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Genotypic and phenotypic identification of olive cultivars from north-western Spain and characterization of their extra virgin olive oils in terms of fatty acid composition and minor compounds

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ABSTRACT

Galicia (NW Spain) is emerging as a new olive-growing region. Galician oil producers are currently striving to recover old autochthonous cultivars with a view to obtaining high quality extra virgin olive oil (EVOO). In this work, a total of 32 trees were studied in order to established their identity and genetic relationships to the main cultivated material in the Iberian Peninsula. The analysis of 11 morphological features of the endocarp and 14 microsatellite markers allowed three different cultivars to be identified among the sampled trees. Comparison with the morphological and molecular profiles available in the World Olive Germplasm Bank of Cordoba (WOGBC) revealed that 24 trees (75%) were of the 'Brava' cultivar and 7 (22%) of the 'Mansa' cultivar. The other tree, labelled as Picuda, matched no specific cultivar in WOGBC. Characterizing the oils obtained from the studied cultivars revealed a high potential for producing high-quality EVOOs of specific origin.

1. Introduction

Virgin olive oil (VOO) is the principal source of fat in the Mediterranean diet and highly appreciated by consumers for its healthy effects and sensory properties (Aparicio and Harwood, 2003). Spain ranks first in the world in olive grove area: 2 584 564 ha, which accounts for about 45% and 60% of all olive production in the world and the European Union, respectively. The average Spanish production from 2007/08 to 2012/13 was 1 215 798t, with a record of 1 615 million in the 2011/12 year (Magrama, 2017). The main olive-growing areas in Spain in terms of production are in the south (Andalusia, 60.4%), centre (Extremadura, 10.2%; Castilla–La Mancha, 15.8%) and northwest (Catalonia, 4.6%; Valencia, 3.7%; Aragón 2.3%). Per capita olive oil consumption in Spain is 9.6 L per year (Martín Cerdeño, 2015).

Galicia (NW Spain), where annual per capita olive oil consumption is 11.4 L (Martín Cerdeño, 2015), is emerging as a new Spanish olive-growing region and a producer of extra virgin olive oil (EVOO). There are two different policies currently in effect to boost the olive sector in Galicia. Thus, some major olive oil producers have focused on promoting Spanish varieties widely used in the world (e.g., Arbequina, Picual) in new plantations. These varieties feature a high pedoclimatic adaptability and productivity. Thus, the Arbequina cultivar adapts easily to extremely dense olive groves, and provides early entry into production, increased productivity and the ability to use modified mechanical vine harvesters (Proietti et al., 2015). Other producers, however, have focused on recovering old autochthonous cultivars from Galicia to obtain high-quality EVOO with specificity of origin, and special, distinctive sensory, nutritional and health-promoting properties (Reboredo-Rodríguez et al., 2016a,b). These two policies represent two different ways of competing on the EVOO market, namely: *competitiveness on cost* (the former approach) and *competitiveness on sensory style* (the latter) (Ilarioni and Proietti, 2014). In the former approach, production is geared towards super-intensive cultivation and highly mechanized methods; in the latter, traditional methods are favoured (Ilarioni and Proietti, 2014). In theory, autochthonous cultivars should be able to adapt to super high-density olive groves for improved production while maintaining sensory differences among oils (Proietti et al., 2012).

Initially, olive cultivars were named for their outstanding morphological traits or utility of production. Denominations are also frequently based on the place of origin of the propagating material (Rallo, 2005). As a result, synonyms (i.e., different names for the same cultivar) and

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homonyms (i.e., the same name for different cultivars) are extremely frequent among and within olive-growing countries (Barranco et al., 2000). Accurate varietal identification is therefore in order before specific olives are preserved and used by growers and producers. Several studies conducted in recent decades have allowed the main cultivated materials within and among countries to be identified and catalogued (Barranco et al., 2000; Barranco and Trujillo, 2000; Barranco et al., 2005; Belaj et al., 2003a, 2003b, 2007, 2012). The diversity of synonyms and homonyms for olives is illustrated by the facts that Lavee (1994) have identified more than 2000 varieties and Bartolini et al. (1998) have documented more than 1200 autochthonous varieties with more than 3000 names. In Galicia, the olive cultivated material is known widely for its homonyms (generic names): *Brava* and *Mansa*. No systematic characterization of this material in the Galician region appears to have been undertaken so far, however.

Recently, a useful protocol for characterizing, identifying and authenticating an olive germplasm bank was established on the basis of morphological and molecular (SSR marker) traits. The protocol was used to set up the World Olive Germplasm Bank of Cordoba (WOGBC), Spain (Trujillo et al., 2014). This is one of the world's largest collections and currently includes more than 500 olive accessions from 21 countries (Trujillo et al., 2014). The protocol also allowed a database including 500 morphological profiles and 332 SSR profiles of potential use for new studies on olives to be created.

Olive cultivars are known to influence the composition of the resulting olive oils, especially with regard to minor compounds such as *phytosterols*, which have nutritional and health effects; *volatile compounds*, which are responsible for oil aroma; and *phenolic compounds*, which have been associated to taste and healthy properties (Angerosa et al., 2004; Gómez-Rico et al., 2008; Inarejos-García et al., 2011). Kyçyk et al. (2015) recently showed the high genetic significance of sterol composition and total sterol, which may be especially useful for new olive breeding projects intended to obtain new cultivars with an improved VOO sterol fraction. Also, Zarrouk et al. (2009) established a classification of eighteen Mediterranean olive varieties based on the abundance of major compounds, fatty acid composition and, more specifically, the monounsaturated/polyunsaturated fatty acid (MUFA/PUFA) and oleic/linoleic acid (C18:1/C18:2) ratios.

The primary aim of this work was to promote the use of autochthonous olive cultivars from Galicia and their monovarietal oils. Available knowledge about the characteristics of Galician olive oils is scant. In previous work, we characterized VOOs obtained by mixing *Brava* and *Mansa* fruits in different proportions similar to those used by producers (Reboredo-Rodríguez et al., 2016a, 2016b) and also in mixtures with Picual and Arbequina fruits (Reboredo-Rodríguez et al., 2015). In this work, monovarietal EVOOs from 'Brava' and 'Mansa' fruits were studied. This required identifying and classifying the material of both varieties cultivated in Galicia in terms of morphological endocarp traits and microsatellite markers. Also, in order to better understand the peculiarities of the resulting autochthonous monovarietal olive oils, these were characterized for quality, stability and chemical composition, which are associated to the nutritional, functional and sensory properties of the oil. To our knowledge, this is the first time these characteristics of monovarietal autochthonous olive oils have been determined, so they may serve as a basis for identifying similarities and differences between cultivars in the future.

2. Materials and methods

2.1. Characterization of cultivars

2.1.1. Plant material

Varieties were identified during the crop year 2014/15 in 11 olive trees of the *Brava*, 20 of the *Mansa* and 1 of the *Picuda* cultivar. The studied material was found at different locations in the valley of River Sil (Lugo, Galicia). Although Galician climate is essentially oceanic and hence highly rainy (Carballeira et al., 1983), the study area has the typical climate of the Mediterranean oceanic domain. Table 1 summarizes the climatic conditions for the area over a period of three years including the crop year (i.e., 2014/16). The rocks in the area are siliceous (granites, schists and slates) and under deep soils (particularly cambisols) (Díaz-Maroto and Vila-Lameiro, 2006). Each tree was given a unique identifier including the name "UVIGO" and two numbers from 01 to 32 corresponding to the entry order in the material collection (Table 2b). Putative local olive varieties from Galicia were related to other olive varieties by using molecular data for the main cultivated material in the Iberian Peninsula, including 23 Spanish cultivars and 6 Portuguese cultivars reported by Trujillo et al. (2014) (Table 3).

Table 1
Climatological conditions of the studied area over the period 2014–2016 (Source: MeteoGalicia, 2017).

Climatological conditions					
Year	R(L/m^2)	T (°C)	TCT_7 (days)	RH (%)	TGR (kJ/m^2)
2014	991.1	12.1	8	80.9	15307
2015	670.8	12.6	19	77.0	16577
2016	1097	12.2	15	81.3	17525

R, total rainfall; **T**, mean air temperature; **TCT_7**, total cold time ($T < 7$ °C); **RH**, mean air relative humidity; **TGR**, total global radiation.

2.1.2. DNA extraction and SSR analysis

Total genomic DNA was extracted from fresh leaves by using the cetyltrimethylammonium bromide (CTAB) method of Murray and Thompson (1980) as modified by de la Rosa et al. (2002). Fourteen olive microsatellite markers including ssrOeUA-DCA3, ssrOeUA-DCA9, ssrOeUA-DCA11, ssrOeUA-DCA15, ssrOeUA-DCA16 and ssrOeUA-DCA18 (Sefc et al., 2000); UDO99-011, UDO99-019, UDO99-024 and UDO99-043 (Cipriani et al., 2002); and GAPU59, GAPU71B, GAPU101 and GAPU103A (Carriero et al., 2002; Cipriani et al., 2002; Sefc et al., 2000) were examined (Table 2a). All were previously found to be highly efficient for olive cultivar identification (Baldoni et al., 2009; Trujillo et al., 2014).

SSR amplification was done in a total volume of 20 µL containing 2 ng genomic DNA, 1 x supplied PCR buffer (Biotools, Spain), 1.5 mM $MgCl_2$, 200 µM dNTPs (Roche), 0.025 U/µL Taq polymerase (Biotools, Spain) and 0.2 µM forward primer (fluorescently labelled) and reverse primer.

Polymerase chain reactions (PCRs) were conducted in a thermal cycler (GeneAmp PCR system 9600, Applied Biosystems, Foster City, CA, USA) using the following sequence: initial denaturation at 95 °C for 5 min and 35 cycles with three steps (95 °C for 20 s for denaturation, 50–52 °C depending on the primer combination for 30 s for annealing, and 72 °C for 30 s for extension). A final extension step at 72 °C for 8 min was also applied. PCR products were separated in an automatic capillary sequencer (an ABI Prism 3100-Avant Genetic Analyser from Applied Biosystems), using appropriate fluorescent dyes. Fragment sizes were determined by using the internal standard GeneScan 400 HD-Rox. The Frantoio and Picual cultivars were used as controls in all runs.

Amplified fragments were analysed and scored using the software GeneMapper 3.0 and GenoTyper 3.7 from Applied Biosystems. A numeric code (1–3) was assigned to each SSR profile (Table 2b).

2.1.3. Morphological traits

The putative cultivars defined by the molecular markers were supplemented with 11 characters of the endocarp, namely: weight, length/width ratio, symmetry at position A, symmetry at position B, position of the maximum transversal diameter B, shape of apex at position A, shape of base at position A, surface roughness, number of grooves, distribution of grooves on the basal end and presence of mucron (Table 4). These traits are the most discriminating and stable —other