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Insight into the European Union community trademarks olive oils traceability: The use of DNA markers as the most effective approach

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ABSTRACT

Background: The high economic value of olive oil has prompted the European Union to support efforts to protect and valorise the olive oil industry through the introduction of designations of origin (PDO/PGI).
Scope and approach: This review provides an overview of the European regulation on certified PDO and PGI extra virgin olive oils highlighting the importance and the impact of these labels. It examines the main fraudulent practices affecting extra virgin olive oil and the methods of analysis used to detect frauds, with a particular focus on DNA-based methods for varietal and sometimes, geographical identification. Moreover, an in-depth study was carried out on the varieties authorized in PDO and PGI extra virgin olive oils in Europe, addressing the issue of synonyms and the availability of SSR marker profiles. Finally, the data collected were used for the detection of private alleles.
Key findings and conclusions: All the data and the information collected represent a useful and reliable tool for the varietal traceability and authentication of European PDO and PGI extra virgin olive oils.

1. Introduction

The olive tree (*Olea europaea* L.) is a species typically found in Mediterranean countries. Olive oil, its main derived product, attracts considerable interest due to its organoleptic properties and health benefits making it an important component of the Mediterranean diet (Centrone et al., 2021; Fernandes et al., 2020). Despite widespread cultivation worldwide, the Mediterranean countries remain the most important olive producers. Consequently, olive trees are cultivated on around 4 million hectares, mainly in the European Union (EU), producing roughly 67% of the world's olive volume, becoming a key economic factor for the development of the agro-industrial sector (https://agriculture.ec.europa.eu/farming/crop-productions-and-plant-based-products/olive-oil_en).

The high economic value of olive oil leads to a variety of fraudulent practices in which olive oil is mixed with low-quality oils of the same species (refined olive oil or olive pomace oil) or other species, and unauthorised production methods are used. According to the 2019 Annual Report of the European Union Food Fraud Network, around 80% of the Italian extra virgin olive oil (EVOO) on the market is fraudulent. Most cases of fraud are due to the addition of cheaper vegetable oils (hazelnut, soya, almond, maize, sunflower and sesame) whether refined or processed (Yan et al., 2020).

The growing demand for high-quality extra virgin olive oil and the resulting increase in counterfeiting has prompted the European Union to support efforts to protect the olive oil industry from adulteration and fraudulent activities. Nowadays, the quality of olive oil is regulated by legislation ensuring the exclusion of contaminants and allergens (http://www.fda.gov/Food/FoodSafety/default.htm), while traceability regulations have only recently been introduced. In order to regulate labelling, ensure product traceability and define the certification of origin, various labels have been introduced. The Protected Designation of

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Origin (PDO) and Protected Geographical Indication (PGI) (European Council Regulation, 2006) labels allow the protection of food products that have distinctive characteristics in terms of their production process, territory, history and traditions, and/or organoleptic properties and, of course, nutritional value. The designations of origin certify the geographical origin and the varieties used as well as the processing methods. The differences between PDO and PGI are primarily linked to the area of origin of the raw materials and the extent to which the production process took place in the specific geographical origin.

The authenticity of extra virgin olive oil is traditionally assessed using chemical methods that can usually detect the presence of contaminants, adulterants, and, in some cases, identify the geographical origin of the product (Lozano-Castellón et al., 2022; Wadood et al., 2020). In the latter case, these methods might be poorly reproducible as they are influenced by environmental conditions and require different statistical data from several years and different areas. This shows remarkable limitations in unmasking the use of varieties not declared on the product label. The assessment of the geographical origin of EVOO takes advantage of metabolic profiling techniques which require the construction of a database of the metabolic profiles of genetically certified monocultivar EVOO, which is not yet available (Calò et al., 2022). Finally, these approaches have proven to be efficient for fresh products, while they tend to lose effectiveness when analysing processed foods (Fanelli et al., 2021).

The PDO and PGI designations are used to label EVOOs whose main characteristics depend on the territory of origin of olives and compliance with strict production rules, such as the varieties to be used in the production process. Therefore, each Member State takes the appropriate administrative and judicial measures to protect this specificity by instituting appropriate Protection Consortia. For these reasons, the correct identification of the cultivars, for which the chemical methods have not proved to be sufficiently reliable, is the primary goal of EVOO certified traceability.

In recent years, the application of DNA-based methods has become a valid support to morphological and biochemical descriptors (Boucheffa et al., 2019; Falek et al., 2021) to achieve the goal of the varietal identification and detection of adulteration in olive oil (Jukić Špika et al., 2022; Pasqualone et al., 2015; Sabetta et al., 2017). Among the wide range of available molecular markers, SSR (Simple Sequence Repeats) and SNP (Single Nucleotide Polymorphism) are the most commonly used for genetic diversity studies (Miazzi, Di Rienzo, et al., 2020; Saddoud Debbabi et al., 2020; Sion et al., 2021). In the case of DNA extracted from a highly processed food matrix, such as olive oil, it can be highly fragmented and may contain compounds that can affect the quality of the PCR. Therefore, the use of SSR and SNP markers, involving a very small portion of DNA, is a powerful tool for discrimination analysis (Crawford et al., 2019; Pasqualone et al., 2016; Pereira et al., 2018; Ben Ayed & Rebai, 2019).

This review aims to provide an overview of the European regulation for PDO and PGI certified EVOO and the main fraudulent practices in the olive oil sector. In addition, a deep check was carried out on the varieties used to produce the PDO and PGI EVOOs currently registered with the European Commission and publicly available SSR profiles for each variety were examined in detail. All identified profiles have been collected here and made available to the scientific community. We were able to highlight many synonymies (different names for the same variety) and homonymies (same name for different varieties), thus partially solving the confusion caused by the misnaming of olive varieties. Finally, the data collected were used to identify private alleles useful to identify the geographical origin of the olive varieties used in the oil production. All the data and information collected here provide a useful and reliable tool for the varietal traceability and the authentication of the PDO and PGI EVOOs.

2. European Union quality food products certification

2.1. Geographical indication (GI)

With "Regulation (EC) No. 2081/92 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs" the European Union aims to protect specific product names that have unique characteristics. Products with a specific link to the place of production can benefit from the designation "Geographical Indication" (GI). GI includes Protected Designation of Origin "PDO" (foodstuffs and wines), Protected Geographical Indication "PGI" (foodstuffs and wines), and Geographical Indication "GI" (spirits drinks). Regulation (EU) No. 1151/2012 defines the required characteristics for these products. Regulation no. 178/2002 defines traceability as "the ability to reconstruct the track of a food through all phases of production, transformation and distribution". The reverse process must also be guaranteed so that we can trace all the information about the food's life cycle from the packaging onwards.

In addition, GI certification establishes intellectual property rights, the recognition of which is becoming increasingly important in trade negotiations between the EU and other Countries. GIs are legally protected against imitations and misuse within the EU and in other Countries with which a specific protection agreement has been signed. The competent national authorities of each EU Country take the necessary measures to protect the names registered in their territory. They should also prevent and stop the illegal manufacture or marketing of products under these names.

2.2. Implementation of the new regulations

The original Community legislative framework has proved inadequate in recent years to respond to European and global changes. In fact, legislative changes, the addition of ten new Countries and the requests of third Countries (Australia and the United States) as well as technical problems in the implementation of the Regulations, have shown that a comprehensive change is necessary. These led to the adoption of the "Regulations 509/2006 and 510/2006" on 20 March 2006, by the EU Council of Ministers. The new regulation speeds up the procedure for recognising Geographical Indications and ensures better coordination between national and Community institutions. In addition, on 31 March 2022, the Commission adopted a proposal aimed at promoting the use of GIs to support the rural economy and achieve a higher level of product protection, especially in the online market. The measures proposed by the Commission to strengthen and improve the existing system include the reinforcement of measures adopted to improve social, environmental, and economic sustainability in the production specifications; greater protection of GIs, especially when sold through online platforms; greater involvement of anti-counterfeiting and customs authorities in all EU Countries; a simplified and shortened registration procedure. To date, there are many registered olive oils resulting from the strong local differentiation. This is based on the climate, the different varieties used, and the production techniques consolidated over time by local producers. Thus, the place of origin is a key factor in defining the identity of the products.

The labelling system is the identity document of food products. The mandatory and optional information that must be included in food labelling is established by the Reg. (EU) no. 1169/2011. In this way, food labelling becomes the tool that allows the consumer to obtain the right information to make a free and informed choice. In addition to the horizontal provisions, envisaged for all foodstuff, Delegated Regulation (EU) 2022/2104 defines the rules for the labelling of packaged products. The mandatory indications for packaged olive oils are the sales denomination (extra virgin olive oil, virgin olive oil, olive oil - composed of refined olive oils and virgin olive oils -, Pomace oil of olive oil), the denomination of origin (only for extra virgin and virgin olive oils), information on the category of the oil and the harvesting campaign (under

special conditions and only for extra virgin and virgin oils). The mandatory information must be clearly legible and indelible. The geographical origin of the oil indicates the State in which the olives were harvested and processed. If the name of a Member State or the European Union is indicated as the origin, both phases of production took place in that member state or the European Union.

In addition to the mandatory indications, the law allows the use of optional indications, provided they are relevant and can be documented such as "first cold pressing", "cold extraction", and other indications relating to organoleptic characteristics (flavour and/or smell) or maximum acidity.

2.3. Certified olive oil market

According to a report published by the Institute of Services for the Agricultural Food Market (Ismea) (https://www.ismeamercati.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/12709), the PDO economy continues to grow even in a difficult macroeconomic scenario that affects agri-food production. After a very positive 2021, the PDO PGI food sector will reach 8.85 billion euros in 2022, representing a growth of +8.8% per year and a +33% compared to 2012, corresponding to 17.35 billion euros. In particular, the value and volume of Extra Virgin Olive Oils with Geographical Indications have increased in recent decades. The market value of PDO and PGI EVOOs has risen steadily to €142 million with a production of 13 thousand tons, corresponding to a 21 percent increase compared to 2012. Exports also increased by 11 percent, reaching €62 million. The sales recorded an increase of +10.6% compared to the previous year and amounted to 32.499 million euros.

In the olive oil market, product differentiation is mainly based on the degree of acidity (extra virgin, virgin, lampante, blend, etc.) (Commission Regulation EC No. 182/2009), olive harvesting, and oil extraction methods. In Countries with a long tradition of olive oil production, such as Italy, this distinction is relevant and market segmentation is mainly based on place of origin and sensory characteristics. In recent years, marketing strategies in the olive oil sector have given greater importance to quality, enhancing the link to the place of origin (Carzedda et al., 2021), the variety of olives used, and sensory attributes (Perito et al., 2019). However, unlike other products such as wine, olive oil prices mainly reflect the area of origin, while designation of origin (PDO/PGI) adds less additional value. This difference compared to wine is probably due to the fact that the designation of origin for oil was only introduced in 1996, whereas it was introduced for wine in 1973. However, a comparison of the results for the two sectors seems to indicate that the importance of the designation of origin for olive oil and the associated certifications is increasing even at the sub-regional level (Cacchiarelli et al., 2016). The registration of certified EVOOs, after an initial increase (1996), still shows a trend for producers to apply for new PDO and PGI EVOOs, indicating an economic benefit of certification for producers and an increased interest from consumer. In Fig. 1 the evolution of the registration of certified extra virgin olive oils from 1996 is illustrated.

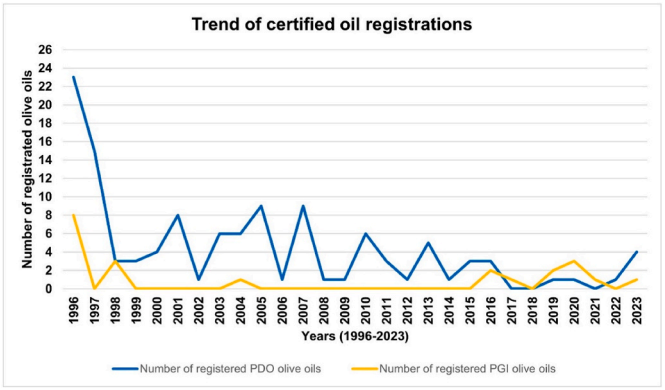

Fig. 1. Number of PDO and PGI EVOOs registered since 1996.

3. Fraudulent practices in extra virgin olive oil

3.1. Fraudulent practices affecting the olive oil sector

Several factors play a role in olive oil fraud. Its high nutritional value and health benefits, which are also due to the use of certain cultivars, contribute to its high market price and encourage fraudulent farmers to make false claims on the product label (Gentile et al., 2020; Rifna et al., 2022). In addition, its liquid state facilitates blending with lower quality (Karbasian et al., 2015) or cheaper oils (Jabeur et al., 2014). These factors, combined with emerging fraud techniques, market fluctuations and different control measures in different countries, make it difficult to detect fraudulent olive oils (Morling & McNaughton, 2016). To ensure consumer safety and increase their confidence in the quality of olive oil, EU Member States have established and applied appropriate standards and anti-fraud measures (Rossi, 2017; Reg. EU 29/2012; Reg. EU 1308/2013). However, in 2019, based on reports from the "EU Network on Food Fraud and the System of Administrative Assistance and Food Fraud", olive oil was found to be one of the most frequently fraudulent products.

There are two main methods of fraudulent practises with EVOO: adulteration and mislabelling (Aparicio et al., 2013). Adulteration refers to mixing EVOO with inferior or other vegetable oils to increase profits; mislabelling occurs when the label contains untrue information. These fraudulent practices deceive consumers and have a negative impact on the credibility and trustworthiness of the EVOO market.

3.2. Analytical methods for detecting fraud

In the second half of the 20th century, extensive research was carried out to develop reliable analytical targeted and non-targeted methods for detecting fraud in the olive oil sector (Bajoub et al., 2018). These techniques contribute significantly to identifying and mitigating the impact of fraud, but do not make it possible to prevent it. Furthermore, these methods have certain limitations: they are unable to determine the specific adulterant, they are ineffective in detecting low levels of adulteration and there is a lack of standardized protocols for monitoring certified and/or mono-varietal olive oils. According to the "EEC European Standards (No./91 of 11 July 1991) on the characteristics of olive oil and methods of analysis", the official quality control system is based on a combination of analytical and sensory approaches. The analytical analysis of numerous physico-chemical parameters specified by the "American Oil Chemists' Society" (AOCS) and the "International Organization of Standardization" (ISO) aims to identify indicators of hydrolytic changes, oxidation and freshness of the olive oil, while the sensory quality is assessed by a panel test. However, considering the drawbacks and limitations of these methods, it seems necessary to improve them with faster and more accurate techniques. For this reason, alternative analytical techniques (including sample preparation, data collection, and processing) for the control of adulteration in virgin olive oil have been proposed over the last decade.

4. The authentication of the geographical and varietal origins of EVOOs

4.1. Analytical strategies for geographical and varietal identification

The authentication of the geographical and varietal origin of virgin olive oil is currently not regulated by any official analytical methods. However, in response to growing consumer interest in the geographical origin of labelled extra virgin oils, considerable efforts have been made to develop robust and reliable analytical strategies for geographical and varietal identification. To achieve this goal, three main strategies have

been applied: targeted analyses, profiling approaches and untargeted methods.

The targeted analysis strategy involves the identification and quantification of one or two specific compounds in olive oil that are related to the variety and/or geographical origin (Gertz et al., 2019; Lukić et al., 2019). These methods, in general, have a greater selectivity and sensitivity than non-targeted methods and are preferable in authenticity issues when the suspected target is a primary marker since it offers direct information about the product authenticity (Ballin and Holst Laursen, 2019). However, targeted approach offers limited information on olive oil authentication since it is not useful in the identification of not declared varieties.

The profiling approach belongs to the category of the targeted methods since it focuses on the qualitative and/or quantitative determination of a broader range of olive oil compounds, taking into account their chemical nature and biosynthetic pathway (Mousavi et al., 2019; Reboredo-Rodríguez et al., 2018). It multiplies the resulting information compared to the other targeted analysis and is often suitable for addressing complex authentication issues (Ballin and Holst Laursen, 2019). Fatty acid profiling showed to have a good efficiency in the discrimination of olive cultivars although a certain level of variability was observed showing a limited reliability (Crawford et al., 2020).

Finally, the untargeted strategies are based on the fingerprinting approach and include different analytical chemistry methods and molecular techniques based on DNA. They provide valuable information through the detection of multiple small changes in the food product and the analysis of these changes through advanced multivariate statistics (Ballin and Holst Laursen, 2019). Some of the chemistry approaches, such as the nuclear magnetic resonance (NMR) and the mass spectrometry (MS), are well-established methods for EVOOs geographic origin assessment representing an effective support for PDO and PGI olive oil validation (Calò et al., 2022).

The untargeted techniques rely on the construction and use of an appropriate database. The database is necessary to compare the obtained sample fingerprint with that of the reference sample. The construction of a database for the chemistry analysis may be a tricky process requiring multiple information, such as the year of olive collection, the environmental conditions, and the methods of extraction (Calò et al., 2022). Molecular approaches, represent an easy and efficient strategy for geographical assessment and varietal identification in olive oil and the database construction is simple since the DNA sequence is not influenced by environmental conditions or olive treatment or processing (Sion et al., 2021). Moreover, the molecular methods showed a higher efficiency in olive varietal discrimination compared to analytical chemistry techniques (Crawford et al., 2020).

4.2. DNA-based methods

Olive is a predominantly allogamous species, resulting in high levels of heterozygosity and DNA polymorphisms among cultivars. This variability, coupled with the ambiguity in olive cultivar names, makes necessary the careful characterization of olive genetic resources since, olive productivity and oil quality are traits strictly related to a variety. DNA-based methods play a key role among the variety of analytical techniques proposed. These provide numerous advantages. Indeed, DNA is not affected by the environment, unlike other olive compounds, and its long-lasting nature allows strong performance even with highly processed matrices (Kalaitzis & El-Zein, 2016; Bohme et al., 2019; Fanelli et al., 2023). These techniques enable the detection of adulterated vegetable oils (Nehal et al., 2021), and varietal identification (Agrimonti & Marmiroli, 2019) and can resolve cases of synonymies, homonymies, and misnaming, providing a valuable approach useful for varietal identification and traceability analysis (Sebastiani & Busconi, 2017; Kalogianni et al., 2015).

The first step in all DNA-based authenticity tests is the extraction of DNA. This step represents an issue because of the high degradation of DNA extracted from olive oil and the presence of phenolic or polysaccharide compounds that can inhibit DNA polymerase in the molecular fingerprinting step (Raieta et al., 2015). For this reason, substantial efforts have been dedicated to the development of reliable effective, fast, and economical DNA extraction methods (Montemurro et al., 2018; Piarulli et al., 2019). For the subsequent identification of varieties in olive oils, several molecular markers have been used over the years. Early approaches for varietal fingerprinting of olive oils were based on AFLP and RAPD molecular markers. The first ones combine the digestion of a target DNA by specific restriction enzymes with Polymerase Chain Reaction (PCR). The discriminating action of restriction enzymes coupled with polymerase amplification activity ensures good reproducibility and a high degree of polymorphism, allowing the simultaneous screening of a large number of loci, without any pre-knowledge of the sequence (Vos et al., 1995). One of the first varietal fingerprinting attempts using AFLP markers was performed by Pafundo et al. (2005) on four Italian olive oils, finding an overlap of molecular profiles of approximately 70% in oils and plants. However, it was shown that DNA extraction from olive oil was the limiting step for the reliability of AFLP profiles, due to the complex matrix analyzed.

As for RAPD markers, genomic DNA is amplified by PCR using a single, short random primer (10 nucleotides) that hybridizes similar sites in the opposite direction, producing amplicons dependent on the length and size of the target genome and primer (Williams et al., 1990). They are characterized by simplicity and applicability, even to genetically unfamiliar species, but are poorly reproducible due to the low annealing temperature in PCR, causing non-specific amplification (Sion et al., 2021). As evidence of their low reproducibility, the varietal characterization study by Martins-Lopes et al., 2008 conducted on Portuguese olive oils showed the presence of only two reproducible bands out of a total of 11 RAPD markers tested. For this reason, RAPD markers are usually used in combination with other molecular markers.

To date, the most commonly used molecular markers are SSRs and SNPs, which are located either in nuclear or organelles DNA (Pereira et al., 2018) and show high efficiency in highly fragmented DNA. In particular, SSRs (Simple Sequence Repeats) are hypervariable patterns of short tandem nucleotide repeats (1–6 bp). Their polymorphisms, which depend on variation in the number of repeats, are detectable by amplification with primers complementary to conserved regions flanking the microsatellite (Powell et al., 1996). Although their development requires the construction of a genomic library, cloning, sequencing, and primer design, the use of SSRs in plant genetics has increased due to the advantages of being codominant, highly distributed throughout the genome, and highly reproducible with low-quantity/quality DNA (Garcia et al., 2004). Moreover, their standardized and straightforward detection systems make these markers the most used in agri-food traceability (Burns et al., 2019). To overcome the limitations associated with discrepancies in the allele sizes assignment between laboratories, a “consensus list” for a set of validated SSR markers has been developed (Baldoni et al., 2009; Debbabi et al., 2021; Doveri et al., 2008), making them very useful in authentication and traceability of olive varieties in monovarietal and blend oils (Fanelli et al., 2021).

In recent years with the advent of next-generation sequencing (NGS) techniques and as a result of the publication of the whole genome sequence of O. europaea (Cruz et al., 2016; Rao et al., 2021; Unver et al., 2017), SNP markers have been widely used in several olive oil traceability studies (Pasqualone et al., 2016; Ben Ayed & Rebai, 2019). A single nucleotide polymorphism (SNP) consists of the variation of a single nucleotide in the DNA sequence of different individuals (Sion et al., 2021). These markers are codominant, abundant, and evenly distributed in the entire genome and their detection is highly reproducible (Bracci et al., 2011). Genotyping by sequencing (GBS) (Kaya et al., 2019) consists of genome reduction by restriction enzymes, followed by fragment sequencing. The technique allows for obtaining thousands of SNP markers, providing a rapid, high-throughput, and cost-effective tool for the study of genetic variability in plants and for the

analysis of the authenticity of table olives and oil, products of high economic value in all Mediterranean countries (Ben Ayed & Rebai, 2019).

For olive varietal identification and cultivars used in the production of EVO oils, other markers AFLP-derived or RAPD-derived, can be used. In this regard, the Sequence-Tagged Sites (STS) is a short DNA sequence present only a single time in the genome whose location and nucleotide sequence are known. Based on the detection method, the STSs are classified into Sequence Characterised Amplified Region (SCAR) or Cleaved Amplified Polymorphic Sequences (CAPS). The first requires two locus-specific oligonucleotide primers designed on the previously sequenced nucleotide sequence of an RAPD or AFLP fragment corresponding to a trait of interest. If the fragment of interest is amplified and digested with restriction enzymes, revealing a restriction fragment length polymorphism caused by SNPs or INDELs, these are called CAPS markers (Sion et al., 2021).

SCAR markers are effective tools in unequivocally discriminating olive varieties (Bautista et al., 2003; Busconi et al., 2006), but they are also applied to oil analysis. However, in the oil fingerprinting study by De la Torre et al., 2004, SCARs developed on leaf DNA are not detectable in oil DNA, because they are too long or not abundant. Therefore, chloroplastic SCAR markers (CP-rpl16T) were isolated from a monovarietal oil AFLP profile (Pafundo et al., 2007).

DNA-based identification studies to verify the cultivar origin of monovarietal olive oils were performed using CAPS markers, which have shown high efficiency in detecting oil blends, proving their utility for testing and verifying the authenticity of monovarietal olive oils, for the certification of olive trees and in germplasm characterization and conservation studies (Pasqualone et al., 2013; Uncu et al., 2015). These studies highlighted that restriction enzyme-based SNP genotyping provides highly reproducible results based on observation of digestion patterns and not on fluorescence intensity measurements or fragment size comparisons (Uncu et al., 2015).

The detection of SSR and SNP markers polymorphism can be efficiently performed through the high-resolution melting (HRM) approach. This technique, based on the different melting temperatures of PCR products, was proved effective in the authentication of monovarietal oils (Batrinou et al., 2020; Chedid et al., 2020; Pasqualone et al., 2015; Xanthopoulou et al., 2017). SSR-HRM and SNP-HRM assays were compared in the traceability study performed by Chedid et al., 2020. Both approaches were proved equally effective in determining the varietal origin of monovarietal olive oils; although the SNP-HRM method showed higher efficiency in discriminating mixtures of olive oils in different ratios compared to the SSR-HRM technique.

4.3. Olive germplasm collections and molecular databases

The "FAO Olive Germplasm Plant Production and Protection Division", estimates that the world's olive germplasm currently comprises more than 2600 different varieties (FAO, "The Second Report on the State of the World's Plant Genetic Resources For Food and Agriculture", Rome, Italy, 2010), and more than 1250 cultivars (Bracci et al., 2011). Nevertheless, the exact number of cultivars is difficult to establish and is probably underestimated due to the lack of harmonized morphological, molecular and agronomic characterization. For this reason, Mediterranean Countries have promoted the establishment of *ex-situ* germplasm collections, including the "World Olive Germplasm Banks" (WOGB) at IFAPA (Cordoba, Spain), Marrakech (Morocco) and the French germplasm collection (Porquerolles Island). Efforts to collect and catalog the great diversity of olive germplasm have led to the implementation of molecular markers databases, which are essential for the application of SSR markers to traceability. However, only a few online databases have been developed for olive trees such as the Italian OLEA database (http://www.oleadb.it/olivodb.html), the Olive Genetic Diversity Database (OGDD) (http://www.bioinfo-cbs.org/ogdd/Methodology.php) and the National Clonal Germplasm Repository – Tree Fruit & Nut Crops & Grapes (https://npgsweb.ars-grin.gov/gringlobal/search.aspx).

5. Olive varieties authorised in the certified EVOOs

5.1. Varietal composition of certified EU EVOOs

A total of 140 certified EVOOs, including 118 PDOs and 22 PGIs from seven different European and one extra-European Country, are currently registered in the eAmbrosia designation register in the product category oils and fats (butter, margarine, oil, etc.) (https://ec.europa.eu/agriculture/eambrosia/geographical-indications-register/). The register is constantly updated and includes products for which applications have already been submitted or which have been published but not yet registered. Among the registered EVOOs Italy stands out with 44 PDOs and 8 PGIs, while Slovenia and Turkey have the lowest number with only one registered EVOO (Table 1). In Supplementary Table S1, the web links to the respective production specifications are given for each registered oil.

The production specifications of the PDOs and PGIs EVOOs list a total of 358 different cultivars (with some cultivars used to produce oils in different Countries), all belonging to the species *Olea europaea* subsp. *europaea* var. *europaea*. The greatest variability is found in the 52 Italian EVOOs with 215 different cultivars, followed by Spain, Greece, Croatia, Portugal, France, Slovenia and Turkey (Table 1). The highest number of Italian cultivars used shows the greater heterogeneity of the Italian germplasm, as evidenced by the recently published MASAF National Register of Fruit Plant Varieties, which includes 734 olive varieties (https://www.politicheagricole.it/flex/cm/pages/ServeBLOB.php/L/IT/IDPagina/10035).

Among the cultivars used for the production of the EVOOs, Italian Leccino, which is included in 35 EVOOs, (33 Italian, one Croatian and one Slovenian), and Frantoio, which is used in 31 EVOOs (29 Italian, one Croatian and one Slovenian), are the most frequently represented. This highlights the high quality of these varieties due to their high content of bioactive compounds and their desirable sensory properties (Di Lecce et al., 2020; Giuffrida et al., 2011).

Information on the cultivars used to produce certified olive oils is freely available in the Olea database (Oleadb), which contains information on 1607 cultivars. Only 20 of the 358 varieties used for the production of certified EVOOs could not be found in the database, probably referring to minor cultivars poorly characterized, or to common cultivars labelled with slightly different/erroneous names (Table 2).

5.2. The problem of synonyms

Over time, hundreds of cultivars have been identified and selected for their adaptation to different ecoclimatic and soil conditions. This has led to many cases of synonymy, homonymy, and molecular variants i.e.

Table 1
Number of registered EVOOs in the different Countries.

Country	Certification		Total number of oils	Number of cultivars admitted in the production process
	PDO	PGI		
Croatia	6	0	6	26
France	9	0	9	19
Greece	20	12	32	25
Italy	44	8	52	215
Portugal	6	0	6	16
Slovenia	1	0	1	7
Spain	31	2	33	66
Turkey	1	0	1	1
TOT	118	22	140	375[a]

[a] The effective total number of cultivars admitted in the production process is 358 considering that some cultivars are used in the production of EVOOs in different countries.

Table 2
Cultivars for which not match were found in the Olea database.

Country	Minor cultivars	Different/erroneous names
France	Capanace - Curtinese - Raspulada	–
Greece	Elia Makris	Caronaiki - Coroneiki Agouromanako - Agouromanakolia
Italy	Galatrese - Marzemino - Pitursello	Sammartinengna - Sammartinenga Tonda di Filocaso - Tonda di Filogaso Giaraffa - Giarraffa Mandanici - Olivo di Mandanici
Croazia	–	Mata - Mata Istarska
Spain	–	Morilla - Morillo Manzanillo de Jaén - Manzanilla de Jaén Verdeal Transmontana - Verdeal Trasmontana Alameña de Montilla - Alameño de Montilla Tempranilla - Tempranillo Hojiblanco - Hojiblanca

intra-cultivar variation (Barranco et al., 2000; Cipriani et al., 2002). As a result, there are currently more than 3000 synonyms reported (Bartolini et al., 1994). However, only a few of these cultivars have been fully characterized by morphological and molecular markers. Therefore, there is still great uncertainty about the names of many olive cultivars.

Over the years, numerous cases of synonymy have been identified among the varieties authorized for certified olive oils. For some cultivars, the synonymy has been proven with the help of molecular markers, as in the case of the Croatian varieties Rošinjola and Rošulja, the Italian varieties Dritta and San Felice or Tonda di Cagliari and Majorca. In most cases, however, the synonyms are supported only by outdated documents, often of a regional scale such as Ocal and Gordal de Archidona, Gremignolo, Mignolo and Mignola, Cannellino and Cannellina, etc. Analysing the existing synonyms sometimes leads to the identification of large groups of cultivars that all seem to be related to each other, e.g. the group including the Italian varieties Frantoio, Frantoiano, Casaliva, Larcianese, Razzola, Rasara and Ogliarola del Bradano, or the group with the Spanish cultivar Verdial, Verdial de Cádiz, Verdial de Huevar, Verdial de Vélez-Málaga, Verdiell and Llei de Cadaqués (Fig. 2).

To make matter worst, the regulations for oil production often give incomplete or generic names to varieties that have different names in different regions. Some examples are the names Nostrale, Nostrana, Olivastro, Royal, or Tonda, which are used as synonyms for a large number of cultivars, or the names Ogliarola, Nocellara, Manzanilla, Lechin and Royal, which are usually followed by an additional name that clarifies the origin (e.g. Ogliarola could be referred to Ogliarola Barese, Ogliarola del Vulture, Ogliarola del Bradano, etc.). Looking at the synonym groups, the number of genotypes used for the registered

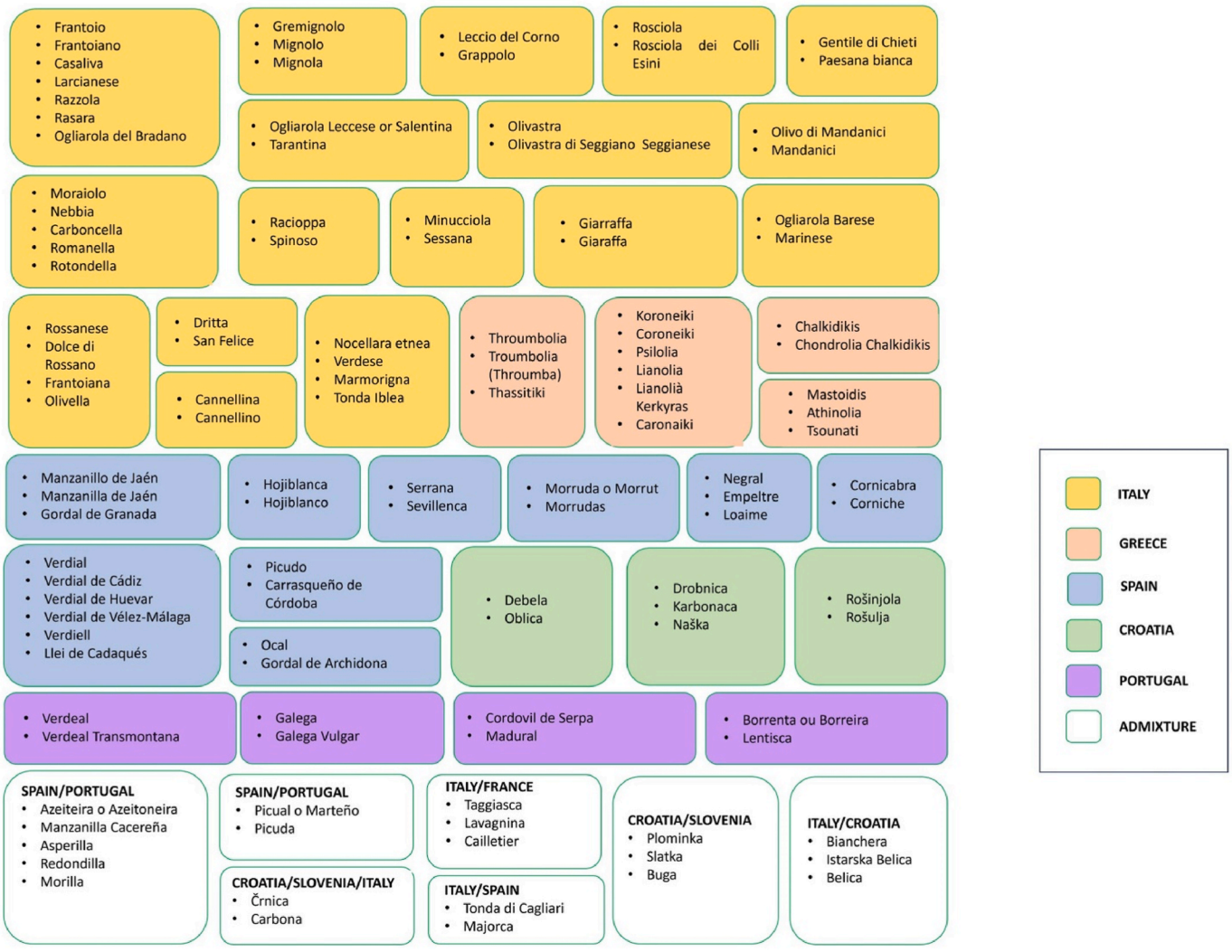

Fig. 2. Synonyms, by EU Country, identified among cultivars used for certified extra virgin olive oil production. In the evaluation of the synonyms, possible inconsistencies reported in Oleadb due to outdated bibliography were discarded.

EVOOs decreases from 358 to 282. Fig. 2 shows 44 synonym groups inferred from the investigation on the Oleadb website.

6. The available SSR marker profiles for the cultivars used in the certified EVOOs

6.1. Oleadb SSR allelic profiles

The Oleadb database currently contains 80 SSR profiles of varieties (15 loci, both alleles) including 39 used in the certified EVOOs and 10 reported as synonyms of these. Other 84 cultivars are listed in the database but lack of the microsatellite profile.

In some cases, the use of SSR markers has solved false cases of synonymy, such as for the Spanish cultivar Cornicabra, which, although reported as a synonym of Cornezuelo de Jaén, proved to be different by using SSR markers. Uncertainty remains for many cultivars, the uncertainty remains such as for the Italian cultivar Raggia, indicated as a synonym of Frantoio and Raia, both characterized by SSR markers and clearly different. Interesting is the case of the cultivar Razzo indicated by historical records as a synonym of Orbetana (Umbria Region, Italy), Frantoio (Tuscany Region, Italy) and Raio, but proved to be different with the SSR genotyping. These examples suggest that there is a general confusion in the labelling of olive varieties', making the identification of the varieties used for the production of certified oils and the detection of adulteration very difficult.

The SSR profiles of cultivars used in the production specifications and available online on Oleadb have been summarised in Supplementary Table S2 and Table 3.

6.2. Other sources of molecular markers profiles

Besides Oleadb, other databases are mentioned in the specialized literature, although they are difficult to access and data comparison is difficult due to the non-uniformity of the SSR sets used. Many scientific papers use microsatellite markers but often only report the allele range and the results obtained from the genetic and population structure analysis (Chiappetta et al., 2017; Marra et al., 2013; Montemurro et al., 2005). Even when the molecular profile is given, they report generic names for the locus and the samples analyzed, or they use different microsatellite sets which do not allow a comparison of the results (di Rienzo et al., 2018; Li et al., 2020; Gómez-Rodríguez et al., 2021). Worth mentioning are the publications by Trujillo et al., 2014; El Bakkali et al., 2019, who report on the molecular characterization of "Worldwide Olive Germplasm Banks" of Marrakech (Morocco) and Córdoba (Spain), in which 672 distinct SSR profiles were identified using 20 SSR markers. These include SSR profiles of many cultivars used for certified extra-virgin olive oils production although only four molecular markers were in common to the Olea database.

Other papers report SSR profiles of cultivars used in the EVOO production already available in the Oleadb (Baldoni et al., 2009; Rotondi et al., 2011), or not yet available, such as the Italian Leccio del Corno, Maurino, Mignolo, Olivastra Segganese, Pendolino, Rossellino, Lazzero, Morchiaio, Maremmano and Razzaio (Girelli et al., 2018), Tonda Iblea, Coratina, Moresca, Nocellara del Belice, Taggiasca, Nocellara Etnea, Itrana, Ogliarola Messinese, Giaraffa and Rosciola (Vietina et al., 2011) and the Spanish cultivars Galega and Carrasquenha (Doveri et al., 2008). However, even in these cases, the SSR markers in common with Oleadb are no more than five.

For this review, the Department of Soil, Plant and Food Sciences (Di.S.S.P.A) of the University of Bari (Italy) has made available the SSR profiles of 40 varieties used in EU-certified oils, referring to 10 SSR markers in common with the Olea database, allowing the comparison of the results (Supplementary Table S3).

Table 3
List of cultivars genotyped by SSR markers and indicated in the production specification of certified EVOOs. Database (Oleadb or Di.S.S.P.A) in which the molecular profile is reported is indicated. Eventual synonyms of each cultivar are also reported.

Genotyped cultivar (Oleadb)	Synonyms	Genotyped cultivar (Di.S.S.P.A.)	Synonyms
Arbequina	–	Aglandau	–
Ascolana tenera	–	Azeteira	–
Biancolilla	–	Bouteillan	–
Blanqueta	–	Buscionetto	–
Bosana	–	Calatina	–
Canino	Caninese	Cerasuola	–
Carolea	–	Ciciarello	–
Cassanese	Grossa di Cassano	Cima di Bitonto	–
Cellina di Nardò	–	Cima di Melfi	–
Changlot real	Royal	Cima di Mola	–
Cornezuelo de Jaen	Cornezuelo	Coratina	–
Cornicabra	Corniche	Dritta	San Felice
Dolce Agogia	–	Drobnica	Karbonaca; Naška
Edremit	Ayvalik	Erbano	–
Empeltre	Negral	Frantoiana	Olivella; Rossanese; Dolce di Rossano
Farga	–	FS17	–
Frantoio	Frantoiano; Larcianese; Rasara; Razzola	Grossane	–
Gentile di Chieti	Paesana bianca	Maiatica	–
Gordal sevillana	Mollar	Marinese	–
Hojiblanca	–	Nerba	–
Istarska Belica	Bianchera	Nocellara del Belice	–
Koroneiki	Caronaiki; Coroneiki; Lianolia; Kerkyras; Psilolia	Nociara	–
Leccino	–	Ogliarola barese	Marina
Lechin de Granada	–	Ogliarola garganica	–
Lechin de Sevilla	–	Olivo di Mandanici	Mandanici
Lucques	–	Pendolino	–
Manzanilla Cacerena	Asperilla; Manzanilla; Redondilla	Peranzana	–
Manzanilla de Jaen	Gordal de Granada	Plominka	Slatka; Buga
Manzanilla de Sevilla	Maçanilha	Provenzale	–
Mastoidis	Athinolia; Tsounati	Racioppa	Spinoso
Moraiolo	Carboncella; Nebbia	Ravece	–
Nostrana di Brisighella	–	Rotondella	–
Oblica	Debela	Sessana	Minucciola
Ogliarola salentina	–	Taggiasca	Cailletier; Lavagnina
Oliviere	–	Tonda di Filogaso	–
Orbetana	–	Tonda di Iblea	Marmorigna; Nocellara Etnea; Verdese
Ottobratica	–	Tondina	–
Piantone di Mogliano	–	Tortiglione	–
Picholine	–	Vaddarica	–
Picual	Picuda	Verdale des Bouches du rhone	–
Picudo	Carrasqueño de Córdoba		
Raia	Raja		
Raio	Rajo		

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Table 3 (*continued*)

Genotyped cultivar (Oleadb)	Synonyms	Genotyped cultivar (Di.S.S. P.A.)	Synonyms
Royal de Cazorla	–		
Semidana	–		
Sevillenca	Serrana		
Verdial de Huevar	–		
Villalonga	–		
Zaituna	–		

6.3. *The feasibility of traceability of certified EVOOs using SSR markers*

The widespread use of microsatellite markers has confirmed their high reproducibility, straightforwardness, and effectiveness in the identification of varieties and olive oil traceability. As regards, the cultivars authorised in the production specifications of EVOOs, the SSR profiles freely available in Oleadb (49) and provided by Di.S.S.P.A. of the University of Bari (40), as well as the main synonymy groups identified in the scientific literature, have made it possible to characterize a considerable number of EVOOs using these markers. In particular, the number of cultivars for which a microsatellite profile would increase available would rise from 89 to 120 (Table 3).

Table 4 shows, for each PDO and PGI oil, the number of cultivars authorised in the production specifications and the number of allelic profiles available for the traceability of the oils. By comparing these two parameters, it is possible to determine what percentage of the varietal composition of an oil can be characterized. Out of 140 oils, 41 EVOOs are fully characterizable (coverage = 100%); only for 14 oils the allelic profiles of the cultivars used in their production are not available (coverage = 0%). In addition, Greece has the highest number of fully characterized oils (16), followed by Spain (11) and Italy (10). However, it should be noted that olive oil production in Greece is based almost exclusively on monovarietal oils produced from the Koroneiki cultivar. For the Portuguese PDO oil "Azeite do Alentejo Interior" the specification does not indicate which varieties are used for production. It is worth noting that of the 140 registered EVOOs, the number of available allelic profiles for 86 oils allows a varietal characterization of more than 50%.

7. Evaluation of private alleles for olive oil traceability

Food traceability makes it possible to trace the origin of food across all stages of production, while genetic traceability determines the genetic identity of plant material used for processed products. Establishing the genetic origin of food products allows for verifying the authenticity of food and reduces adulteration by cheaper and low-quality material (And & Lain, 2005).

The allelic richness and the presence of private alleles (allelic frequency <1%) detectable in a population could reflect the genetic diversity to ease the identification of accessions useful for germplasm management and breeding (Kalinowski, 2004; Breton et al., 2004).

Although the analytical chemistry approaches were demonstrated effective in olive oil's geographic origin assessment, they present some limitations, such as the necessity to construct a comprehensive database for the comparison between the obtained sample fingerprint and those of the reference sample (Calò et al., 2022). The molecular approach based on the detection of private alleles is a straightforward and efficient method for the identification of the product origin allowing the bypass of the limits posed by the chemical techniques. Indeed, the use of private alleles has proven to be a powerful tool for assessing genetic relationships among olive cultivars and assigning them to their geographic populations of origin (Dervishi et al., 2021; Li et al., 2020; Tourvas et al., 2023; Valeri et al., 2022). Moreover, it has been demonstrated the potential of a subset of the analyzed SSR loci, characterized by the highest

Table 4
Certified EVOOs list. The following is given for each oil: Country of origin, oil name, PDO or PGI certification, number of cultivars allowed, number of cultivars genotyped, and percentage of cultivars for which SSR profile is available (Coverage %). NA (not available).

Origin	Oil name	PDO	PGI	N. of admitted cv	N. of genotyped cv	Coverage (%)
CRO	Bračko maslinovo ulje	✔		1	1	**100**
	Istra	✔		18	10	56
	Šoltansko maslinovo ulje	✔		2	1	50
	Korčulansko maslinovo ulje	✔		2	1	50
	Krčko maslinovo ulje	✔		4	3	75
	Ekstra djevičansko maslinovo ulje Cres	✔		2	1	50
SPA	Oli de l'Empordà/ Aceite de L'Empordà	✔		4	1	25
	Aceite de la Comunitat Valenciana	✔		5	4	80
	Aceite Sierra del Moncayo	✔		5	4	80
	Aceite de Lucena	✔		8	3	38
	Aceite de Navarra	✔		3	2	67
	Aceite Campo De Calatrava	✔		2	2	**100**
	Montoro-Adamuz	✔		5	3	60
	Estepa	✔		6	6	**100**
	Aceite Campo de Montiel	✔		5	4	80
	Aceite de La Alcarria	✔		1	0	0
	Aceite del Baix Ebre — Montsià» o « Oli del Baix Ebre — Montsià	✔		3	2	67
	Aceite Monterrubio	✔		7	6	86
	Poniente de Granada	✔		6	4	67
	Gata-Hurdes	✔		1	1	**100**
	Antequera	✔		8	8	**100**
	Montes de Granada	✔		7	4	57
	Aceite de La Rioja	✔		NA	NA	
	Aceite de Terra Alta/Oli de Terra Alta	✔		4	3	75
	Sierra de Cádiz	✔		8	7	88
	"Aceite de Mallorca" or "Aceite mallorquín" or "Oli de Mallorca" or "Oli mallorquí"	✔		4	3	75
	Sierra de Cazorla	✔		2	2	**100**
	Aceite del Bajo Aragón	✔		3	3	**100**
	Montes de Toledo	✔		1	1	**100**
	Sierra Mágina	✔		2	2	**100**

(*continued on next page*)

Table 4 (*continued*)

Origin	Oil name	PDO	PGI	N. of admitted cv	N. of genotyped cv	Coverage (%)
	Priego de Cordoba	✔		3	3	**100**
	Siurana	✔		3	2	67
	Sierra de Segura	✔		4	3	75
	Aceite Villuercas Ibores Jara	✔		6	4	67
	Les Garrigues	✔		2	2	**100**
	Baena	✔		8	4	50
	Aceite de Madrid	✔		7	4	57
	Aceite de Ibiza/Oli d'Eivissa		✔	3	3	**100**
	Aceite de Jaén		✔	7	6	86
POR	Azeite do Alentejo Interior	✔		5	2	40
	Azeites do Ribatejo	✔		3	0	0
	Azeite de Tras-os-Montes	✔		4	0	0
	Azeite de Moura	✔		3	0	0
	Azeites do Norte Alentejano	✔		6	2	33
	Azeite da Beira Baixa o Azeite da Beira Alta	✔		NA	NA	
SLO	Ekstra deviško oljčno olje Slovenske Istre	✔		7	5	71
FRA	Huile d'olive de Provence	✔		4	2	50
	Huile d'olive de Corse/Huile d'olive de Corse – Oliu di Corsica	✔		7	0	0
	Huile d'olive de Nîmes	✔		1	1	**100**
	Huile d'olive de Nice	✔		1	0	0
	Huile d'olive du Languedoc	✔		2	2	**100**
	Huile d'olive d'Aix-en-Provence	✔		7	5	71
	Huile d'olive de Haute-Provence	✔		4	3	75
	Huile d'olive de la Vallée des Baux-de-Provence	✔		5	4	80
	Huile d'olive de Nyons	✔		1	0	0
GRE	Ελαιόλαδο Μάκρης (Elaiolado Makris)	✔		1	0	0
	Γαλανό Μεταγγιτσίου Χαλκιδικής (Galanó Metaggitsíou Chalkidikís)	✔		2	0	0
	Μεσσαρά (Messara)	✔		1	1	**100**
	Αγουρέλαιο Χαλκιδικής (Agoureleo Chalkidikis)	✔		2	2	**100**
	Εξαιρετικό Παρθένο Ελαιόλαδο Σέλινο Κρήτης (Exeretiko Partheno Eleolado Selino Kritis)	✔		2	1	50
	Exairetiko partheno elaiolado Trizinia	✔		3	2	67
	Elaiolado « Finiki Lakonias»	✔		2	2	**100**
	Exeretiko partheno eleolado: «Thrapsano»	✔		1	1	**100**
	Σητεία Λασιθίου Κρήτης (Sitia Lasithiou Kritis)	✔		1	1	**100**
	Αποκορώνας Χανίων Κρήτης Apokoronas Chanion Kritis	✔		1	1	**100**
	Κολυμβάρι Χανίων Κρήτης/ Kolymvari Chanion Kritis	✔		2	2	**100**
	Καλαμάτα (Kalamata)	✔		2	1	50
	Κροκεές Λακωνίας/ Krokees Lakonias	✔		2	1	50
	Κρανίδι Αργολίδας/ Kranidi Argolidas	✔		1	1	**100**
	Πέτρινα Λακωνίας/ Petrina Lakonias	✔		1	1	**100**
	Αρχάνες Ηρακλείου Κρήτης/ Arxanes Irakliou Kritis	✔		1	0	0
	Λυγουριό Ασκληπιείου/ Lygourio Asklipiou	✔		1	1	**100**
	Βιάννος Ηρακλείου Κρήτης/ Viannos Irakliou Kritis	✔		2	1	50
	Βόρειος Μυλοπόταμος Ρεθύμνης Κρήτης/Vorios Mylopotamos Rethymnis Kritis	✔		1	1	**100**
	Πεζά Ηρακλείου Κρήτης/Peza Irakliou Kritis	✔		1	1	**100**

(*continued on next page*)

Table 4 (*continued*)

Origin	Oil name	PDO	PGI	N. of admitted cv	N. of genotyped cv	Coverage (%)
	Κριτσά (Kritsa)		✔	1	1	**100**
	ΑΓΙΟΣ ΜΑΤΘΑΙΟΣ ΚΕΡΚΥΡΑΣ (Agios Mathaios Kerkyras)		✔	1	1	**100**
	Ζάκυνθος/ Zakynthos		✔	2	2	**100**
	Σάμος/Samos		✔	2	1	50
	Λακωνία/ Lakonia		✔	4	2	50
	Πρέβεζα/ Preveza		✔	1	1	**100**
	Ρόδος/Rodos		✔	2	1	50
	Κεφαλονιά/ Kefalonia		✔	3	1	33
	Θάσος/Thassos		✔	1	0	0
	Λέσβος/ Μυτιλήνη/ Lesvos/ Mytilini		✔	1	0	0
	Ολυμπία/ Olympia		✔	2	1	50
	Χανιά Κρήτης/ Chania Kritis		✔	2	2	**100**
ITA	Alto crotonese	✔		6	3	50
	Aprutino pescarese	✔		3	2	67
	Brisighella	✔		1	1	**100**
	Bruzio	✔		4	4	**100**
	Canino	✔		5	4	80
	Cartoceto	✔		10	8	80
	Chianti Classico	✔		4	3	75
	Cilento	✔		6	3	50
	Collina di Brindisi	✔		6	5	83
	Colline di Romagna	✔		5	3	60
	Colline pontine	✔		3	2	67
	Colline salernitane	✔		6	3	50
	Colline teatine	✔		4	3	75
	Dauno	✔		6	5	83
	Garda	✔		4	3	75
	Irpinia – Colline dell'Ufita	✔		8	5	63
	Laghi lombardi	✔		5	3	60
	Lametia	✔		1	1	**100**
	Lucca	✔		4	4	**100**
	Molise	✔		8	2	25
	Monte Etna	✔		7	3	43
	Monti Iblei	✔		6	5	83
	Penisola sorrentina	✔		5	4	80
	Pretuziano delle Colline Teramane	✔		6	5	83
	Riviera Ligure	✔		4	3	75
	Sabina	✔		10	6	60
	Sardegna	✔		4	2	50
	Seggiano	✔		1	0	0
	Tergeste	✔		8	5	63
	Terra di Bari	✔		5	5	**100**
	Terra d'Otranto	✔		2	2	**100**
	Terre Aurunche	✔		4	1	25
	Terre di Siena	✔		17	4	24
	Terre Tarentine	✔		4	3	75
	Tuscia	✔		3	3	**100**
	Umbria	✔		6	6	**100**
	Val di Mazara	✔		6	3	50
	Valdemone	✔		8	2	25
	Valle del Belice	✔		7	4	57
	Valli Trapanesi	✔		2	2	**100**
	Veneto Valpolicella	✔		9	3	33
	Veneto Euganei e Berici	✔		9	4	44
	Veneto del Grappa	✔		7	3	43
	Vulture	✔		10	5	50
	Marche		✔	12	6	50
	Olio di Calabria		✔	24	7	29
	Olio di Puglia		✔	9	9	**100**
	Olio di Roma		✔	12	7	58
	Olio Lucano		✔	32	11	34
	Sicilia		✔	28	13	46
	Toscano		✔	35	6	17
	Olio Campania		✔	19	7	37
TRK	Edremit Zeytinyağı	✔		1	1	**100**

genetic variation and private alleles number, to distinguish among the different populations (Abdessemed et al., 2015; Delgado-Martinez et al., 2012; Sarri et al., 2006). Even in other plant species such as almond, grapevine, and cocoa, the use of private alleles allowed the identification of different gene pools present in the populations (Bustamante et al., 2022; Landjeva et al., 2015; Miazzi, D'Agostino, et al., 2020; Savoia et al., 2022).

Based on this background, the identification of private alleles could verify the compliance of the production with the production specification and prevent food fraud, allowing the traceability of the geographical origin of cultivars. Based on a preliminary analysis using the SSR allele profiles available in Oleadb and Di.S.S.P.A. University of Bari databases, the presence of private alleles useful for identifying the origin of the olives used for the production of a specific oil has been demonstrated (Table 5).

Private alleles were found in French, Greek, Spanish, Turkish and Italian olive cultivars. Table 5 shows more private alleles for Italian and French cultivars (36 and 16 private alleles, respectively). In addition, SSR markers DCA09, DCA13 and DCA17 identified private alleles in four out of five geographical locations, indicating a higher discriminatory power than for the other loci analyzed. The markers DCA03 and EMO90 highlight private alleles belonging to only Italy and France, respectively. A low number of private alleles shows the need to increase the number of available allelic profiles for an accurate genetic characterization of olive oils. In the future, a wider panel of cultivars genetically characterized and the availability of studies that test the unique presence of the identified private alleles in specific regions of origin could provide a powerful and rapid method for olive oil traceability.

8. Conclusion

To date, despite significant progress in assessing the geographical and varietal origin of olive oil, there is no regulation of official analytical methods. Our review aimed to provide a framework for the regulation of the production of PDO and PGI EVOOs and to describe the role of SSR markers in the traceability of certified EVOOs, highlighting the potentiality of this approach and the limits due to the scarce availability of molecular profiles. Specifically, based on the collection of the SSR profiles publicly available and those present in the dataset of the Department of Soil, Plant and Food Sciences (Di.S.S.P.A) of the University of

Table 5
Private alleles expressed in bp identified using SSR profile available on Oleadb and Di.S.S.P.A. database.

Country	SSR Locus	Private allele (bp)	Frequency
FRANCE	DCA09	167	0.143
	DCA13	162	0.071
		184	0.071
		186	0.071
	DCA15	122	0.071
		124	0.071
		140	0.071
	DCA17	105	0.071
		197	0.071
	GAPU71B	126	0.071
		146	0.071
		174	0.143
	GAPU101	143	0.071
		183	0.071
	EMO90	177	0.143
		184	0.071
GREECE	DCA15	264	0.500
	DCA17	217	0.250
	DCA18	163	0.250
ITALY	DCA03	237	0.047
		247	0.028
	DCA05	196	0.019
		200	0.037
		212	0.028
		214	0.009
	DCA09	188	0.009
		198	0.019
		202	0.009
		208	0.009
		210	0.028
	DCA13	142	0.010
		156	0.048
		160	0.010
	DCA15	162	0.066
		166	0.019
		174	0.009
		182	0.009
		194	0.019
		198	0.009
		258	0.019
		269	0.028
	DCA17	107	0.037
		109	0.046
		117	0.111
		129	0.028
		139	0.009
		141	0.019
		165	0.019
		175	0.019
		183	0.009
		185	0.009
	DCA18	187	0.019
	GAPU71B	142	0.011
	GAPU101	192	0.075
		196	0.009
SPAIN	DCA05	210	0.028
	DCA09	180	0.028
	DCA13	118	0.056
	DCA17	194	0.028
TURKEY	DCA09	151	0.500
	DCA13	206	0.500
	DCA18	266	0.500
	GAPU71B	150	0.500
		154	0.500
	GAPU101	159	0.500

Bari (Italy), we attempt to solve and reduce many cases of synonymy among olive cultivars and to identify a set of private alleles for each Country involved in the production of certified oils giving an important instrument for their geographical and varietal traceability. Although the present work shows that it is possible to characterize a large panel of EVOOs, a common genetic approach and an increased number of available allelic profiles could facilitate the traceability of olive oils and protect PDO and PGI-certified products from fraud and adulteration. In this context, the selection of an internationally shared set of microsatellite markers, the systematic sampling of cultivars in the production areas of PDO and PGI oils and the creation of a single dataset publicly available could be decisive for accurate and complete traceability of olive oils.

Author contribution statement

Isabella Mascio: Writing- original draft, reviewing, investigation, visualization. Michele Antonio Savoia: Formal analysis, Writing-review & editing. Monica Marilena Miazzi, Valentina Fanelli. Maria Dellino, Luciana Piarulli: Writing-review & editing. Fabio Grillo Spina, Stefania Carpino: Writing-review & editing, funding acquisition, Cinzia Montemurro: Conceptualization, funding acquisition, writing-review & editing.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tifs.2024.104615.

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