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Polymorphisms of Seed Storage Proteins in *Olea europaea* L. Cultivars

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The use of *Olea europaea* globulins as genetic markers for the identification of the cultivars was perfomed in the laboratory of Genetics of Department of Agriculture, Food and Environment, University of Pisa. The major component of *Olea europaea* L. seed storage proteins is represented by globulins. These fractions were characterised by electrophoresis and compared with the proteins extracted from protein bodies. The biochemical analysis of the olive seed globulins was carried out in sixteen different cultivars coming from several geographical areas of Italy. The electrophoretic patterns in polyacrylamide gels electrophoresis in denaturing conditions (SDS-PAGE) evidenced both qualitative and quantitative differences. It was possible to identify all the cultivars by their electrophoretic spectra. Number and position of the electrophoretic bands allowed the construction of a similarity matrix and of a dendrogram that allowed the separation into groups, according to their phylogenetic relationships. The several clusters seem to be related with agronomic traits such as fruit size or oil production; no relationships were found with the geographical cultivation areas.

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1. INTRODUCTION

At the present, one of the essential problems for an accurate identification of olive (*Olea europaea* L.) cultivars is the development of specific markers.

Traditional cultivar (cv) identification based on agro-morphological traits requires extensive observations of plants in many environmental conditions and lacks definition and objectivity [1]. Furthermore, morphological traits cannot serve as unambiguous markers because of environmental influences. For this reason, it is necessary to develop molecular and biochemical markers useful for cultivar identification and protection and cultivar purity determination.

Standard biochemical marker types and universal database integrated with existing agro-morphological identification could have a significant impact on exact cultivar identification of *Olea*. Protein or isoenzyme variants have been used for evolutionary and taxonomic studies [2-3] and cultivar identification in many crops, for an example in *Glycine max* [4], *Vicia faba* [5], *Pisum sativum* [6], *Phaseolus vulgaris* [7-8], *Phaseolus coccineus* [9]. The use of isoenzyme variants as biochemical markers can give rise to some problems linked to the fact that the related coding genes could be differentially activated under developmental or stress conditions, although several authors have used them [10,11]. The storage proteins of *O. europaea* were characterized [12], but no differences were found by these authors in subunit composition among six olive cultivars examined. Recently, capillary gel electrophoresis (CGE) was applied to separate proteins extracted from olive fruits [13] and the electrophoretic profiles were also evaluated for their capability to characterize olive varieties.

In this paper, we report the characterisation of the major storage proteins of *Olea europaea* L. seeds in sixteen cultivars through SDS-PAGE analysis. We compared the polypeptide composition of the storage proteins in the cultivars and estimated the pattern similarities.

2. MATERIALS AND METHODS

2.1 Plant Materials

The seeds of 16 *Olea europaea* L. cultivars, kindly provided by the Technical Agricultural Institute of Pescia, Pistoia, Italy, were used for electrophoretic studies. The different agro-morphological characteristics of the cultivars are shown in Table 1, according to Bartolini [14].

In this study, the cultivars are identified by numeral codes.

2.2 Protein Extraction and Electro-phoresis

The globulins for the characterisation were extracted from seeds of fully mature fruits of cv Leccino according to [15,16]. Extraction of the globulins from the 16 cvs. for the electrophoretic analyses were carried out from seeds of fully mature fruits according to [15]. Polyacrylamide gels for electrophoresis in denaturing (SDS-PAGE) and in native (PAGE) conditions were prepared according to [17]: acrylamide concentrations were 12% or 15% and 7% respectively. Protein bands on gels were visualised by staining according to [17]. Densitometric readings were performed by direct scanning of the stained gel with QUANTITY ONE Software (BioRad, USA).

The molecular weight (MW) and the amount of protein corresponding to each band were calculated. Similarity indexes between the cultivars were calculated according to [18] and a phylogenetic tree was constructed based on similarity matrix data by applying pair group method averages (UPGMA) cluster analysis using NSYS-pc program (Exeter Software, Setauket, NY).

3. RESULTS

The *Olea europaea* L. globulins extracted with different methods have been compared by electrophoretic analysis. Globulin extractions from seeds [15] and from protein bodies [16] show similar PAGE and SDS-PAGE patterns.

Table 1. Agro-morphological characteristics of the *Olea europaea* cvs under study

N.	Cultivar	Origin	Purpose	Compatibility	Regular production	Productivity	Fruit size	Rooting ability
1	Canino	Lazio	Oil	Self-incompatibility	Alternate	Good	Small	Medium
2	Pendolino	Toscana	Oil	Self-incompatibility	Constant	Medium	Small	Medium
3	Ascolana tenera	Marche	Table	Self-compatibility.	Constant	Medium	Large	High
4	Casaliva	Lombardia	Oil	Partially self-compatibility	Constant	Good	Medium	Medium
5	Nocellara Etnea	Sicilia	Table	Self-incompatibility.	Alternate	Good	Medium	Low
6	Picholine	Francia	Oil/Table	Partially self-compatibility	Constant	Good	Large	Medium
7	Ogliastro C. Stabia	Campania	Oil	Self-incompatibility.	Alternate	Medium	Small	Medium
8	Ogliarola Messinese	Sicilia	Oil	Self-compatibility.	Alternate	Good	Large	Low
9	Maurino	Toscana	Oil	Self-incompatibility	Alternate	Good	Medium	Medium
10	Frantoio	Toscana	Oil	Self-incompatibility	Constant	Good	Medium	High
11	Moraiolo	Toscana	Oil	Self-incompatibility	Constant	Good	Small	High
12	Coratina	Puglia	Oil	Self-incompatibility	Constant	Good	Medium	High
13	Leccino	Toscana	Oil	Self-incompatibility	Constant	Medium	Medium	High
14	Cellina di Nardò	Puglia	Oil	Self-incompatibility	Alternate	Good	Small	High
15	Carolea	Calabria	Oil/Table	Self-incompatibility	Alternate	Good	Medium	High
16	Corniolo	Umbria	Oil	Self-incompatibility	Constant	Good	Small	High

Fruit size: small (< 2 g), medium (2-4 g), large (> 4 g); Rooting ability: low (<33 %), medium (33-66 %), high (>66 %)

Both methods show the presence of two proteins in PAGE with apparent molecular masses of 200 and 360 kDa (Fig. 1A); similarly, after SDS-PAGE the same major subunits were found in the two samples with molecular weight (MW) 45.0, 33.5, 31.0, 27.0, 23.5 and 22.0 kDa (Fig. 1B).

The electrophoretic results after SDS-PAGE of the globulins extracted from the cultivars evidenced qualitative and quantitative differences. The results show a high polymorphism in the analysed olive cultivars: an example is reported in Fig. 2. Moreover, it is possible to distinguish each cultivar by its electrophoretic spectrum (Fig. 3).

A dendrogram constructed on the base of the similarity matrix generated from the analysis of the polypeptide patterns (Table 2) using the NSYS-pc program is shown in Fig. 4.

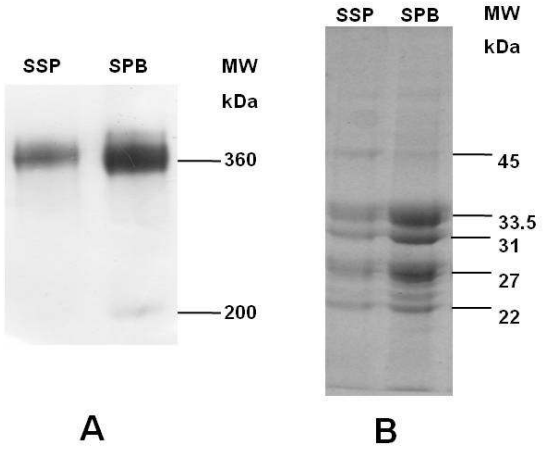

Fig. 1. Electrophoretic patterns of globulins extracted from Leccino total seeds (SSP) and protein bodies (SPB) under native A) and denaturing B) conditions

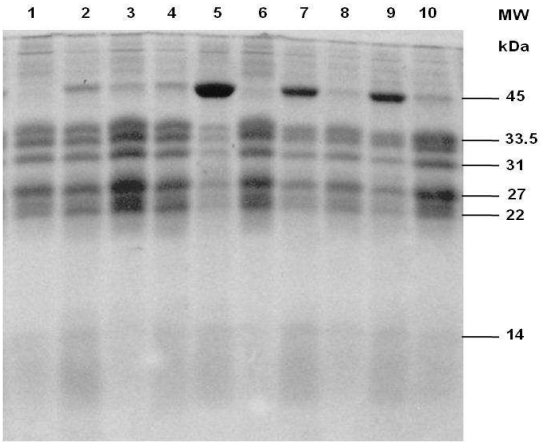

Fig. 2. SDS-PAGE patterns of some representative cultivars

Several clusters were resolved among the 16 olive cultivars. The group A consists of seven cultivars, that can be divided in the two sub-groups A1 (Ascolana tenera, Casaliva, Picholine and Nocellara etnea) and A2 (Ogliarola messinese, Maurino and Frantoio); the group B, consists of six cultivars (Moraiolo, Corniola, Cellina, Coratina, Leccino and Carolea). The other three cultivars (Ogliastro, Canino and Pendolino) cannot be assigned to any group and seem to be the most divergent cultivars.

4. DISCUSSION

Electrophoretic patterns of seed storage proteins have been widely used in several plant species, not only for identification of plant varieties and cultivars, but also for determination of taxonomic affinity and identification of intra- and inter-specific relationships. Similarly, our results in the olive allow the possibility of classifying the cultivars according to the electrophoretic patterns of seed globulins and of joining them in groups according to their phylogenetic relationships. As far as the group A is concerned, olives of the sub-group A1 (Ascolana tenera, Casaliva, Picholine and Nocellara etnea) are primarily grown for table production and have large fruit size, while cvs of the sub-group A2 **(**Ogliarola messinese, Maurino and Frantoio) are primarily grown for production of oil and have small fruit size. Olives of the group B (Moraiolo, Corniola, Cellina, Coratina, Leccino and Carolea) are primarily grown for production of oil and have small fruit size. The other three cultivars (Ogliastro, Canino and Pendolino), cannot be assigned to any group and showed to be the most divergent cultivars: they are grown for production of oil and have small fruit size. These cultivars are very close to the wild type, which could explain their behaviour almost as out-groups in the dendrogram.

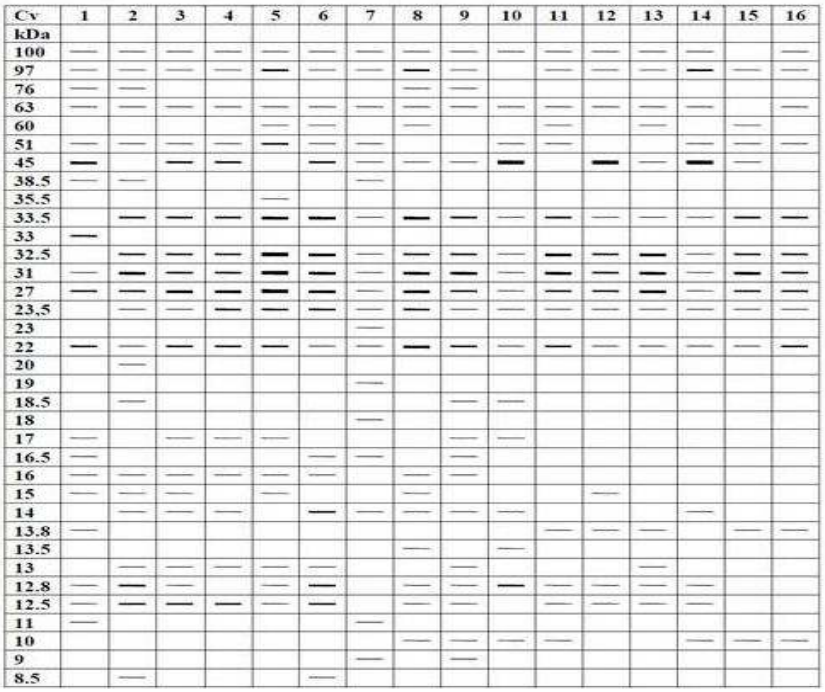

Fig. 3. Schematic electrophoretic patterns of total globulins extracted from seeds of the sixteen cultivars of *Olea europaea*

Table 2. Similarity matrix generated using the Nei's estimate of similarity

CVS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.00															
2	0.65	1.00														
3	0.70	0.82	1.00													
4	0.66	0.78	0.97	1.00												
5	0.64	0.76	0.88	0.85	1.00											
6	0.63	0.80	0.86	0.88	0.81	1.00										
7	0.52	0.60	0.64	0.66	0.54	0.68	1.00									
8	0.63	0.75	0.81	0.77	0.70	0.78	0.57	1.00								
9	0.60	0.76	0.92	0.84	0.71	0.88	0.65	0.80	1.00							
10	0.55	0.63	0.80	0.82	0.74	0.72	0.61	0.83	0.84	1.00						
11	0.58	0.66	0.72	0.75	0.78	0.76	0.58	0.76	0.66	0.75	1.00					
12	0.66	0.68	0.76	0.77	0.75	0.75	0.58	0.78	0.68	0.77	0.82	1.00				
13	0.58	0.68	0.76	0.81	0.78	0.82	0.58	0.76	0.72	0.75	0.80	0.89	1.00			
14	0.58	0.70	0.88	0.84	0.70	0.80	0.72	0.80	0.75	0.84	0.83	0.80	0.82	1.00		
15	0.40	0.45	0.48	0.64	0.55	0.60	0.53	0.66	0.56	0.64	0.76	0.72	0.69	0.66	1.00	
16	0.45	0.62	0.67	0.68	0.66	0.64	0.64	0.64	0.60	0.68	0.81	0.76	0.66	0.78	0.78	1.00

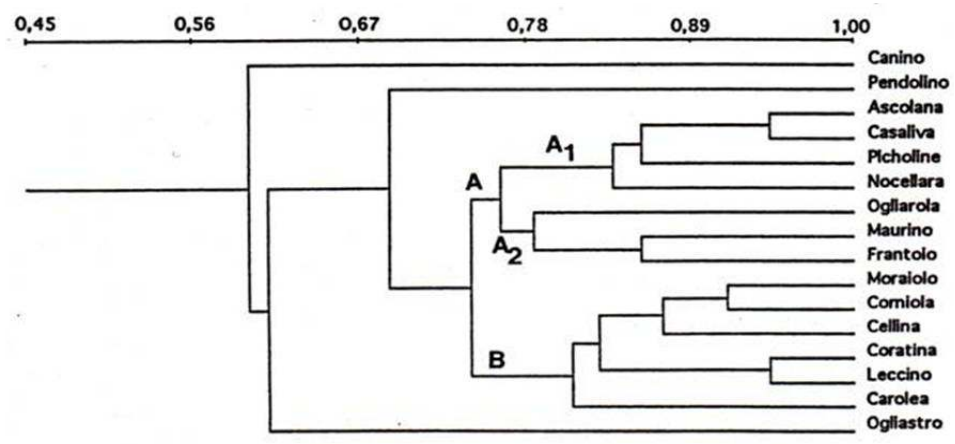

Fig. 4. Dendrogram of 16 olive cultivars generated by UPGMA cluster analysis of the similarity values given in Table 2. The scale indicates the relative genetic similarity of the cultivars

No relationships were found between the geographical cultivation areas of the material and our biochemical data. On the other hand, it should be stressed that in many cases the cultivation area does not coincide with the original selection site (genetic origin) that is often unknown. Another interesting fact is the close genetic proximity between some cultivars that might make synonyms think, but only Moraiolo and Corniolo are actually synonyms confirming the data in the database [14].

The differences in the polypeptide patterns are reproducible when further protein extracts from different seeds of the same cultivar are analysed (data not shown), thus excluding the possibility of altered protein patterns due to proteolytic degradation during protein extraction and/or analysis. Overall, within each group similar agro-morphological characteristics may be found. The results show a good relationship with the fruits characters and they are in accordance with the results reported by [10,19,20] obtained by using different approaches.

5. CONCLUSION

Based on our results, seed storage proteins can be used for the identification of the cultivars. This method could represents a valid and useful approach for olive cvs characterisation since it is rapid and cheap, in comparison to more sophisticated technologies based on molecular markers; moreover, since only a small cotyledonous fragment can be used for protein extraction and analysis without damaging the embryo, the seed can be subsequently reused for plant germination and development. This method can therefore allow the selection of *Olea europaea* plants by using globulins as genetic markers.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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