Vitis 37 (1), 33–38 (1998)

Analysis of the relationship between grapevine cultivars, sports and clones via DNA fingerprinting

by

G. N. YE[1], G. SOYLEMEZOGLU[2], N. F. WEEDEN[3], W. F. LAMBOY[3], R. M. POOL[3] and B. I. REISCH[3]

[1] Monsanto Company, Chesterfield Parkway, St. Louis, USA
[2] Ankara University, Faculty of Agriculture, Department of Horticulture, Ankara, Turkey
[3] Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, USA

S u m m a r y : DNA fingerprinting utilizing RAPD polymorphisms was employed to investigate the relationship among 16 grapevine cultivars and sports thought to have arisen from these cultivars. From 53 primers, a total of 464 bands were generated, of which 29 % were common to all genotypes tested. Cluster analysis classified all tested cultivars into two main groups (*Vitis vinifera* L. and *V.* x *Labruscana* Bailey) as expected. No polymorphism was detected among known clones of Chardonnay (Ch. clone 7, Ch. clone 78 and Ch. Geneva clone) or Pinot noir (P. n. clone 29, P. n. Geneva clone and P. n. Pernand). Pinot Meunier, Pinot gris, and Gamay Beaujolais displayed patterns indistinguishable from Pinot noir. Auxerrois and Melon showed unique patterns and may be classified as distinct cultivars. Chardonnay clone 7 shared 84 % of its bands with Pinot noir. There was more than 97 % RAPD amplicon homology between Niagara and two supposed sports, and between Concord and a red-fruited sport. Taking into account the error rate in scoring RAPD bands, the evidence is against the hypothesis that the three sports are distinct cultivars. While RAPD banding patterns could not distinguish between known clones, they were useful for distinguishing between phenotypically similar cultivars and for assessing the origins of cultivars thought to have originated as sports.

K e y w o r d s : RAPD, PCR, polymorphism, cultivar identification, Vitis, wine grapes, juice grapes.

Introduction

Cultivar identification in grapevine can be very difficult when relying upon ampelographic and botanical characteristics alone. Isozyme techniques have been investigated for the identification of grapevines (WOLFE 1976, BACHMANN and BLAICH 1988, WEEDEN *et al.* 1988, PARFITT and ARULSEKAR 1989, WALTERS *et al.* 1989), and have been successful at distinguishing closely related cultivars derived from sexual crosses. In general, isozyme techniques have not been able to differentiate among asexually produced sports or mutants of a cultivar. Furthermore, the number of isozyme systems available is limited and analyses usually require the use of fresh, young leaf tissues.

DNA polymorphisms appear to be particularly useful tools for distinguishing cultivars because 1) the results directly reflect the genotype; 2) the results are independent of the environment; 3) a large number of potential polymorphic sequences or markers are available; 4) DNA can usually be extracted from nearly every tissue. RFLP analysis has been used to detect DNA polymorphism within *Vitis* using heterologous probes (STRIEM *et al.* 1990, YAMAMOTO *et al.* 1991), and homologous probes (BOURQUIN *et al.* 1992, 1993, MAURO *et al.* 1992, BOWERS *et al.* 1993, BOWERS and MEREDITH 1996). However, RFLP analysis is time-consuming and often requires the use of radioactive isotopes. DNA polymorphisms generated by the RAPD technique (WILLIAMS *et al.* 1990, WELSH and MCCLELLAND 1990) are of great interest because this technique requires only minute amounts of template DNA, it is simple, and it is capable of detecting a high level of genetic variation. There have been numerous reports on the use of RAPD markers for cultivar identification or for the detection of genetic variation among cultivars (HU and QUIROS 1991, CASTIGLIONE *et al.* 1993, MAILER *et al.* 1994, YU and NGUYEN 1994). RAPD and microsatellite polymorphisms have also been shown to be useful in grape cultivar identification (GOGORCENA *et al.* 1993, THOMAS and SCOTT 1993, 1994, CIPRIANI *et al.* 1994, TSCHAMMER and ZYPRIAN 1994, MULCAHEY *et al.* 1995, YE *et al.* 1996).

In this study, RAPD markers were used to detect polymorphisms and to examine relationships among clonally propagated grape cultivars and others commonly believed to be sports and clones of these cultivars. Our goal was to investigate genetic similarity among groups of *V. vinifera* cultivars thought to be sports and/or clones from a single genotypic origin. Pinot noir and Chardonnay and their sports and clones were examined because of their worldwide importance for varietal wine production. Among cultivars of *V. labrusca*, we sought to examine whether genotypes said to have originated from Concord and Niagara were truly sports of these cultivars. The Niagara and the Concord cultivars are important in producing juices and jellies primarily in the Northeastern and Northwestern United States.

Material and methods

P l a n t m a t e r i a l s : Partially expanded leaves were collected from vineyards at the New York State Agricultural Experiment Station. The cultivars, supposed sports, and clones included in this study are listed in Tab. 1. Niagara

Correspondence to: Dr. B. I. REISCH, Department of Horticultural Sciences, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA. Fax: +1-315-787-2216. E-mail: Bruce.Reisch@cornell.edu

Table 1

Grape cultivars and clones used for DNA fingerprinting analysis

Cultivar	Clone	Species
Chardonnay	7	*V. vinifera*
Chardonnay	78	
Chardonnay	Geneva	
Pinot noir	29	*V. vinifera*
Pinot noir	Geneva	
Pinot noir	Pernand	
Supposed sports of Pinot noir		
Pinot Meunier	-	
Gamay Beaujolais	-	
Pinot gris (from France)	-	
Unknown (was labeled Pinot gris, collected in New York)		
Other *V. vinifera* cultivars		
Melon	-	*V. vinifera*
Auxerrois	-	
Niagara and supposed sports		
Niagara		*V.* x *Labruscana*
Niagara Seedless		
Niagara Rosada		
Concord and supposed sport		
Concord		*V.* x *Labruscana*
Damoth 1		

Seedless and Niagara Rosada are said to be seedless and red-fruited sports, respectively, of the normally green-fruited Niagara. Damoth 1 is believed to be a red-fruited sport of the blue-fruited Concord. Niagara and all *V. vinifera* plant material were derived from virus-indexed stock plants. At the time of collection, leaves were apparently free of pests and vines were healthy.

DNA extraction: DNA was extracted according to the method of LODHI *et al.* (1994). DNA concentration was measured on a Spectronic 601 spectrophotometer (Milton Roy Company, Rochester, NY).

Primers: Oligonucleotide primers (10-mers) were purchased from Operon Tech. (Alameda, CA), National Biosciences (Plymouth, MN), Genosys Biotechnologies, Inc. (The Woodlands, TX), University of British Columbia (Canada) and the New York State Center for Advanced Technology in Biotechnology (Cornell University, Ithaca, NY). K primers were kindly provided by Dr. MUHAMMAD LODHI (Cornell University, Geneva, NY). GY primers (containing 17-24 bases) were kindly provided by Dr. SHENG-ZHI PANG (Cornell University, Geneva, NY). The primers and their sequences are listed in Tab. 2.

PCR protocols: RAPD amplification was performed in a reaction volume of 25 µl containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 2 mM $MgCl_2$, 0.1 % Triton X-100, 120 µM of each dNTP, 0.4 µM primer, 100-200 ng

Table 2

Primers used in this study and their sequences

Primer	Sequence (5'-3')
BC301	CGGTGGGGAA
BC302	CGGCCCACGT
BC340	GAGAGGCACC
BC349	GGAGCCCCCT
BC379	GGGCTAGGGT
GTO3	CTCGGTACAC
GTO4	GTGGTTGCGA
GTO5	CTGGACTTAC
GY103	CTACAGCAGGAATATCAC
GY104	GTACTCTGATTCGAGGACT
GY105	CTGTAGCCATGAGCAAAG
GY107	GTTCAGGGCTGTTTATAG
GY109	GATCTTTGCTCAAGCTGG
GY110	ATCAAACACAATCCACACA
GY169	CTAAGCTGCTTTTGTTTGAGC
GY170	AATTATTTGTGTGGATTGTGTTTG
GY171	GCTATGGACTATTACAGTG
GY20	CAGACAGGATTGGAGGCACTGA
GY59	TTCTGGTCTTCTTCAAACTCA
GY60	CCATATATCAAGATGCA
K5	CGCAGGATGG
K7	GGGATGGCTG
OD6	ACCTGAACGG
OD7	TTGGCACGGG
OD8	GTGTGCCCCA
OD11	AGCGCCATTG
OPA1	CAGGCCCTTC
OPA2	TGCCGAGCTG
OPA3	AGTCAGCCAC
OPA4	AATCGGGCTG
P3	GTCCGTTGGG
P105	CAGTCGCGTG
P123	GGGATTCGAC
P143	GCCTCATACC
P161	CGGATGCCTT
P163	ACGCCTACGT
P166	GTGACGGACT
P24	AGCACTGTCA
P25	AATGAAGCCA
P27	ACCTCGAGCA
P33	GTAAAACGACGGCCAGT
P35	TGCGCAACGTTGTTG
P36	ATACCAAACGACGAG
P353	ATACGGGCAA
P382	GAACCGGATC
P394	CGACTCCAAC
P4	GTTAGGTCGT
P402	GCGTTGTCCA
P430	GACCTGTACC
S24	GCGGCATTGT
S31	CTCGACACTG
S66	GCTCACCCTA
S68	GTCGGTTGCG

genomic DNA and 0.5 unit of Taq DNA polymerase. Amplification was performed on a PTC-100 thermocycler (MJ Research Inc., MA) for 35 cycles of 30 s at 94 °C, 1 min at 35 °C and 1 min 45 s at 72 °C, followed by an 8 min extension at 72 °C. Amplification products were separated by electro-phoresis in 2 % agarose gels (1 % agarose /1 % NuSieve GTG agarose, FMC Corporation), visualized by staining with ethidium bromide, and photographed on a transilluminator using Polaroid Type 55 film.

Data analysis: PCR amplified fragments separated on agarose gels were recorded as present (1) or absent (0) and the results were assembled in a data matrix table. Data analysis was restricted to major, easily scored bands. Faint bands and bands that could not be distinguished from other bands of similar sizes were not scored. A pairwise similarity matrix was constructed using the percentage of similarity, which was calculated as the number of shared bands (either present or absent) between two genotypes divided by the total number of bands in the entire group of genotypes analyzed. The percentage similarity values were then used to construct a dendrogram with appropriate software (Numerical Taxonomy and Multivariate Analysis System, Version 1.60; ROHLF 1990).

Results

RAPD phenotypes: From 53 primers, a total of 464 bands were generated from the genomic DNA of 16 genotypes. The total number of bands produced per primer varied from 1 to 23 (Tab. 3), averaging 9 bands per primer. Band sizes ranged from 0.2 to over 2 kb. In general, 17- to 24-mer primers produced twice as many bands as 10-mer primers (YE *et al.* 1996). Fig. 1 A and B shows examples of RAPD patterns obtained by PCR amplification of grapevine DNA with primers BC302 and BC340.

Table 3

Summary of number of RAPD fragments produced by primers used in this study

No. of fragments/primer	No. of primers
1	1
2	4
3	4
4	1
5	3
6	7
7	3
9	7
10	1
11	3
12	6
13	1
14	3
17	2
18	2
20	1
23	1

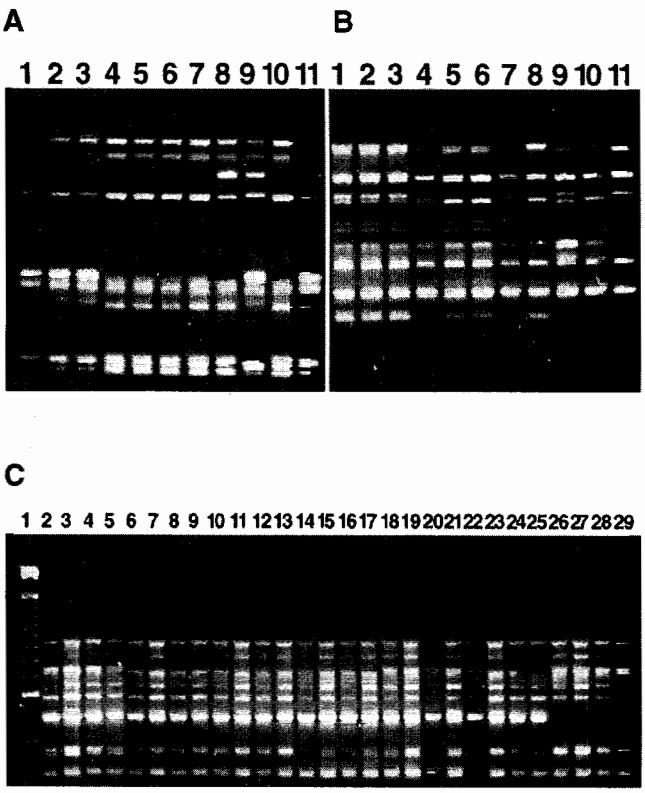

Fig. 1: RAPD profiles for primers BC302 (CGGCCCACGT) (**A**, lanes 1-11) and BC340 (GAGAGGCACC) (**B**, lanes 1-11). The order of genotypes in adjacent lanes is as follows: (1) Chardonnay clone 7; (2) Chardonnay clone 78; (3) Chardonnay Geneva clone; (4) Pinot noir Pernand; (5) Pinot noir Geneva clone; (6) Pinot noir clone 29; (7) Pinot Meunier; (8) unknown, was thought to be Pinot gris, (9) Melon; (10) Gamay Beaujolais; (11) Auxerrois. RAPD fragments were separated on a 2 % agarose gel (1 % agarose/1 % NuSieve GTG agarose). **C**, RAPD profiles generated using primer GY169 show consistency between reactions run with separate DNA extractions and on separate thermocyclers. Two DNA extractions per cultivar were each amplified separately on two thermocyclers. Amplifications of each genotype were electrophoresed in 4 adjacent lanes as follows; lane 1- DNA extraction 1 in thermocycler 1, lane 2 - DNA extraction 1 in thermocycler 2, lane 3, DNA extraction 2 in thermocycler 1, and lane 4 - DNA extraction 2 in thermocycler 2. Lane 1 is the 100 bp DNA ladder. Cultivars are as follows: Lanes 2-5, Chardonnay clone 7; lanes 6-9, Pinot noir Geneva clone; lanes 10-13, Pinot Meunier; lanes 14-17, unknown (was thought to be Pinot gris); lanes 18-21, Melon; lanes 22-25, Gamay Beaujolais; and lanes 26-29, Auxerrois.

Reproducibility of RAPD fingerprinting: A portion of the data collected, generating 147 of the 464 markers within the Pinot/Chardonnay group, was replicated by analyzing two separate DNA extractions per cultivar, each of which was amplified separately on two PTC-100 thermocyclers. The banding patterns obtained from these replicated samples were always identical for each primer/genotype combination (Fig. 1 C).

The relationship between grape cultivars, sports and clones: Chardonnay clones 7, 78 and Geneva and Pinot noir clones 29, Geneva and Pernand were included individually in the reproducibility test described above with 147 RAPD amplicons. No polymorphisms were detected within each group of clones. Therefore, only one clone from each cultivar was chosen for

further analyses. Tab. 4 presents the pairwise percentage similarity between genotypes. The well-defined clones of Chardonnay and Pinot noir were included in the Table as groups. Of all bands analyzed, 29 % were common to all cultivars and clones tested. The dendrogram of Fig. 2 shows that *V. vinifera* and *V. labrusca* cultivars cluster separately.

Sixty percent of the RAPD fragments were shared by all *V. vinifera* cultivars and clones tested. Chardonnay clone 7 shared 84 % RAPD amplicon homology with Pinot noir Geneva (Tab. 4), and Pinot Meunier and Gamay Beaujolais displayed patterns identical to Pinot noir. Surprisingly, however, a clone identified as Pinot gris showed unique patterns ("Unk", Tab. 4), contradicting previous evidence that Pinot gris is a berry color sport from Pinot noir (BOWERS *et al.* 1993, COLLINS and SYMONS 1993, GOGORCENA *et al.* 1993). The Pinot gris sample used, however, proved to be misidentified. A true-to-type Pinot gris DNA sample (kindly supplied by PATRICE THIS, Montpellier, France) had a RAPD profile identical with Pinot noir when tested with a set of 20 primers including all primers which had produced polymorphic bands in the initial tests of the misidentified Pinot gris and true-to-type Pinot noir. Auxerrois and Melon were separate and distinct genotypes according to RAPD profiles. Melon was more closely related to Chardonnay than to Pinot noir.

V. labrusca cultivars shared a minimum of 69 % of all bands tested. Less than 10 polymorphic bands were observed among a total of about 320 bands between Niagara and two supposed sports, and 7 among a total of 370 between Concord and Damoth 1. The polymorphic bands were of intermediate or faint intensity.

Table 4

Percentage similarities[1] between grape cultivars and clones[2]

Ch-7 Ch-78 Ch-G	100											
Pn-G Pn-P Pn-29	84.2 (371)	100										
Pm	84.2 (367)	100 (429)	100									
Unk	77.3 (229)	89.3 (289)	89.3 (289)	100								
Ml	84.7 (378)	80.2 (440)	80.2 (440)	81.1 (286)	100							
Gb	84.2 (378)	100 (440)	100 (441)	89.3 (289)	80.2 (451)	100						
Aux	86.7 (361)	82.0 (423)	82.0 (425)	76.7 (288)	82.9 (432)	82.0 (431)	100					
Nr	61.3 (225)	54.5 (246)	54.5 (249)	54.5 (101)	57.3 (260)	54.5 (256)	56.5 (239)	100				
Ns	62.4 (229)	55.2 (250)	55.2 (250)	55.4 (101)	57.6 (264)	55.2 (260)	56.8 (243)	97.5 (323)	100			
N	62.0 (229)	54.8 (250)	54.8 (250)	55.4 (101)	58.3 (264)	54.8 (260)	57.2 (243)	98.4 (314)	97.2 (316)	100		
Conc	54.8 (230)	50.7 (282)	50.7 (281)	49.2 (132)	49.3 (296)	50.7 (293)	48.0 (275)	81.7 (323)	79.4 (325)	79.4 (316)	100	
Dl	55.8 (231)	51.6 (283)	51.6 (282)	49.2 (132)	50.2 (297)	51.6 (293)	48.9 (276)	82.1 (324)	80.4 (326)	80.4 (317)	98.1 (370)	100
	Ch-7 Ch-78 Ch-G	Pn-G Pn-P Pn-29	Pm	Unk	Ml	Gb	Aux	Nr	Ns	N	Conc	Dl

[1] Percentage similarity was calculated as the number of bands shared by both cultivars divided by the total number of bands analyzed. Numbers in parenthesis indicate the number of bands analyzed.

[2] Cultivar designations: Ch-7: Chardonnay clone 7; Ch-78: Chardonnay clone 78; Ch-G: Chardonnay Geneva clone; Pn-P: Pinot noir Pernand; Pn-G: Pinot noir Geneva clone; Pn-29: Pinot noir clone 29; Pm: Pinot Meunier; Unk: was thought to be Pinot gris; Ml: Melon; Gb: Gamay Beaujolais; Aux: Auxerrois; Nr: Niagara Rosada; Ns: Niagara Seedless; N: Niagara; Conc: Concord; and Dl: Damoth 1.

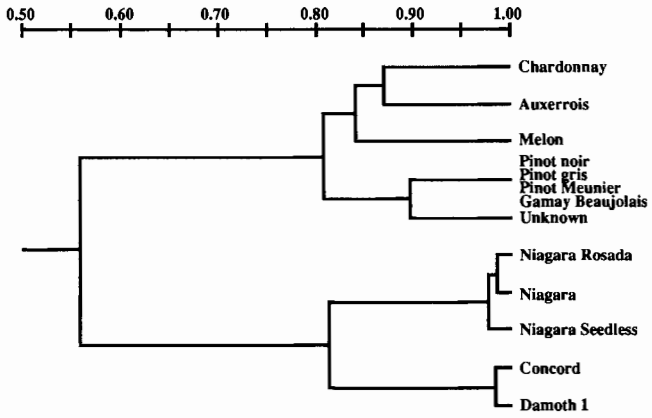

Fig. 2: Dendrogram of grape cultivars based on the similarity percentage from RAPD data, illustrating the genetic relationship among the analyzed cultivars. Scale on top is similarity percentage.

Discussion

RAPD banding patterns were highly polymorphic among the genotypes tested. Cluster analysis classified all tested cultivars into two main groups (*V. vinifera* L. and *V.* x *Labruscana* Bailey) as expected. We were able to distinguish between known cultivars, and our data support the descriptions of Pinot Meunier and Pinot gris as sports of Pinot noir (GALET 1990). BOURQUIN *et al.* (1993) and TSCHAMMER and ZYPRIAN (1994) also showed that Pinot noir and Pinot Meunier could not be distinguished based on molecular markers. Pinot gris and Pinot noir were also indistinguishable in this and other studies (BOWERS *et al.* 1993, CIPRIANI *et al.* 1994, TSCHAMMER and ZYPRIAN 1994). No polymorphisms were detected either among the known clones of Chardonnay or among those of Pinot noir, which is consistent with other recent works on molecular fingerprinting (BOTTA *et al.* 1995, BOWERS *et al.* 1993, CIPRIANI *et al.* 1994, COLLINS and SYMONS 1993, GOGORCENA *et al.* 1993, TSCHAMMER and ZYPRIAN 1994). The inability to separate clones of Chardonnay or clones of Pinot noir may be attributable to the rarity of genetic changes responsible for clonal identity which would make the detection of these changes highly improbable.

In addition to Pinot Meunier, Gamay Beaujolais also produced a RAPD profile identical to that of Pinot noir. This result, in addition to the RFLP data of BOWERS *et al.* (1993), strongly supports the classification of Gamay Beaujolais as a sport of Pinot noir.

Pinot gris is reported to be a berry color sport of Pinot noir, known since at least the 17th century (GALET 1990, VIALA and VERMOREL 1901-1910). Initially, our results did not concur with RFLP fingerprinting studies (BOURQUIN *et al.* 1993, BOWERS *et al.* 1993) which could not distinguish Pinot gris from Pinot noir. The Pinot gris sample used was determined later to not be true-to-type; a new sample obtained resulted in banding patterns identical to Pinot noir, concurring with previous reports.

Auxerrois, considered by some to be a form of or related to Pinot noir, Chardonnay, Melon, or Sylvaner (ROBINSON 1986, AMBROSI *et al.* 1994), is shown here to be distinctly different from Pinot noir, Chardonnay and Melon. This result concurs with the finding by TSCHAMMER and ZYPRIAN (1994) that Auxerrois was clearly different from Chardonnay, but the degree of similarity was much higher (~95 %) based upon bands produced by 20 RAPD primers, as compared to our finding of 86.7 % similarity based on 361 bands scored.

Concord and Damoth 1 were nearly indistinguishable with 98.1 % similarity among RAPD amplicons (Tab. 4). The similarity index among the three Niagara clones was between 97.2 and 98.4 %. Whereas there were no differences found among Pinot noir, and its clones and supposed sports, it becomes more difficult to draw a conclusion when just a few bands representing only 2-3 % of those scored are found to differ between what were thought to be sports. On the one hand, these bands might actually be delineating genetic differences. On the other hand, truly different grapevine cultivars within species show between 80 and 90 % genetic similarity. It is helpful here to note that Niagara arose from a cross of Concord x Cassady (HEDRICK 1908), yet has just 79 % RAPD amplicon similarity with Concord (Tab. 4). Similarity percentages above 97 % seem very high by comparison, and therefore it might be concluded that our evidence is weighted in favor of Niagara Seedless and Niagara Rosada being true sports of Niagara, and Damoth 1 being a sport of Concord. In fact, error rates of 2-7 % in scoring RAPD data have been noted and the greatest error rate usually occurs with bands of faint or intermediate intensity (WEEDEN *et al.* 1992). The bands in this study which distinguished Concord from Damoth 1 and Niagara from its supposed sports were all either faint or intermediate in intensity. Later attempts to reproduce these results failed.

The results in this study showed that RAPD analyses can be used for grape cultivar identification. In general, the RAPD technique allowed for discrimination among phenotypically similar grape cultivars. The results were consistent and reproducible for primer/genotype combinations between different DNA extractions, different amplifications and different thermocyclers. BÜSCHER *et al.* (1993) discussed problems which may occur due to changes in PCR reaction components and tube position in the thermocycler. Taking heed of their cautions, and by strictly controlling conditions, our results as well as those of MULCAHEY *et al.* (1995) show that RAPDs may be used reliably for fingerprinting studies. Error rates may increase among faintly-amplified bands, therefore results must be interpreted with caution (WEEDEN *et al.* 1992). Along with RFLP and microsatellite markers, RAPDs are useful as a sensitive and reliable method for cultivar identification and should help to understand the origins of cultivars thought to have originated as sports.

Acknowledgments

We are grateful to Dr. PATRICE THIS of Montpellier for help with questions of varietal identity and for supplying a true-to-type DNA sample of Pinot gris. We thank Dr. MUHAMMAD A. LODHI for helpful suggestions and discussions, and Dr. SHENG-ZHI PANG for providing the GY primers used in this study. We also thank SHERI WOO and TIM JOHNSON for vineyard assistance. This research was supported by Grant No. US1888-90 from BARD, the US-Israel Binational Agricultural Research and Development Fund.

References

AMBROSI, H.; DETTWEILER, E.; RÜHL, E. H.; SCHMID; J.; SCHUMANN, F.; 1994: Farbatlas Rebsorten, 300 Sorten und ihre Weine. Verlag Eugen Ulmer, Stuttgart, Germany.

BACHMANN, O.; BLAICH, R.; 1988: Isoelectric focusing of grapevine peroxidase as a tool for ampelography. Vitis **27**, 147-155.

BOTTA, R.; SCOTT, N. S.; EYNARD, I.; THOMAS, M. R; 1995: Evaluation of microsatellite sequence-tagged site markers for characterizing *Vitis vinifera* cultivars. Vitis **34**, 99-102.

BOURQUIN, J. C.; SONKO, A.; OTTEN, L.; WALTER, B.; 1993: Restriction fragment length polymorphism and molecular taxonomy in *Vitis vinifera* L. Theoret. Appl. Genet. **87**, 431-438.

- -; TOURNIER, P.; OTTEN, L.; WALTER, B.; 1992: Identification of sixteen grapevine rootstocks by RFLP and RFLP analysis of nuclear DNA extracted from the wood. Vitis **31**, 157-162.

BOWERS, J. E.; BANDMAN, E. B.; MEREDITH, C. P.; 1993: DNA fingerprint characterization of some wine grape cultivars. Amer. J. Enol. Viticult. **44**, 266-274.

- -; MEREDITH, C. P.; 1996: Genetic similarities among wine grape cultivars revealed by restriction fragment length polymorphism (RFLP) analysis. J. Amer. Soc. Hort. Sci. **121**, 620-624.

BÜSCHER, N.; ZYPRIAN, E.; BLAICH, R.; 1993: Identification of grapevine cultivars by DNA analyses: Pitfalls of random amplified polymorphic DNA techniques using 10mer primers. Vitis **32**, 187-188.

CASTIGLIONE, S.; WANG, G.; DAMIANI, G.; BANDI, C.; BISOFFI, S.; SALA, F.; 1993: RAPD fingerprints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. Theoret. Appl. Genet. **87**, 54-59.

CIPRIANI, G.; FRAZZA, G.; PETERLUNGER, E.; TESTOLIN, R.; 1994: Grapevine fingerprinting using microsatellite repeats. Vitis **33**, 211-215.

COLLINS, G. G.; SYMONS, R. H.; 1993: Polymorphisms in grapevine DNA detected by the RAPD PCR technique. Plant Mol. Biol. Reptr. **11**, 105-112.

GALET, P.; 1990: Cépages et Vignobles de France. Tome II. L'Ampélographie Française. (2nd ed.) Imprimerie Charles Déhan, Montpellier, France.

GOGORCENA, Y.; ARULSEKAR, S.; DANDEKAR, A. M.; PARFITT, D. E.; 1993: Molecular markers for grape characterization. Vitis **32**, 183-185.

HEDRICK, U. P.; 1908: The Grapes of New York. New York State Agr. Expt. Sta., Geneva, Ann. Rep. 1907.

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

LODHI, M. A.; YE, G. N.; WEEDEN, N. F.; REISCH, B. I.; 1994: A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant. Mol. Biol. Reptr. **12**, 25-32.

MAILER, R. J.; SCARTH, R.; FRISTENSKY, B.; 1994: Discrimination among cultivars of rapeseed *Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers. Theoret. Appl. Genet. **87**, 697-704.

MAURO, M. C.; STREFELER, M.; WEEDEN, N. F.; REISCH, B. I.; 1992: Genetic analysis of restriction fragment length polymorphisms in *Vitis*. J. Heredity **83**, 18-21.

MULCAHEY, D. L.; CRESTI, M.; LINSKENS, H. F.; INTRIERI, C.; SILVESTRONI, O.; VIGNANI, R.; PANCALDI, M.; 1995: DNA fingerprinting of Italian grape varieties: A test of reliability in RAPDs. Adv. Hort. Sci. **9**, 185-197.

PARFITT, D. E.; ARULSEKAR, S.; 1989: Inheritance and isozyme diversity for GPI and PGM among grape cultivars. J. Amer. Soc. Hort. Sci. **114**, 486-491.

ROBINSON, J.; 1986: Vines, Grapes and Wine. Alfred A. Knopf, New York.

ROHLF, F. J.; 1990: NTSYS-pc Numerical Taxonomy and Multivariate Analysis System, Version 1.60. Applied Biostatistics, New York.

STRIEM, M. J.; SPIEGEL-ROY, P.; BEN-HAYYIM, G.; BECKMANN, J.; GIDONI, D.; 1990: Genomic DNA fingerprinting of *Vitis vinifera* by the use of multi-loci probes. Vitis **29**, 223-227.

THOMAS, M. R.; SCOTT, N. S.; 1993: Microsatellite repeats in grapevine reveal DNA polymorphisms when analyzed as sequence-tagged sites (STSs). Theoret. Appl. Genet. **86**, 985-990.

- -; - -; 1994: Sequence-tagged site markers for microsatellites: simplified technique for rapidly obtaining flanking sequences. Plant Mol. Biol. Reptr. **12**, 58-64.

TSCHAMMER, J.; ZYPRIAN, E.; 1994: Molecular characterization of grapevine cultivars of Riesling-type and of closely related Burgundies. Vitis **33**, 249-250.

VIALA, P.; VERMOREL, V.; 1901-1910: Ampélographie. 7 vol. Masson Editeurs, Paris.

WALTERS, T. W.; POSLUSZNY, U.; KEVAN, P. G.; 1989: Isozyme analysis of the grape (*Vitis*). I. A practical solution. Can. J. Bot. **67**, 2894-2899.

WEEDEN, N. F.; REISCH, B. I.; MARTENS, M. E.; 1988: Genetic analysis of isozyme polymorphism in grape. J. Amer. Soc. Hort. Sci. **113**, 765-769.

- -; TIMMERMAN, G. M.; HEMMAT, M.; KNEEN, B. E.; LODHI, M. A.; 1992: Inheritance and reliability of RAPD markers. In: Joint Plant Breeding Symposia Series: Applications of RAPD Technology to Plant Breeding, 12-17. Crop Sci. Soc. Amer., Madison, Wisconsin, USA.

WELSH, J.; MCCLELLAND. M.; 1990: Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. **19**, 303-306.

WILLIAMS, J. G. K.; KUBELIK, A. R.; LIVAK, K. J.; RAFALSKI J. A.; TINGEY, S. V.; 1990: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**, 6531-6535.

WOLFE, W. H.; 1976: Identification of grape varieties by isozyme banding patterns. Amer. J. Enol. Viticult. **27**, 68-73.

YAMAMOTO, N.; ONO, G.; TAKASHIMA, K.; TOTSUKA, A.; 1991: Restriction fragment length polymorphism of grapevine DNA with the phenylalanine ammonia-lyase cDNA. Jap. J. Breed. **41**, 365-368.

YE, G. N.; HEMMAT, M.; LODHI, M. A.; WEEDEN, N. F.; REISCH, B. I.; 1996: Primers longer than 16 bases are particularly useful for RAPD mapping and fingerprinting of grape and pear. BioTechniques **20**, 368-371.

YU, L. X.; NGUYEN, T. H.; 1994: Genetic variation detected with RAPD markers among upland and lowland rice cultivars (*Oryza sativa* L.). Theoret. Appl. Genet. **87**, 668-672.

Received January 20, 1997