HortScience 30(5):1063–1067. 1995.

DNA Amplified Fingerprinting, A Useful Tool for Determination of Genetic Origin and Diversity Analysis in *Citrus*

François Luro
Station de Recherche Agronomique de Corse, Institut National de la Recherche Agronomique, Centre de Coopération Internationale en Recherche Agronomique pour le Développement—Fruits et Légumes Horticoles, San Giuliano 20230, San Nicolao, France

Frédéric Laigret and Joseph-Marie Bové
Laboratoire de Biologie Cellulaire et Moléculaire, Institut National de la Recherche Agronomique et Université de Bordeaux II, 33883 Villenave d'Ornon, Cedex, France

Patrick Ollitrault
Centre de Coopération Internationale en Recherche Agronomique pour le Développement—Fruits et Légumes Horticoles, BP 5035, 34032 Montpellier, Cedex 1, France

Additional index words. PCR , polymorphism, plant breeding, RAPD

Abstract. **We used three short repetitive nucleotide sequences [(GTG)5, (TCC)5, and (GACA)4] either as radiolabeled probes for hybridization with restricted citrus DNA or as single primers in polymerase chain reaction amplification experiments with total genomic DNA. We tested the ability of the sequences to discriminate between seedlings of zygotic or nucellar origin in the progeny of a Volkamer lemon (*Citrus volkameriana* Ten. & Pasq.) tree. The genetic variability within two species [*Citrus sinensis* (L.) Osbeck (sweet oranges) and *Citrus reticulata* Blanco and relatives (mandarins)] also was evaluated. DNA amplified fingerprinting with single primers was the more successful technique for discriminating between nucellar and zygotic seedlings. Although we were not able to distinguish among 10 cultivars of *C. sinensis*, all 10 *C. reticulata* cultivars tested were distinguishable. However, it still is difficult to identify the putative parents of a hybrid plant when the two parental genomes are closely related.**

To our knowledge, repetitive sequences, called minisatellites or variable number of tandem repeats (VNTR), have been found in the genomes of all eukaryotes tested (Epplen, 1988; Roewer et al., 1990; Tautz and Renz, 1984; Weising and Kahl, 1990). Used as probes, these sequences may yield DNA fingerprints, which are useful for genetic analysis and individual identification (Matsuyama et al., 1992; Nybom et al., 1990; Ryskov et al., 1988; Vassart et al., 1987). Expensive and time-consuming techniques, such as labeling with radioactive probes and purification of large quantities of DNA, may be replaced by using fragments generated by random primers in a polymerase chain reaction (PCR) as markers (Welsh and Mc Clelland, 1990; Williams et al., 1990). For rapid identification of individuals, repetitive sequences scattered throughout the genome seem more appropriate because the probability of revealing some polymorphism likely will be greater. PCR methods based on families of repeated sequences have been tested successfully [e.g., Alu-directed PCR in human genome analysis (Nelson et al. 1989)].

Received for publication 4 Feb. 1994. Accepted for publication 6 Apr. 1995. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

We reported on the occurrence of several minisatellite sequences in citrus genomes by Southern blot hybridization. These sequences were used as primers for PCR amplification. The efficiency of this technique for distinguishing zygotic from nucellar (apomictic) seedlings was evaluated. The phenomenon of facultative apomixis via nucellar embryony, a genetic trait common to many citrus cultivars (Frost and Soost, 1968), is a hindrance in breeding programs already hampered by long generation time, large space requirements by individuals, and little knowledge of inheritance of important traits. Many important citrus cultivars produce a polyembryonic mixture of nucellar and recombinant sexual embryos, and it may be necessary to distinguish the two types of seedlings as early as possible. For example, nucellar seedlings are used for roostock propagation because they are identical to the mother plant, but recombinant zygotic seedlings are of interest in breeding programs. These two types of seedlings are difficult to identify based solely on their morphological characters. The only accurate discriminant morphological character is the trifoliar leaf morphology, seen when *Poncirus trifoliata* (L.) Raf. is the paternal parent of zygotic seedlings. However, using isozyme markers brought a major improvement in the distinction of the two types of seedlings in several citrus progenies (Soost and Williams, 1980; Torres et al., 1982). We also evaluated the VNTR-PCR technique for its ability to distinguish between sweet orange cultivars and to reveal polymorphism among mandarin cultivars.

Materials and Methods

Plant material. Samples from 19 seedlings of *Citrus volkameriana* (numbers 2 to 20) and their mother (number 1), as well as 10 sweet orange [*C. sinensis* (L.) Osbeck] and 10 mandarin cultivars were harvested from the field germplasm collection of Centre de Coopération

Table 1. List of *Citrus* trees studied.

Cultivar	Botanical name	Accession no.	Mode of propagation
Sweet oranges	*C. sinensis* (L.) Osb.		
Pineapple	*C. sinensis* (L.) Osb.	SRA 42	Budding
Tarocco	*C. sinensis* (L.) Osb.	SRA 573	Shoot-tip grafting
Parson Brown	*C. sinensis* (L.) Osb.	SRA 43	Budding
Shamouti	*C. sinensis* (L.) Osb.	SRA 299	Somatic embryo
Doublefine	*C. sinensis* (L.) Osb.	SRA 354	Somatic embryo
Whashington navel	*C. sinensis* (L.) Osb.	SRA 217	Somatic embryo
Valencia late	*C. sinensis* (L.) Osb.	SRA 8	Somatic embryo
Sanguinelli	*C. sinensis* (L.) Osb.	SRA 352	Somatic embryo
Cadenera	*C. sinensis* (L.) Osb.	SRA 232	Somatic embryo
Hamlin	*C. sinensis* (L.) Osb.	SRA 251	Somatic embryo
Mandarins			
Willowleaf	*C. deliciosa* Tenore	SRA 133	Somatic embryo
King of Siam	*C. nobilis* Loureiro	SRA 274	Somatic embryo
Clementina Nules	*C. clementina* Hort. ex. Tan.	SRA 339	Shoot-tip grafting
Dancy	*C. reticulata* Blanco	SRA 594	Shoot-tip grafting
Ponkan	*C. reticulata* Blanco	SRA 585	Shoot-tip grafting
Malvasio	*C. reticulata* Blanco	SRA 427	Somatic embryo
Satsuma Owami	*C. unshiu* (Mack.) Marc.	SRA 145	Somatic embryo
Cleopatra	*C. reshni* Hort. ex. Tan.	B6X19	Somatic embryo
Wilking	*C. nobilis* Loureiro x *C. deliciosa* Tenore	SRA 112	Somatic embryo
Temple	*C. reticulata* Blanco x *C. sinensis* (L.) Obs.	SRA 348	Shoot-tip grafting

Internationale en Recherche Agronomique pour le Développement—Fruits et Légumes Horticoles/Institut National de la Recherche Agronomique, Station de Recherche Agronomique, San Giuliano, France. Total DNA was extracted, according to the procedure described by Doyle and Doyle (1987), from fresh leaves, prewashed with ethanol and distilled water. Sweet orange and mandarin cultivars were chosen to cover a large range of genetic diversity (Table 1).

PCR conditions. The 25-µl PCR reaction mixture contained 0.5 unit of *Taq* DNA polymerase (Gibco-BRL, Gaithersburg, Md.); 2 mM $MgCl_2$; 200 µM each of dATP, dCTP, dGTP, and dTTP; 16.6 mM $(NH_4)_2SO_4$; 67 mM tris HCl (pH 8.0); 10 mM β-mercaptoethanol; 0.01% anionic detergent; 0.4 mg bovin serum albumin/ml; 0.5 µM of a single primer; and sample DNA (5 to 20 ng). The mixture was covered with a drop of paraffin oil, and the reaction was performed in a DNA thermal cycler (model 240 VAC; Perkin-Elmer Cetus, Emeryville, Calif.) with 35 cycles, each consisting of 1 min at 91C, 1 min at annealing temperature (specific to each primer), and 1 min at 72C. After 35 cycles, a final step of 10 min at 72C was allowed to complete the synthesis of DNA strands. Three primers were prepared in a DNA synthesizer (Applied Biosystems, Foster City, Calif.): (GTG)5: 5′-GTGGTGGTGGTGGTG-3′, (GACA)4: 5′-GACAGACAGACAGACA-3′, (TCC)5: 5′-TCCTCCTCCTCCTCC-3′. Annealing temperatures were 47C for (GTG)5 and 45C for (GACA)4 and (TCC)5. Amplification products were analyzed by electrophoresis on 1.5% agarose or 8% polyacrylamide gels, subsequently staining with ethidium bromide and visualization under ultraviolet light.

DNA hybridization procedure. Citrus DNA samples were digested with restriction endonucleases *Hin*fI and *Dra*I according to the manufacturer's recommendations (Gibco-BRL). Digested DNA samples (2.5 µg/lane) were separated on 1.2% agarose gel in tris-acetate-ethylediaminetetraacetic acid (EDTA) buffer (0.04 M tris-acetate; 0.001 M EDTA). The restricted DNA was transferred to nylon membranes N+ (Amersham, Burkinghamshire, U.K.), according to the standard Southern blot procedure (Southern, 1975). The blots were prehybridized and hybridized in the same buffer: 5× saline–sodium citrate (SSC) buffer, 20 mM phosphate buffer (pH 6.5), 10× Denhart's solution, 10% dextran sulfate, and 0.5% skim milk in substitution for salmon sperm DNA (Zeff and Geliebter, 1987). The synthetic oligonucleotides were end-labeled with polynucleotide kinase of bacteriophage T4 (New England Biolabs, Beverly, Mass.); nonincorporated nucleotides were removed by spin-column chromatography on Sephadex G-50 resin (Pharmacia, Uppsala, Sweden) (Maniatis et al., 1982). The hybridization temperatures were the same as those for the annealing step in the amplification reaction. Blots were washed in 1× SSC 0.1% sodium dodecyl sulfate at room temperature and autoradiographed at –80C with intensifying screens.

Estimation of genetic diversity. The presence or absence of amplified fragments in DNA amplified fingerprinting (DAF) of mandarins was scored as 1 (present) or 0 (absent). The pairwise similarities between all individual genotypes were calculated using Nei's estimator (Nei and Li, 1979): $S = 2n_{xy}/(n_x + n_y)$, in which n_x and n_y are the number of fragments in genotypes x and y, respectively, and n_{xy} is the number of fragments shared by the two genotypes.

Results

Identifying zygotic and nucellar seedlings. Hybridization with labeled oligonucleotides and VNTR-directed PCR (PCR with repetitive sequences as primers) were evaluated for their ability to discriminate between nucellar or zygotic seedlings of *C. volkameriana*. The results were compared to those obtained from isozyme analysis using four heterozygous loci (Ollitrault et al., 1994), which showed that *C. volkameriana* individuals 2 to 9 and 13 were nucellar seedlings and individuals 10 to 12 and 14 to 20 were seedlings of zygotic origin.

We have used the minisatellites [(GTG)5, (TCC)5, and (GACA)4] as oligonucleotide probes for Southern hybridizations with restricted DNA from several *C. volkameriana* seedlings. The resulting autoradiography revealed restriction fragment length polymorphism (RFLP), but the high background level and the poor resolution of the bands did not make it possible to use this technique for routine analysis (data not shown). Nevertheless, this experiment showed the presence of numerous minisatellite sequences in *C. volkameriana* and, by extension, in citrus genomes.

When the (GTG)5 sequence was used as primer in the amplification reaction, the resulting DAFs for individuals 2 to 9 and 13 were similar to the mother plant (Fig.1A). The other plants were characterized by the loss of some bands or the presence of new amplified fragments when compared to the mother DAF

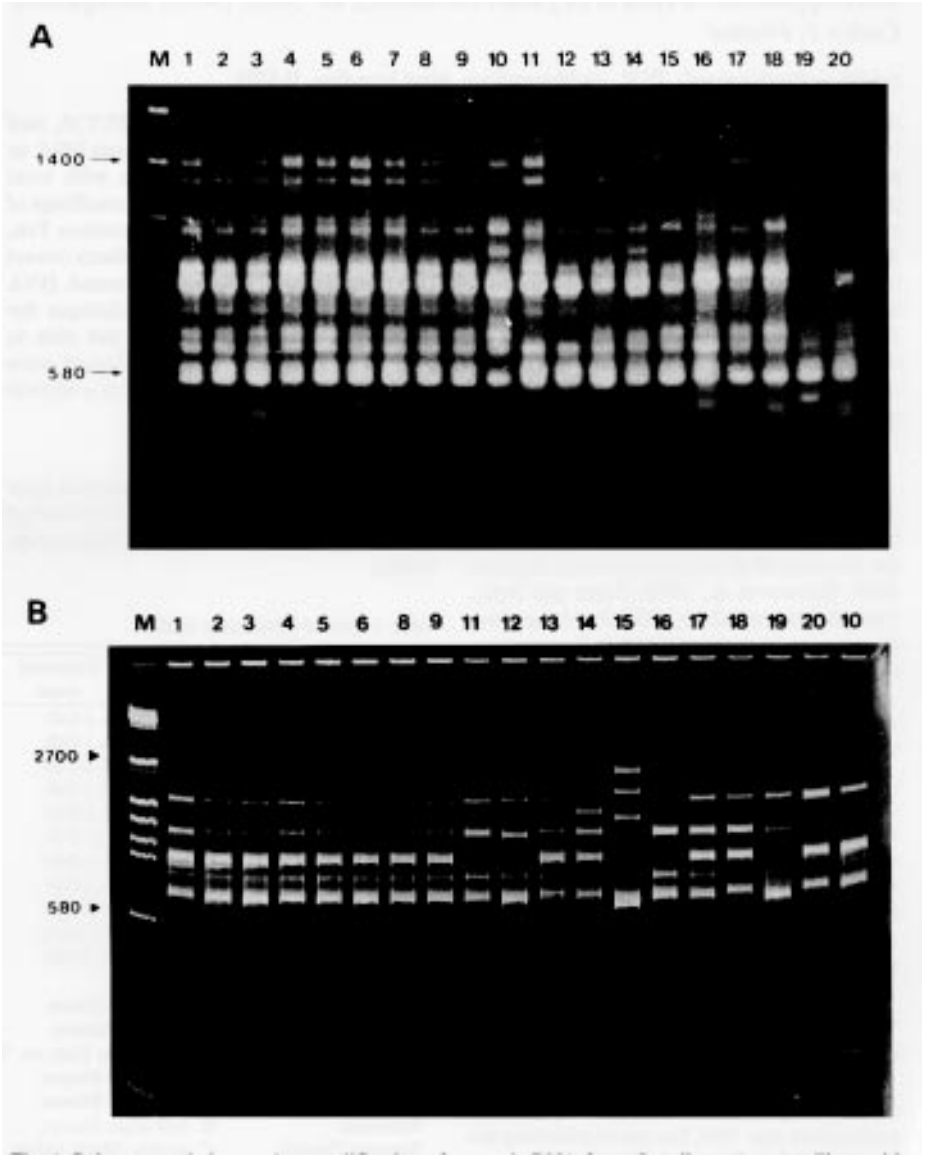

Fig. 1. Polymerase chain reaction amplification of genomic DNA from *C. volkameriana* seedlings with primers (**A**) (GTG)5 and (**B**) (TCC)5. Fragments were separated on (**A**) 1.5% agarose or (**B**) 8% polyacrylamide gel. The upper numbers correspond to the individual designations of *C. volkameriana* seedlings. M = molecular weight markers (in base pairs).

(individual 1). These results fully agree with those obtained with isozymes. However, such clear-cut results were not obtained with primers (TCC)5 (Fig.1B) and (GACA)4 (data not shown). With (TCC)5, three zygotic individuals (17, 18, and 20) had DAFs identical to those of nucellar seedlings, but with (GACA)4 primer, one zygotic sample (20) had a profile similar to that of the mother (data not shown).

Genetic diversity estimation. Using the same VNTR primers, we evaluated the genetic diversity within two groups of citrus plants: sweet oranges (Fig. 2), mandarins, and mandarin relatives (Fig. 3). With these primers, it was not possible to find a single difference among the DAFs of the 10 *C. sinensis* cultivars.

With the mandarin cultivars, combinations of fingerprints obtained with each of the three primers made it possible to distinguish each cultivar from the others (Fig. 3). To quantify the diversity, we constructed a similarity matrix based on 25 polymorphic amplified fragments (Table 2). This similarity is linked to the probability that one amplified fragment from one sample also will be found in another (Nei and Li, 1979).

According to genotype similarities, the mandarin cultivars may be grouped into several clusters. 'Dancy' and 'Ponkan' mandarins (*C. reticulata*) are closely related (0.88 of similarity). 'Malvasio' mandarin (*C. reticulata*) seems more distantly related to these two clones. Another cluster is composed of 'Cleopatra' mandarin (*C. reshni*), 'Nules' clementine (*C. clementina*), and 'Willowleaf' mandarin (*C. deliciosa*). This high relationship (0.9) was expected for 'Nules' clementine and 'Willowleaf' mandarin, which probably originated in China with a possible parentage between the two, but not for 'Cleopatra' mandarin, which originated in India (Hodgson, 1967).

Interestingly, 'Wilking' mandarin, which is a hybrid between *C. deliciosa* and *C. nobilis* (Frost, 1935), shows high similarity with 'King of Siam' mandarin (*C. nobilis*) (0.82) but a lower similarity with 'Willowleaf' mandarin (*C. deliciosa*) (0.67).

The so-called 'Temple' mandarin is generally considered to be a spontaneous tangor [i.e., a hybrid between *C. sinensis* and *C. reticulata* (Hodgson, 1967)]. Consequently, we compared the pattern of 'Temple' mandarin with sweet orange and mandarin profiles (Fig. 3B and not shown). Similarity was high for the amplified DNA patterns of sweet oranges, various mandarin cultivars, and 'Temple'. However, we suggest that the parental *C. reticulata* could be closely related to 'Ponkan' or 'Cleopatra' because 'Temple' DAF fragments, which are not encountered in *C. sinensis* DAF, are present in 'Ponkan' and 'Cleopatra' DAFs.

Discussion

Fingerprints with minisatellites as probes indicated the presence of numerous copies of the three minisatellites (GTG)5, (TCC)5, and (GACA)4 in the genome of citrus species. Consequently, we used these minisatellites as primers for PCR amplification of citrus genomic DNA.

The VNTR-directed PCR technique is simple and rapid and avoids using radioactivity. The DAFs obtained with each primer were highly reproducible. Different DNA preparations from the same individual gave identical results (data not shown). This reproducibility was obvious in the case of DAFs obtained for 10 clones of *C. sinensis* because no variation could be detected among these cultivars after amplification with (GACA)4 or (GTG)5 (Fig. 2).

Identifying nucellar and zygotic seedlings. Successfully using molecular markers to distinguish zygotic from nucellar seedlings depends on the number of loci detected and the degree of plant heterozygosity. Fingerprints with minisatellites as probes indicated the presence of numerous copies of three minisatellites, and consequently, we used these minisatellites as primers for PCR amplification of citrus genomic DNA.

The DAFs obtained with each primer for all the nucellar seedlings were identical. The primers differed in their ability to discriminate between the two types of seedlings and this difference correlated with the number of amplified fragments. The DAFs obtained with (GTG)5 had the most bands and were consequently the most discriminative. Primer (TCC)5 produced fewer bands and was less useful. However, primers giving the most fragments also produced a higher background and pictures of lower quality (Fig. 1A compared to Fig. 1B). Nevertheless, the relatively high level of background does not prevent the reproducibility of the results.

With a single primer (GTG)5 and 5 ng of DNA extracted by a fast procedure, it was possible to distinguish between nucellar and zygotic seedlings; four isozyme systems (aspartate amino transferase, phosphoglucose isomerase, malate dehydrogenase, and isocitrate dehydrogenase) were necessary to accomplish the same task (Ollitrault et al., 1994). The level of efficiency likely will vary with progeny from other citrus species.

Zygotic seedlings may arise by outcrossing or self-fertilization. The individuals resulting from outcrossing were easy to detect because their DAFs were characterized by additional bands compared to nucellar patterns; this was the case of individual 15 with primer (TCC)5 (Fig. 1), where four additional bands were revealed. In contrast, zygotic plants resulting from self-fertilization may have DAFs characterized by the absence of some bands compared to the nucellar pattern. This was the case for individual 11 with primer (TCC)5 (Fig. 1); this distinction was relatively easy with *C. volkameriana* because its genome is highly heterozygous. Most citrus clones are heterozygous at many loci, and analysis of many heterozygous loci can distinguish, with high probability, zygotic seedlings produced by self-pollination from nucellar seedlings (Roose, 1988). The VNTR-PCR technique should be tested in less favorable conditions (i.e., with the mandarins known to be less heterozygous).

Genetic diversity estimation. As demonstrated by isozyme analysis (Ollitrault and Faure, 1992; Torres et al., 1978), the intraspecific diversification processes are fundamentally different for *C. sinensis* and *C. reticulata* and relatives. Sweet orange cultivars arose by successive mutations from an ancestral apomic-

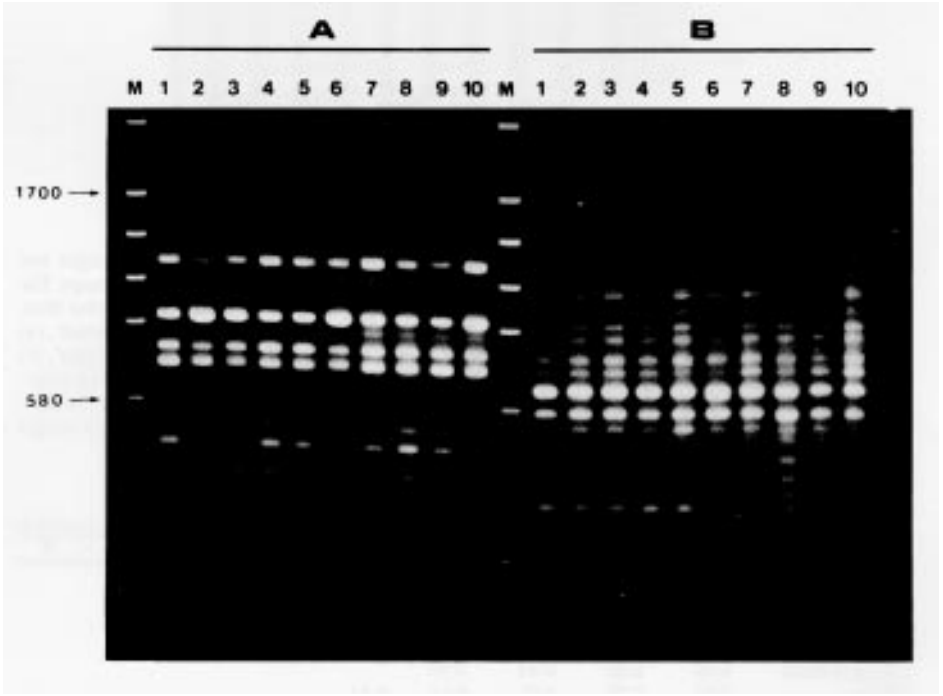

Fig. 2. Analysis of the diversity of *C. sinensis* clones. The DNA of 10 sweet orange cultivars was amplified with primers (**A**) (GACA)4 or (**B**) (GTG)5, and the products were separated on 1.5% agarose gel. The different clones of *C. sinensis* are (1) 'Pineapple', (2) 'Tarocco', (3) 'Parson Brown', (4) 'Shamouti', (5) 'Doublefine', (6) 'Washington Navel', (7) 'Valencia Late', (8) 'Sanguinelli', (9) 'Cadenera', and (10) 'Hamlin'. M = molecular weight markers (in base pairs).

tic genotype, and sexuality and recombination have played a major role in mandarin diversity. By using minisatellites as primers, we hoped to reveal some polymorphism among sweet orange cultivars. Unfortunately, this was not the case; consequently, like other genetic markers, it seems that the minisatellites used as primers are not useful for the detection of point mutations.

In contrast, a great diversity in DAFs was revealed in the mandarin group. The similarities observed were, in general, in good agreement with previous studies. The fragments amplified with short primers, such as RAPD markers, probably are dominant (Williams et al., 1990). This situation also may exist with minisatellites used as primers. With the same primer, the number of amplified fragments likely will vary from one individual to the other, according to the heterozygosity at the corresponding loci. When more loci are detected in a particular genome rather than in another, the similarity values may shift because fragments corresponding to homozygous loci will not be recorded as two fragments. This shift would explain the lower degree of similarity between 'Wilking' mandarin (*C. nobilis* x *C. deliciosa*) and 'Willowleaf' mandarin (*C. deliciosa*) than that observed between 'Wilking' and 'King of Siam' (*C. nobilis*) mandarins, although theoretically, 'Wilking' mandarin should be at equal distance from 'Willowleaf' and 'King of Siam' mandarins. Such a bias likely will be limited with the use of additional primers or by codominant marker analysis (e.g., RFLP or isozymes).

The similar patterns observed with sweet oranges and mandarins agree with the close phylogenetic relationships of these species (Barrett and Rhodes, 1976; Scora, 1975). Consequently, it is difficult to determine whether 'Temple' mandarin is of interspecific origin because of the low level of polymorphism between sweet orange and mandarin. Using additional primers or RFLP may provide more information.

The presence in citrus genomes of three distinct, single-repeat, DNA sequences was demonstrated. Using minisatellites as primers in PCR of genomic DNA is technically easy and rapid and avoids constraints due to limited plant material. Although their use as probes in a classical technique of RFLP fingerprinting is potentially more powerful in detecting polymorphism, the resulting hybridization profiles are more difficult to analyze than electrophoretic profiles of amplified fragments.

The DAFs observed after VNTR–PCR techniques make it possible to distinguish nucellar and zygotic seedlings and to distinguish each of the analyzed mandarins. In contrast, the 10 orange cultivars studied had identical profiles. These results were similar to those obtained with isozymes or RFLPs. Thus, VNTR–PCR techniques can be used for various analyses in citrus breeding, rootstock propagation, and genomic mapping.

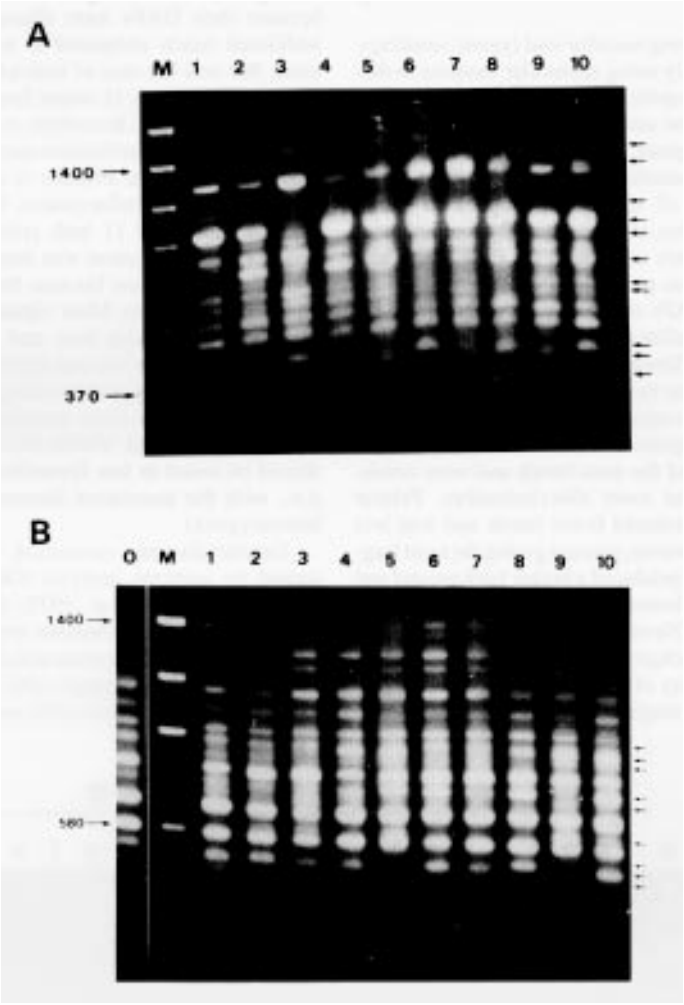

Fig. 3. Genetic diversity within the mandarin group. The DNA of nine mandarins and one tangor was amplified with primer (**A**) (GACA)4 or (**B**) (GTG)5, with an additional sample from a sweet orange. The resulting fragments were separated on 1.5% agarose gel. (0) *C. sinensis* 'Hamlin', (1) *C. reshni* Hort. ex. Tan. 'Cleopatra', (2) *C. clementina* Hort. ex. Tan. 'Nules', (3) *C. deliciosa* Tenore 'Willowleaf', (4) *C. reticulata* Blanco x *C. sinensis* (L.) Osb. 'Temple' tangor, (5) *C. nobilis* Loureiro 'King of Siam', (6) *C. reticulata* Blanco 'Dancy', (7) *C. reticulata* Blanco 'Ponkan', (8) *C. reticulata* Blanco 'Malvasio', (9) *C. nobilis* Loureiro x *C. deliciosa* Tenore 'Wilking', (10) *C. unshiu* (Mack.) Marc. 'Satsuma Owami'. Arrows on the right side indicate polymorphic amplified DNA fragments. M = molecular weight markers (in base pairs).

Table 2. Similarity matrix of mandarin cultivar genotypes.

	1	2	3	4	5	6	7	8	9
1 Cleopatra									
2 Nules	0.91								
3 Willowleaf	0.90	0.91							
4 Temple	0.82	0.76	0.63						
5 King of Siam	0.44	0.60	0.44	0.59					
6 Dancy	0.82	0.74	0.71	0.63	0.53				
7 Ponkan	0.73	0.86	0.74	0.78	0.59	0.88			
8 Malvasio	0.59	0.63	0.47	0.63	0.53	0.57	0.63		
9 Wilking	0.53	0.67	0.53	0.67	0.82	0.38	0.44	0.50	
10 Satsuma	0.67	0.78	0.67	0.70	0.74	0.67	0.70	0.78	0.70

Literature Cited

Barrett, H.C. and A.M. Rhodes. 1976. A numerical taxonomic study of affinity relationships in cultivated citrus and its close relatives. Systematic Bot. 1:105–136.

Doyle, J.J. and J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bul. 19:11–15.

Epplen, J.T. 1988. On simple repeated GA(TC)A sequences in animal genomes: A critical reappraisal. J. Hered. 79:409–417.

Frost, H.B. 1935. Four new citrus varieties—The Kara, Kinnow and Wilking mandarins and the Trovita orange. California Agr. Expt. Sta. Bul. 597.

Frost, H.B. and R.K. Soost. 1968. Seed reproduction: Development of gametes and embryos, p. 290–324. In: W. Reuther, L.D. Batchelor, and H.J. Webber (eds.). The citrus industry. vol. 2. Univ. of California Press, Riverside.

Hodgson, R.W. 1967. Horticultural varieties of *Citrus*, p. 431–591. In: W. Reuther, H.J. Webber, and L.D. Batchelor (eds.). The citrus industry. vol. 1. Univ. of California Press, Riverside.

Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular cloning: A laboratory manual. Cold

Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
Matsuyama, T., R. Motohashi, T. Akihama, and M. Omura. 1992. DNA fingerprinting in *Citrus* cultivars. Jpn. J. Breeding 42:155–159.
Nei, M. and W.H. Li. 1979. Mathematical model for study genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5267–5273.
Nelson, D.L., S.A. Ledbetter, L. Corbo, M.F. Victoria, R. Ramirez-Solis, T.D. Webster, D.H. Ledbetter, and C.T. Caskey. 1989. Alu polymerase chain reaction: A method for rapid isolation of human-specific sequences from complex DNA sources. Proc. Natl. Acad. Sci. USA 86:6686–6690.
Nybom, H., S.H. Rogstad, and B.A. Schaal. 1990. Genetic variation detected by use of the M13 "DNA fingerprint" probe in *Malus*, *Prunus*, and *Rubus* (*Rosaceae*). Theor. Appl. Genet. 79:153–156.
Ollitrault, P. and X. Faure. 1992. Système de reproduction et organisation de la diversité génétique dans le genre *Citrus*, p. 135–151. In: Bureau des Resources Génétiques (ed.). Complexe d'espèces, flux de gènes et ressources génétiques, Paris, France, 8–10 Jan. 1992. Lavoisier, Cachan, France.
Ollitrault, P., X. Faure, and F. Normand. 1994. Citrus rootstocks characterization with bark and leaf isozymes. Application to screen zygotic and nucellar trees, p. 338–341. In: E. Tribulato, A. Gentile, and G. Reforgiato (eds.). Proc. 7th Intl. Citrus Congr., Acireale, Italy, 8–13 Mar. 1992. MCS Congr., Catania, Italy.
Roewer, L., P. Nurnberg, E. Fuhrmann, M. Rose, O. Prokop, and J.T. Epplen. 1990. Stain analysis using oligonucleotide probes specific for simple repetitive DNA sequences. Forensic Sci. Intl. 47:59–70.
Roose, M.L. 1989. Isozymes and DNA restriction fragment length polymorphisms in citrus breeding and systematics, p. 155–165. In: R. Goren and K. Mendel (eds.). Proc. 6th Intl. Citrus Congr., Tel Aviv, Israel, 6–11 Mar. 1988. Margraf Scientific Books, Weikersheim, Germany, and Balaban Publishers, Rehovot, Israel.
Ryskov, A.P., A.G. Jincharadze, M.I. Prosnyak, P.L. Ivanov, and S.A. Limborska. 1988. M13 phage DNA as a universal marker for DNA fingerprinting of animals, plants and microorganisms. FEBS Lett. 233:388–392.
Scora, R.W. 1975. On the history and origin of citrus. Bul. Torrey Bot. Club 102:369–375.
Soost, R.K. and T.E. Williams. 1980. Identification of nucellar and zygotic seedlings of citrus with leaf isozymes. HortScience 15:728–729.
Southern, E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503–517.
Tautz, D. and M. Renz. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res. 12:4127–4138.
Torres, A.M., R.K. Soost, and U. Diedenhofen. 1978. Leaf isozymes as genetic markers in citrus. Amer. J. Bot. 65:869–881.
Torres, A.M., R.K. Soost, and T. Mau-Lastovicka. 1982. Citrus isozymes. Genetics and distinguishing nucellar from zygotic seedlings. J. Hered. 73:335–339.
Vassart, G., M. Georges, R. Monsieur, H. Brocas, A.S. Lequarre, and D. Christophe. 1987. A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science 235:683–684.
Weising, K. and G. Kahl. 1990. DNA fingerprinting in plants—The potential of a new method. Biotech-Forum/Europe 7:230–235.
Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213–7218.
Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531–6535.
Zeff, R.A. and J. Geliebter. 1987. Oligonucleotide probes for genomic DNA blots. Focus (Gibco BRL) 9:1–2.