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Genetic Variability Analysis of *Ficus sycomorus* L. (Moraceae) Species in Syria

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Abstract

Ficus sycomorus L. (Moraceae) as a Mediterranean crop suffers from problematic genotyping identification. It is considered as a local natural resource, is threatened with extinction. Genetic diversity and relationships among its genotypes is fundamentally important in its improvement. Thereby, its conservation and genetic characterization is requested. Molecular characterization of 16 *F. sycomorus* L. genotypes has been assessed using simple sequence repeat (SSR) and inters simple sequence repeat (ISSR) markers. Data revealed that the selected 14 SSR loci produced 32 alleles of which 18 (56.250%) were polymorphic. Whereas, the 11 selected ISSR primers produced 74 bands of which 43 (58.108%) were polymorphic. Cluster analysis of combined SSR and ISSR data together suggests that the 16 studied *F. sycomorus* genotypes are grouped in two main clusters, based on the estimated percent disagreement values (PDV). Based upon combined SSR and ISSR markers together, the current investigation suggests low genetic variation within studied *F. sycomorus* L. genotypes.

Key-words: *Ficus sycomorus* L., SSR, ISSR, genetic diversity.

Introduction

Ficus sycomorus L. (Moraceae) named sycamore fig or the fig-mulberry and it has been cultivated since ancient times. Its genotyping identification has little attention in literature especially in our country Syria. Today, many available molecular PCR-based DNA markers such as: Random amplified polymorphic DNA (RAPD), inter simple sequence repeat (ISSR), inter-retrotransposon-amplified polymorphism (IRAP), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) have been successfully employed for different crops genotyping. Simple sequence repeat (SSR) among them becomes one of the most effective markers in phylogenetic relationship studies of many plant crops due to its highly specify and polymorphic along whole genome. Consequently, it exhibited a privileged position in plant breeding and improvement programs. It is known as microsatellites, consisting of tandem arranged repeats of short DNA motifs (mono, di, tri or tetra nucleotides; 1–6 bp in length). Earlier, it successfully employed for the first time by Tautz and Renz [1] in prokaryotic and eukaryotic genomes researches [2-3].

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It has been demonstrated that *F. carica* L. genotyping has been extensively reported in many investigations using different molecular markers *e.g.*SSR [4-18]; ISSR [7, 19-21]; RAPD [7, 12, 22] and AFLP [10].

It worth noting that genetic diversity in *Ficus* genus mainly focused in *F. carica* species [18] and little less in other related *Ficus* species like, *F. insipida* [4], *F. citrifolia* and *F. eximia*[23], *F. tikoua* [7], *F. hirta* [15], *F. palmata* [21] and *F. virens* [24] species. However, *F. sycomorus* species genetically is poorly investigated. In this respect, Ahmed *et al*. [25] used SSR markers and Saleh [26] used RAPD and IRAP markers for the same destination. More recently, Saleh [27] used Directed Amplification of Minisatellite-region DNA (DAMD) marker for the same porpuse.

Thereby, the current study aimed to sutdy genetic diversity of *F. sycomorus* L. species based on SSR microsatellites and ISSR markers.

Material and Methods

Plant Sampling and DNA Extraction

Leaves samples of sixteen *F. sycomorus* genotypes were collected from coastal regions of Syria at different altitudes (Table 1). For each genotype, 5-10 leaves were harvested and bulked as representative for each genotype. Samples collection has been performed in autumn as previously described by Saleh [26].

Total genomic DNA of each genotype has been performed by a CTAB (cetyltrimethylammonium bromide) protocol as described by Doyle and Doyle [28]. Concentration of DNA was measured by DNA fluorimeter and kept at –80°C until use.

SSR and ISSR Assays

Twenty-four microsatellite loci were employed to detect genetic diversity among 16 genotypes of *F. sycomorus* species. Due to its importance and efficacy in genetic studies, it was successfully employed worldwide. Whereas, eleven ISSR primers designed by University of British Columbia (UBC) were tested for the same purpose.SSR and ISSR amplification PCR reaction was performed in 25 µL reaction final 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 40 and 35 cycles for SSR and ISSR markers, respectively 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 examined primer (Table 2), and an extension step at 72ºC for 2 min, followed by extension cycle for 7 min at 72ºC in the final cycle. PCR products were then separated on a 2.5 and 1.8% ethidium bromide-stained agarose gel (Bio-Rad) in 0.5X TBE buffer, for SSR and ISSR markers, respectively. Electrophoresis was performed for 2.5 h at 75 V and visualized with a UV transilluminator. For estimation molecular weight of SSR loci and ISSR bands size, a VC 100bp Plus DNA Ladder (Vivantis) ladder standard was used.

SSR and ISSR 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 [29]. Then the previous matrix was used to estimate genetic similarity [30]. Polymorphic information content (PIC) value was estimated for each SSR loci and ISSR primer according to the formula:

$$PIC = 1 - \Sigma(Pij)^2$$

Where Pij is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers [31].

Results and Discussion

Twenty-four SSR loci previousely employed for molecular characterization of other *Ficus* species were employed to investigate genetic relationships among 16 *F. sycomorus* genotypes. Data showed that, 10 out of the 24 SSR tested loci were monomorphic with 1 (LMFC14, LMFC22, LMFC25, LMFC28, LMFC30, LMFC37 and MFC8) or 2 (LMFC19, LMFC34a and LMFC35) allele(s)/SSR loci. Thereby, 14 SSR loci were finaly used for estimation DNA variation among the studied 16 genotypes. Data showed that total alleles detected by using the later 14 SSR loci, ranged between 2-3 alleles with an average of 2.3 alleles/SSR loci (Table 3). Whereas, polymorphic alleles ranged between 1-2 alleles with an average of 1.4 alleles/SSR loci (Table 3). Figure 1 shows DNA polymorphism patterns obtained by LMFC30 (a) and LMFC36 (b) SSR loci.

As for ISSR marker, 11 ISSR primers were tested and gave a toltal bands number ranged between 3-10 with an average of 6.727 bands/primer (Table 3). Whereas, polymorphic bands ranged between 1-7 bands with an average of 3.909 bands/primer (Table 3). Figure 2 shows DNA polymorphism patterns obtained by UBC834 (a) and UBC856 (b) ISSR primers.

In the current study, fourteen selected SSR loci and eleven ISSR primers were finally employed for genetic diversity investigation among 16 *F. sycomorus* genotypes. SSR marker produced 32 of which 19 (56.250%) alleles were polymorphic with mean PIC of 0.106. As for ISSR, this marker produced 74 bands of which 43 (58.108%) were polymorphic with mean PIC of 0.165 (Table 3).

Saleh [26] reported 352 bands, of which 252 (71.59%) with PIC of 0.215 and 178 bands, of which 151 (84.83%) were polymorphic with PIC of 0.299 using RAPD and IRAP markers, respectively among 10 *F. sycomorus*genotypes.

Recent observations however, reported that total bands ranged between 4-19 bands with a mean average of 8.083 bands/ primer. Whereas, polymorphic bands ranged between 2-19 bands with a mean average of 6.042 bands/ primer among the same 16 *F. sycomorus* L. genotypes using DAMD marker [27]. The previous study revealed 194 bands of which 145 (74.742 %) were polymorphic with PIC mean average of 0.219 using 24 DAMD primers [27].

In the current study, some of tested SSR loci successfully characterized some *F. sycomorus* genotypes (Table 4). In this regards, LMFC21, LMFC23, LMFC26 F, LMFC27 and MFC2 characterized *F. sycomorus11* genotype;LMFC13, LMFC21, LMFC23, LMFC24, LMFC27, LMFC40, MFC1, MFC2 and MFC6 characterized *F. sycomorus14* genotype. Whereas, LMFC20,

LMFC38, LMFC40 and MFC6 characterized *F. sycomorus15* genotype (Table 4).

As for ISSR, some primers were specific for given genotypes (Table 4). In this respect, 4 ISSR primers (UBC813, UBC836, UBC857 and UBC873) characterized *F.sycomorus 14*, 3 ISSR primers (UBC817, UBC836 and UBC889) characterized *F. sycomorus15*,3 ISSR primers (UBC813, 817, UBC836 and UBC889) characterized *F. sycomorus1*, 3 ISSR primers (UBC836, 857 and UBC873) characterized *F. sycomorus11* and UBC859 ISSR characterized *F. sycomorus5* genotype.

Percent Disagreement Values (PDV) generated by the 14 SSR loci and 11 ISSR primers combination data using UPGMA routine in statistical program, ranged between 0.02-0.35 with a mean average of 0.15 (Table 5).

SSR and ISSR data were combined together to create a matrixes used for determination of genetic similarity among genotypes (Table 6). Thereby, established dendrogram based on SSR and ISSR data combination revealed that studied genotypes were clustered into two main groups (Figure 3). The first group involves *F. sycomorus14* and *F. sycomorus15* that were closed with PDV of 0.15 (similarity 0.90) (Tables 5 and 6). Whereas, the second one was divided into two subgroups, the first subgroup involves 9 genotypes (*F. sycomorus1, 2, 3, 4, 5, 6, 9, 10* and *11*); of which *F. sycomorus2* and *F. sycomorus3* were the most closed genotypes with the lowest PDV of 0.02 and the highest genetic similarity value of 0.99; followed by *F. sycomorus9* and *F. sycomorus10* with PDV of 0.03 (similarity 0.98) (Tables 5 and 6). Whereas, the second subgroup involves 5 genotypes (*F. sycomorus7, 8, 12, 13* and *16*). Of which *F. sycomorus12* and *F. sycomorus16* were genetically closed genotypes with PDV of 0.04 (similarity 0.97) (Tables 5 and 6). Otherwise, *F. sycomorus13* and *F. sycomorus14* were genetically the farthest genotypes by showing the highest PDV of 0.35 followed by *F. sycomorus14* and *F. sycomorus16* with PDV of 0.34 from the PDV mean average of 0.15.

In the current study, cluster analysis revealed that the 16 *F. sycomorus* genotypes were divided in two clusters based on SSR and ISSR data combination. These data were coherent with previous finding reported by Saleh [26] based on RAPD and IRAP data combination and also with the recent findings reported by Saleh [27] based on DAMD marker.

Previously, Ahmed *et al*. [25]applied SSR marker to screen pollination pattern and gene flow in the African fig *F. sycomorus.* The previous study revealednewly loci presented in *F. sycomorus* and a single locus originally developed in *F. carica*. In this regards, 12 loci were polymorphic when tested in between 8 and 79 Namibian *F. sycomorus* samples. Where, three of the new *F. sycomorus* loci were polymorphic in cultivars of the edible fig *F. carica* suggesting that the selection of these loci will be potential for population investigations in other fig species. Whereas, Rout and Aparajita [20]reported 116 as a total bands number of which 106 (91.3%) were polymorphic using 21 ISSR primers among 23 *Ficus* accessions. Whereas, Javed *et al*. [21]reported a total bands number of 29 of which 22 (75.9%) bands were polymorphic. Indeed, total bands number ranged between 5-7 bands with an average of 5.8 bands/primer; whereas, polymorphic bands number ranged between 3-6 with an average of 4.4 bands/primer among 25 Pakistan *F. palmata* genotypes using 5 ISSR primers.

Vignes *et al*. [4]reported SSR marker to identify *F. insipida* species. The previous study revealed that 11 out tested 13 SSR PC combinations produced 1-6 alleles with an average of 3.3 alleles/locus. Indeed, Bandelj *et al*. [5]reported 65 alleles as a total in *F. carica* L. collected from the northern Adriatic coast using 15 SSR PC combinations.

Achtak *et al*. [6]reported 85 alleles and alleles number ranged between 3-9 with an average of 5 alleles/locus among 75 Moroccan fig (*F. carica* L.)accessions using 17 SSR PC combinations. Moreover, Ikegami *et al*. [7]reported 13 ISSR, 19 RAPD, and 13 SSR primers application for genetic diversity of 18 accessions belonging to European and Asian fig (*F. carica* L.). The previous study revealed 258 fragments detected with all markers types. Moreover, mean genetic similarity was recorded to be 0.787, 0.717, and 0.749 for ISSR, RAPD and SSR markers, respectively. Indeed, polymorphism level (P%) was 97.41% for ISSR + RAPD together and 90.18% for SSR.

Oukabli *et al*. [8]reported genetic diversity among 75 Moroccan fig (*F. carica* L.) accessions using 17 SSR PCcombinations. The previous study revealed 25 alleles with an average of 6 alleles/locus. Whereas, Nazareno *et al*. [23]reported genetic diversity in Brazilian*F. citrifolia* and *F. eximia* species using SSR marker. The previous study revealed that 12 out 15 SSR PC combinations successfully discriminate the two species. Moreover, SSR markers produced 11 polymorphic loci along 60 tested individuals for each species (*F. citrifolia* and *F. eximia*). Indeed, SSR markers produced 4-15 alleles/locus and 2-12 alleles/locus for *F. citrifolia* and *F. eximia* species,

respectively. Whereas, Baraket *et al*. [10]reported 351 (342 were polymorphic) and 57 (57 were polymorphic) alleles were detected using AFLP and SSR markers, respectively. Indeed, PIC value was recorded to be 0.94 for SSR marker in Tunisian fig (*F. carica* L.). Moreover, Saddoud *et al*. [9]reported 39 alleles and that alleles/locus number ranged between 5-8 with an average of 6.5 alleles/locus. Indeed, PIC value ranged between 0.67 to 0.85 with an average of 0.79 among 18 Tunisian fig (*F. carica* L.) cultivarsusing 6 SSR PCs combinations. Moreover,

Caliskan *et al*. [12]reported that alleles/locus number ranged between 3-12 with an average of 6.8 alleles/locus. Whereas, RAPD marker produced 68 bands as total of which 55 bands were polymorphic among 76 Turkish fig (*F. carica* L.) accessions using 10 SSR PC combinations and 7 RAPD primers.

Basheer-Salimia*et al*. [22]reported genetic diversity among 9 Palestinian fig (*F. carica* L.) genotypes using 9 RAPD primers. The previous study revealed 57 total bands, of which 40 bands (70.2%) were polymorphic. Indeed, total bands number ranged between 3-9 with an average of 6.33 bands/primer; whereas, polymorphic bands number ranged between 3-7 with an average of 4.44 bands/primer. Whereas, Essid *et al*. [14]reported genetic diversity among 20 Tunisian accessions of fig (*F. carica* L.) using 13 SSR PC combinations. The previous study revealed 37 alleles in total and that alleles/loucs number ranged between 2-6 with an average of 2.85 alleles/loucs. Moreover,

Zheng *et al*. [15]reported application of 16 SSR PC combinations for screening genetic diversity among 4 populations of *F. hirta*. The previous study revealed that 9 out the 16 tested SSR PC combinations produced polymorphic pattern. Indeed, alleles/locus number ranged between 2-15.

Ferraraa *et al*. [16] reported genetic diversity among 24 Italian *F. carica* L. genotypes using SSR marker. The previous study revealed that 39 SSR PCs combinations produced 191 alleles in total of which 142 alleles (74.3%) were polymorphic. Indeed, total number of alleles/locus ranged between 1-9 and polymorphic number of 0-9 and the PIC value ranged between 0.07-0.91. Whereas, Zhang *et al*. [17]reported genetic diversity among 3 Chines *F. tikoua* populations using 14 SSR PC combinations. The previous study revealed 14 polymorphic loci with alleles number ranged between 3-16 in total and between 1-11 within populations.

Recently, Teoman *et al*. [18]reported a total of 82 alleles and that the number of alleles/locus ranged between 2-7 with PIC value ranged between 0.42-0.98 among 47 Turkish *F. carica* L. genotypes using SSR marker. Whereas, Fu et al. [24]reported total alleles number ranged between 3-17 among 85 Chines *F. virens* samples belonged to 3 populations using 15 SSR PC combinations.

Abou-Ellail *et al*. [13]reported 51 alleles in total of which 15 (29.4%) were polymorphic. Indeed, total alelles ranged between 2-15 and polymorphic alelles ranged between 0-13 among 7 *F. carica* L. cultivars using 6 SSR PC combinations. Whereas, Belttar *et al*. [32]reported 25 polymorphic alleles with an average of 1.6 allele/SSR loci with PIC of 0.69 were generated among 89 fig (*F. carica* L.) genotypes collected from Algeria and Turkey using 16 SSR loci. The previous study showed that two populations out of studied genotypes displayed low diversity between the Algerian and Turkish genotypes.

More recently, Aljane and Essid [33]reported 119 total bands of which 95 bands (79.83%) were polymorphic using 11 RAPD primers. Whereas, 13 SSR loci produced 37 total alleles, and total alleles ranged between 2-6 alleles with an average of 2.85 allele/SSR loci among 30 Tunisian fig (*F. carica* L.) cultivars using RAPD and SSR markers.

In the current study, the main average of PIC was 0.106 and 0.165 for SSR and ISSR, respectively was lower than that reported by the other studies mentioned herein. This could explain by the narrow genetic variation among studied *F. sycomorus* L. genotypes. This observation was in agreement with recent finding reported by Aljane and Essid [33]among 39 Tunisian fig (*F. carica* L.) cultivars using RAPD and SSR markers. The weak genetic diversity observed in the current study could be related to the allelic variation among the different *F. sycomorus* tree types through a complex pollination mechanism, involving the symbiotic relationships between the *F. sycomorus* tree and its pollinator and dynamic mutation-recombination operation. This observation was coherent with Ganopoulos *et al*. [34]in *Ficus* sp.

Conclusion

In conclusion, SSR and ISSR DNA-PCR based markers have been employed to investigate phylogenetic relationships among 16 genotypes of *F. sycomorus* species. Cluster analysis based on SSR and ISSR data combination revealed that the studied genotypes were grouped in two main clusters. Moreover, relationships between genetic structure and their geographical distribution was not clear suggesting genetic material exchange among different sites sampling. This observation was

coherent with recent finding in Tunisian fig (*F. carica* L.) using RAPD and SSR markers. Based upon combined SSR and ISSR markers together, the current investigation suggests narrow genetic variation within studied *F. sycomorus* L. genotypes.

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Table 1: Description of the studied *F. sycomorus* in the current study

Genotype code	Original site	Altitude (m)	Annual rainfall (mm))	References
F. sycomor 1	Lattakia	4.5	650-700	[26]
F. sycomor 2	Lattakia	6.5	650-700	Current study
F. sycomor 3	Lattakia	6.3	650-700	[26]
F. sycomor 4	Lattakia	6.5	650-700	Current study
F. sycomor 5	Lattakia	5	650-700	Current study
F. sycomor 6	Lattakia	5	650-700	Current study
F. sycomor 7	Lattakia	5	650-700	Current study
F. sycomor 8	Lattakia	5	650-700	Current study
F. sycomor 9	Lattakia	4	650-700	Current study
F. sycomor 10	Lattakia	6.3	650-700	[26]
F. sycomor 11	Lattakia	5	650-700	Current study
F. sycomor 12	Jableh	11.8	650-700	[26]
F. sycomor 13	Banyas	10	700-750	[26]
F. sycomor 14	Banyas	220	~850	[26]
F. sycomor 15	Banyas	12	700-750	Current study
F. sycomor 16	Banyas	250	~850	[26]

Table 2: SSR and ISSR tested primers in the current investigation in terms of primers sequence and annealing temperature (°C).

Primer name	Primer sequences 5' to 3' (F)	Primer sequences 5' to 3' (R)	Ta (°C)
SSR			
LMFC13	CCTCTTTCTCTCTCTTAATTTT	TTTATCAAACCCACTGATTC	58
LMFC14	CAAAACTCACACCAATAATC	TAATCTGCAAAAAGATGACTA	58
LMFC15	CGGAGAAAGATTTAGAATTTG	ATTCCAGAGACGAAAGGTCT	60
LMFC19	CTTATGAAAACTCGGTAGAAG	AATGAATGGAAATGATCTTG	60
LMFC20	ATGGAGGCTTAGATAGAAAT	ACAACACAAAAAGAAATATCA	60
LMFC21	ATGTCAAAACACCAGCTCTA	AAGAATAGAAAACCTGAAAAAG	60
LMFC22	ATCACGATATAGGTGTTTTAAT	AGACTTGTAATTTTGATTCCT	60
LMFC23	TTTCGTGTCTAACGATCAAAAA	CTCCCATCTCCAACTCCATC	60
LMFC24	ACTTCTTCATATTTGGTATAGG	TTCATAAACTGGTCTAAAAGA	60
LMFC25	GATTCTGATTAAAGGGTATTT	GCTTTCCAAATCTAAAGTAAC	60
LMFC26 F	ATGTTATAGTTGAGTGAGGATAA	AAATAGTGGATCTTGCATGT	60
LMFC27	ATTTCTTCAACTTTTGTAATGA	CCTTTTGTCTACATATACCTTT	58
LMFC28	TGATTCCTTTTACTTGTAGATT	AAGACATTGAGACATACCAG	58
LMFC30	TTGTCCGTTTCTTATACAAT	TCTTTTTAGGCAGATGTTAG	60
LMFC34a	GTTACAAAGTACAGGTAAGCA	GTATTGGATCTTGATTATGTTT	60
LMFC35	CTCAACCCCACCATTTTAAC	AGCTCTTTGTTGCTTCGATT	60
LMFC36	GACTCCTACACCATCAAAGG	CTTCACGTTGTTCCTGTTGT	58
LMFC37	AAGTACATCTTCACCATTGA	ATTAAACTCTTCATTCATCAGT	58
LMFC38	CTCAACGTCCGTACTAACTA	CTAAGGAATAAAAGGAGAAAA	58
LMFC40	TGTCAGTAGTTCCCTGGAGA	CCCGCATCTCTATTATTTGAC	58
MFC1	CTAGACTGAAAAAACATTGC	TGAGATTGAAAGGAAACGAG	58
MFC2	GCTTCCGATGCTGCTCTTA	TCGGAGACTTTTGTTCAAT	58
MFC6	AGGCTACTTCAGTGCTACA	GCCATAAGTAATAAAAACC	52
MFC8	GTGGCGTCGTCTCTAATAAT	TATTCTATGCTGTCTTATGTCA	52
ISSR			
UBC813	CTCTCTCTCTCTCTCTT		50
UBC817	CACACACACACACACAA		52
UBC834	AGAGAGAGAGAGAGAGYT		50
UBC836	AGAGAGAGAGAGAGAGYA		52
UBC840	GAGAGAGAGAGAGAGAYT		55
UBC855	ACACACACACACACACYT		55
UBC856	ACACACACACACACACYA		55
UBC857	ACACACACACACACACYG		55
UBC859	TGTGTGTGTGTGTGTGRC		54
UBC873	GACAGACAGACAGACA		52
UBC889	DBDACACACACACACAC		52

Single letter abbreviations for mixed-base positions: Y = (C, T); R = (A, G) and D = (non C).

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Table 3:Selected SSR and ISSR primers, total number of amplified fragments, number of polymorphic fragments, proportion of polymorphic fragments and PIC values.

SSR loci	Total alleles	Polymorphic alleles	P (%)	PIC
LMFC13	2	1	50	0.052
LMFC15	3	2	66.667	0.093
LMFC20	2	1	50	0.052
LMFC21	2	1	50	0.099
LMFC23	2	1	50	0.099
LMFC24	3	2	66.667	0.183
LMFC26 F	2	1	50	0.052
LMFC27	2	1	50	0.139
LMFC36	3	2	66.667	0.132
LMFC38	3	2	66.667	0.07
LMFC40	2	1	50	0.173
MFC1	2	1	50	0.105
MFC2	2	1	50	0.139
MFC6	2	1	50	0.099
Total	32	18		
Average	2.286	1.286	54.762	0.106
ISSR primer	Total bands	Polymorphic bands	P%	PIC
UBC813	9	6	66.667	0.174
UBC817	6	2	33.333	0.039
UBC834	8	4	50	0.183
UBC836	8	6	75	0.242
UBC840	3	1	33.333	0.164
UBC855	6	3	50	0.121
UBC856	6	3	50	0.238
UBC857	5	4	80	0.197
UBC859	3	1	33.333	0.039
UBC873	10	7	70	0.275
UBC889	10	6	60	0.146
Total	74	43		
Average	6.727	3.909	54.697	0.165

P (%) – Polymorphic %, PIC – Polymorphic information content

Table 4: SSR and ISSR specific primers characterized tested *F. sycomorus* genotypes.

SSR loci	N alleles detected	Specific alleles	Genotypes
LMFC13	2	1	*F. sycomor14*
LMFC15	3	1	*F. sycomor3 & 16*
LMFC20	2	1	*F. sycomor15*
LMFC21	2	1	*F. sycomor11 & 14*
LMFC23	2	1	*F. sycomor11 & 14*
LMFC24	3	1	*F. sycomor14*
LMFC26 F	2	1	*F. sycomor11*
LMFC27	2	1	*F. sycomor1, 11 & 14*
LMFC36	2	1	*F. sycomor8 & 13*
LMFC38	3	1	*F. sycomor15*
LMFC40	2	1	*F. sycomor1, 5, 14 & 15*
MFC1	2	1	*F. sycomor14*
MFC2	2	1	*F. sycomor9, 11 & 14*
MFC6	2	1	*F. sycomor14 & 15*
ISSR Primers	N bands detected	Specific bands	Genotypes
UBC813	9	1	*F. sycomor1,8,12,14 & 16*
UBC817	6	1	*F. sycomor15*
UBC836	8	1	*F. sycomor2,3,9,11,14 & 15*
UBC840	4	1	*F. sycomor1*
UBC857	4	1	*F. sycomor1, 7, 13, 11, 14 & 16*
UBC859	3	1	*F. sycomor1 & 5*
UBC873	10	1	*F. sycomor1,3,9,11 &14*
UBC889	8	1	*F. sycomor15*

Table 5: Percent disagreement values (PDV) among the 16 tested *F. sycomorus*(FS) genotypes yielded by SSR+ISSR combination data.

Genotype	FS1	FS2	FS3	FS4	FS5	FS6	FS7	FS8	FS9	FS10	FS11	FS12	FS13	FS14	FS15	FS16
FS1	0.00															
FS2	0.13	0.00														
FS3	0.13	0.02	0.00													
FS4	0.20	0.08	0.08	0.00												
FS5	0.15	0.06	0.06	0.05	0.00											
FS6	0.15	0.06	0.06	0.07	0.04	0.00										
FS7	0.24	0.16	0.16	0.11	0.12	0.14	0.00									
FS8	0.24	0.18	0.18	0.13	0.14	0.14	0.08	0.00								
FS9	0.18	0.12	0.14	0.15	0.16	0.16	0.13	0.13	0.00							
FS10	0.19	0.13	0.15	0.12	0.15	0.15	0.12	0.12	0.03	0.00						
FS11	0.10	0.12	0.12	0.17	0.12	0.12	0.23	0.23	0.15	0.16	0.00					
FS12	0.21	0.17	0.17	0.10	0.13	0.13	0.10	0.10	0.18	0.15	0.22	0.00				
FS13	0.24	0.22	0.22	0.15	0.18	0.18	0.13	0.11	0.23	0.20	0.26	0.05	0.00			
FS14	0.21	0.19	0.19	0.25	0.21	0.21	0.31	0.29	0.24	0.26	0.16	0.30	0.35	0.00		
FS15	0.19	0.13	0.13	0.20	0.17	0.15	0.24	0.25	0.18	0.19	0.18	0.26	0.31	0.15	0.00	
FS16	0.23	0.21	0.19	0.14	0.17	0.17	0.12	0.14	0.22	0.19	0.25	0.04	0.07	0.34	0.30	0.00

Table 6: Nei and Li similarity index among the 16 tested *F. sycomorus*(FS) genotypes yielded by SSR+ISSR combination data.

Genotype	FS1	FS2	FS3	FS4	FS5	FS6	FS7	FS8	FS9	FS10	FS11	FS12	FS13	FS14	FS15	FS16
FS1	1.00															
FS2	0.91	1.00														
FS3	0.91	0.99	1.00													
FS4	0.86	0.94	0.94	1.00												
FS5	0.89	0.96	0.96	0.96	1.00											
FS6	0.89	0.96	0.96	0.95	0.97	1.00										
FS7	0.83	0.88	0.88	0.91	0.90	0.89	1.00									
FS8	0.83	0.86	0.86	0.89	0.89	0.89	0.94	1.00								
FS9	0.88	0.91	0.90	0.88	0.88	0.88	0.90	0.90	1.00							
FS10	0.87	0.90	0.89	0.90	0.89	0.89	0.90	0.90	0.98	1.00						
FS11	0.93	0.92	0.92	0.88	0.91	0.91	0.83	0.83	0.89	0.89	1.00					
FS12	0.85	0.87	0.87	0.91	0.89	0.90	0.91	0.91	0.86	0.88	0.84	1.00				
FS13	0.83	0.83	0.83	0.88	0.86	0.86	0.89	0.91	0.82	0.84	0.80	0.96	1.00			
FS14	0.87	0.88	0.88	0.82	0.86	0.86	0.78	0.79	0.84	0.82	0.90	0.78	0.75	1.00		
FS15	0.87	0.91	0.91	0.85	0.88	0.89	0.83	0.81	0.87	0.86	0.88	0.80	0.77	0.90	1.00	
FS16	0.83	0.84	0.85	0.88	0.86	0.87	0.90	0.88	0.83	0.85	0.81	0.97	0.94	0.76	0.77	1.00

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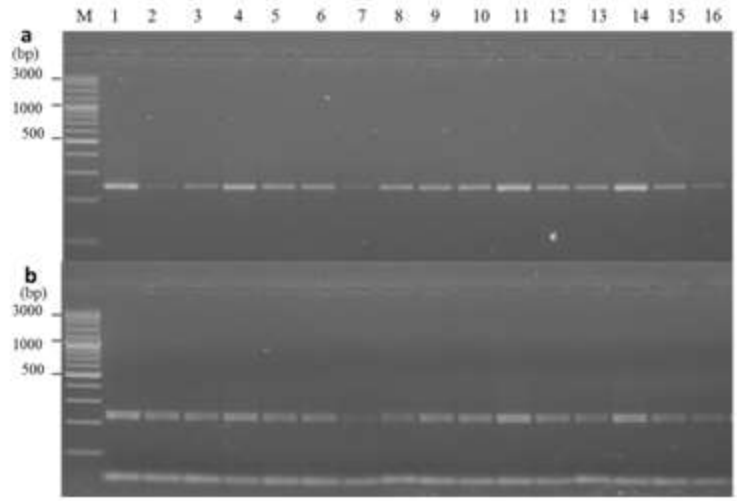

Fig. 1:DNA polymorphism profile in the 16 tested *F. sycomorus* genotypes obtained by LMFC30 (a) and LMFC36 (b) SSR loci. M: A VC 100bp Plus DNA Ladder (Vivantis) ladder standard.

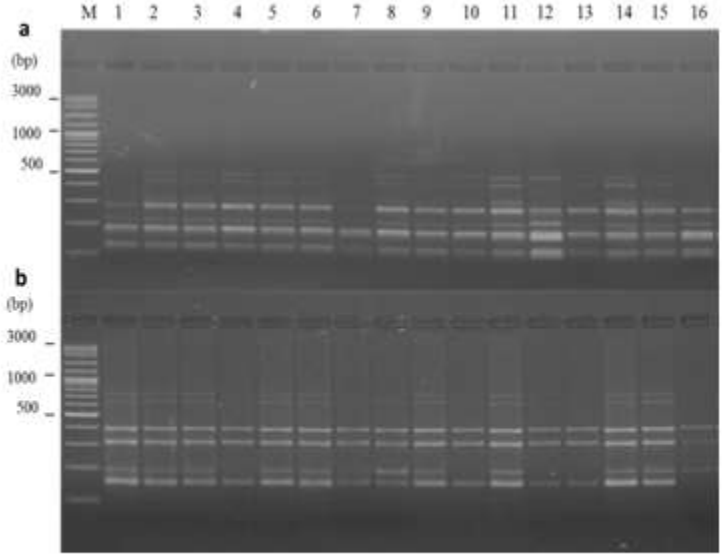

Fig.2: DNA polymorphism profile in the 16 tested *F. sycomorus* genotypes obtained by UBC834 (a) and UBC856 (b) ISSR primers. M: A VC 100bp Plus DNA Ladder (Vivantis) ladder standard.

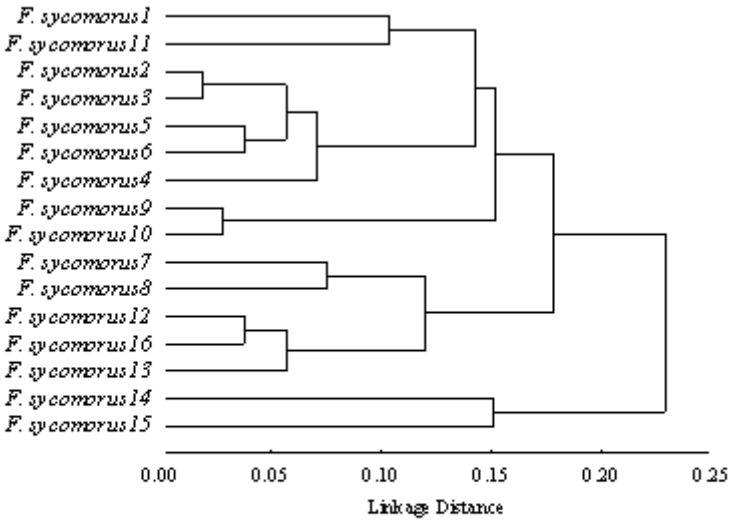

Fig. 3.Cluster analysis of the 16 tested *F. sycomorus* genotypes yielded by SSR+ISSR combination data based on percent disagreement values (PDV)

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