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IDENTIFICATION OF PEACH CULTIVARS USING RAPD AND AFLP MARKERS

Document prepared by experts from France

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IDENTIFICATION OF PEACH CULTIVARS USING RAPD AND AFLP MARKERS

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Introduction

French peach orchards are characterised by a great diversity of varieties with a fast turnover. The ability to distinguish peach cultivars is extremely important for the introduction of new peach varieties in the national trial network (stade A orchards) in order to test the DHS (Distinction-Homogeneity-Stability) necessary for registration into the French official catalogue and applying for protection status. The characterisation of cultivars requires a large set of phenotypic data that is often difficult to assess and sometimes varies due to environmental influences. At present, each variety is observed for 4 or 5 years during which 80 agronomic and botanical traits are evaluated. This number increases continuously in relation with the number of varieties which have to be identified. Consequently, it would be useful to develop molecular markers which could be used in addition to pomological studies. A previous study analysed the genetic distances between 10 peach varieties using isoenzymes (Reynders and Monet, 1987). As most of these varieties have many parents in common, the isoenzyme polymorphism was too low to distinguish them. In the present study, we have tested random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers to evaluate their potential use for detecting intraspecific variations in peach varieties.

Materials and methods

Sixty three peach varieties were chosen in the peach germplasm of Bordeaux for their various genetic and geographic origins (Table 1). They include French, Italian, Canadian and American varieties. Some of them are old varieties (Amsden, Dixired) or newer varieties cultivated all over the world (Catherina for clingstone, Fuzalode and Fantasia for nectarine, Redhaven, Flavorcrest, Robin, Redtop and Ferbar for peach). Forty five peach varieties were analysed by RAPD and 56 by AFLP includind 38 analysed by both RAPD and AFLP).

Genomic DNA was extracted from young leaves following the method of Viruel *et al* (1995) and purified through a CsCl gradient. RAPD reactions were performed as described by Williams et al. (1990) using 100 ten base primers from Operon Technologies. For AFLP analysis, genomic DNA was digested with *Eco*RI and *Mse*I restriction enzymes. A first preselective PCR amplification was performed using *Eco*RI+A and *Mse*I+C primers for 20 cycles of 94°C for 30 s, 56°C for 60 s and 72 °C for 60 s. For selective amplification *Eco*RI and *Mse*I primers with 2 or 3 selective nucleotides were used, one of them being ^{33}P-labelled using T4 polynucleotide kinase. The PCR reaction was done with a regime of denaturation for 30 s at 94°C, annealing for 30 s at 65°C, followed by an extension reaction of 60 s at 72°C for one cycle. For a further 11 cycles the annealing temperature was lowered by 0.7 °C per cycle.

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For a final 24 cycles, the denaturation was 30 s at 90°C, annealing for 30 s at 56°C and extension for 60 s at 72°C. The AFLP reaction products were analysed in 4.5 % denaturing polyacrylamide gels (7.5 M urea) in 0.5 TBE for 2 h. After electrophoresis, gels were dried on a standard slab gel drier for 2 h and exposed for three days to an X-ray film. In this study, 14 primer combinations were tested.

For each genotype, a binary matrix reflecting specific AFLP or RAPD band presence (1) or absence (0) was generated. Similarities between accessions were estimated using Jaccard coefficients. Dendrograms were constructed with the genetic distances matrix using Splus software.

Results and discussion

Out of the 100 primers tested for RAPD, only 47 revealed polymorphism between the 45 peach varieties analysed. This very low level of polymorphism is in agreement with results previously obtained with isoenzymes (Mowrey *et al.* 1990, Monet and Gibault 1991) and with RAPD in genetic mapping studies (Chaparro *et al.*,1994; Dirlewanger and Bodo, 1994; Dirlewanger *et al.*, 1996). With the 47 primers, 131 RAPD markers were identified. By using all of them, every 45 varieties can be identified (Fig.1). The dendrogram confirms the relationship between related varieties: Colomba is a self-pollination of Robin, they are grouped on the dendrogram. The same is true for Merriam and Everts. Minastar is a mutant of Springcrest, July Lady is derived from a cross with J.H.Hale. Harken and Harbrite are full sibs but can be distinguished using all the RAPD markers.

For AFLP, 13 primer combinations out of the 14 tested revealed polymorphism: 212 AFLP markers were identified (Table 2). The number of markers detected is very variable according to the combination primers used i.e. 0 *(Eco*RI AC/*Mse*I CAC) to 32 (*Eco*RI AA/*Mse*I CA). The combinations *Eco*RI AC/*Mse*I CA and *Eco*RI AC/*Mse*I CAC are not selective enough and too many fragments are amplified. By using three selective nucleotides for each primers *(Eco*RI ACC/*Mse*I CAT), the number of amplified fragments is too low and only 22 varieties among the 55 analysed can be distinguished with the 11 identified markers. With the *Eco*RI AA/*Mse*I CA combination, 32 markers were identified and 52 varieties among the 56 analysed were identified, the non-distinguished varieties being Granbo and Sensation, Springtime and Royal Gold (Fig.2). The two latter varieties are geneticaly very close, Royal Gold is issued from a bud mutation of Springtime. By using all the 212 AFLP markers, all the varieties are distinguished including Springtime and Royal Gold which differ only for one marker (Fig.3).

The above results demonstrate that RAPD and AFLP markers can be used as a complement to pomologic studies to distinguish peach varieties even if they are genetically very close i.e. with one or two parents in common or issued from mutations. However, AFLP appears much more effective: with only one analysis, near all the varieties were identified.

Name	Flesh	Type of fruit	Origin	Location	Markers	
783	yellow	peach	Escande	France		AFLP
Amsden	white	peach	L.G. Amsden	(Missouri) USA		AFLP
Andrus	yellow	nectarine	F.W. Anderson	(California) USA	RAPD	AFLP
Anita	white	peach	F. Zaiger	(California) USA	RAPD	AFLP
Babygold 5	yellow	clingstone peach	L.F.Hough&C.H.Bailey	(New Jersey) USA	RAPD	AFLP
Babygold 6	yellow	clingstone peach	L.F.Hough&C.H.Bailey	(New Jersey) USA	RAPD	AFLP
Babygold 8	yellow	clingstone peach	L.F.Hough&C.H.Bailey	(New Jersey) USA		AFLP
Baladin	yellow	clingstone peach	INRA	France		AFLP
Brademberr	yellow	clingstone nectarine	N. & C. Bradford	(California) USA	RAPD	AFLP
Catherina	yellow	clingstone peach	/INRA	New Jersey (USA)/ France	RAPD	AFLP
Colomba	white	peach	INRA	France	RAPD	AFLP
Desertgold	yellow	peach	USDA	(Fresno-California) USA	RAPD	AFLP
Dixired	yellow	peach	USDA	(Fort Valley-Georgie) USA		AFLP
Esgin	yellow	peach	Escande	France		AFLP
Everts	yellow	clingstone peach	University Davis	(Davis-California) USA	RAPD	AFLP
Fairhaven	yellow	peach	S. Johnston	(Michigan) USA		AFLP
Fantasia	yellow	nectarine	USDA	(Fresno-California) USA	RAPD	AFLP
Ferbar	white	peach	INRA	France	RAPD	
Ferradine Orlandine	yellow	peach	INRA	France		AFLP
Flavorcrest	yellow	peach	USDA	(Fresno-California) USA	RAPD	AFLP
Flavortop	yellow	nectarine	USDA	(Fresno-California) USA		AFLP
Fuzalode	white	nectarine	INRA	France	RAPD	AFLP
Genadix4	white	peach	INRA	France	RAPD	
Genadix7	white	peach	INRA	France		AFLP
Grabelle	yellow	peach	G. Merrill	(California) USA	RAPD	AFLP
Granbo	yellow	peach	G. Merrill	(California) USA	RAPD	AFLP
Harbrite	yellow	peach	Station Harrow	(Ontario) Canada	RAPD	AFLP
Harken	yellow	peach	Station Harrow	(Ontario) Canada	RAPD	AFLP
Harko	yellow	nectarine	Station Harrow	(Ontario) Canada	RAPD	AFLP
J.H.Hale	yellow	peach	J.H.Hale	(Connecticut) USA	RAPD	
Julie	white	peach	A. Maillard	(Dordogne) France	RAPD	AFLP
July Lady	yellow	peach	G. Merrill	(California) USA	RAPD	AFLP
Klamt	yellow	clingstone peach	L.D. Davis	(Davis-California) USA	RAPD	AFLP
Maygold	yellow	peach	USDA	(Fort Valley-Georgie) USA		AFLP
Mercil	yellow	peach	G.F.A. Castang	(Dordogne) France	RAPD	
Merriam	yellow	clingstone peach	L.D. Davis	(Davis-California) USA	RAPD	AFLP
Meystar	white	peach	Ner. Meynaud	(Bouches du Rhône)	RAPD	AFLP
Michelini	white	peach	A.Michelini	(Savona) Italie	RAPD	AFLP
Minastar	yellow	peach	W. Y. Minami	(California) USA	RAPD	AFLP
Nectared 9	yellow	nectarine	L.F.Hough&C.H.Bailey	(New Jersey) USA		AFLP
Nectarine Cerise	white	nectarine		(Loiret) France	RAPD	AFLP
Oregian	yellow	nectarine	S.Parnagian	(California) USA	RAPD	AFLP
Pratt's Compact	yellow	peach	Bountiful Ridge	(Maryland) USA		AFLP
Redhaven	yellow	peach	S. Johnston	(Michigan) USA	RAPD	AFLP
Redtop	yellow	peach	USDA	(Fresno-California) USA	RAPD	
Robin	white	peach	D.L. Armstrong	(California) USA	RAPD	AFLP
Royal Gold	yellow	peach	USDA	(Fort Valley-Georgie) USA		AFLP
Senateur Cazeneuve	yellow	peach		France	RAPD	AFLP
Sensation	yellow	peach	A. Maillard	(Dordogne) France	RAPD	AFLP
Sibelle	yellow	peach	A. Maillard	(Dordogne) France	RAPD	AFLP
Snow Queen	white	nectarine	D.L. Armstrong	(California) USA	RAPD	AFLP
Springcrest	yellow	peach	USDA	(Fort Valley-Georgie) USA	RAPD	
Springold	yellow	peach	USDA	(Fort Valley-Georgie) USA		AFLP
Springtime	white	peach	D.L. Armstrong	(California) USA	RAPD	AFLP
Summergold	yellow	peach	USDA	(Fort Valley-Georgie) USA		AFLP
Sundance	yellow	peach	G. Merrill	(California) USA	RAPD	AFLP
Symphonie	yellow	peach	A. Maillard	(Dordogne) France	RAPD	AFLP
Valandine	white	peach	G. Valla	(Drôme) France	RAPD	AFLP
Zaifer	yellow	peach	F. Zaiger	(California) USA		AFLP
Zailina	white	peach	F. Zaiger	(California) USA	RAPD	
Zainara	white	peach	F. Zaiger	(California) USA	RAPD	AFLP
Zairupe	yellow	clingstone nectarine	F. Zaiger	(California) USA		AFLP
Zaitibe	white	peach	F. Zaiger	(California) USA	RAPD	AFLP

Table 1 Name and agronomic characters of the peach varieties analysed for RAPD and AFLP polymorphism

Primer combinations	Nb of detected markers	Nb of varieties analysed	Nb of varieties distinguished	Nb of variety groups not distinguished (nb of varieties in the group)
*Eco*RI AA/*Mse*I CA	32	56	52	2(2)
*Eco*RI AA/*Mse*I CG	28	56	50	3(2)
*Eco*RI AC/*Mse*I CA	5	56	6	4(2)+3(3)+1(4)+1(8)+1(9)+1(12)
*Eco*RI AC/*Mse*I CG	20	56	34	5(2)+4(3)
*Eco*RI AA/*Mse*I CAA	12	55	26	5(2)+4(3)+1(4)
*Eco*RI AA/*Mse*I CAT	26	56	46	5(2)
*Eco*RI AA/*Mse*I CAC	16	52	40	6(2)
*Eco*RI AA/*Mse*I CAG	12	56	31	8(2)+2(3)+1(4)+1(5)
*Eco*RI AC/*Mse*I CAA	9	56	18	9(2)+2(3)+1(5)+1(9)
*Eco*RI AC/*Mse*I CAT	16	56	42	4(2)+2(3)
*Eco*RI AC/*Mse*I CAC	0	56	0	-
*Eco*RI AC *Mse*I CAG	15	54	49	1(2)+1(3)
*Eco*RI AAC/*Mse*I CA	10	55	22	4(2)+3(3)+1(4)+1(5)+1(7)
*Eco*RI ACC/*Mse*I CAT	11	55	22	2(2)+5(3)+1(4)+2(5)
Total	212	56	56	

Table 2 : Number of detected markers and identified varieties according to the primer combination used

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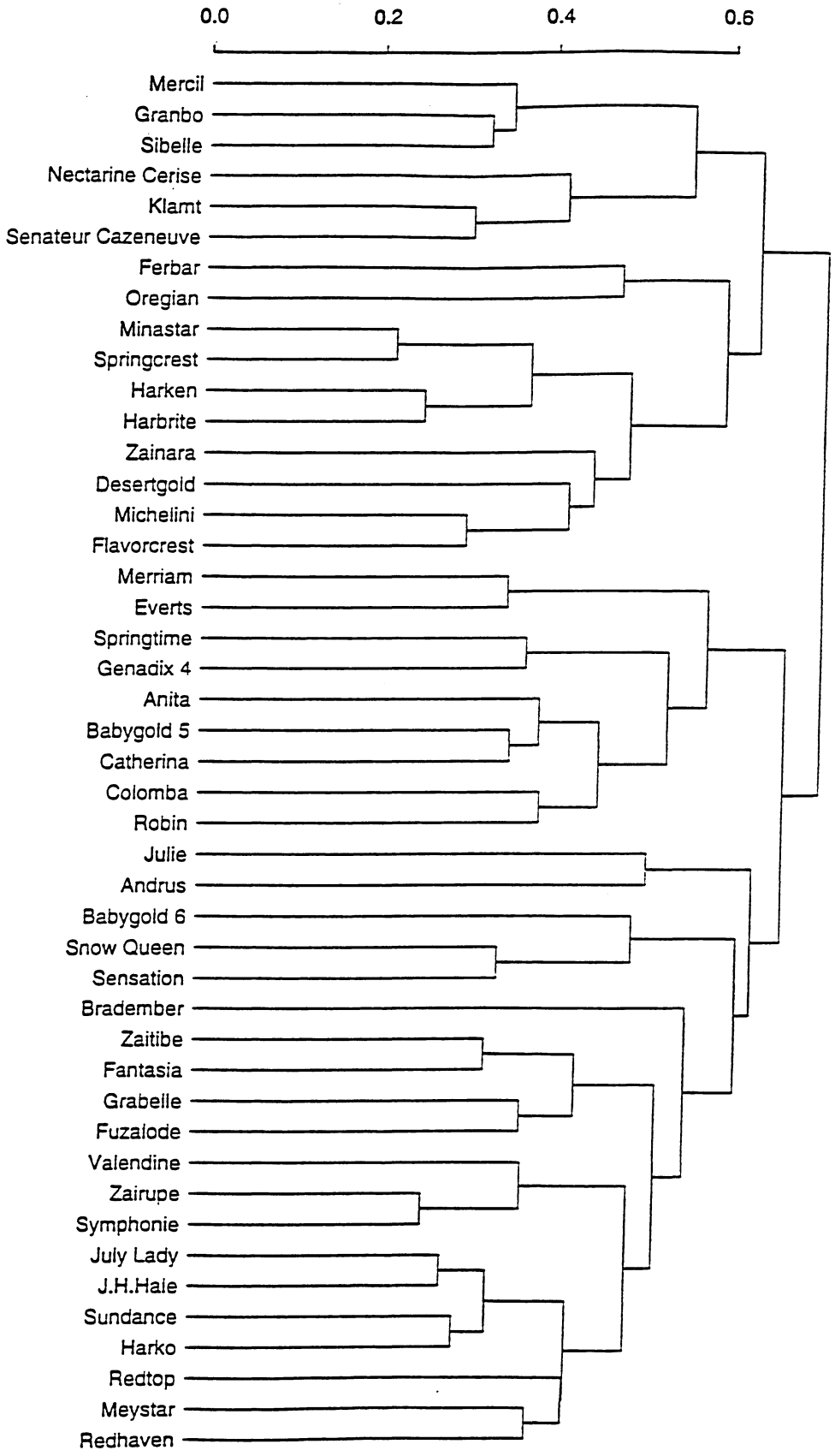

Fig.1 Dendrogram constructed from matrix of RAPD-based genetic distances

420.

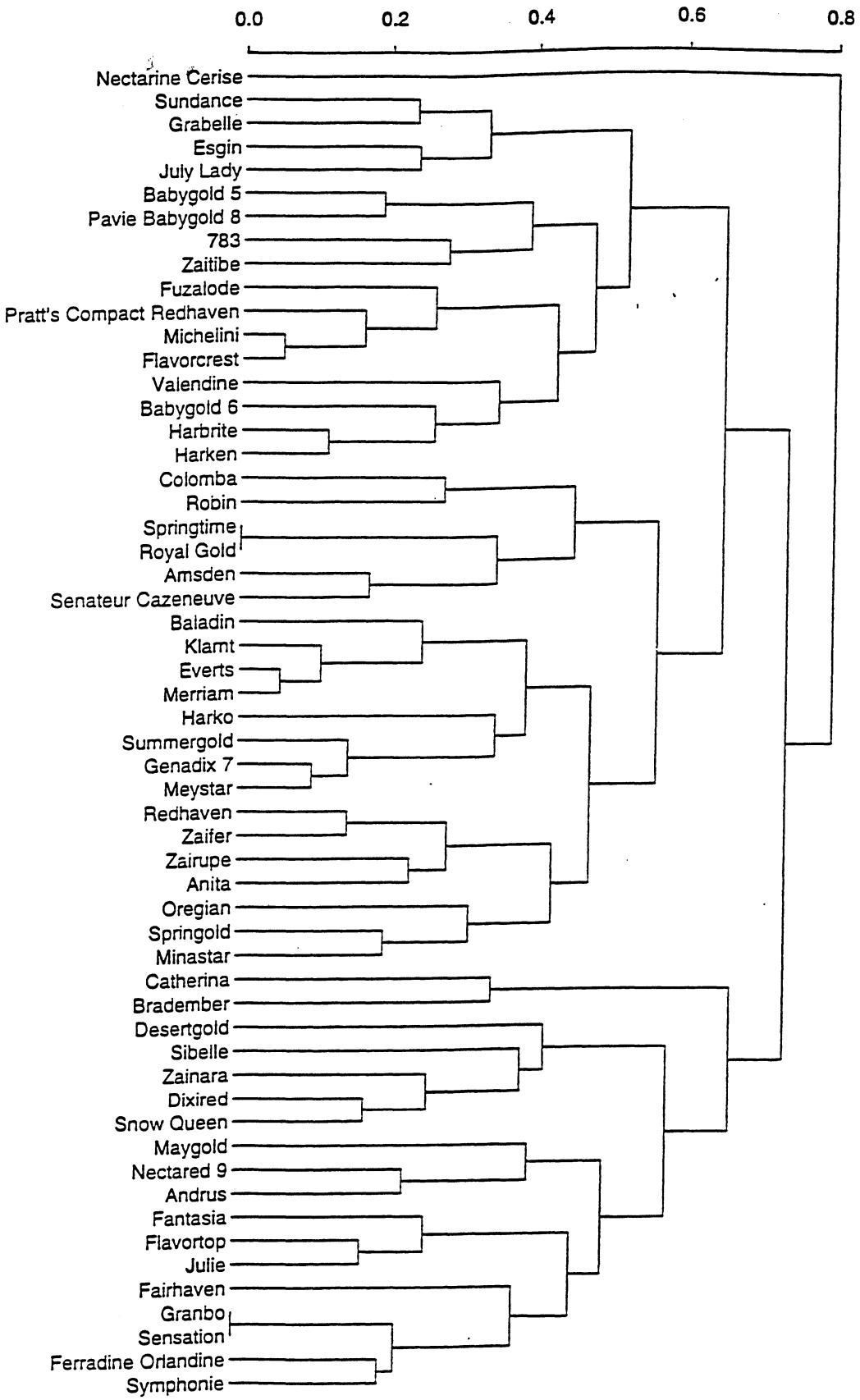

Fig.2 Dendrogram constructed from matrix of the *Eco*RI AA/*Mse*I CA AFLP combination - based genetic distances

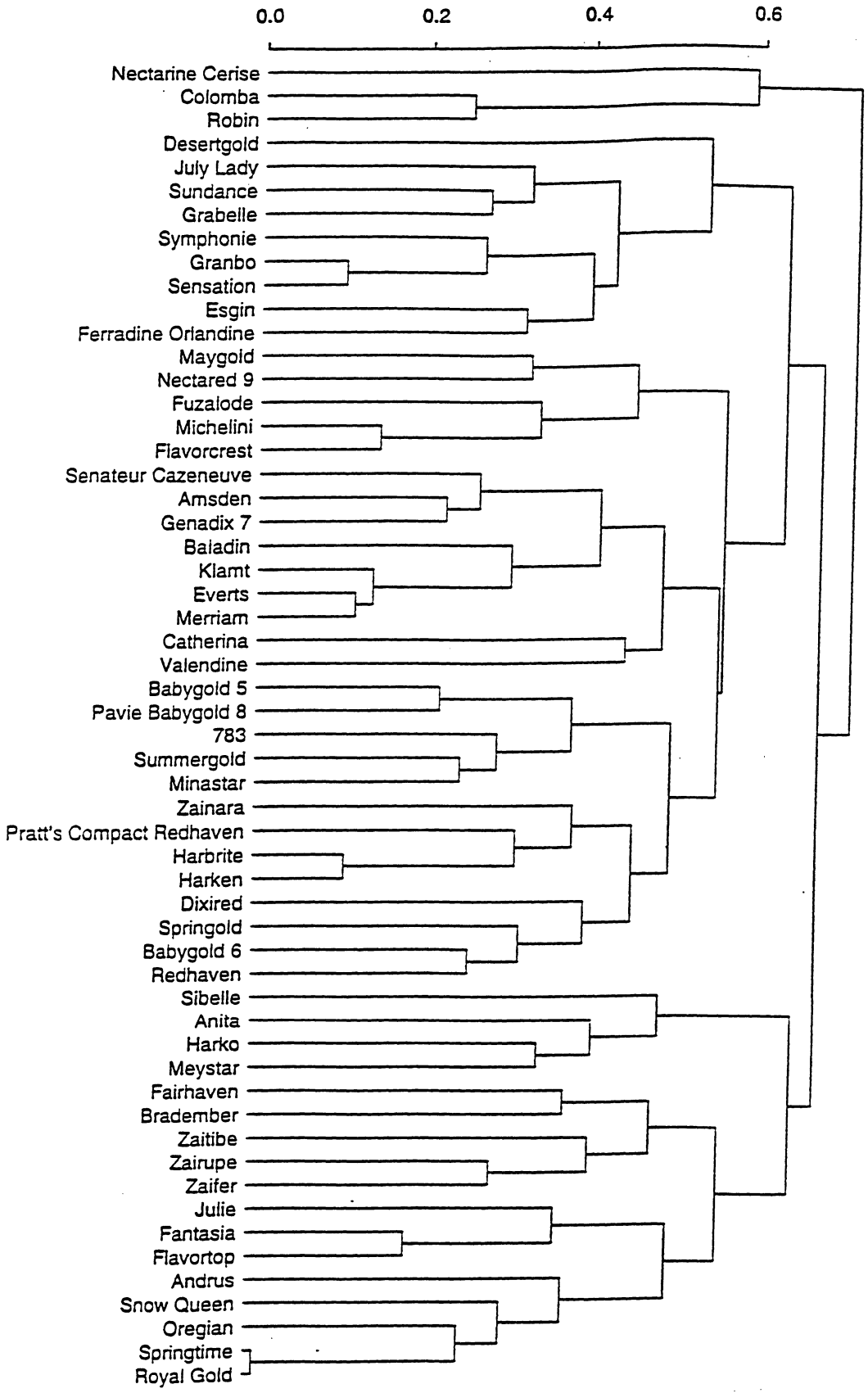

Fig.3 Dendrogram constructed from matrix of AFLP -based genetic distances (212 AFLP markers)

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