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Morphological evaluation of olive germplasm present in Tuscany region

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Summary

This work was done to quantify, evaluate and preserve the genetic variation of the olive (*Olea europaea* L.) from Tuscany and to look for ready-to-use valuable characters. Principal component analysis (PCA) was used to compare fruit, pit, leaf and growth habit characteristics among 83 accessions. Clustering of cultivars would suggest the existence of a strictly related genetic base with little morphological differences. Numerical analysis of phenotypical characters enabled to discriminate among accessions and to reduce the number of cultivars for further studies. It can be used as a rapid method to classify and compare phenotypes within large olive collections. The collected germplasm can be a useful sources of variability for those attributes not traditionally accounted for in olive plant selection. Several late-ripening cultivars, interesting for oil-quality; two cultivars with suited for low-pruning cultivation growth habit as well as four accessions with peculiar leaves, employable as ornamental, were identified. Furthermore, some cultivar with different initial growth potential, susceptible to be used for wood production or high density plantation, were catalogued. Data and photographs of most of the accessions here described were transferred to Internet where are available for consultation at the address http: //www.area.fi.cnr.it/olivo/olivit.htm

Introduction

The Italian olive germplasm is estimated to include about 500 cultivars (Bartolini et al., 1994), most of them landraces vegetatively propagated at the farm level since ancient times. The number is probably underestimated because of the scarce information on minor local varieties widespread in the different olive growing areas. The study of these less-common cultivars is important because they may have traits not considered important in the past but necessary to meet the challenges of modern olive growing. Low vigor, resistance to low temperatures, salinity tolerance (Gucci & Tattini, 1997), adaptability to low-pruning systems (Cantini et al., 1998), late ripening (Montedoro et al., 1989) and fatty-acid content (Cimato et al., 1996) are important traits for olive or olive oil quality. Additionally, morphological characters are sometime correlated or associated with disease susceptibility (Roselli et al., 1997) and can be used as markers in breeding.

In Tuscany, and Central Italy, extensive olive oil production is known since the seventh century. At the present, about 1.5 million olive trees are cultivated in this area, 70 percent of them represented by the cultivars ‘Frantoio’ and ‘Moraiolo’, reported with these names for hundreds of years. Other 20% of the plants are ‘Leccino’ and ‘Pendolino’ cultivars while the rest is represented by many other varieties. Little is known about the olive germplasm of Tuscany; after hundreds of years of uncontrolled propagation the result is that more than 80 different names are used (Cimato et al., 1997), same genotypes are known with different names, phenotypically different trees are identically called and moreover several names are supposed only to represent phenotypical variation of the same cultivar. In the fifties several authors described the morphology of sixty-two local cultivars (Baldini, 1953, 1956; Scaramuzzi & Cancellieri, 1954; Basso, 1958; Basso & Natali, 1962) but the observations were made under different environmental conditions which affected the phenotype. Modern methods for cultivar fingerprinting as isozyme (Trujillo et al., 1995) and RAPD analysis (Bogani et al., 1994; Fabbri et al., 1995) have proved to be useful to distinguish olive varieties but not to separate strictly related genotypes. Microsatellite markers have been successfully used in characterization of germplasm collections of different species (Lamboy & Alpha, 1998; Hokanson et al., 1998) but they have not been tested in olive and we have no information about ongoing research on olive-specific primer design. Morphological characterization continues to be the first step for the description and classification of germplasm and statistical methods like principal components (Hillig & Iezzoni, 1988; Iezzoni & Pritts, 1991) or cluster analysis (Sneath & Sokal, 1973; Peeters & Martinelli, 1989) can be used as useful tools for screening the accessions of a collection.

This research was started with the main purpose of preserving the genetic diversity of the olive of Tuscany. The objectives of the work here reported were to quantify and characterize the Tuscan olive germplasm using phenotypic characters. This information could be used to identify synonyms and reduce confusion among denominations; both important steps to the certification of propagation material recently introduced for olive trees. Furthermore an evaluation of morphological variation was done to identify accessions with possible valuable traits immediately usable by farmers or to exploit in breeding programs.

Materials and methods

Plant material. The collection of one-year-old scions from plants in all the main olive growing areas in Tuscany, Italy, was started in 1991. Eighty-three accessions were collected including cultivars already described in literature, plants identically named but with different phenotypes and several unknown plants. These last were found as single plants in the oldest olive groves, differing from the other plants of the orchards and were not recognized as known cultivars of Italy by morphological traits (Table 1). The scions were grafted on seedling rootstock and the resulting potted plants kept in the nursery for two years. In March 1993 the new trees, four of each accession, were planted at the 'S. Paolina' experimental farm in Follonica (GR), at a spacing of 7 × 5 m. The olive trees were trained as free-bush, with minimum pruning, under non-irrigated standard cultural practices. In

Table 1. List of accessions collected from the local germplasm of Tuscany and analyzed for their morphological traits. Unknowns are accessions with uncertain name

Num	Name	Num	Name
1	Albatro	43	Morchiaio
2	Allora	44	Morchione
3	Americano	45	Morcone 1
4	Arancino	46	Morcone 2
5	Ciliegino	47	Olivastra di Populonia
6	Correggiolo di Pallesse	48	Olivastra Seggianese
7	Cucca 1	49	Olivo di San Lorenzo
8	Cucca 2	50	Pendolino
9	Cuoricino	51	Pesciatino
10	Emilia	52	Piangente 1
11	Firenzuolo	53	Piangente 2
12	Frantoio F46	54	Punteruolo
13	Frantoio ITA	55	Puntino
14	Ginestrino	56	Razzaio
15	Grappolo	57	Razzo 1
16	Gremignolo 1	58	Razzo 2
17	Gremignolo di Bolgheri	59	Rosino 1
18	Gremignolo 2	60	Rosino 2
19	Grossolana	61	Rosino 3
20	Larcianese	62	Rossellino
21	Lastrino	63	Rossello
22	Lazzera Reale	64	Rossino
23	Lazzera Vallescaja	65	S.Caterina
24	Leccino ITA	66	S.Francesco
25	Leccino SPO	67	Scarlinese
26	Leccio del Corno 1	68	Unknown 1
27	Leccio del Corno 2	69	Olivo di Casavecchia
28	Leccio Maremmano	70	Unknown 2
29	Leccione	71	Olivo del Mulino
30	Madonna dell'Impruneta 1	72	Unknown 3
31	Madonna dell'Impruneta 2	73	Filare
32	Mansino	74	Unknown 4
33	Maremmano 1	75	Giogolino
34	Maremmano 2	76	Ornellaia
35	Marzio 1	77	Unknown 5
36	Marzio 2	78	Unknown 6
37	Maurino	79	Unknown 7
38	Mignolo	80	S. Lazzero
39	Mignolo Cerretano	81	Salicino
40	Moraiolo	82	Tondello
41	Moraiolo M11	83	Tondino
42	Morcaio		

Table 2. List of morphological characters used for the multivariate analysis of olive germplasm

Character	Abbreviation
A) Leaf characters:	
1) Blade length (mm)	BL
2) Blade width (mm)	BW
3) Blade length/width	B/B
B) Inflorescence characters:	
4) Inflorescence petiole length (mm)	IP
5) Inflorescence length (mm)	IL
6) Inflorescence width (mm)	IW
7) Inflorescence length/width	I/I
8) Number of flowers	NF
9) Inflorescence density (flowers per mm of length)	ID
C) Fruit characters:	
10) Length (mm)	FL
11) Width (mm)	FD
12) Length/width	F/F
13) Fresh weight of 100 fruits (g)	FW
14) Dry weight of 100 fruits (g)	DW
15) Oil content on dry basis (%)	DO
16) Oil content on fresh basis (%)	FO
17) Water content (%)	WA
18) Green fruit (%)	GF
19) Semi black fruit (%)	SF
20) Black fruit (%)	BF
D) Pit characters:	
21) Number of pit bundles	PB
22) Pit length (mm)	PL
23) Pit width (mm)	PW
24) Pit length/width	P/P
25) Weight of 100 pits (g)	WP
26) Flesh/pit weight ratio	F/P
E) Growth characters:	
27) One-year-old shoot internode length (mm)	IL
28) Canopy height (cm)	CE
29) Canopy volume (m^3)	CV
30) Canopy projection to the soil (m^2)	CP
31) Canopy height/radius of projection to soil	CR
F) Yield capacity:	
32) Fruit yield per plant (Kg)	FY

1996 the plants started fruit production and in 1997 all the accessions had a good fruit yield.

Characters measured. Thirty-two morphological characters and ratios were measured or calculated both in 1996 and 1997 (Table 2). Representative samples of leaves, shoots and inflorescences were taken from each of the four plants. Fifty random samples were evaluated for all characters. Leaf and flowers samples were collected from the mid-shoot portion of the current year's growth, while 1-year-old shoots were taken at random, from fruiting branches, following a rotation around the tree at approximately 1.5 m. from the ground. At harvest time (mid November), two hundred fruits were picked from each group of four plants and then split in two samples, one put in oven to dessicate the other used for fresh fruit and pit analysis. The oil content was determined on dried samples by solvent-extraction and density measurement using the Foss-let device (Foss-electric, Hillerrød, Denmark). The stage of ripeness was determined by assessing the superficial color of the skin according to these classes: green, semi-black, completely black. The data resulting from the two-year study were grouped and the average values used for statistical analysis. To determine plant vigor, in February 1997, canopy height and width of all trees were measured. The canopy spread was calculated considering an averaged circular projection of the trees to the soil, the canopy volume using the formula:

$$r^2\pi(h-r)+2/3\pi r^3$$

where r is the average radius of the canopy spread and h the total height of the tree. To distinguish between canopy growth types upright or weeping, a canopy shape index, considering the ratio between plant height and canopy radius, was calculated. Fruit yield was measured in 1996 and 1997 harvesting the four plants of each accession and the two-year cumulated yield per plant used for statistics.

Data analysis. Principal component analysis (PCA) was used to identify the patterns of morphological variation within the olive germplasm collection. PCA was performed using the PRINCOMP procedure of the SAS statistical package (SAS Institute Inc., North Carolina, USA). The result of PCA was then used to choose the characters to be used in hierarchical cluster analysis since this choice can affect the outcome. Cluster analysis was performed selecting only the variables that accounted more for the total variability, those with eigenvectors higher than 0.3 on the first three PCs. In this way 11 out of 32 variables were selected for clustering, they included: fruit length and width, fresh and dry weight of 100 fruit, pit width, weight of 100 pits, fruit length/width ratio, number

Table 3. Correlations between original variables and the first three principal components (PC) as observed in 83 olive accessions. Only eigenvectors with value equal or higher than 0.2 are shown

	Variables[x]	PC1	PC2	PC3
1.	BL	—	—	—
2.	BW	—	—	—
3.	B/B	—	—	—
4.	IP	—	—	0.35
5.	IL	—	—	0.34
6.	IW	—	—	0.26
7.	I/I	—	—	—
8.	NF	—	—	—
9.	ID	—	—	—
10.	FL	0.36	—	—
11.	FD	0.36	—	—
12.	F/F	—	0.38	—
13.	FW	0.37	—	—
14.	DW	0.36	—	—
15.	DO	—	—	—
16.	5FO	—	—	—
17.	WA	—	—	—
18.	GF	—	0.31	—
19.	SF	—	—	—
20.	BF	—	—	—
21.	PB	—	—	—
22.	PL	0.27	0.27	—
23.	PW	0.31	—	—
24.	P/P	—	0.34	—
25.	WP	0.33	—	—
26.	F/P	0.23	—	—
27.	IL	—	—	—
28.	CE	—	0.25	—
29.	CV	—	0.25	—
30.	CP	—	0.23	—
31.	CR	—	—	0.29
32.	FY	—	0.28	—

[x] See list of abbreviations on Table 2.

of green fruit at ripening, pit length/width ratio, inflorescence and petiole length. Cluster analysis was performed on the selected standardized variables by the CLUSTER procedure of SAS statistical package. The algorithm used for this statistic was the average linkage method, this generally yields results which are the most accurate for classification purposes (Peeters & Martinelli, 1989). The tree-plot of clusters obtained by this procedure was used to decide the ultimate number of clusters by which the accessions could be assessed.

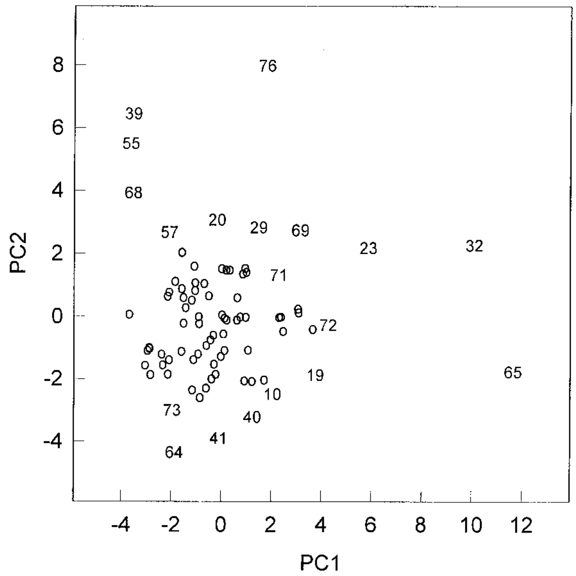

Figure 1. Position of PC scores of the accessions of the olive germplasm of Tuscany. Numerical symbols correspond to the cultivar listed on Table 1. Overlapping numbers are replaced by circles.

Results and discussion

Principal component analysis. The first three PCs of the accessions accounted for 44.1% of the total variance among cultivar means whereas the first 10 accounted for 84.6%. Variables such as fruit and pit length, width, weight, length/width ratio (on PC1) and fruit shape (on PC2) explained the largest portion of the variance (Table 3). Characters as the number of pit bundles, proposed by Barranco and Rallo (1984) or the oil content, were not useful to distinguish among accessions. On PC3 the largest scores were due to characters associated with the size of the inflorescence and the shape of the canopy. The variables correlation matrix obtained from PC analysis, showed few significant values, all of them relating pit to fruit (Table 4). None of the morphological characters were useful markers to estimate the olive oil content or fruit size which are main objectives of the breeding programs.

The measure of the distance among observations can be inferred from the spatial proximity of the observations represented in PC space (Figure 1). Proceeding from negative to positive values of PC1, the means increase in high degree of similarity.

Cluster analysis. The tree-plot obtained by the CLUSTER procedure showed the accessions grouped in clusters with their respective distances (Figure 2). The highest normalized maximum distances usable

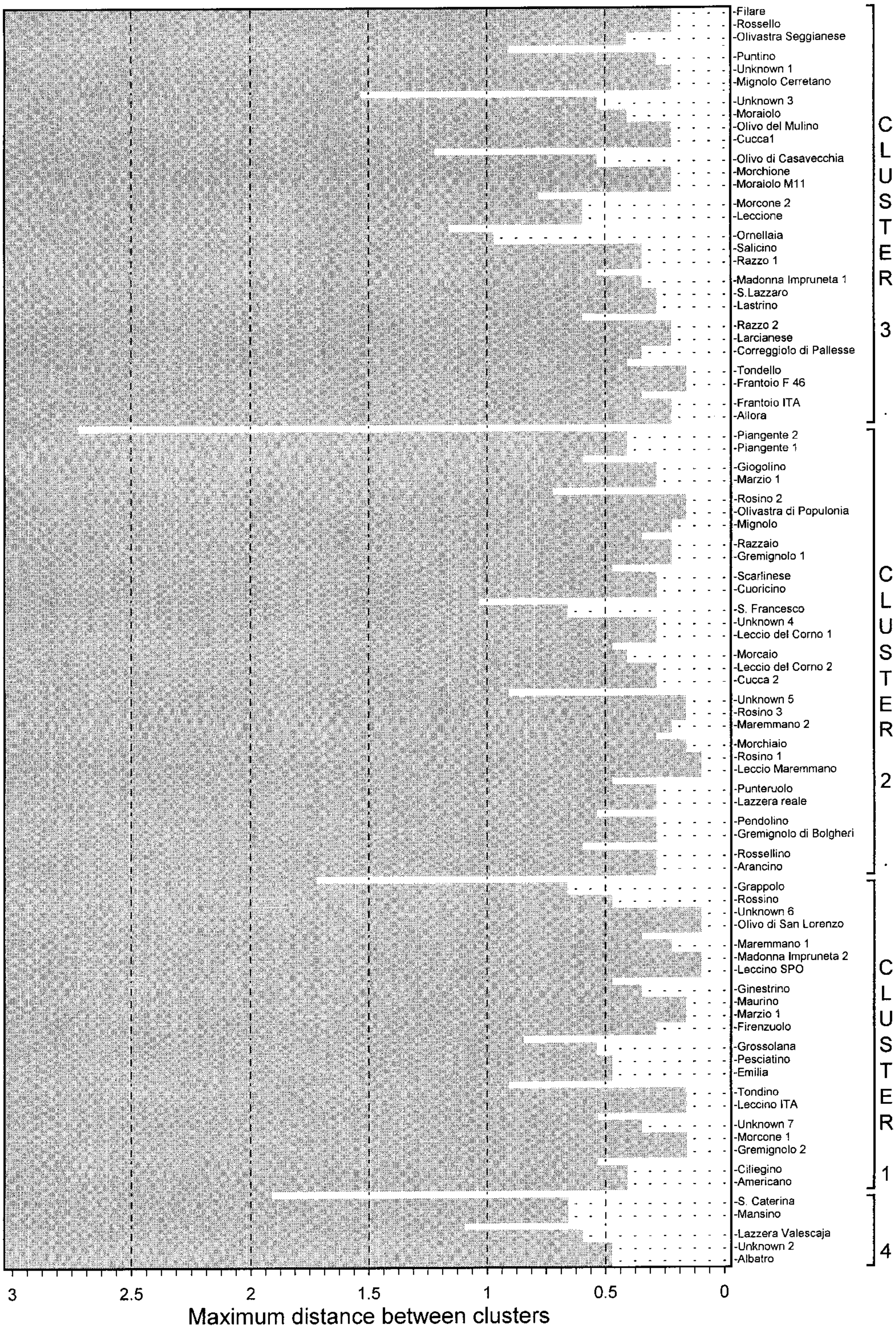

Figure 2. Average linkage cluster analysis tree plot of the olive germplasm accessions.

Table 4. Correlation matrix of the variables used in the PC analysis of olive germplasm. The table reports only correlation coefficients for fruit and pit characters

	[y] FL	FD	F/F	FW	DW	DO	FO	WA	GF	SF	BF	PB	PL	PW	P/P	WP
FD	0.79															
F/F	0.39	–0.25														
FW	0.88	0.93	–0.01													
DW	0.89	0.92	0.01	0.93												
DO	0.06	0.26	–0.31	0.18	0.30											
FO	–0.30	–0.26	–0.09	–0.31	–0.08	0.74										
WA	0.49	0.57	–0.07	0.61	0.35	–0.20	–0.74									
GF	0.04	–0.23	0.45	–0.11	–0.09	–0.10	0.05	–0.16								
SF	0.03	–0.07	0.16	–0.03	–0.01	0.11	0.09	0.02	0.41							
BF	–0.04	0.17	–0.32	0.08	0.06	–0.02	–0.08	0.06	–0.78	–0.89						
PB	0.09	0.19	–0.16	0.17	0.14	0.08	–0.05	0.18	–0.16	–0.08	0.14					
PL	0.87	0.44	0.71	0.60	0.65	–0.15	–0.23	0.22	0.25	0.05	–0.15	0.02				
PW	0.67	0.80	–0.15	0.75	0.80	0.09	–0.18	0.33	–0.17	–0.06	0.13	0.24	0.46			
P/P	0.53	–0.04	0.91	0.17	0.19	–0.23	–0.16	0.04	0.40	0.11	–0.27	–0.14	0.81	–0.13		
WP	0.84	0.74	0.21	0.79	0.84	–0.02	–0.24	0.30	0.00	–0.03	0.02	0.10	0.76	0.83	0.29	
F/P	0.47	0.69	–0.28	0.68	0.05	0.40	–0.21	0.66	–0.21	0.06	0.06	0.13	0.99	0.27	–0.06	0.19

[y] See list of abbreviations on Table 2. Correlation coefficient significant at $p = 0.05$ with value ≥ 0.22.

in clustering was 1.92 but with this value only two clusters were separated, both including a great number of very differing accessions. The reduction of the distance from 1.92 to 1.03 assured the separation of eight clusters but with this limit clones of two cultivars were placed in different groups. A normalized maximum distance of 1.6 was used as ultimate limit for clustering, obtaining four clusters (Figure 2). By this way three of the four clusters included a great number of accessions but was possible to place clones of each cultivar in the same group.

Cluster 1. Cluster 1 included both clones of 'Leccino' together with 19 other accessions. The two original 'Leccino' mother plants, collected two hundred km of distance were morphologically different but the new plants obtained from them resulted to be the identical when put in the germplasm collection at Follonica. The cultivars 'Maurino' and 'Madonna di Impruneta' were included in this group; these two, together with 'Leccino' are diffused in the coldest parts of Tuscany because considered more resistant to frost than other traditional cultivars. Most of the cultivar of this group have a low number of green fruit at harvest time and are known to be less susceptible to the *Cycloconium oleagineum* olive leaf spot disease (Morettini, 1954).

Cluster 2. Twenty-nine accessions belonged to this group, which included cultivars such as 'Pendolino' and 'Piangente' with a weeping type canopy, a traditional cultivar like 'Gremignolo' and one of its supposedly related cultivar 'Gremignolo di Bolgheri'.

Cluster 3. Twenty-eight accessions were in this cluster. The two oldest traditional Tuscan cultivars 'Moraiolo' and 'Frantoio', both with two clones coming from different parts of the region were put in this group; three accessions supposed to be phenotypical variations of 'Frantoio' as 'Correggiolo', 'Larcianese', and 'Razzo' were also comprised in this cluster confirming their proximity to the main cultivar. Two accessions of this group: 'Puntino' and 'Mignolo Cerretano', cultivated in two areas at about 150 km of distance one from the other, seemed to be identical from a morphological point of view; leaf dimension, growth habit and vigor were all the same. Most of the cultivar of this cluster have a medium-high sensibility to leaf spot disease.

Cluster 4. In this cluster was present the only fortable olive Tuscan cultivar 'S.Caterina' and other four accessions with large fruits. The large size character is rare within the collection and table olive production is not traditional of this part of Italy.

Hierarchical cluster analysis allowed the assessment of similarity or dissimilarity and clarified some of the relationships between cultivars, for instance some names appeared in more than one cluster. The two accessions named 'Cucca' corresponded to any of the cultivar described in the past and were completely different one from the other. The cultivar ‘Gremignolo’ was both in cluster number 1 and in number 2, the latter closer to that one described in the past. The cultivar ‘Madonna di Impruneta’, found in two different orchards, was both in cluster 1 with ‘Leccino’ and in cluster 3 with ‘Frantoio’; the latter accession did not correspond to the known cultivar description. The ‘Maremmano’ in cluster 1 was probably a ‘Leccino’ misnamed since the other in cluster 2 was similar to the described one. The name ‘Marzio’ was used for two accessions different in the characters of the fruit, but very similar for all the other characters. The cultivar ‘Morcone’ was also in cluster 1 and 3, but the latter differed from the one described in literature (Scaramuzzi & Cancellieri, 1954) and it is closer to the ‘Frantoio’ fruit type. The three accessions named ‘Rosino’ found in different orchards, were virtually identical and were grouped in the same cluster.

The unknown accessions were placed in the four clusters but each of them was not identical to any other cultivar of their group. It was not possible to identify these accessions with any known cultivar of Tuscany.

This work demonstrates that numerical analysis of olive morphological traits can be used as a rapid and comprehensive method to establish a first order of accessions classification within germplasm collections, it enables cultivar comparison and diversity conservation. Further accumulation of data across the years will result in an increase of the precision of accessions assessment. The result of PC suggest the hypothesis that the olive germplasm of Tuscany has a common, genetically restricted base, the Cluster analysis separated a group of traditional, well known cultivars of Tuscany from the others. It is difficult to say how many of these accessions are really originally produced inside the area by human selection, from seedlings, and how many are instead, cultivars imported, may be as single plant from other part of Italy or from other countries. Olive has been cultivated for long time and in Tuscany is present the greatest olive-nurseries concentration of the world. The industrial propagation of the olive trees has been a tradition of this area since eighteen hundred, scions arrived to the nurseries from many countries and it would not be surprising if some of the plants in collection were the result of this process of exchange.

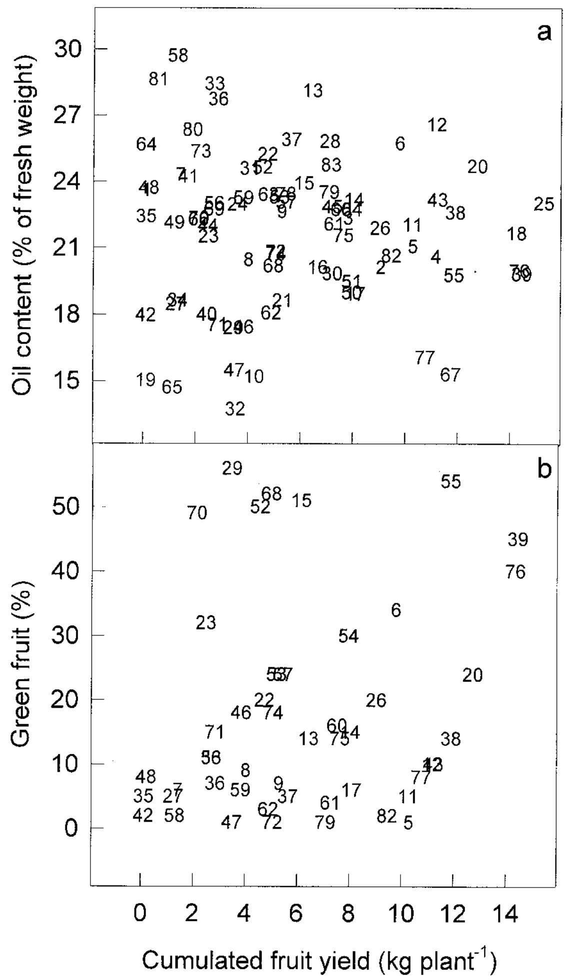

Figure 3. Distance among accessions considering plant oil content (above) and percentage of green fruit (below) vs. two-year cumulated plant yield. In the lower plot cultivars without green olive were not showed. The numbers correspond to the names listed on Table 1.

Variability among accessions. A plot of plant fruit yield versus oil content on a fresh basis showed that the widely cultivated cultivar ‘Frantoio’ (numbers 12 and 13 on the plot) had very good performances (Figure 3a). Oil productivity was used in the past as the main target for selection by farmers. Quantitative production however is no longer the main objective of olive breeding, since olive oil production is increasing in all the Mediterranean countries, as well as in other parts of the world, improving qualitative standard is becoming the pressing aim of the future. Late ripening cultivars, those in which a large percentage of fruit with green skin are present at the beginning of winter time, can insure the achievement of more flavoring olive oil production. Among the accessions of the collection was possible to characterize several late-ripening cultivars as ‘Leccione’, ‘Piangente’, ‘Grap-

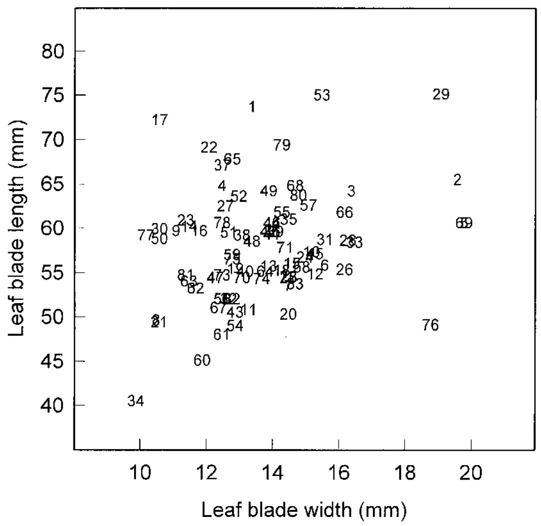

Figure 4. Distance among accessions in relation to leaf blade dimension. Numerical signs correspond to the names listed on Table 1.

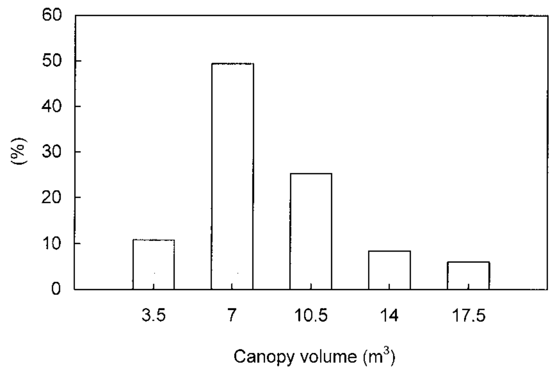

Figure 5. Relative distribution of the accessions in classes of canopy volume.

polo', 'Puntino' and 'Ornellaia', the last two showed a high yield potential compared to the others (Figure 3b). These cultivars are quite interesting because they can be picked at the end of the oil accumulation period, when the temperature are rapidly falling down before winter, obtaining both high oil-content and fine oil chemical characteristics.

The cultivar 'Frantoio' assures good fruiting but it is very sensitive to high humidity and leaf spots-disease. In warm-humid environments it tends to lose the leaves reducing the yield and starting undesirable on-off fruit bearing cycle. The cultivar 'Leccino' is less susceptible to leaf diseases but its fruits ripen very early, they are often already completely black in the middle of October producing a low-tasting olive oil. It might be possible to pick the fruits before complete flesh ripening but this would mean harvesting the olives when the oil accumulation is still in progress. Accessions as 'Leccio del Corno' and 'Leccione' named in a way that resembles the main cultivar 'Leccino', showed different phenotypic characters from the latter: ripening was postponed while the canopy characters were similar. For their 'to the base' growing habit and low susceptibility to leaf spot disease they look very promising for low-pruning cultivation or for high quality oil production.

Olive trees can also be used for ornamental purposes since they are evergreen and can easily bear long periods of drought. We found that three accessions have unusual leaves (Figure 4), long, narrow and light-green as the cultivar 'Gremignolo di Bolgheri' or long, large, dark-green, the cultivars 'Leccione' and 'Allora'. These three genotypes as well as the cultivar 'Maremmano' with tiny leaves, can be immediately used by ornamental gardeners. They do not seem to be more susceptible to leaf diseases than the cultivars usually employed and their unusual pleasant leaves could be more ornamental.

Another character to be taken into consideration was the olive tree growth capacity, determined in this study by canopy volume. In the last years there was an increasing interest in using the olive tree for high-quality wood production as it can grow in hard pedoclimatic condition. For this purpose it is necessary to employ vigorous, fast-growing, cultivars. Five cultivars of the collection had a high growth during the first years: 'Mignolo Cerretano' 'Olivo di Casavecchia', 'Tondello', 'Ciliegino' and 'Mansino', their canopy volume was more than double the average of the accessions and they can be tested as wood producers. On the other side, small canopies would be useful for increasing the number of plants per hectare since there are not size-reducing rootstocks for olive tree. Considering the distribution of the accessions over classes of canopy volume (Figure 5) it was observed that only 10% of the cultivars were in the lowest class. Nine accessions reached a mean canopy volume 50% smaller than the collection average, 3.5 vs. 7 cube meters. The distribution curve revealed a predominance of plants with medium-high canopy volumes in the collection, this could be explained with the fact that the size of the plant was probably one of the first parameters to be considered as a goal of the selection. A high growing plant could assure a good yield potential even in a low-inputs agriculture and olive plants with a low rate of growth were sometime not economically productive even in excellent cultural conditions. Low chemical and fertilizer application, once due to a poor status of the agriculture, is nowadays an objective of farming in order to protect the environment, therefore the performances of these cultivars, especially in terms of yield efficiency, must be further evaluated. We are aware that two years of production are not sufficient to give definitive results since olive culture requires long-term studies but this research gave us good perspective about the methodology and offered clues about germplasm utilization.

In conclusion we have shown that within the olive germplasm of a small geographic area, where this species has been cultivated for a long time, is possible to find cultivars with valuable morphological traits that can be immediately distributed to the farmers or employed in breeding programs. This is true mainly for those attributes that were not taken into consideration during the traditional selection which was for high fruit-oil production. The collection provides material for specific ongoing research, data and photographs of most of the accessions here described are available for consultation at the address http://www.area.fi.cnr.it/olivo/olivit.htm.

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