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Assessment of Genetic Diversity Among Fig *(Ficus carica L.)* Cultivars in Sulaymaniyah Using SRAP Markers

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ABSTRACT

Fig (Ficus carica L.) is a fruit of significant importance worldwide and is considered an essential crop for commercial cultivation. This study investigates the genetic variability of the main fig cultivars in Sulaymaniyah, Kurdistan region of Iraq using Sequence-Related Amplified Polymorphism (SRAP) markers. Over the past decade, molecular markers have become powerful tools for DNA fingerprinting, germplasm characterization, and genetic diversity assessment. The objective of this study was to analyze the genetic fingerprinting of fig varieties using SRAP molecular markers. Ten SRAP primers combinations were applied to detect polymorphisms among the examined cultivars. The selected varieties were cultivated in the Sulaymaniyah governorate, and the experimental work was conducted at the Scientific Research Center, College of Science, University of Duhok.The binary matrix generated was analyzed using NTSYS-PC version 2.10 software to calculate similarity values and construct a phylogram for data interpretation. The highest genetic distance was observed between the Kola and Henzeer Rash cultivars (0.51102), while the lowest genetic distance was recorded between Henjeer Bonndoar and Bakra Joo (0.1157).Cluster (dendrogram) analysis revealed two main groups: the first consisting solely of Henzeer Rash, and the second further divided into two subgroups. The first subgroup comprised only Nejefee, while the second was split again into two clusters—the first including Kola and Galazard, and the second containing Zarda Roon, Henjeer Bonndoar, Bakra Joo, and Sheela.Overall, the results indicate that the analyzed fig cultivars represent a population with high genetic diversity and a broad range of genetic variation.

***Keywords*:** SRAP marker, Ficus carica, genetic diversity, genetic fingerprinting, Sulaymaniyah government.

INTRODUCTION

The fig (*Ficus carica* L.), a member of the Moraceae family, is recognized as one of the earliest domesticated fruit trees linked to the beginnings of horticulture in the Mediterranean region. Archaeobotanical evidence suggests that early humans domesticated Ficus carica from a diverse range of wild populations distributed across the southern and eastern Mediterranean during the early Neolithic period [1]. The cultivated fig (*Ficus carica* L.) exhibits a gynodioecious reproductive system, whereas the species as a whole is functionally dioecious. Pollination is usually performed by small wasps. The relationship between figs and fig wasps is mutualistic: the wasps reproduce inside the figs, and the figs depend on them for pollination [2, 3].The fig fruit consists of an enlarged receptacle, known as a syconium, which encloses and protects the flowers from most parasites, allowing entry only to small insects capable of passing through its natural opening. [4, 5]. Fig wasps belong to the family Agaonidae, a group of parasitic Hymenoptera within the superfamily Chalcidoidea. Each fig species typically possesses a uniquely specialized pollinator wasp [6].

Owing to its nutritional, medicinal and ornamental features, fig is considered a fruit of great importance worldwide; according to FAO figures, more than one million tons of figs are produced annually by the world [7]. *Ficus carica* L. is a highly adaptable woody species displaying considerable variability in its growth habit. It may appear as a compact 23shrub or bush in harsh or dry environments, while under favorable conditions it can develop into a medium or large tree with an extensive canopy [8]. Figs are mostly monoecious plants with a unique inflorescence called a syconium,

containing many small flowers that lack petals.

However, fig trees are commonly propagated through cuttings of mature wood or by grafting, as sexual propagation by seeds is generally not preferred due to the high variability among seedlings. Vegetative propagation, therefore, ensures genetic uniformity, preserves desirable traits of elite cultivars, and enables the rapid multiplication of plants with consistent fruit quality and productivity [9, 10].

Plant fingerprinting depends on the use of DNA Markers tools regarding genetic relatedness among individuals, population structure, and phylogenetic relationships, as well as mapping loci and tracking quantitative traits. Although DNA markers have great potential for generating valuable genetic information, they also have certain limitations, often arising from technical constraints. In the case of Sequence-Related Amplified Polymorphism (SRAP) markers, several studies have reported issues with data reproducibility [11, 12].

SRAP analysis is designed to specifically amplify coding regions of the genome using primers that target GC-rich exons (forward primers) and AT-rich promoters, introns, and spacers (reverse primers). The primers are typically 17–18 nucleotides in length and consist of core sequences of 13–14 bases. The first 10–11 bases at the 5′ end serve as non-specific "filler" sequences, followed by the conserved motifs CCGG- in forward primers or -AATT in reverse primers [13]. SRAP-PCR markers are simple, cost-effective, and highly efficient, allowing the generation of genome-wide fragments with excellent reproducibility and broad applicability across different plant species.

The limitations of SRAP markers are not yet fully understood, as these markers are relatively new and their application is still in its early stages. Similar to other dominant markers, SRAP amplicons cannot be used to estimate heterozygosity or to evaluate genetic parameters such as Hardy–Weinberg equilibrium [14, 15].The objectives of this Study is to evaluate the genetic diversity and phylogenetic relationships among selected fig genotypes using SRAP markers, also to assess the efficiency and reliability of the SRAP marker system in identifying molecular fingerprints for elite cultivars, and finally to provide a molecular basis for germplasm conservation and assist in the selection of parental lines for future breeding programs.

Material and Methods

1. DNA Extraction:

The experiments were conducted in the Plant Molecular Biology Laboratory at the Scientific Research Center, College of Science, University of Duhok. Leaf samples of various fig (Ficus carica L.) varieties were collected from villages surrounding Sulaymaniyah city. Genomic DNA was extracted from the leaf tissues using the CTAB method as described by [16].

2. PCR Assay:

The PCR reaction mixture contained 50 ng/µl of DNA template, 5 pmol of each primer, 10× PCR buffer, 0.25 mM $MgCl_2$, 0.5 mM dNTPs, and 1 U/µl Taq DNA polymerase, in a total volume of 20 µl. The PCR amplification profile consisted of an initial denaturation at 94°C for 5 minutes, followed by five cycles of 94°C for 1 minute (denaturation), 35°C for 1 minute (annealing), and 75°C for 1 minute (extension). This was followed by thirty-five cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute, with a final extension at 72°C for 5 minutes. The PCR products (2 µl) were then separated by electrophoresis on a 2% agarose gel at a constant power of 60 W for 1 hours.

For the present study of genetic diversity among different fig varieties, Ten SRAP combinations were used and produced amplified product. The primers combination and their sequence used during the present study were shown in Table(1):

Table (1): Sequences of forward and reverse SRAP Primers:

Primer	Rivers	Forward
EM16ME8	EM16: 5`-GAC TGC GTA CGA ATT GTC-3`	ME8: 5`-TGA GTC CAA ACC GGA CT-3`
EM16 ME5	EM16: 5`-GAC TGC GTA CGA ATT GTC-3`	ME5: 5`-TGA GTC CAA ACC GGA AG-3`
EM16ME1	EM16: 5`-GAC TGC GTA CGA ATT GTC-3`	ME1: 5`-TGA GTC CAA ACC GGA TA-3`
EM15ME7	EM15: 5`-GAC TGC GTA CGA ATT GAT-3`	ME7: 5`-TGA GTC CAA ACC GGA CG-3`
EM16ME6	EM16: 5`-GAC TGC GTA CGA ATT GTC-3`	ME6: 5`-TGA GTC CAA ACC GGA CA-3`
EM9ME2	ME9: 5`-TGA GTC CAA ACC GGA GG-3`	ME2: 5`-TGA GTC CAA ACC GGA GC-3`
EM15ME8	EM15: 5`-GAC TGC GTA CGA ATT GAT-3`	ME8: 5`-TGA GTC CAA ACC GGA CT-3`
ME13ME2	EM13: 5`-GAC TGC GTA CGA ATT CTG-3`	ME2: 5`-TGA GTC CAA ACC GGA GC-3`
ME13EM1	EM13: 5`-GAC TGC GTA CGA ATT CTG-3`	ME1: 5`-TGA GTC CAA ACC GGA TA-3`
EM15ME5	EM15: 5`-GAC TGC GTA CGA ATT GAT-3`	ME5: 5`-TGA GTC CAA ACC GGA AG-3`

3. Data Analysis

The PCR amplified products were scored as 1 or 0 respectively for the presence or absence of bands across the genotypes to generate a binary matrix. The binary matrix was analyzed using the NTSYS-PC version 2.10 software to calculate the similarity values and to generate the phylogram. Similarity coefficient 2.10 [17]. Cluster analysis was conducted on similarity using the un-weighted pair group method on arithmetic averages (UPGMA).

Results and Discussion

Ten SRAP combinations were used to the study the genetic diversity among the eight Fig varieties Genetic similarity represents coefficient matrix of these fig varieties based on the data of the ten combinations SRAP primers Showed in Table (2), the highest genetic distance was between Kola varieties and Henzeer Rash varieties (0.51102) and lowest genetic distance were between Henjeer Bonndoar and Bakra Joo (0.1157).

Table (2): Genetic similarity of the studied fig varieties:

	Zarda Roon	Sheela	Kola	Nejefee	Gala Zard	Henzeer Rash	Henjeer bonndoar	Bakra Joo
Sheela	0.13042	0.0000						
Kola	0.31576	0.24832	0.0000					
Nejefee	0.42792	0.40053	0.031391	0.0000				
Gala Zard	0.27906	0.34360	0.18453	0.41325	0.0000			
Henzeer Rash	0.51102	0.51102	0.54610	0.41160	0.51763	0.0000		
Henjeer bonndoar	0.26293	0.28235	0.35162	0.37708	0.24624	0.43201	0.000	
Bakra Joo	0.2391	0.2575	0.3005	0.3404	0.2083	0.4689	0.1157	0.0000

Cluster Analysis

A dendrogram was obtained by the UPGMA method using the total number of SRAP bands (Fig. 1). There were two main groups in the dendrogram: the first one consists only of Henzeer Rash; the second group also divided to two subgroups. The first subgroup (Nejefee) appears alone; the second subgroup then consist of another two subgroups; the first one includes Kola and Gala Zard; the second subgroup consist of Zarda Room, Henjeerbonndoar, Bakra Joo and Sheela.

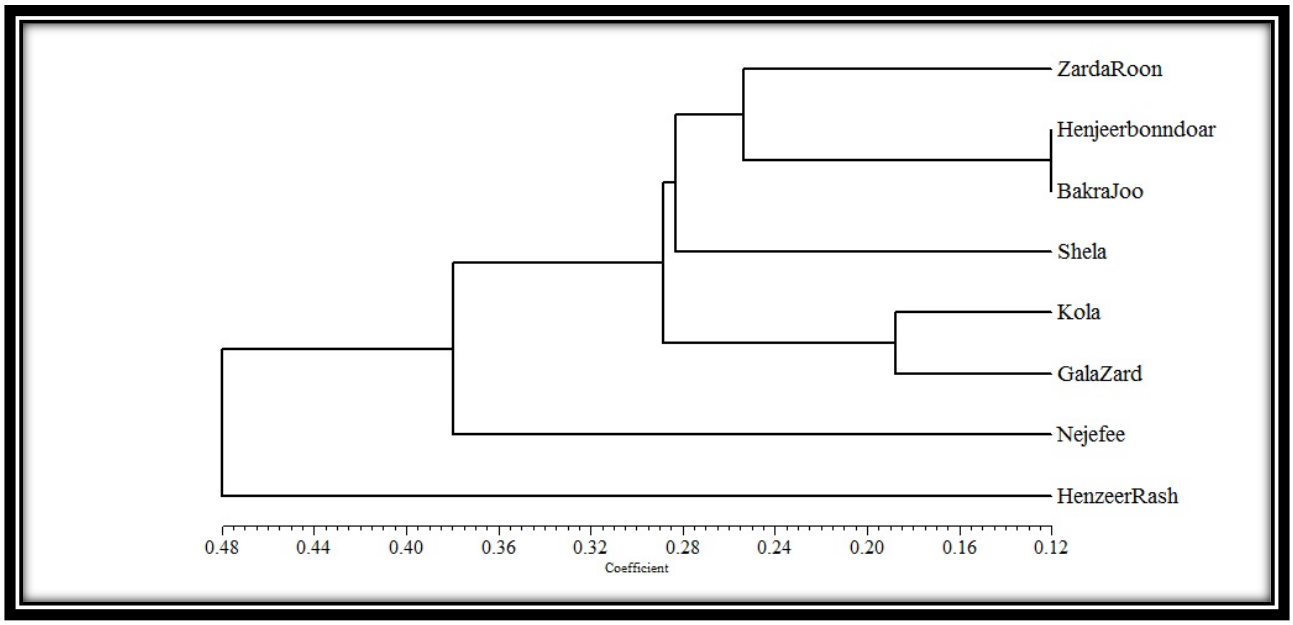

Figure 1: A dendrogram Neighbor─joining tree representing the genetic relationships among studied Fig varieties

In Iraq and the Kurdistan Region, several studies have focused on the genetic fingerprinting of fig cultivars. For instance, molecular identification of figs has been performed using various marker systems, including isozyme markers [18], RAPD markers [18, 19, 20], and SSR markers [21]. Additionally, both SSR and AFLP markers have been applied to investigate genetic diversity and relationships among fig genotypes [22].

The SRAP marker system is becoming the marker of choice for characterization and genetic diversity studies in a wide range of plants. The study described in this paper shows that SRAP analysis is a powerful tool also for the characterization of genetic diversity of fig varieties.

Conclusion

In conclusion, the results obtained from the SRAP analysis exhibited a strong correlation with the morphological classification of the fig cultivars. This alignment confirms that SRAP markers are reliable indicators of the underlying genetic relationships among the analyzed genotypes. Furthermore, the findings demonstrate that SRAP is not only a simple and cost-effective molecular marker system but also a highly informative and versatile tool for assessing genetic diversity, identifying cultivar-specific fingerprints, and elucidating phylogenetic relationships. The high efficiency and reproducibility of SRAP markers underscore their valuable potential for applications in fig breeding programs, germplasm characterization, and the conservation of genetic resource

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تقييم التنوع الوراثي بين أصناف التين *(Ficus carica L.)* في السليمانية باستخدام مؤشرات SRAP

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الخلاصة
تُعدّ التينة ***(Ficus carica L.)*** من الفواكه ذات الأهمية البالغة عالمياً، ومحصولاً أساسياً للزراعة التجارية. تبحث هذه الدراسة في التباين الوراثي لأصناف التين الرئيسية في محافظة السليمانية، إقليم كردستان العراق باستخدام مؤشرات التباين في المواقع المتتابعة المرتبطة ***(SRAP).*** خلال العقد الماضي، أصبحت المؤشرات الجزيئية أدوات قوية للبصمة الوراثية، وتوصيف الأصول الوراثية، وتقييم التنوع الجيني. هدفت هذه الدراسة إلى تحليل البصمة الوراثية لأصناف التين باستخدام مؤشرات ***SRAP*** الجزيئية، حيث تم تطبيق عشرة بادئات ***(Primers)*** للكشف عن التعدد الشكلي ***(Polymorphism)*** بين الأصناف المدروسة. زُرعت الأصناف المختارة في محافظة دهوك، وأُجري العمل التجريبي في مركز الأبحاث العلمية بكلية العلوم بجامعة محافظة السليمانية
ثُم تحليل المصفوفة الثنائية الناتجة باستخدام برنامج ***NTSYS-PC)*** الإصدار 2.10) لحساب قيم التشابه وإنشاء المخطط الوراثي ***(Phylogram)*** لتفسير البيانات. أظهرت النتائج أن أعلى مسافة وراثية كانت بين صنفي ***"Kola"*** و***"Henzeer Rash" (0.51102)*** ، بينما سُجلت أدنى مسافة وراثية بين ***"Henjeer Bonndoar"*** و***"Bakra Joo" (0.1157).*** كشف تحليل العناقيد ***(Dendrogram)*** عن مجموعتين رئيسيتين: ضمت الأولى صنف ***"Henzeer Rash"*** فقط، بينما انقسمت الثانية إلى مجموعتين فرعيتين؛ ضمت المجموعة الفرعية الأولى صنف ***"Nejefee"*** فقط، بينما انقسمت الثانية مجدداً إلى عنقودين: شمل الأول صنفي ***"Kola"*** و***"Galazard"*** ، وضم الثاني أصناف ***"Zarda Roon"*** و ***"Henjeer Bonndoar"*** و ***"Bakra Joo"*** و***"Sheela".***
بشكل عام، تشير النتائج إلى أن أصناف التين التي خضعت للتحليل تمثل مجتمعاً ذا تنوع وراثي عالٍ ونطاق واسع من التباين الجيني.

الكلمات المفتاحية: مؤشر SRAP، التين ((Ficus carica، التنوع الوراثي . محافظة السليمانية.