

# Strawberry cultivars genetic relationship using randomly amplified polymorphic DNA (RAPD) analysis

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**Key words:** dendrogram, DNA fingerprinting, *Fragaria x ananassa* Duch., genetic relationship.

**Abstract:** Genetic diversity of cultivated strawberry was investigated using RAPD markers. Nineteen different strawberry genotypes, including cultivars and breeding parental lines, were analyzed with fifteen 10 bp primers. The amplification patterns revealed a high level of polymorphism. The results obtained with one of the 10 bp primers allowed a characterization of all genotypes. The DNA patterns obtained with three other primers characterized all but two genotypes. Similarity coefficients were calculated based on RAPD patterns obtained from 15 different primers and a dendrogram representing the genetic relationships among the genotypes was constructed. The results obtained in the present study, compared to previous breeding information available on the cultivars analyzed, confirm the genotypes parentage contribution. Therefore, RAPD markers can be considered a useful tool for further strawberry genetic analysis.

## 1. Introduction

The development and application of several molecular marker classes, such as RFLPs (Restriction Fragment Length Polymorphisms) (Botstein *et al.*, 1980; Tanksley *et al.*, 1989), RAPDs (Random Amplified Polymorphic DNA) (Welsh and McClelland, 1990; Williams *et al.*, 1990), AFLPs (Amplified Fragment Length Polymorphisms) (Vos *et al.*, 1995) and microsatellites (Jeffreys *et al.*, 1985; Thomas and Scott, 1993) could be useful also in fruit species in order to investigate genetic diversity, to select germplasm in plant breeding programs, to construct genetic maps (Hemmat *et al.*, 1994; Foolad *et al.*, 1995; Rajapakse *et al.*, 1995) and to isolate genes (Wing *et al.*, 1994), as well as to confirm pedigree notes (Grattapaglia *et al.*, 1992; Skroch *et al.*, 1992; Gepts, 1993). RAPD markers, in particular, have been found to be useful for cultivar identification in several crop species including *Citrus* (Luro *et al.*, 1995), *Rosa*

(Millan *et al.*, 1996), watermelon (Weeden *et al.*, 1992), cauliflower (Hu and Quiros, 1991), wheat (Vierling and Nguyen, 1992), rice (Ramakrishna *et al.*, 1994; Cao and Oard, 1997), tomato (Klein-Lankhorst *et al.*, 1991), peach (Rajapakse *et al.*, 1995), *Prunus* (Foolad *et al.*, 1995) and apple (Koller *et al.*, 1993).

Cultivated strawberry, *Fragaria x ananassa* Duch, is an octoploid ( $x=7$ ,  $2n=56$ ) resulting from natural hybridization of two octoploid species, *F. chiloensis* (L.) Duch. and *F. virginiana* Duch. (Darrow, 1966; Wilhelm and Sagen, 1972). Genetic diversity has been investigated in cultivated strawberry by the statistical analysis of parent contributions (Sjulin and Dale, 1987), by isozyme analysis (Arulsekhar *et al.*, 1981; Bringham *et al.*, 1981) and by RAPD analysis (Hancock *et al.*, 1994; Gidoni *et al.*, 1994; Degani *et al.*, 1998). Fourteen out of 22 cultivars were uniquely characterized using three isozyme systems (Bringham *et al.*, 1981); RAPD analysis, aiming to distinguish newly developed cultivars, revealed that four out of 41 primers characterized each of the eight cultivars tested (Gidoni *et al.*, 1994). Recently, Degani *et al.* (1998)

reported that 15 polymorphic fragments obtained from 10 primers were sufficient to distinguish all 41 genotypes tested. Therefore, it is possible to conclude that the octoploid *Fragaria spp.* showed a high level of polymorphism at biochemical (Arulsekar *et al.*, 1981) and molecular level (Hancock *et al.*, 1994; Gidoni *et al.*, 1994; Degani *et al.*, 1998).

The evaluation of genetic relationships among cultivars by molecular strategies could be useful for planning breeding programs, mainly to select segregating populations and for patent protection.

In this report, we detail the results of DNA fingerprinting using strawberry cultivars of different origin. For this purpose, a unique DNA fingerprint for each of the 19 different strawberry genotypes, including cultivars and parental breeding selections, was obtained using a single primer. The patterns of the amplification reactions using fifteen 10 bp primers were also used to evaluate the genetic relationship between genotypes. The genetic diversity results based on RAPD analysis also revealed a strong relationship with pedigree information of the genotypes involved.

## 2. Materials and Methods

### DNA extractions

Three grams of leaf tissue were collected from the 19 different strawberry genotypes (Table 1). Leaf tissue was ground to a fine powder in liquid nitrogen and stored at -70 °C until total genomic DNA was isolated using a CTAB (hexadecyl-trimethylammonium bromide) procedure (Doyle and Doyle, 1987) modified by Rowland and Nguyen (1993) with the exception that the polyethylene glycole (PEG) precipitation was

omitted. DNA concentrations were determined by an agarose gel electrophoresis using a known concentration of uncut lambda DNA as standard.

### DNA amplification conditions

Amplification reactions were performed in 25 µl volumes using 15 different 10 bp primers (from the Biotechnology Laboratory, University of British Columbia). The Polymerase Chain Reaction (PCR) buffer used was previously described by Barry *et al.*, (1991), showing best results in consistent phenotypes and a high amplification rate of DNA from a variety of fruit crop species (Levi *et al.*, 1993). Reaction conditions were as follows: 1x Barry buffer (20 mM NaCl, 50 mM Tris-Cl pH 9.0, 1% Triton-X-100, 0.1% gelatin), 1.5 mM MgCl<sub>2</sub>, 200 µM each dATP, dCTP, dGTP, and dTTP (Sigma), 0.2 µM 10 bp primer, 1 ng/µl strawberry DNA, and 0.06 units/µl Taq DNA polymerase (Promega, supplied in storage buffer A). DNA was amplified using the original Perkin Elmer thermocycler programmed for 50 cycles of the following program: 60 s at 94°C, 70 s at 48°C, and 120 s at 72°C. Amplified DNA products were separated by electrophoresis on 1.4% agarose gels, stained with ethidium bromide and visualized under UV light.

### RAPDs data analysis

The different DNA patterns obtained using all the 10 bp primers were classified by an alpha-numeric designation (Table 3). Genetic distances between all pairwise comparisons were calculated according to Nei and Li (1979) as:  $GD = 1 - [2N_c / (N_a + N_b)]$  where  $N_c$  is the number of fragments shared by two genotypes (A and B),  $N_a$  and  $N_b$  are the fragments detected by genotypes A and B, respectively. Genetic distances were used to construct a dendrogram by the unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis method with the SAS software package (SAS Institute, 1990).

Table 1 - Strawberry genotypes analyzed (MdUS 4380, MdUS 5071, and NYUS 156 are parental breeding selections, whereas all others are cultivars)

Genotypes	Origin
Cambridge Favourite	England
Redstar	Maryland
Belrubi	France
Rosanne	North Carolina
Rapella	Netherlands
MdUS 4380	Maryland
Tristar	Maryland
MdUS 5071	Maryland
Midland	Maryland
Del Norte	California
NYUS 156	Maryland
Francesco	Italy
Aiko	California
Brighton	California
Earliglow	Maryland
Red Giant	Minnesota
Fort Laramie	Wyoming
Elsanta	Netherlands
Allstar	Maryland

## 3. Results and Discussion

The efficiency of RAPD markers for strawberry cultivar DNA fingerprinting and the estimated genetic similarity were evaluated. Nineteen strawberry genotypes of different origin (Table 1) were previously tested using 28 primers ranging from 50% to 80% of GC contents in order to evaluate the ability to distinguish their pairwise combinations. Finally, fifteen 10 bp primers (Table 2) were exploited in the present study to achieve quite selective RAPD profiles. The remaining 13 primers did not result in amplification of any DNA fragments. The Polymerase Chain Reaction conditions, previously optimized on blueberry (Levi and Rowland, 1997), allowed obtainment of clear and reproducible amplification patterns also on different strawberry cultivars. The size of amplification products ranged from 0.3 to 4.0 kb. The GC content of a

Table 2 - Primer sequences and different profiles observed among the genotypes analyzed

Primer	Sequence				DNA Profiles Observed
A	AGC	GCC	GAC	G	19
B	AAC	GGG	CAC	C	5
C	GGG	GGC	CTC	A	10
D	GTC	ACC	GCG	C	5
E	CCG	CCC	CAC	T	9
F	AGG	AGC	TGG	C	18
G	GCG	GGC	AGG	A	15
H	CCC	GCG	AGT	C	18
I	GAA	GAA	CCG	C	7
J	CGG	CGT	TAC	G	18
K	CAA	GAA	CCG	C	7
L	GCG	CGG	CAC	T	12
M	GGG	CGA	GTG	C	14
N	CAC	CCC	CTG	C	16
O	CCC	ATG	GCC	C	16

primer has been already reported as the best indicator of the amplification efficiency (Fritsch *et al.*, 1993). The experiments on strawberry, as well as previous ones on blueberry, showed that the higher the primer GC content, the higher the probability of it resulting in amplification. The same relationship was observed between GC content and the number of amplified fragments (unpublished data). Therefore, in the present study, almost all selected primers were 70% or 80% GC rich. Since there were not problems in detecting polymorphisms with all the primers, a minimum of five (primers B and D) and a maximum of 19 different DNA profiles (primer A) were obtained on the bases of data set (Fig. 1, Table 3). It is interesting that the DNA patterns achieved by primer A distinguished each genotype, while the patterns obtained with each

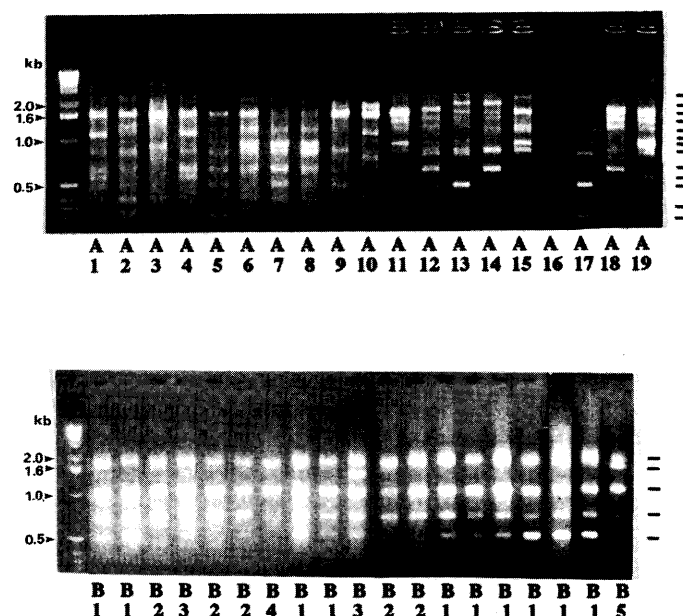


Fig. 1 - Amplification products obtained from all genotypes tested using primer A and primer B. Different patterns are designated with a different number (The black bar reported on the right of the figure indicates the polymorphic bands utilized for cluster analysis).

of the primers F, H, and J discriminated all but two genotypes (Table 3). These results suggest that few different primers could be efficiently utilized to identify strawberry cultivars.

Under our conditions, starting from the selected primers, 142 polymorphic fragments were achieved, while 160 fragments were shared among all genotypes. Sixteen out of 142 polymorphic fragments were unique in specific genotype.

Pairwise comparisons between the cultivars, based on clear unique and shared amplified fragments, were

Table 3 - Alpha-numeric designations for different DNA patterns obtained with each primer (The letter represents the primer used and the numbers indicate each DNA profile obtained)

Genotype	DNA profiles observed														
1	A1	B1	C1	D1	E1	F1	G1	H1	I1	J1	K1	L1	M1	N1	O1
2	A2	B1	C2	D2	E2	F2	G2	H2	I2	J2	K2	L2	M2	N2	O2
3	A3	B2	C3	D2	E3	F3	G3	H3	I3	J3	K2	L3	M3	N3	O1
4	A4	B3	C3	D2	E4	F4	G4	H4	I4	J4	K2	L4	M4	N3	O3
5	A5	B2	C2	D1	E4	F5	G5	H5	I4	J5	K3	L5	M5	N4	O4
6	A6	B2	C4	D2	E2	F6	G6	H6	I3	J2	K2	L6	M3	N5	O5
7	A7	B4	C4	D3	E5	F7	G7	H7	I3	J6	K4	L7	M6	N6	O6
8	A8	B1	C4	D1	E6	F8	G8	H8	I2	J7	K4	L8	M7	N7	O4
9	A9	B1	C5	D2	E7	F9	G9	H9	I4	J8	K5	L3	M8	N8	O7
10	A10	B3	C6	D4	E6	F10	G10	H10	I5	J9	K2	L9	M9	N9	O8
11	A11	B2	C1	D5	E7	F11	G2	H11	I6	J10	K2	L2	M10	N10	O9
12	A12	B2	C7	D2	E6	F12	G2	H12	I4	J11	K6	L10	M11	N11	O7
13	A13	B1	C1	D3	E8	F13	G7	H13	I3	J12	K2	L4	M12	N12	O10
14	A14	B1	C8	D1	E6	F14	G11	H14	I4	J13	K3	L2	M5	N13	O11
15	A15	B1	C1	D2	E6	F15	G12	H2	I7	J14	K7	L4	M13	N14	O12
16	A16	B1	C9	D3	E8	F2	G13	H15	I3	J15	K2	L11	M14	N8	O13
17	A17	B1	C10	D2	E9	F16	G14	H16	I3	J16	K5	L4	M15	N8	O14
18	A18	B1	C1	D2	E7	F17	G2	H17	I4	J17	K5	L10	M16	N15	O15
19	A19	B5	C4	D2	E6	F18	G15	H18	I3	J18	K2	L12	M5	N16	O16

used to generate similarity matrix. A cluster analysis based on the results of all 15 primers was used to generate the dendrogram shown in figure 2A. The dendrogram revealed a low level of similarity (0.49) between the cultivars, although some of them, in the pedigree notes, were reported as closely related (Sjulin and Dale, 1987). The similarity coefficients calculated for all genotypic pairs fell within a relatively narrow range. The dendrogram also showed the different 'Del Norte' origins (a wild selection of *Fragaria chiloensis* L. collected from northern California) according to the parentage analysis (Fig. 3). A different selection of *F. chiloensis* was utilized in a cross combination to obtain 'Cambridge Favourite', an old European cultivar. The remaining genotypes were represented by several near clusters with similarity coefficients ranging from 0.49 to 0.20. Although several genotypes appeared jumbled among the clusters, the analysis of their genetic backgrounds revealed that the parent genotypes are frequently shared (Fig. 3). The genealogical tree of the most similar genotypes, 'Cambridge Favourite' from England and 'Redstar' from Maryland (1<sup>st</sup> cluster 0.49), revealed a common parental ancestry, 'Howard 17' (Fig. 3). The parentage analysis of genotypes included in the other clusters revealed their

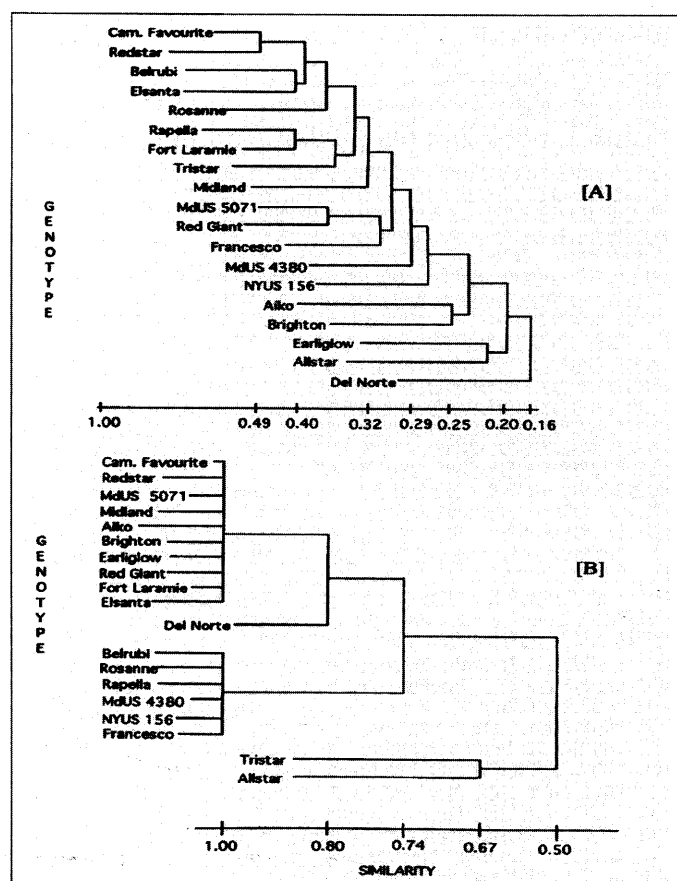


Fig. 2 - Dendrograms obtained by a cluster analysis (logarithmic scale) based on similarity matrix. A) Dendrogram based on data analysis of all 15 primers; B) Dendrogram obtained using only the data coming from primer B.

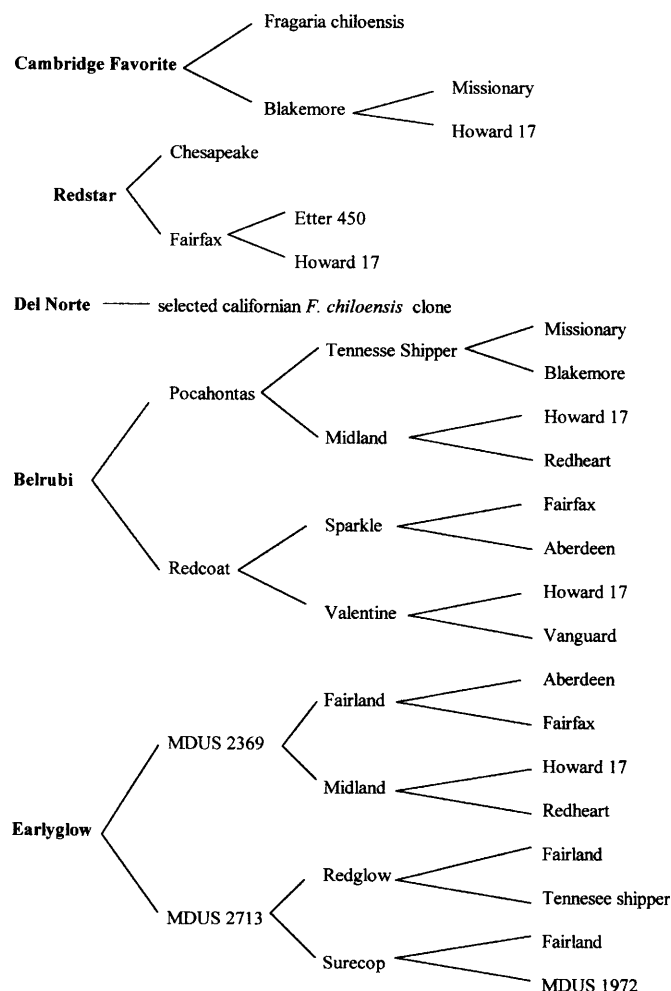


Fig. 3 - Genealogical tree of the main strawberry cultivars analyzed.

common origin since the cultivars 'Midland', 'Fairfax', 'Farland', and 'Howard 17' were utilized in different crosses achieving several genotypes analyzed (Fig. 3).

In contrast, a cluster analysis based on similarity coefficients calculated from the single primer B is shown in figure 2B. All but three genotypes were classified in two major groups and both showed the same similarity coefficients. These results confirmed that not all the primers alone could be useful to distinguish many cultivars.

Interestingly, the similarity coefficients were low in the dendrogram shown in figure 2A, although several genotypes have a similar ancestry. Weeden *et al.* (1992) predicted that the estimation of relative similarity based on RAPDs would achieve erroneously low values as a result of a rather low genetic variation detected by these markers.

Although RAPD markers were reported as not being able to distinguish cultivars in several species (Weeden *et al.*, 1992), RAPD patterns obtained in strawberry using the amplification conditions described in the present study, as well as reported by Gidoni *et al.*, (1994), Hancock *et al.*, (1994) and Degani *et al.*, (1998), were highly reproducible and discriminating

also with closely related cultivars.

In conclusion, RAPD marker analysis proved to be a quite simple and efficient technique in determining relative levels of diversity among strawberry genotypes, especially for cultivar identification. These reliable analyses have been successfully performed also to more closely distinguish related genotypes included in strawberry breeding programs (data not shown). Strawberry genotypes classification, based on RAPD similarity coefficients, could aid in combining and selecting germplasm for plant breeding purposes.

Although the utilization of this technique for DNA cultivar fingerprinting could be interesting for protection of plant breeders' rights (Jondle, 1992), preliminary results in strawberry (data not shown) seem to indicate that new and powerful classes of molecular markers, such as AFLPs and Simple Sequence Repeat (SSRs), in combination with RAPD analysis will be used more successfully for commercial and legal applications.

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