The RAPD analysis of several cultivars of grapevine (*Vitis vinifera* L.) and their clones

H. VLASTNÍKOVÁ, K. MORAVCOVÁ, M. PIDRA

Faculty of Horticulture, Mendel University of Agriculture and Forestry, Brno, Lednice, Czech Republic

ABSTRACT: Nine identification RAPD markers (MORAVCOVÁ et al. 2003) were used to distinguish 24 clones and grapevine cultivars. No polymorphism was detected among all the tested clones of Chardonnay, Pinot gris and Zweigeltrebe from Polešovice. Pinot noir, Pinot gris, Pinot blanc and Pinot Meunier were indistinguishable within clones, they also showed the identical RAPD profile within cultivars (except discussed sample No. 26). On the other hand, Auxerrois as a relative to cultivars of Pinot group showed unique patterns and may be classified as a different cultivar. Some irregularities within the cultivars of Pinot family from Oblekovice were also found, several of them gave different results from those expected: Pinot blanc sample 26 has the RAPD profile typical of Chardonnay. A new abnormal RAPD pattern as a marker of typical Chardonnay and Pinot profiles was observed in two cases. While RAPD banding patterns could not distinguish between the known clones, they were useful for distinguishing between phenotypically similar cultivars and for assessing the origins of cultivars thought to have originated as sports.

Keywords: grapevine; clones; fingerprinting; RAPD

The increasing international trade in grapevine and rootstock plant material as well as in wine necessitates a reliable identification of genotypes. The genetic relationship and identification of clones and cultivars has been one of the points of interest in viticulture. This objective has usually been achieved by the evaluation of morphological characters in different organs of the plant: leaves, grapes, shoot tip, etc. (MORENO 1995) but the cultivar and clone identification can be very difficult when relying only upon ampelographic and botanical characterization itself.

The long period of cultivating traditional varieties was sufficient for varietal diversification into individual genotypes. Nowadays, growers prefer to cultivate the clonal material of traditional cultivars instead of mass-propagated grapevines, so the scientists tried to find markers which are linked to individual clones and which are stable during propagation (REGNER et al. 2000b).

The DNA polymorphism analyzed by RAPD technique (WILLIAMS et al. 1990; WELSH, MCCLELLAND 1990) is of great interest because it requires only very low amounts of template DNA, it is relatively simple and a high level of genetic variation can be detected. Many reports on the use of RAPD markers for cultivars or for detection of genetic variation between cultivars were published (HU, QUIROS 1991; CASTIGLIONE et al. 1993; MAILER et al. 1994; YU, NGUYEN 1994) but on the other hand, some positive results for the identification of some clones are still missing.

The present report summarizes the results of the utilization of RAPD markers for identification of several grapevine clones and cultivars, as well as the level of polymorphism and the reproducibility of the method. A majority of the tested cultivars and their clones were from the Pinot family because of their worldwide importance for varietal wine production.

MATERIAL AND METHODS

Plant material

Partially expanded leaves were collected from healthy plants, free of pests and diseases. Clones of Pinot gris, Zweigeltrebe and some clones of Chardonnay were taken from the collection of the Grapevine Breeding Station in Polešovice. Ten samples of varieties and clones from Pinot family were taken from the collection of the Central Institute for Supervising and Testing in Agriculture, Department of Viticulture in Oblekovice. The cultivars and clones are listed in Table 1.

DNA extraction

Five grams of young leaves were ground to powder in liquid nitrogen. DNA was extracted from 100 mg of powder according to the protocol for DNeasy Plant MiniKit (Qiagen technologies).

RAPD primers

Five previously verified oligonucleotide primers (Operon technologies) providing 8 identification markers for grapevine varieties registered in the Czech Re-

Supported by the Ministry of Agriculture of the Czech Republic, Grant No. QC 1156.

Table 1. Cultivars and clones tested in RAPD analyses

Cultivar	Number	Clone	Cultivar	Number	Clone
Samples from	Polešovice		Samples from	Oblekovice	R-8
	1	68/44	Chardonnay (Italy)	21	no tally
	2	67/25	Chardonnay (OP, Germany)	22	6/22
Pinot gris	3	67/32	Pinot blanc	23	7/36
	4	68/23	Pinot blanc	24	no tally
	5	31/28	Pinot Meunier	25	no tally
	6	158/7	Pinot blanc	26	no tally
	7	155/6	Pinot gris	27	no tally
Chardonnay	8	156/4	Pinot noir	28	no tally
	9	160/1	Chardonnay	29	158/7
	10	161/6	Auxerrois	30	no tally
	16	36/41			
	17	36/46			
Zweigeltrebe	18	36/32			
	19	37/42			
	20	37/33			

public (MORAVCOVÁ 2004) were used. (Primers and markers are listed in Table 2.)

DNA amplification

The RAPD amplification was carried out in 25 µl of reaction mix containing 20 ng DNA, deionized sterile water, 0.5 U of Dy-Nazyme™ II DNA polymerase (Finnzymes), 1x Buffer for Dy-Nazyme™ II DNA polymerase (10 mM Tris HCl, pH 8.8; 1.5 mM MgCl2; 150 mM KCl and 0.1% Triton X-100), 0.2 mM of each dNTP (Promega), 0.4 µM of primer in length of 10 nucleotides (Operon technologies).

DNA was denatured with one cycle of 94°C for 3 min, followed by 45 amplification cycles of 94°C for 1 min for denaturation, 36°C for 1 min for annealing of primers, 72°C for 1 min 30 s for extension. The final step of the program consisted in 72°C for 9 min for finishing the synthesis of all PCR products.

Electrophoresis was performed in 1.5% of TAE agarose gels, stained with ethidium bromide. 100 bp DNA ladder (Biolabs, New England) was used as a molecular marker for approximation of the mass of DNA.

Table 2. Identification primers and markers for tested varieties

		Primer/size of the band (bp)							
Variety	Abbreviation	OPB4/600	OPB4/680	OPO3/380	OPX3/800	OPX4/800	OPO4/1050	OPX6/700	OPX6/1500
Chardonnay	Ch	■					■	■	
Pinot blanc	Pb				■			■	■
Pinot gris	Pg				■			■	■
Pinot noir	Pn				■			■	■
Zweigeltrebe	ZW		■	■	■	■			

RESULTS AND DISCUSSION

Distinguishing of clones by RAPD

Only the intense bands that showed repeatable patterns in all cases were scored. All the used primers gave one or several quality and strong bands that are used for cultivar identification (MORAVCOVÁ 2004). Usually it was the combination of two or three primers discriminating the tested cultivars (Table 2). The combination of primers can determine some varieties, but it is not able to discriminate between their clones. The RAPD pat-

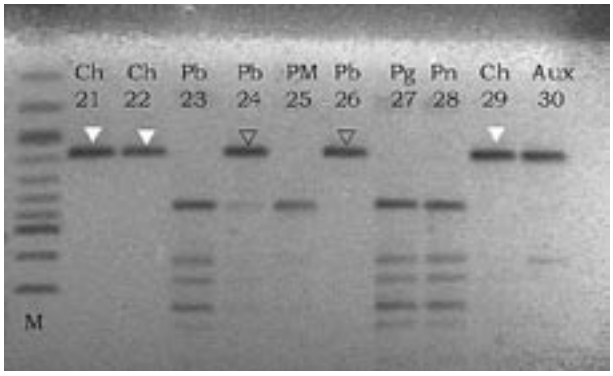

Fig. 1. RAPD patterns obtained with primer OPB 4

Note to the figures: The numbers and abbreviations of cultivar names are listed in Table 1, M – DNA size marker (100 bp ladder)

Arrows show the markers typical of Chardonnay (white arrows) black arrows – of Pinot family

blank – of anomalous products

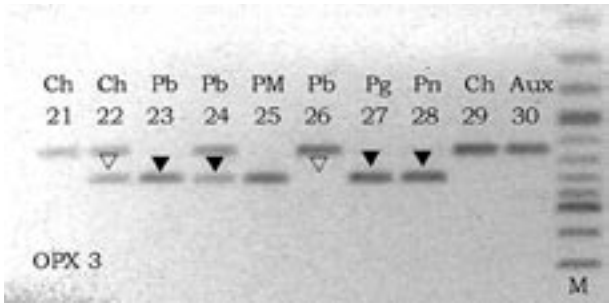

Fig. 2. RAPD patterns obtained with primer OPX 3

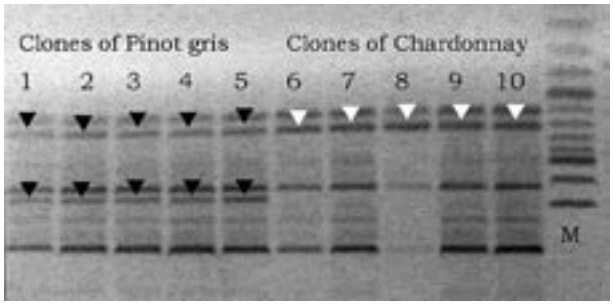

Fig. 4. RAPD patterns obtained with primer OPX 6

terns typical of the tested varieties were obtained in all cases (Fig. 4). The inability to separate clones may be attributable to the rarity of genetic changes responsible for clonal identity which would make the detection of these changes highly improbable (YE et al. 1998).

RAPD study of Pinot family

Pinot family and relative cultivars: Pinot noir, Pinot gris, Pinot blanc, Chardonnay, Pinot Meunier and Auxerrois from Oblekovice were the object of analysis.

Our data support the opinion that the cultivars from the Pinot family (Pinot noir, gris and blanc) are very closely related and it was not possible to distinguish them. These varieties were also indistinguishable in other similar studies (YE et al. 1998; BOWERS et al. 1993).

In our experiment Pinot Meunier produced the RAPD profile identical to that of the Pinot family, this result, in addition to the RAPD data of YE et al. (1998), RFLP data of BOWERS et al. (1993) supports the classification of Pinot Meunier as a sport of Pinot noir.

Auxerrois is shown here to be different from the other cultivars of Pinot family. REGNER (2001a) determined this cultivar (together with Chardonnay) as a product of crossing Burgunder × Heunish.

Some irregularities were found in the analyzed group of the cultivars. The typical RAPD profile for Chardonnay was detected in samples 21, 29, and anomalously also for sample 26, which is registered as Pinot blanc. The ampelographic analysis were performed in Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ) according to the international classifier for grapevine T6/50/8. In the case of sample No. 26 the trait for leaves 27-9 was found (test DUS, ÚKZÚZ, 2003) which corresponds with the characteristics of Chardonnay. We suppose on the basis of these results that the discussed Pinot blanc could be misnamed Chardonnay.

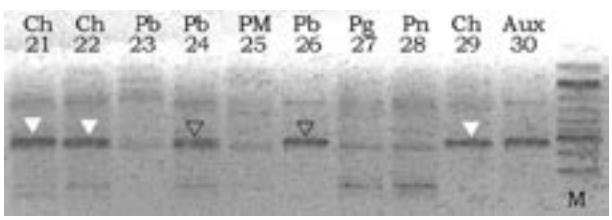

Fig.3. RAPD patterns obtained with primer OPO 4

Based on several publications (REGNER 2001; SEFC et al. 1998) we can assume that the RAPD profiles of Pinot blanc should be identical with the profiles of Pinot noir and Pinot gris. Three RAPD patterns (samples No. 23, 27 and 28) typical of Pinot family were obtained in this study. Illogically, a new RAPD profile was also found that contains identification markers for Chardonnay and for Pinots and that is not typical of any of the cultivars of Pinot family. Anomalously, this new RAPD profile was obtained in two cases – Chardonnay (sample 22) and also Pinot blanc (sample 24).

The next step of testing the cultivars of the Pinot family will be completed by SSR analysis because it is a worldwide respected method and the results of several genetic laboratories are collected in European database (GRANDO 2002). Our first results of the SSR analysis (results are not an object of this study) completely confirm the results from RAPD analysis. Moreover, the microsatellite profiles correspond with profiles published in European Grapevine Microsatellite Collection (GRANDO 1998; REGNER 2000a; SEFC et al. 1998). The present results are very interesting and surprising and they will be verified by other DNA analyses.

CONCLUSION

The results of this study showed that RAPD analysis can be used for the identification of several grapevine cultivars independently of the tested clone of cultivar, but in spite of it we cannot distinguish several cultivars from Pinot family. In general, the RAPD technique allows discrimination between phenotypically similar grape cultivars. This technique is not sufficient for the identification and determination of the clones.

Acknowledgements

We are thankful to the Breeding Station Polešovice and the Central Institute for Supervising and Testing in Agriculture, to the Department of Viticulture in Oblekovice for providing the plant material.

References

BOWERS J.E., BANDMAN X., MEREDITH C.P., 1993. DNA fingerprint characterization of some wine grape cultivars. Amer. J. Enol. Vitic., *44*: 266–274.

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. Theor. Appl. Genet., *87*: 54–59.

GRANDO M.S., FRISINGHELLI C., 1998. Grape microsatellite markers: Sizing of DNA alleles and genotype analysis of some grapevine cultivars. Vitis, *37*: 79–82.

GRANDO M.S., 2002. Grape Microsatellite collection (online database) San Michele all'Adige – Italy: Istituto Agrario San Michele all'Adige (IASMA) <http://217.222.71.209/genetica/query.html >

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

MAILER R.J., SCARTH R., FRISTENSKY B., 1994. Discrimination among cultivars of rapeseed *Brassica napus* L. using DNA polymorphism amplified from arbitrary primers. Theor. Appl. Genet., *87*: 697–704.

MORAVCOVÁ K., BARÁNEK M., PIDRA M., 2004. The use of RAPD markers for differentiation of grapevine cultivars registered in the Czech Republic. Hort. Sci., *31*: 102–107.

MORAVCOVÁ K., BARÁNEK M., SOTOLÁŘ R., PIDRA M., 2003. Identification of grapevine varieties by molecular-genetic method RAPD. Science of the Youth, 6.–7. 9. 2003, Coll. of Abstracts, B48: 276.

MORENO S., GOGORCENA Y., ORTIZ J.M., 1995. The use of RAPD markers for identification of cultivated grapevine (*Vitis vinifera* L.). Sci. Hort., *62*: 237–243.

REGNER F., SEFC K.M., GLÖSSL J., STEINKELLNER H., 2000a. Parentage analysis and pedigree reconstruction of vine cultivars using microsatellite markers. Acta Hort., VII International Symposium on Grapevine Genetics and Breeding: 177–183.

REGNER F., WIEDECK E., STADLBAUER A., 2000b. Differentiation and identification of White Riesling clones by genetic markers. Vitis, *39*: 103–107.

REGNER F., STADBLAUER A., EISENHELD C., 2001. Molecular markers for genotyping grapevine and for identifying clones of traditional varieties. Acta Hort., *546*: 331–341.

SEFC K.M., REGNER F., GLÖSSL J., STEINKELLNER H., 1998. Genotyping of grapevine and rootstock cultivars using microsatellite markers. Vitis, *37*: 15–20.

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 polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res., *18*: 6531–6535.

YE G.N., SOYLEMEZOGLU G., WEEDEN N.F., LAMBOY W.F., POOL R.M., REISCH B.I., 1998. Analysis of the relationship between grapevine cultivars, sports and clones via DNA fingerprinting. Vitis, *37*: 33–39.

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 for publication April 8, 2004
Accepted after corrections June 7, 2004

Použití RAPD markerů k identifikaci vybraných kultivarů révy vinné (*Vitis vinifera* L.) a jejich klonů

ABSTRAKT: Devět identifikačních RAPD (Random Amplified Polymorphism DNA) markerů (MORAVCOVÁ 2004) bylo vyzkoušeno pro odlišení 24 klonů a odrůd révy vinné. Nebyl nalezen polymorfismus mezi testovanými klony jednotlivých odrůd Chardonnay, Rulandského šedého a Zweigeltrebe, izolovaných z rostlinného materiálu pocházejícího ze šlechtitelské stanice v Polešovicích. Analýza rulandských odrůd (Rulandské modré, šedé a bílé) a Pinot Meunier (Mlynářka) neodhalila žádný výrazný rozdíl v RAPD spektrech jednotlivých klonů. Rovněž nebyl mezi odrůdami nalezen žádný významný rozdíl (s výjimkou diskutabilního vzorku 26). Odrůda Auxerrois, blízce příbuzná burgundské skupině, vykázala polymorfní RAPD profil, umožňující její odlišení od ostatních rulandských odrůd. Zároveň byly odhaleny jisté nesrovnalosti v rámci testovaných kultivarů burgundské skupiny odrůd z Oblekovic. Některé z nich měly odlišné profily od profilů očekávaných: při testování Rulandského bílého (vzorek 26) bylo získáno RAPD spektrum typické pro odrůdu Chardonnay. Ve dvou případech byl objeven zcela nový, abnormální profil složený z charakteristických markerů jak pro Chardonnay, tak pro rulandskou skupinu odrůd. Přestože pomocí RAPD analýzy nelze odlišit jednotlivé klony odrůd, je možné ji použít pro identifikaci fenotypově podobných odrůd a k posouzení jejich původu.

Klíčová slova: réva vinná; klony; fingerprinting; RAPD

Corresponding author:

Ing. KATEŘINA MORAVCOVÁ, Mendelova zemědělská a lesnická univerzita, Brno, Zahradnická fakulta, Mendeleum, Valtická 334, 691 41 Lednice, Česká republika
tel.: + 420 519 367 313, fax: + 420 519 367 202, e-mail: xmoravc2@node.mendelu.cz