Multivariate Analysis of the Tuscan Olive Germplasm

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Abstract
This work was done to evaluate the genetic variation of the olive germplasm of the Tuscany region, central Italy, and to search for valuable characters. Principal component analysis (PCA) was used to compare fruit, pit, leaf and growth habit characteristics among 76 accessions. Clustering of cultivars suggested the existence of a strictly related genetic base with little morphological differences.

The germplasm proved to be a useful sources of variability for those attributes not traditionally accounted for in olive plant selection such as: late-ripening, interesting for the quality of the oil; leaf characteristics, for gardening; plant growth, for wood production or high density plantation. Photographs and data of most of the accessions were transferred to Internet where they are available at the address http://www.area.fi.cnr.it/olivo/olivit.htm

INTRODUCTION

Little is known about the olive germplasm of Tuscany after hundreds of years of uncontrolled propagation operated mostly at the farm level. More than 80 different names are used (Cimato et al., 1997), often without a certainty about the real identity and several appellations are supposed only to indicate phenotypic variation of the same cultivar. In the fifties several authors described the morphology of sixty-two local cultivars but the observations were made under different environmental conditions which affected the phenotype.

Methods for DNA fingerprinting as RAPD and microsatellite analysis can be successfully used in characterisation of germplasm collections but morphological information is the primary needed step for description and classification of germplasm. Statistical methods like principal components (Hillig and Iezzoni, 1988) or cluster analysis (Peeters and Martinelli, 1989) can be applied to the accessions for a first screening. The present study was done to better characterise and describe the Tuscan olive germplasm at a phenotypic level and to identify accessions with valuable traits susceptible to be used immediately or implemented by ordinary breeding programs and biotechnology.

MATERIALS AND METHODS

The germplasm collection localised at the “S. Paolina” experimental farm in Follonica, Italy was established in March 1993 planting four trees of each accession. During the period 1996-1999 samples of leaves, shoots and inflorescences were taken from each of the four plants. Fifty random samples were evaluated for all characters. Two hundred fruits were picked from each group, half of them put in oven to dessicate the other half used for fresh fruit and pit analysis. The oil content was determined on dried samples by solvent-extraction. 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 four-year study were grouped and the average values used for statistical analysis.

To determine plant vigor, in February 1999, 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: $r2\pi(h-r)+2/3\pi r3$ 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 harvesting the four plants of each accession and the four-year cumulated yield per plant used for statistics.

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 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.

RESULTS AND DISCUSSION

The first three PCs of the accessions accounted for 47.5 % of the total variance among cultivar means whereas the first 10 accounted for 89.5%. 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. On PC3 the largest scores were due to characters associated with the size of the inflorescence and the shape of the canopy. By the CLUSTER procedure was possible to obtain six clusters. Cluster 1 included both clones of “Leccino” together with 17 other accessions. 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. In cluster 2 were present twenty- seven accessions with “Pendolino” and “Piangente” characterised by a weeping canopy. Thirteen accessions were in cluster 3 including two clones of “Frantoio”, ”Correggiolo”, “Larcianese”, and “Razzo” the last three sometime considered only a phenotypical variation of the "Frantoio" cultivar-population. The accessions in cluster 5 included “Puntino” and “Mignolo Cerretano” belonging probably to the same genotype. In cluster 6 are present all accessions with large fruits. Several late-ripening cultivars as “Leccione” and “Grappolo” are quite interesting because they can be picked at the end of the oil accumulation period obtaining oil with positive chemical characteristics. “Leccio del Corno” and “Leccione” showed “to the base” growing habit and low susceptibility to leaf spot disease looking very promising for low-pruning cultivation. Four accessions with unusual leaves: long, narrow and light-green the “Gremignolo di

Bolgheri"; long, large, dark-green, the "Leccione" and "Allora"; very tiny the "Maremmano" can be used in gardening. The canopy growth of cultivars such us "Mignolo Cerretano" "Olivo di Casavecchia" and "Tondello" 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. Nine accessions reached a mean canopy volume 50 % smaller than the collection average the performances of these cultivars, especially in terms of yield efficiency, must be further evaluated.

CONCLUSIONS

This numerical analysis of olive morphological traits proved to be useful as a rapid and comprehensive method to establish a first order of accessions classification within germplasm collections, it enabled cultivar comparison and diversity conservation although the maximum distance among clusters presented low values showing the existence of a strictly related genetic base.

Even within the olive germplasm of a small geographic area like Tuscany, where the olive culture has been present since ancient time, it was 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, especially studying fruit and oil characteristics or secondary compounds in leaves and fruit pulp. Data and photographs of most of the studied accessions are available for consultation at the address http://www.area.fi.cnr.it/olivo/olivit.htm.

Literature Cited

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Tables

Table 1. List of morphological characters used for the multivariate analysis of olive germplasm.

Character
A) Leaf characters:

1) Blade length (mm) 2) Blade width (mm) 3) Blade length/width

B) Inflorescence characters:

4) Inflorescence petiole length (mm) 5) Inflorescence length (mm)
6) Inflorescence width (mm)
7) Inflorescence length/width

8) Number of flowers
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:

F) Yield capacity:
32) Fruit yield per plant (Kg) FY

Abbreviation

BL BW B/B

IP IL IW I/I NF