranged from 0.305085 to 0.846939 as shown in Table 2. The two most closely related genotypes were Golden and Dejentle with the smallest genetic distance (0.305085) whereas the largest genetic distance was 0.846939 between *F. religiousa* and *F. sycamours* (Table 2). RAPD primers developed markers for the identification of each species under investigation like RAPDA2, RAPDA1 and UBC211 (Fig. 1). The primers RAPDA1, RAPDA4 and RAPDA5 produced the highest number of bands also showed a higher level of polymorphism and while others showed lower polymorphism. The dendrogram based on genetic distance Unweighted Pair Group Method of Arithmetic Means (UPGMA) (Fig. 2) indicated that the *Ficus* species could be grouped into twins: *F.* Golden and *F. Dejentle*, *F. sycamorus* and *F. benjamina*, *F. trigonata* and *F. carica* and *F. religiousa* and *F. retusa*. The dendogram also showed that the furthest species was *F. Decora*. The twining of species in genetic studies of *Ficus* species using DNA barcodes showed similar pattern in Mexico [20] and in Egypt (Elansary, unpublished). The genetic characters might be reflected on morphological and physiological properties [21] and might not be the case [22]. Morphorlogically, *F.* Dejentle *and F.* Golden are very similar but this assumption was not applicable in other cases.

Table 2: Genetic distance based on mean character difference

Items	*F. retusa*	*F. Dejentle*	*F. Golden*	*F. religiousa*	*F. trigonata*	*F. carica*	*F. sycamorus*	*F. Decora*	*F. benjamina*
F. retusa	0								
F. Dejentle	0.701299	0							
F. Golden	0.613333	0.305085	0						
F. religiousa	0.662162	0.766234	0.792683	0					
F. trigonata	0.680556	0.71831	0.716216	0.783784	0				
F. carica	0.726027	0.797297	0.776316	0.794521	0.707692	0			
F. sycamorus	0.783505	0.76087	0.795918	0.846939	0.802198	0.784091	0		
F. Decora	0.793478	0.836957	0.806452	0.822222	0.840909	0.837209	0.762376	0	
F. benjamina	0.734043	0.678161	0.677778	0.824742	0.705882	0.786517	0.585106	0.764706	0

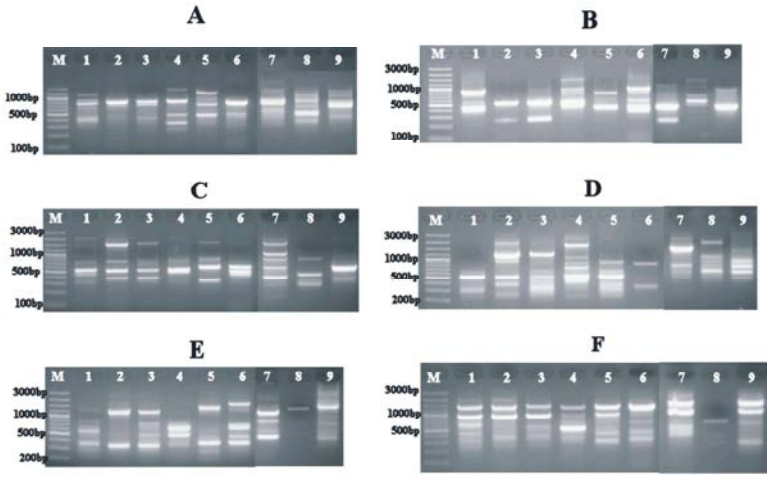

Fig. 1: RAPD profiles of 5 accessions of *Ficus* sp. A)RAPDA6 B)RAPDA2C)RAPDA3 D)UBC211 E)RAPDA1 F)OPH-14M)Molecular weight marker

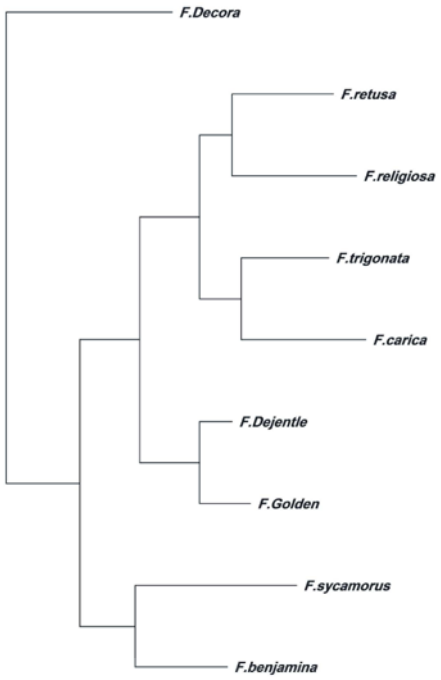

Fig. 2: Dendrogram of the nine *Ficus* sp. in Egypt based on UPGMA analysis

Antioxidant Capacity: Free radical scavenging activity using DPPH results presented as TAA % (Total Antioxidant Activity) were 37, 5, 14, 67, 6, 16,14, 81, 70 and 39% for *F. retusa* L., *F.* Dejentle, *F.* Golden, *F. religiosa, F. trigonata, F. carica, F. sycamorus, F. elastica* Decora and *F. benjamina*, respectively (Table 3). The highest antioxidant activity in both of leaves and fruits methanolic extracts was found in *F. elastica* Decora followed by *F. benjamina*. The lowest leaves extract TAA was found in *F.* Dejentle. TAA% in fruit extracts was 20, 65, 32, 21 and 39 for *F. retusa, F. religiousa, F. carica*, *F. sycamorus* and *F. lyrata* respectively. TAA% was higher in fruit extracts of each of *F. carica* and *F. sycamorus* than their leaves, in the other hand; it was lower in fruits extracts of *F. retusa* and *F. religiousa* than their leaves extracts. DPPH free radical scavenging activity SC_{50} (µg/ml) was also determined for each extract (Table 3), where SC_{50} is the concentration in µg/ml required for scavenging the DPPH radical (20 µg/ml) by 50%. The lowest value of SC_{50} was achieved in *F. elastica* Decora (5.45) indicating powerful antioxidant components. *F. religiousa* fruits showed the highest total antioxidant activity among examined fruits but

Table 3: %TAA and free radical scavenging activity (antiradical) of methanol extracts of *Ficus* species.

Items	% TAA	DPPH free radical scavenging activity SC50 (µg/ml)[a]
Leaves		
F. retusa	37	12.44 ±1.2
F. Dejentle	5	88.75 ±1.5
F. Golden	14	30.90 ±1.4
F. religiousa	67	6.64 ±1.5
F. trigonata	6	71.77 ±1.6
F. carica	16	28.42 ±1.2
F. sycamorus	14	32.35 ±1.5
F. Decora	81	5.45 ±1.6
F. benjamina	70	6.38 ±1.4
Fruits		
F. retusa	20	21.79 ±1.6
F. religiousa	65	6.89 ±1.4
F. carica	32	13.73 ±1.3
F. sycamorus	21	21.63 ±1.6
F. lyrata	39	11.30 ±1.2

Values of SC50 are expressed as mean of duplicate determinations ±standard deviation.

[a]SC50, Concentration in lg/ml required scavenging the DPPH radical (20 µg/ml) by 50%.

Table 4: Total phenolics content of methanol extracts of fresh leaves and fruits of *Ficus* species

Items	Total phenolics content (using MeOH80%) (mg GAE/g ext.)
Leaves	
F. retusa	123
F. Dejentle	60
F. Golden	70
F. religiousa	201
F. trigonata	35
F. carica	69
F. sycamorus	140
F. Decora	71
F. benjamina	58
Fruits	
F. retusa	80
F. religiousa	171
F. carica	50
F. sycamorus	130
F. lyrata	134

unfortunately neither has acceptable taste characteristics as in *F. carica* nor even low acceptable taste as in *F. sycamorus*. *F. retusa* fruits don't have sugary taste as in *F. carica* and *F. sycamours*. The only two fruits of the selected species in this study favored by consumers are *F. sycamorus* and *F. carica*. Most of these *Ficus* species are used in landscape gardening of public and private gardens. Some of *Ficus* are preferred as street trees like *F. retusa, F. elastica* Decora and *F. benjamina*.

In Egypt, there are millions of *F. retusa* grown in streets, resorts in the Red Sea or the Mediterranean North coast and in compounds scattered across the country. Other species might be grown in public gardens or in some streets like *F. religiousa, F. carica*, *F. sycamorus*, *F. trigonatata*, *F.* Golden and *F.* Dejentle. There is an annual pruning for all these large trees but the ultimate destination of the pruning remaining is either lost or burned. The economic utilization of large amount of fruits produced and the annual remaining of pruning process (leaves and branches) of the main street trees in Egypt as the case of *F. retusa* might be of great value if used as a natural source of antioxidant materials. *F. carica* and *F. sycamours* fruits are already sold in Egypt and favored by Egyptians and our results recommend the consumption of these fruits as natural resources of antioxidants. The use of some species of *Ficus* in traditional medicine or as a natural source of antioxidant had been studied in other countries [23, 24, 25]. The antioxidant activity is mainly due to the presence of phenolic compounds and flavonoids which scavenge free radicals, chelate metal-ions and may inhibit enzymes [26, 27]. The antioxidant activities depend on the species and environmental conditions [28] and the search for new natural resources for antioxidants will continue in this genus because of the large genetic diversity within it. Genetically, the furthest species in this study was *F. elastica* Decora. It was found that the highest antioxidant activity was in *F.* Decora leaves extract. The genetic twin of *F. religiousa and F. sycamorus* fruits extracts showed the highest antioxidant activities.

Phenolic Content in Leaves: Phenolic compounds play a role in antioxidant activity in many species consequently affecting the medicinal value of each plant part. Phenolic compounds in leaves extracts of nine species of *Ficus* and fruit extracts of five species were investigated using a Folin-Ciocalteau colorimetric method. Total phenolic contents in leaves extracts expressed (mg GAE/g ext.) were 123, 60, 70, 201, 35, 69,140, 71, 58 and 134 for *F. retusa* L., *F.* Dejentle, *F.* Golden, *F. religiosa, F. trigonata, F. carica, F. sycamorus, F. elastica* Decora and *F. benjamina*, respectively. The highest value achieved was in *F. religiosa* (201 mg GAE/g ext.) and the lowest value was found in *F. trigonata.* Top three species in leaves phenolic contents were *F. religiousa, F. sycamorus* (140) and *F. retusa* (123) which may suggest the possible utilization of these leaves as source of natural phenols. *F. retusa* and *F. religiousa* was genetic twin and showed close genetic distances might be

reflected on their phenolic contents of leaves as the case here. The genetic twin of *F.* Dejentle & *F.* Golden which showed the closest genetic distances had narrow difference in their leaves extract phenolic contents. Phenolics are major factors affecting fruit quality due to relatedness to taste, color and nutritional value. In fruits extracts, total phenolic contents were 80, 171, 50, 130 and 134 for *F. retusa, F. religiousa, F. carica*, *F. sycamorus and F. lyrata,* respectively. *F. carica* fruits showed low phenolic contents which indicate acceptable taste for its fruits compared to other fruits of the same genus showing higher content of phenols like *F. religiosa* and *F. lyrata*. *F. sycamorus* is not favored as *F. carica* in Egypt due to taste characteristics and important reason is "phenolics" were *F. sycamorus* showed higher content of phenols as found here. The consumption of fruits rich in phenolic compounds may prevent the diseases in which homeostasis is impaired by oxidative features, also leaves proven to have high amounts in phenolic compounds may be used in pharmaceutical industries and the treatment of dermatological diseases [29]. Studies conducted on other species of *Ficus* in other countries showed variable amounts of phenolic content [30, 31].

CONCLUSION

Single marker identification system was developed using RAPD markers. Genetically, the furthest species in this study was *F.* Decora. Also, we found that the highest antioxidant activity was in *F. elastica* Decora leaves extract. Top three species in leaves phenolic contents were *F. religiousa, F. sycamorus* and *F. retusa* which may suggest the possible utilization of these leaves as source of natural phenols. Fruits consumption of *F. carica* and *F. sycamorus* is highly recommended for their richness in antioxidants. Indeed, genetic relationships within *Ficus* sp. still need additional work for applying on the rest of the genus.

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