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RAPD fingerprints for identification and genetic characterization of fig (*Ficus carica* L.) genotypes

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ABSTRACT

Identification of 21 fig accessions (*Ficus carica* L.) representing different varieties was performed using the RAPD (Random Amplified Polymorphism DNA) technique. The prescreening of 85 primers on four genotypes allowed to select 12 primers which revealed polymorphism and gave reproducible results. The 19 RAPD markers used provided 17 different banding patterns. The analysis revealed a labelling error for one accession and confirmed a synonymy for two others. The RAPD markers showed sufficient polymorphism for genotype discrimination, clonal stability, environmental stability and experimental reproducibility. The genetic variability observed in the fig genotypes was not structured into distinct subgroups probably because an important gene flow occurred in the natural populations from which cultivars originated.

Key words: Genetic variability, Genotype characterization, PCR, Polymorphism.

INTRODUCTION

The common fig (*Ficus carica* L.) is a gynodioecious plant species which grows and reproduces in the wild throughout the Mediterranean Basin (KJELLBERG *et al.*, 1987). Widely cultivated in different Mediterranean countries for its edible fruits, figs are multiplied through cuttings and a fig variety is defined as all cultivated individuals resulting from the same genotype. Generally, this genotype was originally located in the wild and chosen for its agronomic features (large, pulpy and sweet fig, high fruit yield etc). A number of fig varietal collections are kept by nurserymen. Error of varietal identification and synonymy constitute an important problem of collection maintenance.

Classical varietal identification is based on morphological traits. Using the size and the shape of fruits and leaves, the colour of fruit epiderm, etc CONDIT (1955) classified more than 600 fig varieties. However, these characters are sensitive to environmental conditions (CONDIT, 1950) and do not correspond in general to alternative criteria which lead to the separation of the phenotypes into distinct groups (VALDEYRON and VALIZADEH, 1976). Isozyme electrophoresis has been used in many species (BAILY, 1983; WEEDEN and LAMB, 1985; ATKINSON *et al.*, 1986) including the common fig (VALIZADEH *et al.*, 1977). An analysis of 61 fig varieties using four polymorphic isozyme loci allowed the characterization of 41 multilocus genotypes (VALIZADEH *et al.*, 1977). The success of varietal identification by isozyme electrophoresis depends on the number of isozyme systems and alleles studied. Unfortunately, this number is usually limited, which leads to insufficient polymorphism among closely related genotypes. In addition, the isozyme patterns may vary among tissues, developmental stages, and environments (BECKMAN and SOLLER, 1983). Restriction fragment length polymorphism based on the use of mini-satellites DNA sequences (DALLAS, 1988; NYBOM and HALL, 1991) or micro-satellites (WEISING *et al.*, 1991) as probe allowed to discriminate genotypes even in the case of a narrow genetic basis. However, the RFLP technology is a laborious procedure and usually requires the use of radioisotopes.

Recently, a novel technique based on the amplification of random DNA sequences by the polymerase chain reaction (PCR) with arbitrary primers has been developed by WILLIAMS *et al.* (1990) and WELSH and MCCLELLAND (1990). The

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random amplified DNA fragments are issued from repeated DNA or low-copy number sequences (WILLIAMS *et al.*, 1990; WILDE *et al.*, 1992). This technique allows to detect extensive polymorphisms resulting from the unlimited number of arbitrary primers. Specific amplified products (RAPDs) segregate in a Mendelian fashion (CARLSON *et al.*, 1991; HUNT and PAGE, 1992) and, therefore, can be used as genetic markers to study hybrid populations or species (ARNOLD *et al.*, 1991), to analyse paternity and kinship relationships (WELSH *et al.*, 1991; HADRYS *et al.*, 1992), and to develop genetic linkage maps (KLEIN-LANKHORT *et al.*, 1991; REITER *et al.*, 1992). The RAPD markers can also be used in the study of the genetic variability of species or natural populations (CHALMERS *et al.*, 1992; VAN HEUSDEN and BACHMANN, 1992; MOSSELER *et al.*, 1992; HUFF *et al.*, 1993; LASHERMES *et al.*, 1993; WILKIE *et al.*, 1993) and in the identification of different genotypes (HU and QUIROS, 1991; WILDE *et al.*,1992; WOLFF and PETERS-VAN RIJN, 1993; KOLLER *et al.*, 1993).

In this work, we developed and characterized RAPD markers in order to fingerprint fig genotypes. Our aim was to demonstrate that fig varietal identification using the RAPD technique is feasible, and to get a set of RAPD markers in order to subsequently study genetic variability in natural populations.

MATERIALS AND METHODS

Plant material

The analysis was performed on 21 fig accessions representing 19 denominations (Table 1). These samples were chosen to represent the collections maintained by nurserymen in France and to include economically important varieties. The accessions were from four different localities: Baud nursery (Vaison la Romaine), the varietal collection of Porquerolles (Botanic conservatory), a grower from Perpignan and the campus of CEFE/CNRS Montpellier.

In order to study clonal stability, five samples representing cvs: Col de Dame blanche, Col de Dame grise and Col de Dame noire were chosen. These varieties have the same enzymatic spectrum (VALIZADEH *et al.*, 1977) and identical morphological traits, the only difference being the colour of the fruit epiderm probably resulting from somatic mutations in a fig clone (VALIZADEH *et al.*, 1977). The analysis included two samples of cvs Col de Dame blanche and Col de Dame noire. The first clone originated from the varietal collection of Porquerolles and the second from the traditional area of cultivation at Perpignan. Each of these two clones has been grown separately in two different geographic localities since long.

TABLE 1

List of fig accessions used in this study

Number	Name	Collection
1	Dauphine	Porquerolles
2	Violette Dauphine	Baud nursery
3	Col de Dame grise	Porquerolles
4	Col de Dame blanche (accession n° 1)	Porquèrolles
5	Col de Dame noire (accession n° 1)	Porquerolles
6	Col de Dame noire (accession n° 2)	Perpignan
7	Bourgeassotte noire	Porquerolles
8	Violette de Solliès	Baud nursery
9	Marseillaise	Baud nursery
10	Verte d'Argenteuil	Baud nursery
11	Longue d'aout	Baud nursery
12	Osborn Prolific	Baud nursery
13	Medeleine des deux Saisons	Baud nursery
14	Grise de Saint Jean	Baud nursery
15	Tardive Aout Turkey	Baud nursery
16	Sultane	Baud nursery
17	Noire voisine de la Sultane	Baud nursery
18	Grise ronde à chair rose	Baud nursery
19	Grosse longue verte	Baud nursery
20	Roscoff	CEFE/CNRS, Montpellier
21	Col de Dame blanche (accession n° 2)	Perpignan

Genomic DNA isolation

Total DNA was extracted from dormant vegetative buds according to DELLAPORTA *et al.* (1983) with the following modifications: the extraction buffer contained 50 mM beta-mercaptoethanol, precipitations were carried out at −70°C for 30 min and nucleic acids solutions were treated three times with a mixture of phenol (1 vol.), chloroform (1 vol.) and meta-cresol (0.2 vol.) for protein removal. The estimation of genomic DNA concentration was performed by spectrophotometry and by ethidium bromure coloration after electrophoresis.

PCR technique and electrophoresis

Arbitrary decamer oligonucleotides purchased from Operon Technologies (USA) were used for the amplification of random DNA sequences. Amplificaton reactions were done in 25 µl volumes containing 10 mM tris-HC1 pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 100 µm of each dATP, dCTP, dGTP and dTTP (Boehringer), about 10 ng of genomic DNA, 0.4 µM of primer and 1 unit of Taq polymerase (Perkin Elmer Cetus). The reaction mix was overlaid with two drops of mineral oil (Sigma) to prevent evaporation. PCR reactions were perfomed using the Trio-ThermoBlock apparatus (Biometra/Germany). Samples were kept at

94°C for 5 min and then subjected to 45 repeats of the following cycle: one minute at 94°C, one minute at 35°C, two minutes at 72°C, and then a final step of six minutes at 72°C. Amplification products were analysed by electrophoresis in 1.5% agarose gels in TBE buffer at 4 volt/cm for 4h and detected by staining with ethidium bromide. The fragment patterns were photographed (667, Polaroid) for further analysis.

Data analysis

For each primer, the consistent amplified products were recorded. The polymorphic fragments (RAPD) were named by the primer code followed by the size of the amplified fragment in base pairs. The presence of a specific product was noted whatever the intensity of the band. Each RAPD marker was assumed to correspond to a locus with two alleles (presence or absence of the band). A similarity index S, expressing the probability that a RAPD in one accession is also found in another was calculated according to Nei and Li (1979) for all possible pairwise comparisons between accessions: $S = 2 N_{AB} / (N_A + N_B)$ where N_{AB} is the number of amplified products common to both A and B, and N_A and N_B correspond to number of amplified products in A and B respectively. The probability of finding two individuals with the same pattern can be calculated as the mean similarity index to the power of the mean number of bands (Nybom and Hall, 1991). The Phylip computer program (Felsenstein, 1987) was used for a hierarchical clustering analysis based on the unweighted pair group method with arithmetic mean (Upgma; Benzecri, 1973) to generate a dendrogram and to describe relationships among genotypes.

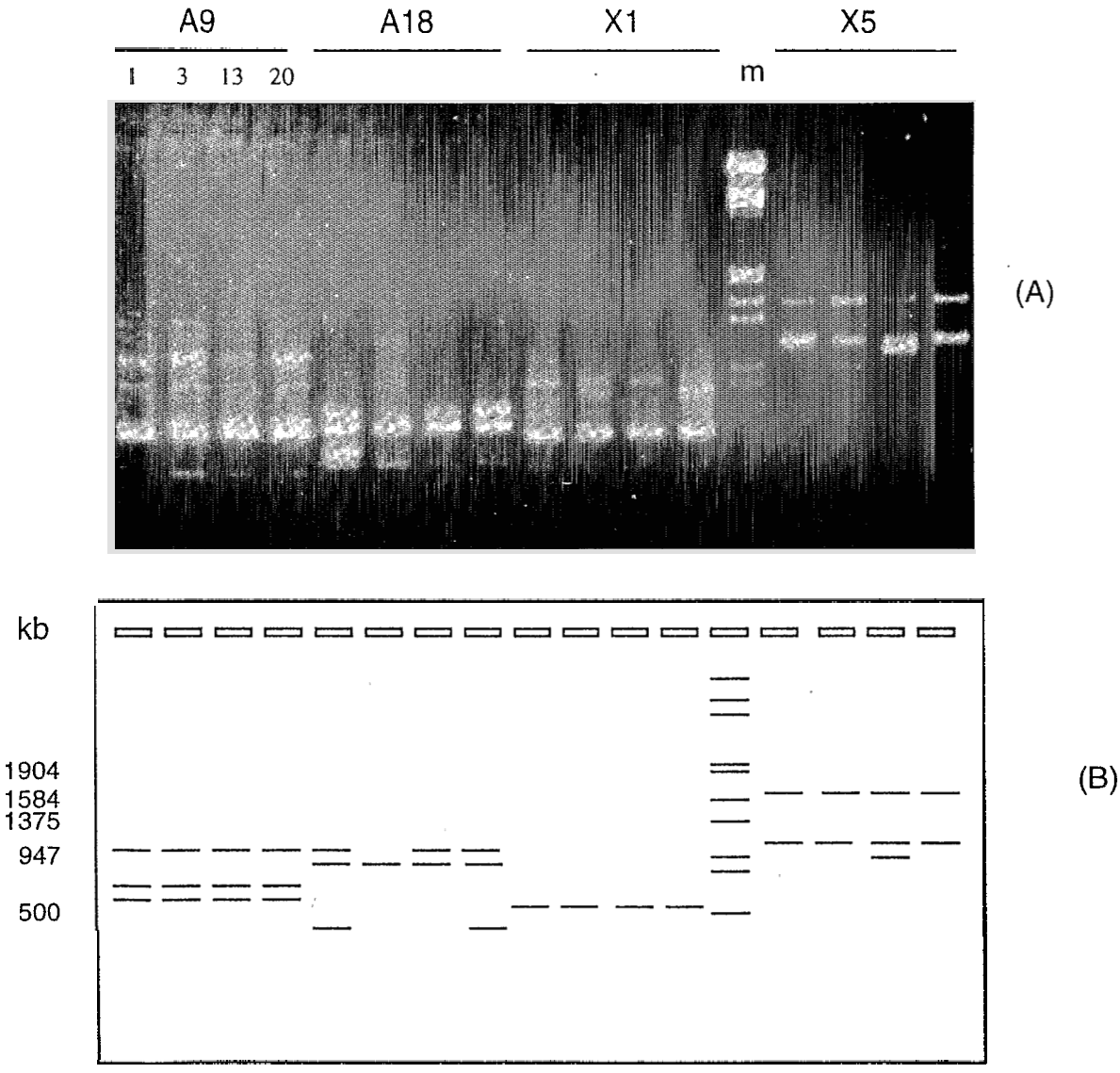

Figure 1 - Agarose-gel electrophoresis of fragments obtained by PCR amplification of genomic DNAs from four fig accessions with four primers (numbers and codes on top refer to the fig accessions and the primers listed in Table 1 and 2). (A) Photograph of gel stained with ethidium bromide, (B) diagram showing bands scored. m = markers.

RESULTS AND DISCUSSION

Selection and validation of RAPD markers

A set of 85 primers was tested on four accessions *i.e.*: Dauphine, Col de Dame grise, Madeleine des deux saisons and Roscoff, and 21 primers (25%) were then excluded because their amplified products were unstable. Of the remaining 64 primers, 27 revealed a polymorphism (Fig. 1). Each of these primers was then tested on all samples studied, and 12 of them were selected for genotype analysis because their patterns were reproducible (Fig. 2). Among the 64 bands generated by the 12 selected primers, 16 (25%) were unstable and were therefore excluded. Of the remaining 48 amplified products, 29 (45%) were constant among all the samples analysed and corresponded to monomorphic loci. The remaining 19 variable bands were then selected as RAPD markers (Table 2). Although the number of bands for each primer varied from 2 to 6 with an average of 4 bands per primer, each of the 12 selected primers produced only one to two RAPD markers (Table 2).

The use of short sequence primers makes the RAPD reaction very sensitive to amplification conditions. Indeed, the high proportion of unstable bands (25%) obtained here shows the importance of using multiple runs and strict control of the experimental conditions (HADRYS *et al.*, 1992; WIL-

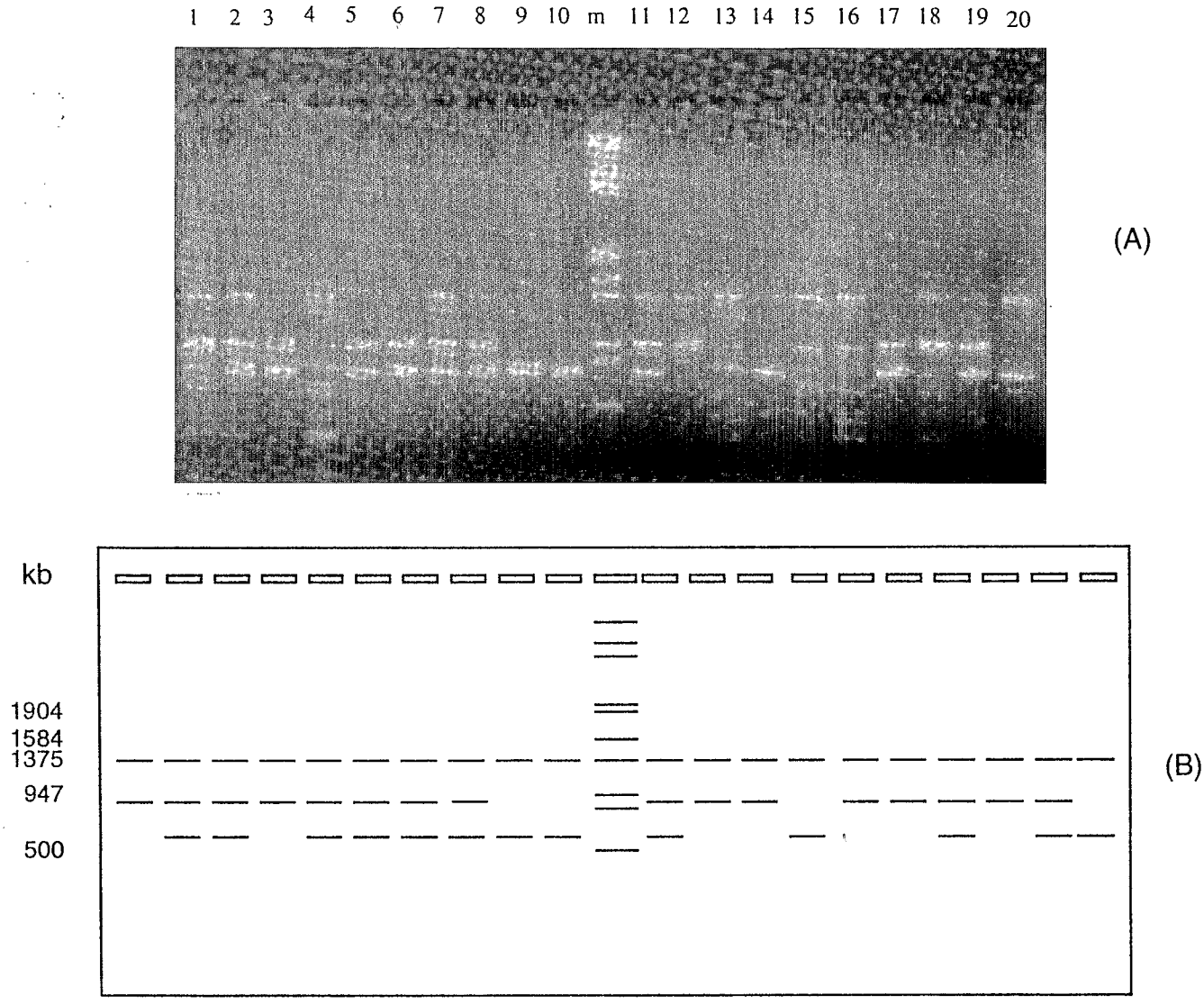

FIGURE 2 - DNA polymorphism of 20 fig accessions obtained by amplification from fig genomic DNA using random primer OPA16 (see Table 2). Lane numbers 1-20 correspond to fig numbers in Table 1. (A) Photograph of gel stained with ethidium bromide, (B) diagram showing bands scored. m = markers.

TABLE 2

Nucleotide sequence of selected primers with the number of amplified products and RAPD markers

Code	Sequence 5'—3'	Number of amplified products	Number of RAPD markers
OPA11	CAATCGCCGT	3	1
OPA16	AGCCAGCGAA	3	2
OPA18	AGGTGACCGT	4	2
OPH11	CTTCCGCAGT	5	2
OPK17	CCCAGCTGTG	6	2
OPX5	CCTTTCCCTC	3	1
OPX9	GGTCTGGTTG	4	1
OPX11	GGAGCCTCAG	6	2
OPY4	GGCTGCAATG	3	1
OPY11	AGACGATGGG	6	2
OPY14	GGTCGATCTG	2	1
OPZ12	TCAACGGGAC	3	2

LIAMS *et al.*, 1993; YANG and QUIROS, 1993). Two distinct amplified products of similar size may co-migrate on gel; however, this event can be easily detected by eluting individual PCR products from gel and reprobing the products via Southern analysis (WILLIAMS *et al.*, 1990). The RAPD markers used in our study have not been verified according to this method. However, the OPY14 marker was found to segregate in the Mendelian fashion (unpublished data). It was not possible to test segregation for the other markers because the parental trees in the available crosses were monomorphic. Nevertheless, there is only a low probability of finding a co-migration for individuals within the same species as in our study (KAZAN *et al.*, 1993).

Polymorphism and genotypes identification

The analysis of 21 fig accessions using the 19 selected RAPD markers allowed us to distinguish 17 different banding patterns (Fig. 3). The same band combinaton occurred in all the Col de Dame samples except Col de Dame blanche originating from the Porquerolles varietal collection. The similarity index between the two accessions of Col de Dame blanche genotypes was 0.57, which corresponds to a difference for nine markers (Table 3). Therefore, our results clearly indicate a labelling error in the varietal collection. Bourgeassotte noire and Violette de Solliès also had a common banding pattern. Violette de Solliès has always been suspected to be synonymous with Bourjassotte noire, and our results confirmed this synonymy. The genetic similarity between the three Col de Dame varieties demonstrates the clonal stability of genotypes obtained from the same original clone and grown

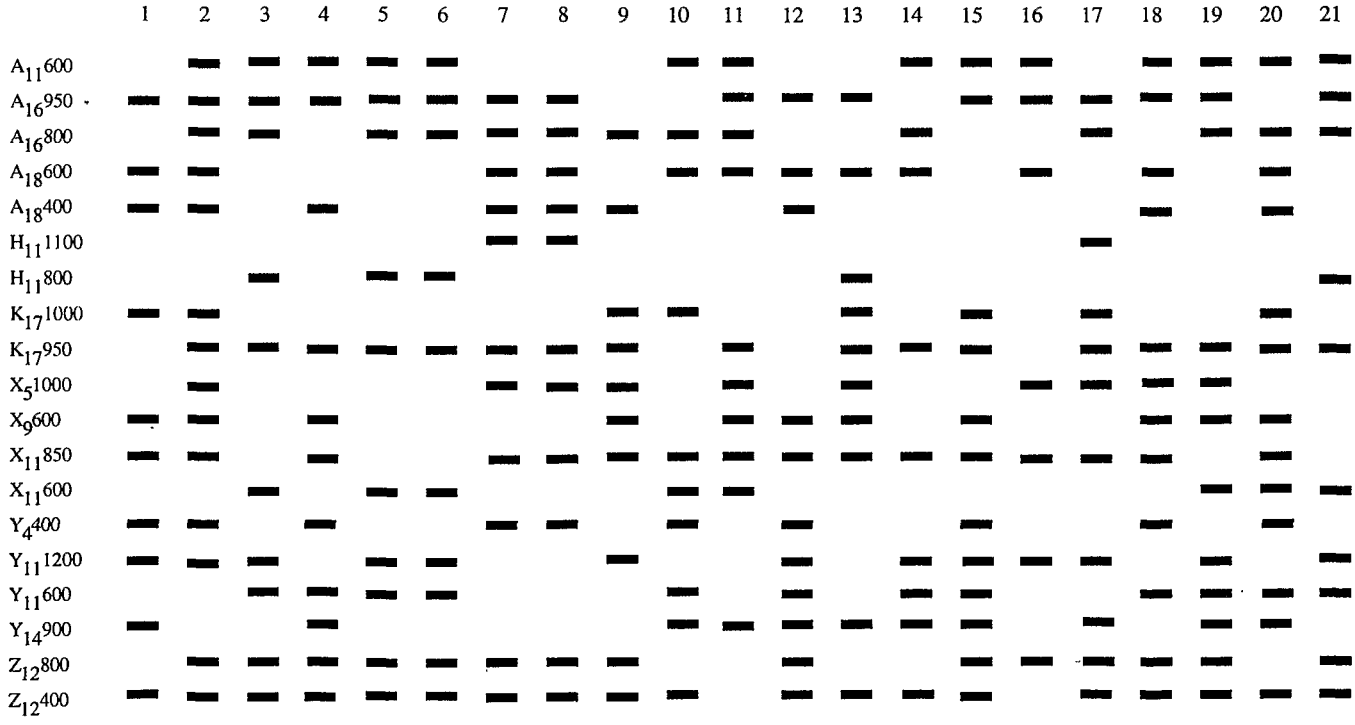

FIGURE 3 - Banding patterns resulting from 19 RAPD markers for 21 fig accessions.

TABLE 3

Matrix of Nei and Li's (1979) similarity index

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Dauphine	1.00																				
Violette Dauphine	0.75	1.00																			
Col de Dame grise	0.30	0.58	1.00																		
Col de Dame blanche (n° 1)	0.67	0.72	0.57	1.00																	
Col de Dame noire (n° 1)	0.30	0.58	1.00	0.57	1.00																
Col de Dame noire (n° 2)	0.30	0.58	1.00	0.57	1.00	1.00															
Bourgeassotte noire	0.57	0.80	0.48	0.64	0.48	0.48	1.00														
Violette de Solliès	0.57	0.80	0.48	0.64	0.48	0.48	1.00	1.00													
Marseillaise	0.60	0.83	0.50	0.57	0.50	0.50	0.67	0.63	1.00												
Verte d'Argenteuil	0.60	0.58	0.50	0.57	0.50	0.50	0.48	0.48	0.40	1.00											
Longue d'aout	0.50	0.67	0.50	0.57	0.50	0.50	0.57	0.57	0.50	0.60	1.00										
Osborn Prolific	0.86	0.72	0.48	0.82	0.48	0.48	0.64	0.64	0.57	0.57	0.48	1.00									
Madeleine des deux Saisons	0.70	0.67	0.40	0.57	0.40	0.40	0.57	0.57	0.60	0.50	0.70	0.57	1.00								
Grise de Saint Jean	0.53	0.60	0.63	0.60	0.63	0.63	0.50	0.50	0.53	0.74	0.63	0.60	0.53	1.00							
Tardive Brown Turkey	0.73	0.77	0.64	0.91	0.64	0.64	0.52	0.52	0.64	0.64	0.55	0.78	0.64	0.67	1.00						
Sultane	0.47	0.67	0.47	0.47	0.47	0.47	0.56	0.56	0.47	0.35	0.59	0.56	0.47	0.50	0.53	1.00					
Noire voisine de la Sultane	0.57	0.72	0.57	0.57	0.57	0.57	0.73	0.73	0.76	0.48	0.57	0.50	0.67	0.60	0.73	0.56	1.00				
Grise ronde à chair rose	0.64	0.85	0.54	0.91	0.54	0.54	0.78	0.78	0.64	0.54	0.64	0.78	0.63	0.57	0.75	0.63	0.52	1.00			
Grosse longue verte	0.45	0.69	0.82	0.73	0.82	0.82	0.52	0.52	0.64	0.54	0.73	0.61	0.55	0.67	0.75	0.53	0.70	0.67	1.00		
Roscoff	0.70	0.74	0.52	0.78	0.52	0.52	0.58	0.58	0.61	0.87	0.70	0.67	0.61	0.80	0.72	0.30	0.50	0.72	0.64	1.00	
Col de Dame blanche (n° 2)	0.30	0.58	1.00	0.57	1.00	1.00	0.48	0.48	0.50	0.50	0.50	0.48	0.40	0.63	0.64	0.47	0.57	0.54	0.82	0.52	1.00

Mean = 0.61.
SD = 0.14.

separately in two different geographic localities. Wolff and Peters-Van Rijn (1993) found an identical banding pattern in 13 cultivars of Chrysanthemum (*Dendranthema grandiflora* Tzvelev) resulting from the same original clone by somatic mutation or by irradiation.

The 17 banding patterns obtained here were different in at least three alleles (Table 3, Fig. 3). With a mean similarity index of 0.61 (Table 3), the probability of finding the same pattern in two individuals is 5.10^{-3} (*i.e.* $0.61^{10.67}$). The use of 19 independent RAPD markers, would allow the theoretical differentiation of 524,288 (*i.e.* 2^{19}) banding patterns, which is very difficult to obtain using enzymatic polymorphism because of the low number of loci and alleles (Valizadeh *et al.*, 1977; Weeden and Lamb, 1985; Atkinson *et al.*, 1986; Ouazzani *et al.*, 1993). The characterization of the 21 fig accessions could potentially be performed by a choice of 5 to 6 independent markers. However, our analysis required 17 markers because two genotypes with a similarity index as high as 0.91 shared 16 alleles (Table 3 and Fig. 3). The fact that the number of markers required was three times higher than expected suggests a linkage of some loci and/or the absence of some band combinations resulting from sampling. The number of markers to be used in these studies is very important because it affects the cost of the analysis (Kresovich *et al.*,1992). Bailey (1983) pointed out three basic criteria for cultivar identification: distinguishable intervarietal variation, minimal intravarietal variation, environmental stability and experimental reproducibility. The level of variation detected in the fig samples studied here with RAPD markers was sufficient for the characterization of all genotypes whereas the clonal stability was demonstrated by the genetic similarity of the Col

de Dame accessions. Moreover, the experiments were highly reproducible. By fulfilling these three criteria, the RAPD markers used in our study are therefore useful for genotype identification.

Genotype relationship and genetic variability

The 12 selected primers generated 48 consistent amplified products, with an average number of four bands per primer. This number is relatively low compared with those obstained in other species (Hu and Quiros, 1991; Mossler *et al.*, 1992; Yang and Quiros, 1993; Koller *et al.*, 1993; Wolff and Peters-Van Rijn, 1993) and was one of major factors limiting the average number of RAPD markers per primer to 1.5 in our study (Table 2). This can be compared with a study of Koller *et al.* (1993) in which 14 markers were generated by a single primer. The use of polyacrylamide gels may

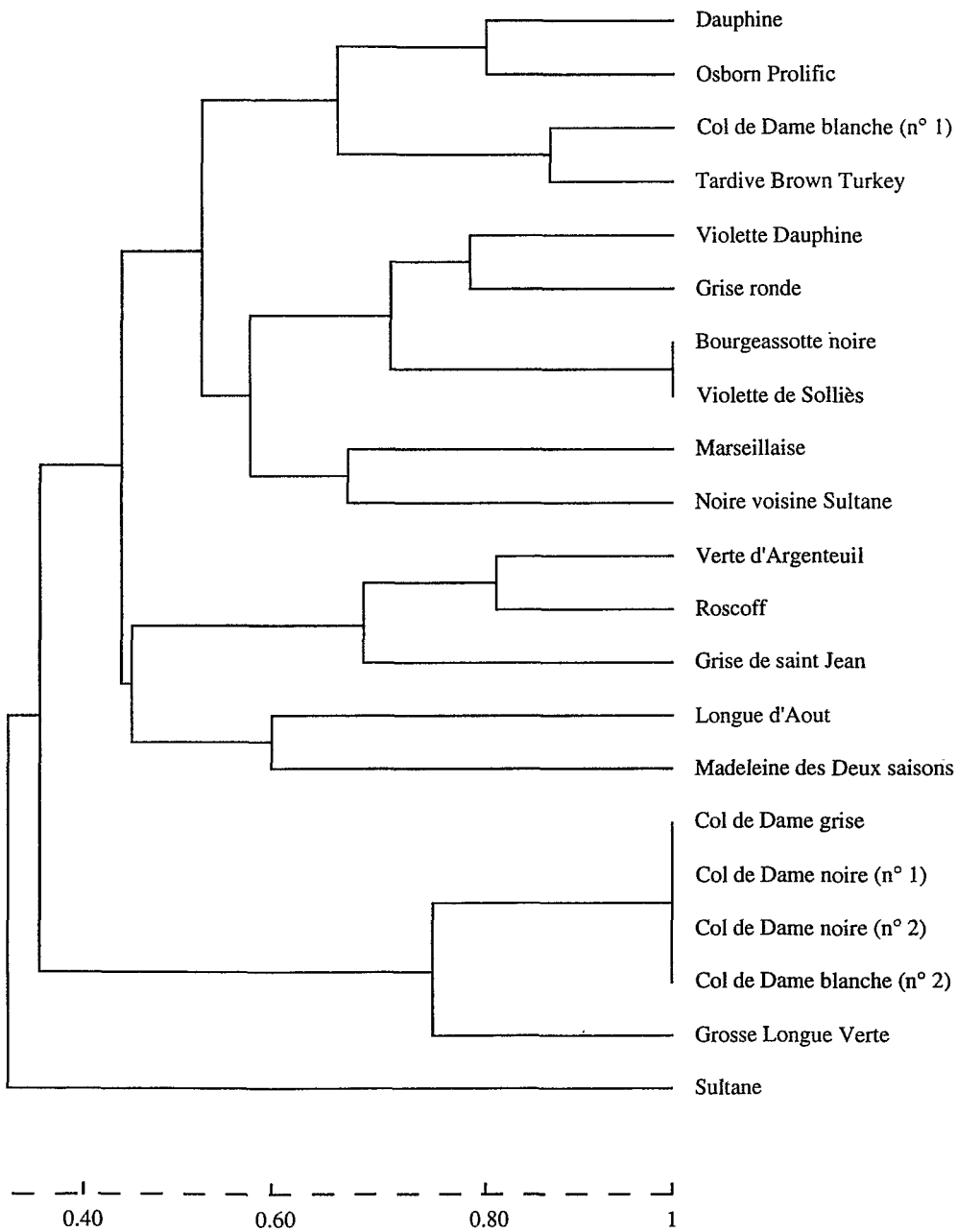

Figure 4 - Dendrogram based on Nei and Li's (1979) similarity index showing relationships among 21 fig accessions. The dendrogram was built up according to the unweighted pair-group method with arithmetic mean (UPGMA).

improve the band separation and prove to be usefull in generating more RAPD markers per primer (CAETANO-ANOLLES *et al.*, 1991; DWEIKAT *et al.*, 1993). Among the 85 primers tested, only 27 (29%) revealed polymorphism. The prescreening of primers for their informativeness was thus justified. The advantage of analysing only four genotypes rather than all the accessions was to reduce time and cost. Obviously, the polymorphism generated by the selected primers reflected the genetic variability among the four genotypes only. However, the average similarity index calculated for these genotypes was 0.54 which is not significantly different from the average similarity index (0.61) for all the accessions suggesting that the genetic variability of the genotypes used in the prescreening corresponded to that observed in all the accessions analysed.

The matrix calculated for all possible pairwise comparisons between accessions (Table 3) showed that the similarity index varied from 0.30 to 0.91 with an average value of 0.61 and a variation of 23%. However, the relationship among individuals illustrated by the dendrogram in fig. 4 showed the absence of distinct genotype grouping. The analysis of ten individual from ten different natural populations of *Microseris elegans* showed similarity indexes of 0.48 to 0.91 (mean = 0.64) and a variation of 20% (VAN HEUSDEN and BACHMANN, 1992). The separation of these individuals into four groups was related to the geographic distribution of *Microseris elegans* populations and to relatively limited gene flow. Therefore, it is suggested that the absence of distinct group for the fig genotypes studied here may result from an important gene flow amongst them.

CONCLUSION

This study showed that the use of RAPD markers is a useful tool for fig varietal identification. The advantages of this technique are its ability to detect extensive polymorphisms, simplicity, rapidity and no need for radioisotopes (NEWBURY and FORD-LLOYD, 1993). The principal limitation of RAPD fingerprinting arises from its sensitivity to the reaction conditions, which may affect the reproducibility of patterns. The low number of individuals analysed did not allow to verify whether gene flow was responsible for unstructured genetic variability in fig. On the other hand, RAPDs are not expected to identify heterozygous loci and, therefore, may have some limitation for estimation of gene flow (CARLSON *et al.*, 1991; HADRYS *et al.*, 1992). The first studies of genetic differentiation among natural populations have shown that RAPD markers are useful tools to identify areas of maximum diversity and may be used to estimate genetic variability in natural populations (CHALMERS *et al.*, 1992; VAN HEUSDEN and BACHMANN, 1992; HUFF *et al.*, 1993).

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REFERENCES

ARNOLD, M.L., BUCKNER, C.M. and ROBINSON, J.J., 1991. Pollen-mediated introgression and hybrid speciation in Louisiana irises. *Proc. Natl. Acad. Sci. USA*, **88:** 1398-1402.

ATKINSON, M.D., WITHERS, L.A. and SIMPSON, M.J.A., 1986. Characterisation of cacao germplasm using isoenzyme markers. *Euphytica*, **35:** 741-750.

BAILEY, D.C., 1983. Isozymic variation and plant breeders' rights. In: S.D. TANKSLEY and T.J. ORTON eds., *Isozymes in plant genetics and breeding*, Part A. Elsevier, Amsterdam, pp. 425-441.

BECKMANN, J.S. and SOLLER, M., 1983. Restriction fragment length polymorphism in genetic improvement: methodologies, mapping and cost. *Theor. Appl. Genet.*, **67:** 33-43.

BENZECRI, J.P., 1973. *L'analyse des données, tome 1, La taxonomie*, Eds. Dunod, Paris, 616 pp.

CAETANO-ANOLLES, G., BASSAM, B.J. and GRESSHOFF, P.M., 1991. DNA amplification fingerprinting using very short arbitrary primers. *Bio/Technology*, **9:** 553-557.

CARLSON, J.E., TULSIERAM, L.K., GLAUBITZ, J.C., LUK, V.W.K., KAUFFELDT, C. and RUTLEDGE, R., 1991. Segregation of random amplified DNA markers in F1 progeny of conifers. *Theor. Appl. Genet.*, **83:** 194-200.

CHALMERS, K.J., WAUGH, R., SPRENT, J.I., SIMONS, A.J. and POWELL, W., 1992. Detection of genetic variation between and within populations of *Gliricidia sepium* and *G. maculata* using RAPD markers. *Heredity*, **69:** 465-472.

CONDIT, I.J., 1950. Figs characters as affected by climate. *Amer. Soc. Hort. Sci. Proc.*, **44:** 211-214.

CONDIT, I.J., 1955. Fig varieties: A monograph. *Hilgardia, Berkeley*, **23:** 323-538.

DALLAS, J.F., 1988. Detection of DNA "fingerprints" of cultivated rice by hybridization with a human minisatellite probe. *Proc. Natl. Acad. Sci. USA*, **85:** 6831-6835.

DWEIKAT, I., MACKENZIE, S., LEVY, M. and OHM, H., 1993. Pedigree assessment using RAPD-DGGE in cereal crop species. *Theo. Appl. Genet.*, **85:** 497-505.

DELLAPORTA, S.L., WOOD, J. and HICKS, J.B., 1983. A plant DNA preparation. Version II. *Plant Mol. Biol. Rep.*, **4:** 19-21.

FELSENSTEIN, J., 1987. *Phylip, ver. 3.0 (computer sofwàre and manual)*. Department of Genetics, University of Washington, Seattle, W.A. 98195, USA.

HADRYS, H., BALICK, M. and SCHIERWATER, B., 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. *Molec. Ecol.*, **1:** 55-63.

HU, J., and QUIROS, C.F., 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. *Plant Cell. Rep.*, **10:** 505-511.

HUFF, D.R., PEAKALL, R. and SMOUSE, P.E., 1993. RAPD variation within and among natural populations of outcrossing buffalograss (*Buchloë dactyloides* (Nutt.) Engelm.). *Theor. Appl. Genet.*, **86:** 927-934.

HUNT, G.J. and PAGE, R.E.J., 1992. Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee. *Theor. Appl. Genet.*, **85:** 15-20.

KAZAN, K., MANNERS, J.M. and CAMERON, D.F., 1993. Genetic variation in agronomically important species of *Stylosanthes* determined using random amplified polymorphic DNA markers. *Theor. Appl. Genet.*, **85:** 882-888.

KJELLBERG, F., GOUYON, P.H., IBRAHIM, M., RAYMOND, M. and VALDEYRON, G., 1987. The stability of the symbiosis between dioecious figs and their pollinators: a study of *Ficus carica* L. and *Blastophaga psenes* L. *Evolution*, **41:** 693-704.

KLEIN-LANKHORST, R.M., VERMUNT, A., WEIDE, R., LIHARSKA, T. and ZABEL, P., 1991. Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). *Theor. Appl. Genet.*, **83:** 1549-1551.

KOLLER, B., LEHMANN, A., MCDERMOTT, J.M. and GESSLER, C., 1993. Identification of apple cultivars using RAPD markers. *Theor. Appl. Genet.*, **85:** 901-904.

KRESOVICH, S., WILLIAMS, J.G.K., MCFERSON, J.R., ROUTMAN, E.J. and SCHAAL, B.A., 1992. Characterization of genetic identities and relationships of *Brassica oleracea* L. via a random amplified polymorphic DNA assay. *Theor. Appl. Genet.*, **85:** 190-196.

LASHERMES, PH., CROS, J., MARMEY, PH. and CHARRIER, A., 1993. Use of random amplified polymorphic DNA markers to analyse genetic variability and relationships of *Coffea species. Crop Evol. Genet. Res.*, **40:** 91-99.

MOSSELER, A., EGGER, K.N. and HUGHES, G.A., 1992. Low levels of genetic diversity in red pine confirmed by random amplified polymorphic DNA markers. *Can. J. For. Res.*, **22:** 1332-1337.

NEI, M. and LI, W.H., 1979. Mathematical model for studying genetical variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA*, **74:** 5267-5273.

NEWBURY, H.J. and FORD-LLOYD, B.V., 1993. The use of RAPD for assessing variation in plants. *Pl. Grow. Reg.*, **12:** 43-51.

NYBOM, H. and HALL, H.K., 1991. Minisatellite DNA "fingerprints" can distinguish Rubus cultivars and estimate their degree of relatedness. *Euphytica*, **53:** 107-114.

OUAZZANI, N., LUMARET, R., VILLEMUR, P. and DI GIUSTO, F., 1993. Leaf allozyme variation in cultivated and wild olive trees (*Olea europea* L.). *J. Heredity*, **84:** 34-42.

REITER, R.S., WILLIAMS, J.G.K., FELDMANN, K.A., RAFALSKI, J.A., TINGEY, S.V. and SCOLNIK, P.A., 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. *Proc. Natl. Acad. Sci. USA*, **89:** 1477-1481.

VALDEYRON, G. and VALIZADEH, M. 1976. L'identification variétale du figuier, *Ficus carica* L. par l'étude du polymorphisme enzymatique par électrophorèse. *C.R. Acad. Agri.*, **62(3):** 170-175.

VALIZADEH, M., RIVALS, P. and VALDEYRON, G., 1977. Utilisation du polymorphisme protéique pour l'étude des variétés de figuier (*Ficus carica* L.). *C.R. Acad. Agri.*, **63(10):** 647-655.

VAN HEUSDEN, A.W. and BACHMANN, K., 1992. Genotype relationships in *Microseris elegans* (Asteraceae, Lactuceae) revealed by DNA amplification from arbitrary primers (RAPDs). *Pl. Syst. Evol.*, **179:** 221-233.

WEEDEN, N.F. and LAMB, R.C., 1985. Identification of apple cultivars by isoenzyme phenotypes. *J. Am. Soc. Hortic. Sci.*, **110:** 509-515.

WEISING, K., RAMSER, J., KAEMMER, D., KAHL, G. and EPPLEN, J.T., 1991. Oligonucleotide Fingerprinting in Plants and Fungi. In: T. BURKE, J. DOLF, A.J. JEFFREYS and R. WOLFF eds., *DNA fingerprinting: Approaches and applications*, Birkhauser Verlag Basel/Switzerland, pp. 312-329.

WELSH, J. and MCCLELLAND, M., 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucl. Ac. Res.*, **18:** 7213-7218.

WELSH, J., HONEYCUTT, R.J., MCCLELLAND, M. and SOBRAL, B.W.S., 1991. Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction (AP-PCR). *Theor. Appl. Genet.*, **82:** 473-476.

WILDE, J., WAUGH, R. and POWELL, W., 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. *Theor. Appl. Genet.*, **83:** 871-877.

WILKIE, S.E., ISAAC, P.G. and SLATER, R.J., 1993. Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium. Theor. Appl. Genet.*, **86:** 497-504.

WILLIAMS, J.G.K., KUBELIK, A.R., KENNETH, J.L., RAFALSKI, J.A. and TINGEY, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nuc. Ac. Res.*, **18:** 22, 6531.

WILLIAMS, J.G.K., HANAFEY, M.K., RAFALSKI, J.A. and TINGEY, S.V., 1993. Genetic analysis using random amplified polymorphic DNA markers. *Methods Enzymol.*, **51:** 704-740.

WOLFF, K. and PETERS-VAN RIJN, J., 1993. Rapid detection of genetic variability in chrysanthemum (*Dendranthema grandiflora* Tzvelev) using random primers. *Heredity*, **71:** 335-341.

YANG, X. and QUIROS, C., 1993. Identification and classification of celery cultivars with RAPD markers. *Theor. Appl. Genet.*, **86:** 205-212.