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GENETIC DIVERSITY IN *FICUS SYCOMORUS* L. SPECIES (*MORACEAE*) USING RAPD AND IRAP MARKERS

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This study was conducted in order to assess accuracy, repeatability and reproducibility of the RAPD and IRAP techniques for determining the genetic variability in 10 *Ficus sycomorus* L. genotypes grown in the coastal regions of Syria. Thirty-six RAPD primers applied gave 352 discernible loci, of which 252 (71.59%) were polymorphic. Polymerase chain reaction (PCR) amplification with 36 RAPD primers gave an average of 9.778 selected markers/primers, with a maximum of 21 (OPA18) and a minimum of five (OPG11, OPK12 and OPT18). The amplification with 22 IRAP primers (single or combination) generated 178 bands, of which 151 (84.83%) were polymorphic, with an average of 11.125 selected markers/primer, with a maximum of 17 (IRAP-TDK11F) and a minimum of seven (BREP1F+BREP1R, IRAP-TDK1F+IRAP-TDK1R and IRAP-TDK2F+IRAP-TDK2R). In the present investigation, the IRAP marker was more efficient than the RAPD assay, where the latter exhibited a lower marker index (MI) average (1.629) compared with the IRAP technique (2.941). Otherwise, *F. sycomor4* genotype showed the highest dissimilarity compared with other genotypes studied in this investigation. Based upon the estimated percent disagreement values (PDV), we can suggest that there are three subspecies present among the 10 samples tested.

Key words: *Ficus sycomorus* L.; genotype; IRAP; marker index; polymorphism; RAPD

Ficus sycomorus L. species arranged as a subgenus of the fig, belongs to the *Moraceae* family. Within the genus *Ficus*, approximately 400 monoecious and 800 gynodioecious species are in existence (Al Malki & Elmeer 2010). The name, *sycomorus*, comes from the Greek syca-morus which means mulberry fig. The trees are not as cold-hardy as the common fig *Ficus carica* L. They are usually grown in the warmer regions of the Middle East and Africa. *F. sycomorus* L. is a rare perennial tree or sub-tree. It was originally brought from Ethiopia and Central Africa. It has been planted since antiquity in Egypt, Palestine, Lebanon and Syria. It is presently becoming rare because of urban development. Thus, the rest of this species can be found in Sida and Syrian littoral zones (Mouterde 1966). It is known as Sycmore as a common English name, and as Al-Joumayz as a common Arabic name. This species is becoming one of the endangered species leading to extinction, among other plant genetic resources in Syria.

However, there are some efforts for characterisation and conservation of the genetic diversity to prevent their potential extinction and further utilisation. This species shows a good tolerance for an abiotic stress (salinity) in addition to being traditionally appreciated for its ripe fruits. It plays an important role in traditional treatment of many diseases and ailments. In coastal regions from Syria, the stem bark of *Ficus sycomorus* L. is traditionally used to treat fungal diseases.

Syria is an important region for different plant genetic resources (wild and cultivated species) in the world because of its diverse ecosystems and cli-

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matic conditions. Syria is considered to be a centre of origin and biodiversity for many crops, feeds and fruit trees (Zohary 1962, 1973). It is one of the few core centres where numerous species of temperate-zone agricultural specimens originated thousands of years ago, and where third-world relatives and land races of enormous genetic diversity are still present. Estimates indicated that Syrian flora includes about 3,150 species arranged in 919 genus and 133 families (Barkoudah *et al*. 2000).

Different marker systems are currently available for monitoring and assessing genetic diversity. Random amplified polymorphic DNA (RAPD) markers established by Williams *et al*. (1990) are DNA fragments from PCR amplification of random segments of genomic DNA with a single primer of arbitrary nucleotide sequences, which are able to differentiate between genetically distinct individuals. This technique is simple to use, and does not need any sequence information. The RAPD marker becomes one of the fewer molecular techniques for assessing genetic variation in fig in many countries; for example, in Italy (De-Masi *et al*. 2005), in Tunisia (Salhi-Hannaci *et al*. 2006; Baraket *et al*. 2011), in Egypt (Hadia *et al*. 2008), in Japan (Ikegami *et al*. 2009), and also in Turkey (Akbulut *et al*. 2009; Dalkilic *et al*. 2011). New techniques based on DNA profiling provide novel approaches to identification of the varieties, which offer advantages over traditional morphological comparisons. Inter-retrotransposon amplified polymorphism (IRAP) displays insertional polymorphisms by amplifying the segments of DNA between two retrotransposons. The IRAP marker detects high levels of polymorphism which does not need DNA digestion, ligations, or probe hybridisation to generate marker data, thus increasing the reliability and robustness of the assay. Mobile genetic elements, retrotransposons, generally show widespread chromosomal dispersion, variable copy number, and random distribution in the genome.

The IRAP marker is one among other retrotransposon-based markers which are a new group of markers applied successfully in genetic variation studies in various plant crops, for example, in banana (Nair *et al*. 2005), in barley (Alavi-Kia *et al*. 2008), in *Aegilops* (Saeidi *et al.* 2008), in potato (Novakova *et al*. 2009) and recently in flax (*Linum usitatissimum* L.) (Smýkal *et al*. 2011).

IRAP could be performed as an accurate, repeatable and reproducible marker compared with the RAPD system (Biswas *et al*. 2009). As yet, our knowledge of the *Ficus* genus breeding system and its evolution has been shaped by taxonomy, anatomy, ecology, pollinator behaviour and genetic variability. However, phylogenetic studies in *F. sycomorus* L. species have not yet been examined in detail. Therefore, this investigation was performed to assess genetic variation in *F. sycomorus* L. species in Syria using RAPD and IRAP markers. Comparative assessment of PCR-based markers (RAPD and IRAP) was carried out, and their potential application as marker systems was investigated for their utility in phylogenetic relationships within this species.

MATERIAL AND METHODS

Plant materials

Ten samples were collected from their natural habitats along the coastal regions of Syria (Table 1). These geographical regions consist of altitudes ranging from 4.5 m to 250 m from the wet coastal regions. Sampling was carried out in autumn from trees spread on clay soil, and where annual rainfall ranged from 650 mm to 850 mm.

Total DNA isolation

The genomic DNA of the plant was extracted from young leaves of 10 samples of *F. sycomorus* species in Syria (bulk of 5 leaves/tree for each representative genotype) by a CTAB (cetyltrimethylammonium bromide) protocol as described by Doyle and Doyle (1987) with minor modifications. Leaf tissue (150 mg) was ground in liquid nitrogen, the powder was transferred to a 2-ml Eppendorf tube, mixed with 900 µl of extraction buffer (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 0.0018 ml ß-mercaptoethanol, 2% CTAB), and incubated at 65°C for 20 min. DNA was extracted with one volume of a chloroform:isoamyl alcohol mix (24:1, v/v), and centrifuged at 12,000 g for 10 min at 4°C. The aqueous phase was transferred to a fresh tube, and the DNA was precipitated with an equal

volume of cold isopropanol and kept at −20°C for 10 min, then centrifuged at 12,000 g for 10 min at 4°C, the supernatant was discarded; DNA was then spooled out and washed with 1 M ammonium acetate and 100% ethanol. The cleaned DNA pellet was air dried and dissolved in 100 µl of 0.1X TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). After addition of 5 µl of RNase (10 mg/ml), and incubation for 30 min at 37°C, the DNA was washed with 1 M ammonium acetate and 100% ethanol. Then it was centrifuged at 12,000 g for 30 min at 4°C, and the supernatant was discarded; DNA was rinsed twice with 70% ethanol. The cleaned DNA pellet was air dried and dissolved in 100 µl of 0.1X TE buffer. The DNA concentration was quantified by DNA fluorimeter and kept at –80°C until use.

RAPD assay

Thirty-three RAPD primers belonging to Operon Technologies Inc., USA, and three primers from the University of British Columbia were tested for genetic variability detection within *F. sycomorus* species. The amplification reaction was carried out in a 25 µl reaction volume containing 1X PCR buffer, 2 mM $MgCl_2$, 0.25 mM dNTPs, 25 pmol primer, 1.5 U of Taq DNA polymerase and 50 ng template DNA. PCR amplification was performed in a T-gradient thermal cycler (Bio-Rad; T-Gradient). It was programmed to fulfil 42 cycles after an initial denaturation cycle for 4 min at 94ºC. Each cycle consisted of a denaturation step for 1 min at 94ºC, an annealing step for 2 min at 35ºC, and an extension step at 72ºC for 2 min, followed by an extension cycle for 7 min at 72ºC in the final cycle. The PCR products were separated on a 1.5% ethidium bromide-stained agarose gel (Bio-Rad) in 0.5X TBE buffer. Electrophoresis was performed for 2.5 h for RAPD at 85 V and visualised with a UV transilluminator. A 1 kb DNA ladder standard was used to estimate the molecular weight of the amplification products.

IRAP assay

For the IRAP marker, 22 (single, or in combination) primers were also examined, as previously described in other species (Guo *et al*. 2006; Alavi-Kia *et al*. 2008; Novakova *et al*. 2009). The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 2 mM $MgCl_2$, 0.25 mM dNTPs, 25 pmol primer, 1.5 U of Taq DNA polymerase and 50 ng template DNA. PCR amplification was performed in a T-gradient thermal cycler (Bio-Rad; T-Gradient). It was programmed to fulfil 35 cycles after an initial denaturation cycle for 4 min at 94ºC. Each cycle consisted of a denaturation step for 1 min at 94ºC, an annealing step for 2 min at Tm varied according to each primer examined, and an extension step at 72ºC for 2 min, followed by extension cycle for 7 min at 72ºC in the final cycle. The PCR products were separated on a 2% ethidium

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Geographical regions, and description of original sites where the 10 *F. sycomorus* genotypes were collected

Genotype code	Original site	Accompanied species	Altitude [m]	Annual rainfall [mm]
F. sycomor1	Lattakia	*Eucalyptus* ssp and *Azadarachtx indica*	4.5	650–700
F. sycomor2	Lattakia	*Azadarachtx indica*	4.5	650–700
F. sycomor3	Lattakia	None	6.3	650–700
F. sycomor4	Lattakia	*Cupressus sempervirens*	6.3	650–700
F. sycomor5	Lattakia	None	6.3	650–700
F. sycomor6	Lattakia	*Ailanthus altissima*	6.3	650–700
F. sycomor7	Jableh	*Azadarachtx indica*	11.8	650–700
F. sycomor8	Banyas	None	10.0	700–750
F. sycomor9	Banyas	*Juglans regia* L.	220.0	~850
F. sycomor10	Banyas	None	250.0	~850

bromide-stained agarose gel (Bio-Rad) in 0.5X TBE buffer. Electrophoresis was performed for 1.5 h at 85 V and visualised with a UV transilluminator. A 1 kb DNA ladder standard was used to estimate molecular weight of amplification products.

RAPD and IRAP data analysis

The presence or absence of each size class was scored as 1 or 0, respectively. The percent disagreement values (PDV) found were used to generate a matrix via the Unweighted Pair Group Mean Arithmetic average (UPGMA) using Statistica program (Statistica 2003). This matrix was used to calculate genetic similarity (Jaccard 1908). Polymorphic information content (PIC) values were calculated for each RAPD or IRAP primer according to the formula

$$PIC = 1 - \Sigma(P_{ij})^2$$

where P_{ij} is the frequency of the i^{th} pattern revealed by the j^{th} primer summed across all patterns revealed by the primers (Botestein *et al*. 1980). The marker index (MI), as a universal metric to represent the amount of information obtained per experiment for a given kind of markers, as reported by Powell *et al*. (1996), was also calculated for each RAPD and IRAP primer as

$$MI = PIC \times \eta\beta$$

where PIC is the mean PIC value, η the number of bands, and β is the proportion of polymorphic.

RESULTS AND DISCUSSION

PCR amplification produced by RAPD and IRAP primers are listed in Tables 2 and 3 in terms of the percentage of PCR products appearing in the genotypes studied. The RAPD analysis carried out on the 10 genotypes of *F. sycomorus* produced a large number of distinct fragments for each primer. The 36 selected arbitrary primers generated a total of 352 scorable bands, of which 252 (71.59%) were polymorphic, with an average of 9.778 amplicons/primer (Table 2). OPA18 gave the highest number of fragments (21 amplicons), while OPG11, OPK12 and OPT18 primers revealed the lowest number (5 amplicons). Figure 1 shows the RAPD profile for the 10 genotypes yielded by OPK17, UBC132 and OPD20 primers.

As for IRAP analysis, a total of 178 bands were detected, among which 151 bands (84.83%) were polymorphic, with the mean of 11.125 bands/primer or primer combination (Table 3). The highest number of fragments (17) was obtained with the IRAP-TDK11F, whereas, the lowest (7) was revealed by (BREP1F+BREP1R, IRAP-TDK1F+IRAP-TDK1R and IRAP-TDK2F+IRAP-TDK2R) primer combinations. PCR amplification products obtained from Sukkula + Nikita, IRAP-TDK11F and Sukkula + LTR6150-BARE1 primer combinations are illustrated in Figure 2.

In this study, two PCR-based systems (RAPD and IRAP) were employed to investigate the genetic diversity among 10 genotypes of *F. sycomorus* grown in Syria.

Our study showed moderate polymorphism level values of 71.59% (RAPD marker), and high values of 84.83% (IRAP marker) among the genotypes examined. Other researches, however, reported a good degree of polymorphism in neotropical *Ficus* (80%) (Nazareno *et al*. 2009), in *F. carica* L. (81.1%) (De-Masi *et al*. 2005) using the same marker; in *F. carica* L. (97.5% and 100% using AFLP and SSR markers, respectively) (Baraket *et al*. 2011) and 84.96% and 90.91% in *Arthrocnemum macrostachyum* (Saleh 2011) using RAPD and ISSR markers, respectively. Hadia *et al*. (2008) reported values of 62.4% and 61.2% using RAPD and ISSR markers, respectively, in *F. carica*, however, Dalkilic *et al*. (2011) reported a lower value for the same species (27.9% using RAPD marker). Singh *et al*. (2011) also reported a low polymorphism level (P%) (54.33% and 56.02% using, respectively, RAPD and ISSR markers in *Morinda* spp). Whereas, our results were in agreement with other findings in *F. carica* (Salhi-Hannaci *et al*. 2006; Akbulut *et al*. 2009; Ikegami *et al*. 2009).

Moreover, Aradhya *et al*. (2010) used 15 microsatellite loci for genetic diversity in cultivated fig (*F. carica* L.) to examine the genetic structure and differentiation. Their results revealed weak genetic structure, and they proposed that this was probably due to an inherently narrow genetic base from which the fig was domesticated, combined with historical migration of germplasm, and the outcrossing mode of pollination, all of which have countered human selection in different fig-growing regions of the

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Selected 36 RAPD primers tested in this study

Primer name	Primer Sequence 5‘ to 3‘	Tb	Pb	P [%]	PIC	MI
OPA02	TGCCGAGCTG	9	7	77.778	0.258	1.806
OPA04	AATCGGGCTG	10	8	80.000	0.256	2.048
OPA18	AGGTGACCGT	21	16	76.190	0.243	3.888
OPB01	GTTTCGCTCC	10	8	80.000	0.288	2.304
OPB04	GGACTGGAGT	16	10	62.500	0.225	2.250
OPB05	TGCGCCCTTC	10	9	90.000	0.220	1.980
OPB12	CCTTGACGCA	10	4	40.000	0.110	0.440
OPB17	AGGGAACGAG	10	6	60.000	0.200	1.200
OPC02	GTGAGGCGTC	10	8	80.000	0.296	2.368
OPC07	GTCCCGACGA	6	5	83.333	0.250	1.250
OPC08	TGGACCGGTG	6	4	66.667	0.210	0.840
OPC13	AAGCCTCGTC	7	4	57.143	0.169	0.676
OPC15	GACGGATCAG	8	6	75.000	0.310	1.860
OPD08	GTGTGCCCCA	9	3	33.333	0.060	0.180
OPD20	GGTCTACACC	14	10	71.429	0.190	1.900
OPE04	GTGACATGCC	8	6	75.000	0.190	1.140
OPE07	AGATGCAGCC	11	9	81.818	0.347	3.123
OPE15	ACGCACAACC	9	7	77.778	0.260	1.820
OPE18	GGACTGCAGA	11	8	72.727	0.230	1.840
OPG11	TGCCCGTCGT	5	2	40.000	0.168	0.336
OPJ01	CCCGGCATAA	12	11	91.667	0.202	2.222
OPJ07	CCTCTCGACA	11	10	90.909	0.330	3.300
OPK08	GAACACTGGG	10	9	90.000	0.286	2.574
OPK12	TGGCCCTCAC	5	3	60.000	0.136	0.408
OPK13	GGTTGTACCC	8	4	50.000	0.190	0.760
OPK17	CCCAGCTGTG	13	10	76.923	0.190	1.900
OPQ01	GGGACGATGG	10	6	60.000	0.138	0.828
OPQ18	AGGCTGGGTG	7	4	57.143	0.171	0.684
OPR12	ACAGGTGCGT	10	3	30.000	0.106	0.318
OPT18	GATGCCAGAC	5	2	40.000	0.072	0.144
OPV03	CTCCCTGCAA	12	11	91.667	0.313	3.443
OPW17	GTCCTGGGTT	10	9	90.000	0.236	2.124
OPY10	CAAACGTGGG	10	8	80.000	0.226	1.808
UBC132	AGGGATCTCC	10	6	60.000	0.186	1.116
UBC159	GAGCCCGTAG	12	10	83.333	0.248	2.480
UBC702	GGGAGAAGGG	7	6	85.714	0.217	1.302
Total		352	252	–	–	–
Mean		9.778	7	69.990	0.215	1.629

Tb – Total bands; Pb – Polymorphic bands; P [%] – Polymorphic %; PIC – Polymorphic information content; MI – Marker index

T a b l e 3

Selected 22 IRAP primers tested in this study

Primer name	Primer Sequence 5' to 3'	Ta [ºC]	Tb	Pb	P [%]	PIC	MI
BREP1 F BREP1 R	AAGTATTCGGTGTCCAAAATC ACTCCCTGTTGAAAATTCTGA	45	7	5	71.429	0.269	1.345
IRAP-TDK1F IRAP-TDK1R	TCAATCGGACTTGTTCAAAACCCCA TACAGACCAAATGCTCACCATCACT	45	7	3	42.857	0.197	0.591
IRAP-TDK2F IRAP-TDK2R	GAAGTTAGTGGGAGCAAAAGATGT TACCAATGTCGGGAGGCTTGTGTCA	45	7	6	85.714	0.254	1.524
BREP F BREP R	TTCAAGATTTCTGACCTTTCG CCAGTGGCACATCAAAACAAAA	45	10	10	100.000	0.330	3.300
IRAP-TDK10F	CTTTGTGATAGAACTTGGGTTTGCT	45	12	10	83.333	0.305	3.05
IRAP-TDK11F	AGGTATGGTTTCAAGATGATGGATG	45	17	15	88.235	0.282	4.23
IRAP-TDK12F IRAP-TDK12R	ATACAACAGACTCAATGCCGACCCT ACCTGCCAACCAACTTCTTTTCCTC	45	8	6	75.000	0.240	1.440
IRAP-TDK13F IRAP-TDK13R	TCCTGATGGGAACTTCGTTGCTCGT CCTGACACCTCAAAACCTTCTGGCT	45	12	11	91.667	0.295	3.245
Sukkula 5′LT R2-BARE-1	GATAGGGTCGCATCTGGGCGTGAC ATCATTCTCTAGGGCATAATTC	45	14	13	92.857	0.339	4.407
Sukkula LT R6150-BARE-1	GATAGGGTCGCATCTGGGCGTGAC CTGGTTCGGCCATGTCTATGTATCACACATGTA	45	13	11	84.615	0.342	3.762
Sukkula Nikita	GATAGGGTCGCATCTGGGCGTGAC CGCATTGTTCAAGCCTAAACC	45	15	14	93.333	0.387	5.418
3′LT R-BARE-1 5′LT R2-BARE-1	TGTTCATGCGACGTTCAACA ATCATTCTCTAGGGCATAATTC	44	12	11	91.667	0.337	3.707
3′LT R-BARE-1 Nikita	TGTTCATGCGACGTTCAACA CGCATTGTTCAAGCCTAAACC	44	11	10	90.909	0.362	3.620
3′LT R-BARE-1	TGTTCATGCGACGTTCAACA	44	13	8	61.539	0.223	1.784
P-Tst-6	ACTAAATCTGCCTACTCATTCAACACTC	55	9	8	88.889	0.327	2.616
P-Tst-1 P-Tst-3	ATGACTAAATCTGCCTACTCATTCAACA ACTAAAAATCTGCCTACTCATTCAACACTC	45	11	10	90.909	0.302	3.020
Total			178	151	–	–	–
Mean			11.125	9.438	83.309	0.299	2.941

Ta [ºC] – Annealing temperature; Tb – Total bands; Pb – Polymorphic bands; P [%] – Polymorphic %; PIC – Polymorphic information content; MI – Marker index

world. Moreover**,** in Weiblen's (2000) investigation, separated and combined phylogenetic analyses of ITS and morphological characters indicate that monoecious *Sycomorus* is monophyletic and nested in a clade of functionally dioecious *Ficus*.

In the present investigation, the PIC value estimated with RAPD assay ranged from 0.060 to 0.347 with an average of 0.215, whereas, this index varied between 0.197 and 0.387 with an average of 0.299 using an IRAP marker. Dalkilic *et al*. (2011), obtained values that ranged from 0.16 to 0.50 in male fig (*Ficus carica caprificus* L.) genotypes using an RAPD marker, whereas, this value was recorded to be 0.79 and 0.94 in fig (*F. carica* L.) using AFLP and SSR markers, respectively (Baraket *et al*. 2011).

For determining the overall usefulness of a given marker system, the MI was calculated for both the marker systems examined. IRAP markers showed the highest MI (2.941; Table 3), which is higher than the estimated value for the RAPD (1.629; Table 2). This analysis highlights the distinctive nature of the IRAP assay. Indeed, the MI, considered to be an overall measurement of the efficiency to detect polymorphism, was higher (2.941) for IRAP than (1.629) for RAPD marker systems (Tables 2 and 3). According to the formula used, the high-value MI calculated for the IRAP assay, makes the IRAP marker system suitable for estimating the level of genetic diversity in *F. sycomorus* genotypes compared with the RAPD system. Consequently, IRAP fingerprinting was more efficient than the RAPD assay. The present results were in accordance with the observation by Biswas *et al*. (2009) in citrus, while, this value was 45.2 and 0.94 for AFLP and

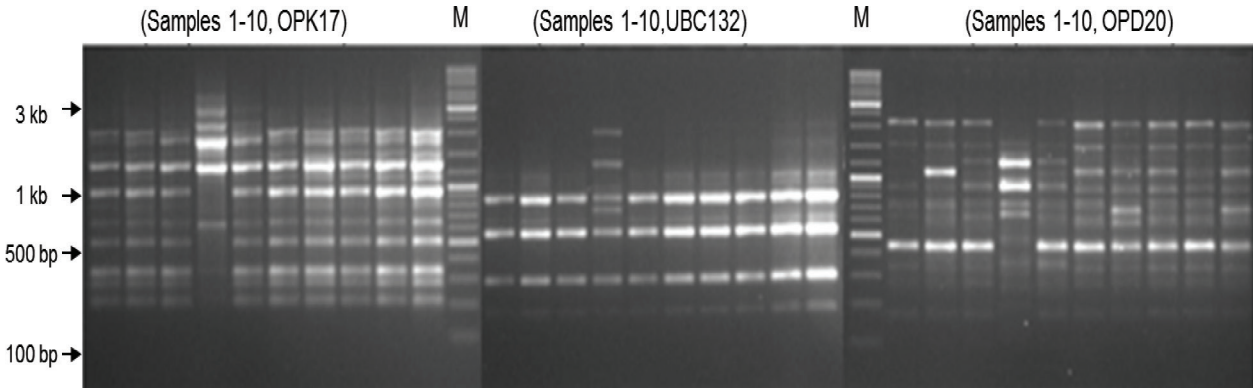

Figure 1. Polymorphism resultant from the use of OPK17, UBC132 and OPD20 RAPD primers for *F. sycomorus* genotypes 1, 2, 3 and 10, Lane M, DNA marker 1 kb

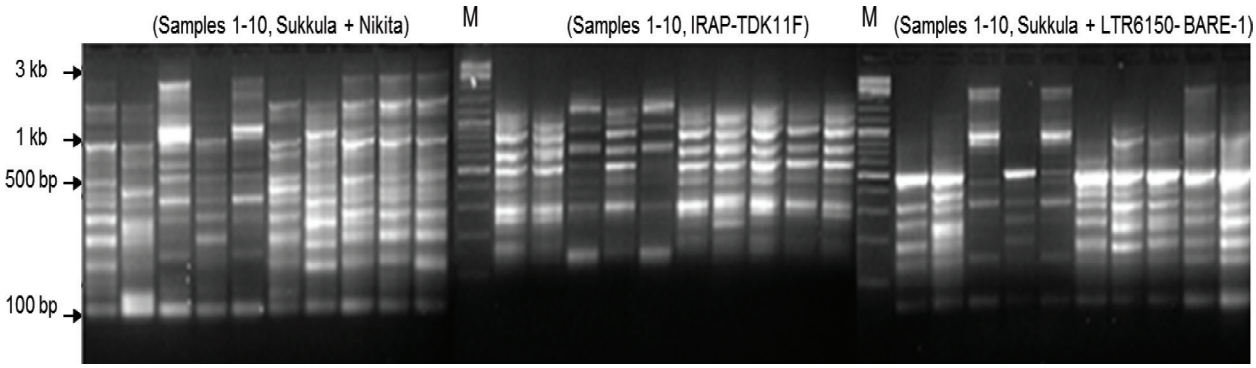

Figure 2. Polymorphism resultant from the use of Sukkula + Nikita, IRAP-TDK11F and Sukkula + LTR6150-BARE-1 IRAP primer combinations for *F. sycomorus* genotypes 1, 2, 3 and 10, Lane M, DNA marker 1 kb

SSR markers, respectively, in *F. carica* L. (Baraket *et al*. 2011).

Genetic similarity estimated among genotypes was scaled between 0.401 and 0.897, with an average of 0.705 in the case of RAPD, and between 0.205 and 0.976 with an average of 0.623 in the case of IRAP markers. While, this value was higher than that previously reported using the same technique in *F. carica* L., it was estimated to be (0.21–0.62) with an average of 0.468 in the Akbulut *et al*. (2009) investigation with RAPD markers, ranging between 0.04 and 0.59, with an average of 0.32 (for AFLP) and between 0.017–0.546 with an average of 0.281 (for SSR) in the Baraket *et al*. (2011) study, where-

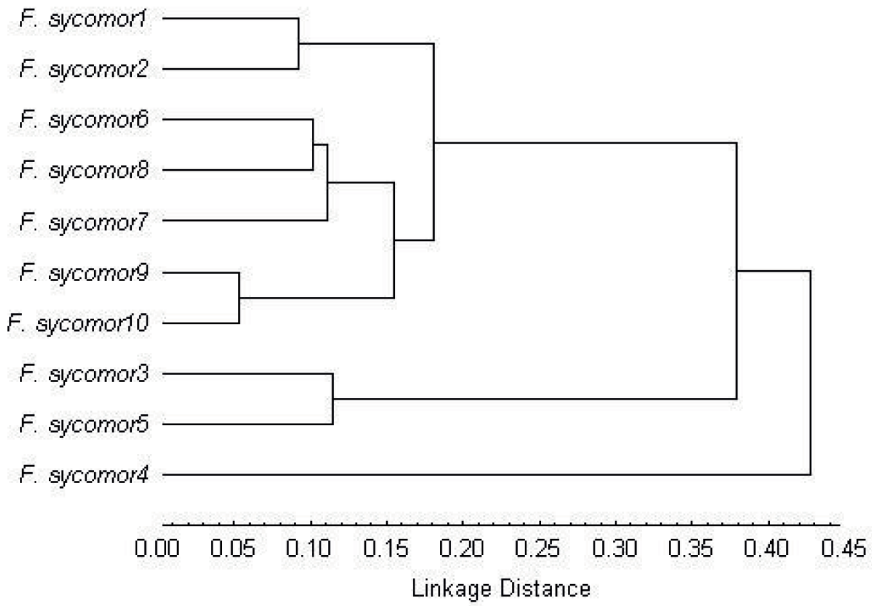

Figure 3. UPGMA cluster analysis-based on the percent disagreement value for RAPD and IRAP data combination showing genetic relationship among the 10 genotypes of *F. sycomorus* L. species

T a b l e 4

Percent disagreement values (PDV) produced by RAPD and IRAP (single or in combination) data combination using the UPGMA routine in statistical program

Genotype	*F. sycomor1*	*F. sycomor2*	*F. sycomor3*	*F. sycomor4*	*F. sycomor5*	*F. sycomor6*	*F. sycomor7*	*F. sycomor8*	*F. sycomor9*	*F. sycomor10*
F. sycomor1	0									
F. sycomor2	0.09	0								
F. sycomor3	0.34	0.37	0							
F. sycomor4	0.40	0.42	0.38	0						
F. sycomor5	0.36	0.36	0.12	0.38	0					
F. sycomor6	0.17	0.15	0.39	0.45	0.36	0				
F. sycomor7	0.21	0.19	0.41	0.44	0.37	0.11	0			
F. sycomor8	0.19	0.16	0.39	0.46	0.37	0.10	0.12	0		
F. sycomor9	0.17	0.16	0.40	0.45	0.38	0.15	0.15	0.11	0	
F. sycomor10	0.20	0.19	0.41	0.46	0.40	0.18	0.19	0.14	0.05	0

T a b l e 5

Jaccard's similarity matrix produced by RAPD and IRAP (single or combination) data combination for the 10 *F. sycomorus* tested genotypes

Genotype	*F. sycomor1*	*F. sycomor2*	*F. sycomor3*	*F. sycomor4*	*F. sycomor5*	*F. sycomor6*	*F. sycomor7*	*F. sycomor8*	*F. sycomor9*	*F. sycomor10*
F. sycomor1	1									
F. sycomor2	0.857	1								
F. sycomor3	0.529	0.506	1							
F. sycomor4	0.485	0.477	0.473	1						
F. sycomor5	0.508	0.516	0.798	0.483	1					
F. sycomor6	0.747	0.789	0.481	0.447	0.518	1				
F. sycomor7	0.704	0.740	0.471	0.465	0.517	0.843	1			
F. sycomor8	0.734	0.770	0.495	0.454	0.516	0.850	0.836	1		
F. sycomor9	0.755	0.781	0.492	0.464	0.512	0.783	0.790	0.840	1	
F. sycomor10	0.722	0.739	0.485	0.461	0.501	0.759	0.753	0.805	0.924	1

as, it was varied between 0 and 0.78 with an average of 0.39 (Salhi-Hannaci *et al*. 2006), between 0.20 and 0.85 with an average of 0.51 (Cerqueira-Silva *et al*. 2010) in *Passiflora cincinnata*, between 0.181 and 0.562 with an average of 0.543 using an RAPD marker in *Arthrocnemum macrostachyum* (Saleh 2011), and in the study reported by Ikegami *et al*. (2009) on *F. carica* L., the average was 0.717.

Combined RAPD and IRAP data produced genetic distances ranging from 0.05 to 0.46 with a mean of 0.28 (Table 4), and the resultant dendrogram (Figure 3) demonstrated that the 10 *F. sycomorus* genotypes phylogenetics fell into two main groups. The first cluster consisted of *F. sycomor4* genotype that formed a distinct cluster with a PDV estimated value higher than 0.38 with other tested genotypes, especially with *F. sycomor8* and *F. sycomor4*, and with *F. sycomor10* and *F. sycomor4* (PDV = 0.46), whereas, the second cluster included the remaining genotypes. Subsequently, the last cluster was further divided into four subclusters containing the remaining tested genotypes. The first subcluster involved genotypes *F. sycomor1* and *2* that were closely related at PDV = 0.09 (similarity 0.857, Table 5). While, the second subcluster included *F. sycomor6* and *8*, that were closely related at PDV = 0.1 (similarity 0.850, Table 5). This subcluster was closed to *F. sycomor7* at PDV = 0.11 (similarity 0.843, Table 5) and with *F. sycomor8* at PDV = 0.12 (similarity 0.836, Table 5), whereas, the third subcluster consisted of *F. sycomor9* and *10* that were the most related genotypes with a PDV = 0.05) similarity 0.924, Table 5). The fourth and last subcluster involved *F. sycomor3* and *F. sycomor5* that were also closely related at PDV = 0.12 (similarity 0.798, Table 5).

Our results demonstrated that the genotypes studied are clustered independently from their geographical origin. Taking into account that *F. sycomorus* genotypes aggregated together in the same cluster, this indicated a possible common origin of these genotypes.

This is in agreement with the monoecious origin of *Ficus* that has evolved into two gynodioecious forms as suggested by Machado *et al*. (2001). It is important to note that similar data have been reported in Tunisian fig using RAPD markers (Salhi Hannachi *et al*. 2006).

CONCLUSION

Overall, this study clearly demonstrates a relatively high diversity among *F. sycomorus* genotypes found in the coastal regions of Syria. Moreover, the two techniques applied may provide useful information on polymorphism levels as well as diversity in *F. sycomorus* L. species, but the IRAP marker differentiates accessions much better than the RAPD marker system. Consequently, the IRAP technique could be performed as a high-accuracy approach

compared with the RAPD tool. Moreover, *F. sycomor4* genotype showed the highest dissimilarity compared to the other genotypes studied in this investigation. According to the dendrogram based on a combination of RAPD and IRAP data, it could be suggested, that *F. sycomor4* genotype belongs to a subspecies that is different from that of the remaining genotypes.

In this investigation, it was not easy to predict the exact number of subspecies studied herein, especially since the exact number of *F. sycomorus* subspecies found in Syria is not known. Based upon the previous observation, and according to the position of genotypes presented by UPGMA cluster analysis, it may be postulated, however, that there are three subspecies present among the 10 samples tested. The first subspecies involved *F. sycomorus3* and *F. sycomorus5* genotypes, the second involved *F. sycomorus4* which was being suggested as presenting a distinct subspecies from the other genotypes tested, whereas, the third involved the remaining tested genotypes. Based upon the results obtained herein, it is satisfying to confirm the previous data using more potential techniques such as, for example, SSR and universal cytoplasmic molecular markers. Thereby, further analyses are required to confirm the number of subspecies to which *F. sycomorus* trees growing in Syria belong.

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