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Genotyping of fig (*Ficus carica* L) via RAPD markers

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Abstract: The genetic diversity among 15 fig accessions (*Ficus carica* L), belonging to 9 renowned cultivars of the Calabrian fig collection, and 24 unidentified genotypes of figs also located in the Calabrian region of Southern Italy, was investigated by random amplified polymorphic DNA (RAPD) analysis. The genetic similarity values were calculated for the 39 samples, and a dendrogram was elaborated by cluster analysis according to the UPGMA algorithm. The generated DNA fragments grouped the samples into two main clusters of RAPD profiles. Most of the 24 unknown samples, coming from the Luzzi area, formed a unique cluster with high degree of genetic similarity. This indicates that it is possible to distinguish, at DNA level, the fig trees with an already well-known potential to produce figs suitable for the drying process and possibly to specify cultivars with suitable features for industrial transformation.

Keywords: common fig; PCR; DNA fingerprint; arbitrary primer; random amplified polymorphic DNA; RAPD

INTRODUCTION

The common or edible fig (*Ficus carica* L), belonging to the *Moraceae* family, has been cultivated in Southern Italy since ancient times. The fruit is important in human alimentation[1,2] under various forms, ie fresh, dried and canned, stuffed with nuts, covered with chocolate or aromatized in different ways.[3] The importance of figs in nutritional and therapeutic fields has recently emerged.[4] The province of Cosenza in the Calabrian region (Fig 1) is naturally suited for fig cultivation and is especially important in the production of dried figs, reaching a yearly production of 800 tons in the year 2000.[5] In particular, the area of Luzzi is the most important (approximately 15%) for the dried product. Traditionally, the 'Dottato' cultivar has been most suitable for industrial transformation owing to its excellent qualitative features, even for fresh consumption. These traits have been accentuated by the climatic conditions of the Cosenza region, which is exceptionally favourable for fig cultivation.[5] The edible fig is a currently widespread species with a large number of local cultivars whose identity is poorly studied. However, plant characterization is an important aspect in the food industry. Growers and breeders have an interest in the accurate identification of cultivars and their clones because the quality of the final product greatly depends on the origin and the identity of the employed cultivar. Pavone and Bruno[5] reported that some cultivars located in Cosenza are specially produced to meet current industrial transformation. At present, there are modest morphological and agronomic studies on fig accessions used for the dried products.[6] Consequently, reliable techniques are required to verify the distinctiveness of these cultivars and clones that constitute a germplasm resource that is unique in the world.

Plant identification and estimation of their relationships and diversity are traditionally established on the basis of morphological and agronomic characteristics. Since there are substantial intra-species variations in vegetative traits, it is difficult to differentiate genotypes only on the basis of their external structure (phenotype), especially for leaf and fruit characters, because these may vary according to development conditions. This could lead to misidentification; consequently the lack of a way for safely assessing genetic relationships makes it necessary to use stable markers. Advances in molecular biology, especially the introduction of the polymerase chain reaction (PCR) for the *in vitro* amplification of desired DNA fragments, has led to innovative techniques for plant genetic identification.[7] PCR-based technologies have been successfully developed and rely on stochastic amplification of genomic DNA.[8,9] The use of

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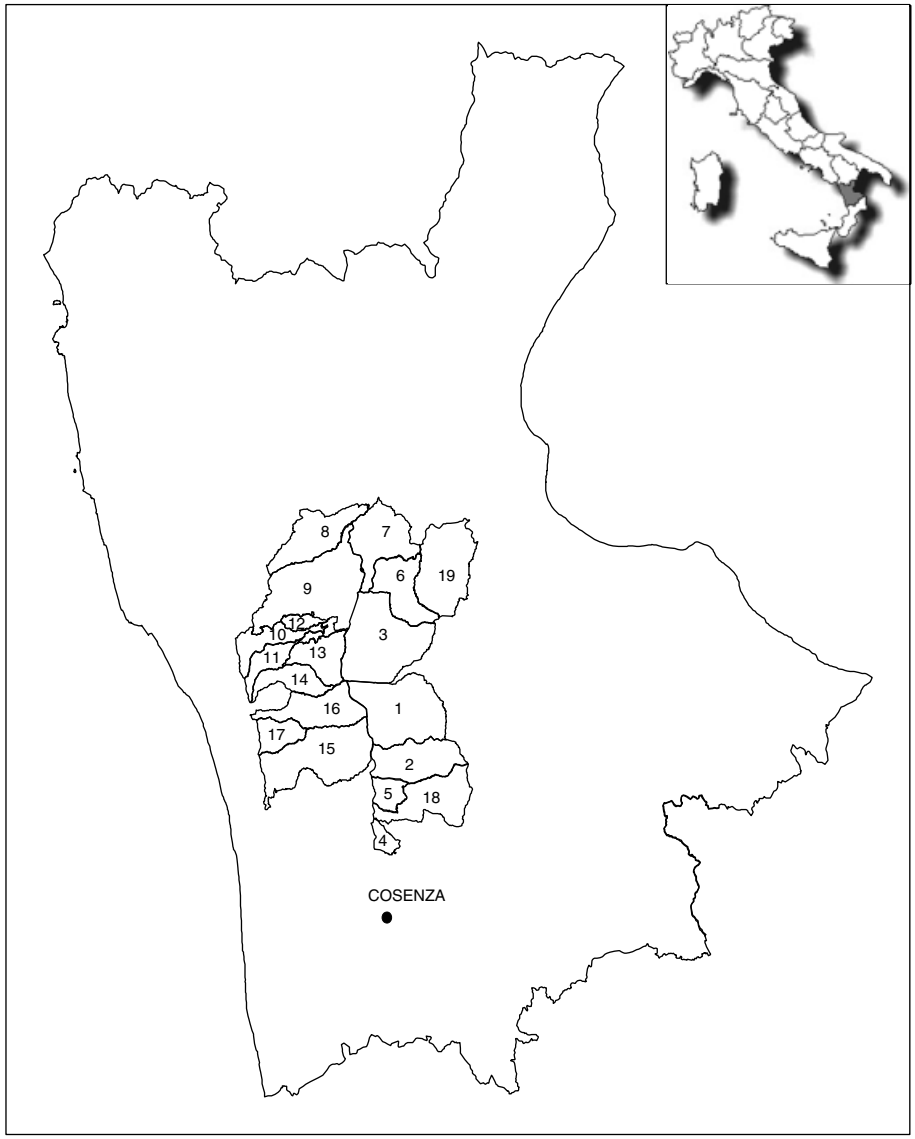

Figure 1. The renowned zones of dried fig production in Cosenza province are indicated: (1) Luzzi; (2) Rose; (3) Bisignano; (4) Zumpano; (5) Castiglione Cosentino; (6) S Sofia d'Epiro; (7) Tarsia; (8) Roggiano Gravina; (9) S Marco Argentano; (10) Mongrassano; (11) Cerzeto; (12) Cervicati; (13) Torano Castello; (14) S Martino di Finita; (15) Montalto Uffugo; (16) Lattarico; (17) S Benedetto Ullano; (18) S Pietro in Guarano; (19) S Demetrio Corone.

DNA-based identification procedures enables plant identification by generating genotype-specific DNA banding profiles.[10] In particular, random amplified polymorphic DNA (RAPD) analysis employs a single arbitrary primer that produces anonymous DNA fragments by PCR. Polymorphic DNA fingerprints can be created for typifying cultivars and clones since specific DNA products will be amplified in one sample but not in another. The major advantage of the RAPD technique is that it can be applied without the knowledge of the species genomic DNA base sequence.[10,11] RAPD analysis has been applied to detect molecular differences at intra- and inter-variety levels in a number of fruit trees including pear,[12] apple,[13] peach,[14] plum[15] and fig.[16–19]

In related papers the experimental conditions for the cultivar identification of common fig by RAPD-PCR were carried out on a limited number of fig samples.[17,20,21] In this work the CTAB-based DNA isolation protocol was followed by RAPD-PCR screening and applied to a number of important Calabrian fig cultivars which were selected on the basis of their geographical proximity, morphological similarity and industrial potential for drying.

Our investigation focused on the genetic similarities among cultivars and among their clones to evaluate the presence of redundancy in the Calabrian collection. The obtained DNA fingerprints were used to classify the natural fig population cultivated in the important economic area of Luzzi.

MATERIALS AND METHODS

Plant material

A total of 39 samples of *F carica* were analyzed in this study. Of these, 15 accessions were clones of nine cultivars (Table 1), selected from the reference Calabrian fig collection located at the

Table 1. The 15 accessions of nine *Ficus carica* cultivars from Calabrian collection tested for RAPD analysis

Number	Cultivar	Accession number	Synonymous
1	Citrolara	3	Corno
2	Columbra Bianca	9C, 3D, 12	Columbro
3	Columbra Nera	6	Columbro
4	Dottato	5, 7, 8	Ottato
5	Gnurella	1	Zingarella
6	Granato	4, 4c, 4a	Malosso
7	Natalise	11	Vernile
8	Noreglia	9	Scavedda
9	Paradiso	2	Paravis

Table 2. The 24 unclassified *Ficus carica* genotypes from Luzzi area tested for RAPD analysis

Number	Sample[a]	Firm	Place of origin (Luzzi)
1	M1	Marchese	Pagliarella
2	M2	Marchese	Pagliarella
3	M3	Marchese	Pagliarella
4	P1	Pingitore	Marzi
5	P2	Pingitore	Marzi
6	P3	Pingitore	Marzi
7	P4	Pingitore	Marzi
8	P5	Pingitore	Marzi
9	BM1	Bria M	Valle Leotta
10	BM2	Bria M	Valle Leotta
11	BM3	Bria M	Valle Leotta
12	BM4	Bria M	Valle Leotta
13	BM5	Bria M	Valle Leotta
14	BM6	Bria M	Valle Leotta
15	BU1	Bria U	Valle Leotta
16	BU2	Bria U	Valle Leotta
17	F1	Fazio	Caprella
18	F2	Fazio	Caprella
19	F3	Fazio	Caprella
20	F4	Fazio	Caprella
21	C1	Caloiero	Fosse D'Orsola
22	C2	Caloiero	Fosse D'Orsola
23	C3	Caloiero	Fosse D'Orsola
24	C4	Caloiero	Fosse D'Orsola

[a] Internal sample codes.

Centro Sperimentale Dimostrativo (CSD)—Agenzia Regionale per lo Sviluppo e per i Servizi in Agricoltura (ARSSA)—Casello San Marco, Cosenza, Italy. The remaining 24 genotypes of *F carica* (Table 2) came from plants located in the Luzzi area of Calabria, Southern Italy, as shown in Fig 1. Young leaves were taken from adult trees of each cultivar or genotype and kept chilled during transport. The leaves were quickly frozen at −80 °C and stored until DNA extraction.

Plant DNA isolation

The genomic DNA of all the samples was extracted and purified in duplicate, according to the method of Doyle and Doyle,[22] with some modifications as described in a previous work.[20] Briefly, 100 mg of young fig leaves were ground to powder in a mortar in the presence of liquid nitrogen. The powder was dissolved in preheated (60 °C) lysis buffer consisting of 1.4 M NaCl, 2% (w/v) CTAB, 200 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 2% (v/v) 2-mercaptoethanol, and 5 mM ascorbic acid. The suspension was incubated at 60 °C for 30 min and then subjected to chloroform:butanol (24:1) extraction. One volume of cold isopropanol was added to the aqueous phase. The precipitate was collected, washed in 70% ethanol and dissolved again in the lysis buffer. The procedure was repeated to achieve additional purification. RNase A treatment (5 $\mu g\,ml^{-1}$) at 37 °C for 60 min was necessary to eliminate the co-extracted RNA. Finally, the DNA, after a further precipitation with 5 M ammonium acetate (1/10 volumes) and cold ethanol (3 volumes), was dissolved in double-distilled sterile water to a final concentration of 10 $ng\,\mu l^{-1}$, calculated from the absorbance at 260 nm. Purity and quality of DNA template was checked both from the 260/280 nm absorbance ratio and gel electrophoresis analysis.

RAPD-PCR procedure

Optimization

The parameters of the genomic DNA extraction process and RAPD-PCR procedure were investigated and established in previous works.[17,20,21] The quality of DNA template is a crucial factor for successful PCR. This is important in fig species because they contain high levels of plant secondary metabolites which are powerful PCR inhibitors. Instead, by employing our method, the DNA purified from fig leaves amplified easily, thus showing that PCR inhibitors were effectively removed. Sensitivity and reproducibility of the RAPD method were examined on fig genomic DNA from 0.1 to 100 ng using the Stoffel Fragment of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA, USA). A 10-ng DNA template sample was used in the present study. Identical RAPD profiles were obtained using a wide range of magnesium concentrations in different DNA extraction from the same fig clone.[17]

Amplification

The arbitrary primers tested in the PCR reaction had 60% G + C content and were 10 nucleotides long. An initial screening of 20 arbitrary oligodeoxyribonucleotide primers (M-Medical, Florence, Italy) allowed us to select seven of them which clearly differentiated the fig trees: U1: 5′-AGG GGT CTT G-3′, U3: 5′-GGG TTT AGG G-3′, U4: 5′-GAC AGA CAG G-3′, U5: 5′-CGA CAG ACA G-3′, U11: 5′-CGA AGC TAC C-3′, U13: 5′-CCA GTG CTC T-3′, and U19: 5′-TGG GAA CGG T-3′ (Table 3). The PCR reactions were carried out in a final volume of 50 µl. Each RAPD-PCR mixture contained 10 mM Tris-HCl pH 8.3, 10 mM KCl, 3 mM $MgCl_2$, 200 µM of each dNTPs, 20 pmols of the unique primer, 2.5 Units of AmpliTaq DNA polymerase Stoffel Fragment (Applied Biosystems, Foster

Table 3. Results of the seven selected arbitrary primers in RAPD analysis of fig genome

Primer name	5′-Sequence-3′	Total DNA fragments (nr 53)	Polymorphic DNA fragments (nr 43)	Polymorphic DNA fragments (%)
U1	AGG GGT CTT G	10	9	90
U3	GGG TTT AGG G	8	6	75
U4	GAC AGA CAG G	6	4	67
U5	CGA CAG ACA G	9	9	100
U11	CGA AGC TAC C	4	2	50
U13	CCA GTG CTC T	9	8	89
U19	TGG GAA CGG T	7	5	71

City, CA, USA) and 10 ng of fig genomic DNA. The DNA template amplification was performed by the 'cold start' procedure: the PCR mix was assembled on ice and then transferred at 4 °C to a PTC-100 Programmable Thermal Controller with heated lid (MJ Research, Inc, Waltham, MA, USA). The cycling profile used a program that started with an initial DNA template melting by incubation for 3 min at 94 °C, followed by 45 cycles of denaturation for 1 min at 94 °C, primer annealing for 1 min at 40 °C and synthesis for 1 min at 72 °C. The program ended with a final extension conducted for 7 min at 72 °C. The reaction products were stored at 4 °C. Each reaction was repeated at least three times to validate the reproducibility of the method.

Gel electrophoresis

The RAPD-PCR products (25 µl) were separated by electrophoresis on 2% (w/v) agarose gel containing 0.5 µg ml^{-1} ethidium bromide and 1X TBE buffer (89 mM Tris-borate pH 8.4, 2 mM EDTA) at 100 V. A 100-bp DNA ladder was used as standard marker of known molecular weights (Amersham Pharmacia Biotech Inc, Piscataway, NJ, USA). Amplicons were visualized with UV transilluminator and digitalized by the Electrophoresis Documentation and Analysis 120 System (Kodak ds-digital science- Rochester, NY, USA).

Data analysis

Reproducible RAPD fragments were scored 1 or 0 for each sample: 1 was assigned for the presence of an amplicon and 0 for its absence. The data were used to calculate the pairwise similarity matrix between genotypes according to Nei and Li's genetic similarity coefficient (NLc): $2N_{ij}/(2N_{ij} + N_i + N_j)$, where N_{ij} is the number of bands common to samples i and j, N_i and N_j are the number of bands unique to sample i and j, respectively.[23] The genetic coefficient of Nei and Li represents the fraction of shared DNA fragments between two samples. It ranges from 0 to 1, the complete genetic identity corresponding to 1. The clustering program of Multi-Variate Statistical Package (MVSP) version 3.1 was then used to group the genotypes.[24] Relationships among the tested samples was calculated by the Unweighted Pair Group Method using Arithmetic Average (UPGMA) and was visualized through a genetic relatedness dendrogram.

RESULTS AND DISCUSSION

In order to identify primers useful in distinguishing genotypes, we examined 15 accessions of nine reference cultivars from the Calabrian collection (Table 1). The optimal number of primers, required to discriminate among genomic DNA of two or more cultivars, depends on the level of polymorphism in the RAPD-PCR experiments. Suggestions about the amount of genetic distance required to classify two correlated fig accessions as distinct cultivars have been reported by several authors.[16–19] For our purpose 7 out of 20 primers (35%) with informative patterns were selected on the basis of the number of amplification products and the stability of the patterns (Table 3). As a consequence 13 primers were excluded (65%). We extended the RAPD analysis with the chosen primers to characterize 24 unidentified commercial fig clones (Table 2) from the Luzzi area (Fig 1). A total number of 53 DNA bands, separated by electrophoresis on agarose gel, for both the reference and the unidentified groups, were obtained. Of these, 43 bands were polymorphic (81%) and 10 were monomorphic (19%), with an average of 8 bands per primer (Table 3). Previous works demonstrated that a low number of amplicons per primer was sufficient to produce useful fingerprints for cultivar and clone discrimination.[17,18] In particular, Galderisi *et al*[17] have reported that only two primers were sufficient to distinguish *F carica* cultivars.

Figure 2 is an example of RAPD fingerprints, obtained using U1 primer on the nine reference cultivars, which demonstrate the cultivar diversity and the intra-variety homogeneity. RAPD amplification patterns, obtained after electrophoretic separation,

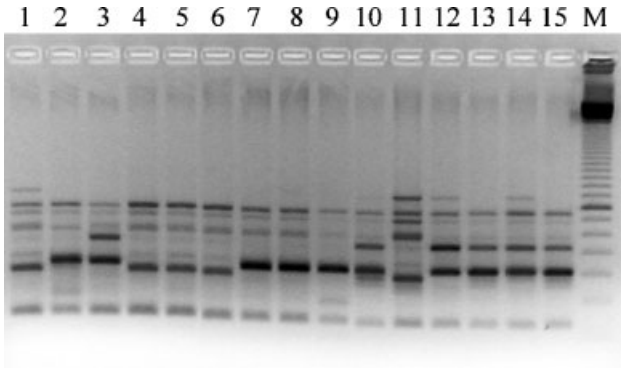

Figure 2. Comparison of the RAPD profiles obtained with U1 primer on genome of the nine fig cultivars. Lane 1: 'Gnurella' clone 1; lane 2: 'Paradiso' clone 2; lane 3: 'Citrolara' clone 3; lanes 4–6: 'Granato' clones 4, 4a, 4c; lanes 7–9: 'Dottato' clones 5, 7, 8; lane 10: 'Noreglia' clone 9; lane 11: 'Natalise' clone 11; lane 12: 'Columbra Nera' clone 6; lanes 13–15: 'Columbra Bianca' clones 12, 3D, 9C; lane M: 100 base-pair ladder as molecular weight marker, the darkest band corresponds to 800 bp.

refer to the nine cultivars belonging to the collection. The polymorphism is easily seen among the nine cultivars examined from the electrophoretic patterns. A high similarity is observed among clones of the cultivars which showed three clones, ie 'Granato', 'Dottato' and 'Columbra Bianca'.

The results, acquired with the seven selected primers (Table 3), revealed differences among the nine reference cultivars. The absence of repeated accessions in the Calabrian germplasm, called 'redundancy absence', leads to establish the validity of the analyzed genetic resources and allows: (a) individual identification, (b) marker-assisted selection and (c) phylogenetic analyses.[16–21] In our case, the examined accessions represent unique genotypes with certainty to use as references to genetically identify unknown fig trees.

Moreover, RAPD analysis can be an invaluable tool for safeguarding and improving fig species. In fact, the biodiversity of the *F carica* L species can be safeguarded efficiently only after characterizing the genetic diversity of the present fig population with RAPD markers or other means. Selection assisted by RAPD markers may also be helpful to produce new cultivars with improved productivity, organoleptic aspects, and pest resistance.

In order to describe the relationships among samples, a pairwise distance matrix, based on the Nei and Li coefficient (NLc), was calculated from RAPD data (Table 4). The resulted level of intra-cultivar clonal similarity was very high and homogeneous, in agreement with Galderisi *et al*.[17] In fact the 'Dottato' accessions showed elevated similarity values: NLc = 0.99 for clone DoF 5 versus DoF 7 and DoF 8, NLc = 0.97 for clone DoF 7 versus DoF 8.

The 'Granato' accessions showed similarity values close to those of the 'Dottato': NLc = 0.96 for the clone GrF 4 versus GrF 4a and GrF 4c clones, NLc = 0.97 for the GrF 4a versus GrF 4c clone. These high similarity values indicate that the three clones GrF 4, Gr F4a and GrF 4c belong to the 'Granato' cultivar and confirm the phenotypical classification made when collecting the fig germplasm.

In the same way, the 'Columbra Bianca' accessions showed very close similarity coefficients (NLc > 0.9). In particular, NLc = 0.96 for CBF 12 versus CBF 3D clone, NLc = 0.94 for CBF 3D versus CBF 9C and NLc = 0.93 for CBF 9C versus CBF 12. This confirms the classification made when collecting the germplasm and indicates that the three clones CBF 12, CBF 3D and CBF 9C belong to the 'Columbra Bianca' cultivar.

In contrast, the anonymous samples (Table 2) showed very heterogeneous similarity coefficients from a minimum NLc = 0.57 for the BM3 versus GrF 4a and 4c clones, to a maximum of NLc = 1 for M2 versus M3 and the ten clones C1, C2, C3, C4, F2, F3, F4, BU1, P2 and P4 (Table 4). The parameter NLc = 1 indicates the complete genetic identity of the ten samples C1, C2, C3, C4, F2, F3, F4, BU1, P2 and P4 and in the other pair of clones M2 and M3.

The genetic relationships among genotypes is illustrated by a dendrogram, generated by UPGMA clustering method (Fig 3). The dendrogram was divided into two main clusters. The first cluster (I) included the eight cultivars with 12 accessions from Calabrian collection: the three accessions of 'Granato'; the accession of 'Gnurella'; the three accessions of 'Dottato'; the accession of 'Paradiso'; the accession of 'Columbra Nera'; the accession of 'Noreglia'; the

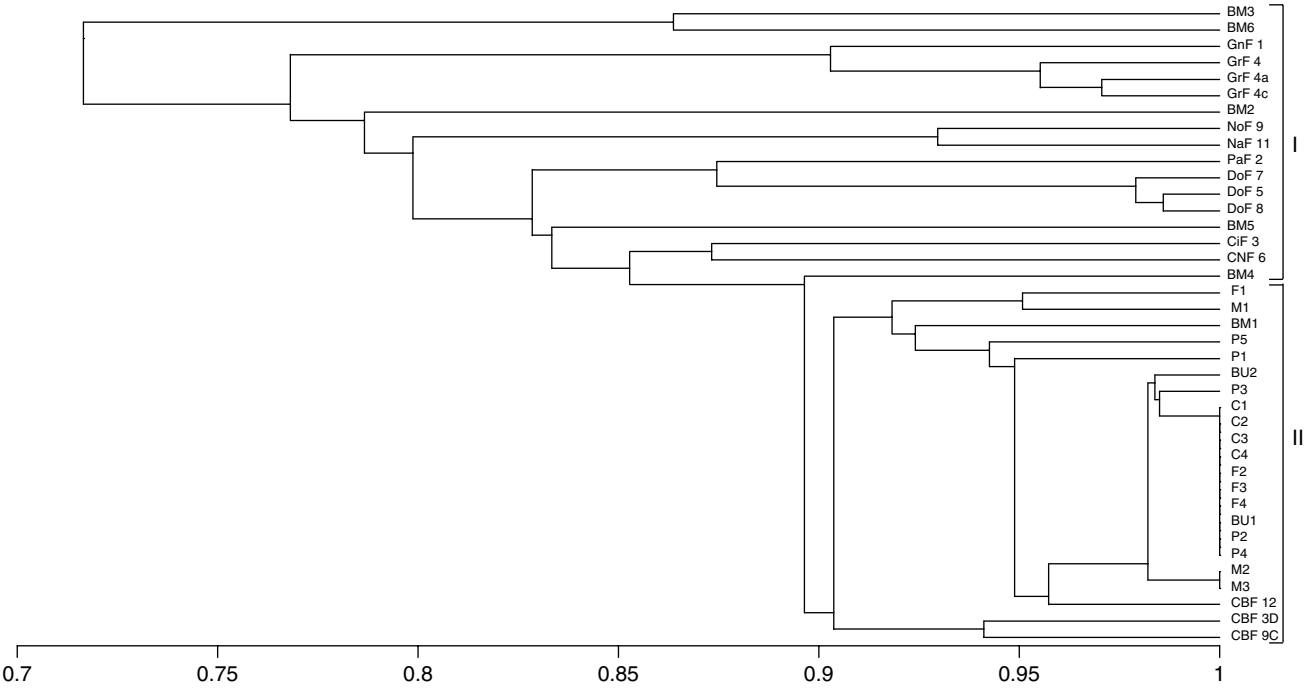

Figure 3. UPGMA-based dendrogram generated from 53 RAPD markers. The numerical scale indicates the Nei and Li coefficient of genetic similarity. Samples fit into clusters I and II.

Table 4. Similarity matrix based on Nei and Li's coefficient

Samples	CBF 9C	CBF 3D	CBF 12	CNF 6	NaF 11	NoF 9	DoF 8	DoF 7	DoF 5	GrF 4c	GrF 4a	GrF 4	CiF 3	PaF 2	GnF 1	M 3	M 2	M 1	P 5	P 4	P 3	P 2	P 1	BM 6	BM 5	BM 4	BM 3	BM 2	BM 1	BU 2	BU 1	F 4	F 3	F 2	F 1	C 4	C 3	C 2	C 1
CBF 9C	1																																						
CBF 3D	0.94	1																																					
CBF 12	0.93	0.96	1																																				
CNF 6	0.82	0.85	0.86	1																																			
NaF 11	0.85	0.86	0.84	0.85	1																																		
NoF 9	0.87	0.87	0.86	0.87	0.93	1																																	
DoF 8	0.78	0.76	0.80	0.81	0.73	0.75	1																																
DoF 7	0.81	0.79	0.83	0.81	0.76	0.78	0.97	1																															
DoF 5	0.79	0.77	0.81	0.82	0.74	0.76	0.99	0.99	1																														
GrF 4c	0.78	0.81	0.79	0.75	0.81	0.77	0.80	0.83	0.81	1																													
GrF 4a	0.75	0.78	0.77	0.75	0.78	0.74	0.83	0.80	0.81	0.97	1																												
GrF 4	0.79	0.82	0.81	0.79	0.82	0.78	0.81	0.81	0.82	0.96	0.96	1																											
CiF 3	0.88	0.88	0.90	0.87	0.82	0.84	0.81	0.81	0.82	0.81	0.81	0.82	1																										
PaF 2	0.81	0.85	0.86	0.81	0.73	0.75	0.89	0.86	0.87	0.80	0.83	0.84	0.81	1																									
GnF 1	0.77	0.80	0.78	0.82	0.83	0.79	0.82	0.82	0.83	0.90	0.90	0.91	0.82	0.82	1																								
M3	0.89	0.93	0.97	0.86	0.81	0.82	0.82	0.82	0.84	0.76	0.76	0.80	0.89	0.88	0.78	1																							
M2	0.89	0.93	0.97	0.86	0.81	0.82	0.82	0.82	0.84	0.76	0.76	0.80	0.89	0.88	0.78	1	1																						
M1	0.86	0.89	0.94	0.79	0.77	0.79	0.82	0.85	0.83	0.78	0.75	0.79	0.86	0.85	0.74	0.94	0.94	1																					
P5	0.86	0.86	0.91	0.79	0.74	0.76	0.79	0.79	0.80	0.69	0.69	0.73	0.83	0.82	0.71	0.94	0.94	0.87	1																				
P4	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1																			
P3	0.90	0.90	0.94	0.83	0.78	0.83	0.83	0.83	0.84	0.74	0.74	0.78	0.87	0.86	0.75	0.97	0.97	0.91	0.94	0.99	1																		
P2	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1	0.99	1																	
P1	0.89	0.90	0.91	0.80	0.75	0.79	0.79	0.79	0.81	0.73	0.73	0.77	0.83	0.85	0.75	0.94	0.94	0.87	0.90	0.95	0.97	0.95	1																
BM6	0.69	0.70	0.75	0.67	0.60	0.62	0.69	0.69	0.70	0.61	0.61	0.66	0.73	0.69	0.60	0.78	0.78	0.81	0.85	0.80	0.79	0.80	0.74	1															
BM5	0.85	0.79	0.83	0.75	0.75	0.77	0.77	0.81	0.79	0.70	0.67	0.71	0.81	0.74	0.69	0.83	0.83	0.89	0.79	0.85	0.83	0.85	0.79	0.83	1														
BM4	0.86	0.86	0.88	0.79	0.77	0.79	0.76	0.76	0.77	0.72	0.72	0.76	0.86	0.79	0.71	0.90	0.90	0.83	0.87	0.92	0.91	0.92	0.87	0.85	0.86	1													
BM3	0.73	0.67	0.71	0.70	0.67	0.66	0.66	0.66	0.67	0.57	0.57	0.62	0.76	0.62	0.60	0.70	0.70	0.73	0.77	0.73	0.71	0.73	0.67	0.86	0.79	0.73	1												
BM2	0.78	0.85	0.83	0.81	0.73	0.81	0.66	0.66	0.67	0.71	0.71	0.72	0.81	0.75	0.73	0.83	0.83	0.75	0.75	0.81	0.83	0.81	0.86	0.60	0.67	0.75	0.60	1											
BM1	0.87	0.90	0.94	0.83	0.78	0.83	0.77	0.77	0.78	0.77	0.77	0.78	0.87	0.83	0.75	0.94	0.94	0.88	0.88	0.93	0.94	0.93	0.91	0.71	0.77	0.84	0.68	0.89	1										
BU2	0.93	0.93	0.97	0.83	0.81	0.83	0.83	0.86	0.84	0.77	0.74	0.78	0.87	0.86	0.75	0.97	0.97	0.94	0.94	0.99	0.97	0.99	0.94	0.79	0.87	0.91	0.71	0.80	0.91	1									
BU1	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1	0.99	1	0.95	0.80	0.85	0.92	0.73	0.81	0.93	0.99	1							
F4	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1	0.99	1	0.95	0.80	0.85	0.92	0.73	0.81	0.93	0.99	1	1						
F3	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1	0.99	1	0.95	0.80	0.85	0.92	0.73	0.81	0.93	0.99	1	1	1					
F2	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1	0.99	1	0.95	0.80	0.85	0.92	0.73	0.81	0.93	0.99	1	1	1	1				
F1	0.88	0.88	0.89	0.78	0.76	0.78	0.84	0.84	0.85	0.77	0.77	0.81	0.88	0.84	0.73	0.92	0.92	0.95	0.89	0.94	0.92	0.94	0.89	0.83	0.88	0.89	0.76	0.74	0.86	0.92	0.94	0.94	0.94	0.94	1			
C4	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1	0.99	1	0.95	0.80	0.85	0.92	0.73	0.81	0.93	0.99	1	1	1	1	0.94	1		
C3	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1	0.99	1	0.95	0.80	0.85	0.92	0.73	0.81	0.93	0.99	1	1	1	1	0.94	1	1	
C2	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1	0.99	1	0.95	0.80	0.85	0.92	0.73	0.81	0.93	0.99	1	1	1	1	0.94	1	1	1
C1	0.91	0.91	0.96	0.85	0.79	0.81	0.84	0.84	0.85	0.75	0.75	0.79	0.88	0.87	0.77	0.99	0.99	0.92	0.95	1	0.99	1	0.95	0.80	0.85	0.92	0.73	0.81	0.93	0.99	1	1	1	1	0.94	1	1	1	1

Fig cultivars are presented as acronyms: CBF = Columbra Bianca fig; CNF = Columbra Nera fig; NaF = Natalise fig; NoF = Noreglia fig; DoF = Dottato fig; GrF = Granato fig; CiF = Citrolara fig; PaF = Paradiso fig; GnF = Gnurella fig.

accession of 'Natalise'; and the accession of 'Citrolara'. The three accessions of 'Columbra Bianca' were not included in the first cluster. All the fig cultivars from Calabrian collection clearly showed different genotypes, confirming the morphologic differences observed in the same collection. The fig samples indicated by the label BM (Table 2), excepting BM1, belong to the cluster I, but show genetic heterogeneity with respect to the eight cultivars of the same cluster. Thus, those samples maintained a certain genetic proximity, for example BM3 and BM6 with NLc = 0.86, as to come together into the cluster I. Some of the low NLc values (below 0.9) are due to rare change seedlings that have been propagated rather than natural somatic mutations. Nevertheless, the figs are exclusively reproduced asexually by scion or layer thus the hereditary characters of the mother-plant are retained with a consequent low genetic variability.[19]

The RAPD technique is usually employed to discriminate cultivars. The results of this study show that it can also be used to distinguish clones of the same cultivar. The genetic analysis of the cluster I demonstrated the existence of clonal variability at intra-cultivar level for both 'Dottato' and 'Granato' cultivars (Fig 3). This can be explained by the fact that intensive and rational fields of fig trees do not exist in Calabria, where the cultivation techniques still follow domestic and rural traditions and the areas devoted to fig cultivation generally have little extension and are very spread on the region. This necessarily implies a strong genetic variability.

The second cluster (II) was composed of all commercial fig samples under study together with the three accessions of 'Columbra Bianca', except BM2, BM3, BM4, BM5 and BM6. The dataset obtained by RAPD marker analysis on the samples showed identical or very similar DNA patterns among 19 out of the 24 unidentified genotypes (NLc > 0.9). This demonstrates their genetic homogeneity in accordance with their common potential for industrial drying process, a choice which was likely made by the factories without previous morphological and agronomic characterization.[5] For many centuries, the dried fig production of Cosenza province has preferred cultivars producing fruits with average size, with an intact, thin and elastic peel, with a sweet honey like pulp and empty and slim achenes. In the province of Cosenza, fig cultivars are mainly represented by the 'Dottato' and to less extent by the 'Paradiso' and 'Citrolara' cultivars.[5] On the basis of the information derived from RAPD data for the genotypes belonging to the cluster II, a direct correlation between genotype and potential to drying process was evident. We suggest that the cultivars employed nowadays for the production of dried figs from the industrial firms in Luzzi area are no longer the 'Dottato', 'Paradiso' and 'Citrolara' cultivars. As depicted in the dendrogram, the 'Columbra Bianca' was within cluster II. This indicates that 'Columbra Bianca' is similar to the 19 commercial figs examined and likely they represent the main source for the production of the Calabrian dry fig.

The high level of genetic homogeneity of the fig clones in the Luzzi zone is not surprising. In fact the present propagation and selection techniques lead to reduced biodiversity. The cultivated figs are propagated by vegetative reproduction and the growers maintain the genotypes by selecting trees for their potential to produce dried fruits.

RAPD analysis provides a reliable, specific and highly sensitive method for DNA typing and genetic characterization of a multiplicity of fig genotypes. The PCR-based procedure applied here represents an improvement of our understanding of genetic diversity within *F carica* species and allowed us to authenticate and monitor the identity of fig source used to obtain dried products. The polymorphism observed was sufficient to differentiate all the samples we analyzed and provide an innovative method to identify fig trees, even those very similar from a phenotypic point of view such as the clones of the same cultivar. In general, our results are in agreement with similar studies on fig cultivars.[16–19]

In RAPD analysis the polymorphisms of cultivars and their clones are a direct consequence of the differences existing at genomic DNA level. Since RAPD markers are transmitted by inheritance because they are DNA associated,[25] they constitute a better typifying system than those based on phenotypic determination. In the future they may represent an alternative and routine tool to verify the identity and the quality of fig cultivars, fruits and fig-derived products. Furthermore, the molecular marker-assisted selection may become important in the assessment of the genotypes used in the *F carica* improvement programmes of the Mediterranean countries.

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