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Genetic and Molecular Evolutionary Analysis of Iraqi Figs Using ITS Sequences Compared to some International Cultivars

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Abstract

This study aimed to investigate the genetic variability and molecular evolution of Iraqi figs (*Ficus carica* L., family Moraceae) compared to some worldwide reference sequences. Upon internal transcribed spacer (ITS) sequence data analysis, the results indicated variation in the guanine-cytosine content (GC-content) ranging from 61.83% to 64.52%, with an average of 63.34%. Transitions seemed to occur less frequently than transversions in this region, where the overall bias R for transition/transversion has been calculated to be 0.551. Eight haplotypes have been identified from the 17 matched reference sequences under study, and the aligned sequences associated with the ITS allowed us to identify 64 polymorphic sites, 60 of which were singleton variables and 4 of which were parsimony- informative. A random distribution independent of provenance was observed in overall fig accessions in the neighbour-joining (NJ) tree. Argentine, Tunisian, and United Kingdom (UK) figs have the most genetic diversity (substitution), with a divergence distance of 0.05. This finding was further supported by principal components analysis (PCA) and the haplotype network. The first three of the PCA described 64.36% of the total variance. Molecular evolution tests revealed that the worldwide figs had suggested demographic expansion under a perfectly neutral model in a population that was in equilibrium until the 9th generation.

Keywords: Fig, GC content, Haplotypes, Mismatch, Nucleotide composition, PCA

تحليل التباين الوراثي والتطور الجزيئي لتسلسل التين العراقي مقارنة ببعض الأصناف العالمية (*Ficus carica* L.) باستخدام مناطق الفاصل النسخ الداخلي (ITS)

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الخلاصة

هدفت هذه الدراسة إلى دراسة التباين الوراثي والتطور الجزيئي في التين العراقي مقارنة ببعض التسلسلات المرجعية العالمية لهذا النوع (*Ficus carica* L.), حيث تم تسجيل التباين في محتوى الجوانين والسيتوسين (محتوى GC) في منطقة ITS بنسبة تتراوح بين 61.8 –64.52٪ و بمتوسط 63.34٪, وبين التحليل أن

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التحولات تحدث بشكل أقل من التبدالات في هذه المنطقة، حيث تم حساب التحيز الكلي R للتحول/التبدال بمقدار 0.551, تم تحديد ثمانية أنماط وراثية فردانية من تطابق ال 17 تسلسلًا مرجعياً قيد الدراسة، كما وسمحت لنا المحاذاة المرتبطة بالفاصل النسخ الداخلي بتحديد 64 متعدد الأشكال (متغاير)، 60 منها كانت متغيرات مفردة و 4 منها كانت معلوماتية. لوحظ توزيع عشوائي مستقل عن الموقع (الموطن) في مجموعات التين بواسطة تحليل شجرة الربط بالجوار (NJ), كما تبين ان أشجار التين الأرجنتينية والتونسية والبريطانية هي الأكثر استبدالًا، بتباعد جيني بلغ 0.05, وقد تم دعم هذا الاستنتاج من خلال تحليل المكونات الأساسية (PCA) وشبكة النمط الفرداني, وقد كشفت المكونات الثلاثة الأولى عن 64.36٪ من التباين الكلي, في حين بينت اختبارات التطور الجزيئي أن التين في جميع أنحاء العالم شهد توسعًا ديموغرافيًا في ظل نموذج محايد تمامًا في مجموعة سكانية كانت في حالة توازن حتى الجيل التاسع.

1. Introduction

The fig tree (*Ficus carica* L.) belongs to the Moraceae family (2n=26). It is cultivated worldwide for its highly valued fruit, which can be eaten fresh or dried, and offers significant nutritional and medicinal benefits. In addition to the nutritional significance of this important crop, many researchers, including Hajam and Saleem [1] and Li *et al*. [2], have identified that some fig tree extracts have a biological impact that boosts immunity and exhibit antimicrobial activity against various microorganisms. As a result, there is growing interest in the cultivation of this tree. Apart from its nutritional utility, almost all fig plant parts represent a good source of latex. Functionally dioecious, the fig tree exhibits a unique separation into two plant types: the hermaphrodite Capri fig and the female fig [3]. Some authors believe that figs originated in the Transcaucasian region, while others locate their origin in the eastern Mediterranean B asin or southern Arabia. From its birthplace, figs are believed to have migrated to the Mediterranean region, reaching the New World and eastern regions, such as China, Japan, and Southeast Asia in the sixteenth century [4].

Genetic erosion, which refers to the loss of biological genetic diversity, is a major concern for fig cultivation, primarily due to the consequences of urbanisation, monoculture, and the propagation of plants from remote regions. Fig agriculture takes on new dimensions due to the growing demand for fig fruit. Therefore, considerable genetic variety seen in established fig populations should serve as the foundation for improving fig cultivars, given that figs are not subjected to intensive breeding [5]. Molecular markers provide a practical approach for genotypic characterization and the assurance of highly accurate assessments of genetic relationships among various species.

The internal transcribed spacer (ITS) of nuclear ribosomal DNA sequences has the potential to exhibit a high degree of evolutionary differentiation at the species level, adding to the diversity assessment of fig cultivars around the world. ITS1 and ITS2 are two internal transcribed spacers found in eukaryotic nuclear ribosomal DNA (nrDNA). The ITS region, which is made up of the two spacers and the 5.8S subunit, has recently been considered a very diverse informative locus in flowering plants' nuclei for studying molecular systematics [6]. A remarkable feature of nrDNA (including the ITS) genes, as a result of concerted evolution, is that their paralogs are quite homogenous within individuals. The ITS region is very relevant in the systematic comparison of very recently divergent taxa due to the transcribed spacers' comparatively high rate of nucleotide substitution [7].

The study aimed to assess the organization of the fig population by allocating each of the accessions to groups based on genetic diversity and genetic evolution that occurred. This

could aid in identifying and conserving these genetic resources, offering a useful baseline for future breeding initiatives.

2. Materials and methods

2.1. Resource of fig sequences

All the studied reference sequences of fig had previously been deposited in GenBank, National Centre for Biotechnology Information (NCBI). Accession numbers of the assessed sequences are listed in Table 1. They were all extracted from the internal transcription spacer region (ITS), which is located between the 18S and 5.8S rRNA genes of the small subunit ITS. The large subunit ITS2 region is located between the 5.8S and 28S in eukaryotic rRNA genes.

2.2. Sequence analysis

The MEGA version 12.0.7 [8] was used to align and evaluate the nucleotide sequences of interest. The alignment had been manually verified, and a Maximum Composite Likelihood (MCL) methodology was applied to calculate the pairwise sequence difference among cultivars [9]. The GC content of each sequence was determined using DAMBE version 6 [10], and a chart diagram for each sequence was generated online by Biologics Corp. (https://www.com/tools/GCcontent/).

The "neighbour-joining (NJ) and maximum parsimony (MP)" trees were built using 1000 bootstrapping repetitions that were used to generate phylogenetic trees. The consistency indexes (CI) have been determined, along with the scores for retention indexes (RI) and composite indices (CI). The equation R = [A*G*k1 T*C*k2]/[(A+ G)*(T + C)] was applied to figure out the ratio of transitions/transversions (ti/tv), with A, G, C, and T representing the four nucleotide frequencies [8]. The analysis displays the number of nucleotide substitutions present at each position throughout the sequences.

Utilizing the DnaSP program version 5.10.01 [11], the aligned sequences were analyzed to determine polymorphism indices, demographic histories, and haplotype diversity (Hd), as well as nucleotide substitution ratios, to evaluate genetic diversity among sequences. In the entire ITS region, the diversity (Pi), along with its respective standard deviation. Likewise, the estimation of a mean pairwise nucleotide difference (K) was performed. Tajima's D [12] and Fu and Li's D* and F* [13] approaches were used to examine selective neutrality. In addition, the assessment of demographic parameters included the analysis of population size change, the allelic frequency distribution at a site [12], and the mismatch distribution of pairwise sequencing differences. The genetic relationship among some worldwide figs, as identified by the detection of haplotypes, was accomplished through network analysis by using the NETWORK program version 4.6.1.0 [14]. Additionally, PAST version 5.0.2 [15] was used to include the scatter chart plot produced by the multivariate principal component analysis (PCA).

Table 1: Length of figs sequences and GC contents of the ITS region of nuclear ribosomal DNA

Accession	GenBank accession number	GC content%	Sequence Length bp
Fig UK	EU091645.1	62.23	593
Thai fig	PQ182244.1	63.08	669
Iraqi fig	PQ182246.1	63.62	668
Costa Rican figs	KJ579172.1	63.95	613
Salvadoran fig	KJ579169.1	63.78	613
Argentine fig	KJ579170.1	62.78	618
Colombian fig	KJ579168.1	64.01	614
Turkish fig	GQ504297.1	63.43	648
Saudi fig	LC375796.1	61.83	820
Tunisian fig	GQ395458.1	61.96	723
Spanish fig	OP314383.1	64.52	637
Palestinian fig	OP314384.1	64.52	637
Indian fig	HM368190.1	62.99	608
Chinese fig	MT227783.1	63.00	719
French fig	MN960123.1	64.38	640
South Korean fig	KX572966.1	63.00	727
American fig	FJ916994.1	63.79	671
Average		63.34	659.88

3. Results and Discussions

3.1. Genetic diversity analysis

3.1.1. Length variation of ITS sequences

The sequences achieved presented variations in both their lengths and their nucleotide compositions for all fig cultivars. Notably, the size of the entire ITS ranges from 593 bp in the UK fig to 820 bp in the Saudi fig, with an average of 659.88 (Table 2). The analysis of 17 fig sequences from various countries worldwide revealed that the average transcribed intergenic spacer (ITS) length is 659.88 bp. In Tunisian fig, the total ITS region is 697.5 bp long [7]. This is quite similar to that of other plant species such as *Durio* spp. [16] (683 to 747 bp), *Lycium* [17] (532 to 691 bp), and Persian clover [18] (695 bp). Indeed, in the Egyptian *Ficus* species, ITS sequences ranged from 344 to 727 bp [19]. The variation of the entire ITS length in the Asteraceae family ranged from 650 to 750 bp [20]. According to Kehie *et al.,* [21], the average length of Naga King Chilli was 620 bp. However, among angiosperms, the ITS region varied in size from 565 to 700 bp. On the other hand, it is larger than those found in Tunisian date palms, which range from 441 bp to 445 bp [22].

3.1.2. Nucleotide frequencies

The nucleotide frequency analysis of the worldwide fig revealed 19.29%, 17.02%, 32.89%, and 30.79% for A, T, C, and G, respectively. This finding is completely compatible with the Tunisian figs [7], where the entire ITS region has a base composition of 19.7%, 18.6%, 31.4%, and 30.2% for A, T, C, and G, respectively. In other plant species, similar results were reported, such as Naga King Chilli, with nucleotide compositions of 18.85%, 17.56%, 33.95%, and 29.64% for A, T, C, and G, respectively [21]. The Egyptian *Ficus* species showed nucleotide frequencies of 20.24% (A), 17.03% (T/U), 31.87% (C), and 30.86% (G) [19]. On the other hand, dissimilar other outcomes have been reported in the Asteraceae family: 25%, 24%, 26%, and 25% for A, T, C, and G, respectively [20]. In Tunisian date palm [22], the nucleotide frequencies of the ITS are 24.76% for A, 25.67% for T, 27.22% for C,

and 21.95% for G. In Persian clover [18], the nucleotide distribution is as follows: the average ratios are 26.8%, 22.5%, 23.7%, and 26.9% for T, C, A, and G, respectively.

3.1.3 GC content

the GC percentage in the entire ITS sequence varied from 61.83% in Saudi figs to 64.52% in Spanish and Palestinian figs, with an average of 63.35% (Table 1 and Figures. 1 and 2). For the ITS 1 and ITS 2 regions of the Tunisian figs, the ratio was observed to range between 62 and 63.2, as reported by Baraket *et al.,* [7]. The Egyptian *Ficus* species had GC levels that ranged from 60.44 to 63.88% [19]. Other plant species, such as Naga King Chilli, have similar value (63.59%) [21]. The *Lycium* putative functional genes had higher values (50.9% to 60.8%), while putative pseudogenes had lower values (43.4% to 52.6%). [17]. On the other hand, the GC content was higher than that reported in previous study of other plant species study including Persian clover of 49.41% [18], Tunisian date palm at 49 to 49.7 [22] and *Robinia pseudoacacia* at 52.72–56.56% [23], while less than that of *Durio* spp of 68.71% [16]. This suggests that both have separate evolutionary origins for ITS [6]. Notably, there is limited variability in size, and the GC content of the ITS region reflects homogeneity between different cultivars. The different and widely separated environments confirm the high degree of conservation of ribosomal genes found in eukaryotes.

Notably, the nitrogenous base guanine was constant across almost all cultivars under investigation (30%), except in the case of the Costa Rican, Spanish, and Argentine figs, which were 31%, while the guanine base percentage of figs cultivated in Saudi Arabia and the UK is 29%. It seems that the variation in GC content may be entirely attributed to variations in the nitrogenous base cytosine. Similar to the results of Cucurbitaceae, as described by Hemleben *et al*., [24], the fig's rDNA appears to have been more affected by methylation.

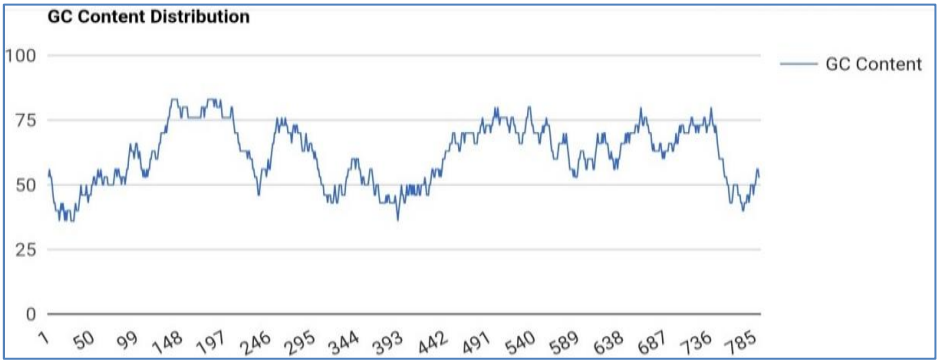

Figure 1: GC percentage of Saudi figs with variation in the GC distribution of 61.83% among nrDNA sequences at 820 bp.

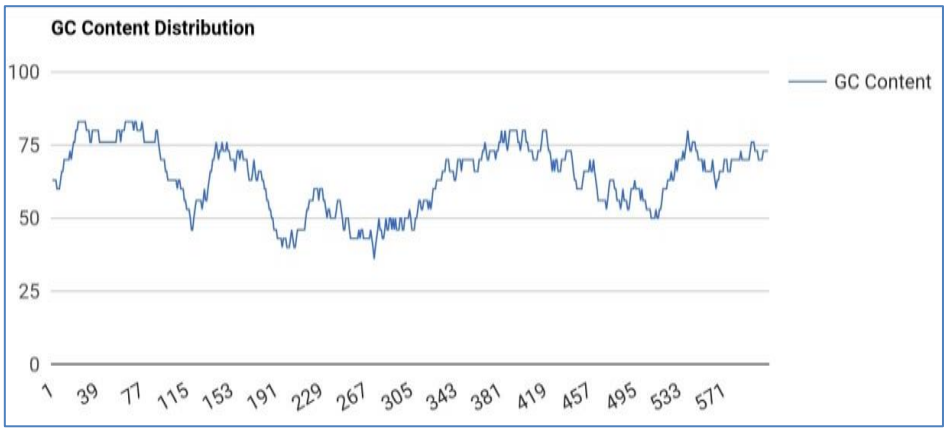

Figure 2: GC percentage of Palestinian figs with variation in the GC distribution of 64.52% among nrDNA sequences at 637 bp.

3.1.4 Nucleotide composition variance, mutational event

Following the analysis of entire ITS sequences of the assessed fig cultivars, the purine bases exhibited a transition/transversion ratio of $K_1 = 0.484$, whereas the pyrimidine bases had $K_2 = 1.948$. All the bases were calculated to have a transition/transversion ratio (R) of 0.551. A total transition/ transversion ratio obtained in the entire ITS region is less than those recorded in Tunisian figs (ti/tv) of 0.7 [7] and in the Egyptian *Ficus* species of 1.224 [19]. However, it was much less than that found for the sequence analysis of the entire ITS region in the Tunisian date palm (R = 4.375) [22], *Capsicum* sp. (ti/tv = 3.746) [21], and the Asteraceae family (ti/tv = 1.43) [20]. Table 2 lists the substitutions that were noticed. The study demonstrates that within the intergenic spacer ITS sequences of the fig of some countries under study, transitions occurred less frequently than transversions. According to the results presented in Table 2, transitions involving G/A and C/T transitions are more common than A/G and T/C transitions.

Table 2: Relative frequencies of nucleotide substitutions estimated from the fig sequences of the entire ITS region.

	A	T	C	G
A	-	*5.3*	*10.23*	**4.64**
T	*6*	-	**19.93**	*9.58*
C	*6*	**10.32**	-	*9.58*
G	**2.91**	*5.3*	*10.23*	-

NOTE*: Each entry in the provided data represents the probability of substitution (r) from one base (row) to another base (column). Transitional substitutions are denoted in bold, while transversional substitutions are shown in italics.

It is worth noting that the composition of the nucleotide sequence alignment within the entire ITS show remarkable diversity, as revealed by a character matrix of 837 base pairs (bp), and reveals the presence of 432 conserved sites alongside 64 sites that exhibit variations (Table 3). Among these variable sites, 60 are singletons at site positions 224, 225, 275, 292, 293, 302, 303, 331, 332, 333, 340, 375, 409, 411, 412, 413, 434, 474, 489, 491, 507, 511, 514, 523, 530, 531, 542, 545, 553, 559, 560, 561, 569, 579, 583, 589, 590, 595, 596, 600, 622, 634, 637, 643, 644, 650, 652, 659, 660, 663, 667, 670, 671, 674, 678, 681, 684, 686, 699 and 710, while 4 sites at positions 334, 544, 578 and 682, are considered as parsimony informative.
However, a considerable sequence variation in the intergenic region of the ribosomal DNA's ITS was observed. Indeed, 8 haplotypes were found among the 17 examined reference sequences. According to estimates, the nucleotide diversity (Pi) and haplotype diversity (Hd) were estimated to have standard deviations of 0.01663±0.00739 and 0.728±0.114, respectively, for the entire ITS sequences. In comparison to Tunisian figs, the world *Ficus carica* seem to have lesser nucleotide and haplotype diversities (Hd = 0.996, Pi = 0.072) [7]. In contrast, Tunisian palm and *Capsicum* sp. exhibit higher haplotype diversity (Hd of 0.552 and 1) and lower nucleotide diversity (Pi of 0.00155 and 0.01499), respectively [22 and 21].
Analysis of the whole ITS region of ribosomal DNA reveals significant genetic variation, with an average number of pairwise differences (K) estimated at 8.250, showing a high level of polymorphism (Table 3). The average pairwise nucleotide differences (K) observed in Tunisian figs (35.34) and *Capsicum* sp. (9.267) are notable, indicating a significant level of variability within the ITS sequences of these species [7,21]. On the other hand, the Tunisian and Iraqi date palms demonstrated minimal genetic diversity, with K values of 0.686 [22], 3.889, respectively [25].

Table 3: Neutrality and sequence polymorphism tests based on the ITS region

Figs sequences	The value
No. of sequences	17
Monomorphic characters	432
Polymorphic characters	64
Singleton variable sites	60
parsimony	4
H	8
Variance of Hd	0.01308
Pi(s) ± SD	0.01663±0.00739
Hd ± SD	0.728±0.114
Pi(JC)	0.01722
K	8.250
Probability	0.149
Minimum number of recombination (Rm)	1
Ramos-Onsins and Rozas (R2)	0.1274
Raggedness statistic (r)	0.0804
Tajima's D	-2.43202(s)***, P < 0.001
Fu and Li's D	-3.40171(s)**, P < 0.02
Fu and Li's F	-3.61749(s)**, P < 0.02
Fu's Fs statistic	1.859

3.2 Genetic relationships of entire ITS sequences

Based on a comparison of the entire ITS sequences, the Maximum Likelihood Composite (MCL) estimate of genetic distance ranges from 0.00 to 0.05, with an average of 0.02. This result suggests a significantly higher degree of genetic variation. There was no discernible difference (0.00) between the Palestinian, American, Turkish, and Spanish figs and between the Chinese, French, Costa Rican, and South Korean figs which also do not differ in the genetic distance from the Salvadoran and Colombian figs, indicating that these cultivars have the highest degree of homology. Nonetheless, there was a significant genetic distance (0.05) between the UK, Argentine, and Tunisian figs. However, the two Saudi and Thai figs were with a divergence distance of 0.02, while the Iraqi fig diverged at 0.01. A most parsimonious tree with 139 steps was identified through the parsimony analysis, which revealed 7 trees. The consistency index (CI) of 0.714286, retention index (RI) of 0.764706 and composite index (CI) of 0.546218, highlight the presence of homoplasic characters in the analyzed sequences.

The Neighbor-Joining dendrograms (NJ) divided fig sequences into two major groups, as seen in Figure 3. UK figs make up the first one, whereas all other cultivars belong to the second group. The second group is stratified into two subgroups; the first subgroup is composed of only Argentine fig. While all the other figs form the second subgroup, with a notable similarity between them and a notable difference from the Tunisian fig, it is crucial to remember that these classifications show the ongoing variability that defines the cultivars under study over generations. In fact, cultivars are not grouped according to the plant's geographical origin (continent). The findings of our study support the conclusions drawn from the Tunisian figs study [7].

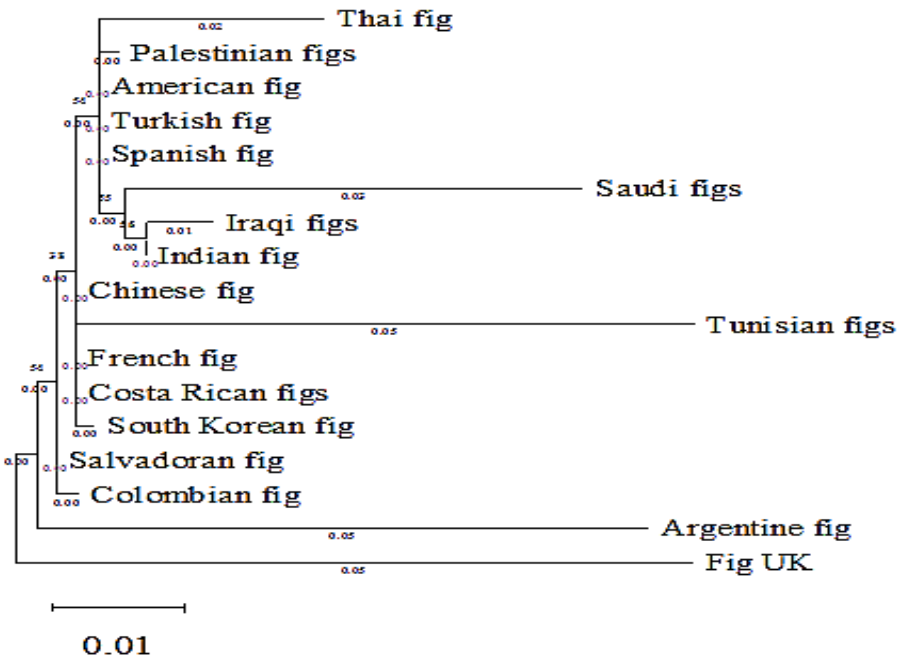

Figure 3: Neighbour joining tree according to the ITS sequences of the nrDNA; describes the tree and distance among some worldwide figs.

3.3 Molecular evolution

3.3.1 Recombination event

The intraspecific genetic characteristics found in figs are an excellent source to assess diversity among species. Additionally, the minimum recombination rate is a significant factor influencing DNA polymorphism patterns. The full data sequences revealed one recombination event in *F. carica*. Recombination events impacted the following sites: 334 and 544 (Table 3). This value is equivalent to Tunisian palm trees with a recombination event of 1 [22] and less than Tunisian figs with a recombination event of 20 [7].

3.3.2 Tests for selective neutrality

To assess whether the observed diversity patterns in fig nuclear ribosomal DNA sequences worldwide significantly departed from a neutral equilibrium hypothesis, a variety of statistical techniques were used. The null hypothesis was assessed by applying both methodologies of Tajima [12] and Fu and Li [13] to test the selective neutrality of the rare mutations. The findings of this analysis demonstrated that Tajima D had negative and statistically significant values (D*** = -2.43202, $P < 0.001$) in the sequences overall. Similarly, Fu and Li's statistical tests showed that the entire ITS region also had negative and significant values (D** = -3.40171, $P < 0.02$) and F** = -3.61749, $P < 0.02$). Therefore, all parameter statistics reject neutrality in the ITS region (Table 3).

Tajima and Fu & Li's analysis reveals a divergence from selection neutrality due to an excess of rare mutations in singleton sequences analysed. This amount is effective in detecting deviations from neutrality, as well as assessing population growth and sample size. Analysis of Fu & Li parameters rejects neutrality in the analysed regions, indicating a recent demographic expansion of the worldwide fig (Table 3 and Figure 4). Upon inspecting the findings in Table 3, it can be seen that Fu's Fs parameter has a positive value, reported as 1.859.

3.3.3 Mismatch distribution

On the other hand, the mismatch distribution among the 17 fig reference sequences examined. This analysis showed that the frequency graph between alleles exhibits

demographic equilibrium rather than a recent population expansion (Figure 4). In worldwide figs sequences (Figure 5), Harpending's raggedness and the Ramos-Onsins statistic (r = 0.1274; P = 0.149, R2 = 0.0804) support these findings. Fu's Fs value, which is a positive value, shows no signs of recent population expansion of some worldwide fig sequences studied (Table 3). The internal transcribed spacer of the nr DNA (ITS) of fig references sequences demonstrated positive values, however insignificant, confirming the null hypothesis of selective neutrality and suggesting that fig evolved in a drift-mutation equilibrium. These results match those recorded in Tunisia and Iraqi date palm cultivars using sequences of intergenic spacer nr DNA [22, 25]. The recent population expansion of these sequences does not seem to be supported by the multimodal nature of the mismatch curves (Figure 4). ITS sequences seem to indicate an early evolutionary stage [7]. The mismatch distribution usually appears as multimodal, but it can also appear as unimodal in a population that has not recently experienced a demographic expansion.

This suggests that it is likely attributed to the widespread of these species and/or may be due to gene conversion and unequal crossing over. Alternatively, the reasons may include mechanisms, such as genetic drift accompanied by limited gene flow, natural selection, small population size, and geographical isolation as factors associated helplessly with reduced variability of the nr DNA spacer. The mismatch distribution is an indicator of expansion time [22].

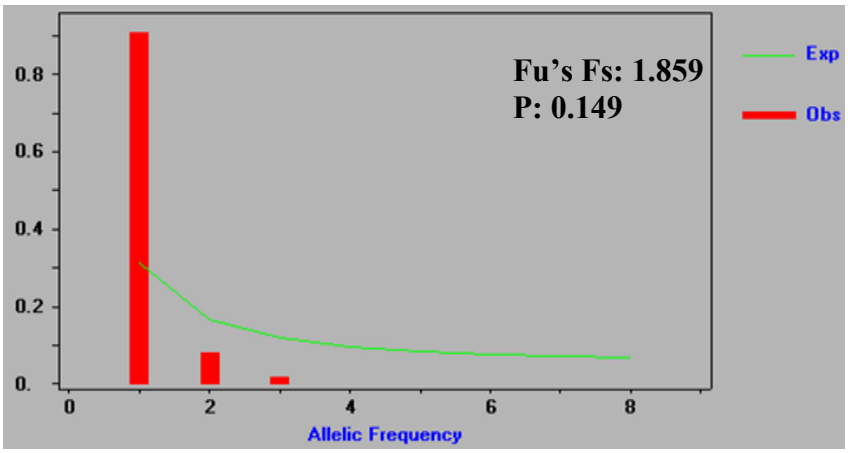

Figure 4: The frequency spectrum of worldwide examined figs nrDNA sequences detected in the ITS region. The solid lines in the spectrum reflect the distributions noticed under neutrality and equilibrium (mutation drift).

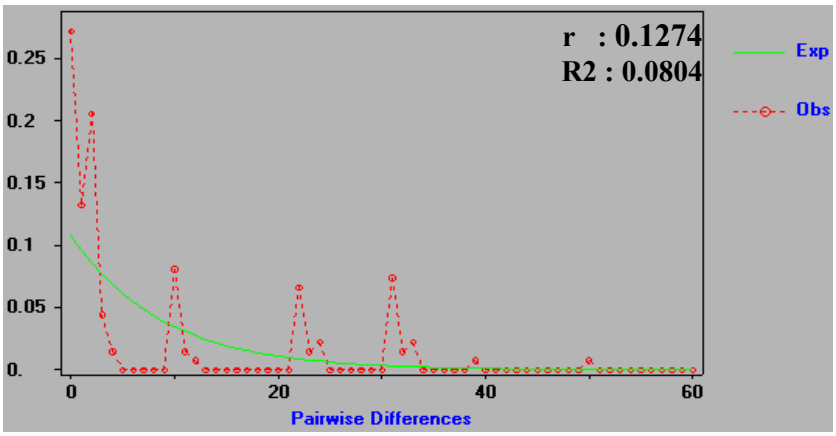

Figure 5: Mismatch distribution of figs population, displaying the observed distribution and expected values for population size changes with initial theta (11.359) and final tau (1000.000).

3.3.4 Distribution of haplotypes according to ITS sequencing

Based on the entire ITS spacer sequence, the haplotype network illustrated in Figure 6 clearly demonstrates the evolution and population expansion of figs related to an ancestor haplotype. The founder haplotype, Hap 2: is represented by the Thai, Costa Rican, Salvadoran, Turkish, Spanish, Chinese, French, South Korean, and American figs. This may be due to the fig being more preserved in the genetic makeup of the original, where a network exhibits radial form within its core structure and is considered an ancestor of the rest. Thus, it can be concluded that haplotype Hap 2 is the ancestral sequence from which the other sequences have evolved through mutations. The substitutions within the figs were comprised of only one mutant in both of the Hap 5 and Hap 8, representing the Colombian and Palestinian figs, respectively, and two mutants with Hap 3: representing both the Iraqi and Indian figs together, and Hap 7: representing the Tunisian figs. The remaining figs were listed in the subsequently: ten mutants with Hap 6: represent the Saudi figs, while both Hap 4: and Hap 1 were represented by both the Argentine figs and Fig UK, with 22 and 31 mutants, respectively. Furthermore, the hatch marks in Figure 6 indicate the number of mutational events identified, which reflects the age of fig trees worldwide. A network of links between the haplotypes is also presented in the study, with a set of hatch marks indicating the number of mutations. This result is consistent with the phylogenetic tree in Figure 3.

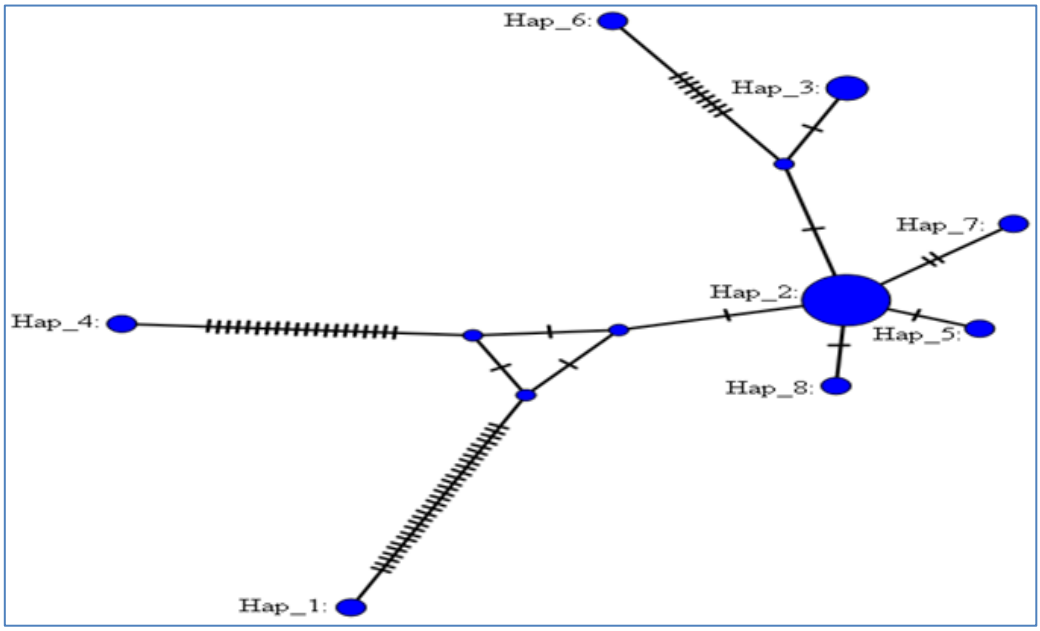

Figure 6: The network of haplotypes inferred from ITS sequences shows that the hatch marks on connected lines referred to the number of mutations, H1: represent Fig UK. H2: representation of the Thai, Costa Rican, Salvadoran, Turkish, Spanish, Chinese, French, South Korean and American figs. H3: representing both the Iraqi and Indian figs. H4: refers to Argentine figs. H5: representing the Colombian fig. H6: representation of the Saudi figs. H7: representing the Tunisian figs. H8: representing the Palestinian figs. The blue circles represent distinct haplotypes, with diameters indicating the frequency of the corresponding sequences, while inferred ancestral nodes are shown by small, unlabeled blue circles**.**

3.4 Principal Component Analysis (PCA)

The scatter plot obtained from the first three components from the ITS region using a multiple-variate principal component analysis revealed that PC1 had a variance of 24.521 with an eigenvalue of 7.81238, PC2 had a variance of 21.271 with an eigenvalue of 6.77704, and PC3 had a variance of 18.572 with an eigenvalue of 5.91698. Collectively, they (PC1-3)

accounted for 64.364% of the total genetic variance. Noteworthy is that, except for the Argentine and the UK figs, they were in two separate groups due to the high number of mutations (Figure 7). The population structure and dendrogram findings are in agreement with other analyses. There was no clustering based on the collecting site, similar to the other analyses. Based on physiological, morphological, fruit morphological, or biochemical traits (genetic or phenotypic traits), the percentage of variance appears to be either relatively high or low in comparison to the percentages obtained from fig plants, or it is nearly equal if only the first two components are taken into account. According to the majority of earlier research, the average variance ratio and component count were as follows: three components explained 52.99% of the total variation [26]; ten components accounted for 84.11% of the total variation [27]; four components explained 83.67% variation [28]; 5 components with a variability of 75.40% [29]; five components exhibited a cumulative contribution rate of 91.5% [30]. Moreover, the first two components explained 56.14%, 91.04%, 49.15%, 77%, 72.8%, 62.41%, 86.25%, and 65% of the total variance in *Ficus carica* [31; 32; 33; 34; 35; 36; 37; 38], respectively.

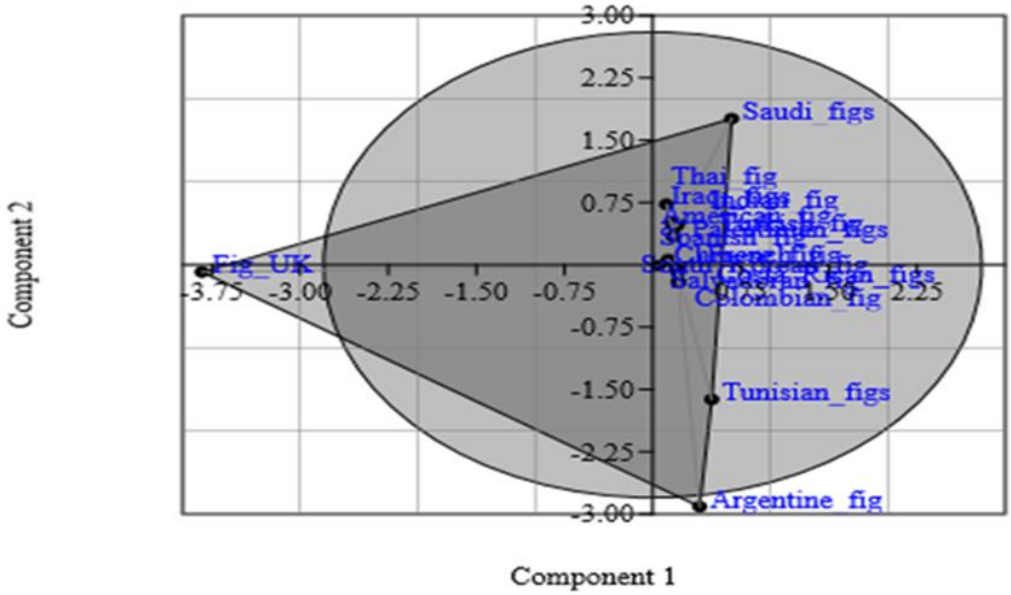

Figure 7: A scatter plot diagram for principal component analysis demonstrates the relative distribution of 17 figs sequences based on the entire ITS region.

4. Conclusion

Despite the presence of unique genetic distinctions, the investigation concludes that all of the analysed cultivars are descended from a common ancestor. Such an outcome highlights the utmost importance of preserving diversity to protect species that possess valuable traits. The fig reference sequences analysed suggest there has not been a significant increase in genetic diversity during the course of our miscellaneous investigations. Notably, most investigations and testing indicate that Iraqi figs did not differ considerably, as is the case with most figs worldwide. Argentine, Tunisian, and United Kingdom (UK) figs have the most genetic diversity among the reference sequences studied. Moreover, a more comprehensive and detailed evaluation of the available genetic diversity is required. To determine the long-term effects of these variations on the diversity of the fig population, further research in this region, as well as in other regions, using different markers, is necessary. As a result, a thorough understanding of the breadth and distribution of genetic variability is essential for determining a cultivar's genetic predisposition. In the future, it is preferable to choose Iraqi wild plants, which have few studies.

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Conflict of interest statement

The authors have declared that no competing interests exist.

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