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Polymorphic Analysis and Genetic Similarity of Genus *Ficus* L. (Moraceae) in Egypt

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NINTEEN SPECIES belonging to genus *Ficus* were obtained from three Egyptian gardens to study the information derived from ISSR fingerprinting, and to estimate the level of polymorphism and genetic similarity. Five ISSR primers were used to estimate the level of polymorphism among the different species. This study indicates that, the total number of bands detected by the different ISSR primers was 229 all of them were polymorphic, representing a level of polymorphism of 100% and an average number of 46 polymorphic bands per primer. The ISSR analysis revealed the highest genetic similarity (85%) between *F. afzelii* and *F. benghalensis*, while the lowest genetic similarity (58%) was observed between *F. carica* and *F. sycomorus*. The obtained results clearly revealed a high level of similarity among the investigated *Ficus* species, ensuring the highest degree of homology and the narrow genetic background of these species. The two studied taxa of subgenus *Ficus*, viz., *F. carica* and *F. deltoidea* were widely separated and showed relations with the taxa of subgenus *Urostigma*. Also, the three studied taxa of subgenus *Sycomorus* possessed relations with members of subgenus *Urostigma.*

Keywords: *Ficus*, ISSR, Polymorphism, Fingerprinting.

Ficus L. (Moraceae) constitutes one of the largest genera of angiosperms (Frodin, 2004), consisting of about 1000 species from pantropical, subtropical origins, several of which are desirable interior foliage plants. *Ficus* includes a large number of indoor ornamental plants and garden and roadside trees (Wagner *et al*., 1999), and formed a distinctive monophyletic clade within the family (Judd *et al.*, 1999). The classification of *Ficus* emphasized on two items; the first is whether the species is monoecious or functionally dioecious (gynodioecious), the second is on the tight coevolutionary relationship that exists between *Ficus* species and their specific wasp pollinators (Weiblen, 2000). One of the most widely adopted infrageneric classification of *Ficus* is that of Corner (1965). In that classification, *Ficus* is divided into four subgenera (*Urostigma*, *Pharmacosycea*, *Sycomorus* and *Ficus*), with the functionally dioecious species united under the subgenus *Ficus*. Yet, in the most recent classification by Berg and Corner (2005), *Ficus* is divided into six subgenera and a number of sections. Several DNA-markers (RAPD, RFLP, SSR, and ISSR) have available to identify the varieties / accessions. These markers can be effectively used to answer the phylogenetic

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relationship between *Ficus* accessions (Chatti *et al.*, 2007). Inter simple sequence repeat (ISSR) overcomes many of the limitations faced by different marker system and has a higher reproducibility (Guasmi *et al.*, 2006). For instance ISSR markers may offer considerable variation among varieties and have been widely used in cultivated species (Wolfe and Liston, 1998). ISSR has been described as a powerful technique to assess genetic diversity among closely related species and to detect similarities between and within plant species levels (Ghariani *et al.*, 2003). The optimal utilization of diversity ISSR-PCR has been used widely in plants for the analysis of genetic relationships between and within species (Martin and Yelamo, 2000), assessment of hybridization in natural populations (Wolfe *et al*., 1998 a&b) and germplasm analysis (Gillbert *et al*., 1999). Further, ISSR-PCR is useful in fingerprinting and characterization of accessions (McGregor *et al*., 2000) and identification of cultivars and varieties (Kumar *et al.*, 2001). Occasionally, it has been used to study relationships at the interspecific level (Huang and Sun, 2000).

Salhi-Hannachi *et al* (2005) compared the genetic diversity in two Tunisian fig cultivars by using RAPD and ISSR markers. As *Ficus* species are represented by a large number of varieties / accessions which are facing genetic erosion. Rout and Aparajita (2009) proved that clear cut separation of the 23 *Ficus* accessions and were in broad agreement with the morphology. Both molecular and morphological markers will be useful for preservation of the *Ficus* germplasm. They demonstrated that information for accession identification and the presence of accessions in the natural distribution of parental species for *Ficus* have been confirmed with ISSR markers. This analysis is quick and reproducible, can generate sufficient polymorphism to identify the *Ficus* accessions, although most ISSR alleles are dominant rather than co-dominant. Using some of the co-dominant markers like SSR can further check the findings.

Nabil and Abou-Ellail (2013) proved that RAPD markers are useful for germplasm discrimination as well as for investigation of patterns of variation in seven Fig (*Ficus carica*) cultivars. These results indicated that RAPD is useful, rapid and accurate technique for studying genetic diversity and germplasm characterization of *Ficus carica* some cultivars. There is a wide spectrum genetic variation among studied fig varieties, these variation could be an effective factor in breeding program.

The main objectives of the present study are; study the taxonomic information through the investigation of DNA cretiria, compare and bind out the relationships between the studied species on the bases of DNA fingerprint using ISSR-PCR analysis, estimate the level of polymorphism and genetic similarity and identify some moleculer genetic markers which help in identification of the taxa under investigation.

Material and Methods

The studied nineteen *Ficus* species are outlined in the following Table:

TABLE 1. Data collection.

No	Species	Subgenus	Source
1	***Ficus afzelii*** G. Don., In J. C. Loudon, Hort. Brit. ed. 1: 416.1830 = *F. saussureana* DC.	*Urostigma*	OBG
2	***F. benghalensis*** L. in Sp. Pl. 2: 1059. 1753 = *F. indica* L.	*Urostigma*	OBG
3	***F. benjamina*** var. ***comosa*** (Roxb.) Kurz, In Forest Fl. Burma 2: 446. 1877.	*Urostigma*	AG
4	***F. carica*** L. In Sp. Pl. 2: 1059. 1753 = *F. carica* L. var. *rupestris* Hausskn	*Ficus*	AG
5	***F. cordata*** Thumb. subsp. ***salicifolia*** (Vahl) = *F. salicifolia* Vahl, In Symb. Bot. 1: 82. 1790.	*Urostigma*	AG
6	***F. cunninghamii*** Miq., In Ann. Mus. Bot. Lugd. Bat. iii, 286-Austral.	*Urostigma*	OBG
7	***F. deltoidea*** Jack, In Malayan Misc. 2(7):71.1822. = *F. diversifolia* Blume	*Ficus*	AG
8	***F. elastica*** Roxb. ex Hornem, In Hort. Bot. Hahn. Suppl. 7.1819 = *F. decora* Hort.	*Urostigma*	AG
9	***F. infectoria*** Roxb., In Ann. Bot. Gard. Calcutta, i.l.t. 75, 84(1887). = *F. virens* Aiton	*Urostigma*	ZG
10	***F. laurifolia*** Hort. ex. Lam., In Encycl. Meth. (Bot.) 2: 495. 1786. = *F. inspida* wika = *F. glabrata* H. B. K.= *F. anthelemintica* Mart.	*Urostigma*	ZG
11	***F. macrophylla*** Desf. ex Pers., In Syn. Pl. 2: 609. 1807 = *F. magnolioides* Borzi	*Urostigma*	OBG
12	***Ficus mysorensis*** B.Heyne ex Roth, In J. J. Roemer & J. A. Schultes, Syst. Veg. 1:508.1817(A. W. Roth, Nov. Pl. Sp. 390.182 = *F. drupacea* var. *pubescens* (Roth.) corer	*Urostigma*	OBG
13	***F. palmata*** Forsk. = *F. pseudosycomorus* Decne., In Ann. Sc. Nat. Ser. II.ii. (1834) 242.	*Sycomorus*	OBG
14	***F. platypoda*** (Miq.) A. Cunn. ex Miq., In, Ann. Mus. Bot. Lugduno-Batavum 3: 287. 1867 = *Urostigma platipodum*	*Urostigma*	OBG
15	***F. racemosa*** Wall-Cat. 1799 = *F. glomorata* Roxb., In Pl. Coromandel 2: 13, t. 123.	*Sycomorus*	OBG
16	***F. religiosa*** L., In Sp. Pl. 2:1059. 1753	*Urostigma*	AG
17	***F. retusa*** L. = *F. nitida* Thunb. In *Ficus* 10.1786.	*Urostigma*	AG
18	***F. sprague****ana* Mildbr. & Burret, In, Bot. Jahrb. Syst. 46: 253. 1911.	*Urostigma*	OBG
19	***F. sycomorus*** L., In Sp. Pl. 1059. 1753.	*Sycomorus*	OBG

Author citation and synonymy were verified according to W^3 TROPICOS (2008), GRIN(2008) and IPNI (2008). OBG=Orman Botanical Garden, Ministry of Agriculture, Dokky, Giza, Egypt, AG=Botanical Garden of Ain Shams University, Faculty of Science, Abbasia, Cairo, Egypt, ZG=Zoo-Garden, Dokky, Giza, Egypt.

Extraction and purification of genomic DNA

The genome DNA of studied species was extracted using CTAB (hexadecyl trimethyl ammonium bromide) assay as described by Porebski *et al*. (1997).

Inter Simple Sequence Repeats (ISSRs)

ISSR markers involve PCR amplification of DNA using a single primer composed of microsatellite sequence Primer Code IS3, IS4, IS6, IS10 and A9 (Bioneer, sequencing service Daedeok-gu, Daezeon 306-220, South Korea) such as (CA)7 anchored at the 3` or 5` end by 2-4 arbitrary, often degenerate nucleotides. The sequences of repeats and anchored nucleotides were randomly selected. The technique was carried out according to Adawy *et al*. (2002 and 2004a). Five oligonucleotides composed wholly of defined, short tandem repeat sequences with anchor, and representing different microsatellites (di- and tri-repeats) were used as generic primers in PCR amplification of inter simple sequence repeat regions. Oligonucleotide primers to microsatellite repeats (Table 2) were synthesized on an ABI 392 DNA/RNA synthesizer at Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt.

TABLE 2. Name and sequence of the primers used in ISSR detection.

Primer Code	Sequence (5`- 3`)
IS3	$TTT(TCC)_5$
IS4	$CAT(CA)_7T$
IS6	$(GA)_8CG$
IS10	$(TCC)_5AC$
A9	$(AGC)_4AC$

ISSR-PCR reaction and thermo-cycling profile

PCR was performed in 25 µl reaction volume containing 1X PCR buffer, 1.75 mM $MgCl_2$, 5 mM of each dNTPs, 40 pM oligonucleotide primer, 25 ng genomic DNA and one unit of *Taq* DNA polymerase. The PCR amplification conditions were performed as follows: (1) an initial denaturation step at 94ºC for 30 sec, 65ºC for 45 sec and 72ºC for 1 min, (2) the annealing temperature was lowered each cycle 1ºC during nine cycles, which gave a touch down phase of ten cycles, (3) thirty-five cycles performed at 94ºC for 30 sec, 55ºC for 45 sec. and 72ºC for 1 min, and an extension cycle at 72ºC, (4) the PCR products were separated on 1.5% agarose gel in 1X TBE buffer containing ethidium bromide and photographed with a Polaroid camera.

Data analysis

The banding patterns generated by ISSR markers were used to determine the genetic relatedness of 19 *Ficus* species. Clear and distinct amplification products were scored as (1) for presence and (0) for absence of the developed bands. The similarity coefficient between two genotypes was estimated according to Jaccard's coefficient (Jaccard, 1908).

Jaccard's formula: $GS= N_{AB} / (N_{AB}+N_A+N_B)$, where
GS: is the measure of genetic similarity between two samples
N_{AB}: is the number of bands shared by A and B,
N_A: is the number of bands present in sample A,
N_B: is the number of bands present in sample B.

The similarity matrix was used in the cluster analysis by using the NTSYS-pc software version 2.02 (Exeter Software, NY, USA; Rohlf, 1998), where the SIMQUAL program was used to calculate Jaccard's coefficients. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomies. At the first step, when each species represents its own cluster, the distance between these species are defined by the chosen distance measure (Jaccard's coefficient). However, once several species have been linked together, the distance between two clusters is calculated as the average distance between all pairs of species in the two different clusters. This method is called Unweighted Pair Group Method using Arithmatic Average (UPGMA) using Sequential Agglomerative, Hierarchical and Nested cluster (SAHN) (Sneath and Sokal, 1973).

Results and Discussion

ISSR diversity as revealed by ISSR markers

The studied species were analyzed using five Inter Simple Sequence Repeat (ISSR) primers. These primers were anchored either at the 5` end or at the 3` end or at both ends. The amplification results of the ISSR primers used in this investigation are presented in (Table 2). The five primers including two dinucleotide repeat and three tri-nucleotide repeat produced good reproducible and scorable patterns and the amplification profiles were screened for the presence of polymorphisms among the studied nineteen *Ficus* species (Fig. 1. A-E).

As shown in (Table 3), a total of 229 fragments were generated by the five primers with an average of 46 fragments / primer. Trinuceotide 3' anchored primer IS10 yielded the highest number of products (52 fragments), while trinucleotide 5' anchored primer IS3 detected the lowest number of products (36 fragments). On average, one primer was amplified 46 fragments. The numbers of polymorphic bands were 229 with 100% of polymorphism. Moreover, the size of the amplified fragments varied with different primers, ranging from 2402 to 175 bp. Among different species, *F. platypoda* showed the highest number of polymorphic bands (62), whereas *F. benghalensis* showed the lowest number of polymorphic bands (31).

Genetic relationships as revealed by DNA marker

Detection of genetic variation and determination of genetic relationships between species is an important consideration for the efficient conservation and utilization of plant genetic resources. Once the morphological traits or the generated molecular marker profiles have been evaluated, there are different strategies to estimate the similarity between the analyzed individuals. Similarity indices measure the amount of closeness between two species, the larger the value the more similar are the two species.

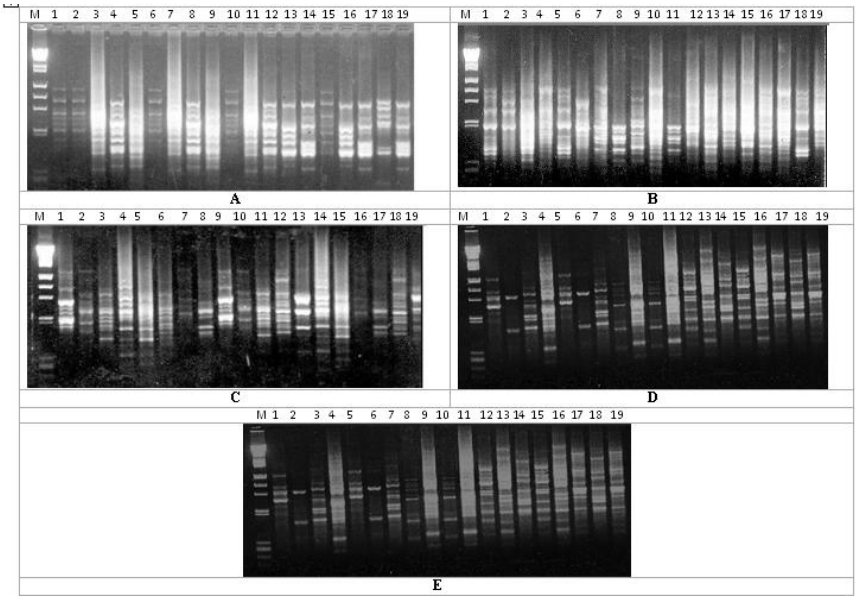

Fig. 1. ISSR profiles obtained by different primers on the studied 19 *Ficus* species A:IS3, B: IS4, C: IS6, D: IS10 and E: A9.

TABLE 3. Total number of bands, polymorphic bands, species-specific bands and percentage of polymorphism revealed by ISSR markers among the studied *Ficus* species.

Primer	sequence	Length of amplification product (bp)	No. of bands	Unique bands	Polymorphic bands	Species-specific percentage for primer
IS3	$TTT(TCC)_5$	1340-211	36	10	36	27.8
IS4	$CAT(CA)_7T$	1768-221	44	8	44	18.18
IS6	$(GA)_8CG$	1845-175	49	8	49	16.32
IS10	$(TCC)_5AC$	2402-196	52	15	52	28.8
A9	$(AGC)_4AC$	1462-193	84	14	48	29.1
Total			229	55	229	
Mean			46	11	46	
Percentage					100	24.04

Genetic relationships as revealed by ISSR markers

The scored data obtained from five primers were used to determine the genetic similarity among the studied species using Jaccard's coefficient (Table 4). The highest similarity percentage (85%) was observed between *F. afzelii* and *F. benghalensis*. This was followed by genetic similarity of (81.7%) between *F. benghalensis* and *F. deltoidea*. *F. infectoria, F. laurifolia, F. benjamina* v *comosa* and *F. cunninghamii* have genetic similarity (80%). *F. elastica* and *F. macrophylla* have genetic similarity (77%). Also, *F. carica* and *F. cunninghamii* have the same genetic similarity. The lowest genetic similarity (58%) was detected between *F. carica* and *F. sycomorus*.

In the present study, five ISSR primers were used for fingerprinting, estimating genetic diversity and relationships of *Ficus* species. By using these primers, 229 discernible DNA fragments were generated with 229 polymorphic ones. The present study revealed quite high polymorphism (100%). The high percentage of polymorphism is common for ISSR amplified products. Prevost and Wilkinson (1999), Hess *et al.* (2000) and Manimekalai and Nagarajan (2006) obtained similar results in *Potato* 90%, *Olea europaea*, 100%, and *Cocos nucifera* 77.4%.

The variation of the polymorphism in the different species can be explained by the hypothesis that the microsatellites, whose sequences are complementary to the primer, were abundant or rare in the genome of the studied species, these microsatellites occupied some sites sufficiently distant not allowing the synthesis of sequences that separating them (Guasmi *et al.*, 2006).

TABLE 4. Genetic similarity matrices among *Ficus*species as computed according to Jaccard's Coefficient.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	1.000																		
2	0.852	1.000																	
3	0.751	0.742	1.000																
4	0.721	0.712	0.742	1.000															
5	0.742	0.786	0.808	0.777	1.000														
6	0.747	0.817	0.734	0.712	0.760	1.000													
7	0.734	0.769	0.694	0.672	0.712	0.725	1.000												
8	0.734	0.803	0.677	0.664	0.712	0.716	0.764	1.000											
9	0.734	0.769	0.703	0.655	0.729	0.681	0.729	0.808	1.000										
10	0.742	0.786	0.703	0.672	0.712	0.760	0.773	0.755	0.755	1.000									
11	0.686	0.729	0.681	0.659	0.707	0.721	0.707	0.751	0.690	0.742	1.000								
12	0.655	0.699	0.659	0.594	0.659	0.655	0.651	0.712	0.659	0.686	0.664	1.000							
13	0.694	0.677	0.664	0.616	0.646	0.668	0.664	0.690	0.672	0.690	0.633	0.664	1.000						
14	0.694	0.721	0.664	0.616	0.681	0.703	0.638	0.707	0.699	0.716	0.694	0.638	0.677	1.000					
15	0.721	0.747	0.681	0.642	0.707	0.747	0.707	0.742	0.699	0.734	0.721	0.716	0.677	0.747	1.000				
16	0.694	0.738	0.646	0.616	0.672	0.686	0.716	0.699	0.725	0.734	0.642	0.655	0.712	0.651	0.668	1.000			
17	0.725	0.742	0.642	0.664	0.677	0.716	0.703	0.747	0.729	0.729	0.655	0.659	0.672	0.734	0.734	0.707	1.000		
18	0.747	0.764	0.672	0.624	0.699	0.738	0.681	0.681	0.672	0.716	0.721	0.664	0.633	0.712	0.721	0.677	0.681	1.000	
19	0.646	0.690	0.598	0.585	0.651	0.646	0.616	0.651	0.624	0.712	0.690	0.633	0.629	0.699	0.672	0.655	0.712	0.672	1.000

ISSR primers based on di-nucleotide repeats reveal high polymorphism (Nagaoka and Ogihara, 1997; Blair *et al.*, 1999, Joshi *et al.*, 2000 and He *et al*., 2009). In this study, ISSR markers revealed high levels of polymorphism with an average of 46 polymorphic bands per primer. At the same time, ISSR primers based on di-nucleotide repeats generated more polymorphic bands than those based on tri-nucleotide 5' anchored repeats but tri-nucleotide 3' anchord repeats generated the highest numbers of polymorphic bands. According to ISSR results, the most closely related species were *F. afzelii* and *F. benghalensis* with the highest similarity index (0.85). On the other hand, the most distantly related species were *F. carica* and *F. sycomorus* with low similarity index (0.58). Danuta *et al*. (2006) and Heikal *et al.* (2008) proved that ISSR is a good tool to assess the genetic similarity and relationships between species.

Unique markers as revealed by ISSR

Unique markers (species-specific markers) were identified, which could easily discriminate between the studied species. Unique markers are defined as bands that are present or absent and specifically identify samples from the others. The bands that present in a sample but not found in the others are termed positive unique markers (PUM) in contrast negative unique markers (NUM), which are absent bands. These bands are used for genotype identification.

In the present study, fifty five amplified fragments were considered as unique markers. The highest number of species-specific marker was 15 markers generated with primer IS10, while the lowest number of species-specific marker was 8 markers generated with primer IS4 and IS6. On the other hand, the highest number of ISSR unique marker was scored for *F. carica* (8 markers) followed by *F. retusa* (7 markers), *F. palmata* (6 markers), *F. elastica*, *F. platypoda*, *F. sycomorus* (5 markers), *F. deltoidea*, *F. infectoria*, *F. laurifolia*, *F. racemosa*, *F. religiosa*, *F. cordata* and *F. spragueana* (2 markers), while the lowest number (1 marker) was scored for *F. benjamina* v. *comosa*, *F. cunninghamii*, *F. macrophylla* and *F. mysorensis*. Seventeen species out of nineteen species could be identified by the use of positive unique marker products. These markers ranged in size from 175 to 2403 bp. A total number of 55 unique markers were identified by all primers used in this investigation.

Ficus benjamina var. *comosa*, *F. cunninghamii* and *F. macrophylla* could be distinguished by the presence of one unique band $IS4_{1084bp}$, $IS6_{421bp}$ and $IS3_{1247bp}$, respectively. Eight species could be distinguished by the presence of two unique bands which were absent in all other species. *Ficus deltoidea* $IS3_{699bp}$ and $IS3_{751bp}$, *F. infectoria* $IS3_{236bp}$ and $IS3_{1003bp}$, *F. laurifolia* $IS3_{480bp}$ and $IS3_{849bp}$, *F. mysorensis* $A9_{745bp}$ and $A9_{1462bp}$, *F. racemosa* $A9_{193bp}$ and $IS6_{201bp}$, *F. religiosa* $IS3_{1293bp}$ and $IS4_{1768bp}$, *F. cordata* $IS10_{1842bp}$ and $IS10_{2402bp}$ and finally *F. spragueana* $IS3_{211bp}$ and $A9_{216bp}$.

TABLE 5. *Ficus* species characterized by unique positive ISSR markers, marker size and total number of the marker identifying each species.

Characters / Species	Unique Positive Marker		
	Primer code	Size of the marker band (bp)	Total no. of unique marker
Ficus afzelii	-----	-----	---------
F. benghalensis	-----	-----	---------
F. benjamina var. *comosa*	IS4	1084	1
F. carica	IS3	1160	8
	IS4	221	
	IS6	175	
		1015	
	IS10	862	
		2064	
		2227	
	A9	665	
F. cordata subsp. *salicifolia*	IS10	1842	2
		2402	
F. cunninghamii	IS6	421	1
F. deltoidea	IS3	699	2
		751	
F. elastica	IS3	1118	5
	IS6	1393	
		1603	
	IS10	196	
		404	
F. infectoria	IS3	236 1003	2
F. laurifolia	IS3	480 849	2
F. macrophylla	IS3	1247	1
F. mysorensis	IS3	1247	1
F. palmate	IS4	240	6
		1329	
	IS10	547	
		1004	
	IS9	224	
		512	
F. platypoda	A9	201 802 1083 1167 1306	5
F. racemosa	IS6 A9	201 193	2
F. religiosa	IS3 IS4	1293 1768	2
F. retusa	IS3	304	7
	IS4	250	
	IS6	1344	
		1845	
	IS10	220	
		2313	
	IS9	493	
F. spragueana	IS3	221	2
	A9	216	
F. sycomorus	IS10	374 1360 1773 1987	5
	A9	1356	

Three species could be recognized by the presence of five unique bands. *F. elastica* $IS3_{1118bp}$, $IS6_{1393bp}$, $IS6_{1603bp}$, $IS10_{196bp}$ and $IS10_{404bp}$, *F. platypoda* $A9_{201bp}$, $A9_{802}$, $A9_{1083bp}$, $A9_{1167bp}$ and $A9_{1306bp}$ and The last one was *F. sycomorus* $A9_{135bp}$, $IS10_{374bp}$, $IS10_{1360bp}$, $IS10_{1773bp}$ and $IS10_{1987bp}$. *Ficus palmata* was characterized by the presence of six unique bands $IS4_{240bp}$, $IS4_{1329bp}$, A9224bp, $A9_{512bp}$, $IS10_{547bp}$ and $IS10_{1004bp}$, whereas *F. retusa* was identified by the presence of seven unique bands $IS3_{304bp}$, $IS4_{250bp}$, $IS6_{1344bp}$, $IS6_{1845bp}$, $IS10_{220bp}$, $IS10_{2313bp}$ and $A9_{493bp}$. *Ficus carica* was identified by the highest number of unique marker. It was characterized by the presence of eight unique bands $IS3_{1160bp}$, $IS4_{221bp}$, $IS6_{175bp}$, $IS6_{1015bp}$ $IS10_{862bp}$, $IS10_{206bp}$,$IS10_{2227bp}$ and $A9_{665bp}$ which were absent in all other species under the study. The remaining two species *Ficus afzelii* and *F. benghalensis* couldn't distinguished by any positive or negative unique marker. This study provides evidence that ISSR polymorphisms could be used as efficient tools for the detection of similarities, fingerprinting and phylogenetic relationships of the studied species. The same conclusion was obtained by Abdel-Tawab *et al*. (2001); Alexander *et al.* (2002); Arnau *et al*. (2003); Ghariani *et al.* (2003); Rajesh *et al.* (2003); Heikal *et al*. (2007) and Aparajita *et al*. (2008).

Numerical analysis based on ISSR characters

Matrix of similarity between pairs of individuals may be used as starting point for statistical procedures such as cluster analysis. In the cluster analysis, relatively homogenous groups of individuals cluster together in a hierarchical way and this clustering is visually displayed in a dendrogram. The denderogram is based on the information obtained from (Table 4) which has been used as a data matrix for measuring the genetic similarity among the examined taxa.

The UPGMA cluster analysis was carried out to represent graphically the genetic similarity among 19 taxa studied (Fig. 2). The dendrogram was separated into two main clusters; Cluster I included *F. sycomorus* (subgenus *Sycomorus*) which is split from the other species at 0.65. Cluster II was divided into six groups: *F. platypoda* (subgenus *Urostigma*), *F. retusa* (subgenus *Urostigma*) and *F. palmata* (subgenus *Sycomorus*.) formed the first group in cluster II. *F. platypoda* showed 0.67 JSI with *F. palmata* and *F. retusa* in the group while *F. palmata* and *F. retusa* showed 0.71 JSI between them. The second group included *F. carica* (subgenus *Ficus*), *F. cunninghamii* (subgenus *Urostigma*) and *F. benjamina* var. *comosa* (subgenus *Urostigma*). *F. carica* showed 0.77 JSI with *F. cunninghamii* and *F. benjamina* v. *comosa*, while *F. cunninghamii* and *F. benjamina* var. *comosa* showed 0.80 JSI between them.Third group included *F. mysorensis* (subgenus *Urostigma*) and *F. spragueana* (subgenus *Urostigma*)

at 0.72 JSI. Three species formed the fourth group in cluster II, in this group, *F. cordata* (subgenus *Urostigma*) showed 0.73 JSI with *F. religiosa* (subgenus *Urostigma*) and *F. racemosa* (subgenus *Sycomorus*) while *F. religiosa* and *F. rasemosa* showed 0.75 JSI between them. Fifth group showed 0.80 JSI between *F. laurifoila* (subgenus *Urostigma*) and *F. infectoria* (Subgenus *Urostigma*). The sixth group included five species, including *F. macrophylla* (Subgenus *Urostigma*) had 0.77 JSI with *F. elastica* (subgenus *Urostigma*) in the same group the two species had 0.81 JSI with *F. deltoidea*. *F. deltoidea* (subgenus *Ficus*) showed 0.81 JSI also with *F. benghalensis* (subgenus *Urostigma*) and *F. afzelii* (subgenus *Urostigma*). *Ficus benghalensis* and *F. afzelii* showed maximum similarity (0.85 JSI) between them indicated that these two species are closely related to each other.

The two studied taxa of subgenus *Ficus* viz *F. carica* and *F. deltoidea* were widely separated and didn't cluster together and showed relations with the taxa of subgenus *Urostigma*. Also, the three studied taxa of subgenus *Sycomorus* possessed relations with members of subgenus *Urostigma*.

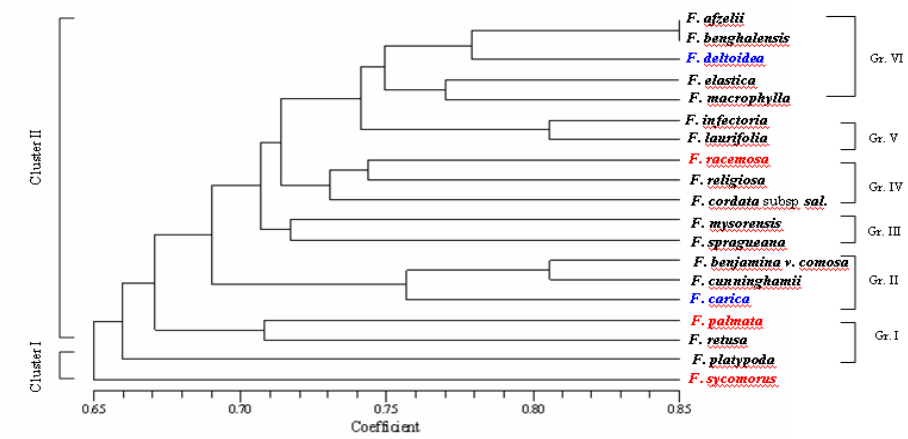

Fig. 2. UPGMA- dendrogram based on 229 ISSR characters illustrating genetic similarity between the studied taxa.

Conclusions

Although the genus as a whole represents a strictly monophyletic linkage, the study didn't support its traditional infrageneric classification by Corner (1965) based on syconium morphology. The studied taxa were distributed across the constructed phenograms, independent of the previous infrageneric classification of the genus. These results were in consistence with previous studies on the

genus utilizing molecular criteria and reproductive biology (Rønsted *et al.*, 2008). However, these studies showed clearly that the accepted infrageneric classification of the genus required a thorough revision by large number of primers and different techniques.

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تحليل التباين والتماثل الوراثى لجنس فيكس (الفصيلة التوتية) فى مصر

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تم دراسة تسعة عشر نوعاً لجنس فيكس جمعت من ثلاث حدائق مصرية على اساس المعلومات المشتقة من البصمة الوراثية باستخدام التكرار التتابعى البينى البسيط ISSR لتقدير مستوى تحليل الشكل الظاهرى والتماثل الجينى. تم دراسة التباين الوراثى بين التسعة عشر نوعاً تحت جنس الفيكس باستخدام الواسم الجزيئى لل ISSR حيث أستخدمت خمسة بادئات ل ISSRلتقدير مستوى التباين بين الأنواع المختلفة0 و قد أظهرت الدراسة 229 شظية د ن أ وكانت كلها مظهرة للتباين بين الأنواع و تمثل نسبة تباين 100% و كان متوسط عدد الشظايا المظهرة للتباين بالنسبة للبادئ الواحد 46 شظية0 وأظهرت واسمات ال ISSR أعلى نسبة تشابة وراثى (85%) بين *F. afzelii* و *F. benghalensis* بينما كانت أقل نسبة تشابة وراثى (58%) بين *F. carica* و *F. sycomorus*0 وبذلك أظهرت النتائج نسبة عالية من التشابة الوراثى بين الأنواع محل الدراسة مما يدل على درجة عالية من التماثل و محدودية الأساس الوراثى لهذة الأنواع . أظهرت الدراسة تباعد النوعان المنتميان إلى تحت جنس *Ficus* و هما *F. carica* و *F. deltoidea* و فى نفس الوقت أظهرت النتائج أن الأنواع المنتمية إلى تحت جنس يوروستجما (Urostigma) لها علاقات وثيقة مع بعض الأنواع التى تنتمى إلى تحت جنس فيكس (*Ficus*) وتحت جنس سيكومواراس (*Sycomorus*) .