Characterisation of Local Olive (*Olea europaea* L.) Accessions by Oil Composition, Morphological and Molecular Markers Methods

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Abstract

Olive accessions collected in Lake Garda, one of the northernmost olive growing districts in the world, were characterised by a combination of morphological traits, chemical analysis of oils and AFLP-DNA markers. Among the more relevant accessions, other than the very well-known cultivars 'Casaliva', 'Frantoio', 'Leccino' and 'Pendolino', the local 'Baia', 'Mitria' and 'Regina' should be regarded as interesting for both horticultural and oil quality traits.

INTRODUCTION

Olive cultivars outnumber one thousand accessions, although the situation is complicated by a three-fold number of synonyms (Bartolini et al., 1994). Therefore, the need for dependable tools to ensure positive identification has a long tradition in olive cultivar description and identification (Baldini and Scaramuzzi, 1955). Advances in statistical data processing has enabled numerical analyses of many morphological and phenological traits, showing the potential of some of them in cultivar characterisation (Barone et. al., 1994), despite occasional little overall variability (Cantini et al., 1999). Oil composition too has been employed in cultivar characterisation and identification, with sound results (Guinda et al., 1996; Stefanoudaki et al., 1999). Fingerprinting by DNA analysis has been extensively tested in olive and proved to be very good at inter-cultivar discrimination and synonym identification (Mekuria et al., 1999; Perri et al., 2000; Testolin et al., 2000). The present study attempted a more comprehensive characterisation of olive germplasm by taking into account morphological, chemical (oil composition) and DNA marker analyses.

MATERIALS AND METHODS

Olive fruits of the putative local accessions 'Baia', 'Casaliva 1', 'Cornarol', 'Favarol 2', 'Gargnà', 'Less', 'Miniol 2', 'Mitria', 'Raza', 'Regina' and 'Rossanello' (Carocci-Buzi, 1937) and the cvs. 'Frantoio', 'Leccino', 'Maurino' and 'Pendolino' were harvested in 1997 and 1998 from a germplasm collection located at Puegnago (Lake Garda, northern Italy). Thirty fruits, pits and leaves from the 1997 harvest were measured for length and width and their ratios calculated. Eight-ten kg of olives per accession were processed by a standard discontinuous procedure: the olives were crushed with an inox hammer crusher, malaxed for 30 min at 28°C and the oil was extracted by hydraulic press (max 200 bars) and separated by centrifugation at 2000 g. Oils were analysed for aliphatic and triterpenic alcohols, sterols, triglycerides and fatty acids according to Council Regulation (EC) no. 2568/91 of 11 July 1991, tocopherols according to Andrikopoulos et al. (1989), volatile and phenolic according to Angerosa et al. (2000). To investigate the level and pattern of DNA variation at the entire genome level, AFLP (amplified fragment length polymorphism) analysis was conducted. Total DNA was extracted from leaves of mature plants using the NucleoSpin Plant kit (Clontech). AFLP analysis was carried out after Vos et al. (1995). Total DNA (200-400 ng) was digested with *Eco*RI and *Mse*I restriction enzymes. Primer combinations E32-M47 and E36-M32 were employed for selective amplification. Detection was achieved by labelling *Eco*RI selective primers with ^{33}P-γ-ATP, followed by electrophoresis and autoradiography. The presence and absence of AFLP bands was scored as 1 or 0 and the data analysed according to the Dice index =

n/[n+2(N-n)], where n = traits present or absent in both accessions; N = total traits. For each homogeneous set of phenotypic traits, accessions were grouped by cluster analysis using between-group linkage and the square Euclidean distance computed on z-standardised values. Accessions were then classified by visual inspection of dendrograms into comparable groups. To consider the different phenotypic traits together, each was scored as a binary character (0=absence, 1=presence). Accessions were classified following this dichotomy approach and a general dendrogram was built using the Dice index.

RESULTS AND DISCUSSION

Two pairs of selective primers were able to detect a total of 92 polymorphic bands (43 and 49 respectively); most bands corresponded to fragments <500 bp long. All the accessions were univocally identified (Figure 1). When morphological parameters and oil compounds were taken into account as a whole, the accessions grouped in a very different fashion, the only similarity with the AFLP grouping being 'Casaliva 1' and 'Raza' (Figure 2). While these two accessions showed a rather good agreement between genetic and phenotypic similarity (0.86 and 0.55, respectively), the comparison between 'Raza' and 'Frantoio' yielded 0.88 and 0.18 only, respectively (Table 1).

CONCLUSIONS

The marked disagreement found in the present study between reliable DNA markers (AFLP) and phenotypic expression, although it was based on one year data only, needs further investigation. However, putative clones showing horticultural and oil trait differences are not always separated by DNA markers (Parlati et al., 2000; Perri et al., 1998). Furthermore, it should be underscored that while DNA markers detect genetic identity, it is still questionable how this uniqueness also reflects the cultivar phenotype. Since the cultivar is primarily a horticultural entity, efforts must be made to identify DNA markers linked to phenotypic traits, as differences in DNA only may not justify cultivar differentiation.

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Literature Cited

Andrikopoulos, N., Hassapidou, M. and Manoukas, A. 1989. The tocopherol content of Greek olive oil. J. Sci. Food Agric. 46: 503-509.

Angerosa, F., Mostallino, R., Basti, C. and Vito, R. 2000. Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. Food Chemistry 68: 283-287.

Baldini, E. and Scaramuzzi, F. 1955. Uteriori indagini sulla validità del metodo bio-statistico nella descrizione e classificazione delle cultivar di olivo. Ann. Sper. Agraria, Roma.

Barone, E., Di Marco, L., Motisi, A. and Caruso, T. 1994. The Sicilian olive germplasm and its characterisation by using statistical methods. Acta Hort. 356: 66-69.

Bartolini, G., Messeri, C. and Prevost, G. 1994. Olive tree germplasm: descriptor lists of cultivated varieties in the world. Acta Hort. 356: 116-118.

Cantini, C. Cimato, A. and Sani, G. 1999. Morphological evaluation of olive germplasm present in Tuscany region. Euphytica 109(3): 173-181.

Carocci-Buzi, C. 1937. Le varietà di olivo coltivate nel Garda. In: Federazione Nazionale dei Consorzi per l'Olivicoltura ed.: Le varietà di olivo coltivate in Italia: 165-184.

Guinda, A., Lanzòn, A. and Albi, T. 1996. Differences in hydrocarbons of virgin olive oils obtained from several olive varieties. J. Agric. Food Chem., 44, 1723-1726.

Mekuria, G.T., Collins, G.G. and Sedgley, M. 1999. Genetic variability between different

accessions of some common commercial olive cultivars. J. Hort. Sci. & Biotech. 74(39: 309-314.
Parlati, M.V. e Iannotta, N, Maiolo, B. and Pandolfi, S. 2000. Selezione clonale dell'olivo nel Lazio: valutazioni agronomiche e sulla composizione degli oli dei cloni 'Carboncella Pianacce A, B C', osservati in apposito campo di comparazione. Atti 'V Giornate Scientifiche S. O. I.', Sirmione (I): 437-438.
Perri, E., Parlati, M.V., Palopoli, A. and Godino, G. 1998. Valutazione di presunti cloni di olivo (Olea europaea L.) cv Carboncella, mediante marcatori RAPD. Atti 'IV Giornate Scientifiche S.O.I.', Sanremo (I): 519-520.
Perri, E., Lombardo, N., Godino, G., Alfei, B and Pannelli, G. 2000. Caratterizzazione molecolare del germoplasma olivicolo delle Marche. Italus Hortus (7), 2: 7-11.
Stefanoudaki, E., Kotsifaki, F. and Koutsaftakis, A. 1999. Classification of virgin olive oils of the two major Cretan cultivars based on their fatty acid composition. J. Am. Oil Chem. Soc. 76 (5): 623-626.
Testolin, R., Cipriani, G., Marrazzo, T., Marconi, R. and Cimato, A. 2000. Identificazione genetica delle varietà di olivo del Garda. L'Informatore Agrario, 35:71-76.
Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van der Lee, T., Hornes, M., Frijters, A., Pot J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Tables

Table 1. Examples of comparisons among phenotypic and genotypic similarity based on Dice index.

Accession	Similarity	
	Phenotypic	Genetic
Casaliva 1 vs. Raza	0.55	0.86
Raza vs. Frantoio	0.18	0.88

Accession	Similarity	
	Phenotypic	Genetic
Mitria vs. Rossanel	0.18	0.82
Maurino vs. Less	0.00	0.49

Figures

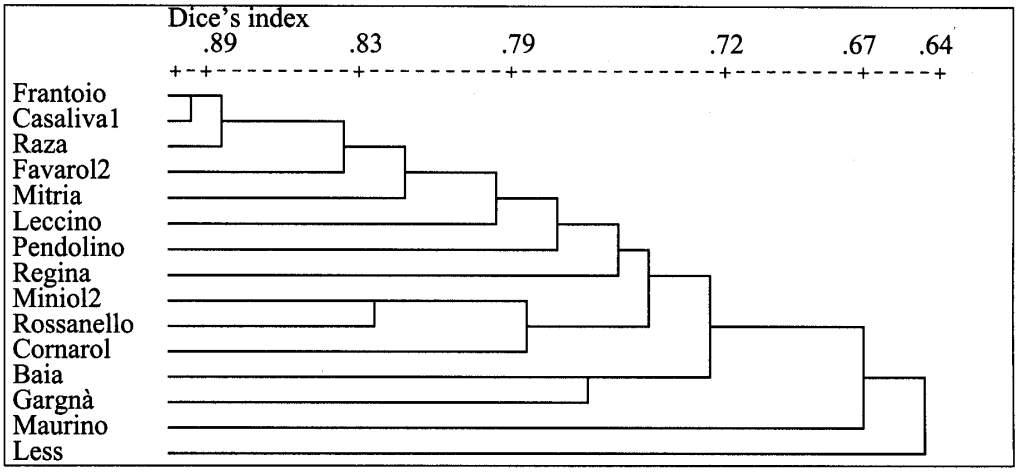

Fig. 1. Similarity dendrogram based on AFLP data.

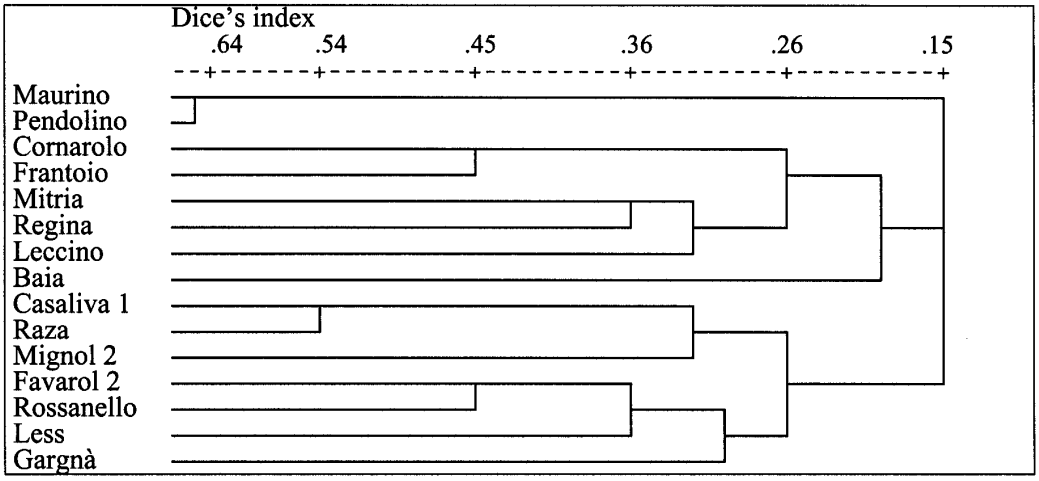

Fig. 2. Similarity dendrogram based on phenotypic data.