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A comparison of methods for the estimation of genetic diversity in strawberry cultivars

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Abstract RAPD markers were used to examine the genetic relatedness of eight strawberry cultivars released from four breeding programmes around the world. Ten random primers successfully amplified DNA fragments from each cultivar and specific fingerprints were generated from the molecular marker data. The cultivars were traced back to founding clones and the relationships between the cultivars were examined from both the molecular and the pedigree data.

Key words Strawberry · RAPDs · PCR

Introduction

The cultivated strawberry *Fragaria* × *ananassa* Duch. (2n = 56) is a result of hybridisation between two native American species, *F. chiloensis* (L) Duch. (2n = 56) and *F. viriginiana* Duch. (2n = 56), and is the most widely planted strawberry. Two other species, *F. vesca* L. (2n = 14) and *F. moschata* Duch. (2n = 42), are also grown commercially but on a much smaller scale. Breeding practices such as selection and inbreeding have narrowed the germ plasm base (Darrow 1966; Sjulin and Dale 1987). This is of concern because inbreeding and a lack of genetic diversity can result in vulnerability to diseases, pests and environmental stresses. In general, all major strawberry cultivars have arisen from common ancestors (Scott and Lawrence 1975). The successful development of new combinations of traits in future cultivars may be limited by a lack of genetic diversity and so necessitate the use of exotic gene sources. It is unclear at present how much genetic diversity is available in breeding programmes. Pedigree information does not give an indication of genetic diversity, since some of the original founding clones, although given different names, can actually be the same or else genetically very similar.

Molecular markers can be used to provide an estimate of the similarity or diversity of existing cultivars and new releases (Graham et al. 1994). Germplasm can be screened to determine the available genetic diversity and, depending on the results, this can highlight the need to widen the genetic base in a particular breeding programme or in a part of any one programme.

PCR can randomly amplify polymorphic DNAs (RAPD) in conjunction with random 10-bp primers (Williams et al. 1990). This technique allows differences at the DNA level to be detected by using small amounts of genomic DNA, ng–μg, as a template for PCR, with a set of random primers and under a specific set of conditions, to generate RAPD markers.

The present study sets out to use RAPD markers to examine eight strawberry cultivars released from four different breeding programmes, two in the UK, one in The Netherlands, and one in the USA. Through a pedigree search the cultivars were also traced back to the founding clones in order to determine how this data correlated with the molecular data.

Materials and methods

Eight strawberry cultivars (Cambridge Favourite, Melody, Rhapsody, Symphony, Pegasus, Honeoye, Elsanta and Evita) were chosen for this study as being representative of diverse breeding programmes (Table 1).

DNA extraction technique

From each cultivar, 1 g of leaf material from a number of young glasshouse-grown plants was ground in liquid nitrogen. Hexadecyltrimethylammoniumbromide (CTAB) solution (5 ml) was added and incubated at 65 °C for 30 min prior to the addition of 4 ml of chloroform/isoamyl alcohol. The mixture was agitated for 15 min followed by a spin of 6 min at 5000 g. The aqueous layer was filtered through sterile muslin and an equal volume of ice-cold propan-2-ol was added, mixed and incubated at room temperature for 15 min to precipitate the DNA. The DNA was hooked out if possible, or

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Table 1 Parents and country of origin of strawberry cultivars studied

Cultivar	Parents	Country of origin
Cambridge Favourite	(Etter seedling × Avent Tout) × Blakemore	England
Melody	Sengana × 66M1 (60BQ8 US2700)	Scotland
Pegasus	Redgauntlet × Gorella	England
Honeoye	Vibrant × Holiday	USA
Elsanta	Gorella × Holiday	Holland
Rhapsody	Cambridge Favourite × 61G51 (Talisman × 57C23)	Scotland
Symphony	Rhapsody × Holiday	Scotland
Evita	Chandler × (Brighton × Gorella)	England

pelleted by spinning at 500 g for 10 min, and re-suspended in 1 ml of Tris-EDTA buffer. In our experience, co-precipitation of polysaccharide is a problem with strawberry DNA extraction, leading to an inhibition of the PCR reaction. One or two salt precipitation stages are therefore included before suitable DNA can be obtained. A one-quarter volume of 5 M sodium chloride was added to the DNA, re-suspended in Tris-EDTA buffer and incubated at −20 °C for 30 min. This was then spun at top speed to pellet the polysaccharide and the DNA re-precipitated in 100% ethanol. If at this stage the DNA was still 'gel-like', the salt precipitation stage was repeated. The DNA was then washed twice in 70% ethanol and dissolved in 500 µl of TE; 5–10 µl of Rnase was added and the extract then stored at 4 °C until required.

DNA amplification (RAPD)

For PCR reactions the DNA was precipitated with 5 vol. of 100% ethanol; then DNA was hooked out, dried under vacuum and re-suspended in 200 µl of Tris-EDTA buffer. PCR reactions were carried out in 50 µl volumes containing 15 ng of genomic DNA, 5 µl of dNTP solution (2 mM), 5 µl of primer (2 µM), 5 µl of 10 × *Taq* buffer and 0.08 µl of *Taq* DNA polymerase (5 U/µl). Each reaction was overlaid with 40 µl of mineral oil to prevent evaporation. PCR reactions were carried out in a Hybaid OmniGene thermal cycler(Middlesex, England) under the following programme conditions: 45 cycles of 92 °C

Table 2 Percentage contribution of founding clones

Clone	Cultivar							
	Honeoye	Symphony	Elsanta	Rhapsody	Melody	Pegasus	Cambridge Favourite	Evita
Aberdeen	23	23.4	18	23	18.8	28	–	3.0
Missionary	16	22.6	16.4	12.5	–	–	25	4.4
Hudson Bay	3.7	2.1	1.6	1.8	0.4	1.1	2.3	1.9
White Caroline	3.7	2.1	1.6	1.8	0.4	1.1	2.3	1.9
NJ Scarlet	12.7	7.4	5.3	6.2	–	3.8	8.6	7.0
Ettersburg 450	6.2	–	–	–	–	–	–	–
Lupton	3.1	3.1	3.1	–	–	–	–	–
Belmont	4.5	2.6	1.9	2.2	–	1.4	3.0	2.3
Native Iowa	5.4	3.3	3.3	2.9	6.2	2.6	1.6	4.2
Jacunda	2.2	1.3	13.5	1.1	–	13.2	1.6	7.4
British Queen	1.1	0.7	0.5	0.6	–	0.3	–	0.6
Neunans	1.6	1.0	1.2	0.9	3.0	1.0	–	1.7
Ross Phoenix	0.8	0.5	0.6	0.4	1.6	0.5	–	0.7
Black Prince	3.1	2.0	2.3	1.8	–	2.0	–	2.9
Elton	3.1	2.0	2.3	1.8	–	2.0	–	2.9
Etters seedling	–	6.2	–	12.5	–	–	25	–
Avant Tout	–	6.2	–	12.5	–	–	25	–
Pearl	–	2.3	–	4.7	–	9.4	–	–
TD-8(Frith)	–	3.1	–	6.2	–	6.3	–	–
Ekey	–	1.6	–	3.0	–	–	–	–
US-3763	–	–	25	–	–	25	–	13
Markee	–	–	–	–	25	–	–	–
K. Sachsen	–	–	–	–	6.2	–	–	–
F. virginiana	–	–	–	–	18.8	–	–	1.6
F. vesca	–	–	–	–	–	–	–	0.7
F. chiloensis	–	–	–	–	–	–	–	1.0
US1854	–	–	–	–	12.5	–	–	–
Marshall	–	–	–	–	–	–	–	9
Nich Ohmer	–	–	–	–	–	–	–	16
Wm Belt	–	–	–	–	–	–	–	1.7
CAO.11	–	–	–	–	–	–	–	1.9
53.10–2	–	–	–	–	–	–	–	3
Cape Mendocino 1	–	–	–	–	–	–	–	0.4
Cape Mendocino 2	–	–	–	–	–	–	–	0.6
Perry	–	–	–	–	–	–	–	0.1
Shesapeake	–	–	–	–	–	–	–	0.1
Ett 1144	–	–	–	–	–	–	–	0.6
Cal BH 14	–	–	–	–	–	–	–	0.4
Unknown	–	4.2	3.2	3.6	7	2.2	4.7	9

Table 3 Random primers used for the detection of polymorphism

Primers	No. markers generated	No. cultivars identified
1. GGTAGCAGTC	14	8
2. GGTCCTCAGG	9	2
3. CAGTTCGAGG	9	8
4. TACCGACACC	9	3
5. ACTCAGGAGC	11	3
6. CCACCGCCAG	14	4
7. AGAGATGCCC	9	2
8. CAGTTCTGGC	11	5
9. AGCCAGCGAA	15	8
10. AATCGGGCTG	15	6

for 1 min, 35 °C for 3 min and 72 °C for 2 min, followed by one cycle of 72 °C for 5 min. All PCR reactions were carried out in triplicate. Random primers (see Table 3) were obtained from Pharmacia. Amplification products were resolved on a 1.5% agarose gel run in 0.5 × TBE buffer, stained with ethidium bromide, and visualised by illumination with ultraviolet radiation (312 nm).

Pedigree information

The Pedigree information shown in Table 2 was generated by tracing each cultivar back as far as possible to their founding clones (Darrow 1966; breeders' records; J.J. Luby and R.A. Harrison personal communication) and calculating the percentage contribution of each. This, however, can only be regarded as an estimate due to conflicting pedigree information, breeders' codes for which no information could be traced, and a lack of some early records.

Marker data analysis

The marker data are summarised in a bandmap (Powell et al. 1991). Genetic distance was calculated by the method of Nei and Li (1979), and the dendrogram was based on the average linkage clustering (UPGMA).

Pedigree data analysis

Genetic distances were based on the 'ecological' coefficient, namely:

$$dij = \sum_{k} |\chi ik - \chi jk| / 100$$

where χik and χjk are the percentage contributions of the k^{th} founding clone to the i^{th} and j^{th} genotype. When both χik and χjk are zero the contribution of the k^{th} founding clone is ignored. The dendrogram was derived by the UPGMA method.

Results and discussion

One-hundred-and-sixteen bands were generated from the ten primers, of which 79 (68%) were polymorphic and 37 (32%) were monomorphic. Figure 1 gives examples of the bands generated. All reactions were carried out in triplicate and it was found that the bands produced were generally consistent across the three reactions carried out for every primer and cultivar combination.

The bandmap (Powell et al. 1991), shown in Fig. 2, gives a graphical representation of all the markers generated. The ordering of cultivars on the bandmap is determined from the dendrogram (Fig. 3) based on the similarity index (see Fig. 5), and those cultivars with the greatest similarity are found to be next to each other on the bandmap. The bandmap also ordered the markers by their frequency of occurrence, whereby the commonest RAPD markers are placed to the top of the bandmap and the rarest to the bottom. The number of positive scores for the cultivars ranged from 62 to 76, indicating that the RAPD markers were evenly distributed throughout the germplasm. All cultivars had distinct profiles.

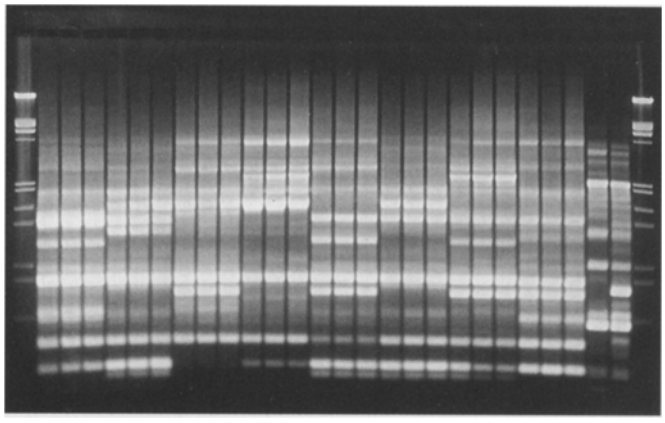

Fig. 1 PCR products amplified from strawberry DNA using primer 9

Dendrograms (Figs. 3 and 4) and similarity matrices (Figs. 5 and 6) were generated using both molecular data and by an estimate of the percentage contribution of each founding clone. The degree of similarity calculated from pedigree information (Fig. 6) is lower than that calculated from the molecular data (Fig. 5). It was anticipated that a lower similarity would be generated from pedigree information as it is likely that some of the founding clones, although given different cultivar names, were in fact the same or else very similar genetically. The use of molecular markers to assess genetic variability is therefore likely to be more accurate than pedigree analysis.

From the analysis of both pedigree and molecular data, Symphony and Rhapsody were most closely related with approximately 80% and 96% similarity respectively. This level of relatedness can be explained as Symphony is a first generation direct descendant of Rhapsody. Pegasus and Elsanta group together as the next most closely related (70% pedigree data and 87% molecular data) with Honeoye joining them. Pegasus and Elsanta both have Gorella as a parent and Honeoye has Holiday, the other parent of Elsanta.

Melody appears as the most distinct on the basis of pedigree information with only 25% similarity to the other cultivars. However, 12.5% of Melody was a breeders's code for which we could trace no further data and 18.8% was attributed to *F. viginiana*. This code will have some of the common ancestors represented and this is reflected in the molecular data which links

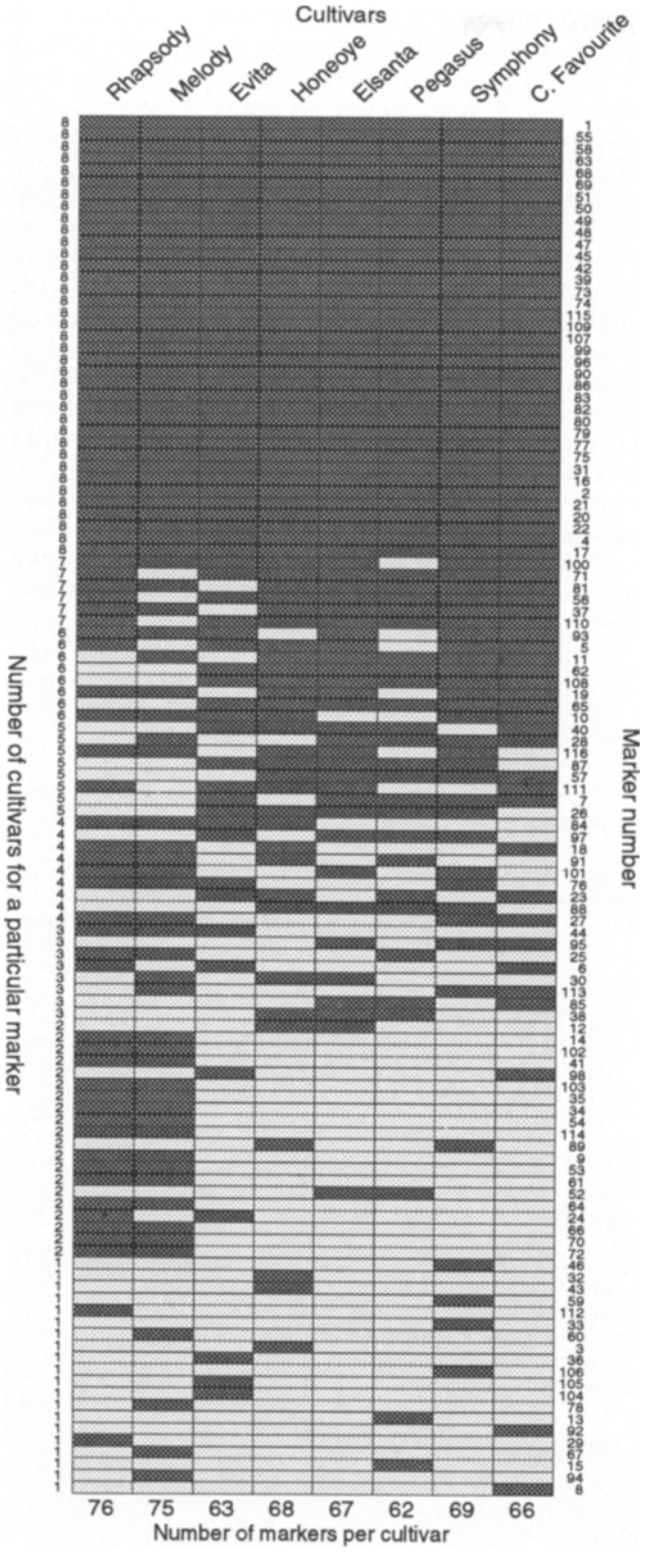

Fig. 2 Bandmap of RAPD markers detected in strawberry. A *dark box* represents the presence of a marker, a *light grey box* the absence of a marker

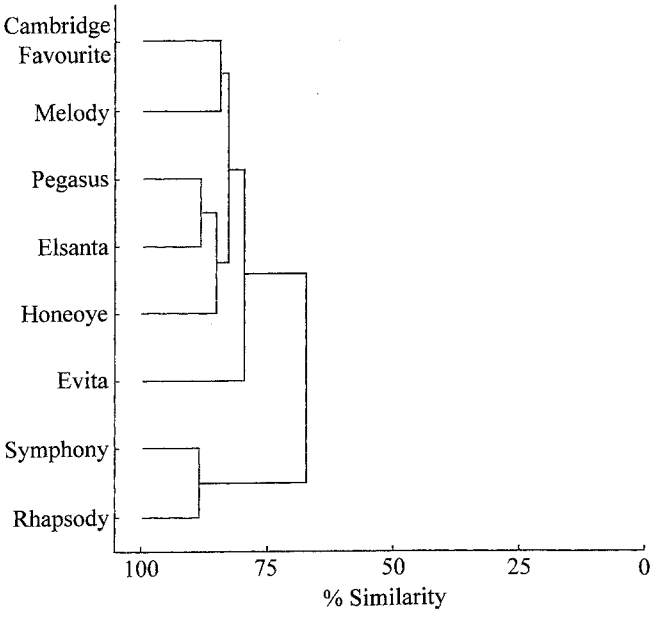

Fig. 3 Dendrogram of strawberry cultivars based on RAPD data

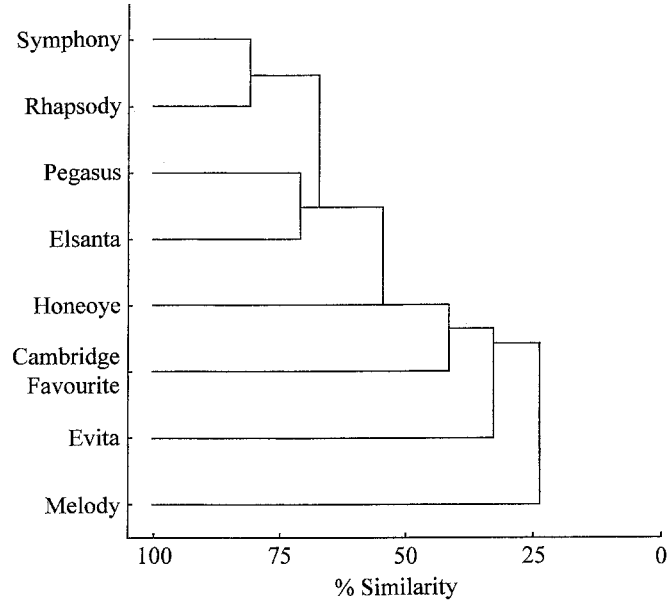

Fig. 4 Dendrogram of strawberry cultivars based on pedigree information

Melody with Cambridge Favourite then Honeoye, Pegasus and Elsanta.

Evita is the next most distinct on the basis of pedigree information with 23–42% similarity with the other cultivars. For Evita, 36% is attributed to Nich Ohmer (Table 4) and other founders not represented anywhere else. On the basis of molecular data, Evita has approximately 62–82% similarity with the others.

It is interesting and unexpected that Symphony and Rhapsody form a distinct group on the basis of molecular data, though still with 70% similarity to the others. A possible explanation is that these two cultivars were released from a strawberry breeding programme with the major objective of producing cultivars resistant to red core (*Phytophthora fragariae* var *fragaria*). Hence,

C. Favourite	100							
Melody	82	100						
Pegasus	83	80	100					
Elsanta	87	89	87	100				
Honeoye	83	84	83	86	100			
Evita	80	79	78	83	79	100		
Symphony	71	69	67	70	72	64	100	
Rhapsody	75	96	69	72	74	67	71	100
	C. Favourite	Melody	Pegasus	Elsanta	Honeoye	Evita	Symphony	Rhapsody

Fig. 5 Similarity matrix based on 116 markers

C. Favourite	100							
Symphony	60	100						
Pegasus	37	61	100					
Elsanta	44	75	70	100				
Honeoye	33	65	35	55	100			
Evita	29	42	35	40	32	100		
Melody	20	40	32	33	21	23	100	
Rhapsody	57	80	67	63	50	39	38	100
	C. Favourite	Symphony	Pegasus	Elsanta	Honeoye	Evita	Melody	Rhapsody

Fig. 6 Similarity matrix based on percentage contribution of founding clones

much of the resistant germplasm was derived by selection from the old cultivar Aberdeen.

The similarity matrix based on molecular data indicates the restricted genetic base of these strawberries with overall 70% similarity between all eight cultivars. The range of similarities for the strawberry cultivars was between 62% and 89% indicating their closely related nature even though many resulted from breeding programmes that were widely separated geographically. This can be compared to other soft fruit, such as closely related European red raspberry cultivars (*Rubus idaeus*), where similarities ranged from 48% to 87% (Graham et al. 1994), and in blackcurrant cultivars (*Ribes nigrum*), with similarities from 24% to 77% (Lanham et al. 1995).

The present study indicates the need to determine the genetic diversity of parents in breeding programmes and to increase the use of unrelated or unexploited germ plasm from other parts of the world. The use of RAPDs in strawberry will now be applied to determine and increase genetic diversity and to derive specific links between RAPDs, and other markers, with genes controlling agronomically important traits such as pest-resistance genes. The incorporation of markers for characteristics which are difficult to assess will represent a major advance in the genetic improvement of strawberry.

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