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Characterization of olive germplasm by chemical oil components and morphological descriptors in Basilicata region (Italy)

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Abstract

In this paper, we describe variations among autochthonous olive cultivars from five different areas in Basilicata (Southern Italy) classified according to 33 chemical oil components and morphological traits. While all examined descriptors show no significant differences among cultivars, means and coefficients of variations have been highlighted. Principal component analysis has then been used to reduce the number of descriptors. Cultivars have been classified by cluster analysis into three groups. Following a discussion of cultivar group similarities, results suggest that an '*a priori*' classification of cultivars according to growing area does not strictly correspond to phenotypic grouping. From the spatial distribution of cultivars, however, it has been possible to identify 'superior' genotypes in terms of olive oil composition.

Keywords: chemical oil components; diversity; *Olea europaea* L.; olive cultivars; morphological traits

Introduction

The olive tree, which is the cultivated form of wild oleaster and which, together with wild oleaster, belongs to the subspecies *europaea* of *Olea europaea*, is widely distributed over the Mediterranean basin. The general belief is that olive was domesticated in the East of the Mediterranean basin (Near East) and subsequently spread further west by the Phoenicians, Greeks and Romans. Several hundred geographically diverse olive cultivars exist in the Mediterranean basin (Breton *et al*., 2006, 2008, 2009). The wide genetic patrimony and the large number of synonyms and homonyms in olive require precise methods of discrimination for cultivar identification and classification. While morphological, agronomical and biochemical characterization are important in evaluating olive diversity (La Mantia *et al*., 2004; Breton *et al*., 2008), molecular markers have recently made it possible to study the diversity of olive trees and relationships between cultivars (Breton *et al*., 2006).

In recent years, increasing attention has been paid to the niche products of European brands and the protection and exploitation of autochthonous cultivars in sustainable production systems. Olive cultivation has a crucial commercial role in many Italian regions. This has involved increasing exploitation of cultivar diversity and refining agronomic practices and processes of transformation (Alba *et al*., 2011a, b). In the coastal and mountainous regions of Basilicata, Southern Italy, there are, according to Rotundo and Marone (2002), about 32,000 hectares comprising over 4,000,000 olive trees from a variety of autochthonous cultivars. Distributed in five main areas: 'Colline Materane', 'Medio Agri-Basento', 'Melandro', 'Pollino' and 'Vulture' (Fig. 1), this germplasm is an important genetic resource

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for identifying high-quality ecotypes and recovering traditional cultivars at risk of disappearing.

More recently, sensory characteristics, morphology of drupes, oil composition and DNA molecular markers (Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR)) have been used to identify cultivar origins (Besnard *et al.*, 2001; Belaj *et al.*, 2007, 2010; Galtier *et al.*, 2007; Hannachi *et al.*, 2008). Pattern analysis has enabled researchers to define cultivars according to markers and so retain the best varieties for olive production. Chemical parameters such as composition of volatile compounds, total phenols and fatty acids have also been used to differentiate oils obtained from different varieties (Allouche *et al.*, 2009; Benito *et al.*, 2010; Vekiari *et al.*, 2010; Ouni *et al.*, 2011).

In this study, chemical oil and morphological descriptors have been used to identify superior cultivars in the Basilicata olive germplasm.

Materials and methods

As reported in Fig. 1 and Table S1 (available online only at http://journals.cambridge.org), five olive-growing areas and a total of 27 autochthonous cultivars have been identified by morphological and bio-agronomic descriptors in a preliminary survey on the spread of olive germplasm in Basilicata (Rotundo and Marone, 2002). By analyzing soil and climatic conditions, and olive plant distribution in relation to cultivated areas, it has been possible to identify three main cultivation areas. In the Vulture (Potenza province), the main cultivar is 'Ogliarola del Vulture'; in Medio Agri-Basento and Colline Materane, the cultivars are 'Maiatica di Ferrandina' and 'Ogliarola del Bradano', respectively. Pollino and Melandro, because of their being mountainous, are marginal to olive production. Five productive and homogeneous trees at the same growing stage have been labelled according to area and cultivar. A total of 100 drupes chosen at random from different parts of foliage were collected before harvest time. Also, 200 leaves from the middle of yielding branches were measured using a portable area meter (Lambda Instruments Corporation, LI-COR Biosciences Lincoln, NE, USA). For each sample, drupes and relative endocarps were weighed before shattering to obtain a homogeneous olive paste. Lipid content was measured using standard AOAC (1995) (Soxhlet extraction by Petroleum Ether 40–60) and fatty acid composition was obtained using standard CEE 2568/91 and subsequent modifications. Total phenols were measured using Folin–Ciocalteau colorimetric method at 720 nm and caffeic acid as external standard.

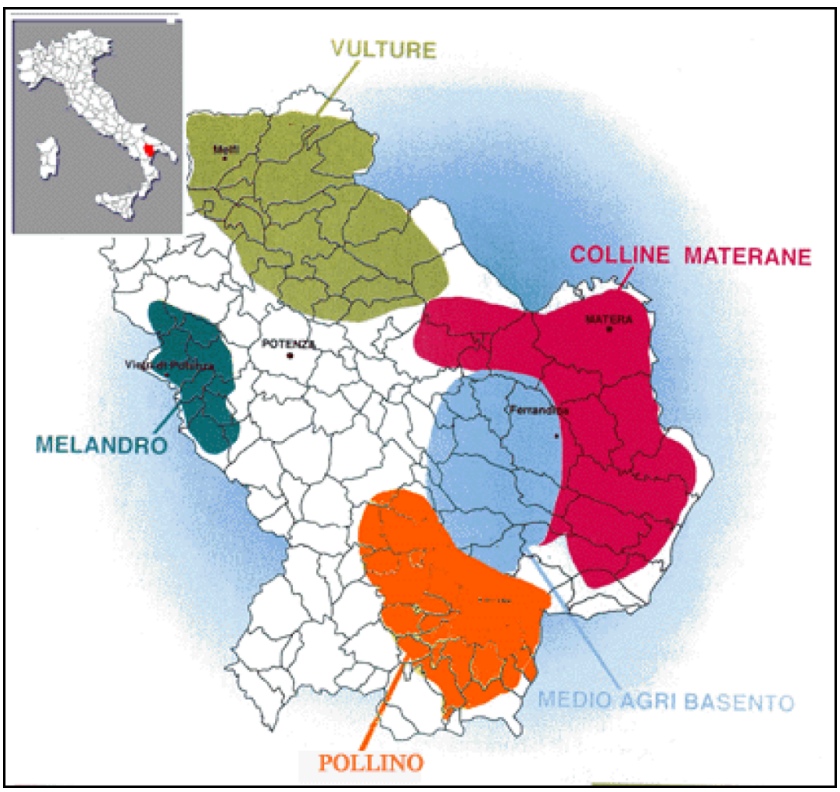

Fig. 1. Map of the olive-growing areas in Basilicata region (A colour version of this figure can be found online at http://www.journals.cambridge.org/pgr).

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Main phenols were identified using the method described by Montedoro *et al.* (1992) on 5 g of oil utilizing for separation water/acetonitrile (methyl cyanide) (acidified by formic acid 1%) as gradient eluent. The same method was adopted for sterols, which were measured by HPLC.

Data analysis followed four steps:

(1) evaluation of general statistics for each cultivar (means, standard deviation and coefficient of variation (CV));
(2) calculation of correlation coefficient between each pair of descriptors using mean values for each cultivar;
(3) derivation of orthogonal uncorrelated descriptors;
(4) clustering cultivars into similarity groups using uncorrelated descriptors.

Biometrical analysis was undertaken using 'Statistical Analysis System' (SAS Institute, 1987): MEANS, ANOVA, PRINCOMP and CLUSTER and then plotted using STATISTICA® (StatSoft, 1995). Principal components were identified from a correlation matrix using PRINCOMP. With eigenvalues indicating the 'useful' number of new components and eigenvectors showing 'loadings' on each descriptor, it was possible to reduce the original number of descriptors. Hierarchical clustering was then carried out using uncorrelated descriptors. Following Besnard *et al.* (2001), Ward's minimum variance was used to define cultivar groups and to establish similarity and dissimilarity by computing a matrix of Euclidean distances from group means and producing a dendrogram to show successive fusions of cultivars. This hierarchical method starts from a situation in which there are n clusters of a

Table 1. Means, standard error and coefficients of variation (CV) of 33 quantitative descriptors observed in 27 olive cultivars from Basilicata region (Southern Italy)

Descriptor	Mean ± SD	CV
Fatty acids (%)		
Palmitic acid	13.04 ± 1.80	13.80
Palmitoleic acid	0.96 ± 0.44	45.83
Stearic acid	2.29 ± 0.92	40.17
Oleic acid	73.48 ± 4.22	5.74
Linoleic acid	8.39 ± 2.95	35.16
Linolenic acid	0.75 ± 0.13	17.33
Arachic acid	0.33 ± 0.11	33.33
Fatty acids rate (no.)		
Oleic/palmitic	5.78 ± 1.05	18.16
Oleic/palmitoleic	91.99 ± 36.05	39.19
Linoleic/palmitoleic	10.27 ± 5.07	49.37
Unsaturated/saturated fatty acids	5.36 ± 0.78	14.55
Phenols (mg/1.000 g oil)		
Total phenols (mg caffeic acid/1.000 g oil)	236.24 ± 113.07	47.86
Hydroxy-tyrosol	0.07 ± 0.06	85.71
Tyrosol	1.93 ± 1.56	80.83
Dialdehydic form of elenolic acid linked to hydroxy-tyrosol (OHTyEDA)	62.64 ± 34.87	55.67
Dialdehydic form of elenolic acid linked to tyrosol (TyEDA)	11.81 ± 9.52	80.61
Oleuropeine-aglicon isomer (3,4-DHPEA-EA)	17.77 ± 7.21	40.57
Sterols (%)		
Cholesterol	0.31 ± 0.15	48.39
Campesterol	2.00 ± 0.33	16.50
Stigmasterol	0.57 ± 0.31	54.39
Clerosterol	0.88 ± 0.54	61.36
β-Sitosterol	68.89 ± 8.91	12.93
Δ-5-Avenasterol	19.97 ± 7.37	36.91
Δ-5,24-Stigmastadienol	0.96 ± 1.13	85.43
Δ-7-Stigmasterol	0.42 ± 0.23	54.76
Δ-7-Avenasterol	0.65 ± 0.44	67.69
Total β-sitosterol	94.95 ± 1.29	1.36
β-Sitosterol/Δ-5-avenasterol (no.)	4.56 ± 3.67	80.48
Total sterols (mg/1.000 g oil)	3923.96 ± 2134.68	54.40
Morphological traits		
Leaf surface (cm^2)	6.69 ± 0.96	14.35
Drupe weight (g)	3.27 ± 1.17	35.77
Endocarp weight (g)	0.58 ± 0.17	29.31
Oil yield (% fresh weight)	20.66 ± 1.78	8.62

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single cultivar to achieve, through successive fusion of the clusters less distant from each other, to a situation in which there is only one cluster that contains all the *n* cultivars. The most appropriate number of clusters was found by looking for consensus from statistics R^2 (RSQ), cubic clustering criterion (CCC), pseudo-F (PSF) and pseudo-t^2 (PST^2). Clustering results were combined with results from principal component analysis as a visual aid for discerning clusters in subsequent graphical representations (Polignano *et al.*, 1993, 2005; Granati *et al.*, 2003; Allouche *et al.*, 2009).

Results

Means, standard error and CV for all descriptors observed are presented in Table 1. Of the fatty acids, oleic acid has the lowest CV value (5.74%), while palmitoleic acid has the highest value with 45.83%. All main phenols have high or medium-high CVs ranging from 40.57% for oleuropein-aglicon isomer to 85.71% for hydroxy-tyrosol. Similarly, sterols range from the lowest CV value for total β-sitosterol (1.36%) to the highest CV for Δ-5,24-stigmastadienol (85.43%). Lower CV values

Table 2. Eigenvalues, eigenvectors and percentage of variation accounted by the first three principal components for 33 quantitative descriptors observed in 27 olive cultivars from Basilicata region (Southern Italy)

Descriptors	PRIN 1	PRIN 2	PRIN 3
Eigenvectors			
Palmitic acid	−0.34	0.06	−0.16
Palmitoleic acid	−0.18	−0.07	−0.31
Stearic acid	0.02	0.20	0.04
Oleic acid	0.23	−0.29	0.17
Linoleic acid	−0.12	0.32	−0.14
Linolenic acid	−0.17	0.14	0.05
Arachic acid	0.01	0.33	0.05
Oleic/palmitic	0.33	−0.13	0.16
Oleic/palmitoleic	0.25	0.11	0.28
Linoleic/palmitoleic	0.09	0.35	0.11
Unsaturated/saturated fatty acids	0.31	−0.18	0.12
Total phenols	−0.20	−0.06	0.24
Hydroxy-tyrosol	−0.07	−0.03	−0.05
Tyrosol	0.06	0.02	0.11
OHTyEDA	−0.18	−0.03	0.26
TyEDA	−0.17	−0.06	0.16
3,4-DHPEA-EA isomer	0.09	0.13	0.29
Cholesterol	0.13	0.11	0.06
Campesterol	−0.14	0.10	−0.01
Stigmasterol	0.01	−0.01	−0.22
Clerosterol	0.01	0.05	−0.15
β-Sitosterol	−0.23	−0.02	0.28
Δ-5-Avenasterol	0.19	−0.02	−0.28
Δ-5,24-Stigmastadienol	−0.05	0.13	−0.04
Δ-7-Stigmasterol	0.14	0.16	−0.09
Δ-7-Avenasterol	0.09	0.14	−0.22
Total β-Sitosterol	−0.16	−0.13	0.14
β-Sitosterol/Δ-5-avenasterol	−0.19	0.04	0.23
Total sterols	−0.15	−0.12	0.12
Leaf surface	−0.08	0.27	−0.09
Drupe weight	0.11	0.34	0.13
Endocarp weight	0.14	0.28	0.17
Oil yield	−0.01	−0.18	−0.10
Eigenvalues	6.84	4.37	4.15
Variation %	21	13	12
Variation comm%		34	46

OHTyEDA, dialdehydic form of elenolic acid linked to hydroxy-tyrosol; TyEDA, dialdehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropeine-aglicon isomer.

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have been recorded for morphological descriptors such as leaf surface (14.35%) and oil yield (8.62%). Medium-low values, 35.77 and 29.31%, respectively, have been reported for drupe and endocarp weights.

Eigenvalues, percentage of variation and load coefficients of the first three components have been calculated for the 33 descriptors using principal component analysis. This accounts for 46% of the total variation (Table 2). The percentage of variation explained by the three components is 21, 13 and 12%, respectively. Low or moderate coefficients of association have been reported for all descriptors. The first three principal components do not appear to be drastically affected by the 33 descriptors. Considering coefficients with absolute value up to 0.30, palmitic acid (−0.34), oleic:palmitic ratio (0.33) and unsaturated:saturated fatty acid ratio (0.31) are the primary determinants of the first principal component. The second principal component is characterized by linoleic acid (0.32), arachic acid (0.33), linoleic:palmitoleic ratio (0.35) and drupe weight (0.34). The third principal component only involves palmitoleic acid (−0.31). From the first axis, cultivars 'Ogliarola del Bradano', 'Scarpetta' and 'Cannellina' are clearly differentiated from cultivars 'Orazio', 'Roma' and 'Rotondella'. From the second axis, coordinates relating to the second principal component show that cultivars 'Cannellina', 'Provenzale' and 'Lardaia' are phenotypically different from cultivars 'Russulella' and 'Palmarola'. From the third axis, the most extreme cases are cultivars 'Provenzale' and 'Palmarola' (cluster II) and 'Lardaia' and 'Russulella' (cluster I) (Fig. 2).

Following these results, all principal components have been considered in the subsequent cluster analysis using Ward's minimum variance. The most appropriate number of clusters was found by looking for consensus from RSQ, CCC, PSF and PST^2 (Table 3). CCC has a local peak at three clusters, compared to PSF and PST^2 at five clusters. At three clusters, the percentage of variance accounted for by clusters is 91% of the original phenotypic variation; the remaining 9% can be ignored. The relative distance of cultivars, which can in turn be linked to the relative overall phenotypic differences, is shown in Fig. 2 where the 27 cultivars have been plotted for the first two principal components. The position of each point represents the adjusted mean for each cultivar. Thirteen of the 27 olive cultivars have been included in the largest cluster I, while clusters II and III include nine and five cultivars, respectively. Cluster I includes cultivars from Colline Materane ('Nociara', 'Ghiannara' and 'Ogliarola del Bradano'), Medio Agri Basento ('Augellina'), Melandro ('Cornacchiola' and 'Romanella'), Pollino ('Sammartinegna' and 'Spinoso')

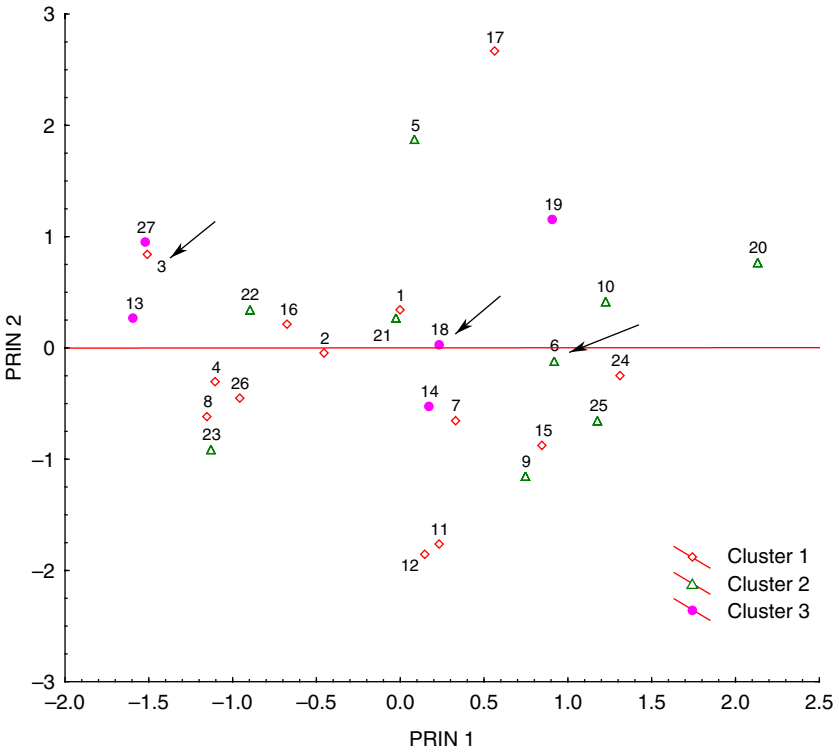

Fig. 2. Results of the principal component analysis showing a plot of the first two components, together with the results of the clustering procedure. Each cluster is represented by a different symbol (arrows indicate cvs: 3. 'Ogliarola del Bradano'; 6. 'Maiatica di Ferrandina'; 18. 'Ogliarola del Vulture') (A colour version of this figure can be found online at http://www.journals.cambridge.org/pgr).

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Table 3. Values of the four statistics obtained by Ward's minimum variance cluster analysis

Cluster	RSQ	CCC	PSF	PST^2
5	0.97	−0.73	*157.0*	14.6
4	0.95	−0.48	134.0	26.6
3	*0.91*	−0.06	115.0	29.9
2	0.72	*−0.87*	65.9	*32.9*
1	0.00	0.00	–	65.9

RSQ or R^2, Squared multiple correlation (Significance: is the proportion of variance accounted for by the clusters); CCC, Cubic clustering criterion (Significance: The CCC and approximate expected R^2 are given missing values when the number of clusters is greater than one-fifth the number of observations); PSF, Pseudo F statistic (Significance: measuring the separation among all the clusters at the current level); PST^2, Pseudo t^2 statistic (Significance: measuring the separation between the two clusters most recently joined). Italic significance: values useful to establish the significant number of clusters (3) by their consensus.

and Vulture ('Lardaia', 'Roma', 'Fasolina', 'Fasolona' and 'Russulella'). The smallest cluster III only includes cultivars from the Vulture ('Scarpetta', 'Cannellina', 'Cima di Melfi', 'Olivo da mensa' and 'Ogliarola del Vulture'). Remaining cultivars from the Vulture ('Provenzale', 'Orazio', 'Rotondella', 'Racioppa' and 'Palmarola') Pollino ('Faresana' and 'Carpinegna') and Medio Agri Basento ('Maiatica di Ferrandina' and 'Justa') have been grouped in cluster II.

Further considerations can be made on the strength of cluster analysis. Although not supported by significant differences in descriptors, different trends can be discerned in the three clusters. In particular, cluster I, with cv. 'Ogliarola del Bradano' widespread in Colline Materane, is characterized by higher monounsaturated fatty acid content (oleic acid 74.15% and palmitoleic acid 1.05%) than cultivars in cluster III (oleic acid 71.35% and palmitoleic acid 0.76%) such as the highly representative cv 'Ogliarola del Vulture'. While cluster III gives the highest values for saturated (stearic acid 3.40%) and polyunsaturated (linoleic acid 9.56%) fatty acids, total phenols (304.66 mg equivalent of caffeic acid per kg of oil, with a medium bitterness intensity) and total sterols (4934 mg/kg of oil), cluster II is characterized by the highest sterol compound values (tyrosol 3.18%, stigmasterol 0.68%, clerosterol 1.15%). The most representative cultivar in this group is 'Maiatica di Ferrandina' from the Medio Agri-Basento area, which is characterized by higher endocarp and drupe weight (0.55 and 3.84 g, respectively).

Discussion

To produce high-quality olive oil attractive to consumer tastes, not only must farmers focus on the growing environment but also on improving the cultivars. In recent decades, organoleptic properties and nutritional quality of olive oil have been subject to intense analysis. Quality olive oil depends as much on a complex interaction between genotype potential and environmental, agronomic and technical factors that contribute to fruit growth and ripening as on oil extraction processes and storage (Sanz-Cortés *et al.*, 2003; Haddada *et al.*, 2007; Benito *et al.*, 2010; Youssef *et al.*, 2011; Inglese *et al.*, 2011). The analytical and sensory profiles of most of olive oil produced by the most important cultivars worldwide have been largely described using combined data of fatty acids composition and minor compounds (Ollivier *et al.*, 2003, 2006). Here, it is important to underline that the quality of olive oil is heavily dependent on the content of single chemical components: high content of monounsaturated fatty acids such as oleic acid and palmitoleic acid, as opposed to low content of polyunsaturated fatty acids such as linoleic acid and linolenic acid. In the present paper, results suggest that there is no significant link between olive cultivar-growing areas and the phenotypic variations we have analyzed. In future studies, the use of molecular markers such as RAPDs, AFLP and SSR with eco-geographic parameters could improve the identification and characterization of Basilicata region cultivars as reported by several authors (Belaj *et al.*, 2007, 2010; Galtier *et al.*, 2007; Hannachi *et al.*, 2008). Interestingly, most of these studies did not show a clear relationship between the genetic diversity of the cultivars and its area of origin apart from Besnard *et al.* (2001) which is the focus in a broad geographical area. While similarity trends have enabled us to identify three main cluster groups, none of these appear to be related to cultivar-growing areas. Figures clearly show the absence of overlapping growing area-clusters; cultivars from the same growing area cluster in different phenotypic groups. In other words, the '*a priori*' classification of the cultivars according to growing area does not strictly correspond to phenotypic grouping. From the spatial distribution of olive cultivars, however, it has been possible to identify superior genotypes in terms of olive oil composition. In fact, the available and potential phenotypic variability described here may be of interest to potential users of the olive germplasm to identify variants with fruits characterized by improved levels of specific chemical components such as monounsaturated and polyunsaturated fatty acids and total phenols. While 'Ogliarola del Vulture', 'Ogliarola del Bradano' and 'Maiatica di Ferrandina' are the widest and most adaptable autochthonous cultivars in the Vulture, Colline Materane and Medio Agri-Basento growing areas, respectively, they are, as already noted, characterized by different morphological and chemical components. This information can be used to improve and standardize olive oil production in the local area, and thereby ensure the survival of these cultivars.

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