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Oil Quality and Morphological, Phenological, Bio-agronomical and Molecular Characterization of Syrian *Olea europaea* L. germplasm

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SUMMARY – The oil, morphological, phenological, bio-agronomical and genetic molecular characteristics of the main Syrian olive cultivars (Dan, Doebli, Hemplasi, Insassy, Karamani, Khoderi, Jlot, kaissy, Sorani, Safrawy, Souri and Zaity) were evaluated in the period 2004-06, within the Italian cooperation project "Technical Assistance for the Improvement of Olive Oil Quality in Syria". The molecular analyses, using SSR and AFLP markers, were carried out on 42 accessions, selected from six distinct collections located in Syria (Damascus, Tartous, Aleppo, Lattakia, Idleb and Hama), in order to verify the presence of inter- and intra-varietal genetic differences. The cultivars showed marked differences in all the fruit characteristics studied (detachment force, weight, pulp/pit ratio, water and oil content, pigmentation and morphology). The oleic content in the oils of most of the cultivars was about 70% or higher. The Karamani cultivar had a relatively lower oleic value (63-64%) and relatively higher amounts of palmitic and linoleic acids. The values of all the parameters (acidity, peroxide number, absorbencies in ultraviolet, fatty acid composition, sterol composition and content) used to evaluate oil quality were within the IOOC trade standards, with the exception of the Δ-7-stigmastenol values that were sometimes too high in the cultivars Khoderi and Doebli (> 0.5%) and of the Campesterol content that was sometime too high in the cultivar Insassy (> 4.0%). Regarding the molecular characterization, no ambiguous cases of synonymy among the considered cultivars were found. The notable genetic variability observed among the 42 samples (accessions) indicates a situation of "cultivar populations", that is, the presence of different clones within the same cultivar.

Keywords: *Olea europaea* L., AFLP, microsatellites, genetic diversity, oil quality, olive germoplasm

RESUME - *Pendant la période 2004-2006 dans le cadre du projet de coopération intitulé "Assistance technique pour l'amélioration de la qualité de l'huile d'olive en Syrie les caractéristiques morphologiques, phenologiques, bio agronomiques, de l'huile et génétiques des principales variétés Syriennes (Dan, Doueibli, Hemplasi, Insassy, Karamani, Khodeiri, Jlot, Qaisi, Sorani , Safrawi , Souri, Zeiti), ont été évaluées. Les analyses moléculaires, effectuées à travers la méthode SSR et AFLP ont été réali-sées sur 42 accessions, sélectionnées à partir de 6 différentes collections génétiques localisées en Syrie (Damas, Tartous, Alep, Latakia, Idleb et Hama), afin de vérifier la présence des différences inter et intra variétales. Les cultivars ont montré des différences importantes dans tous les fruits analysès pour leurs caractéristiques morphologiques (force de détachement, poids, rapport entre pulpe et noyau, contenu en eau et huile, pigmentation et morphologie). L'huile de la majorité des variétés avait un pourcentage d'acide oléique environ du 70% et plus, seulement la cv Karamani a montré une valeur inférieure (63-64%) qu'a été mis en relation avec une valeur élevé d'acides palmitique et linoléique. Tous les paramètres qu'ont étè examinés (l'acidité, les peroxydes, absorbance dans l'ultra violet, la composition en acides gras, le contenu en stérols et la composition stérolique) ont été conformes*

aux limites imposées par les paramètres de qualité du COI, avec l'exception du ∆-7- Stigmastérol, dans les cultivars Khodeiri et Doebli, que quelque fois a été trop élevé (>0,5%). En ce qui concerne la caractérisation génétique, il n'y a pas eu aucun cas de l'ambiguïté ou de cas de synonymie entre les cultivars considérées. La grande et importante variabilité génétique observée entre les 42 sélections utilisées (accession) ont indiqué une situation plus semblable à quelle d'une "population des cultivars) donnée par la présence des différentes clones dans la même cultivar.

Mots-clés: *Olea europaea L., AFLP, microsatellites, diversité génétique, qualité de l'huile, germoplasme*

Introduction

In Syria, the olive sector is very important for the economy of the country. Many new olive orchards have been planted in recent years (about 57% of the olive trees in Syria are less than 20 years old) and Syria is now the fourth largest olive oil producing country in the world (Al Ibrahem, 2006).

Syria is part of the original habitat of *Olea europaea* L. and has a very rich germoplasm. There are more than seventy varieties of olive cultivated in the different areas of the country (Nseir et al., 1985). However, only a few varieties have been extensively cultivated; in fact, five cultivars, Zaity, Sorani, Doebli, Khoderi and Kaissy, represent about 90% of the total olive trees cultivated in Syria (Al-Ibrahem, 2006). The others are local varieties that have a limited distribution.

To date, very few studies have evaluated the morphological, phenological, bio-agronomical and productive characteristics of Syrian olive varieties (Nseir et al., 1985; Tubeileh et al., 2004 a,b), and very little has been done to assess the characteristics of their oils (Tubeileh et al., 2004 b) and to characterise their genetic variability by using molecular markers (Belaj et al., 2003a). More information about these aspects is needed in order to: identify the different cultivars and possible clones present in Syria; choose the best varieties for the establishment of new olive orchards; carry out programmes for the genetic improvement of olive; develop procedures for certifying the genetic identity (variety) and sanitary state (absence of diseases and pests) of plants produced in nurseries, as is done in the European Union and other countries where olive is cultivated; characterise the quality of olive oils produced in Syria and determine if they meet the quality standards demanded by the international market. This last point is particularly useful considering that with the many new olive orchards that have been planted in the last decades, there is an increasing amount of oil available for export.

Within the framework of the Italian cooperation project "Technical Assistance for the Improvement of Olive Oil Quality in Syria" carried out in Syria from 2004 to 2006, studies have been conducted to characterise the oil quality and morphological, phenological, bio-agronomical and genetic molecular characteristics of the main Syrian olive cultivars. The main results of these studies are reported.

Project related activities

Oil quality and morphological, phenological and bio-agronomical characterization

The aim of this part of the project was to evaluate the oil quality and morphological, phenological and bio-agronomical characteristics of most of the main Syrian olive cultivars. The cultivars considered were: Kaissy and Zaity in the Aleppo area (where about 30% of Syria's olive cultivation is concentrated), Dan, Hemplasi and Souri in the Damascus area (about 3.0%), Insassy, Karamani and Sorani in the Idleb area (about 22%), Khoderi in the Lattakia area (about 7%), Safrawi in the Hama (Mousiaf) area (about 6%) and Doebli in the Tartous area (about 13%). The environmental conditions of the areas differ especially for the annual rainfall: 350-450 mm in Aleppo and Hama, 200-250 mm in Damascus, 450-500 mm in Idleb and 800-900 mm in Lattakia and Tartous. In each area, in germoplasm collections or on private farms, 5 trees per cultivar were selected and labelled. These trees were then used for morphological, phenological and bio-agronomical characterisation and to have the oil for oil quality evaluation.

The morphological investigation was carried out by using the "methodology for primary characterisation of olive varieties" proposed by the International Olive Oil Council (IOOC) (Barranco et al., 2000).

Observations were made on the tree (vigour, growth habit, canopy density), the fruiting shoot (internode length), the leaf (dimension, shape, longitudinal curvature of the blade), the inflorescence (dimension and number of flowers), the fruit (weight, shape, symmetry, colour, etc.) and the endocarp (weight, shape, symmetry, etc.).

The phenology was characterised by direct observations carried out with a frequency that depended on the phenological phase (i.e. every 1 week during flowering, every 1-2 weeks during fruit growth, every 4 weeks during the tree rest period). The phases recorded were: vegetative (winter) rest, beginning of vegetative growth (bud bursting), beginning of inflorescence development, complete inflorescence development, beginning of bloom, full bloom, fall of petals (end of bloom), fruit set, fruit growth – 1st phase, fruit growth – 2nd phase – pit hardening, fruit growth – 3rd phase, fruit turning (veraison) and fruit ripening.

In order to characterise fruit ripening and oil quality, from mid-September to December, periodically (every 2-3 weeks), fruit detachment force was measured (on about 50 olives/tree) and fruit samples were collected from the labelled trees and used to determine fruit weight (50 drupes/tree), pulp/pit ratio (30 drupes/tree), pigmentation of the pulp (by using 50 olives/tree and a 'pigmentation index', ranging from 0 to 7, with 0 for green olives and 7 for olives with superficial pigmentation of 100% of the epicarp and 100% pigmentation in the pulp), water (by drying the samples used for fresh weight determination in an oven until constant weight) and oil content (by using a Soxelet apparatus and one sample of olives/tree). The samples were also used to extract the oil on which, according to IOOC procedures indicated within parentheses, acidity, peroxide number, spectrophotometer absorbency in ultra-violet (COI/T.20/Doc. n.19), fatty acid composition (COI/T.20/Doc.n.24), sterol composition and content (COI/T.20/Doc.n.10) and organoleptic characteristics (panel test) were determined. The oil was extracted from 2-3 kg of olives using a lab hammer mill; the mash was malaxed for 20 minutes, then it was centrifuged and the oil was filtered through a filter plate. Oil characteristics were evaluated by comparing them with the International Olive Oil Council (IOOC) trade standards for extra virgin olive oils (COI/T.15/NC no. 3/Rev. 1 - 5 December 2003).

The self-compatibility of the different cultivars was also evaluated by isolating some small branches with inflorescences with bags from just before the beginning to the end of flowering.

Results

To simplify the presentation, only the main fruit and oil characteristics that were evaluated in November, which is within the normal harvesting time (October – December) in Syria, are reported.

Notable differences between the cultivars were observed in all the fruit characteristics considered (Table 1). Fresh fruit weight was high for the Kaissy and Safrawi cultivars, medium for Zaity, Dan, Karamani, Sorani and Doebli, and medium-low for Hemplasi, Souri, Khoderi and Doebli. The highest pulp/pit ratios values were recorded for Kaissy, followed by Sorani and Karamani. The level of pigmentation differed among the cultivars. The oil content was high in Zaity, Sorani, Khoderi and Insassy, medium in Karamani, Safrawi and Doebli and relatively low in the other cultivars.

The acidity and peroxide number of the oils of all cultivars were very low (Table 2). The spectrophotometer absorbencies in ultra-violet were also low. Most cultivars had an oleic content of about 70% or higher (Table 3). Only the Karamani cultivar had a lower value (63-64%) that was associated with relatively high amounts of palmitic and linoleic acids. The sterol composition and content were quite different in the cultivars (Table 4). The Khodeiri and Doebli cultivars had a relatively high value of Δ-7stigmastenol. All of the parameter values (acidity, peroxide number, absorbencies in ultra-violet, fatty acid composition, sterol composition and content) used to evaluate oil quality were within the IOOC trade standards. The only exceptions were in cultivars Khodeiri and Doebli that sometimes had Δ-7-stigmastenol values that were higher than 0.5% and in cultivar Insassy that sometimes had a Campesterol content higher than 4.0%. In all the oils the content of β-sitosterol + Δ-5-avenasterol + Δ-5-23-stigmastadienol + clerosterol + sitostanol + Δ-5-24-stigmastadienol was higher than 93.0%, the IOOC trade standard for this parameter (data not shown).

The data collected for each cultivar gave some indications about the best time to harvest in order to optimise both oil quantity and quality (data not shown).

Table 1: Fruit characteristics of the different cultivars. Average values of the two-year period 2004-05 ± standard error.

Area	Cultivar	Fresh weight (g)	Pulp/pit ratio (n)	Pigmentation index (0-7)	Water content (%)	Oil Content (% f.w.)	Oil content (% d.w.)
Aleppo	Kaissy	4.7± 1.1	5.9±1.7	1.0±0.2	56.0±2.0	16.5±4.0	37.5±8.1
	Zaity	2.2±0.3	4.6±0.6	2.2±0.3	43.2±2.1	27.3±2.4	48.1±6.2
Damascus	Dan	2.9±0.3	4.0±0.3	2.2±1.3	53.2±1.5	14.8±2.2	31.6±2.0
	Hemplasi	2.0±0.4	4.5±0.8	1.9±0.6	55.8±2.5	12.7±1.4	28.7±2.8
	Souri	1.9±0.4	3.6±0.4	2.1±0.3	45.6±2.0	16.8±1.9	30.9±2.6
Idleb	Insassy*	2.0±0.3	4.4±0.4	1.9±0.4	50.8±2.3	24.2±2.3	49.9±3.6
	Karamani	3.5±0.4	5.3±0.6	1.6±0.3	52.9±2.8	19.5±4.3	41.4±6.7
	Sorani	3.1±0.4	5.5±0.4	1.9±0.2	44.7±2.4	26.3±1.4	47.6±4.8
Lattakia	Khoderi	2.1±0.5	3.5±0.3	1.8±0.3	38.5±4.5	25.4±3.3	41.3±5.0
Mousiaf	Safrawi*	5.0±0.5	4.4±0.3	2.9±0.4	48.4±2.1	21.6±1.9	41.8±3.1
Tartous	Doebli*	3.4±0.4	3.5±0.5	2.7±0.5	41.3±2.0	21.2±2.2	36.1±4.0

* Average values of 2005, only.

Table 2: Free acidity, peroxide number, spectrophotometer absorbencies in ultra-violet (K 232, K 270, Δk) of oils of the different olive cultivars. Average values of the two-year period 2004-05 ± standard error. The IOOC trade standard (TS) values for extra virgin olive oils are reported in the last line.

Area	Cultivar	Free acidity (%)	Peroxide number (meq O_2/kg oil)	Abs 232	Abs 270	Δk
Aleppo	**Kaissy**	**0.3±0.1**	**4.2±0.2**	**1.54±0.02**	**0.12±0.02**	**0.00±0.00**
	Zaity	**0.3±0.1**	**3.8±0.3**	**1.59±0.13**	**0.13±0.04**	**0.00±0.00**
Damascus	**Dan**	**0.3±0.1**	**5.1±1.2**	**2.04±0.02**	**0.18±0.03**	**0.00±0.00**
	Hemplasi	**0.2±0.0**	**4.0±0.5**	**1.71±0.08**	**0.19±0.02**	**0.00±0.00**
	Souri	**0.3±0.1**	**2.5±0.0**	**1.91±0.27**	**0.17±0.06**	**0.00±0.00**
Idleb	**Insassy***	**0.4±0.1**	**4.8±0.5**	**1.62±0.10**	**0.13±0.03**	**0.00±0.00**
	Karamani	**0.4±0.1**	**5.9±1.5**	**2.15±0.01**	**0.20±0.01**	**0.00±0.00**
	Sorani	**0.4±0.1**	**4.4±0.7**	**1.90±0.16**	**0.20±0.02**	**0.00±0.00**
Lattakia	**Khoderi**	**0.6±0.2**	**6.3±2.1**	**1.78±0.22**	**0.21±0.01**	**0.00±0.00**
Mousiaf	**Safrawi***	**0.6±0.1**	**2.7±0.5**	**1.62±0.05**	**0.12±0.03**	**0.00±0.00**
Tartous	**Doebli***	**0.6±0.1**	**4.7±1.0**	**1.66±0.10**	**0.12±0.05**	**0.00±0.00**
IOOC-TS		**< 0.8**	**≤ 20.0**	**≤ 2.50**	**≤ 0.22**	**≤ 0.01**

*Average values of 2005, only.

Table 3: Fatty acid composition of oils of the different olive cultivars. Average values of the two-year period 2004-05 ± standard error. The IOOC trade standard (TS) values for extra virgin olive oils are reported in the last line.

Area	Cultivar	Palmitic (%)	Palmitoleic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Linolenic (%)	Arachidic (%)
Aleppo	Kaissy	11.9±0.7	0.8±0.3	3.0±0.6	73.2±3.5	9.4±1.8	0.7±0.1	0.4±0.1
	Zaity	14.0±0.2	0.7±0.1	3.6±0.2	74.3±2.1	6.4±2.2	0.6±0.1	0.4±0.1
Damascus	Dan	13.9±0.1	0.6±0.1	2.4±0.1	70.2±0.2	10.1±0.5	0.8±0.1	0.4±0.1
	Hemplasi	12.3±0.1	0.8±0.1	3.0±0.2	74.0±2.9	7.4±2.5	0.9±0.1	0.5±0.1
	Souri	12.4±0.8	0.8±0.3	2.9±0.5	72.6±1.1	9.0±0.3	0.7±0.1	0.5±0.1
Idleb	Insassy*	12.3±0.2	0.4±0.1	3.7±0.2	70.4±0.3	11.7±0.2	0.5±0.1	0.5±0.1
	Karamani	17.7±1.5	2.0±0.5	2.5±0.3	63.5±4.5	12.5±3.3	0.8±0.1	0.5±0.1
	Sorani	14.2±0.9	0.7±0.2	3.7±0.1	69.6±1.9	10.0±1.1	0.8±0.1	0.5±0.1
Lattakia	Khoderi	13.4±1.4	0.4±0.1	4.4±1.1	69.3±0.6	10.4±1.0	0.7±0.1	0.6±0.1
Mousiaf	Safrawi*	12.9±0.3	1.0±0.1	2.7±0.1	70.6±0.6	11.1±0.2	0.5±0.1	0.5±0.1
Tartous	Doebli*	15.4±0.2	0.9±0.1	3.0±0.1	70.0±0.1	12.1±0.1	0.7±0.1	0.5±0.1
IOOC-TS		7.5-20.0	0.3-3.5	0.5-5.0	55.0-83.0	3.5-21.0	≤ 1.0	≤ 0.6

* Average values of 2005, only.

Table 4: Sterol composition and content of oils of the different olive cultivars. Average values of the two-year period 2004-05 ± standard error. The IOOC trade standard (TS) values for extra virgin olive oils are reported in the last line.

Area	Cultivar	Cholesterol (%)	Brassicast-erol (%)	Campesterol (%)	Stigmasterol (%)	β-sitosterol (%)	Δ - 7 - S t i g - mastenol (%)	Total sterol (mg/kg oil)
Aleppo	Kaissy	0.28±0.22	0.03±0.03	3.04±0.03	0.67±0.13	74.0±8.2	0.26±0.06	1356±50
	Zaity	0.08±0.03	0.00±0.00	3.65±0.05	0.55±0.05	85.7±3.1	0.39±0.09	1371±311
Damascus	Dan	0.13±0.08	0.00±0.00	2.98±0.08	0.87±0.17	88.7±3.5	0.24±0.06	1559±295
	Hemplasi	0.07±0.03	0.05±0.05	3.44±0.16	0.42±0.02	81.0±6.8	0.31±0.09	1980±276
	Souri	0.08±0.03	0.00±0.00	2.95±0.05	0.58±0.18	89.8±2.7	0.39±0.02	1388±219
Idleb	Insassy*	0.05±0.03	0.00±0.00	4.35±0.45	0.80±0.10	90.0±1.2	0.44±0.02	1276±110
	Karamani	0.07±0.04	0.00±0.00	2.95±0.16	0.76±0.04	87.5±2.0	0.36±0.04	1238±104
	Sorani	0.13±0.08	0.00±0.00	2.43±0.23	0.81±0.10	85.4±3.1	0.47±0.01	1254±214
Lattakia	Khoderi	0.13±0.08	0.00±0.00	2.53±0.13	1.76±1.15	87.5±2.2	0.74±0.36	1130±25
Mousiaf	Safrawi*	0.05±0.01	0.00±0.00	3.45±0.20	0.72±0.10	90.1±1.6	0.28±0.02	1866±170
Tartous	Doebli*	0.05±0.01	0.00±0.00	2.38±0.20	1.15±0.10	88.8±1.0	0.52±0.05	1267±103
IOOC-TS		≤ 0.50	≤ 0.10	≤ 4.00	< campesterol		≤ 0.50	≥ 1000

* Average values of 2005, only.

Molecular characterization

In recent years, the development of molecular biology has allowed the genetic variation in olive to be examined in much greater detail through the use of the large array of DNA (Fabbri et al., 1995; Angiolillo et al., 1999; Sefc et al., 2000) molecular-marker types and the availability of linkage maps based on DNA markers. Molecular markers such as restriction fragment length polymorphism (RFLP) and the polymorphism of DNA fragments amplified by polymerase chain reaction (RAPD, AFLP, AP-PCR, ISSR, microsatellites), provide a way to discriminate between olive cultivars, independent of any environmental influences or the developmental stage.

The aim of this part of the Cooperation project was to accurately identify the cultivars using suitable molecular markers, such as SSR (Simple Sequence Repeats) and AFLP (Amplified Fragment Length Polymorphism). The molecular analyses were carried out on 42 accessions belonging to 12 olive cultivars selected from six distinct collection sites located in Syria (Damascus, Tartous, Aleppo, Lattakia, Idleb and Hama) in order to verify the presence of inter- and intra-varietal genetic differences. The use of different accessions of the same cultivar allows possible intra-cultivar polymorphism to be identified (i.e. different clones within the same cultivar).

The DNA extraction was performed following a simple protocol called the “DellaPorta protocol” that allows a good quality of DNA to be obtained with simple operations and without the use of many chemical products. The DNA concentration was determined by electrophoresis on 0.8% agarose gel with λDNA standard. About 1000 ng DNA was extracted from 50 µg of leaf tissue. Twenty-two primer pairs of microsatellite markers were tested: DCA1, DCA3, DCA4, DCA5, DCA7, DCA8 (Sefc et al., 2000); GAPU11, GAPU14, GAPU19, GAPU47, GAPU59, GAPU62, GAPU71A, GAPU71B, GAPU72, GAPU82, GAPU89, GAPU90, GAPU101, GAPU103A, GAPU108 and GAPU113 (Carriero et al., 2002). For the AFLP analysis, two primer combinations (P-AGC/M-ACT; P-AGG/M-ACA) with three selective nucleotides were used. PstI primers were radiolabelled with γ-[33P]-ATP. SSR amplification reactions and the AFLP analysis were essentially conducted as described in a previous paper (Montemurro et al., 2005).

The genetic similarity analysis was performed with the NTSYS programme (Rohlf, 1992). Both AFLP and SSR polymorphic bands were scored as either present (1) or absent (0). The SIMQUAL function, was used to compute similarity coefficients for qualitative data using the Jaccard similarity index (Sneath, 1957). The similarity matrix was analysed by the Unweighted Pair Group Method (UPGMA), and the similarity tree was obtained by clustering the similarity data with the SAHN clustering programme.

Results

Twenty-two microsatellite primer pairs were first screened in a panel of eight cultivars to select the markers characterised by a clearly detectable polymorphism under the analysis conditions. Ten primer pairs were then chosen and analysed among the entire set of olive accessions. A total of 45 alleles ranging from two to eight alleles per locus were recorded. Direct count of heterozygosities ranged from 0 (GAPU-108) to 1.00 ssr-DCA4).

The AFLP analysis revealed a total of 120 bands, only 48 of which were considered unambiguous. The average percentage of polymorphism was 36.25%. The AFLPs were able to distinguish all of the selected accessions by using only two primer combinations, excluding: Jlot-1 and Jlot-2.

To compare the efficiency of SSRs and AFLPs, some variability indices were calculated (Table 5) (Kloosterman et al., 1993; Russell et al., 1997). The ratio of polymorphic bands/total bands ranged from a maximum of 100% (SSR) to 36.25% for AFLP. The average number of polymorphic bands per AFLP primer combination was 45, compared to a mean of 4 bands for each SSR primer pair. The Diversity Index (DI) was calculated by $DI=1-\Sigma Pi^2$, where Pi is the genotypic frequency for each assay unit (Russell et al., 1997). The efficiency of AFLPs in providing many bands with just one electrophoretic analysis is reflected in the higher Diversity Index value (87.1%), compared to a D.I. of 72.1% for SSRs, according to Belaj et al. (2003 b).

Table 5: Characteristics of the SSR and AFLP markers used to analyse genetic variability among 42 olive accessions.

Marker	No of primer pairs or primer combinations	Total number of bands	Polymorphic bands/total bands (%)	No of polymorphic bands per assay unit	No of genotypic classes per assay unit	Diversity index (%)
SSRs	10	45	100	4	8.1	72.1
AFLPs	2	120	36.25	24	38.6	87.1

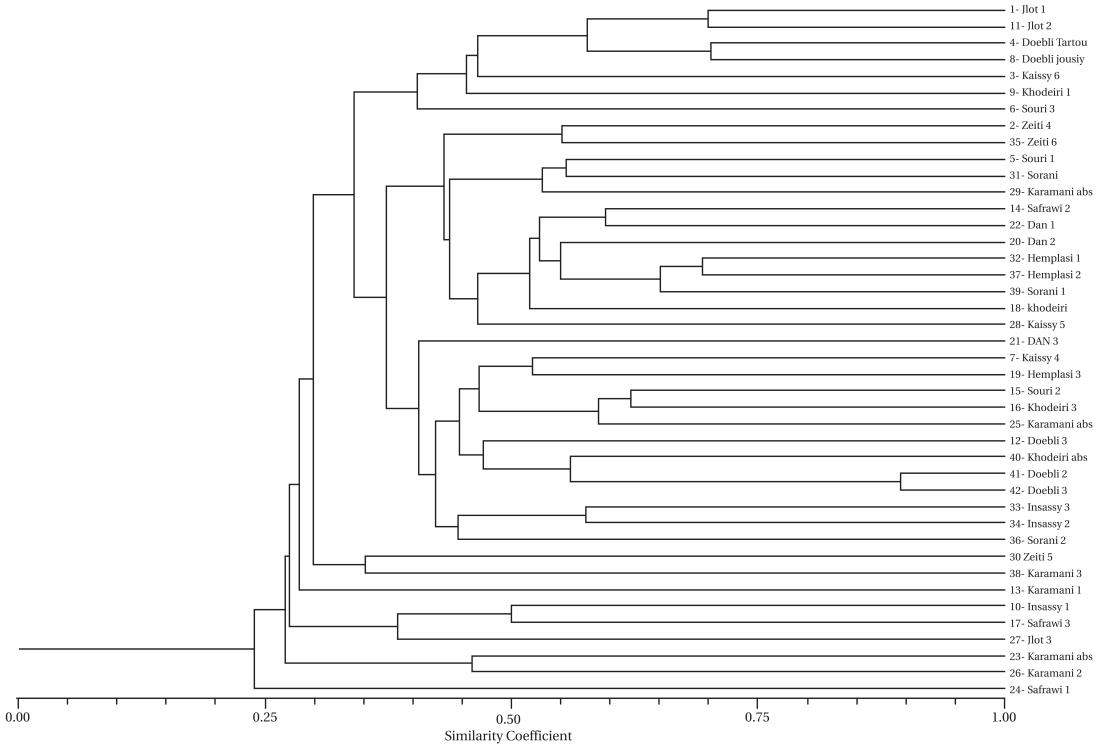

Fig. 1: UPGMA dendrogram obtained using the Jaccard similarity index of 42 Syrian olive accessions.

Considered together the SSR and AFLP data provided a clear distinction among all 42 samples. The phenetic dendrogram (Figure 1) showed clustering among 12 olive cultivars shared in a different sub-cluster. At 0.30, it is the largest cluster comprised of three sub-clusters. The first one in the upper part of the dendrogram grouped seven cultivars from the Jlot1 to Souri 3. The second one is comprised of thirteen samples from Zaity 6 to Kaissy 5. The third group included thirteen cultivars from Dan 3 to Sorani 2. Beside this large cluster in the lower part of the figure, there are several sub-clusters made-up of two or four cultivars (i.e. Zaity 5 and Karamani 1; Insassy 1, Safrawi 3 and Jlot 3; Karamani-absh and Karamani 2). The mean genetic similarity among all 42 accessions tested was 0.62. Individuals that could belong to the same cultivar were not identical and, in a few cases, were grouped together at an average similarity level of 0.70 (‘Jlot 1-2’, ‘Zaity 6-4’, ‘Hemplasi 2, ‘Doebli 2-3’, ‘Insassy 2-3’).

Conclusions

The fruit fresh weight and oil content in Zaity, Sorani and Kaissy were similar to values reported in the literature for the same cultivars (Nseir et al., 1985; Tubeileh et al., 2004 a,b,c). The cultivar Dan showed a lower value than that reported by Tubeileh et al. (2004 c). The high oil content in Zaity, Sorani and Khoderi shows their high efficiency in accumulating oil in the fruit and confirm the high ability of these cultivars in producing oil and is in agreement with the fact that these cultivars together with Doebli are the main ones for oil production in Syria (Al Ibrahem, 2006). The medium or high fresh fruit weight and pulp/pit ratio as well as the moderate oil content of the varieties Kaissy, Karamani and Safrawi

confirm their suitability to be used as dual purpose cultivars. The relatively low oil content in the cultivars Dan, Hemplasi and Souri are probably due to the dry environmental conditions of the Damascus area. However, for these cultivars, in some cases, increases in the oil content can be observed with later harvesting dates (data not shown).

It should be noted that all the oils produced by the olive cultivars met the IOOC trade standards applied to extra virgin olive oils. The only exceptions were cultivars Khoderi and Doebli that sometimes had excessively high Δ-7-stigmastenol levels and cultivar Insassy which sometimes had excessively high Campesterol contents. Further evaluation to determine if environment and/or harvesting time affect Δ-7-stigmastenol content are in progress. The overall results on oil characteristics are very important considering that in Syria increasing amounts of oil will be available for export in the next years.

As far as molecular characterization is concerned, no ambiguous cases of synonymy were found. This means that all of the cultivars examined were different from each other. The cultivars were able to be distinguished even when they originated from the same area. In fact, the dendrogram did not show any particular aggregation related to the geographic site of origin of the considered olive cultivars, as was reported by Carriero et al. (2002), Angiolillo et al. (1999) and Claros et al. (2000). On the other hand, the marked genetic variability observed among the 42 samples indicated a situation of "cultivar populations", that is, the presence of different clones within the same cultivar. This situation was found in all the cultivars considered. The results of this study have provided important information about Syrian olive germplasm. Until now, only a few studies on very limited sample sets have been carried out (Belaj et al., 2002; Hatzopoulos et al., 2002; Belaj et al., 2003 a; Owen et al., 2005).

All the data and information collected for the characterization of the main Syrian olive varieties have been used to produce a book "Characterization of the main Syrian olive cultivars" in which a detailed description of each cultivar is given. Part of data were also published in Jibara et al. (2006) and Montemurro et al. (2006).

Based on the ample amount of data and information collected, the following observations and recommendations can be made.

i) The cultivars taken into account give fruit that presents good characteristics to be used for the production of oil (high oil content) and/or to be processed as table olives (high fruit size and pulp/pit ratio and, in some cases, moderate oil content). Moreover, the oil quality of most of the cultivars considered meet the IOOC trade standard applied to extra virgin olive oils and so the oil can be easily exported. Finally, the medium-sized fruit of all the cultivars utilizable for oil production make them suitable for mechanical harvesting. This is important because the availability of manpower will probably decrease in the future, while the cost will increase. All these good characteristics make most of these cultivars still highly recommended in the establishment of new olive orchards (for details see the book "Characteristics of the main Syrian olive cultivars").

ii) Since the values for Δ-7-stigmastenol were sometimes too high in cultivars Khoderi and Doebli, samples must be carefully checked when the oils are going to be marketed. If the value is too high, the oils should be blended with other oils. In Lattakia and Tartous areas, where Khoderi and Doebli are the main cultivars, experiments to evaluate also the behaviour of other varieties that could allow to overcome the Δ-7-Stigmastenol problem could be useful.

iii) Since the values for Campesterol were sometimes too high in cultivar Insassy, samples must be carefully checked when the oils are going to be marketed. If the value is too high, the oils should be blended with other oils. However, the cultivar Insassy is a minor variety that usually is cultivated together with other varieties and so it is not frequent to have pure Insassy oil.

iv) The collected morphological and molecular data and information allow a clear identification of the considered cultivars. This represents a very important step towards the certification, from a genetic point of view, of the new plants produced in olive nurseries.

v) The presence of different clones within the same cultivar (resulted from molecular analysis) indicates that it is important to select within the "cultivar tree population" the best trees to produce the mother plants from which to collect materials to propagate new trees. This situation also suggest that programmes for a full characterization (taking into account morphological, phenological, bio-agronomical and oil quality traits) and selection of the different clones should

be carried out in order to individuate the best ones, that then should be preferred at propagation level and so in the establishment of new orchards.

vi) The high quantity of collected data and information on morphological, phenological, bio-agronomical, oil quality and molecular characteristics allows the drawing of a good picture of each cultivar, giving the opportunity to use in a more profitable way the different varieties within programmes for genetic improvement of the olive.

vii) Considering the good results obtained with the main Syrian olive cultivars, it would be useful to extend the investigation to minor varieties that are very numerous in Syria, because these genotypes could have peculiarities useful from an agronomical point of view (resistance to biotic and abiotic stresses particularly useful for integrated and organic cultivations, high production of oil, etc.) and/or from a commercial point of view (i.e. oils with peculiar qualitative characteristics that could provide a variety of production) and so they could represent a very important resource for a positive evolution of the olive sector in the future. It would also be useful to extend the investigation to varieties mainly utilisable for table olive production.

In conclusion, the activities carried out within the cooperation project "Technical Assistance for the Improvement of Olive Oil Quality in Syria" have provided a good characterization of the main Syrian olive cultivars, that is useful for the improvement of the entire sector. As a matter of fact the results give useful indications for the cultivar identification, the establishment of new olive orchards, the obtainment of oils that meet the qualitative standards of international markets, the choice of trees to use as mother plants in nurseries, the design of programmes for olive genetic improvement (clone selection, breeding). The knowledge of the genetic variation and genetic relationships between olive cultivars by means of molecular markers are particularly useful for a more efficient management of germplasm resources and for breeding purposes. Further investigations to characterise also minor Syrian varieties and cultivars for the production of table olives would be useful for an optimal evolution of the olive sector in Syria.

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