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Molecular characterization and identification of olive genotypes (*Olea europaea* L.) from the NW region of the province of Córdoba, Argentina and determination of the intravarietal diversity of genotypes associated with the reference cultivars Arauco, Manzanilla and Arbequina

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ABSTRACT
Córdoba (Argentina) has 4,484 ha dedicated to olive cultivation. The orchards established in the last century were characterized by low planting density and heterogeneous varietal constitution with dubious names. To solve this problem, it is possible to use genetic markers that allow materials to be characterized and identified. The objectives of the work were: to characterize and identify, using microsatellites, local olive genotypes from the Department of Cruz del Eje and to determine the intravarietal diversity of plants called Arbequina, Arauco and Manzanilla varieties. Olive plants, selected for yield, fruit quality and/or good response to biotic and abiotic stress, were analyzed together with accessions from the Olive Collection of INTA Junín (Mendoza, Argentina) and the World Germplasm Bank (Córdoba, Spain) with known identity. The diversity of genotypes associated with the reference cultivars Arauco, Manzanilla and Arbequina was determined with seventeen microsatellites. Six local genotypes were identified. The most polymorphic microsatellites were Gapu 103A and UDO43 and the least informative Gapu 71B and EMO 90. The Gapu 71B microsatellite differentiated Nevadillo Negro from the rest of the materials. The genotype called "Arauco Olly" presented the same genetic profile as the "Arauco Centenario" cultivar and the genotype locally called "21 Kg" was identified.

Keywords: genetic markers, microsatellites, genetic diversity

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la región NO de la provincia de Córdoba, Argentina y determinación de la diversidad intravarietal de genotipos asociados a los cultivares de referencia Arauco, Manzanilla y Arbequina. *Agriscientia, 41(2),* 1-18

RESUMEN

Córdoba (Argentina) cuenta con 4.484 ha dedicadas al cultivo del olivo. Las huertas establecidas en el siglo pasado se caracterizaron por baja densidad de plantación y una constitución varietal heterogénea con nombres inciertos. Para solucionar este problema, es posible utilizar marcadores genéticos que permitan caracterizar e identificar materiales. Los objetivos del trabajo fueron: caracterizar e identificar, mediante microsatélites, genotipos locales de olivo del Departamento Cruz del Eje y determinar la diversidad intravarietal de plantas denominadas variedades Arbequina, Arauco y Manzanilla. Se analizaron plantas de olivo, seleccionadas por rendimiento, calidad de fruto y/o buena respuesta a estreses bióticos y abióticos, junto con accesiones de la Colección de Olivo del INTA Junín (Mendoza, Argentina) y del Banco Mundial de Germoplasma (Córdoba, España), de identidad conocida. La diversidad de genotipos asociados a los cultivares de referencia Arauco, Manzanilla y Arbequina se determinó con diecisiete microsatélites. Se identificaron seis genotipos locales. Los microsatélites más polimórficos fueron Gapu 103A y UDO43 y los menos informativos Gapu 71B y EMO 90. El microsatélite Gapu 71B diferenció a Nevadillo Negro del resto de materiales. El genotipo denominado "Arauco Olly" presentó el mismo perfil genético que el cultivar "Arauco Centenario" y se identificó el genotipo denominado localmente "21 Kg".

Palabras clave: marcadores genéticos, microsatélites, diversidad genética

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INTRODUCTION

The olive tree *(Olea europaea* L.) is one of the iconic tree species of the Mediterranean basin. Six subspecies are currently recognized according to their morphological characters and geographic distribution (Green, 2002). The subspecies *cuspidata* is widespread in Africa and Asia, subsp. *laperrinei* is present in the Sahara Desert, *maroccana* mainly in Morocco, *guanchica* in the Canary Islands, *cerasiformis* in Madeira and the subspecies *europaea* is distributed throughout the Mediterranean basin. The latter is divided into two botanical varieties: the var. *europaea* to which the cultivated olive belongs and the var. *sylvestris* which corresponds to the wild olive or wild olive tree (Fanelli et al., 2022; Green, 2002) and which gave rise to the former about 6000 years ago in the northeastern region of the Mediterranean Levant (Fanelli et al., 2022).

Cultivated olives are intended for the production of oil and table or canned olives, as a consequence of the domestication and selection of more productive trees adapted to different environmental conditions, there are currently more than 2000 cultivars. However, the International Olive Council (IOC) estimates that 85 % of world olive production is explained by the cultivation of only 139 cultivars in 23 countries (Fanelli et al., 2022; IOC, 2000).

Argentina is a country with an important commercial activity in olive growing, this country being the main producer of table olives and oil in Latin America (Gómez del Campo et al., 2010), this is partly due to its favorable agro-climatic conditions. In addition, Argentina has olive germplasm banks in different official institutions, among which the collections of the National Institute of Agricultural Research (INTA), located in the provinces of Mendoza, Catamarca, La Rioja and San Juan, stand out for their age and number of accessions.

Recently, the germplasm bank of San Juan has been recognized by the IOC, incorporating it into the world network together with those of Spain (for the European continent), Morocco (for Africa) and Turkey (for Asia) (IOC, 2023). The collection has three nuclei and more than 100 cultivars of different origin and commercial destination (oil and table olives), which are distributed over fifteen hectares, making it the reference germplasm bank for the entire American continent and the southern hemisphere (IOC, 2023). (Figure 1).

The olive collection of INTA Junín Mendoza, started in the middle of the last century, although smaller in size, has accessions from Argentina, the USA and the Mediterranean basin, also constituting an important site for the reference of varieties (Banco et al., 2020). These collections contain excellent materials and constitute a valuable safeguard of the biodiversity of the species in the country.

The objective of these germplasm banks is to avoid the loss of variability and to guarantee the survival of the species in front of possible catastrophes of various kinds, such as that currently caused by the *Xylella fastidiosa* bacterium and the effects of climate change. The protection of germplasm collections is important, as they are the genetic reservoir of exotic species and landraces. Also, the genetic characterization of their accessions allows the determination of type conformity and detection of gene flow, the establishment of reference profiles, the identification of duplicates and errors in labeling, the detection of diversity and the determination of the coefficient of relationship in a collection. In addition, characterization highlights the expression of highly heritable morphological, physiological and agronomic traits, including agrobotanical traits. This information is essential to distinguish the accessions of a collection from each other (Food and Agriculture Organization of the United Nations [FAO], 2014). According to Baccino et al. (2013), the IOC has defined morphological and molecular markers as the varietal identification methodology for olives. This allows not only to corroborate the varietal identity of imported material, but also to postulate the identity of those materials present in olive groves of ancient date. Currently, in order to maintain international quality standards for oil and table olive production, it is necessary to certify and guarantee the varietal identity of the raw material from which they have been produced, particularly those that are internationally recognized.

Argentina is the leading producer of olive oil and table olives in South America, with an estimated production for the 2020/2021 season of 30,000 tons of oil and about 78,000 tons of table olives (IOC, 2019).

Although the most cultivated varieties in the country come from Spain and Italy, and in many cases have kept their original names, there are still planted genotypes whose origin and varietal identity is uncertain (Cavagnaro et al., 2001). The uncertainty of identity originates in part from the anthropic action of plant multiplication and selection in search of olive genotypes of higher agronomic and commercial value, but of doubtful varietal identity (Gómez del Campo et al., 2010). In the Argentinian

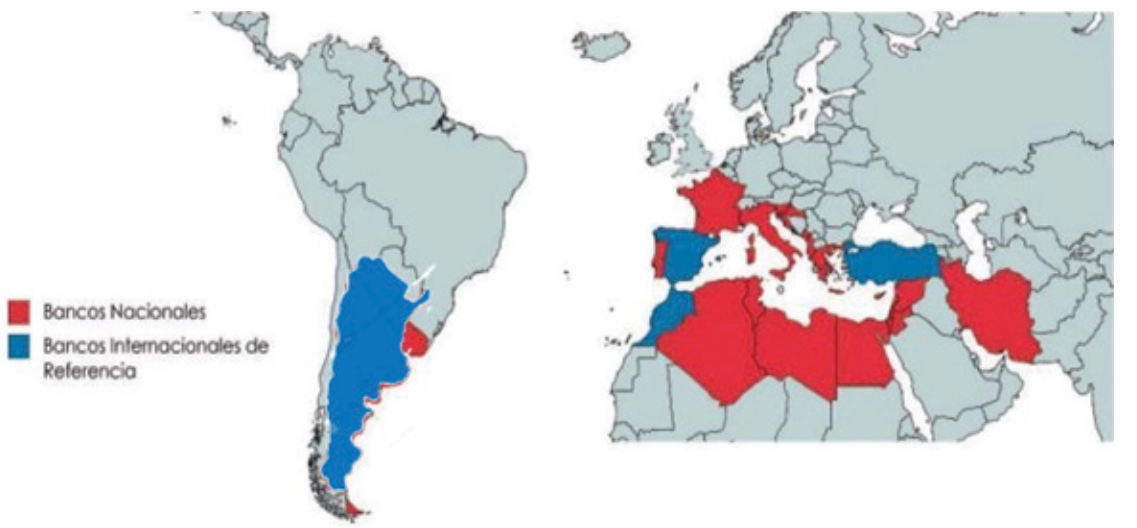

Figure 1. International Olive Council Network, adapted from Rallo (2020)

territory, the province of Córdoba devotes 4,484 ha to olive cultivation, of which 2,999 ha (66.88 %) are allotted to oil varieties and 1485.2 ha (33.12 %) to table olives (Instituto Nacional de Estadísticas y Censos de la República Argentina [INDEC], 2021). Cruz del Eje department, northwest of the province (30° 43' 22'' S -64° 48' 30"' W), concentrates the largest area, coexisting orchards with traditional type plantations from the middle of the last century, with more recent ones of higher planting density and varietal homogeneity. Arbequina, Frantoio and Picudillo are among the varieties destined for oil production, while Manzanilla, Arauco, Nevadillo, Farga, Empeltre and Ascolano, are the main varieties destined for table and canned olive production (Consejo Federal de Ciencia y Tecnología [COFECYT], 2016). Arbequina, Arauco and Manzanilla are cultivars that are particularly important due to their international acceptance given the quality of their oils and/or table olives (Gómez-Escalonilla et al., 2006).

In Argentina, the Arauco cultivar has been valued since ancient times and its origin dates back to the Spanish colonization stage, being the town of Arauco, province of La Rioja, the place where the first plantations were made and where it found the best conditions for its development. The "quatricentennial" olive tree, declared a National Historic Monument in 1980, is a witness to that era (Matías et al., 2010). At present, the area planted with Arauco has decreased in relation to other cultivars that make up the "Manzanilla" varietal complex, which includes several types that could be different varieties, such as "'Manzanilla de Sevilla", "Manzanilla Criolla", "Manzanilla Fina", "Manzanilla Reina", "Manzanilla Común" "Manzanilla Aceitera", "Manzanilla Denett", "Manzanilla Californiana", "Manzanilla israelí" (Gómez del Campo et al., 2010). Although Arauco maintains its importance due to the strong demand in the Argentine and Brazilian markets, sanitary factors that affect it and official measures to promote varieties of international recognition explain these changes.

Given the wide range of olive tree varieties and the fact that it is a woody species that begins to bear fruit from the second year after planting, it is essential to make the right choice of cultivar and to guarantee the varietal identity of the starting plant material in order to meet the interests of the producer. This makes it possible, for example, to certify and guarantee the traceability of oils, differentiating them from those that arise from a non-specific composition of varieties (Montealegre et al., 2010). Thus, in northwestern Argentina, where high temperatures can sometimes affect fat yield and oleic acid content in oils produced from cultivars such as Arbequina, blending with other high content oils from certified cultivars, for example Coratina and Picual, makes it possible to meet the requirements of the IOC (Gómez del Campo et al., 2010).

The characterization and identification of olive cultivars can be done with genetic markers, both morphological and molecular. The former are often only possible to evaluate at the level of the whole plant when it reaches its adult stage and can be strongly influenced by the environment. For this reason, methods have been developed that allow the identification and characterization of varieties based on the use of molecular markers (Fendri, 2011).

In the particular case of olive, DNA markers have been used for the characterization and identification of cultivars and for studies of inter and intra-cultivar genetic variation (Abdelhamid et al., 2013; Las Casas et al., 2014; Sastre et al., 2015), of determination of genetic diversity (Beghè et al., 2015) and of construction of linkage maps (De la Rosa et al., 2003), among others. The IOC genebank network currently has more than 1700 accessions, of which more than 1000 have been authenticated both morphologically and molecularly, using microsatellites (SSR) (Abdelkrim et al., 2021). The use of this type of molecular markers has contributed to solve problems of frequent homonymies and synonymies in the olive varietal panorama (Barranco et al., 2000).

In the traditional orchards of Cruz del Eje department, it is possible to find a set of different cultivars in the same plot whose denomination is confusing and there are even genotypes derived from seed rootstocks, due to loss of grafting, whose origin is uncertain.

The objectives of the present work were to molecularly characterize and identify olive (*Olea europaea* L.) genotypes from the NW region of the province of Córdoba, Argentina, and to determine the intravarietal diversity of genotypes associated with the reference cultivars Arauco, Manzanilla and Arbequina.

MATERIALS AND METHODS

Plant material

Characterization of local genotypes

Ten individuals corresponding to genotypes of uncertain identity were analyzed. The set of plants was located in different orchards in the department of Cruz del Eje. These orchads were

called: El Cóndor (EC), 4 Soles (4S), Ollantay (Olly) and Las Playas (LP). This set was named "local genotypes" and their names had been assigned by the producers based on the phenotypic similarity of its fruits with commercial cultivars of known name and their location in the different orchards. All the genotypes studied have been assigned names by the producers for their phenological characteristics. These are the names and their location: Genotype 1: Arbequina 21 Kg (EC); Genotype 2: Arbequina (4S). The remaining genotypes were named Genotype 3, G3 (Olly) for their similar characteristics to Arauco. Genotypes G4 (EC) and G5 (LP) had previously been called Ascolano (EC) and Empeltre (LP). Genotypes G6 (LP) and G7 (4S) had been named Farga (LP) and Frantoio (4S) by the producers. Genotypes G8 (4S) and G9 (4S) were named Manzanilla 4 Soles and Manzanilla Gigante (4S), respectively. Finally, G10 (4S) had previously been assigned the name Nevadillo 4 Soles.

In addition to the above genotypes, a specimen located on the premises of the National University of Córdoba (UNC) (Córdoba, Argentina), Oblonga (UNC), was studied together with seven plants with confirmed identity belonging to the Colección de Olivos de Junín, Mendoza (COM) (Agricultural Experimental Station of the National Institute of Agricultural Technology), and one DNA sample from the World Olive Germplasm Bank of Córdoba (WOGBC), Spain (Table 1).

Intravarietal diversity

In order to determine the intravarietal diversity of genotypes closely phenotypically related to the Arauco, Manzanilla and Arbequina varieties, three groups were formed. The "Arauco complex" included local genotypes G3 (Olly) and G11 (EC), and the varietal reference Arauco Centenario (AraCen) and the clones related to Arauco from Junín, Mendoza (AraJM.COM) Criolla Salvarredi (CSa.COM) and Criolla San Martín (CSM.COM) deposited in the collection of Junín, Mendoza.

Table 1. Olive genotypes/cultivars from Cruz del Eje, Córdoba, and reference varieties from the World Bank (WOGBC) of Córdoba (Spain) and the Junín Experimental Station (Mendoza) of INTA (COM)

Local Genotypes/cultivars			**Reference varieties**		
Denomination	**Acronymo**	**Location**	**Denomination**	**Acronymo**	**Location**
Arbequina-21 Kg Arbequina 4S	G1(EC) G2(4S)	El Cóndor (30° 48' 50'' S, 65° 6' 7'' W) 4 Soles (30° 48' 50'' S, 65° 6' 7'' W)	Arbequina	Arb. WOGBC Arb.COM	Córdoba- España Junín- Mendoza
Arauco Ollantay Arauco EC	G3(Olly) G11(EC)	Ollantay (30° 38' 52" S 64° 55' 11" W) El Cóndor (30° 48' 50'' S, 65° 6' 7'' W)	Arauco Centenario Criolla Salvarredi Criolla San Martín	Ara.Cen CSa.COM CSM.COM	La Rioja- Argentina Junín- Mendoza Junín- Mendoza
Ascolano EC	G4(EC)	El Cóndor (30° 48' 50'' S, 65° 6' 7'' W)	Ascolano		
Empeltre LP	G5(LP)	Las Playas (30° 39' 41" S, 64° 54' 33" W)	Empeltre		
Farga LP	G6(LP)	Las Playas (30° 39' 41" S, 64° 54' 33" W)	Farga	Fa.COM	Junín- Mendoza
Frantoio 4S Oblonga-FCA	G7(LP) Oblonga (FCA)	Las Playas (30° 39' 41" S, 64° 54' 33" W) FCA (31° 26' 27,82" S; 64° 11' 05,76" W)	Frantoio	Fr. WOGBC Fr.COM	Córdoba- España Junín- Mendoza
Manzanilla 4S Manzanilla Gigante 4S	G8(4S) G9(4S)	4 Soles (30° 48' 50'' S, 65° 6' 7'' W)	Manzanilla Sevilla Manzanilla Aceitera Manzanilla Imperial Manzanilla 2 Hermanas Manzanilla Española Manzanilla Carmona	MaSev. WOGBC MaAc.COM Ma.Im.COM Ma.2H.COM Ma.Es.COM MaCa.COM	Córdoba- España Junín- Mendoza
Nevadillo 4S	G10(4S)	4 Soles (30° 48' 50'' S, 65° 6' 7'' W)	Nevadillo Blanco Nevadillo Negro	NevB.COM NevN.COM	Junín- Mendoza

References: local genotypes/cultivars from Cruz del Eje: G1 (EC), G2 (4S), G3 (Olly), G4 (EC), G5 (LP), G6 (LP), G7 (LP), G8 (FCA), G9 (4S), G10 (4S). Varieties: (WOGBC) Worldwide Olive Germplasm Banks Córdoba Spain; Olivos Collection, Junín, Mendoza, Argentina COM.

The “Manzanilla complex” consisted of genotypes G8 (4S) and G9 (4S) and the varietal references Manzanilla de Sevilla from the World Bank of Olive Germplasm, Córdoba, Spain (MaSe.WOGBC), Manzanilla Imperial (MaIm.COM), 2 Hermanas (Ma2H.COM), Española (MaEs.COM), Carmona (MaCa.COM) and Manzanilla Aceitera (MaAc.COM) from the Junín collection, Mendoza. The “Arbequina Complex” comprised the local genotypes G1 (EC) and G2 (4S), together with the Arbequina references from the Junín collection, Mendoza (Arb.COM) and from the World Bank of Olive Germplasm, Córdoba, Spain (Arb.WOGBC) (Table 1).

DNA extraction

Plant material for molecular characterization was collected in spring and early summer. Branches of 15-20 cm were kept at 4 ºC for one or two days, to be processed in the laboratory. DNA extraction of all materials was performed from young leaves using the protocol of Doyle and Doyle (1990). DNA from Arbequina (Arbe.WOGBC), Frantoio (Fr. WOGBC) and Manzanilla Sevilla (MaSe.WOGBC) were provided by WOGBC Cordoba, Spain.

The quality of DNA was evaluated by electrophoresis in 1 % agarose gels with TAE 1X buffer at 100V (cte.) for 30 min. The DNA was visualized under UV light, after staining in ethidium bromide solution (Br. Et.: 1mg/ml) and the concentration was determined by spectrophotometry (DeNovix Inc., Wilmington, USA).

Microsatellites and Polymerase Chain Reaction (PCR)

The genetic characterization of the materials under study was performed using ten consensus microsatellites from the DCA (03, 05, 09, 14, 18) (Sefc et al., 2000), GAPU (71B, 101, 103A) (Carriero et al., 2002), EMO90 (De la Rosa et al., 2004) and UDO43 (Cipriani et al., 2002) series. These microsatellites were selected for their repeatability, consistency, and effectiveness for cultivar discrimination (Baldoni et al., 2009).

To explore intravarietal diversity in materials closely phenotypically related to the reference varieties, the “Arauco complex” was analyzed with microsatellites of the DCA series (03, 05, 09, 14, 18). The “Manzanilla” and “Arbequina” complexes were also analyzed. The Manzanilla complex was studied with a group of thirteen highly polymorphic microsatellite loci from the DCA (03, 08, 09, 10, 16), IAS-Oli (22, 23, 26, 27), UDO (43) and GAPU (71B, 101, 103A) series (Baldoni et al., 2009 and Díaz et al., 2006); while the “Arbequina complex” was analyzed with fourteen microsatellites from the DCA series (05, 08, 09, 10, 11, 14, 16,) IAS-Oli (22, 23, 26, 27), GAPU (101, 103A) and EMO 90.

Table 2 details the sequence of the microsatellites used in this work, the hybridization temperature used and the size in bp (range) obtained in the cited bibliography. Amplifications by PCR, were performed in a final reaction volume of 20 µl according to the following concentrations of each component: 1 µl template DNA (10-20 ng/µl); Taq polymerase buffer, 1X; dNTP, 0.2 mM; primers, 1 µM of each; 1U of Taq polymerase enzyme (Promega).

The cycling conditions for the primers or SSR DCA 03-05-09-14-18 were those cited by Sefc et al. (2000). For the SSR EMO 90, the PCR temperatures were described by De la Rosa et al. (2003). For SSR GAPU 71B, 101 and 103, the cycling values were stated by Carriero et al. (2002) and for UDO 43 the conditions were those cited by Cipriani et al. (2002). For primers DCA 03, 05 and 09, slight modifications in the number of cycles were introduced in order to resolve the appearance of nonspecific bands.

All amplifications were performed on a PCR MasterCycler Thermal Cycler model 5333 (Eppendorf Hamburg, Germany). PCR reactions were repeated at least twice from different DNA extractions.

The amplified products were separated by vertical electrophoresis on discontinuous polyacrylamide gels (SDS-PAGE) with a final concentration of 15 % for the resolving gel and 5 % for the stacking gel, in 1X Tris glycine buffer at 100 V (constant voltage) for 16 h. The amplified DNA was visualized under UV light, after staining with ethidium bromide (1mg/ml), and the results were documented using an image capture system (DigiDoc-It [UVP] Analytik Jena US LLC, Upland, California).

The processing of the images and the determination of the size of each band, in base pairs (bp), was performed with the free software PB2 (Verga, 2007). The assignment of the value of each band was performed considering previous work by Doveri et al. (2008), Baldoni et al. (2009) and data published at http://www.oleadb.it.

References: SSR repeat motifs, primer sequences (5’-3’), and sizes of cloned alleles according to Bibliography. Ta: Hybridization temperatures used in this work.

Table 2. Microsatellites used for the study of olive genotypes in the northwest of the province of Córdoba (Argentina)

	Repeated motif	Primer sequences (5' to 3') Forward	Primer sequences (5' to 3') Reverse	Ta	Allele size (range in bp)	Bibliographic reference
DCA03	(AT)0-1(GA)11-19	CCCAAGCGGAGGTGTATATTGTTAC	TGCTTTTGTCGTGTTTGAGATGTTG	50 °C	250 (228-250)	Sefc et al. (2000); Baldoni et al. (2009)
DCA05	A3-5 (GA)5-11	AACAAATCCCATACGAACTGCC	CGTGTTGCTGTGAAGAAAATCG	50 °C	211 (195-211)	
DCA08	(GA)18	ACAATTCAACCTCACCCCCATACCC	TCACGTCAACTGTGCCACTGAACTG	55 °C	135 (123-139)	Sefc et al. (2000)
DCA09	(GA)7-29	AATCAAAGTCTTCCTTCTCATTTCG	GATCCTTCCAAAAGTATAACCTCTC	55 °C	191 (161-205)	Sefc et al. (2000); Baldoni et al. (2009)
DCA10	TA)14(GA)17	CGTGACCACCTAAATCCGCCCC	CTGTCCAGAGCTAAAGGTTTCG	55 °C	158 (138-258)	Sefc et al. (2000)
DCA11	(GA)26(GGGA)4	GATCAAACTACTGCACGAGAGAG	TTGTCTCAGTGAACCCTTAAACC	55 °C	179 (125-161)	
DCA 14	(AC)9-18(A)2-9 (TAA)5	AATTTTTTAATGCACTATAATTTAC	TTGAGGTCTCTATATCTCCCAGGGG	50 °C	187 (170-187)	Sefc et al. (2000); Baldoni et al. (2009)
DCA 16	(GT)6–16 (GA)9–15	TTAGGTGGGATTCTGTAGATGGTTG	TTTTAGGTGAGTTCATAGAATTAGC	50 °C	178 (120-178)	Sefc et al. (2000)
DCA18	(CT)10-20(TG)1-4 (AG) (TG)4-6	AAGAAAGAAAAAGGCAGAATTAAGC	GTTTTCGTCTCTCTACATAAGTGAC	50 °C	178 (168-184)	Sefc et al. (2000); Baldoni et al. (2009)
EMO90	(AC)10-15	CATCCGGATTTCTTGCTTTT	AGCGAATGTAGCTTTGCATGT	55 °C	184 (180-197)	De la Rosa et al. (2002); Baldoni et al. (2009)
GAPU71B	(AG)7-8 (AAG)5-12GA(AG)6(AAG)8	GATCAAAGGAAGAAGGGGATAAA	ACAACAAATCCGTACGCTTG	60 °C	285	Carriero et al. (2002); Baldoni et al. (2009)
GAPU101	(AG)3-21	CATGAAAGGAGGGGGACATA	GGCACTTGTTTGCAGATTG	60 °C	264	
GAPU103	(TCTTTCATGGTGGATCAG ACG)0-1 (TC)8-32	TGAATTTAACTTTAAACCCACACA	GCATCGCTCGATTTTTATCC	60 °C	245	
IAS-oli22	(GA)6(GT)5GACCT(GA)	CAAGAAGAGAGAGAAAATGAGAGCC CCCAT	TCCGACTTATGAGCTGTTTTTTCTTTGGA A	68 °C	134	Rallo et al. (2000)
IAS-oli23	(AG)18	CCCCCCATGTTTTTAATTCAATGAAAA ATTAAGTGG	ACCACCACCGGTCTGGAAGGGATG	55 °C	213–238	Díaz et al. (2006)
IAS-oli26	(GA)14	TCGGAGAGTTGCTACAGAACA	CCCTTCCAAGTCGAGCAATAAA	55 °C	168-210	
IAS-oli27	(T)5GA(T)10CA(T)3	GGTGGTATTGCCCTCGACA	CCGCCATCTATTCAACCATC	55 °C	115-140	
UDO 43	(GT)12	TCGGCTTTACAACCCATTTC	TGCCAATTATGGGGCTAACT	57 °C	174	Cipriani (2002)

Analysis of genetic variability

Data were statistically analyzed using the InfoGen program (Balzarini and Di Rienzo, 2018). The molecular information obtained was evaluated with the following summary measures: number of alleles per locus, allele frequencies, genotypic frequencies, observed heterozygosity (Ho) or by direct counting. Nei's unbiased or expected heterozygosity (He) and polymorphic information content (PIC) were also calculated.

Analysis of local genotypes

To perform an exploratory study of the genetic structure present in the group of genotypes under examination from the data obtained with the ten microsatellites, a Roger's distance matrix was generated and multivariate statistical techniques, such as principal coordinate analysis (PCoordA), principal component analysis (PCA) and cluster analysis, were applied.

Intravarietal diversity of genotypes

In order to know the intravarietal diversity in the Arauco, Manzanilla and Arbequina complexes, the local genotypes/cultivars were compared with the respective reference materials to establish distance relationships, principal coordinate analysis and principal component analysis.

RESULTS AND DISCUSSION

Characterization of local genotypes by microsatellite markers

The province of Córdoba has traditional olive groves planted in the middle of the last century and characterized by their heterogeneous composition and uncertain varietal origin given that there are also genotypes derived from seed rootstocks, which had graft loss. According to Gómez del Campo et al. (2010), the uncertainty of identity originates in part from the anthropic action of multiplication and selection of plants in search of olive genotypes of greater agronomic and commercial value, but of doubtful varietal identity. Local producers assigned names to the material present in the commercial orchards based on fruit quality, regularity in production and better response to stress caused by adverse biotic and abiotic factors, compared to other plants of known varietal identity in the same plot. This situation generated uncertainty about the correct denomination of the local accessions. Because of this, the study of the genetic variability of the olive tree (*Olea europaea* L.) is important both for the design of breeding programs and for conservation and to avoid duplications that lead to increased maintenance time and costs. Therefore, to distinguish the accessions of a collection from each other and for the varietal identification of olive trees, the IOC determined that this should be done by morphological and molecular markers (FAO, 2014; Baccino et al., 2013). In the present work, the molecular characterization and subsequent identification of olive (*Olea europaea* L.) genotypes from the NW region of the province of Córdoba (Argentina) was carried out and the intravarietal diversity of genotypes associated with the reference cultivars Arauco, Manzanilla and Arbequina was determined.

From the DNA corresponding to the individuals analyzed, the selected primers amplified SSR's with bands of equal size or within the expected range as proposed by the bibliography and data published in the Olea database. Table 3 shows the allelic profiles obtained for each local genotype and for the reference varieties using ten consensus microsatellites. For all of them, amplifications were obtained in the 20 DNA samples analyzed. In the reference varieties Arb.COM, Fa.COM, Fr.WOGBC, Fr.COM, MaSe.WOGBC and in the local genotypes related to them, size bands in bp similar to those published in Olea database were obtained for the microsatellites Gapu71B, EMO90, Gapu101 and Gapu103A loci. The UDO43 loci amplified band sizes (alleles) in a range between 160 and 214 bp, similar to those obtained by Torkzaban et al. (2015). In the remaining five loci (DCA 03, 05, 09, 14 and 18), band sizes similar to those were published by Bandelj et al. (2002), Mantia, et al. (2005), Montemurro et al. (2005), Sarri et al. (2006), Muzzalupo et al. (2008) and Rekik et al. (2008).

Local genotypes/cultivars: from Cruz del Eje: Genotypes EC, 4S, Olly, LP; from Córdoba: Genotype Oblonga, FCA, UNC. Reference varieties from Worldwide Olive Germplasm Banks Córdoba, Spain (WOGBC): Frantoio (Fr.WOGBC), Manzanilla de Sevilla (MaSe.WOGBC), from Olivos Collection, Junín, Mendoza, Argentina (COM): Arbequina (Arbe.COM), Arauco Centenario (AraCen), Farga (Fa.COM), Frantoio (Fra.COM), Manzanilla Aceitera (MaAc.COM), Nevadillo Blanco, (NeB.COM), Nevadillo Negro (NeN.COM). In this analysis, reference DNA from Arbequina referring to the (WOGBC) was not included.

Summary measures of genetic variability: He (expected heterozygosity), Ho (observed heterozygosity) and PIC (polymorphic information content) of local genotypes of Cruz del Eje olive

Table 3. Allelic profiles, expressed in base pairs, of the olive genotypes from Cruz del Eje and the reference varieties analyzed with 10 SSR

Genotypes/cultivars and reference varieties	DCA03	DCA05	DCA09	DCA14	DCA18	GAPU71B	EMO90	GAPU101	GAPU103	UDO43
Arbe.COM	241-253	217-217	177-198	196-231	162-171	123-141	184-189	183-204	147-155	178-214
G1 (EC)	241-253	217-217	177-198	196-231	162-171	123-141	184-189	183-204	147-155	178-214
G2 (4S)	241-253	217-217	177-198	196-231	162-171	123-141	184-189	183-204	147-155	178-214
AraCen	235-261	250-255	177-187	196-231	167-177	123-141	181-181	196-206	133-149	178-214
G3 (Olly)	235-261	250-255	177-187	196-231	167-177	123-141	181-181	196-206	133-149	178-214
G4 (EC)	247-264	255-255	187-200	196-231	159-169	123-141	181-181	200-206	143-155	178-214
G5 (LP)	259-264	220-220	177-200	163-185	167-171	129-129	184-189	200-216	172-183	178-214
Fa.COM	253-259	220-255	181-200	163-185	167-174	125-125	189-189	198-198	149-172	182-206
G6 (LP)	253-259	220-255	181-200	163-185	167-174	125-125	189-189	198-198	149-172	182-206
Fr.WOGBC	241-259	217-217	174-196	188-216	169-187	123-141	184-189	186-200	159-170	172-206
Fra.COM	241-259	217-217	177-198	190-218	169-187	123-141	184-189	186-200	159-170	172-203
G7 (4S)	241-259	217-217	177-198	190-218	169-187	123-141	184-189	186-200	159-170	172-203
Oblonga-FCA	241-259	217-217	177-198	190-218	169-187	123-141	184-189	186-200	159-170	172-203
MaSe.WOGBC	253-264	250-255	158-198	193-216	169-177	123-141	186-186	200-216	135-149	160-197
MaAc.COM	253-264	225-225	187-198	157-157	169-177	125-141	184-184	196-216	159-177	170-170
G8 (4S)	253-264	225-225	187-198	163-190	169-177	125-141	184-184	196-216	159-177	172-203
G9 (4S)	247-264	253-253	187-198	185-221	159-169	123-141	186-186	200-206	139-177	172-197
NeB.COM	247-253	248-255	158-198	185-185	169-177	123-141	186-186	200-216	135-149	157-197
NeN.COM	247-264	248-248	158-177	190-221	169-177	121-127	186-186	200-216	135-149	170-194
G10 (4S)	257-299	217-217	158-198	188-211	169-169	123-141	184-184	200-206	170-181	157-197

Local genotypes/cultivars: from Cruz del Eje: Genotypes EC, 4S, Olly, LP; from Córdoba: Genotype Oblonga, FCA, UNC. Reference varieties from Worldwide Olive Germplasm Banks Córdoba, Spain (WOGBC): Frantoio (Fr.WOGBC), Manzanilla de Sevilla (MaSe.WOGBC), from Olivos Collection, Junín, Mendoza, Argentina (COM): Arbequina (Arbe.COM), Arauco Centenario (AraCen), Farga (Fa.COM), Frantoio (Fra.COM), Manzanilla Aceitera (MaAc.COM), Nevadillo Blanco, (NeB.COM), Nevadillo Negro (NeN.COM). In this analysis, reference DNA from Arbequina referring to the (WOGBC) was not included.

trees and COM reference plants are presented in Tables 4 and 5.

At loci DCA05 and EMO90 of Cruz del Eje genotypes, Ho was lower than He. The same trend was observed for cultivars from the olive collection (COM) for primers DCA05, DCA14, EMO90 and UDO43. Regarding the differences found between Ho and He in the loci corresponding to DCA05 and EMO90 in local EC genotypes, and in the loci DCA05, EMO90, DCA14 and UDO43 of the .COM varieties, these could be attributed to the presence of null alleles, i.e., the allele is present but does not amplify probably due to the occurrence of a mutation at the site of hybridization with the primer and the individual is classified as homozygous (Bello Pigem, 2001).

A lower value of Ho with respect to that expected for microsatellites DCA05 and EMO90 was also observed by El Bakkali et al. (2019), while Gómes et al. (2009) cite a similar value of Ho for DCA05, in studies of genetic diversity of olive cultivars in Portugal. Considering the PIC values in both Cruz del Eje genotypes and .COM varieties, the average values were 0.78 y 0.76 respectively. Considering the PIC values in both Cruz del Eje genotypes and .COM varieties, the average values were 0.78 y 0.76, respectively. The observed polymorphism presented a total of 74 alleles in the Cruz del Eje olive population ranging from four loci (EMO90 and GAPU71B) to twelve loci (GAPU103A). In the set of varieties from the olive collection of Junín, Mendoza, 64 alleles were detected. In all the materials analyzed, the most polymorphic loci were GAPU103A, which revealed twelve alleles in local genotypes and UDO43 with ten alleles in the reference materials, while EMO90 was the least polymorphic with four alleles per loci in the Cruz del Eje olive population and three in the varieties from the olive collection of Junín, Mendoza.

The results obtained with the most polymorphic microsatellites, GAPU103A and UDO43, showed similar results to those previously observed by

Table 4. Summary measures of genetic variability of local genotypes/cultivars of olive trees from Cruz del Eje, Province of Córdoba

SSR	Repeated motif	Rank obtained (pb)	N.° of alleles	He	Ho	PIC
DCA03	(AT)0-1(GA)11-19	235-299	9	0.91	1.00	0.84
DCA05	A3-5 (GA)5-11	217-258	6	0.79	0.20	0.72
DCA09	(GA)7-29	158-200	6	0.82	1.00	0.75
DCA14	(AC)9-18(A)2-9 (TAA)5	163-231	9	0.90	1.00	0.84
DCA18	(CT)10-20(TG)1-4 (AG) (TG)4-6	159-187	8	0.87	0.92	0.81
EMO90	(AC)10-15	181-189	4	0.74	0.42	0.65
GAPU71B	(AG)7-8 (AAG)5-12	123-141	4	0.72	0.82	0.63
GAPU101	(AG)3-21	183-216	8	0.88	0.90	0.83
GAPU103	(TCTTTCATGGTGGATCAGACG) 0-1 (TC) 8-32	133-183	12	0.95	1.00	0.89
UDO43	(GT)12-18	148-214	8	0.88	1.00	0.80
Promedio			8	0.84	0.83	0.78

He (expected heterozygosity), Ho (observed heterozygosity) and PIC (polymorphic information content).

Table 5. Genetic variability of reference cultivars from the Junín, Mendoza olive collection (.COM)

SSR	Repeated motif	Rank obtained (pb)	N.° of alleles	He	Ho	PIC
DCA03	(AT)0-1(GA)11-19	240-299	5	0.84	1.00	0.76
DCA05	A3-5 (GA)5-11	217-258	5	0.82	0.29	0.73
DCA09	(GA)7-29	158-200	6	0.85	1.00	0.75
DCA14	(AC)9-18(A)29(TAA)5	157-231	8	0.92	0.67	0.83
DCA18	(CT)10-20(TG)1-4(AG) (TG)4-6	159-187	8	0.84	1.00	0.77
EMO90	(AC)10-15	181-189	4	0.79	0.29	0.69
GAPU71B	(AG)7-8 (AAG)5-12	121-141	6	0.83	0.75	0.75
GAPU101	(AG)3-21	183-216	8	0.86	0.88	0.78
GAPU103	(TCTTTCATGGTGGATCA GACG)0-1 (TC)8-32	135-183	10	0.94	1.00	0.87
UDO43	(GT)12-18	157-214	10	0.94	0.86	0.87
Average			7	0.86	0.77	0.78

He (expected heterozygosity), Ho (observed heterozygosity) and PIC (polymorphic information content).

Costero et al. (2021).

For these markers Abdelhamid et al. (2013) obtained similar results (0.56 for EMO90 and 0.86 for GAPU103A) in studies of genetic similarity and identification of Tunisian cultivars. The present study also counted the unique alleles belonging to each genotype and to the reference varieties and it was observed that allele 127 of marker GAPU71B was unique for Nevadillo Negro from Junín, Mendoza and was discriminant to differentiate this genotype from the rest of the genotypes evaluated in this work. Therefore, this marker could be used as a specific marker for the identification of this particular material. According to Fendri (2008) unique alleles acquire importance when determining the origin or traceability of a given material.

In the principal coordinates analysis, the first three coordinates (PCO1, PCO2, and PCO3) explained 65 % of the total variability studied and the first two (PCO1 and PCO2) explained 46 % of the variability Figure 2.

The PCO1 with a value of 25.5 %, allowed differentiating two groupings, one was formed by the varieties Arbequina and Frantoio and the genotypes/local cultivars G1 (EC), G2 (4S) and G7 (4S) related to them together with the cultivar Oblonga. The other grouping consisted of Arauco, Farga, Manzanillas and Nevadillos, and the genotypes G3 (Olly), G4 (EC) and G5 (LP), known by the local names of Arauco Ollantai, Ascolano El Cóndor, and Empeltre Las Playas, respectively by their characteristics and origin (Figure 2). In the analysis of the second principal coordinate, PCO2 (with a value of 21.3 %), two groups of materials could be clearly differentiated. One group consisted of Farga, genotype G6 (LP), genotype G5 (LP), Arauco Centenario, genotype G3 (Olly), genotype G4 (EC), Arbequina, and genotypes G1 (EC) and

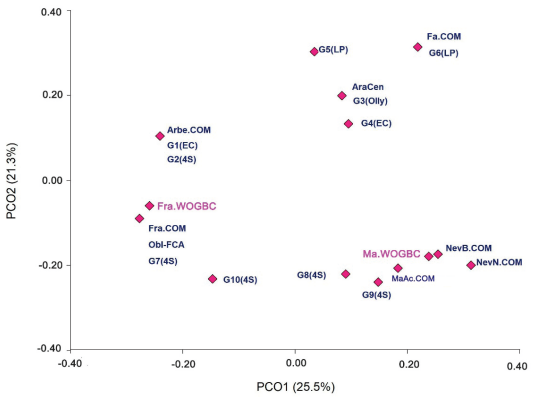

Figure 2. Scatter plot of the analysis of principal coordinates (PCO1 and PCO2), based on the Rogers distance matrix for the set of local genotypes and reference varieties analyzed with ten microsatellites. PCO1 vs. PCO2

References: Local genotypes from Cruz del Eje: Genotypes EC, 4S, Olly, LP; from Córdoba: Oblonga- FCA (Cba). Reference varieties from Worldwide Olive Germplasm Banks Córdoba, Spain (WOGBC): Frantoio (Fr.WOGBC), Manzanilla de Sevilla (MSe. WOGBC). From Olivos Collection, Junín, Mendoza, Argentina (COM): Arbequina (Arbe.COM), Farga (Fa.COM), Frantoio (Fra. COM), Manzanilla Aceitera (MaAc.COM), Nevadillo Blanco (NeB. COM), Nevadillo Negro (NeN.COM). From La Rioja, Arauco Centenario (AraCen)

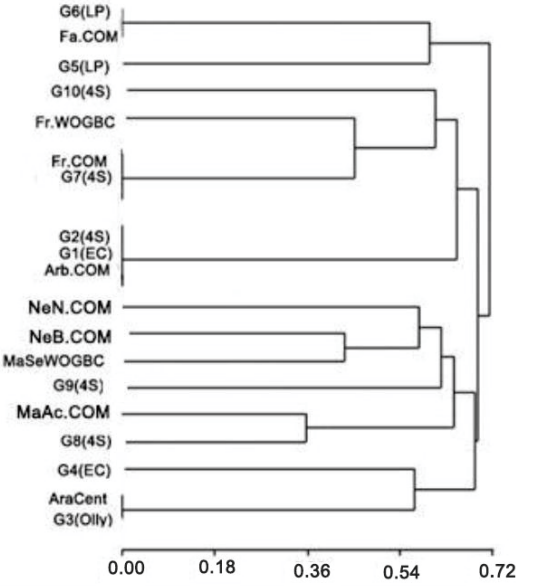

Figure 3. Dendrogram generated by UPGMA clustering from the Roger distance matrix of ten SSR markers, in local genotypes from Cruz del Eje and olive varieties from the olive collections of Junín, Mendoza and the Instituto AgroSostenible, Córdoba, Spain

References: Local genotypes from Cruz del Eje: Genotypes EC, 4S, Olly, LP; from Córdoba: Oblonga- FCA (Cba). Reference varieties from Worldwide Olive Germplasm Banks Córdoba, Spain (WOGBC): Frantoio (Fr.WOGBC), Manzanilla de Sevilla (MSe. WOGBC). From Olivos Collection, Junín, Mendoza, Argentina (COM): Arbequina (Arbe.COM), Farga (Fa.COM), Frantoio (Fra. COM), Manzanilla Aceitera (MaAc.COM), Nevadillo Blanco (NeB.COM), Nevadillo Negro (NeN.COM). From La Rioja Arauco Centenario (AraCen).
Arbequina reference DNA from the WOGBC was not included in this analysis.

G2 (4S); and the other consisted of the varieties called Frantoio, Manzanilla, Nevadillos and the associated genotypes (Figure 2). The cultivars of local genotypes that presented zero distance with the reference varieties are located at the same point of the scatter plot. The UPGMA hierarchical clustering technique generated a dendrogram (Figure 3) and the obtained cophenetic correlation coefficient value of 0.96 suggested an adequate representation of the distance matrix in the dendrogram.

The results obtained confirmed the genetic relationship between the individuals, in agreement with the principal coordinates analysis. The multivariate analysis confirmed the identity of six genotypes/cultivars from the Cruz del Eje locality, which presented the same allelic profiles with respect to the plants used as varietal references. Thus, the materials called locally by the producers Arbequina 21 Kg [G1 (EC)] and Arbequina 4 Soles [G2 (4S)] were assigned to the Arbequina variety, the genotype called Arauco [G3 (olly)] to Var. Arauco and the genotype G6 (LP) named Farga Las Playas to Var. Farga. The genotypes G7 (4S), locally called Frantoio 4 Soles, and the cultivar Oblonga. UNC from Córdoba showed identical amplification patterns to the reference variety Frantoio, thus reconfirming the genetic similarity of Oblonga (Cba) previously reported by Barranco et al. (2000) who used 22 primers to perform an analysis with RAPDS molecular markers.us characteristics and origin.

Intravarietal diversity

Table 6 summarizes the allelic profiles corresponding to the local genotypes G3 (Olly) and G11 (EC) analyzed with the highly polymorphic loci of the DCA series, together with its ancestral referent AraCen and other materials associated with the Arauco Complex (abreviated in this work as AraJM.COM, Csa.COM and CSM.COM). Genotype G3 (Olly) showed 0 (zero) genetic distance in relation to the native Arauco variety, to which the Centenario olive tree belongs (Matías et al., 2010). Similarly, Criolla San Martín and AraucoJM, both from the COM collection, were identical to each other (Table 7).

Table 6. Allelic profiles of genotypes of the "Arauco complex" analyzed at the level of five polymorphic microsatellite loci

Genotipo	DCA03	DCA05	DCA09	DCA14	DCA18
CSM.COM	252-283	241-258	177-185	196-231	162-172
CSa.COM	254-285	241-258	177-185	196-231	162-172
AraJM.COM	252-283	241-258	177-185	196-231	162-172
AraCen	235-261	250-255	177-187	196-231	167-177
G3(Olly)	235-261	250-255	177-187	196-231	167-177
G11(EC)	254-285	241-258	177-185	196-231	162-172

Genotypes from Cruz del Eje: G3 (Olly); G11 (EC). Reference varieties: from Olivos Collection, Junín, Mendoza, Argentina (COM): Criolla San Martín (CSM.COM); Criolla Salvarredi (CSa.COM); Arauco Junín, Mendoza (AraJM.COM). From La Rioja, Arauco Centenary (AraCent).

Table 7. Roger's genetic distance of the "Arauco complex" analyzed with five SSR markers. (Lower triangular of the genetic distance matrix calculated with Roger's similarity index)

Genotipo	CSM. COM	CSa. COM	AraJM. COM	AraCen	G3 (Olly)	Genotipo11 (EC)
CSM.COM	0.00					
CSa.COM	0.14	0.00				
AraJM.COM	0.00	0.14	0.00			
AraCen	0.52	0.52	0.52	0.00		
G3(Olly)	0.52	0.52	0.52	0.00	0.00	
G11(EC)	0.14	0.00	0.14	0.52	0.52	0.00

Genotypes from Cruz del Eje: G3 (Olly); G11 (EC). Reference varieties: from Olivos Collection, Junín, Mendoza, Argentina (COM): Criolla San Martín (CSM.COM); Criolla Salvarredi (CSa. COM); Arauco Junín, Mendoza (AraJM.COM). From La Rioja, Arauco Centenary (AraCent).

The main purpose of the Arauco Centenario variety is for both oil and canning. Therefore, G3 (Olly) is a material of high genetic value because it is identical to Arauco Centenario, considered as a varietal reference. While genotype "G11 (EC)" was identical to Criolla Salvarredi and very close to Criolla San Martín, showing genetic distance values of 0 and 0.14, respectively. The greatest genetic distance (0.52) was observed between Arauco Centenario and the members of this group belonging to the COM. This study also confirmed, at the molecular level, the genetic similarity of Arauco.COM, Criolla Salvarredi and Criolla San Martín. However, they presented a distance value of 0.52 with the Arauco Centenario plant, considered a varietal reference. Based on the analysis with morphological markers of accessions from the olive collection of the EEA of INTA Junín, Mendoza, in 2011 Trentacoste and Puertas reported little morphological variation between the Arauco cultivar and the two clones Criolla San Martín and Criolla Salvarredi selected from this collection. These results could be explained by the fact that, in traditional plantations in the Cordilleran valleys, plant material often came from seed or vegetative propagation of certain individuals mainly from the "Arauco" table variety (Gómez del Campo et al., 2010). Possible discrepancies or confusion in the denomination of local plants with respect to the reference varieties that were supposed to have given rise to the cuttings could be attributed to factors such as the handling of olive trees from the national or international collections and nurseries from which they were obtained, as well as unintentional errors in identification (Rehman et al., 2012).

Currently the area planted with Arauco has decreased in relation to other cultivars that make up the "Manzanilla" varietal complex, valued mainly for the production of table olives (Gómez del Campo et al., 2010). This complex, under whose denomination several types that could be different varieties are included (Barranco et al., 2000), represents a case of interest with respect to varietal denomination, given that at least sixteen homonyms and twelve synonymies are cited for this cultivar (Barranco et al., 2005; Gómez-Escalonilla et al., 2006). Table 8 presents the alleles corresponding to the analysis, with thirteen polymorphic microsatellites of the DCA, GAPU, IAS-Oli and UDO series, of the group formed by the genotypes called Manzanilla 4 Soles (G84S) and Manzanilla Gigante 4 Soles (G94S) together

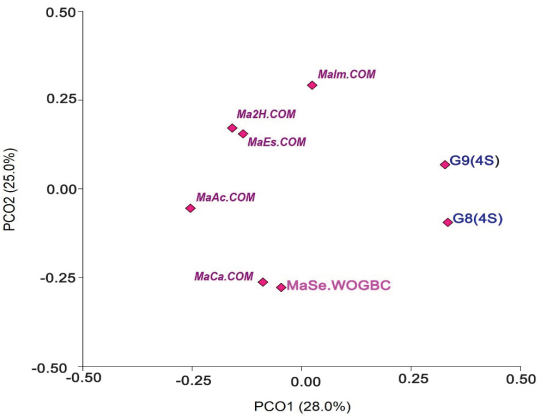

Figure 4. Scatter plot of the analysis of principal coordinates (PCO1 and PCO2), based on the Rogers distance matrix for the set of local genotypes related to the reference varieties of the Manzanilla complex analyzed with thirteen microsatellites

References: local genotypes: G8 (4S), G9 (4S). Reference varieties: Manzanilla de Sevilla from Spain (MaSe.WOGBC); Manzanilla Imperial Junín, Mendoza (MaIm.COM). Genotype: Manzanilla 2 Hermanas (Ma2H.COM), Manzanilla Española (MaEs.COM), Manzanilla Carmona (MaCa.COM), Manzanilla Aceitera (MaAc. COM).

Table 8. Allelic composition of thirteen microsatellite loci that make up the "Manzanilla complex"

α) **DCA Series SSR**

Varieties/genotypes	DCA 03	DCA 08	DCA 09	DCA 10	DCA 16
MaSe.WOGBC	270-287	139-150	187-198	124-218	176-176
G8 (4S)	270-287	137-137	187-198	124-218	192-275
G9 (4S)	270-287	137-147	180-192	178-230	239-173
MaIm.COM	270-272	139-147	182-172	178-230	135-204
Ma2H.COM	257-284	139-150	149-189	124-178	135-204
MaEs.COM	270-270	137-150	149-189	124-178	135-204
MaCa.COM	270-287	139-139	180-192	124-218	187-187
MaAc.COM	270-270	139-139	180-192	124-178	208-208

β) **IAS-Oli, Udo and Gapu Series SSRs**

Varieties/ genotypes	Gapu 71	Gapu101	Gapu 103	IAS-Oli 22	IAS-li23	IAS-Oli 26	IAS-Oli 27	Udo 43
MaSe. WOGBC	119-134	192-212	136-146	132-146	226-242	185-190	118-118	146-190
G8 (4S)	125-141	196-216	159-177	132-144	221-264	193-212	120-140	162-200
G9 (4S)	123-141	200-206	139-177	138-148	221-264	183-202	110-136	146-190
MaIm.COM	130-142	192-219	146-146	138-148	224-240	183-202	120-140	146-196
Ma2H.COM	116-119	186-212	136-146	134-146	224-242	183-205	120-140	162-212
MaEs.COM	118-136	186-212	136-146	134-146	224-242	183-205	120-140	190-190
MaCa.COM	119-134	189-212	136-146	136-148	227-236	185-190	120-120	146-190
MaAc.COM	121-134	186-212	152-167	136-146	227-236	180-191	124-144	146-190

References for local genotypes: G8 (4S), G9 (4S). Reference varieties: Manzanilla de Sevilla from Spain (MaSe.WOGBC); Manzanilla Imperial Junín, Mendoza (MaIm.COM) Genotype: Manzanilla 2 Hermanas (Ma2H.COM), Manzanilla Española (MaEs.COM), Manzanilla Carmona (MaCa.COM), Manzanilla Aceitera (MaAc.COM).

Table 9. Roger's genetic distances between local genotypes and reference varieties of the "Manzanilla complex". Distance matrix

Varieties/ genotypes	MaSe. COM	G8 (4S)	G9 (4S)	MaIm. COM	Ma2H. COM	MaEs. COM	MaCa. COM	MaAc. COM
MaSe.COM	0							
G8 (4S)	0.62	0						
G9 (4S)	0.62	0.5	0					
MaIm.COM	0.65	0.66	0.51	0				
Ma2H.COM	0.54	0.63	0.68	0.52	0			
MaEs.COM	0.57	0.62	0.63	0.53	0.28	0		
MaCa.COM	0.39	0.62	0.62	0.62	0.59	0.54	0	
MaAc.COM	0.59	0.71	0.65	0.61	0.59	0.54	0.5	0

References for local genotypes: G8 (4S), G9 (4S). Reference varieties: Manzanilla de Sevilla from Spain (MaSe.WOGBC); Manzanilla Imperial Junín, Mendoza (MaIm.COM) Genotype: Manzanilla 2 Hermanas (Ma2H.COM), Manzanilla Española (MaEs.COM), Manzanilla Carmona (MaCa.COM), Manzanilla Aceitera (MaAc.COM).

with the referent varieties called Manzanillas (de Sevilla, Aceitera, Imperial, 2 Hermanas, Española and Carmona). Table 9 shows the genetic distance matrix obtained. With the molecular information from this complex, an ACoordP and an ACP were performed.

PCO1 clearly differentiated two groups: one composed of the genotypes G8 (4S), G9 (4S) and another composed of the reference varieties MaSe. WOGBC, MaCa.COM, MaAc.COM, Ma2H.COM, MaEs.COM and MaIm.COM. On the other hand, PCO2 also discriminated two groups, one formed by the reference varieties Ma2H.COM, MaEs.COM and MaIm.COM and the other by genotype G9 (4S), related to the variety commonly called Manzanilla Gigante. Finally, the other differentiated group was made up of the rest of the varieties used as references in this complex. In this study, it should be noted that the analysis at the level of thirteen microsatellite loci showed the high genetic diversity in the complex formed by the selected local

genotypes and the Manzanilla varieties with which they are related by phenotypic and agronomic characteristics. Both genotypes presented a genetic distance value of 0.5 between them, while, for the same genotypes, Costero et al. (2021) with a lower number of SSR markers observed a higher distance value.

In the principal components analysis, the axes corresponding to PC1 and PC2 explained 41.7 % of the total genetic variability and the relative importance of the classification variables (Figure 5).

The autovectors that presented the highest absolute value were those corresponding to alleles 198 and 212 of the markers DCA09 and GAPU101, respectively. These components allowed the definition of two groups represented in 2D, a group formed mainly by referent varieties (MaSe.WOGBC, MaCa.COM, MaAc.COM, Ma2H.COM, MaEs. COM and MaIm.COM) and another composed of Genotypes 8 and 9. PC2 with the lowest value (18.9 %) showed that alleles 183 of Oli26 and 218 of the DCA10 marker were relevant to relate the materials under study. Costero et al. (2021) observed in the ACP that 38.3 % of the total genetic variability was represented by PC1 (23 %) and PC2 (15.6 %). In the present work it was observed that, regarding the relationship of the local germplasm with the reference varieties, there was a greater presence of the genome of the Imperial cultivar, although the two local genotypes analyzed showed a genetic distance between them smaller than that previously obtained by Costero et al. in 2021, who analyzed six SSR loci. Local genotypes have their own germplasm conformation that could be partly explained by the phenotypic difference in fruit size, which gave rise to the local name of G9 (4S) as "Manzanilla Gigante". The analysis of the variability of the Arbequina complex carried out by means of 14 SSR's of the DCA series (14, 16, 10, 05, 08, 09, 11), IAS-Oli (22, 23, 26, 27), GAPU (101, 103A) and EMO 90, showed homogeneity in the allelic composition of the group (Table 10 and Table 11), observing a genetic distance of 0.2 for G1 (EC) (Arbequina 21 Kg) with respect to the Spanish reference variety (Arb.WOGBC) and a value of 0.09 with respect to the Arb.COM variety (Tables 10 and 11) differentiating it from the rest of the materials. A distance value of 0.15 was obtained for genotype G2 (4S).

The genetic distance values were low (0.10) and the identity of G1 (EC) with the reference variety Arb.WOGBC was confirmed. The genotype G1 (EC), also locally referred to as "21 Kg", was distinguished from the rest. A distance value of 0.15 was obtained for G2 (4S) with respect to the Spanish reference variety (Arb.WOGBC) and a value of 0.09 with respect to the Arb.COM variety.

In the present work, the varieties used as references not only allow verification of identity, but also provide information on the relationship between them and related genotypes. Faced with the wide varietal panorama, it is essential to make a correct choice of cultivar, which responds to the interest of the producer, and to guarantee the varietal identity of the starting plant material in order to establish the oleic acid content in oils such as Arbequina (Montealegre et al., 2010).

Table 10. Allelic profile of genotypes that make up the "Arbequina complex" analyzed with 14 SSRs that were polymorphic in the study of other olive genotypes SSR from series DCA

a) DCA Series SSR

Genotype	DCA05	DCA08	DCA09	DCA10	DCA11	DCA14	DCA16
Arbe.WOGBC	217-217	145-155	177-198	130-164	123-133	231-196	122-139
G2 (4S)	217-217	145-155	177-198	130-164	123-133	231-196	122-139
G1 (EC)	217-217	143-151	177-198	130-164	123-133	231-196	122-139
Arbe.COM	217-217	143-135	177-198	130-164	123-133	231-196	122-139

b) SR EMO, GAPU and IAS-Oli series

Genotype	EMO90	GAPU103	GAPU101	IAS-Oli22	IAS-Oli23	IAS-Oli26	IAS-Oli27
Arbe.WOGBC	184-189	147-155	183-204	135-175	226-240	183-205	124-128
G2 (4S)	184-189	147-155	183-204	135-175	226-240	183-205	124-128
G1 (EC)	184-189	147-155	183-204	138-177	220-263	183-205	124-128
Arbe.COM	184-189	147-155	183-204	138-177	226-240	183-205	124-128

References: Local genotypes: G2 (4S); G1 (EC). Reference varieties: Arbequina reference Spain (Arbe.WOGBC), Arbequina from Junín, Mendoza (Arbe.COM).

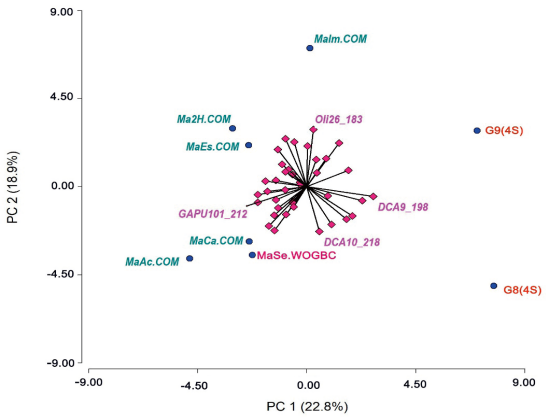

Figure 5. Scatter plot of the analysis of principal component (PC1 and PC2), the set of local genotypes related to the reference varieties of the Manzanilla complex analyzed with thirteen microsatellites

References: local genotypes: G8 (4S), G9 (4S); reference varieties: Manzanilla de Sevilla from Spain (MaSe.WOGBC); Manzanilla Imperial Junín, Mendoza (MaIm.COM). Genotype: Manzanilla 2 Hermanas (Ma2H.COM), Manzanilla Española (MaEs.COM), Manzanilla Carmona (MaCa.COM), Manzanilla Aceitera (MaAc.COM).

CONCLUSIONS

The results obtained in this study confirmed the importance of the use of molecular markers for the correct identification and characterization of local genotypes and olive collections. The allelic constitution of local olive genotypes selected from Cruz del Eje and reference varieties from the Olive Collection of the EEA-INTA Junín (Mendoza) and the Institute of Sustainable Agriculture (CSIC), Córdoba, Spain, was obtained and registered in a database of SSR. The multivariate analysis confirmed the identity of six genotypes that were found to be the same as the reference cultivars Arbequina, Arauco, Frantoio and Farga. It was clarified that the genotype called "Arauco Olly" from the orchard named Ollantay had the same genetic profile as "Arauco Centenario", and the identity of the genotype called "21 Kg" was determined. It was also possible to clarify the genetic diversity existing among the reference genotypes and cultivars associated with the Arauco and Arbequina denominations and to confirm the variability of the Manzanilla varietal complex.

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Table 11. Roger genetic distances between local genotypes and reference varieties of the Arbequina complex analyzed with fourteen microsatellites

Genotype	Arbe. WOGBC	G2 (4S)	G1 (EC)	Arbe. COM
Arbe.WOGBC	0			
G2 (4S)	0.15	0		
G1 (EC)	0.2	0.2	0	
Arbe.COM	0.1	0.09	0.1	0

References: Local genotypes: G2 (4S); G1 (EC). Reference varieties: Arbequina reference Spain (Arbe.WOGBC), Arbequina from Junín, Mendoza. (Arbe.COM).

REFERENCES

Abdelhamid, S., Grati-kamoun, N., Marra, F., and Caruso, T. (2013). Genetic similarity among Tunisian cultivated olive estimated through SSR markers. *Scientia Agricola, 70*(1), 33-38. https://doi.org/10.1590/S0103-90162013000100006

Abdelkrim, A., Bairrao, C., Barranco, D., Morello, P., and Rallo, L. (2021). La Red de Bancos de Germoplasma de Olivo del Consejo Oleícola Internacional. In *Fundación de Estudios Rurales ANNUARIO 2021* (114-148). Fundación de Estudios Rurales, UPA.

Baccino, E, Scaltritti, J., Silveira, A., López, S., Gándara, J., and Pereira, J. (29 de octubre, 2013). *Evaluación de los descriptores morfológicos y moleculares de variedades de Olivo en Uruguay* (9-21). In *Jornada de Divulgación Programa de Investigación en Producción Frutícola.* Serie Actividades de Difusión N.º 721.

Baldoni, L., Cultrera, N. G., Mariotti, R., Ricciolini, C., Arcioni, S., Vendramin, G. G., Buonamici, A., Porceddu, A., Sarri, V., Ojeda, M. A., Trujillo, I., Rallo, L., Belaj, A., Perri, E., Salimonti, A., Muzzalupo, I., Casagrande, A., Lain, O., Messina, R., and Testolin, R. (2009). A consensus list of microsatellite markers for olive genotyping. *Molecular Breeding, 24*(3), 213-231. https://doi.org/10.1007/s11032-009-9285-8

Balzarini, M. G. and Di Rienzo, J. A. (2018). InfoGen version (2018) [computer program]. Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina. http://www.info-gen.com.ar

Banco, A. P., Trentacoste, E. R., and Calderón, F. J. (Eds.)

(2020). *Colección de olivos en Mendoza: Investigación y caracterización de variedades.* Instituto Nacional de Tecnología Agropecuaria.

Bandelj, D., Jakše, J., and Javornik B. (2002). DNA fingerprinting of olive varieties by microsatellite markers. *Food Technology and Biotechnology, 40*(3), 185-190. https://www.ftb.com.hr/archives/678-dna-fingerprinting-of-olive-varieties-by-microsatellite-markers

Barranco, D., Trujillo, I. and Rallo, L. (2005). Elaiografía Hispánica. In: L., Rallo, D., Barranco, J. M., Caballero, C., Del Rio, A., Martin, J,. Tous, and I., Trujillo (Eds.), *Variedades de olivo en España* (45-231). Ediciones Mundi-Prensa.

Barranco, D., Trujillo, I., and Rallo, P. (2000). Are 'Oblonga' and 'Frantoio' Olives the Same Cultivar? *HortScience*, *35*(7), 1323-1325. https://doi.org/10.21273/HORTSCI.35.7.1323

Beghè, D., García Molano, J. F., Fabri, A., and Ganino, T. (2015). Olive biodiversity in Colombia. A molecular study of local germplasm. *Scientia Horticulturae, 189*, 122-131. https://doi.org/10.1016/j.scienta.2015.04.003

Bello Pigem, N. (2001). *Desarrollo de marcadores moleculares en el avestruz (Struthio camelus)* [Doctoral thesis in veterinary medicine] Universidad Autónoma de Barcelona. Bellaterra, Spain.

Carriero, F., Fontanazza, G., Cellini, F., and Giorio, G. (2002). Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). *Theorical and Applied Genetics, 104*(2), 301-307. https://doi.org/10.1007/s001220100691

Cavagnaro, P., Juarez, J., Bauza, M., and Masuelli, R. W. (2001). Use of morphological and molecular markers for discrimination among olive varieties. *Agriscientia, 18*, 27-35. https://revistas.unc.edu.ar/index.php/agris/article/view/2638/1604

Cipriani, G., Marrazzo, M. T., Marconi, R., Cimato, A., and Testolin, R. (2002). Microsatellite markers isolated in olive (*Olea europaea* L.) are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars. *Theorical and Applied Genetics, 104*(2), 223-228. https://doi.org/10.1007/s001220100685

Consejo Federal de Ciencia y Tecnología, Ministerio de Ciencia y Tecnología (COFECYT-MINCYT) (2016). *Olivicultura: Debilidades cuya superación implica un desafío científico tecnológico.*

Costero, B., Teich, I., Taborda, R. J., Toro, A., Prenol, L., and Torres, L. (2021). Intravarietal diversity of Manzanilla Olive cultivar (*Olea europaea* L.) and related selected genotypes from the INTA Catamarca collection and orchards of Cruz del Eje, Córdoba (Argentina). *Agriscientia*, *38*(2), 75-88. http://doi.org/10.31047/1668.298x.v38.n2.30288

De la Rosa, R., Angiolillo, A., Guerrero, C. A., Pellegrini, M., Rallo, L., Besnard, G., Bervillé, A., Martin, A., and Baldoni, L. (2003). A first linkage map of olive (*Olea europaea* L.) cultivars using RAPD, AFLP, RFLP and SSR markers. *Theorical and Applied Genetics, 106*(7), 1273-1282. https://doi.org/10.1007/s00122-002-1189-5

De la Rosa, R, James, C. M., and Tobutt, K. R. (2004). Using microsatellites for paternity testing in olive progenies. *HortScience, 39(2),* 351-354. https://doi.org/10.21273/HORTSCI.39.2.351

Díaz, A., De la Rosa, R., Martin, A. and Rallo, P. (2006). Development, characterization and inheritance of new microsatellites in olive (*Olea europaea* L.) and evaluation of their usefulness in cultivar identification and genetic relationship studies. *Tree Genetics and Genomes*, *2*(3), 165-175. https://doi.org/10.1007/s11295-006-0041-5

Doveri, S., Sabino Gil, F., Díaz, A., Reale, S., Busconi, M., da Cámara Machado, A., Martín, A., Fogher, C., Donini, P., and Lee, D. (2008). Standardization of a set of microsatellite markers for use in cultivar identification studies in olive (*Olea europaea* L.). *Scientia Horticulturae, 116*(4), 367-373. https://doi.org/10.1016/j.scienta.2008.02.005

Doyle, J. J. and Doyle, J L. (1990). Isolation of plant DNA from fresh tissue. *Focus, 12*(1), 13-15.

El Bakkali, A., Essalouh, L., Tollon, C., Rivallan, R., Mournet, P., Moukhli, A., Zaher, H., Mekkaoui, A., Hadidou, A., Sikaoui, S. and Khadari, B. (2019). Characterization of Worldwide Olive Germplasm Banks of Marrakech (Morocco) and Córdoba (Spain): Towards management and use of olive germplasm in breeding programs. *PLoS ONE*, *14*(10): e0223716. https://doi.org/10.1371%2Fjournal.pone.0223716

Fanelli, V., Mascio, I., Falek, W., Miazzi, M. M., and Montemurro, C. (2022). Current Status of Biodiversity Assessment and Conservation of Wild Olive (*Olea europaea* L. subsp. europaea var. sylvestris). *Plants, 11*(4), 480. https://doi.org/10.3390/plants11040480

Fendri, M. (2008). Uso de marcadores microsatélites (SSRs) para el análisis de la variabilidad molecular y la identificación de las variedades de olivo del banco de germoplasma de "Boughrara" (Sfax, Túnez). [Master's thesis]. University of Cordoba, Spain.

Fendri, M. (2011). *Varietal discrimination in olive (Olea europaea L.) pollen by morphological characteristics and microsatellite markers*. [PhD thesis]. Consejo Superior de Investigaciones Científicas and University of Granada, Granada, Spain.

Food and Agriculture Organization of the United Nations (FAO) (2014). *Standards for germplasm banks of plant genetic resources for food and agriculture*. Revised edition. FAO.

Gómes, S., Martins Lópes, P., Lópes J., and Ghedes-

Pinto, H. (2009). Assessing genetic diversity in *Olea Europaea* L. using ISSR and SSR markers. *Plant Molecular Biology Reporter, 27*(3), 365-373. https://doi.org/10.1007/s11105-009-0106-3

Gómez del Campo, M., Morales-Sillero, A., Vita Serman, F., Rousseaux, M. C. and Searles, P. S. (2010). El Olivar en los Valles áridos del Noroeste de Argentina (provincias de Catamarca, La Rioja y San Juan). *Olivae*, *114*, 23-45.

Gómez-Escalonilla, M., Sánchez-Heredero, M. and Vidal Hernández, J. (2006). Variedades del olivar, *Hojas Divulgadoras*, *2117 HD*. Ministry of Agriculture, Fisheries and Food, Government of Spain.

https://www.mapa.gob.es/ministerio/pags/biblioteca/hojas/hd_2006_2117.pdf

Green, P S. (2002). A revision of *Olea* L. (Oleaceae). *Kew Bulletin, 57*, 91-140. http://www.e-publicacoes.uerj.br/index.php/geouerj

International Olive Council (IOC) (2000). *World Catalogue of Olive Varieties*. International Olive Council.

International Olive Council (IOC) (2019). *Book of the IOC network of germplasm banks.* International Olive Council. https://www.internationaloliveoil.org/wp-content/uploads/2021/08/ANNEXE-1-A-BOOK-OF-THE-IOC-NETWORK-OF-GERMPLASM-BANKS-Completa.pdf

International Olive Council (IOC) (January 27th, 2023). *Argentina repository of the new international olive plant collection recognised by the IOC.* https://www.internationaloliveoil.org/argentina-repository-of-the-new-international-olive-plant-collection-recognised-by-the-ioc/

Mantia, L, Lain, T., Caruso T., and Testolin, R. (2005). SSR-based fingerprints reveal the genetic diversity of Sicilian olive (*Olea europaea* L.) germplasm. *The Journal of Horticultural Science and Biotechnology 80*(5), 628-632 https://doi.org/10.1080/14620316.2005.11511989

Las Casas, G., Scollo, F., Distefano, G., Continella, A., Gentile, A., and La Malfa, S. (2014). Molecular Characterization of Olive (*Olea europaea* L.) Sicilian Cultivars using SSRs markers. *Biochemical Systematics and Ecology, 57*, 15-19. https://doi.org/10.1016/j.bse.2014.07.010

Matías, A. C., Toro, A. A., Montalván, L. D., and Molina, M. S. (2010). Variedades de olivo cultivadas en las provincias de Catamarca y La Rioja, Argentina. Ediciones INTA.

Montealegre, C., Alegre, M., and García- Ruiz, C. (2010). Traceability markers to the botanical origin in olive oils. *Journal of Agricultural and Food Chemistry*, *58*(1), 28-38. https://doi.org/10.1021/jf902619z

Montemurro, C., Simeone, R., Pasqualone, A., Ferrara, E., and Blanco, A. (2005). Genetic relationships and cultivar identification among 112 olive accessions using AFLP and SSR markers. *The Journal of Horticultural Science and Biotechnology, 80*(1), 105-110. https://doi.org/10.1080/14620316.2005.11511899

Muzzalupo, I., Lombardo, N., Salimonti, A., and Perri, E. (2008). Molecular characterization of Italian olive cultivars by microsatellite markers. *Advances in Horticultural Science, 22*(2), 142-148. https://www.jstor.org/stable/42882630

Instituto Nacional de Estadísticas y Censos (INDEC). (2021). Censo Nacional Agropecuario 2018. Resultados definitivos. https://www.indec.gob.ar/ftp/cuadros/economia/cna2018_resultados_definitivos.pdf

Olea database Molecular Database. *Olea database*. http://www.oleadb.it

Rallo, L. (2020). El futuro del germoplasma del olivo. *Agro Mercacei, 102*, 160-176.

Rehman, A. U., Mailer, R. J., Belaj, A., De la Rosa, R. and Raman, H. (2012). Microsatellite marker-based identification of mother plants for the reliable propagation of olive (*Olea europaea* L.) cultivars in Australia. *The Journal of Horticultural Science and Biotechnology, 87*(6), 647-653. https://doi.org/10.1080/14620316.2012.11512925

Rekik, I., Salimonti, A., Grati Kamoun, N., Muzzalupo, I., Lepais, O., Gerber, S., Perri, E., and Rebai, A. (2008). Characterization and identification of Tunisian olive tree varieties by microsatellite markers. *HortScience, 43*(5), 1371-1376. https://doi.org/10.21273/HORTSCI.43.5.1371

Sarri, V., Baldoni, L., Porceddu, A., Cultrera, N. G. M., Contento, A., Frediani, M., Belaj, A., Trujillo, I., and Cionini, P. G. (2006). Microsatellite markers are powerful tools for discriminating among olive cultivars and assigning them to geographically defined populations. *Genome, 49*(12), 1606-1615. https://doi.org/10.1139/g06-126

Sastre, B., De Lorenzo, C., Pérez, M. A., and Cabezas, J. A. (2015). *Variabilidad inter e intra-cultivar del olivar de la Comunidad de Madrid*. In: *XVII Actas Simposio científico-técnico Expoliva*. Jaen, Spain. Fundación del Olivar.

Sefc, K. M., Lopes, M. S., Mendonça, D., Rodrigues Dos Santos, M., Laimer Da Câmara Machado, M., and Da Câmara Machado, A. (2000). Identification of microsatellite loci in olive (*Olea europaea L.*) and their characterization in Italian and Iberian olive trees. *Molecular Ecology, 9*(8), 1171-1173. https://doi.org/10.1046/j.1365-294x.2000.00954.x

Trentacoste, E. R., and Puertas, C. M. (2011). Preliminary characterization and morpho-agronomic evaluation

of the olive germplasm collection of the Mendoza province (Argentina*). Euphytica, 177*(1), 99-109. https://doi.org/10.1007/s10681-010-0270-4

Torkzaban, B., Kayvanjoo, A. H., Ardalan, A., Mousavi1, S., Mariotti, R., Baldoni, L., Ebrahimie E., Ebrahimi, M., and Hosseini-Mazinani, M. (2015). Machine Learning Based Classification of Microsatellite Variation: An Effective Approach for Phylogeographic Characterization of Olive Populations. *PLoS ONE*, *10*(11), e0143465. https://doi.org/10.1371/journal.pone.0143465

Verga, A. (2007). PB2 [Software]. Córdoba, Argentina: Instituto Nacional de Tecnología Agropecuaria, Instituto de Virología y Fitopatología Vegetal (IFFIVE).