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Cultivar Identification in Olive Based on RAPD Markers

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Abstract. One hundred and thirteen olive (*Olea europaea* L.) accessions were characterized using randomly amplified polymorphic DNA (RAPD) markers. Forty-five polymorphic RAPD markers were obtained enabling us to distinguish 102 different RAPD profiles. The approximate estimation of the probability of obtaining the same RAPD profile for two different trees was between 6.75×10^{-5} and 4.82×10^{-14}. A dendrogram was constructed using Ward's minimum variance algorithm based on chi-square distances. This led to a more clear-cut classification of profiles than the classical approach of unweighted pair group method with arithmetic average. Twenty-four clusters of RAPD profiles were shown in Ward's dendrogram. Reliability of the dendrogram structure was checked using variance analysis. RAPD data exhibited an acceptable resolving power for cultivar identification. A combination of three primers was proposed for rapid molecular identification of cultivars in collections and in nurseries.

Olive (*Olea europaea*) is of great socioeconomic importance in the Mediterranean basin. Its cultivation is expanding because of increased demand for olive oil. A great number of olive cultivars (presumed clones) are grown throughout the world. Several hundred supposedly clonal accessions are described in the main countries of the Mediterranean basin. In France about 150 cultivars are registered (Andlauer, 1997). During the history of olive cultivation, as for other tree species, different cultivars may have been given the same name, whereas a cultivar may have been named differently in different countries. Therefore, cultivar and denomination (usual or local name) are ambiguous terms.

Vegetative propagation of trees of agronomic interest has produced numerous clones (Zohary and Hopf, 1994). Consequently, genetic uniformity within a given cultivar, and thus, within a denomination, is expected. Cultivar identification based on phenological and morphological phenotypes from field or nursery observations may not be adequate to assign cultivar identity due to environmental effects on traits. Moreover, the mode of inheritance for most traits used for characterization is not known. Traditionally, fruit traits appeared the most efficient for cultivar differentiation and identification. However, identification of young trees is difficult because of juvenility and the absence of fruit, which provide the best morphological descriptors. The usual identification method is phenotypic characterization following the principles of pomology (Barranco and Rallo, 1984; Prevost et al., 1993), however, biochemical markers (Ouazzani et al., 1995; Pontikis et al., 1980; Trujillo et al., 1995), and molecular markers such as RAPD markers (Bogani et al., 1994; Fabbri et al., 1995) or amplified fragment length polymorphism (AFLP) markers (Angiolillo et al., 1999) might be used for germplasm characterization in olive. Furthermore, Gregoriou (1996) and Wiesman et al. (1998) showed that genetic variability could occur within some cultivated populations, sometimes called landraces. For example, under one denomination, several RAPD profiles corresponding to different clones have been shown for 'Nabali' olive (Wiesman et al., 1998). Heterogeneity in production and quality traits may result from this kind of variable genetic basis of cultivars.

Today, the prevailing goal of quality in oleiculture demands development and control of high quality appellations for canning and oil production. New appellations are undergoing registration in European Community countries. Production by specific denominations corresponding to well-defined cultivars is thus necessary. Quality control requires identification of orchard-derived clones, distributed by nurseries. Furthermore, in olive breeding programs, it is important to identify the parents accurately, and further, to distinguish new cultivars for registration purposes. Lastly, in the management of cultivar collections, it is necessary to identify each clone in order to detect any possible synonyms, mislabeling, and mutants. Thus, cultivar identification is a prerequisite to starting and managing a breeding program, and ensuring against illegal use of cultivars. Identifying the cultivar also makes it possible to determine, most of the time, in which country or region the oil was produced.

The present investigation was undertaken using RAPD markers on a set of olive cultivars sampled from different countries around the Mediterranean basin. These markers enabled us to differentiate and to characterize cultivars. An optimum strategy combining a subset of markers is proposed for rapid molecular identification of clones in collections and in nurseries.

Materials and Methods

Plant material used in genetic diversity study. One hundred and thirteen accessions including 263 from different collections and from various orchards were sampled (Table 1) and analyzed. More detailed information about this material can be obtained from the authors. An accession was defined by the common name given to a clone (cultivar) or a group of clones. To assess genetic diversity in some cultivars, several trees were characterized in 24 accessions (Table 1).

Marker procedures. The DNA extraction protocol has been described by Besnard et al. (2000).

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Table 1. Codes of the clones identified with RAPD markers.

Profile code	Clone and origin[z]
53	Aglandau (Fr) (2)[v,u]
66	Amellau (Fr) (3)[p]
6	Amygdalolia (Gr)[u]
85	Arbequina (Sp) (3)[x,u,p]
20	Ascolana Tenera (It) (2)[x,w]
60	Aubenc (Fr)[v]
9	Ayvalik (Tk)[x]
95	Azeradj (Al)[o]
14	Barnea (Is)[s]
100	Barouni (Tu)[u]
48	Berdaneil (Fr)[v], Poumal (Fr)[v]
34	Biancolilla (Its)[w]
43	Blanquetier d'Antibes (Fr)[p]
76	Blanquetier de Nice (Fr)[p]
41	Bouteillan (Fr)[x]
44	Cailletier (Fr)[u]
82	Capanacce (Frc)[q]
5	Carolia (Gr)[u]
29	Cassanese (It)[w]
57	Cayet Rouge (Fr)[v]
45	Cayon (Fr) (2)[v,u]
54	Celounen (Fr)[v]
92	Chemlal (Al) (3)[x,u,o]
93	Chemlal (Al)[o]
94	Chemlal Mechtrass (Al)[o]
101	Chemlali (Tu)[v]
97	Chetoui (Tu)[x]
63	Colombale (Fr)[v]
67	Corniale (Fr)[p]
83	Cornicabra (Sp)[x]
56	Coucourelle (Fr)[v]
55	Courbeil (Fr)[v]
62	Curnet (Fr)[v]
25	Dolce Agogia (It)[w]
8	Domat (Tk)[x]
71	Dorée (Fr)[p]
86	Empeltre (Sp)[x]
78	Filayre Rouge (Fr)[p]
22	Frantoio (It) (2)[x,w], Cellina (It), Ghjermana (Frc)[t]
3	Gaïdourolia (Gr)[x]
91	Galega (Pt)[x]
23	Giarraffa (It)[w]
52	Grapié (Fr)[v]
77	Grossane (Fr)[u]
15	Kaissy (Sy)[x]
1	Kalamata (Gr) (2)[x,w]
4	Koroneiki (Gr) (3)[x,v,u]
26	Leccino (It)[w]
84	Lechin de Sevilla (Sp) (3)[x,u,t]
30	Leucocarpa (It)[w]
40	Lucques (Fr) (20)[x,u,p]
59	Malaussena (Fr)[v]
89	Manzanilla (Sp)[u]
17	Merhavia (Is)[x], Belgentier (Fr)[u]
99	Meski (Tu)[u], Bid el hamam (Tu)[u]
28	Moraiolo (It)[w], Cayet Bleu (Fr)[v], Ghjermana (Frc)[q], Aliva Nera (Frc)[q]
36	Moresca (Its)[w]
13	Nabali Mohassen (Is)[r]
50	Négrette (Fr)[v]
24	Nocellara del Belice (It)[w]
38	Nocellara Etnea (Its)[w]
51	Noirette (Fr)[v]
19	Oblica (Yu)[x]
37	Ogliarola Messinese (Its)[w]
42	Olivière (Fr) (10)[u,p]
35	Passalunara (Its)[w]
21	Pendolino (It) (2)[x,w]
39	Picholine (Fr) (80)[x,u,p]
73	Picholine de Rochefort (Fr)[p]
102	Picholine Marocaine (Mo)[x], Sigoise (Al)[x], Shimlali (Is)[s], Canivano Blanco (Sp)[x]
87	Picual (Sp)[x]
72	Pigale (Fr) (8)[p]
64	Poulo (Fr)[v]
58	Rascasset (Fr)[v]
49	Redouneil (Fr)[v]
61	Reymet (Fr)[v]
68	Rougette de Pignan (Fr)[p]
65	Rousset (Fr)[v], Verdale de l'Hérault (Fr)[p]
79	Sabina (Frc) (2)[t,q], Aliva Bianca (Frc)[q], Biancaghia (Frc)[q]
80	Sabina (Frc)[q]
46	Salonenque (Fr) (2)[v,u]
27	San Felice (It)[w]
32	Santagatese (Its)[w], Nabali Baladi Baka (Is)[r], Souri Cadouri (Is)[r]
75	Sauzin (Fr)[p]
90	Sevillenca (Sp)[x]
10	Sofralik (Tk)[u]
11	Souri (Is) (9)[x,r], Nabali (Is) (2)[r]
12	Souri Mansi (Is)[r]
96	Taksrit (Al)[o], Limli (Al)[o]
74	Tanche (Fr) (2)[u,p]
18	Toffahi (Eg)[x]
33	Tonda Iblea (Its)[w]
7	Uslu (Tk)[x]
2	Vallanolia (Gr)[x]
47	Verdanel (Fr)[p]
70	Verdelé (Fr)[p]
69	Vermillau (Fr)[p]
88	Villalonga (Sp)[x]
31	Zaituna (Its)[w]
16	Zaity (Sy)[x]
98	Zarazi (Tu)[u]
81	Zinzala (Frc) (3)[q]

[z]The origin of the accessions is in parentheses: Al = Algeria; Eg = Egypt; Fr = France; Frc = Corsica, France; Gr = Greece; Is = Israel; It = Italy; Its = Sicily, Italy; Mo = Morocco Pt = Portugal; Sp = Spain; Sy = Syria; Tu = Tunisia; Tk = Turkey; and Yu = Yugoslavia).

[y]The number in parentheses represents the number of trees analyzed for each accession and the following footnotes indicate the source of trees or DNAs.

[x]OGB C = Olive Germplasm Bank, Cordoba, Spain.

[w]IRO P = Institute of Olive Research, CNR, Perugia, Italy.

[v]CBNMP = Conservatoire Botanique National Méditerranéen de Porquerolles, France.

[u]INRA M = Institut National de Recherche Agronomique, domaine de Melgueil, Montpellier, France.

[t]INRA-CIRAD SG = Institut National de Recherche Agronomique and CIRAD, St. Guilano, Corsica, France.

[s]NYRC = Newe-Ya'ar Research Center, Ramat Yishay, Israel.

[r]Collected by R. Assaf (Newe-Ya'ar Research Center).

[q]DNAs provided by V. Bronzini de Caraffa (Corte University).

[p]Collected in production orchards in France.

[o]Collected by A. Ouksili (Tizi Ouzou University).

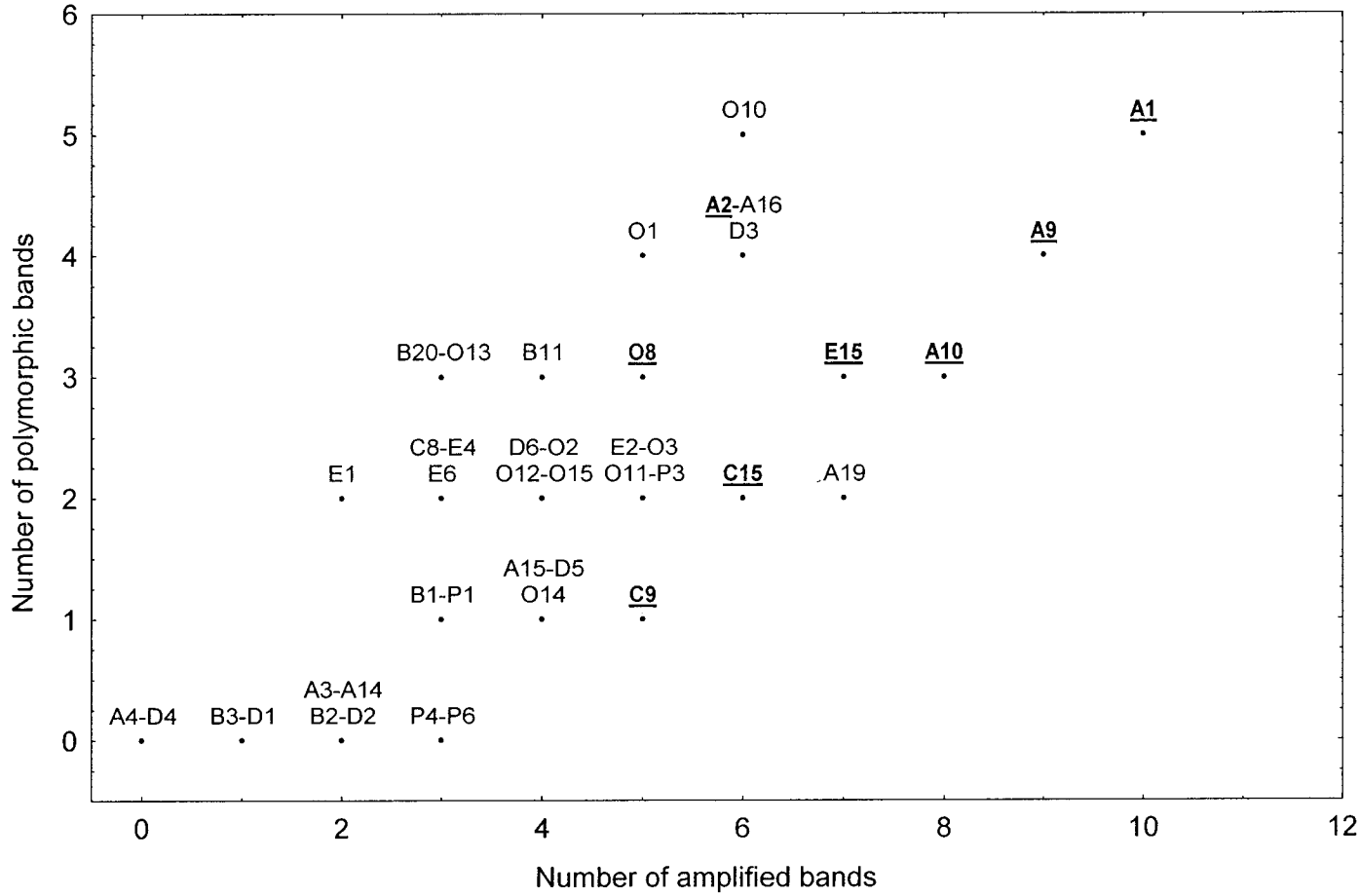


Fig. 1. Number of amplified bands plotted against the number of polymorphic bands for each primer applied on the DNAs from 'Olivière ', 'Lucques', 'Domat', 'Giarraffa' and 'Arbequina' olive. The primers retained in our study are in bold and underlined.

RAPD ANALYSIS. The procedure for RAPD markers has been described by Quillet et al. (1995). Forty-three decamer primers from Bioprobe (Paris, France) (Fig. 1) were tested on five cultivars: Olivière, Lucques, Giarraffa, Arbequina and Domat. The choice of these cultivars was based on their distinct geographic origins and on their morphological variability. The choice of primers, for further analysis of all the samples, was based on the number of amplified fragments with polymorphisms and on the clarity of the electrophoretic profiles. Eight decamers (A1, A2, A9, A10, C9, C15, E15, and O8) were used to characterize all samples (Table 2). We verified that several DNA preparations from one tree and several independent amplifications from one DNA sample led to similar RAPD profiles. Two amplifications for each accession were performed and only the reproducible, well-separated and intense fragments were retained.

MITOTYPES. The method used to display mtDNA restriction fragment length polymorphism (RFLP) markers has been described previously in Besnard et al. (2000). Two restriction enzymes, HindIII and XbaI, and two mitochondrial probes were used in pairwise combination to screen for polymorphisms: cox3 from *Oenothera biennis* L. (Hiesel et al., 1987) and atp9 from maize (*Zea mays* L.) (Dewey et al., 1985).

DATA ANALYSIS. OPEP software (Baradat and Labbé, 1995) and SPAD software, release 3.5 (Lebart et al., 1997), were used for data analyses.

DENDROGRAM CONSTRUCTION AND STABILITY OF THE CLUSTERS. Two RAPD profile dendrograms were constructed and compared using two different approaches. First, Nei and Li (1979) distances (D_{ij}) between pairs of individual were computed: $D_{ij} = n_{ij}/n_i + n_j$, where n_{ij} is the number of common bands in individuals i and j, and n_i and n_j are the number bands in individuals i and j, respectively. At first, we used the unweighted pair group method with

Table 2. Codes and sequences of the RAPD primers used and fragment sizes of the generated RAPD markers.

Primer	Sequence	Fragment size (bp)
A1	CAGGCCCTTC	225, 275, 300, 525, 800, 825, 850, 1000, 1200
A2	TGCCGAGCTG	450, 475, 480, 500, 650
A9	GGGTAACGCC	225, 275, 625, 650, 675, 700, 950
A10	GTGTCGCAG	400, 625, 750, 875, 1050, 1250
C15	GACGGATCAG	400, 425, 675, 950, 1100
E15	ACGCACAACC	700, 950
C9	CTCACCGTCC	450, 500, 750, 1000, 1050, 1100, 1150
O8	CCTCCAGTGT	200, 550, 1025, 1050

arithmetic average (UPGMA) (Benzécri, 1973) to aggregate Nei and Li distances (Nei and Li, 1979) between accessions to obtain a dendrogram. The clustering of profiles was also performed and displayed in a dendrogram following the minimum variance algorithm of Ward (1963) based on the 44-dimensional space chi-square distances computed by a multiple correspondence analysis. Saporta (1990) and Lebart et al. (1997) have described this algorithm in a generalized form. Its principle is to cluster profiles or groups at each step by keeping a maximum value of the ratio intergroup sum of squares : total sum of squares.

Stability of the overall dendrogram pattern of Ward (1963) was assessed by 1) subdividing the position of its nodes into four different nested classes (a, b, c, and d by decreasing order, e being the error term) and 2) using a nonorthogonal analysis of variance model for partitioning the sum of squares of the coordinates of the 102 profiles on the 44 axes of the correspondence analysis where $y_{ijklm} = \mu + a_i + b_{ij} + c_{ijk} + d_{ijkl} + e_{ijklm}$, with the corresponding variances: σ^2_a, $\sigma^2_{b/a}$, $\sigma^2_{c/b}$, $\sigma^2_{d/c}$, and σ^2_e. The three following intraclass correlation coefficients, t_1, t_2, and t_3, express the stability of the structure corresponding to each hierarchical level, when the upper levels are considered as fixed. As shown by Kempthorne (1957), t, is the expectation of the usual correlation between two profiles randomly chosen within the same class and observed for same trait.

$t_1 = \sigma^2_a/(\sigma^2_a + \sigma^2_{b/a} + \sigma^2_{c/b} + \sigma^2_{d/c} + \sigma^2_e)$

$t_2 = \sigma^2_{b/a}/(\sigma^2_{b/a} + \sigma^2_{c/b} + \sigma^2_{d/c} + \sigma^2_e)$

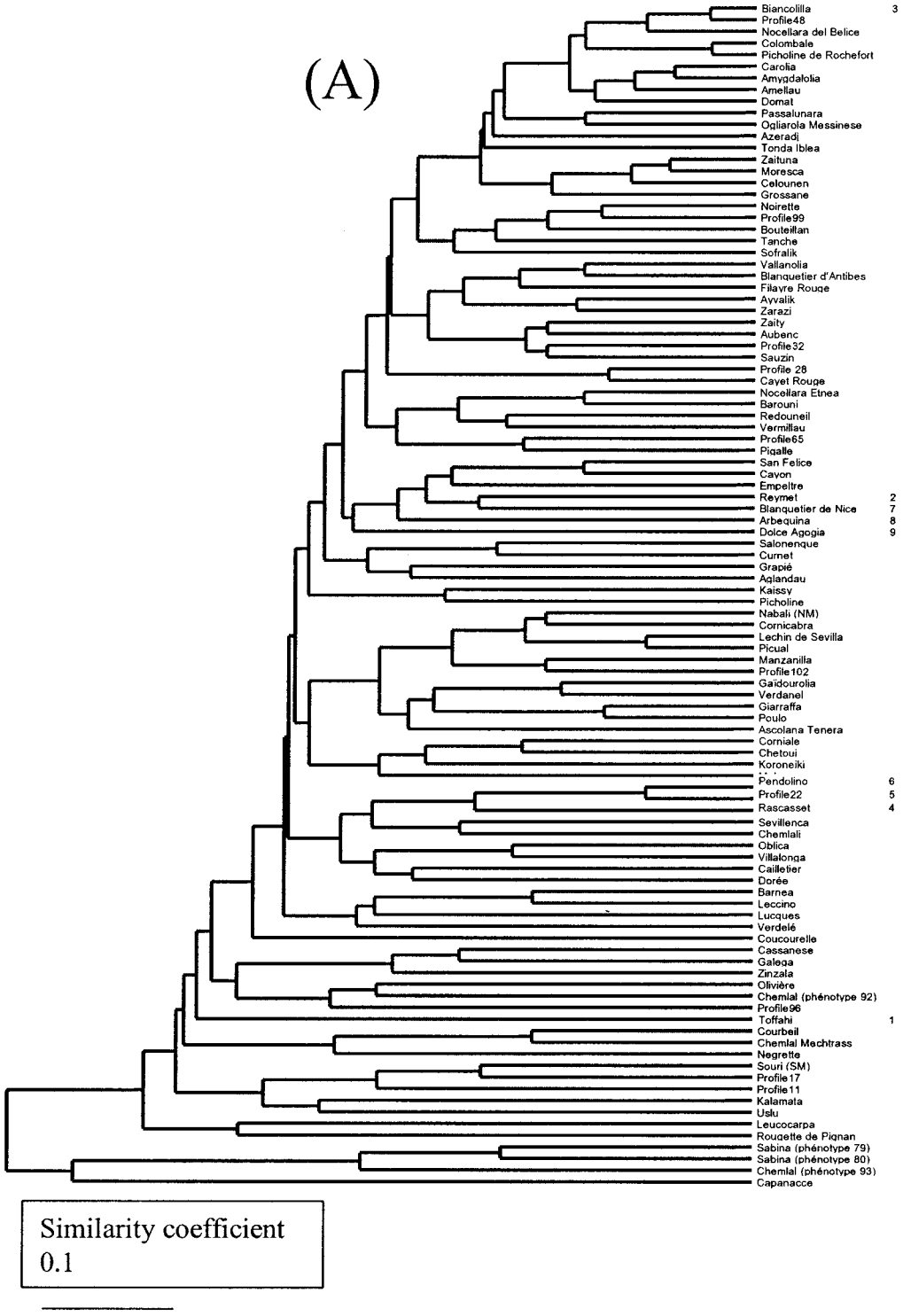
$t_3 = \sigma^2_{c/b}/(\sigma^2_{c/b} + \sigma^2_{d/c} + \sigma^2_e)$

$t_4 = \sigma^2_{d/c}/(\sigma^2_{d/c} + \sigma^2_e)$

SE values on the estimates of t, were computed by the jackknife method (Lebart et al., 1997; Shao and Tu, 1995) with 101 degrees of freedom. This method was developed to allow comparison with other techniques comparing overall topology of dendrograms as with the procedure described by Zharkikh and Li (1995). Such methods are more adapted to choosing the most likely structures among a series of relatively simple dendrograms for testing the likelihood of a limited number of phylogenetic hypotheses. Our purpose herein was to depict the most stable portions of a quite complex tree and not to test alternative hypotheses.

Probability of no discrimination between clones. The probability, $P\ i/C\ i\ \varepsilon\ G\ k$, was estimated that the i^{th} RAPD profile could be met in the same group, G_k. This is the probability of no distinction of different genotypes on the basis of their RAPD profiles, and is the

Fig. 2. Dendrogram of the cultivars (**A**) based on Nei and Li (1979) distances and constructed with the UPGMA algorithm and (**B, see page 672**) based on chi-square distances was constructed with the minimum variance algorithm (Ward, 1963). The two main branches defined the two groups 1 and 2. The numbers on the extreme right indicate which profiles are compared in the text. The origin of the accessions is in brackets: Al = Algeria; Eg = Egypt; Fr = France; Frc = Corsica, France; Gr = Greece; Is = Israel; It = Italy; Its = Sicily, Italy; Pt = Portugal; Sp = Spain; Sy = Syria; Tu = Tunisia; Tk = Turkey; Yu = Yugoslavia; and ? = uncertain origin.



product of the average frequencies of presence (F_j) or absence ($1 - F_j$) of each marker within the group of profiles considered: $P\ i/C\ i\ \varepsilon\ G\ k = \pi\ jk\ f\ jk$ where f_{jk} is the marker frequency within the group G_k: either F_{jk}, if it is present in the profile C_i, or $1 - Fjk$ if it is absent in this profile. This formula assumes an independent association between markers in the considered group of profiles. This may be considered only as an approximation due to correlation between some markers (Besnard et al., 2001).

Discriminating power of the markers. Discriminating power (D) for each primer and for each mitotype was determined according to Tessier et al. (1999). D is the probability that two different randomly chosen profiles of a given group would appear different. A higher value of D corresponds to a better discriminating efficiency. For the j[th] primer, we have $D\ j = 1 - \sum_i p_i(\mathrm{N}\ p_i - 1)/(N - 1)$, where p_i is the frequency of the i^{th} profile and N is the number of final profiles.

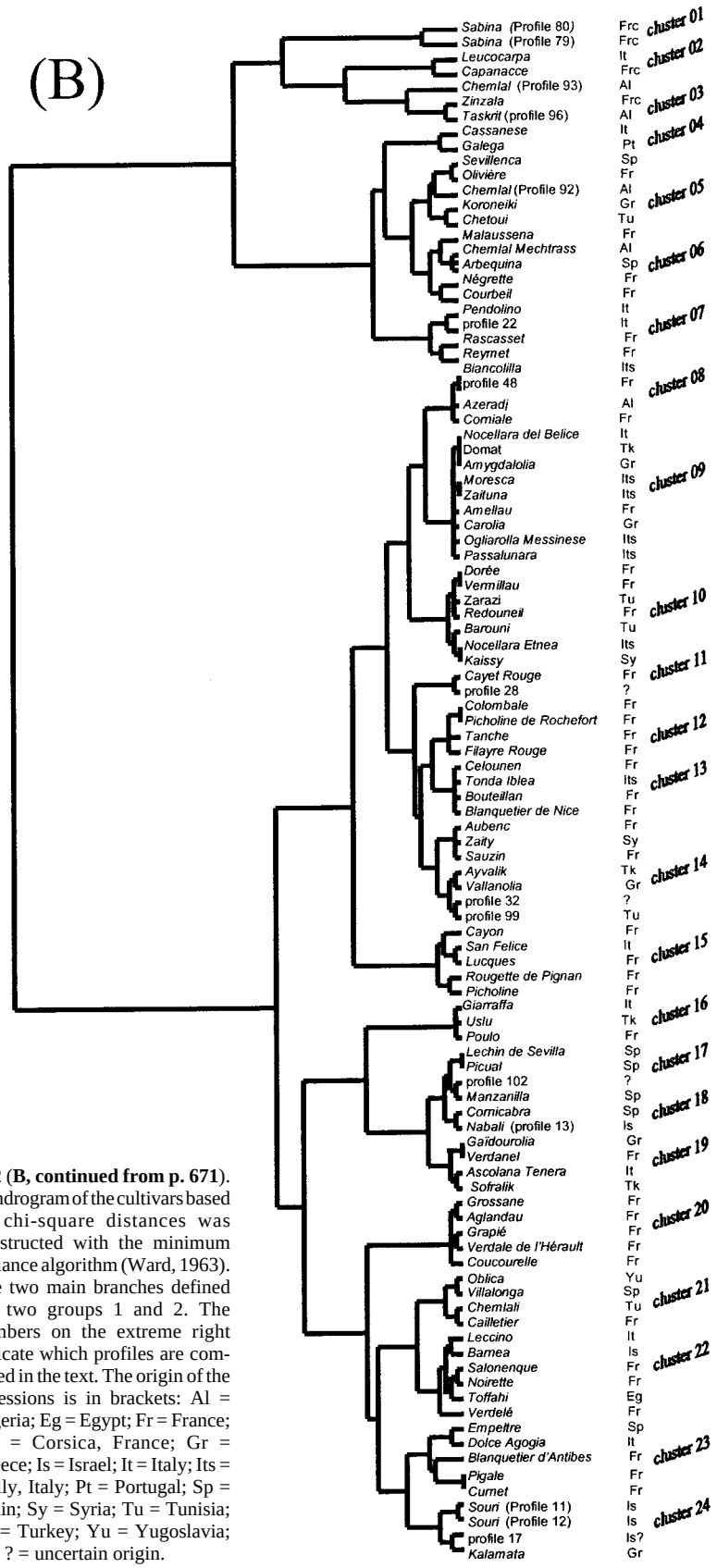


Fig. 2 (**B, continued from p. 671**). Dendrogram of the cultivars based on chi-square distances was constructed with the minimum variance algorithm (Ward, 1963). The two main branches defined the two groups 1 and 2. The numbers on the extreme right indicate which profiles are compared in the text. The origin of the accessions is in brackets: Al = Algeria; Eg = Egypt; Fr = France; Frc = Corsica, France; Gr = Greece; Is = Israel; It = Italy; Its = Sicily, Italy; Pt = Portugal; Sp = Spain; Sy = Syria; Tu = Tunisia; Tk = Turkey; Yu = Yugoslavia; and ? = uncertain origin.

Results

Choice of primers, characterization, and clustering of cultivars

Primer screening. Out of 43 primers utilized, 33 displayed polymorphisms. One hundred and seventy-seven fragments were amplified with an average of 4.1 fragments per primer. They led to 82 RAPD markers (46% of the total fragments). Eight primers, i.e., A1, A2, A9, A10, C9, C15, E15, and O8, were retained for their high level of polymorphism and for their profile cleanliness, i.e., when fragments were intense and well-separated (Table 2, Fig. 1). Difficulties in reading some fragments of E15 profiles were also observed with different DNA samples. Consequently, we eliminated those fragments and therefore only two E15 RAPD markers were retained. Forty-five RAPD markers were finally used to differentiate the profiles in this study. Each of the three fragments was unique to one cultivar in our sample: A2-475 for 'Zaity', A9-625 for 'Lechin de Sevilla', and E15-950 for profile 80 ('Sabina'). Consequently, the 113 accessions including 262 trees displayed 102 RAPD profiles.

Clustering cultivars by their RAPD profiles. One hundred and two profiles were distinguished (Table 1). Several profiles were found in 'Sabina', 'Ghjermana', 'Chemlal', 'Souri' and 'Nabali' olive. In contrast, a single RAPD profile was found in different accessions. This occurred for profiles 11, 17, 22, 28, 32, 47, 48, 65, 79, 99, and 102 (referenced in Table 1). Dendrograms of the 102 profiles are shown in Fig. 2A and B, and were constructed according to the UPGMA and the method of Ward (1963), respectively. However, the clusters were different: the clear-cut separation into two groups with the method of Ward (1963) is not present with UPGMA. Furthermore, some accessions grouped tightly with Ward's (1963) method [i.e., 'Reymet' (2), 'Rascasset' (4), and 'Pendolino' (6)] are separated in different clusters with UPGMA; 'Toffahi' (1) that clustered with five other accessions (cluster 22) using Ward's (1963) is isolated with UPGMA. Also, in contrast to the UPGMA tree, a cluster including 'Arbequina' (8), 'Dolce Agogia' (9) and 'Blanquetier de Nice' (7) was not present in the dendrogram generated by Ward's (1963) method.

Table 3. Comparison of the discriminating efficiency (*D*) for RAPD markers, mitotypes, and isozymes.

Marker technology	Markers (no.)	Profiles (no.)	*D*	Reference
Primer (RAPD				
A1	9	62	0.977	From 102 profiles (this study)
A2	5	15	0.887	
A9	7	25	0.894	
A10	6	19	0.872	
C9	7	14	0.528	
C15	5	10	0.622	
E15	2	3	0.368	
O8	4	10	0.808	
Mitotypes	4	4	0.406	From 102 profiles (Besnard et al., 2001)
Isozymes 1				
LAP1	3	5	0.463	From 47 cultivars (Ouazzani et al., 1995)
EST1	3	3	0.162	
EST2	2	2	0.342	
ADH1	4	3	0.376	
MDH2	2	2	0.511	
PGI2	4	6	0.692	
PXI	2	2	0.194	
Isozymes 2				
ADH	2	2	0.055	From 143 cultivars (Trujillo et al., 1995)
ME	17	25	0.922	
EST	8	22	0.895	
GPI	14	23	0.913	
LAP	6	9	0.751	

However, 'Biancollila' (3) was still grouped with the same profiles using the two methods.

Consequently, the two approaches of clustering led to very different structures. We tried to find different biological properties, i.e., fruit for canning, or fruit for oil, or country of origin for cultivars. So, we verified whether a priori groups sharing such a trait could be revealed in the clusters of the two trees. Ward's (1963) method tended to group cultivars from the same geographic origin. We also preferred this method because the approach eliminates information redundancy by use of correspondence analysis and defines homogeneous groups. For assessing stability with Ward's (1963) method, the following three levels were defined on the basis of the 24 elementary clusters (fourth level); and Level 1: (1 to 7) and (8 to 24); Level 2: (1 to 3), (4 to 7), (8 to 15), (16 to 19), and (20 to 24); Level 3: (1), (2 and 3), (4 to 6), (7), (8 to 10), (11 to 14), (15), (16), (17 to 19), (20), (21,22), and (23,24). The intraclass correlation coefficients corresponding to the four levels are given with their 95% confidence intervals in brackets. Level 1: $t_1 = 0.128$ (0.057–0.199); Level 2: $t_2 = 0.047$ (0–0.110); Level 3: $t_3 = 0.131$ (0.02–0.243); and Level 4: $t_4 = 0.244$ (0.152–0.336). This shows that the most reproducible parts of the structure are the extreme levels of hierarchy (the two main classes and the 24 elementary clusters). This conclusion was also verified after building of the 102 partial dendrograms obtained by deleting one profile at a time (data not presented). In the dendrogram, some groups of profiles corresponding to limited geographic zones clustered together. This occurred for four profiles from Corsica, five from Andalusia, and six from Sicily. In contrast, the profiles from other countries appeared scattered into subgroups with rather low similarities. For instance, the profiles from continental France were spread in most of the 24 elementary clusters. Other countries showed an intermediate pattern; for instance, Spain, with eight profiles in four elementary clusters.

Probability of nondiscrimination of a profile. The probabilities of no distinction of a particular profile within a group, $P\ i/C\ i\ \varepsilon\ G\ k$, were computed within the two main groups of Ward's (1963) dendrogram (Fig. 2B), which exhibited great stability. The P_i values were comprised between 6.75×10^{-5} (profile 36) and 4.82×10^{-14} (profile 80). For the profiles for which several accessions were attributed, P was comprised between 1.08×10^{-5} and 9.94×10^{-11}. They probably refer to the same clone.

Definition of a routine tool for germplasm characterization. The discriminating efficiency (*D*) was computed for each primer, for each mitotype, and also for each isozyme developed by Ouazzani et al. (1995) and Trujillo et al. (1995) (Table 3). For primers (*D* mean = 0.745) and isozymes developed by Trujillo et al. (1995) (*D* mean = 0.870 without ADH), it was higher than for isozymes developed by Ouazzani et al. (1995) (*D* mean = 0.391) and for mitotype (*D* = 0.406). With the combination of primers (A1-A9-A10) and a mean number of 8.76 RAPD markers/clone, no confusion appeared among the 102 clones.

Discussion

Synonymy. RAPD markers enabled us to distinguish most of the accessions. The probability of finding the same profile for two different cultivars is low, and multiple accessions with the same profile are probably synonymous or mislabeled. 'Meski' and 'Bid el Hamam' were not discriminated using RAPD markers but were clearly identified on the basis of fruit morphology. This means that difference between the two cultivars is small at the DNA level. Similar observations have been reported with RAPD markers for vegetatively propagated grape (*Vitis vinifera* L.) (Tessier et al., 1999) and fig (*Ficus carica* L.) (Khadari et al., 1995). Another reason for finding several accessions with the same profile is that the environment might influence the phenotypic descriptions used in different countries, i.e., leaf shape, fruit

mucron more or less acute, and tree vigor. 'Shimlali' from Israel displayed the same profile as the accessions of 'Picholine Marocaine' (Morocco), 'Sigoise' (Algeria), and 'Canivano Blanco' (Andalusia, Spain). This apparent synonymy is probably due to local and long distance human displacements in the Mediterranean basin. In 'Ghjermana' from Corsica, two clones were found (V. Bronzini, unpublished data) that display profiles of two Italian cultivars, Frantoio (profile 22) and Moraiolo (profile 28). In all likelihood, this reflects the introduction into Corsica of Italian cultivars by the Genoeses.

Polyclonal accessions. Accessions such as 'Picholine', 'Lucques', and 'Olivière' were found to be monoclonal. This is in agreement with pomological and very discriminating traits such as stone morphology, thus avoiding possible confusion between cultivars. In contrast, several profiles, and therefore several clones, were found for 'Sabina' and 'Ghjermana'. We confirmed that 'Nabali', 'Souri', and 'Chemlal' are also polyclonal accessions (Loussert and Brousse, 1978; Wiesman et al., 1998). Phenotypic characterization of each clone from these accessions is insufficient to distinguish one from the other. 'Nabali' and 'Souri' are considered as ancient cultivated populations in the Near East. Their propagation probably occurred following multiple collections from wild populations allowing selection of different genotypes. We therefore had doubts about their identification as 'Nabali' or 'Souri' since in orchards these cultivars are characterized by a mixture of different trees propagated vegetatively and by seeds (Wiesman et al., 1998). Two profiles (11 and 32) have been attributed both to 'Nabali' and 'Souri'. That is another reason for the increasing uncertainty about the identity of cultivars in this region. Moreover, 'Sabina' and 'Chemlal' were revealed as polyclonal and we suggest that both seed propagation and grafting of such old cultivated trees probably occurred. Since out-crossing is prevalent in olive (rates close to 100%; Villemur et al., 1984), seed dissemination should generate heterogeneous progenies. In Kabylie, Algeria), rootstock production by seeds harvested from 'Chemlal' (Loussert and Brousse, 1978 may have resulted in accidental propagation of new genotypes, erroneously considered as identical to the original clone.

Clustering of cultivars. The cultivars that cluster in the dendrogram may share a common trait(s). It is not always obvious to find which trait(s). Furthermore, with the UPGMA method, any of the clusters may display a known common trait, such as country of origin for cultivars, or use of fruit for oil or canning. In contrast, with Ward's (1963) method, some clusters in the dendrogram fit the geographic origin of cultivars. For instance, several Sicilian cultivars are grouped in cluster 9, and the Andalusian cultivars are grouped in cluster 17. This structure suggests that local selection has been performed (Besnard et al., 2001). We wondered whether the two main groups using the method of Ward (1963) might have biological significance. We observed that the main difference between the two clusters might be based upon olive use as already reported by Besnard et al. (2001). The method based on Ward's (1963) minimum variance algorithm is considered by Saporta (1990) as the best one for hierarchical classification on Euclidean distances. This method is formally very close to a discriminant analysis as it maximizes at each step the ratio of intergroup on intragroup inertia. Therefore, it ensures definition of homogeneous groups, for all markers and in particular for markers that bring original information.

Cultivar identification. The discriminating power of primers ($0.368 < D < 0.977$) is, on average, higher than that of isozyme polymorphisms ($0.055 < D < 0.922$) and higher than that of mitotypes (0.406) developed by Besnard et al. (2001) (Table 3). The comparison of RAPD/isozyme identification confirmed the inability to distinguish between 'Canivano Blanco' and 'Picholine Marocaine' (Ouazzani et al., 1995). 'Sigoise' was discriminated using isozymes from the other two but not using RAPD markers. The distinction is based on only one allele for the esterase (EST) system (Trujillo et al. 1995). Isozyme expression was shown to be dependent on the environment and this has been already proposed for the EST system (Loukas and Pontikis, 1981). Nevertheless, Ouazzani et al. (1996) showed that 'Picholine Marocaine' is a polyclonal accession. It is possible that several trees with different profiles belonging to the same cultivar are maintained in the Cordoba collection explaining the disparity between isozyme and RAPD results.

RAPD markers are not influenced by the environment and could lead to more reliable clone identification than by using isozymes. Recently, AFLP marker used by Angiolillo et al. (1999) appeared more efficient than RAPD discriminating between cultivars and, more widely, between wild trees. However, using such a marker is still not realistic. This is due to the difficulty in identifying elementary AFLP fragments in each pattern in order to determine which band is common to two profiles. AFLP markers or microsatellites generating a higher number of patterns could prove, in future studies, to be more efficient methods, as shown for grape (Tessier et al., 1999). Mitotypes are less efficient in distinguishing cultivars compared with RAPD markers. However, they enabled us to trace maternal lineages in order to study the structure of genetic diversity of olive (Besnard and Bervillé, 2000). Combined with nuclear markers (such as RAPD markers), they enabled us to trace the origin of the cultivars and to detect gene flow. For instance, nuclear markers could reveal crosses between cultivars, which have led to new ones, as for grape (Bowers et al., 1999), but maternal lineage could reveal the direction of the crosses.

In conclusion, RAPD markers are efficient tools for characterization of olive germplasm collections. Taken together, the three primers (A1, A9, and A10) make it possible to discriminate all the trees with a low probability of confusion among cultivars. To increase efficiency, we propose using two additional primers, C9 and C15, since their profiles are easily scored. The French cultivar collection at Porquerolles, France, which contains about 120 accessions (five trees per accession), is currently under characterization with these markers (Khadari et al., unpublished data). Data herein demonstrate that the nuclear RAPD profiles of olive trees are efficient for highly accurate cultivar identification.

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