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INITIAL GENETIC DIVERSITY ASSESSMENT OF ENDEMIC HYRCANIAN WILD FIG POPULATIONS IN AZERBAIJAN

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Abstract. *This study presents the first genetic assessment of wild Ficus carica populations from the Hyrcanian forests of Azerbaijan using Inter Simple Sequence Repeat (ISSR) markers. Fifteen genotypes collected from Hirkan National Park were analyzed using five ISSR primers, which revealed a moderate level of genetic diversity (GDI = 0.62). All accessions were successfully differentiated, highlighting the high discriminatory power of the selected primers. Jaccard similarity indices ranged from 0.14 to 0.89, with an overall mean of 0.53. Cluster and Principal Coordinates Analyses revealed three major genetic clusters and significant intra-population diversity, with no correlation to geographic proximity. Unique bands were observed in several genotypes, and variation in genetic diversity indices across primers emphasized the importance of marker selection. These findings underscore the substantial genetic variation among wild fig populations in Azerbaijan and confirm the utility of ISSR markers for conservation, breeding, and taxonomic studies of underutilized fig germplasm.*

Keywords: Ficus hyrcana, genetic diversity, ISSR, wild fig.

INTRODUCTION

The genus *Ficus* L. (Moraceae) is one of the largest among flowering plants, comprising over 800 species that are predominantly distributed across tropical and subtropical regions. *Ficus carica* L., commonly known as the common fig, holds significant economic and cultural importance and is widely cultivated throughout the Mediterranean basin and West Asia, owing to its broad ecological adaptability [1]. Cytogenetically, it is diploid, with a somatic chromosome number of 2n = 2x = 26 [2].

Despite its long-standing cultivation and domestication, the exact origin of *F. carica* remains debated. Some researchers propose western Asia or southern Arabia as the center of origin, where both wild and caprifig populations may be found [3]. Others suggest that the species may have originated along the Caspian coastlines or in northwestern Turkey, where dense natural populations are still present [2]. Over time, fig trees became naturalized across a broad area, encompassing the Mediterranean, Iran, Central Asia, and the Transcaucasus [4]. This broad distribution, coupled with long-term cultivation, has led to the emergence of a rich diversity of local cultivars, many of which are believed to have arisen from centuries of unconscious selection among wild seedlings [5]. Wild fig populations continue to serve as vital reservoirs of genetic diversity, especially in regions such as Turkey and Transcaucasia.

In this context, Azerbaijan, a Transcaucasian country, represents an important but underexplored center of fig diversity. The wild fig populations native to the Hyrcanian forests in southeastern Azerbaijan are currently recognized as rare and underutilized genetic resources within the *F. carica* complex. First described by the botanist Alexander Grossheim in 1939 as *Ficus hyrcana*, these trees are now commonly regarded as a local ecotype or synonym of *F. carica* subsp. *carica* [6]. Notably resilient to both abiotic and biotic stressors, Hyrcanian wild figs may possess valuable alleles with potential applications in fig breeding. Their comprehensive study could improve conservation efforts and support their use as rootstocks or grafting material for cultivated *F. carica* varieties.

To effectively evaluate and conserve such genetic resources, molecular markers are increasingly favored over conventional morphological approaches. Among these, Inter Simple Sequence Repeat (ISSR) markers are particularly useful due to their reproducibility, cost-effectiveness, and ability to detect polymorphisms without requiring prior sequence information. ISSR markers amplify regions between microsatellites, providing high levels of resolution for genetic diversity studies in various plant species, including figs [7].

Despite their ecological importance and potential genetic value, Hyrcanian wild figs remain largely neglected and are classified as a vulnerable plant group in need of conservation. Previously, we conducted a comprehensive morphological assessment of these trees [8], which provided the groundwork for subsequent molecular investigations. The objective of the present study is to provide the first and an initial assessment of the genetic diversity and relationships among rare and underutilized wild fig populations from the Hyrcanian forests of Azerbaijan using ISSR markers.

MATERIALS AND METHODS

During a field expedition to Hirkan National Park in Azerbaijan, in May 2025, young leaf samples were collected from wild *Ficus carica* L. (syn. *F. hyrcana* Grossh.) trees and placed into sterile tubes for preservation. Genomic DNA was extracted at the Department of Molecular Genetics and Genomics, Genetic Resources Institute, Ministry of Science and Education of Azerbaijan, using a modified cetyltrimethylammonium bromide (CTAB) method as described by Doyle and Doyle [9]. The purity and quantity of the extracted DNA were evaluated with a NanoDrop spectrophotometer (Thermo Scientific, 2000). Five ISSR primers were selected based on their ability to produce clear, reproducible, and polymorphic amplification profiles (Table 1). PCR amplification and subsequent separation of amplified fragments were carried out using 1.5% agarose gel electrophoresis, following the protocol described by Qurbanova et al. [10].

Only clearly distinguishable and reproducible bands were manually scored as present (1) or absent (0), forming a binary matrix for analysis. To evaluate the informativeness of each ISSR primer, various genetic diversity metrics were calculated, including the genetic diversity index (GDI) [11], polymorphism information content (PIC), effective multiplex ratio (EMR), marker index (MI), resolving power (RP), and mean resolving power (MRP). Genetic relationships among the fig accessions were inferred through unweighted neighbor-joining (NJ) clustering and principal coordinate analysis (PCoA), both performed using the Jaccard similarity coefficient in DARwin 6.0 software [12].

RESULTS AND DISCUSSIONS

A total of five ISSR primers were used to evaluate genetic diversity in 15 Hyrcanian wild fig genotypes collected from the Hirkan National Park in Azerbaijan. The primers generated 41 bands in total, of which 17 (41.2%) were polymorphic, indicating a moderate level of genetic variability within the studied accessions (Table 1). The number of bands per primer ranged from 7 (UBC 818) to 9 (UBC 834 and UBC 835), with the number of polymorphic bands (Np) ranging from 2 to 5. These results are consistent with the findings of Khadari et al. [13], who reported a total of 37 markers generated by 8 ISSR primers, with the number of markers per primer ranging from 2 to 8, and an average of 4.6 markers per primer. In contrast, a study on 24 cultivated and wild fig accessions from Palestine using 15 RAPD markers revealed an average of 6.1 alleles per primer [14].

Among the primers, UBC 835 exhibited the highest polymorphism percentage (56%), followed closely by UBC 823 (50%), suggesting these primers were more effective in detecting variation among the accessions. In contrast, UBC 818 showed the lowest polymorphism (29%), indicating relatively lower variability

captured by this primer.

Table 1. Genetic diversity parameters in wild fig accessions from Azerbaijan based on ISSR markers

Primer	N_t	N_p	P, %	MI	EMR	RP	MRP	PIC	GDI
UBC 818	7	2	29	0.21	0.57	0.94	0.47	0.36	0.42
UBC 823	8	4	50	0.76	2	2.8	0.70	0.38	0.76
UBC 834	9	3	33	0.45	1	2.14	0.71	0.45	0.72
UBC 835	9	5	56	0.50	2.8	1.1	0.22	0.18	0.66
UBC 840	8	3	38	0.36	1.1	1.32	0.44	0.33	0.52
Total	41	17	-	-	-	-	-	-	-
Mean	8.2	3.4	41.2	0.46	1.5	1.53	0.34	0.34	0.62

Despite the limited number of studied genotypes, unique bands were observed in some accessions with certain primers. For example, using the UBC 835 primer (Figure 1), a unique band of 1210 bp was amplified in the Hyrcan 2 accession, while a distinct 860 bp fragment was observed in Hyrcan 12. Additionally, a 950 bp fragment was present in all accessions except Hyrcan 3, making this absence a unique profile feature for that tree.

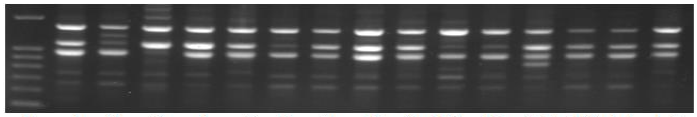


Figure 1. Genetic profile of wild Hyrcanian fig accessions generated using ISSR primer UBC 835

Several genetic parameters were calculated for the ISSR primers used. Marker index values, reflecting both informativeness and multiplexing capacity, ranged from 0.21 (UBC 818) to 0.76 (UBC 823), supporting the high utility of UBC 823 in genetic studies of Hyrcanian wild figs. The effective multiplex ratio ranged from 0.57 to 2.8, with an average of 1.5. In the study by Khadari et al. [7], ISSR markers demonstrated higher efficiency compared to RAPD and SSR markers when evaluated based on the EMR across 30 fig cultivars. The primers UBC 823 and UBC 818 exhibited the highest and lowest resolving power values (2.8 and 0.94), respectively. Similarly, UBC 834 and UBC 823 showed the greatest MRP, whereas UBC 835 displayed the lowest.

One of the main parameters used to evaluate the efficiency of molecular markers, the GDI, showed considerable variation among the primers, ranging from 0.42 for UBC 818 to 0.76 for UBC 823. On average, the genetic diversity within the collection was 0.62, indicating a moderate level of variation among the samples. PIC values ranged from 0.18 (UBC 835) to 0.45 (UBC 834), with an average of

0.34. These values fall within the typical range for dominant markers and reflect moderate informativeness of the primers. UBC 823, with the highest PIC and resolving power (RP = 2.8), was particularly efficient in differentiating among genotypes. Overall, the ISSR marker system proved to be a valuable tool in assessing genetic diversity among Hyrcanian wild fig accessions.

Jaccard similarity indices ranged from 0.14 (between Hyrcan 5 and Hyrcan 13) to 0.89 (between Hyrcan 10 and Hyrcan 12), with an overall mean of 0.53. This moderate average similarity indicates a substantial degree of genetic variability within the studied population, consistent with the expectation for wild fig populations that have evolved under varying ecological conditions. Cluster analysis using UPGMA revealed the formation of three major clusters, each comprising several subclusters (Figure 2). Notably, no clear clustering based on geographic proximity was observed. Genotypes from different villages and distant areas of the national park often grouped together, while others collected from adjacent locations were placed in separate clusters. This pattern suggests that geographic distance is not the primary driver of genetic similarity in these populations.

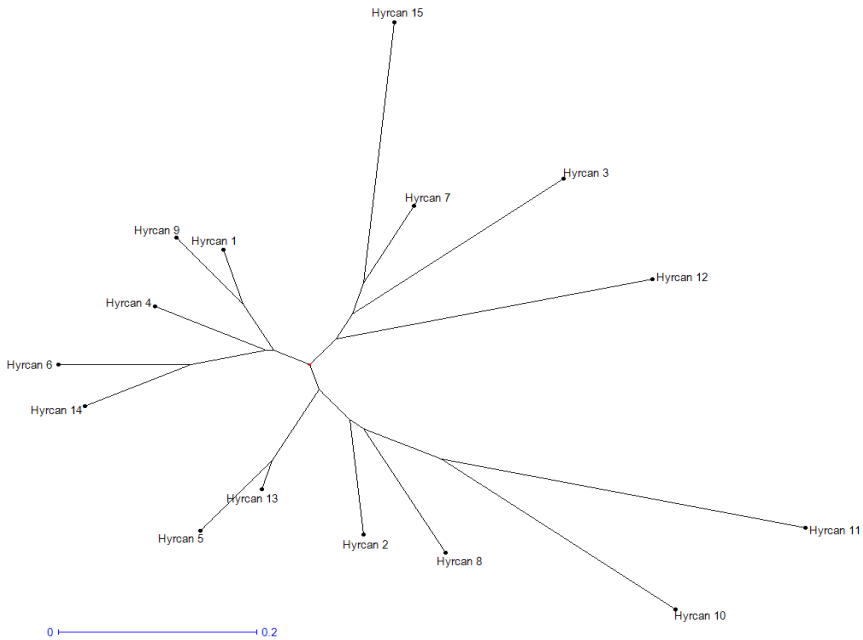


Figure 2. Genetic relationships among wild fig accessions revealed by ISSR markers

Cluster I included four genotypes, among which Hyrcan 3 and Hyrcan 15 were noteworthy. Despite being sampled from different regions, both were located near rivers. Cluster II was composed of five genotypes, with Hyrcan 1 and Hyrcan 9 showing the greatest genetic similarity within this group. The remaining

genotypes were assigned to Cluster III, within which, Hyrcan 10 and Hyrcan 11 exhibited the greatest divergence, with pairwise genetic distances ranging from 0.50 to 0.71 when compared to other members of the cluster.

The PCoA further supported these findings (Figure 3). The first two axes explained 49.4% of the total genetic variation, while the first five axes accounted for 85.2%, indicating a high resolution of the genetic structure within the dataset. The scattered distribution of genotypes in the PCoA plot, without the formation of tight clusters, reflects a high level of genetic differentiation among individuals, supporting the hypothesis of significant intra-population diversity. Interestingly, only Hyrcan 10 and Hyrcan 11 were placed distinctly apart from the main group of genotypes on the PCoA plot, underscoring their unique genetic profile. Additionally, Hyrcan 15, collected from the highest elevation site (287 m a.s.l.), was positioned at the extreme edge along the negative Y-axis, suggesting possible altitudinal effects on genetic differentiation.

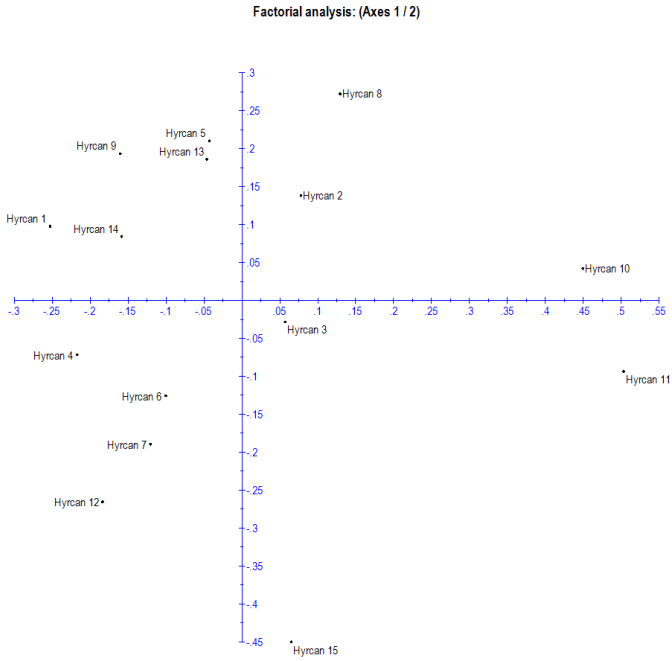


Figure 3. PCoA plot showing genetic variation among wild Hyrcanian figs of Accessions

Previous studies have focused predominantly on cultivated figs, with limited attention given to wild populations. For example, Khadari et al. [13] analyzed 75 fig accessions using eight ISSR and six SSR markers but were unable to fully discriminate between all cultivars due to genetic heterogeneity and naming

inconsistencies. In contrast, the current study successfully achieved complete discrimination of all 15 wild fig accessions using only five ISSR primers, demonstrating the high discriminatory power and efficiency of the selected markers in assessing genetic diversity among wild individuals. Our findings align with recent work by Haffar et al. [15], who reported clear genetic differentiation between cultivated and wild fig gene pools using RGA markers. Their results suggested that distinct RGA loci, potentially associated with adaptive traits such as resistance to biotic and abiotic stresses, have accumulated in wild populations. This further highlights the evolutionary and ecological significance of conserving wild fig germplasm as a reservoir of genetic diversity and resilience traits.

CONCLUSIONS

This first genetic study of Hyrcanian figs demonstrated that ISSR markers are highly effective for assessing genetic diversity in wild *F. carica* populations. Using just five primers, all 15 genotypes were successfully differentiated, revealing moderate overall diversity and distinct genetic profiles. Cluster and PCoA analyses indicated substantial intra-population variation, unstructured by geographic proximity, suggesting the influence of gene flow and environmental heterogeneity. These results highlight the genetic variation among wild figs in Azerbaijan and emphasize the value of ISSR markers for their conservation, breeding, and taxonomic study.

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