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Review

Decoding Genetic Diversity in Subspecies *cuspidata* of Wild Olives

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Abstract: *Olea europaea* subsp. *cuspidata*, a wild olive, is widely distributed in various regions. It faces challenges from climate change, habitat loss, and human activities. Understanding and addressing these challenges is crucial for conservation efforts. Decoding the genetic diversity of this wild plant is vital to preserving its identity. This review examines the genetic diversity of *Olea europaea* subsp. *cuspidata* using morphological, biochemical, and molecular markers. Biochemical markers, like fatty acids and phenolic compounds, are vital for assessing olive oil quality. Molecular markers, particularly SNPs, have superseded isozyme analysis, revealing correlations with biochemical markers and effectively evaluating genome-wide diversity for cultivar classification and phylogenetic analysis. Regional studies of *Olea europaea* subsp. *cuspidata* indicate high genetic diversity but low population differentiation. Marker-dependent analyses (SSRs, ISSRs, RAPDs) reveal variations in population structure. Gene flow, geographical proximity, and habitat fragmentation influence genetic differentiation, creating complex biogeographic patterns. Parameters like gene diversity (H) and genetic differentiation coefficients (FST, GST) are key for assessing genetic variability. This review provides insights into the genetic diversity of *Olea europaea* subsp. *cuspidata*, emphasizing its importance for conservation and sustainable use amidst environmental challenges and its contribution to crop genetic diversity studies.

Keywords: *Olea europaea*; genetic diversity; molecular markers; biochemical; gene flow

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1. Introduction

Genetic diversity is the quantitative measure of variability in a population, reflecting the equilibrium between mutation and genetic variation loss (Hughes et al., 2008). Plant diversity in

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tropical and subtropical regions faces various challenges, including climate change due to anthropogenic activities like overexploitation, habitat destruction, and fragmentation, which have led to the continuous loss of genetic variability in many species, including wild olives (Ortega et al., 2024; Gufi et al., 2024). Altered precipitation and temperature patterns can disrupt plant morphology, physiology, and life history, affecting seed viability, germination efficiency, and phenotypic variability (Tang et al., 2023; Fanelli et al., 2022). Prolonged El Nino-induced drought has exacerbated these challenges, impacting plant diversity (Slik, 2004). Developing countries like Ethiopia have witnessed extensive deforestation for agriculture, urban expansion, and fuel wood, further affecting plant populations (Jacob et al., 2015).

Wild olive (*Olea europaea* subsp *cuspidata*) is one of six subspecies of wild olives distributed across several regions, including South Africa, Egypt, the Middle East, and Ethiopia (Green, 2002). The other wild olive subspecies include *Olea europaea* subsp. *europaea*, *Olea europaea* subsp. *cerasiformis*, *Olea europaea* subsp. *guanchica*, *Olea europaea* subsp. *laperrinei*, and *Olea europaea* subsp. *marocanna*. Subspecies *cuspidata* inhabit distinct environments including dry regions, rocky hillsides, poor soils and can withstand harsh environmental conditions (Ourge et al., 2018). It is diploid (2n = 46) with a genome size of approximately 1.32 GB (Wu et al., 2022), predominantly allogamous, monoecious, and reproduces sexually (Alcantara and Rey, 2003).

Wild olive plays a vital role in developing countries' economies, serving purposes from crafting farm implements to producing household items (Legesse, 2010). The trees' leaves, bark, stems, and fruit are utilized in numerous ways, including in traditional medicine, ceremonial practices, and food preparation (Abayneh et al., 20204). Particularly notable is the use of wild olive smoke, which serves as a key element in rituals, insect control, fumigation, and even flavouring food and beverages (Alemu & Kuyu, 2024). Additionally, the wild olive tree is embedded in the region’s cultural heritage, often linked to beliefs about purification, protection, and healing (Abayneh et al., 2024). The fruit is traditionally used in soup preparation, particularly in some African communities (Hashmi et al., 2015).

Efforts to assess olive germplasm and genetic diversity have utilized various markers, including morphological, biochemical, and molecular markers. DNA-based markers like SSR and SNPs have become popular due to their wide genomic distribution and other favorable characteristics (Islam et al., 2021). Furthermore, genotyping-by-sequencing (GBS) technology has been adopted for high-throughput sequencing of olive germplasm from different countries (Zhu et al., 2019; Julca et al., 2023).

Wild olive offers significant ecological benefits, such as thriving in challenging environments and aiding in natural regeneration (Aerts et al., 2008). Despite its ecological importance, it has received limited attention in terms of conservation and improvement. Although *Olea europaea* subspecies *cuspidata* experiences disease resistance, resilience, and stress tolerance, they have limited adaptive variation in a fragmented habitat (Colombo and Villanueva, 2017). According to Serrano-Garcia et al. (2022), wild olive produces small fruits with little oil content, presenting challenges for local oil production and the establishment of agroforestry systems. Furthermore, wild olive populations experience poor germination rates, and their root systems, which are adapted to highland soils, may struggle in a degraded or compacted environment (Legesse, 2010). As a result, improvement efforts are essential. This review provides an overview of key morphological and genetic studies on wild olive germplasm, summarizing their main findings and conclusions. This leads to correct cultivar identification and the application of such knowledge in olive breeding. The review is intended to assist researchers who are working on the genetic diversity of *Olea europaea* subspecies *cuspidata*, highlighting the relevance and utility of morphological, biochemical, and molecular markers in diversity identification.

2. Genetic Diversity Analysis in Plants

Genetic diversity, encompassing heritable variations within a species, is pivotal for plant breeding, conservation, and evolution, serving as the foundation for adaptation and improvement (Arif et al., 2010). Analyzing genetic diversity in plants involves various methods, including morphological, biochemical, and molecular markers (Table 1).

2.1. Morphological Marker Analysis in Wild Olives

Morphological markers, observable traits such as leaf shape and fruit size, offer insights into plant adaptation and selection effects (Chesnokov et al., 2020). However, they have limitations like low viability, environmental influence, and subjectivity (White et al., 2007). Morphological evaluation, fundamental for olive characterization, has evolved to integrate with molecular markers due to developmental stage reliance, subjective analysis, and environmental impacts (Sion et al., 2021). Wild olives, particularly in isolated populations, exhibit substantial diversity, emphasizing characteristics like drupe and pit morphology, oil content, and composition (Guodong et al., 201). Notably, wild olive varieties show more diversity than cultivated ones, featuring smaller and harder drupes, narrow leaves with leathery texture, taller up growth habit and low pulp-to-seed ratio percentages (Klepo et al., 2013; Hamid et al., 2022; Besnard et al., 2023). They also have a stronger capacity for natural hybridization, more genetic robustness, significantly enhanced allelic richness, and reduced oil content (Tourvas et al., 2023). In a recent study, Dehghan-Seresht et al. (2024) investigated the genetic diversity among Iranian olive (*Olea europaea* L.) cultivars, classifying them based on various traits such as tree growth, leaf characteristics, shoot coloration, and stone attributes. Their findings revealed significant variability in pomological traits, including fruit weight, shape, ripeness color, pulp-to-stone ratio, and ripening duration. Notably, they observed a high coefficient of variation (CV) of 75.00% for fruit density. In another study by Khadivi et al. (2023), 59 subspecies of *cuspidata* accessions from Iran were analyzed, examining 62 traits. The study found high morphological variability, with an average coefficient of variation (CV) of 37.30%, indicating substantial diversity among the accessions.

Utilizing morphological markers, Nikpeyma (2023) explored the genetic diversity of Turkish olive varieties, revealing significant differences in leaf, flower, fruit, and seed characteristics among the varieties. Blazakis et al. (2017) proposed a semi-automatic methodology using computational tools for precise morphological parameter detection, emphasizing the importance of characterizing phenotypic diversity in crop species, with olives as a notable example. Falek et al. (2022) investigated the morphological diversity of wild olives in North Algeria, demonstrating high trait variation across different bioclimatic habitats. Similarly, Boucheffa et al. (2018) assessed Algerian olive diversity through molecular, morphological, and chemical traits, noting distinct separation between wild and cultivated olives.

Atrouz et al. (2021) emphasized morphological differences between Algerian olive germplasm and Central-Western Mediterranean varieties, with leaf and seed characteristics being key discriminators. Genetic diversity in Spanish wild olive trees, revealing high variation in agro-morphological traits and SSR markers. According to Belaj et al. (2010). Wild olives have significantly higher allelic richness than cultivated varieties, with values of 3.974 and 3.324, respectively ($P < 0.01$). Furthermore, Belaj et al. (2011) found that wild olives possess a notably higher expected heterozygosity (HE) than cultivated varieties ($P < 0.01$). The findings of Belaj et al. (2010) also underscore the importance of wild olive genetic resources, which constitute a valuable gene pool in comparison to cultivars from the same region and to wild olive populations from other locations.

Morphological descriptors are very useful in identifying olive cultivars and offer an economical alternative to molecular markers. Combining morphological and molecular marker characterization offers a powerful complementary approach to understanding genetic diversity. Morphological characteristics offered significant insights into phenotypic variation, but molecular markers facilitated accurate genetic separation among accessions. This integrated approach revealed substantial genetic diversity in wild olive accessions for comprehensive characterization (D'Imperio et al., 2011). Despite their limitations, they are nonetheless useful methods for measuring and understanding genetic diversity in wild olives. The observed morphological polymorphism in wild olives provides beneficial traits for breeding, domestication and conservation (Hannachi et al., 2012; Tadic et al., 2021; Falek et al., 2022).

However, current morphological assessments are restricted to a limited geographic location and sample size, which restricts our understanding of the complete range of wild olives' morphological diversity (Khadivi et al., 2023; Nikpeyma, 2023). To achieve a more comprehensive representative assessment, future studies should focus on the whole distribution area of wild olives and a wider range of morphological traits. Such an approach would provide deeper insight into morphological diversity found in wild olive populations for more comprehensive analysis.

2.2. Biochemical and Molecular Markers

Genetic diversity in olive is vital for its nutritional and economic significance, as well as its adaptability to diverse environments. Biochemical markers, such as fatty acids and phenolic compounds, play a crucial role in assessing olive oil quality and health benefits. The markers vary based on cultivar, environmental factors, and processing methods.

2.2.1. Biochemical marker analysis

Wild olives (*Olea europaea* subsp. *cuspidata*) exhibit remarkable biochemical diversity in their fruits, leaves, and oils, reflecting their adaptation to various environmental conditions (Rafaqat et al., 2020; Tadic et al., 2021; Irakli et al., 2024). It arises from differences among varieties and geographic variations in phenolic compounds, fatty acids, and volatile organic compounds (Irakli et al., 2024). Understanding the relationship between molecular and biochemical markers is essential for revealing genetic diversity, adaptive traits, and breeding potential (Abood et al., 2017). For instance, drought-resistant wild olives, identified through specific molecular markers, often exhibit elevated levels of protective polyphenols, facilitating the selection of promising accessions for domestication and crop enhancement (Adi et al., 2025).

Several studies have highlighted the molecular and biochemical relationships within olive cultivars. Alhaithloul et al. (2024) reported significant genetic diversity among eight Saudi Arabian olive cultivars, which formed distinct clusters based on chemical and anatomical traits, as determined using ISSR and SCoT markers. Similarly, Tunc et al. (2024) reported substantial differences in total phenolic content (ranging from 2.86 to 12.58 mg GAE/100 g) and flavonoid levels (between 1.88 and 3.48 mg QE/100 g) among wild olive genotypes sourced from Mesopotamia. Furthermore, Sisodiya et al. (2025) identified a high degree of polymorphism (88.23%) alongside notable biochemical variations in protein, fat, carbohydrate, and phenolic, flavonoid, and antioxidant levels across seven foreign olive cultivars in India. Additionally, Abood et al. (2017) discovered 199 chemical compounds in Saudi Arabian wild olives, including fatty acids, aldehydes, and phenols, and reported a strong correlation between genetic and biochemical distances.

Isozyme analysis, which uses electrophoresis to examine genetic variation in proteins encoded by genes, has played a notable role in early olive genetic research (Trujillo et al., 1995). Although they were once extensively used, isozyme markers have mostly been replaced by more sophisticated molecular markers because of their drawbacks, including sensitivity to environmental conditions and non-neutral behavior (Ramesh et al., 2020). Nevertheless, isozymes remain useful for identifying cultivars and assessing isozyme polymorphism in open-pollinated olive seedlings (Seker et al., 2008). Allozyme markers have revealed significant genetic differentiation in wild olive (oleaster) populations, revealing significant genetic distinctions between eastern and western oleaster populations in Mediterranean basin, providing insights into the genetic diversity of wild olive subspecies (Lumaret et al. 2004). The findings indicated substantial genetic differences between eastern and western oleaster populations. Eastern populations were found to be genetically closer to cultivated olive clones, whereas western populations exhibited stronger connections to wild Canarian varieties. Furthermore, cultivated olives showed lower heterozygosity in comparison to oleasters, highlighting the impacts of intensive selection and inbreeding. This genetic differentiation is reflective of historical biogeographic events and adaptations to the diverse Mediterranean environments, along with limited gene flow between the regions (Besnard et al., 2013). The associated biochemical diversity in wild olives is crucial for their survival, adaptation, and evolutionary

Table 1. Summary of different metrics used to measure morphological, biochemical and molecular diversity of wild olive

Marker Type	**Genetic Diversity Metric**	**Study Reference**
Morphological Markers	Coefficient of Variation (CV)	Khadivi et al., 2023
Morphological Marker	Fruit and stone traits: PCA, and clustering.	Lazovic et al., 2018
Morphological Markers	Various morphological characteristics	Nikpeyma, 2023
Morphological and Biochemical Markers	Fruit size and oil content	Leon et al., 2018
Biochemical Markers	Fatty acids and phenolic compounds	Lafka et al., 2013
Biochemical Marker Analysis	Correlation with molecular markers	Abood et al., 2017
Isozyme Analysis	Isozyme polymorphism	Seker et al., 2008
SSR and ISSR markers	Inter-population genetic diversity	Noormohammadi et al., 2012
AFLP and SSR markers	Construction of Genetic Linkage Map	Aabidine et al., 2010
SSR marker	Gene flow dynamics	Di Rienzo et al., 2018
SSR marker	Gene flow and admixture	Diez et al., 2014
SNP Markers	Genetic structure and phylogenetic analysis	Zhu et al., 2019
EST-SNP marker	Genetic diversity in cultivated and wild Olives	Mariotti et al., 2020
Genotyping-by-sequencing	Assessment of genetic diversity	Taranto et al., 2018
Microsatellite Markers	High genetic diversity	Rahmani et al., 2019
SSR markers	Diversity & differentiation	Habib et al., 2020

resilience, providing a genetic reservoir for stress resistance and other beneficial traits (Tadic et al., 2021). Therefore, the conservation and further study of these genetically diverse wild populations are essential, not only for preserving their evolutionary legacy but also for supporting future olive breeding and improvement initiatives (Leon et al., 2018).

2.2.2. Genetic diversity analysis using molecular markers

DNA-based markers revolutionized species identification in plants, particularly olives (Madesis et al., 2013; Ramesh et al., 2020). Various molecular markers such as RAPD, AFLP, RFLP, SNP, and microsatellites (SSR) provide different methods for evaluating the genetic diversity of olives (Sheidai et al., 2014; De Boer et al., 2021; Bidyananda et al., 2024).

SNPs, high-throughput and naturally abundant markers, have become essential in olive diversity assessments. Zhu et al. (2019) utilized SNP data to classify olive cultivars into groups, revealing genome-wide diversity and phylogenetic relationships. SNP markers were also identified using ESTs, providing insights into olive germplasm diversity (Mariotti et al., 2020). Utility of high-throughput EST-SNP markers for olive germplasm management has been exploited, demonstrating consistent results across different plant materials and propagation events. For instance, Taranto et al. (2018) used genotyping-by-sequencing to identify SNPs and assess genetic diversity in olive cultivars, supporting future genome-wide association mapping studies. Likewise, Zhu et al. (2019) applied genotyping-by-sequencing to analyze the genetic diversity of 57 olive cultivars, identifying two groups with no clear geographical distribution. This study emphasized the effectiveness of SNP data in evaluating genome-wide diversity. The study characterized SNP polymorphism in ESTs of *Olea europaea*, identifying over 1000 transcript-specific SNP markers (Mariotti et al. 2020). This approach offers advantages in identifying functional SNPs for diversity analysis. Additionally, microsatellite markers were used to analyze genetic diversity in local olive trees in Tunisia, revealing high genetic diversity and facilitating molecular fingerprinting of local varieties (Rahmani et al., 2019). Genomic SSRs were employed to assess the genetic diversity of 79 Mediterranean olive accessions, leading to the identification of two subpopulations based on computational models (Ayed et al. 2021). Furthermore, the study involved the development of SNP markers for USDA olive germplasm, uncovering moderate genetic diversity and population structure (Islam et al., 2021). Overall, the research highlights the importance of SNP markers in advancing olive genetic studies.

Few researches investigated the genetic diversity of a specific olive subspecies, *Olea europaea* subsp. *cuspidata*, and found that there is a moderate level of genetic diversity in the Hajar Mountain of Oman within the areas sampled for the study (Habib et al. 2021). Yet another study explored the landscape genetic structure of this subspecies in the Ethiopian highlands (Kassa et al. 2017). Despite the challenges of habitat fragmentation, the findings indicated both high genetic diversity and low differentiation among populations. GBS-driven SNP catalogues were used to examine the genetic variability and geographical relationships among Italian olive cultivars D'Agostino et al. 2018). The study provided insights into the distribution of genetic variation and allele recovery in Italian olive cultivars (Table 2). Studies using various markers showcase the multifaceted nature of genetic diversity in *Olea europaea* L. For example, Noormohammadi et al. (2012) reported that ISSR markers demonstrated a mean value of polymorphism of 81.74%. Meanwhile, RAPD markers exhibited a mean value of polymorphism of 73.71% in Hormozgan Province located in southern Iran.

Existing studies on the molecular diversity and structure of *Olea europaea* subsp. *cuspidata* are constrained by limited sample sizes, a narrow geographic focus, and less effective molecular techniques (Sheidai et al., 2014; Kassa et al., 2017; Mariotti et al., 2020). As a result, these studies may not adequately capture the full spectrum of genetic variation or the evolutionary dynamics across the entire subspecies range. To achieve a more accurate and comprehensive understanding of diversity patterns and evolutionary relationships within *cuspidata*, it is crucial to conduct more extensive sampling and utilize advanced molecular techniques that encompass the complete geographic distribution of the subspecies. This method will enable researchers to uncover regional differentiation, historical gene flow patterns, and potential cryptic lineages that might be overlooked in narrowly focused studies. However, both biochemical and molecular approaches are instrumental in providing a thorough comprehension of olive genetic diversity, which supports its conservation and sustainable use.

3. Genetic Diversity and Differentiation of *Olea europaea* Subsp. *cuspidata*

Olea europaea subsp. *cuspidata*, a wild olive tree, exhibits a wide distribution across from South Africa to southern Egypt, the Mascarenes, western Asia, the Indian subcontinent, and western China (Green, 2002). Variation in climate, soil, and evolutionary adaptations lead to variations in leaf dimensions, fruit traits, and growth patterns throughout these areas (De Casas et al., 2006). For example, African varieties flourish in dry forests and savannas, demonstrating adaptations for drought resistance (Aerts et al., 2008; Besnard et al., 2023). In contrast, varieties found in the Middle East and north-western Asia thrive in semi-arid, mountainous terrains and are frequently utilized as rootstock (De Casas et al., 2006).

Molecular analyses employing Random Amplified Polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) methodologies indicate that the Arabian forms of this subspecies possess a genetic profile that is intermediate between the Eastern/Southern African and north-western Asian varieties (Abood et al., 2017). Eastern African olives exhibit similarities in terms of leaf morphology, fruit traits, and genetic lineage with Arabian olives, a likeness attributed to their geographic proximity (Sarwar et al., 2023). Despite the distinct barrier presented by the Red Sea, there is compelling evidence indicating that seed-mediated gene flow may have occurred between these populations (Besnard et al., 2007). Additionally, within the olive complex, the subspecies *cuspidata* ranks as the second most diverse, with a genetic diversity index of (Nei, H=2.6×10–3) (Julca et al., 2023).

Genetic analysis has identified distinct lineages within the subspecies *cuspidata*, specifically African and Asian varieties (Julca et al., 2023). The African lineage encompasses populations from South Africa and Réunion. In contrast, the Asian lineage, located in Iran, is characterized by the plastid "C" type, which differs from the "A" type found in Africa. Notably, Iranian specimens of *cuspidata* from southeastern provinces demonstrate gene flow with European olives (Mousavi et al., 2014). Hybridization between *Olea europaea* subsp. *cuspidata* and *Olea europaea* subsp. *europaea* occurs in both Iran and South Africa, including instances of unintentional hybrids in South Africa (Julca et al., 2023). Additionally, DNA variations have been documented within *cuspidata* populations from Kenya, the Mediterranean, and Italy (Loureiro et al., 2006). Subspecies *cuspidata* is genetically distinct from Mediterranean olives, with geographic distance playing a more significant role in influencing genetic variation in *cuspidata* compared to the Mediterranean olive (Besnard et al., 2001).

Table 2. Summary of key findings from various studies, comparing genetic diversity in different Olive subspecies or populations

Study Reference	Genetic Marker Type	Key Findings
Dong et al., 2021	Molecular Markers	The genus Olea is polyphyletic.
Falek et al., 2022	Morphological Marker	High genetic variability among Algerian wild olive
Julca et al., 2023	Molecular Markers	Genetic admixture and differentiation of the *Olea europaea* complex.
Mariotti et al., 2020	EST–SNP Markers	Genetic differentiation of cultivated and wild olives.
Ayed et al., 2022	Genomic SSRs	Mediterranean olives show high genetic diversity due to geographic origin and domestication history.
Wu et al., 2022	SNP marker	High-quality De novo genome assembly of *Olea europaea* subsp. *cuspidate.*
Tuniç et al., 2024	ISSR marker	Very high genetic polymorphism 92.94%.
D'Agostino et al., 2018	GBS-driven SNP catalogues	Genetic variability and differentiation of fruit weight in Italian olives.
Dehghan-Seresht et al., 2024	Morphological marker	High morphological and pomological diversity exists among the 10 Iranian olive cultivars.
Gomez-Rodriguez et al., 2020	New set of SSR markers	New SSR markers differentiate 36 olive varieties in the germplasm bank of Cordoba.
D'Imperio et al., 2011	Molecular and morphological markers	Endocarp traits in molecular and morphological data enable cultivar discrimination.

Genetic diversity was lower in invasive *cuspidata* populations than in source populations (Besnard et al., 2006). The molecular diversity of invasive subspecies *cuspidata* and Mediterranean forms, as well as their source populations outside of their natural range in Australia and Hawaii, where assessed using nuclear SSRs. The findings from Besnard et al. (2007, 2014) indicated that due to founder effects following multiple introductions, two invasive *cuspidata* populations (He = 0.41, 0.51) exhibited lower genetic diversity compared to the original populations (He = 0.70).

In Oman, Habib et al. (2020) utilized microsatellite markers to study *Olea europaea* subsp. *cuspidata*, uncovering significant genetic diversity within the Hajar mountain range. Their research indicated high genetic diversity and minimal differentiation among populations, suggesting considerable gene flow. The clustering of populations showed patterns that align with the northern and southern regions, pointing to historical connections and susceptibility to present-day environmental stressors. The research also showed that despite significant genetic differentiation, there was minimal evidence of population substructure, indicating that long-distance seed and pollen dispersal had been effective. This finding is crucial for the conservation and management of these species, especially considering the human-induced disturbances in the region.

3.1. Phylogenetic Insights and Biogeography

Several studies used phylogenomic data from genome skimming to resolve relationships within the genus Olea and identify molecular markers for species identification. Phylogenetic relationships among the subspecies of Olea have been elucidated using complete plastomes, nuclear ribosomal DNA (nrDNA), plastid DNA and mitochondrial DNA markers. These analyses employed a variety of markers, including Amplified Fragment Length Polymorphism (AFLP) (Angiolillo et al., 1999), Internal Transcribed Spacer (ITS)-1 sequences (Besnard et al., 2007), Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSRs) (Hess et al., 2000), Restriction Fragment Length Polymorphisms (RFLPs) (Besnard et al., 2002), and Single Nucleotide Polymorphisms (SNPs) (Dong et al., 2021), with next-generation sequencing also being employed (Julca et al., 2023). The results show an inconsistent phylogenetic pattern among the different subsp. *cuspidata* forms. The likely reason for the inconsistence of results is reticulate evolution and different analysis methods (e.g., type of markers, organelles studied), as well as different numbers of samples analyzed in the various studies (Bensard et al., 2006; Beiko et al., 2008). However, results also demonstrate a clear separation between *cuspidata* and the other wild olive subspecies. The nuclear phylogeny and split network analysis reveal a highly reticulated structure, particularly among the five subspecies (excluding *cuspidata*), indicating significant historical gene flow within the complex (Julca et al., 2023).

Recent phylogenomic analyses leveraging whole-genome sequencing from 15 individuals within the *Olea europaea* complex have provided compelling evidence of genetic admixture and supported the recognition of seven distinct subspecies (Julca et al., 2023). Notably, these studies identified *Olea europaea* subsp. *ferruginea* as a genetically distinct lineage associated with Asian populations, clearly separating it from the African subsp. *cuspidata*. This finding, however, stands in contrast to the accepted taxonomy based on morphological characteristics and geographic distribution (Green, 2002), which recognizes only six subspecies: *cuspidata*, *laperrinei*, *maroccana*, *guanchica*, *europaea*, and *cerasiformis*. The discordance between phylogenomic data and classical taxonomy underscores the complexity of subspecies delimitation within the *O. europaea* complex. It also reveals intricate biogeographic patterns shaped by historical gene flow, ecological adaptation, and long-term evolutionary processes (Diez et al., 2014). These findings highlight the need to reconcile genomic insights with morphological and ecological data to achieve a more integrative and accurate understanding of olive diversity and evolution.

3.2. Marker-Dependent Genetic Diversity

Estimates of genetic diversity in wild olive populations exhibit considerable variation, largely depending on the molecular markers used. This variation is primarily due to differences in inheritance patterns, mutation rates, genome coverage, and the resolution capacity of each marker type (Belaj et al., 2003). Among the most commonly employed markers are simple sequence repeats (SSRs), with a particular focus on nuclear SSRs and, more recently, expressed sequence tag-derived SSRs (EST-SSRs), which have gained significant attention in recent studies (Gomez-Rodriguez et al., 2020; Diez et al., 2011; Mariotti et al., 2016) (Figure 1). Microsatellites play a crucial role in the cataloging, authentication, and traceability of olive germplasm (Sheidai et al., 2014). Their utility arises from several advantageous features, including high levels of polymorphism, codominant inheritance, multi-allelic nature, and reliable reproducibility. These characteristics make SSR markers particularly well-suited for genotyping, cultivar identification, and evaluating the genetic structure of both wild and cultivated olive populations (Diez et al., 2015; Kassa et al., 2017; Mousavi et al., 2017; Li et al., 2020).

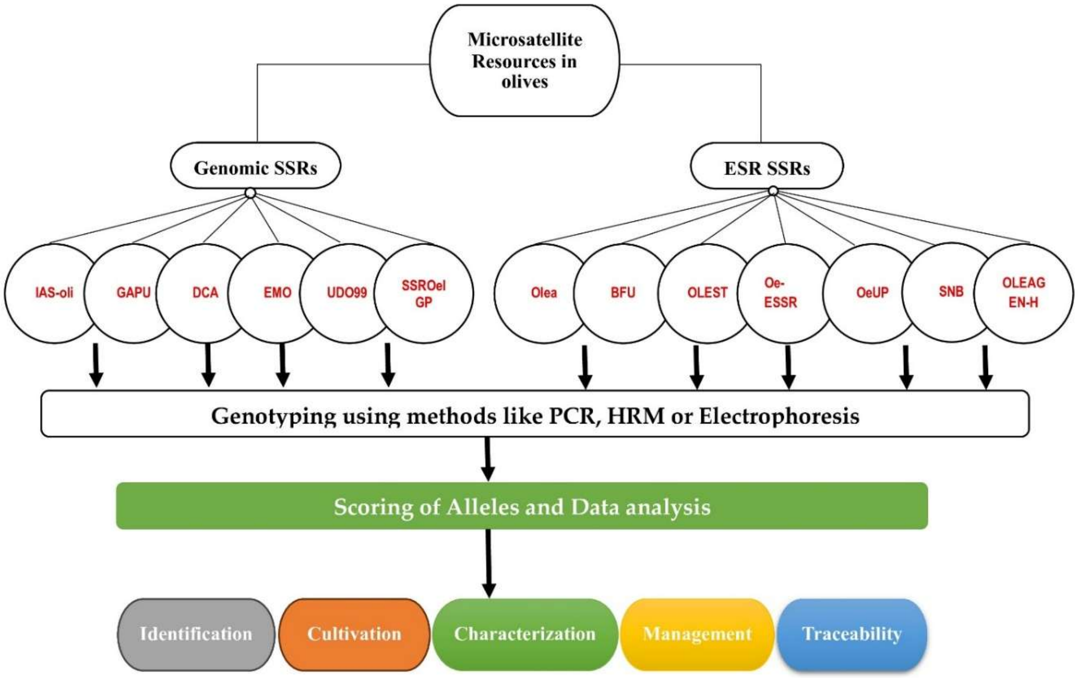

Figure 1. SSR and EST based Genomic Markers Employed for Profiling Genetic Diversity in Olive Plants

4. Factors Influencing Genetic Differentiation

Genetic differentiation among subsp. *cuspidata* populations can be influenced by factors such as effective gene flow, geographical proximity, habitat fragmentation, isolation by distance, isolation by environment, and selection (Figure 2).

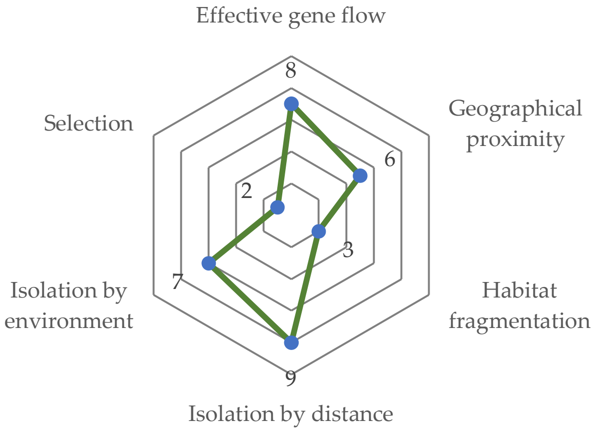

Figure 2. Factors Influencing Genetic Differentiation in Subsp. *cuspidata* Populations. A radar chart illustrating the influence of various factors on genetic differentiation among *Olea europaea* subsp. *cuspidata* populations. Each spoke represents a factor, including effective gene flow, geographical proximity, habitat fragmentation, isolation by distance, isolation by environment, and selection. The length of each spoke indicates the degree of influence, providing a comprehensive view of the factors shaping the genetic diversity landscape in subsp. *cuspidata* populations.

A reported low genetic differentiation (FST = 0.016) in some cases, indicating effective gene flow, while other studies found significant differentiation among widely distributed populations (Kassa et al., 2017).

4.1. *Impact of habitat loss on genetic diversity*

Habitat loss is one of the major drivers of species extinctions and declines of species richness at local scales (Hald-Mortensen, 2023). Habitat loss, exemplified by invasive forms of subspecies *cuspidata*, can lead to genetic diversity reduction. Genetic studies using eight nuclear DNA microsatellites, plastid DNA markers as well as ITS-1 sequences have confirmed invasive *Olea europaea* subspecies *cuspidata* populations in Australia and Hawaii showed lower genetic diversity compared to source populations due to founder effects (Besnard et al., 2007).

In a study by Besnard et al. (2013), the invasive African olive trees in Australia and Hawaii were analyzed using chloroplast and nuclear microsatellites. The researchers found that African olives in New South Wales experienced a 57.7% reduction in allelic richness, indicating a significant loss of genetic diversity compared to their native regions. Notably, the population in Maui, Hawaii exhibited the lowest genetic diversity when compared to those in South Africa and New South Wales, showing significant declines in both allelic richness and heterozygosity ($P < 0.01$). This decrease is attributed to the sequential introduction of African olives from South Africa to New South Wales and then to Hawaii, with each introduction resulting in a genetic bottleneck (Besnard et al., 2013).

A genetic bottleneck occurs when a small subset of individuals from diverse native populations is introduced into a new environment, resulting in a reduction of allelic richness and heterozygosity within the invasive range (Aronne, 2017). Historical records indicate that *Olea europaea* subspecies *cuspidata* was introduced to both Hawaii and Australia for purposes such as hedging, with a limited number of founding individuals (Cuneo and Leishman, 2006; Besnard et al., 2007). This lack of gene flow has likely contributed to the maintenance of a low-diversity genetic structure in these populations (Stevens et al., 2018).

4.2. *Impact of geographic proximity on genetic diversity*

Geographic proximity significantly influences the genetic diversity of plant populations (Salgotra and Chauhan, 2023). Plants relay on mechanisms like wind, insects, and animals for the dispersal of seeds and pollen. Geographic barriers such as mountains and water bodies can limit gene flow leading to genetic differentiation between populations. For example, Eastern African (Ethiopian) olives are more closely related to Arabian olive populations due to their geographical proximity. Despite the barrier of the Red Sea, there may have been minimal isolation, possibly because of seed-mediated gene flow between Arabian and Eastern African populations (Besnard et al., 2007). Limited gene flow, environmental and climatic factors (Perez-Alquicira et al., 2023), and the boundaries of a species' geographic range (Vitorino et al., 2020) can lead to isolation and genetic structuring, often resulting in greater genetic differentiation. This significantly impacts the genetic diversity within plant populations (Navas-Lopez et al., 2019).

5. Conclusions and Future Perspective

Advancements in molecular marker technology have enabled the characterization of germplasm and facilitated genetic improvement based on specific markers. Both morphological and molecular markers, particularly DNA markers, have been instrumental in unravelling genetic variability within the wild olive subspecies complex.

While significant progress has been made in utilizing molecular markers for phylogeographic and phylogenetic investigations, a more comprehensive dataset is needed to fully characterize the phylogeny of the olive complex. Numerous studies have explored molecular diversity patterns within various wild olive tree species, revealing that the majority of variability exists within populations rather than between them. High outcrossing and traits related to animal seed distribution play pivotal roles in maintaining high gene diversity, particularly in fragmented environments like Ethiopian *cuspidata* and Saharan *laperrinei* populations. Subspecies *cuspidata* is distinguished as the earliest diverging and the second most diverse among the subspecies.

Despite strides in molecular genetics, unanswered questions remain, especially regarding domestication and phylogenetics in the olive tree. The release of cultivated cultivar and oleaster genomes opens avenues for using modern molecular technologies, such as Next-Generation Sequencing, to address these challenges. Subspecies *cuspidata*, despite its wide distribution and ecological significance, has received relatively less research attention.

Wild olives thrive in diverse climatic and agro-ecological conditions. Subspecies *europaea* and *cuspidata* exhibit distinct niche needs, suggesting high variability in ecological requirements. Reforestation initiatives, considering climate change, should account for these diverse ecological needs. Genetic studies should move beyond neutral molecular variation, incorporating quantitative features and integrating genetic research with ecological and population genomic methodologies. While our understanding of the impacts of fragmentation, distribution range, and olive cultivation on genetic diversity is incomplete, evidence from large-scale studies should theoretically guide effective wild olive conservation efforts, particularly in local contexts.

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