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Identification of *Feijoa sellowiana* Berg accessions by RAPD markers

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Abstract

Random amplified polymorphic DNAs (RAPDs) were used to discriminate among 25 *Feijoa sellowiana* cultivars and accessions. Fifty ten-mer oligonucleotide primers were tested on five accessions. Twenty-two primers, showing clear polymorphic patterns, were chosen to amplify the DNAs of all 25 genotypes; each amplification was repeated three times separately. Only 11 primers were finally analysed, yielding a total of 23 polymorphic RAPD markers.

Twenty-one genotypes were fully distinguished, with as few as five primers and nine RAPD markers. The remaining four genotypes grouped into pairs ('Edenvale late' and 'Edenvale supreme', Mangiapane 1 and Mangiapane 3) could not be further distinguished, even when the number of primers examined was increased to 100.

A cluster analysis based on 49 RAPD markers was performed to construct a phenetic similarity dendrogram. The low number of rare bands and the lack of apparent relation between clusters and the geographical origin of the accessions suggest that most of the plant materials of this study share a common genetic ancestry, possibly coming from relatively few introductions. A larger study, analysing Brazilian type (undomesticated) accessions of *F. sellowiana*, would be necessary to confirm this hypothesis and to evaluate more accurately the diversity available for feijoa breeding.

Keywords: *Acca sellowiana*; Pineapple guava; DNA fingerprinting; Similarity

1. Introduction

Traditional cultivar identification, based on morphological and phenological traits, requires extensive observations of mature plants, and can take several years

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in fruit trees. Furthermore, morphological traits can vary due to environmental influences, and their use as markers can be ambiguous (Liu and Furnier, 1993). For these reasons, the development of cultivar-specific genetic markers is desirable for cultivar identification.

Proteins and isoenzymes have been used in many crops, including trees, for identification purposes, but for many species, including feijoa (unpublished data), insufficient polymorphism is a problem. Additionally, some proteins can exhibit spatial and temporal variation as well as variation due to environments (Beckman and Soller, 1983).

With the development of DNA markers based on PCR techniques (e.g., RAPDs and SSRs), researchers have a high number of available markers for identification purposes. These systems have been successfully used in many plant species for fingerprinting and paternity testing.

RAPD markers provide a fast and easy approach to the problem of cultivar identification. Many horticultural crops have been fingerprinted using RAPD markers, including some fruit (Koller et al., 1993; Quarta et al., 1994; Lu et al., 1996; Warburton and Bliss, 1996; Oliveira et al., 1999).

Feijoa sellowiana is a subtropical species, belonging to the Myrtaceae family. It is native to South Brazil with a secondary dispersion in Uruguay (Nodari et al., 1997). The species was introduced mainly as an ornamental plant in many mild-climate regions, such as California, Florida, and the Mediterranean basin. In Italy, its introduction dates from the beginning of this century. Recently, the species assumed some economic relevance, with orchards planted in the Central and Southern regions of the country. Feijoa cultivars are extremely difficult to identify using traditional identification methods, and can show, within a single plant, a wide variability in morphological traits (Monastra and Paesano, 1990b).

The aim of this study was to investigate the efficiency of RAPD markers to fingerprint *F. sellowiana* accessions and to evaluate the variability available within domesticated feijoas.

2. Materials and methods

The cultivars and accessions used in this study are listed in Table 1; this material, obtained from several countries, was part of a *F. sellowiana* collection maintained at the Istituto Sperimentale per la Frutticoltura in the fields of Rome (Monastra and Paesano, 1990a). The pedigree of the cultivars and accessions is unknown.

Young, unexpanded leaves were collected from a single tree for each genotype, immediately frozen in liquid nitrogen and stored at -80°C. Genomic DNA was isolated from the leaf samples by a CTAB method (Doyle and Doyle, 1990). RNAse treatment and two phenol–chloroform purifications followed the

Table 1
Plant material

Accession	Country of origin
Apollo	New Zealand
Calinge	Unknown
David	New Zealand
Edenvale late	USA
Edenvale supreme	USA
Edenvale improved	USA
Gemini	New Zealand
Grossa di Sicilia	Italy
Inderst	Italy
Large oval	Australia
Mammoth	New Zealand
Mangiapane 1	Italy
Mangiapane 2	Italy
Mangiapane 3	Italy
Marian	Unknown
Moore	Unknown
Nazemetz	USA
Pagliaia	Italy
Robert	New Zealand
Robert 3f	Suspected spontaneous mutation of 'Robert'
Roundjon	New Zealand
Russo	Italy
Slor	Israel
Smith	USA
Triumph	New Zealand

extraction. A mini fluorometer (TKA100, Hoefer Scientific) was used to measure DNA concentrations. DNA quality was evaluated by agarose gel electrophoresis. Working solutions of the DNAs were prepared by diluting the stocks at 10 ng μl^{-1} in sterile distilled water.

The amplifications followed the protocol of Williams et al. (1990) with few modifications. PCR was performed in a total volume of 25 μl, containing 20 ng of template DNA, 0.2 μM of a decamer oligonucleotide primer (Operon Technologies, Alameda, CA), 0.625U Taq DNA polymerase (Boehringer Mannheim), 100 μM each dNTP (Boehringer Mannheim), 2 mM $MgCl_2$, 10 mM Tris–HCl, 50 mM KCl, pH 8.3. Each reaction was overlaid with two drops of mineral oil (Sigma–Aldrich).

DNA amplification was carried out in an MJ Research PT100 thermal cycler and followed the thermal cycling adopted by Williams et al. (1990). An initial denaturation cycle of 1 min 30 s at 94°C followed by 44 cycles comprising 1 min at 94°C, 1 min at 36°C, 2 min at 72°C. An additional cycle of 7 min at 72°C was

used for final extension. Amplification products were separated by electrophoresis ($\Delta V \sim 6\ V\ cm^{-1}$) in 1.5% agarose gels (TBE) and stained in ethidium bromide. A photographic record was taken for each PCR run. The DNA size reference standard was the 1 Kb ladder (Gibco BRL).

The DNAs of five cultivars, randomly chosen for each amplification, were amplified with 50 primers (belonging to kits A, B, C, D, E, F, G, H). Twenty-two primers, showing clear polymorphic patterns in these preliminary trials, were selected to amplify the DNAs of all the accessions: A3, A5, A9, A11, A12, A16, A17, A18, A20; B4, B6, B8; D1, D3; E1; F1, F6, F8, F13; G18; H1, H5. Each amplification was repeated at least three times. Bands on agarose gels were scored as present (1) or absent (0) and the fragment readings were entered in a computer file as a binary matrix. All the first group of 50 primers and another group of 50 additional primers were used to amplify the DNA of two cultivars and two Italian accessions, not fully distinguished by the first 22 primers ('Edenvale late', 'Edenvale supreme', Mangiapane 1 and Mangiapane 3).

NTSYS-pc, version 1.80 (Numerical Taxonomy System, Exeter Software, New York, USA), was used to perform a cluster analysis of the complete data set and of a subset containing only polymorphic RAPD fragments.

A pairwise similarity matrix was constructed by using the Dice similarity index $SD=2N_{ab}/(N_a+N_b)$ (Sneath and Sokal, 1973), where N_{ab} is the number of shared bands between a pair of genotypes 'a' and 'b', N_a the number of scored bands in genotype 'a' and N_b the number of scored bands in genotype 'b'. Similarity estimates were analysed by the unweighted pair-group method arithmetic averages (UPGMA); the resulting clusters were expressed as dendrograms. A cophenetic matrix was obtained from the tree matrix and the cophenetic correlation coefficient was calculated between the similarity matrix and the cophenetic matrix to evaluate how well the phenograms represented the relations among cultivars and to compare the dendrograms obtained by the full and the reduced data sets.

3. Results and discussion

To increase confidence in the fragments included in the matrix, the scoring of RAPD bands was done very conservatively, excluding weak bands or bands that were ambiguous for some accessions. It is possible that some useful polymorphisms were discarded, but our main aim was to obtain easily reproducible data for fingerprinting.

Although many primers, out of the 50 employed in the first trials, produced polymorphic banding patterns, only 22 primers were selected for fingerprinting all the accessions, and among them, 11 showed useful bands (e.g., reproducible, clearly resolved and unambiguously scorable). An example is shown in Fig. 1.

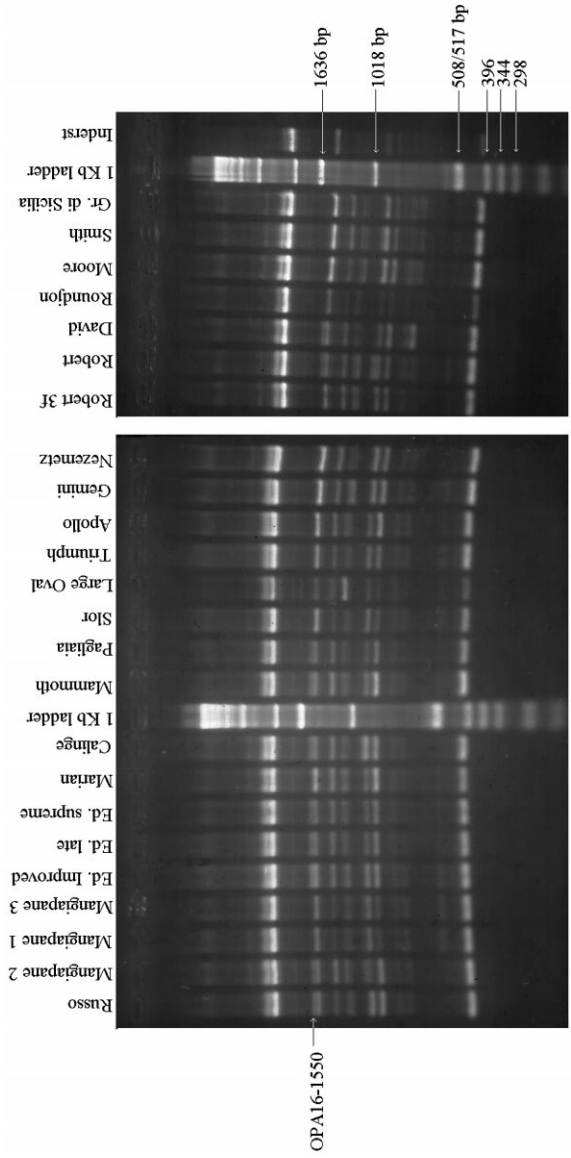

Fig. 1. RAPD markers produced by primer A16.

Table 2
Description of eleven ten-mer primers chosen for fingerprint analysis of feijoa

Operon kit[a]	*N*	Sequence 5′–3′	Resolved bands	Scored bands		Fragment size of resolved bands (kilobases)[b]		Polymorphic scored RAPD markers (base pairs)[b]	Accessions showing the polymorphic selected marker	
				Monomorphic	Polymorphic	Min	Max		*N*	%
A	11	CAATCGCCGT	17	2	5	0.4	3.8	2350	23	(92)
								1400	1	(4)
								1000	2	(8)
								740	19	(76)
								510	21	(84)
	12	TCGGCGATAG	6	1	1	0.7	3.0	1200	12	(48)
	16	AGCCAGCGAA	15	6	1	0.4	2.3	1550	10	(40)
	17	GACCGCTTGT	13	3	3	0.5	2.4	1800	3	(12)
								1400	11	(44)
								750	20	(80)
	18	AGGTGACCGT	8	1	2	0.6	2.1	1400	9	(36)
								1250	1	(4)
	20	GTTGCGATCC	10	5	2	0.7	3.1	1750	9	(36)
								1500	18	(72)
B	6	TGCTCTGCCC	12	4	1	0.5	2.5	2500	18	(72)
	8	GTCCACACGG	14	0	1	0.4	4.0	770	15	(60)

D	3	GTCGCCGTCA	14	1	4	0.7	2.5	2450	9	(36)
								2100	23	(92)
								1650	17	(68)
								930	16	(64)
F	1	ACGGATCCTG	13	1	2	0.4	1.4	560	9	(36)
								470	7	(28)
	6	GGGAATTCGG	14	2	1	0.5	2.8	670	16	(64)
Total	–	–	136	26	23	–	–	–	–	–
Average (for each primer)	–	–	12	2.4	2.1	0.5	2.7	–	13	(50.2)

[a] Operon primers notation: the letter refers to the kit; each kit is composed of 20 primers, each identified by the number following the kit letter.
[b] Based on the 1 Kb ladder marker.

These 11 primers showed an average of 12 resolved bands each, but few of them (4.5 on an average) were judged strong and clear enough in all the accessions to be used for fingerprinting. A total of 23 informative polymorphic fragments (Table 2) and 26 monomorphic ones were finally considered. The monomorphic fragments were added to the final matrix because excluding them could provide a misleading representation of the phenetic diversity present in the germplasm.

The fragments ranged from about 340 to 4000 base pairs (bp), but bands were scorable only from 510 to 2500 bp, as fragments out of this range were rarely reproducible.

The binary matrix of polymorphic primers is presented in Table 3. The number of rare RAPD markers was very low; only two cultivar-specific markers were found (A11 — 1400 bp marker and A18 — 1250 bp marker, both specific for cv. Smith) and only four polymorphic bands were common to less than 28% of the accessions. All the others were shared from a minimum of 28 to a maximum of 92% of the accessions, 50% being the mean value. These RAPD data discriminated 23 patterns. Twenty-one patterns characterised a single accession each. The remaining two patterns grouped a pair of accessions each: 'Edenvale late' and 'Edenvale supreme', Mangiapane 1 and Mangiapane 3. Discriminating these accessions was impossible, even with a total of 100 primers.

All the accessions named 'Mangiapane' (1, 2 and 3), of unknown origin, were collected in the same garden in Sicily. It is therefore possible that 'Mangiapane 1' and 'Mangiapane 3' are genetically identical. Nothing is known about the pedigree of the cultivars named 'Edenvale', except that they were obtained by the same breeder in California (Dr. F. Monastra, personal communication). The RAPD technique is rarely able to discriminate between very closely related genotypes (Demeke et al., 1993; Quarta et al., 1994; Warburton and Bliss, 1996; Oliveira et al., 1999), such as sports, clonal variants, somatic variants, and this could be the case for these two genotypes.

Our data show that the accessions 'Robert' and 'Robert 3f', the latter a putative bud sport of the former, differ for five bands out of 23 polymorphic fragments. In other species, such as potatoes, sport mutants or clonal variants have been separated only by one band out of 43 polymorphic fragments examined, or have not been separable by this technique (Demeke et al., 1993; Sosinski and Douches, 1996). Therefore, it seems unlikely that the accession called 'Robert 3f' could be a sport of 'Robert'.

All 23 patterns were identified by as few as five of the 11 informative primers and a total of nine bands (A11 — 1000, 740, 510; A12 — 1200; A17 — 1400; D3 — 2450, 1650, 930; F6 — 670).

Fig. 2 shows the dendrogram produced from the elaboration of the full matrix of 23 polymorphic and 26 monomorphic RAPD markers. The dendrogram obtained analysing the reduced matrix (not reported) showed few minor differences with the one presented. These differences did not affect the overall

Table 3
RAPD markers presence (1) or absence (0) for 25 feijoa genotypes

Primer	bp	Apollo	Calinge	David	Edenvale improved	Edenvale supreme	Edenvale late	Gemini	Grossa di Sicilia	Inderst	Large oval	Mammoth	Mangiapane 1	Mangiapane 2	Mangiapane 3	Marian	Moore	Nazemetz	Pagliaia	Robert	Robert 3f	Roundjon	Russo	Slor	Smith	Triumph
A11	2350	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1
	1400	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
	1000	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	740	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1
	510	1	0	1	1	1	1	1	1	1	0	1	0	1	0	1	1	1	1	1	1	1	1	1	1	1
A12	1200	1	0	0	0	0	0	1	0	1	1	1	0	1	0	0	1	1	1	1	0	0	0	0	1	1
A16	1550	0	1	1	1	1	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	0
A17	1800	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0
	1400	1	0	0	0	0	0	1	0	0	1	1	1	0	1	1	0	0	0	0	0	1	1	0	1	1
	750	1	0	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1	1	1	1	1	1	1
A18	1400	1	1	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0	0
	1250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
A20	1750	1	0	0	1	1	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	1
	1500	0	1	1	0	0	0	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1
B6	2500	1	0	1	1	1	1	1	0	0	1	1	1	1	1	0	0	1	1	0	0	1	1	1	1	1
B8	770	1	1	1	1	1	1	0	1	0	0	1	1	0	1	1	0	1	0	0	0	1	0	1	1	0
D3	2450	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	1	0	1	1	1	0	1	0	0	0
	2100	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	1650	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	1	1	0
	930	1	1	1	0	0	0	1	0	0	1	0	1	1	1	1	1	1	0	0	0	1	1	1	1	1
F1	560	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	1	1	1	0	1	1	0	0
	470	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0
F6	670	0	1	0	1	0	0	0	1	1	1	0	1	1	1	1	1	1	0	1	0	1	1	0	1	1

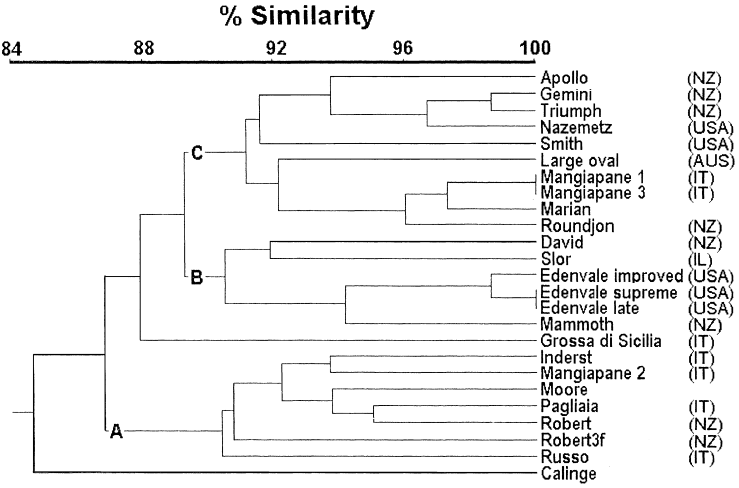

Fig. 2. Dendrogram of *F. sellowiana* accessions based on similarity index.

composition of the major clusters, indicated in Fig. 2 as A, B and C. The correlation coefficient between the dendrograms and their similarity matrix is of r=0.82 in both cases. This value indicates that both matrices are a good representation of the relationships among the accessions. A correlation coefficient of r=0.98 holds between the similarity matrix constructed from all RAPDs vs. only polymorphic RAPDs, indicating that the addition of monomorphic markers does not change the information provided by the polymorphic ones.

The distribution of the accessions within the clusters has no apparent relation with the geographical origin. Both the dendrograms contain three major clusters and two single-clustering accessions. Clusters B and C, which branch very close to each other, and are thus quite similar, contain accessions coming from all the countries considered in this study. Among them, four are widespread among cultivated varieties: Apollo, Gemini, Triumph and Mammoth. The first three cluster in the same group (C; Fig. 2), and are very close to each other; the fourth, cv. Mammoth, clusters in the nearby group B. The similarity values calculated between these cultivars are very high, both in the complete and reduced data sets (from 0.91 to 0.99 in the former case, from 0.70 to 0.96 in the latter). Cluster A groups four Italian accessions, two accessions from New Zealand and one of unknown geographical origin.

The low number of rare bands and the lack of evident relation between clusters and the geographical origin of the accessions suggest that most of the plant material analysed in this study shares a common genetic ancestry. Particularly, it is possible that relatively few accessions were used for breeding. This is in agreement with a study (Nodari et al., 1997) about feijoa germplasm in which feijoa accessions of Brazilian type (undomesticated) and Uruguay type

(domesticated) were analysed for morphologic characters and isoenzyme variability. The authors concluded that the diversity of domesticated types is low.

In recent years, molecular markers have been widely used as a tool to provide an estimate of germplasm similarity (or diversity) and the data derived from banding patterns were judged as more informative than those coming from pedigree analyses (Stiles et al., 1993; Graham et al., 1994, 1996). Our results demonstrate that RAPD markers are useful to identify feijoa genotypes and suggest that most of the accessions investigated in this study share a common parentage. A larger study, analysing also Brazilian type (undomesticated) accessions of *F. sellowiana*, would be necessary to confirm this hypothesis and to evaluate more accurately the diversity available for feijoa breeding.

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