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Chromosomal Architecture, Karyotype Profiling and Evolutionary Dynamics in Aleppo Oak (*Quercus infectoria* Oliv.)

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Abstract

Aleppo oak (*Quercus infectoria*) is among the most industrially and ecologically significant oak species, valued for its medicinal properties and considerable genetic importance. Cytogenetic analysis provides critical insight into evolutionary history, interspecific relationships, and karyotypic differentiation. This study investigated the chromosomal architecture and karyotypic diversity of five natural populations of this species in western Iran (Sardasht, Oramanat, Baneh, Paveh, and Marivan) using actively dividing root meristems and a high-resolution image-based cytogenetic system. All examined cells displayed a basic chromosome number of x = 12 and a diploid condition, and chromosome lengths ranged from 0.90 to 2.12 µm. ANOVA and mean comparisons of five chromosomal parameters (Long Arm, Short Arm and Total Length, Arm Ratio, and Centromeric Index) revealed significant interpopulation differences in chromosome length and arm dimensions. All populations shared the karyotype formula 12 m and were classified into Stebbins' Category B, indicating a moderately symmetrical, relatively primitive cytogenetic structure. Principal component analysis reduced the dataset to two major axes explaining 99.93% of the total variance, predominantly influenced by SA and TL on PC1 and by LA, AR, and CI on PC2. Hierarchical clustering grouped the populations into three distinct lineages, with Sardasht–Oramanat–Baneh showing the greatest divergence. Biplot vector patterns further clarified trait correlations, highlighting genomic structuring and potential breeding utility.

Keywords: Aleppo oak; *Quercus infectoria*; chromosomal morphology; cytogenetic diversity; principal component analysis

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1. Introduction

The oak tree is a member of the Quercus genus of the beech family (Fagaceae), which is one of the substantial and ecologically and economically diverse genera of deciduous and evergreen forest ecosystems throughout the Northern Hemisphere [1,2] which comprises nearly 450 species [3,4]. There is noteworthy evidence from the literature that these species can adapt to different environments and potentially to rapid climate change [5–9]. Oak tree or oak shrub, which is a member of the Quercus genus, is the most important and diverse component of forest ecosystems, many of which are common or even dominant

species in different habitats, from temperate deciduous forests to subtropical and tropical savannas [10–13]. The beech Fagaceae family has nine genera worldwide [14–16] and 3 genera in Iran, namely beech, oak and chestnut [17,18]. Due to the distribution of oak species in a wide area of the country and their presence in different climates and weather conditions, the species of this genus are of special importance. In addition to its economic and environmental importance, oak has value as a cultural and heritage resource in many countries.

In Iran and Turkey, the range of species of this genus is also wide from the northernmost part of the country to the south of the Zagros Mountain range and the northern areas of the Alborz Mountain range in Iran [19,20] and in Türkiye exhibit a remarkably broad biogeographical distribution, extending from the humid, temperate formations of the Black Sea region to the sub-Mediterranean and Mediterranean xeric woodlands of western, southern, and southeastern Anatolia [21]. Encompassing approximately one quarter of the nation's total forest area, these stands represent one of Türkiye's most extensive and ecologically influential forest formations, shaped by the country's pronounced topographic heterogeneity and its position at the intersection of the Euro-Siberian, Irano-Turanian, and Mediterranean phytogeographical regions [22]. The spatial configuration and species richness of Turkish oak forests further underscore their evolutionary significance, with northwestern Anatolia identified as a major center of Quercus diversity in multiple floristic evaluations [23]. Oak forests regenerate very quickly after being exposed to fire and are resistant to harsh climatic conditions [1].

Among the globally distributed oak species, *Quercus infectoria* stands out as one of the most industrially significant and ecologically indispensable taxa, owing to its exceptional adaptive capacity, diverse phytochemical profile, and substantial contributions to both ecosystem functioning and bio-based industries [24]. It is one of the representative species of Zagros vegetation region. It is main species of Zagros forests that grows in West Azarbaijan, Kurdistan, Kermanshah and Lorestan provinces in Iran [25]. The local names for this species of oak are Darmazoo or Mazoo in Iran and Mazı in Turkey.

Within the field of plant breeding, the examination of chromosomal organization and cytomorphological features constitutes an essential initial phase, since both the numerical complement and structural integrity of chromosomes are pivotal in guiding the selection of effective genetic enhancement approaches strategies [26–28].

Beyond elucidating the karyotypic architecture of a species, chromosomal characterization and cytogenetic analyses provide a robust framework for assessing the extent of genetic differentiation among populations of the same taxon. Given that the genome of each individual embodies the totality of its genetic information, and that phenotypic traits ultimately arise from gene expression, any alteration in chromosomal structure or size (being the physical repositories of genes) can manifest in discernible phenotypic variation. Therefore, karyotypic studies within species populations are particularly significant, as distinct populations often exhibit unique genomic adjustments and evolutionary accommodations to the specific environmental conditions under which they persist [29].

Variation in chromosome number, morphology, size, and mitotic behavior (collectively reflected in karyotypic structure) can serve as a powerful indicator of underlying genetic differentiation. In general, cytotaxonomic investigations not only elucidate relationships and degrees of affinity among populations but also provide valuable insights into the genetic resources present within a region, thereby supporting the development and conservation of national gene banks. Consequently, conducting cytogenetic analyses on plant species and their respective populations (particularly wild and indigenous tax) is of exceptional significance, as such studies yield quantitative information on evolutionary trajectories, interspecific affinities, karyological attributes, and other essential cytogenetic characteristics [30].

Among the oak species worldwide, this taxon is recognized as one of the most industrially important, endowed with substantial ecological value [31]. Unfortunately, in recent years, the abundance of this species within its natural habitats has been declining due to various biotic and anthropogenic pressures; also, the Zagros forests are facing serious threats and severe degradation due to excessive livestock grazing, habitat destruction, increasing aridity, soil depletion, and unsustainable exploitation practices [32].

At present, the systematic and evolutionary studies of the genus Quercus in Iran remain comparatively underexplored, and no definitive consensus exists regarding the exact number of species within the country [20]. Rechinger (1997), in Flora Iranica, reported seven species, three subspecies, and three varieties of Quercus in Iran, recognizing a single variety for *Q. infectoria* (a classification largely grounded in leaf morphology and other macromorphological descriptors [33]. In contrast, Khoie-Djavanchir (1967) documented 28 species, seven subspecies, and seven varieties for the genus, attributing two varieties to *Q. infectoria* and basing his taxonomic delineation on traits such as leaf characteristics, bud morphology, fruit structure, cupule form, cupule-scale morphology, and, in certain cases, the morphology of male and female flowers [34]. Sabeti (2002) proposed yet another scheme, identifying 16 species, five subspecies, and two varieties of Quercus in Iran, and recognizing three subspecies within *Q. infectoria*, using leaf morphology, cupule architecture, scale configuration, and a suite of auxiliary morphological characters as diagnostic criteria [25]. More recently, Mozafarian (2004) [35] reported eight species, three subspecies, and three varieties, attributing a single subspecies to *Q. infectoria*, with species boundaries predominantly inferred from leaf characters, reproductive-organ morphology, and fruit traits. Notably, many of these taxonomic assessments suffer from incomplete geographical sampling, often covering only limited portions of the country [35]. Consequently, given the lack of taxonomic unanimity within classical systematics, the integration of molecular systematics alongside traditional morphological taxonomy is essential for resolving the persistent ambiguities surrounding the species delimitations within the Iranian oaks [36].

The Zagros oak forests exhibit a pronounced ecological, climatic, and vegetation gradient extending from the northwestern to the southwestern regions of Iran, transitioning from relatively humid and dense forests to progressively drier, more open, and sparse woodlands. In the northwestern Zagros (West Azerbaijan and northern Kurdistan provinces, including Sardasht, Baneh, and Marivan), climatic conditions are strongly influenced by Mediterranean rainfall systems, with relatively high annual precipitation (approximately 700–800 mm), colder winters, and milder summers. These conditions promote higher soil moisture availability, resulting in denser, more continuous, and structurally complex forest stands with comparatively higher species diversity, deeper soils, and greater organic matter content. Moving toward the central Zagros region (including the Oramanat area and Paveh in Kermanshah Province), annual precipitation gradually decreases and the dry season becomes longer. Consequently, forest structure shifts toward a semi-dense and patchy configuration, forming a mosaic of woodland, grassland, and rocky habitats. Although oak remains the dominant tree component, individuals are typically shorter, more widely spaced, and characterized by more open canopies, reflecting increasing environmental stresses such as water limitation, steep slopes, and shallow soils. Overall, this northwest–southwest gradient is associated with declining precipitation, forest density, and species diversity, accompanied by increasing drought stress, heat, and land degradation, which collectively play a key role in shaping differences in forest structure, physiological performance, and potentially genetic and cytogenetic variation among Zagros oak populations.

Quercus infectoria Oliv. (Aleppo oak) is a small to medium-sized deciduous tree or occasionally a large shrub of the family Fagaceae, typically reaching 2–10 m in height. It is characterized by leathery, ovate to elliptic leaves with serrated margins, a glossy adaxial

surface, a pubescent abaxial surface, and acorn fruits enclosed in a narrow, scaly cupule. The species is widely distributed across the eastern Mediterranean and Middle Eastern regions, including Türkiye, Iran, Iraq, Syria, and Cyprus, and constitutes a dominant component of the Zagros forests, particularly in West Azerbaijan, Kurdistan, Kermanshah, and Lorestan provinces. The studied populations occur in mountainous areas of the Zagros range at mid to high altitudes under continental to semi-humid climatic conditions characterized by cold winters, moderate precipitation, and dry summers, typically growing on calcareous and rocky soils and exhibiting considerable tolerance to edaphic and climatic stresses.

Based on this ecological and geographical framework, we hypothesize that (i) despite overall karyotypic stability in *Q. infectoria*, quantitative chromosomal parameters (e.g., chromosome length and arm dimensions) exhibit measurable variation among geographically separated populations, and (ii) such variation is associated with ecogeographical differentiation driven by moisture and temperature gradients along the Mediterranean climatic continuum of the Zagros Mountains, reflecting local environmental conditions and long-term evolutionary adaptation rather than changes in chromosome number or gross chromosomal morphology.

2. Materials and Methods

2.1. Materials

The five population of *Quercus infectoria* from several northern forests in Iran during the years 2020–2021 were studied. The area studied in this research was vast areas of Zagros forests of Iran. Some of the one-year registered seedlings of the studied populations, which were obtained from the herbarium of the "Research Institute of Forests and Rangelands of Iran" and "Agricultural and Natural Resources Research Center of West Azarbaijan Province" were transferred to two-story pots at the beginning of the growing season. Then, the growing place of the samples was identified and most of the samples were collected from the natural habitat during several trips from different areas of Zagros. The investigated samples were prepared from these regions: 1: Sardasht; 2: Oramanat; 3: Baneh; 4: Paveh and 5: Marivan.

2.2. Methods

After the collection of samples was completed, they were homogenized and young roots were taken from the samples of five different regions. Karyological observations were based on the material collected from natural populations of these regions. Root tips were continuously sampled from oaks. To prevent damage to the tips of the roots inside the soil, the lower part of the desired pot was completely cut and placed on the second pot containing sand. In this way, 1–2 cm fresh roots grew from the lower part of the upper pot and were cut before sinking completely into the lower pot for karyological investigations (Figure 1).

Root tips were pretreated with 1% α-bromo-naphthalene, fixed in Lewitsky's fixative, hydrolyzed in 1 N HCl at 60 °C for 30 min, and stained with aceto-orcein. Permanent slides were prepared using the squash technique, and well-spread metaphase plates with clear staining were examined at 1000× magnification and documented by microphotograp. Chromosome numbers were subsequently determined by counting chromosomes in several cells from each population. For each population, a minimum of five well-spread and morphologically intact metaphase plates were selected for detailed karyotype analysis following the screening of a larger number of preparations. The analyzed metaphases were obtained from multiple individuals per population to capture intra-population variability and to avoid genotype-specific bias. Only metaphase cells meeting strict quality criteria were retained for quantitative measurements, thereby minimizing artifacts associated with

poorly spread chromosomes. This sampling strategy, which is commonly accepted in cytogenetic studies of taxa characterized by high chromosomal stability, provides reliable and reproducible estimates of karyological parameters while ensuring measurement accuracy and robustness of the comparative analyses.



Figure 1. Steps for sampling from pots and transferring roots to the pretreatment solution.

Chromosomal morphometrics, including long-arm length, short-arm length, and total chromosome length in micron (µm), were quantified using MicroMeasure 3.3 software. In addition, key architectural indices such as the arm ratio and the centromeric index were calculated for each chromosome. Using the mean values of long- and short-arm lengths, haploid idiograms were constructed for all populations. These idiograms were generated in Excel and organized by arranging chromosomes from left to right according to descending chromosomal size, beginning with the largest and ending with the smallest. This approach enabled a precise visualization of chromosomal architecture and allowed for comparative assessment of karyotype patterns among the studied populations. Subsequently, to assess interpopulation variation based on the aforementioned chromosomal parameters, a one-way analysis of variance (ANOVA) followed by appropriate multiple comparison tests was conducted. All statistical analyses were performed using STATGRAPHICS Centurion 19 [37]. To evaluate several parameters including long arm length (LA), short arm length (SA), satellite length (SAT), total chromosome length (TL), arms ratio (AR), total karyotypic form percentage (%TF) and for karyotypic symmetry, the following cytological indices were employed: intrachromosomal asymmetry index (A1) and interchromosomal asymmetry index (A2) were calculated [38] (Table 1).

Table 1. Cytogenetic parameters used to analyze chromosomes and karyotypes parameters.

Parameters	Formula
Short arm (S)	Distance between the end of the short arm to centromere (µm)
Long arm (L)	Distance between the end of the long arm to centromere (µm)
Total length (TL)	TL = LAi + SAi (µm)
Centromer index (CI)	$CI = \frac{SA_i}{TL_i} \times 100$ (%)
Intrachromosomal Asymmetry Index (A1)	$A1 = 1 - \frac{\sum_{i=t}^{n} \frac{S_i}{L_i}}{n}$
Interchromosomal Asymmetry Index (A2)	$A_2 = \frac{Sx}{\overline{X}}$

n: chromosome number

The Total form of % TF, which is: the ratio of the total length of the small arms to the total length of the chromosomes multiplied by a hundred. When TF% is equal to 50%, it means that the centromeres are located in the middle of the chromosome. The difference in the range of the relative length of the chromosome DRL, which represents the difference between the minimum and maximum relative length of the chromosomes in a karyotype, was used for karyotypic symmetry comparisons. The higher the value of this parameter in a species or variety, it indicates that the corresponding karyotype is more asymmetric and that species or variety is in a higher degree of karyotypic evolution. The classification of chromosomes of each karyotype was performed according to the method of Levan method [39] (Figure 2). Karyotype symmetry was also compared according to the method of Stebbins [40] (Table 2).

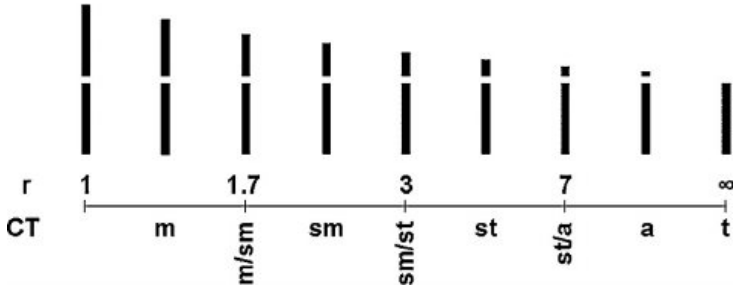


Figure 2. Chromosomes categories on the basis of Arms Ratio, [39].

Table 2. The classification of karyotypes in relation to their degree of asymmetry according to [40].

Ratio	Proportion of Chromosomes with Arm Ratio > 2:1			
Largest/Smallest	**1.00 (1)**	**0.99–0.51 (2)**	**0.50–0.01 (3)**	**0.00 (4)**
<2:1 (A)	1A	2A	3A	4A
2:1–4:1 (B)	1B	2B	3B	4B
>4:1 (C)	1C	2C	3C	4C

Subsequently, to elucidate the extent of morphological variation among populations, multivariate statistical procedures were employed, most notably Principal Component Analysis (PCA) and hierarchical cluster analysis. The utility of PCA and related multivariate techniques in karyological investigations has been well established; for example, Almas [41,42] employed these methods to characterize chromosomal affinities and discriminate among closely related populations. Principal Components Analysis (PCA) was conducted to group the populations based on their respective localities. The objective of the analysis was also to derive a limited set of linear combinations of the five variables that explain the majority of the variability observed in the data. The statistical software package JMP version 16 was utilized to conduct principal component analysis (PCA).

3. Results

3.1. Determining and Optimizing the Appropriate Guidelines for Better Viewing of Chromosomes

The appropriate instructions for the cytogenetic studies of *Quercus infectoria* were optimized, according to which sampling is performed at 8–9 in the morning, alpha-bromonaphthalene is used for 3 h at 4 °C as pretreatment [43–56]. Root tips were immersed in 1 N NaOH at 60 °C for 10 min in Ben-Marie water bath to hydrolyze and % 2 aceto-orcein for 48 h at 4 °C for staining.

3.2. The Results of Microscopic Examination, Chromosomal Counting and Determination of Karyotype

The results of chromosomal counting showed that the chromosomal number of all the studied cells from each population of this species was 24 and all of them were diploid (Figure 3; Figure 4)

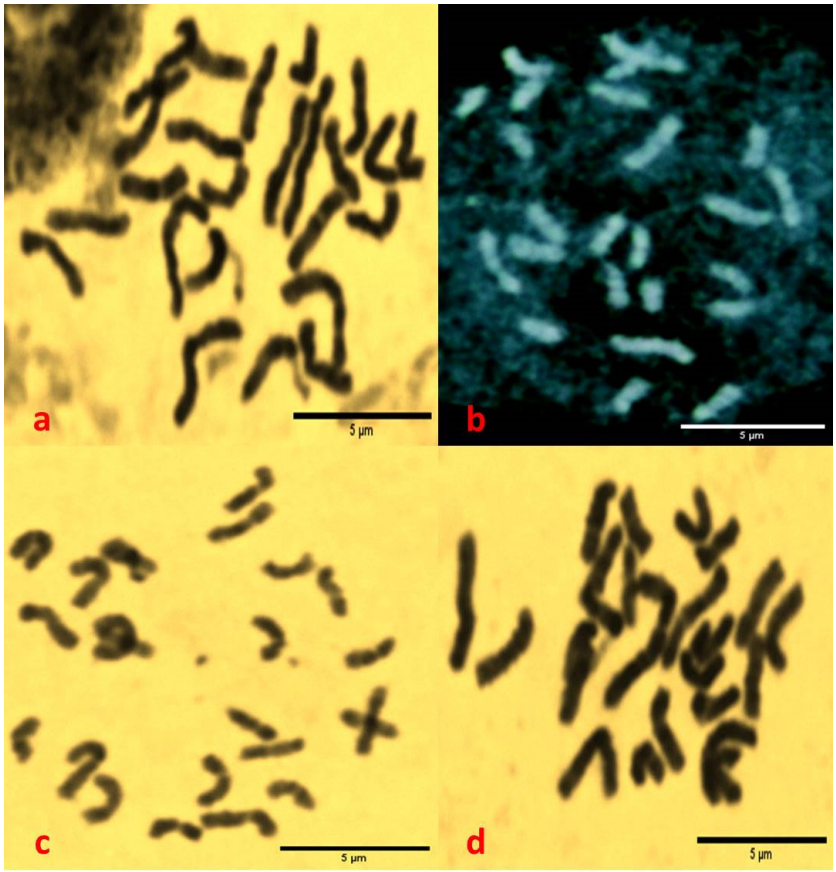


Figure 3. Microscopic image of metaphase plates of cells; (**a**). Sardasht; (**b**). Oramanat; (**c**). Baneh; (**d**). Paveh.

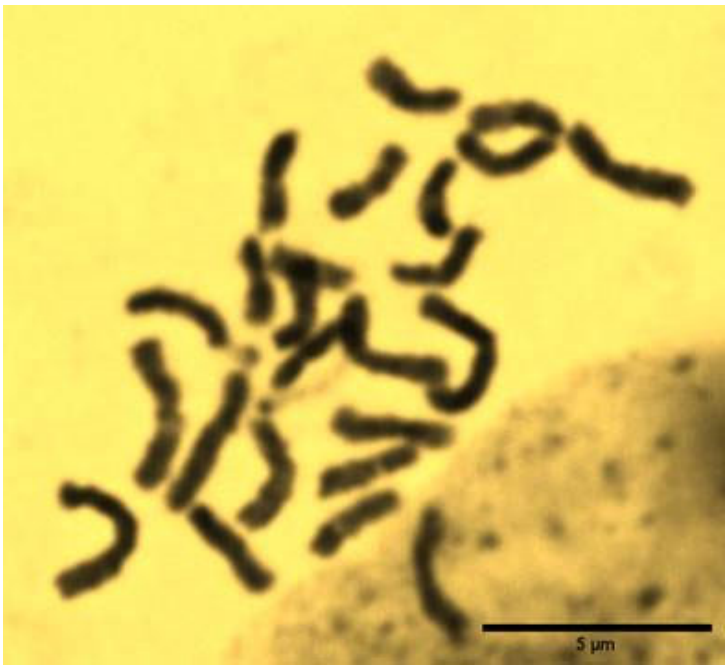


Figure 4. Microscopic image of metaphase plates of cells; Marivan.

The Sardasht population exhibited a distinctly symmetrical and evolutionarily conserved karyotypic architecture, with total chromosome lengths ranging from 1.14 to 2.12 μm, reflecting a compact chromosomal system. The proportionality between long- and short-arm measurements and the arm ratios (1.00–1.31) confirmed that all chromosome pairs were metacentric, a finding reinforced by centromeric index values clustering tightly around 43–50%. The r-values approaching unity demonstrate intrachromosomal equilibrium, while the absence of satellite chromosomes indicates a structurally stable complement devoid of secondary constrictions. Collectively, these parameters underscore a primitive, highly balanced genome with minimal asymmetry chromosomal divergence (Table S1).

The Oramanat population displayed a highly coherent chromosomal spectrum, with TL values spanning from 0.98 to 2.00 μm and consistent LA–SA proportionality across all chromosome pairs. Arm ratios fluctuated narrowly around the metacentric threshold (1.00–1.50), supported by centromeric indices ranging from 40% to 50%, firmly situating the karyotype within a symmetrical cytotype. The r-value pattern corroborated a stable proportionality between arms, with no satellites detected throughout the complement. This uniformity signifies a genome of high cytogenetic integrity, reflecting conservative evolutionary dynamics and limited structural differentiation (Table S2).

The Baneh population revealed a compact chromosomal organization, with TL values ranging from 0.90 to 1.95 μm and balanced long- and short-arm lengths reflected in arm ratios (1.00–1.39). Centromeric indices consistently within the 41–50% range validated the uniformly metacentric nature of all chromosome pairs. The r-values demonstrated strong proportional similarity between L and S arms, and the complete absence of satellites indicated a structurally conservative genome. The slightly greater compression of TL values relative to other populations suggests a minor reduction in chromosomal size without altering the fundamental symmetrical karyotype, thereby maintaining a primitive and evolutionarily stable chromosomal constitution (Table S3).

The Paveh population manifested a highly ordered chromosomal architecture, with TL measurements between 1.13 and 2.10 μm and arm ratios sustaining the metacentric classification (1.01–1.31). The centromeric indices (43–49%) showed tight clustering, attesting to the structural symmetry of the entire complement. A consistently proportional r-value pattern and uniform RL distribution illustrated minimal intrachromosomal asymmetry. As with other populations, no satellite structures were observed, reinforcing the preserved genomic architecture and indicating a stable cytogenetic lineage with limited structural rearrangement across chromosome pairs (Table S4).

The Marivan population exhibited a uniformly symmetrical karyotypic configuration, with TL values spanning 0.91–1.98 μm and arm ratios ranging from 1.00 to 1.39, supporting the exclusive metacentric constitution of all chromosome pairs. Centromeric indices predominantly between 42% and 50% confirmed the pronounced symmetry of the complement. The proportional arm dimensions (LA vs. SA) and r-values near unity reflect extremely balanced chromosomal morphology. Similarly, to the other populations, the lack of satellite chromosomes signifies a primitive, evolutionarily conserved genome. Overall, the Marivan karyotype is structurally coherent, cytogenetically stable, and indicative of negligible evolutionary deviation (Table S5).

The averaged karyotypic profile of the five *Quercus infectoria* populations revealed a remarkably uniform and symmetrical chromosomal architecture across sampled regions: total chromosome length (TL: 1.01–2.03 μm) and arm metrics (LA = 0.53–1.14 μm; SA = 0.49–0.98 μm) exhibited limited dispersion, and arm ratios (1.00–1.29) together with centromeric indices (CI = 36–41%) unequivocally classify all pairs as metacentric. The r-value (S/L) proximate to unity for most chromosomes corroborates proportional arm distribution and the absence of secondary constrictions or structural aberrations; likewise,

the dearth of satellite chromosomes attests to a conserved genomic organization. Stability in relative length (RL), TF% and %S further signifies low intragenomic asymmetry. Crucially, the asymmetry indices reinforce this interpretation: the low A1 (intrachromosomal asymmetry) indicates minimal heterogeneity in centromere position within chromosome pairs (centromeric placement is consistently median across the complement) whereas the low A2 (interchromosomal asymmetry or coefficient of variation in chromosome lengths) denotes negligible disparity in chromosome sizes between pairs, confirming homogeneity of chromosomal dimensions at the karyotype level. Collectively, these metrics indicate an evolutionarily primitive, cytogenetically stable and morphologically homogeneous karyotype consistent with Stebbins' Category B, with both intrachromosomal and interchromosomal asymmetry indices underscoring the species' conserved chromosomal integrity (Table S6).

3.3. Idiogram of Each Population

Following the quantification of chromosomal metrics, idiograms for each population were meticulously constructed by integrating the mean lengths of their respective long and short chromosomal arms (Figures 5–9).

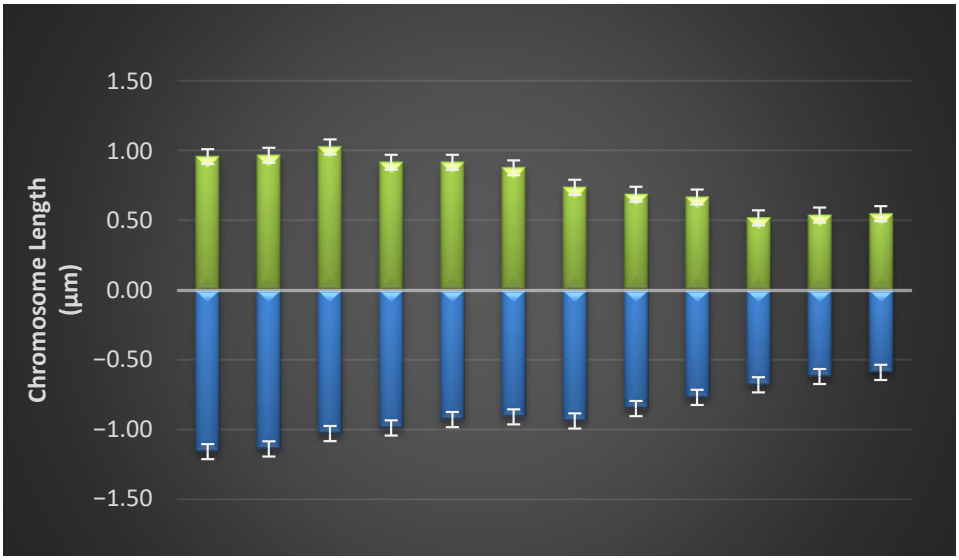


Figure 5. Haploid idiogram of Sardasht population.

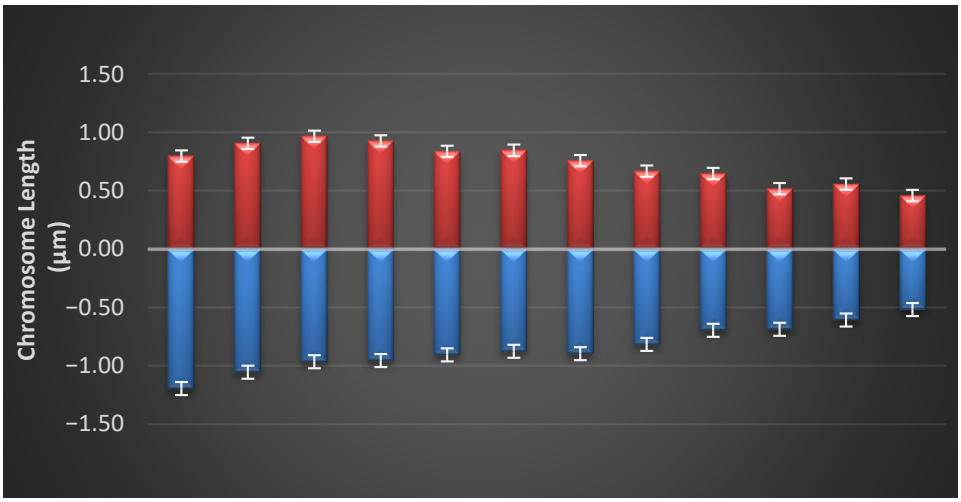


Figure 6. Haploid idiogram of Oramanat population).

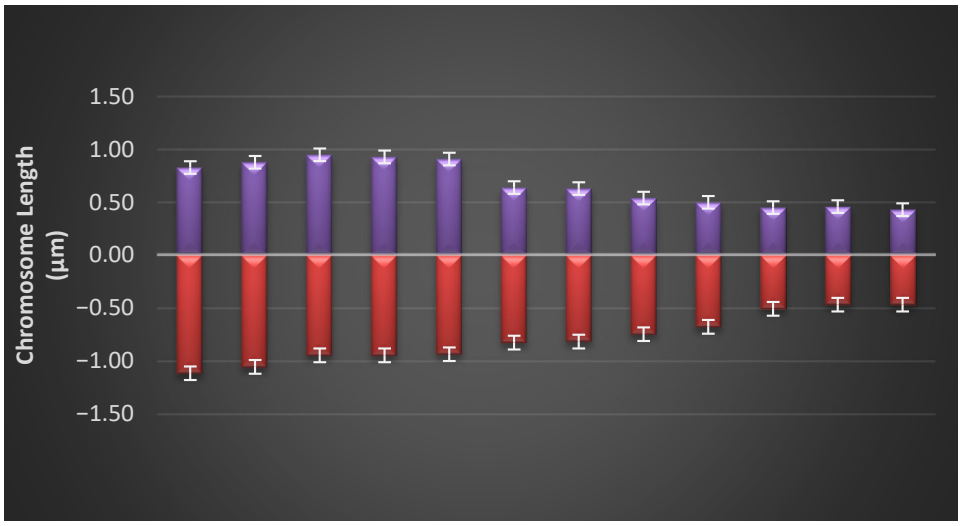


Figure 7. Haploid idiogram of Baneh population.

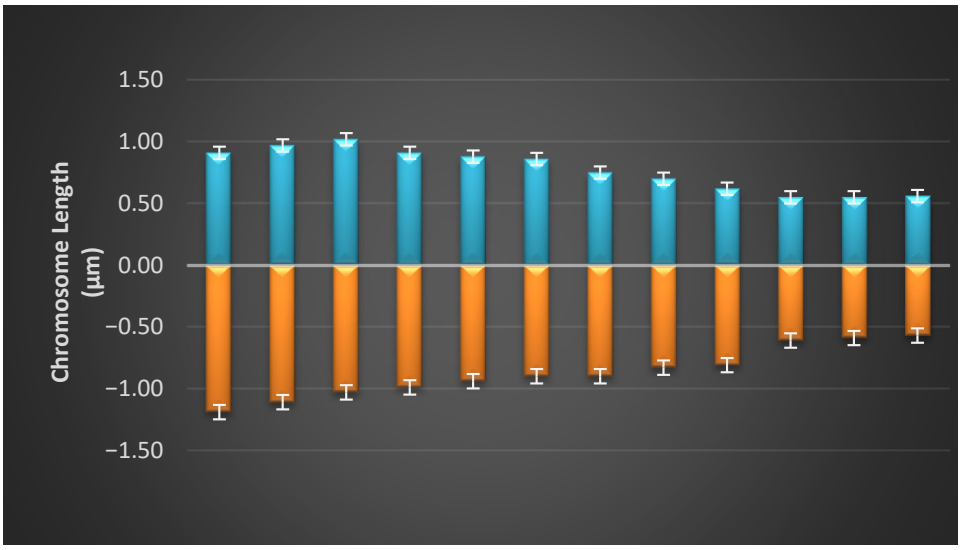


Figure 8. Haploid idiogram of Paveh population.

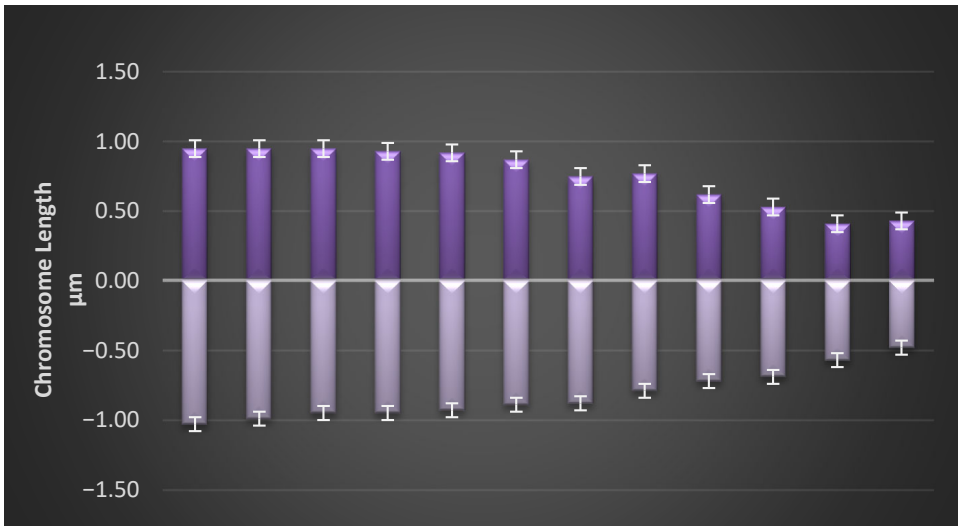


Figure 9. Haploid idiogram of Marivan population.

3.4. Variance Analysis of Karyotype Morphological Parameters

The analysis of variance performed on the karyotypic parameters of the examined populations revealed highly significant interpopulation differentiation at the $p < 0.01$ threshold for total chromosomal length (TL), long-arm length (LA), short-arm length (SA), and centromeric index (CI). In contrast, arm ratio (AR) exhibited no statistically discernible variation among populations (Table 3).

Table 3. Analysis of variance of chromosome morphological traits in studied populations of *Quercus infectoria*.

S.O.V	df	Mean of Square				
		TL	LA	SA	Arm Ratio	CI
Location	4	5.68 **	1.096 **	1.96 **	0.008 ^{ns}	3.69 ^{ns}
Error	10	0.49	0.22	0.31	0.007	3.57
Total	14					

** Significant at $p < 0.01$; ^{ns} Not significant.

3.5. Mean Comparisons of Karyotype Morphological Traits

The average classification of the studied populations in terms of karyotypic characteristics by Duncan's test showed that the populations of Oramanat and Baneh and Marivan in terms of the traits of chromosome length and short arm length, the averages are lower compared to the populations of Sardasht and Paveh assigned to themselves (Table 4).

Table 4. Comparison of the average of different chromosomal parameters in *Q. infectoria* populations.

Location	TL	LA	SA
1	21.43 a	11.27 a	10.17 a
2	19.19 bc	10.08 ab	9.11 b
3	17.63 c	9.61 b	8.01 c
4	19.72 ab	10.20 ab	9.52 ab
5	19.00 bc	10.24 ab	8.76 bc

Averages with the same letters are not significantly different at $p < 0.01$ based on Dunkan Multiple Range Test (DMRT).

The results further revealed that the Sardasht population exhibited the greatest mean chromosomal length (21.43 µm), whereas the Baneh population displayed the smallest (17.63 µm). With respect to long-arm metrics, Sardasht again possessed the maximum arm length (11.27 µm), while Baneh registered the minimum (9.61 µm). A parallel pattern was observed for the short-arm dimension, in which Sardasht attained the highest value and Baneh the lowest. From the perspective of the centromeric index, the Pavah population manifested the highest magnitude of this parameter, whereas Baneh presented the lowest value (Table 4).

3.6. Principal Component Analysis Based on All Characteristics of Each Population

Results revealed that, first two components have been successfully retrieved as per the given request. Collectively, these two factors explain 99.93% of the variance observed in the original dataset. In the current study, it was shown that the first two principal components, which had eigenvalues greater than one, accounted for a cumulative variability of 99.93% among the five populations that were assessed for five karyotypic characteristics. The first principal component explained 66.17% of the variance, whilst the second component accounted for 33.76% of the overall variability. Hence, it can be deduced that the primary

characteristics of the dataset were captured in the initial two principal components. The traits SA (0.477) and TL (0.463) accounted for the highest amount of variance in the first principal component (PC1). Principal Component 2 (PC2) contributed to 33.76% of the overall variance and exhibited greater variances for LA (0.555), AR (0.553), and CI (−0.506), indicating their significant relevance in the context of oak populations. Moreover, the loading of various traits, as determined by the first two principal components, revealed that the primary component (PC1) of divergence among the five populations comprise the most significant traits. Conversely, the other traits made relatively less contributions to the overall divergence (Table 5).

Table 5. Analysis of five characteristics: component loadings, eigenvalues, proportion of total variance explained by the initial two principal components (PC), and cumulative variance across five *Q. infectoria* populations. * Significant at $p < 0.05$.

	PC1	PC2
TL	0.463	0.328
LA	0.428	0.555
SA	0.477	0.144
AR	−0.428	0.554
CI	0.437	−0.506
Eigenvalue	3.308	1.688
Percent	66.169	33.762
Cumulative Percent	66.169	99.931
Chi Square	144.125	109.86
DF	9.444	9.349
Prob > ChiSq	<0.0001 *	<0.0001 *

Principal components analysis is a robust multivariate technique utilized for data reduction. It effectively eliminates interrelationships among components and is particularly useful in identifying structures within data sets, categorizing genotypes, and estimating genetic diversity in breeding materials [57,58].

The application of hierarchical cluster analysis led to the categorization of five oak populations into three separate clusters, as seen in Figure 10. The early cluster included solely of one population, namely the Marivan population. The second cluster was comprised solely of the Paveh population. The third cluster comprised two distinct sub-clusters. The first sub-cluster encompassed a single population, namely Sardasht. The second sub-cluster, on the other hand, included two populations, namely Oramanat and Baneh. The results of the cluster analysis revealed that individuals classified inside the third cluster exhibited heightened values for notable karyological features in oak. Therefore, the analysis of clustering patterns seen in the dendrogram will be beneficial in identifying suitable oak varieties for future breeding programs in Iran.

The biplot diagram also incorporates an examination of vector angles, a factor highlighted by [59]. According to his findings, vector angles serve as indicators of correlations between vectors, and by extension, among traits. In Figure 11, certain trait vectors exhibit small angles, signifying positive correlations. Conversely, the AR vector takes an entirely opposite direction, indicating a negative correlation with other traits. Consequently, a smaller angle between vectors signifies a stronger positive correlation among associated traits, while a larger angle suggests a more negative correlation.

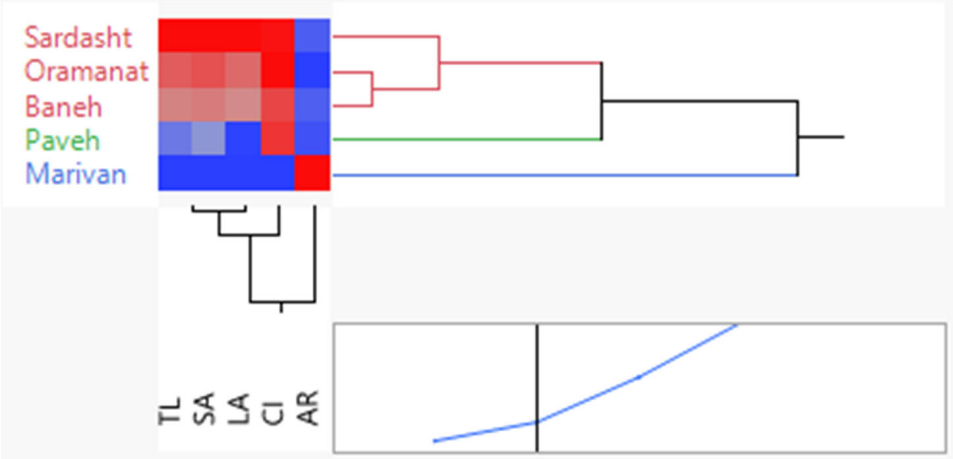


Figure 10. Hierarchical clustering analysis led to the grouping of five oak populations.

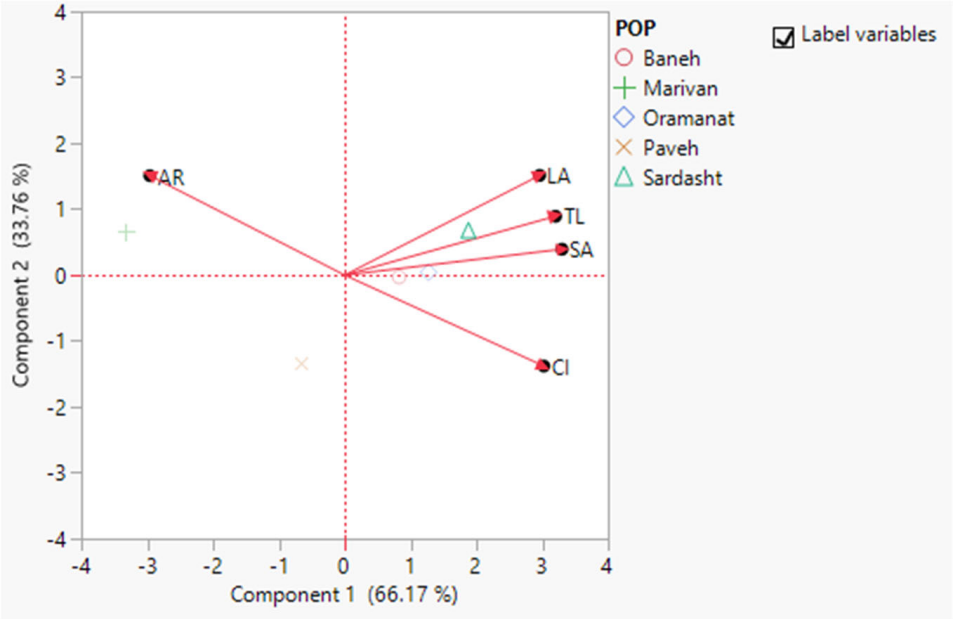


Figure 11. Biplot diagram of five oak populations based on first two principal components.

4. Discussion

Cytogenetic and karyomorphological investigations have long been regarded as indispensable tools in elucidating phylogenetic affinities, genomic divergence, and evolutionary trajectories within and among plant taxa. Systematic scientists posit that chromosomal attribute (when interpreted alongside genetic and morphological criteria) serve as highly reliable indicators for assessing kinship relationships among congeneric species, given that the structural configuration of chromosomes often mirrors their evolutionary proximity [60]. As chromosomes represent the fundamental carriers of hereditary information, any fluctuation in their architecture (including number, form, and proportional dimensions) inevitably reflects underlying genomic asymmetries, thereby manifesting as phenotypic disparities across populations and species [29]. In this sense, cytogenetically similar taxa, particularly those exhibiting high concordance in karyotypic parameters, tend to possess closer interspecific affinities; such proximity significantly enhances the likelihood of successful hybridization when desirable traits are sought in breeding programs [30].

Beyond enabling the identification of chromosomal complements and structural organization, cytogenetic approaches also provide invaluable insights into a species' evolu-

tionary history, biogeographical differentiation, and intra-populational diversity. This is especially crucial because populations inhabiting different ecological niches often develop unique genomic adaptations shaped by long-term environmental pressures [61]. For genera with unresolved taxonomic delimitations (such as Quercus) the integration of chromosomal systematics with classical morphological criteria becomes even more critical. Indeed, the systematics and evolutionary classification of Iranian oaks remain unsettled, with considerable ambiguity surrounding the recognition of species, subspecies, and varieties [20]. Historical classifications relied predominantly on leaf morphology, reproductive structures, fruit architecture, and cupule characteristics [35], yet these criteria alone have proven insufficient due to both morphological plasticity and incomplete field sampling across the species' geographical range. Consequently, cytogenetic and molecular systematics are indispensable for resolving these longstanding ambiguities and establishing a robust evolutionary framework for Iranian oaks [19].

Oak species are characteristically diploid with 2n = 2x = 24 chromosomes, a feature consistently documented across a broad array of studies [62–68]. Findings from the present investigation reaffirm this genomic stability: all examined cells across all studied populations were unequivocally diploid (2n = 24), with a basic chromosome number of x = 12. This uniformity is consistent with earlier reports indicating that polyploidy is exceedingly rare in this genus and that diploidy predominates throughout its evolutionary lineage [69–71].

The karyotypic assessment of the five studied populations (Sardasht, Oramanat, Baneh, Paveh and Marivan) revealed a remarkable degree of morphological similarity, echoing the structural congruence previously observed in related investigations [72]. Chromosomes of Quercus species are characteristically small, and in the present study their lengths ranged from 0.90 to 2.12 µm a pattern highly consistent with the values reported by Tabande Saravi (2012) [61], who recorded chromosome lengths between 0.97 and 3.75 µm in the same genus. The comparative karyotype analyses further demonstrated that all populations possessed highly symmetrical complements, with all chromosomes classified as metacentric; neither telocentric nor acrocentric chromosomes were detected. This structural harmony aligns closely with the findings of Tabande Saravi (2012), who likewise reported predominantly symmetric and morphologically similar karyotypes across multiple populations [61].

Parallel observations have been reported in broader geographic contexts. A comprehensive karyotypic analysis of 18 species across three genera of the Fagaceae family in northern Thailand revealed that metaphase chromosomes were uniformly small and predominantly metacentric or submetacentric [73]. Although arm ratios varied across species (highlighting interspecific differences) the overall symmetrical nature of tropical oak karyotypes contrasted sharply with the asymmetry more commonly reported in European oaks. These authors concluded that tropical and temperate oaks likely represent distinct evolutionary lineages, shaped by divergent chromosomal trajectories.

Similarly, a detailed karyological assessment of four oak species in Türkiye (*Q. libani*, *Q. coccifera*, *Q. petraea*, *Q. infectoria*) reported exclusively metacentric chromosome pairs, with length variations ranging from 0.81 to 2.18 µm [74]. These findings reinforced the notion that Turkish oaks possess exceptionally symmetrical karyotypes, surpassing even their tropical and European counterparts.

Against this comparative backdrop, the present study demonstrates that the species under investigation exhibits an even more symmetrical karyotype than any previously documented Quercus species. All chromosomes across all examined populations were metacentric, with no exceptions, implying an extraordinarily stable and primitive cytogenetic architecture. This heightened symmetry strongly suggests that this species occupies an evolutionarily basal position within the genus. Further supporting this hypothesis is the geological context: *Quercus castaneifolia* is believed to originate from the Tertiary period,

whereas European oak species trace their origins to the Quaternary. As such, it is plausible (based on cytogenetic evidence and evolutionary chronology) that European oaks may have diverged from ancestral lineages represented by *Q. castaneifolia*.

Comparative cytogenetic evidence from other Quercus species suggests that pronounced karyotypic stability may be indicative of ancestral genomic configurations. In this regard, the high degree of chromosomal symmetry observed in the present study supports the hypothesis that the investigated taxon may occupy a pivotal position within the evolutionary history of the genus, potentially representing a progenitor or evolutionarily conservative lineage.

Collectively, the results of this study highlight the critical role of cytogenetic approaches in transcending purely descriptive taxonomy by providing deeper insights into evolutionary processes, population structuring, and genome-level adaptations. Although the species examined here exhibits a remarkably conserved karyotype characterized by pronounced symmetry and structural uniformity, such chromosomal stability should not be interpreted as evolutionary stasis. Rather, within cytogenetically conservative taxa, evolutionary divergence is often encoded in subtle quantitative variations in chromosomal metrics and genome organization. In this context, the fine-scale differences detected in chromosome size parameters, asymmetry indices, and the non-random separation of populations revealed by PCA analyses constitute meaningful evolutionary signals that reflect microevolutionary dynamics operating within a stable chromosomal framework. These patterns suggest that population differentiation and potential adaptive divergence may proceed through incremental genomic reorganization without necessitating changes in chromosome number or gross morphology. Furthermore, the exceptional chromosomal congruence observed in this species not only reinforces its evolutionary distinctiveness within the genus Quercus, but also supports hypotheses regarding its potential role as a progenitor lineage. Given that chromosomal compatibility is a key prerequisite for successful hybridization [75], the high degree of karyotypic stability documented here carries important implications for understanding evolutionary relationships within the genus, as well as for future applications in conservation genetics, phylogenomic inference, and breeding strategies aimed at preserving or exploiting adaptive genetic variation.

5. Conclusions

The present study demonstrates that *Quercus infectoria* possesses an exceptionally stable and highly symmetrical diploid karyotype (2n = 24), with exclusively metacentric chromosomes across all examined populations, reaffirming the species' remarkable chromosomal conservatism within the genus. The narrow range of chromosome lengths, strong inter-populational congruence, and absence of structural asymmetry collectively suggest an evolutionarily basal cytogenetic architecture that may reflect the species' ancient lineage and limited genomic divergence. These findings not only clarify long-standing taxonomic uncertainties surrounding Iranian oaks but also highlight the critical role of chromosomal data in reconstructing evolutionary relationships, guiding conservation priorities, and informing future breeding programs through the identification of cytogenetically compatible genotypes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d18010059/s1, Table S1: Quercus infectoria Sardasht population; Table S2. Quercus infectoria Oramanat population; Table S3. Quercus infectoria Baneh population; Table S4. Quercus infectoria Paveh population; Table S5. Quercus infectoria Marivan population; Table S6. Quercus infectoria populations (Average).

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