The use of RAPD markers for differentiation of grapevine varieties registered in the Czech Republic

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ABSTRACT: Fifty-one grapevine cultivars registered in the Czech Republic were a subject of RAPD analysis. Nine grapevine varieties which were supposed to be genetically distant, were analysed by means of 120 Operon primers during the initial screening of RAPD method. Sixteen primers producing reproducible and polymorphic bands were then used for amplification of all registered varieties. A dendrogram of genetic relationships and a methodology of identification of each variety were created. The resultant dendrogram mostly corresponds with known information about the origin and genetic relationships of the varieties. It is necessary to use the combination of RAPD markers for identification of each variety. It was possible to identify 26 out of 51 varieties registered in the Czech Republic by RAPD markers without any problems. Nine varieties were identifiable only on the basis of present and absent markers. Some varieties were very close relatives – they formed remote clusters. It was not possible to distinguish between them by RAPD method. Two varieties were not identifiable by the designed key.

Keywords: grapevine; registered cultivars; fingerprinting; RAPD

Grapevine is one of the oldest agricultural crops that the mankind has used to produce table fruit, raisins, juice and wine (OLMO 1976). *Vitis vinifera* L. is cultivated worldwide and many important cultivars have been selected through the centuries. An inventory of grapevine cultivars described in the literature revealed the existence of more than 14,000 putative varieties. Non-*Vitis vinifera* species and interspecific hybrids are widely used as rootstocks. They are also a source of genes for breeding of both table and wine cultivars (ALLEWELDT et al. 1990).

The genetic relationships of traditional grapevine cultivars have always been a matter of speculation. Neither classic ampelography nor analytical chemistry could resolve the disorder of grapevine cultivars or offer any valuable information about heterozygosity for grapevine breeding (REGNER et al. 2001). Identification of grapevine cultivars can be difficult when relying only on ampelographic and botanical characteristics.

DNA polymorphism appears to be a particularly useful tool for distinguishing cultivars because the results directly reflect the genotype. Results are independent of the environment. A large number of potential molecular markers is available and DNA can usually be extracted from nearly every tissue (YE et al. 1998). The RAPD (Random Amplified Polymorphic DNA) technique is fast and relatively simple since it does not require any preliminary knowledge of the sequences of the markers and can be resolved using agarose gels. This technique has already proved its usefulness for the identification of cultivars in numerous plant species (ARUNA et al. 1995; KOLLER et al. 1993). RAPD produces more amplified products with a problem of its reproducibility. However, some primers yield more stable profiles than others and especially intensive bands in RAPD profiles are usually stable and repeatable (THIS et al. 1997).

In this study RAPD was used to characterise grapevine varieties registered in the Czech Republic.

MATERIAL AND METHODS

Plant material: 51 grapevine varieties and rootstocks registered in the Czech Republic were obtained from the collection of the Central Institute for Supervising and Testing in Agriculture, Department of Viticulture in Oblekovice.

DNA extraction: DNA was isolated from young leaves by Dneasy Plant Mini Kit of Qiagen and quantified on 1% agarose gel stained with ethidium bromide by visual comparison with known quantities of λDNA.

Primers: Seven sets (OPA, OPB, OPE, OPF, OPM, OPO, OPX) of oligonucleotide primers (10 – mers) were purchased from Operon Technologies (Alameda, CA, USA).

PCR protocol: RAPD amplification was performed in a reaction volume 25 µl containing 1 X buffer (10 mM Tris HCl, pH 8.8; 1.5 mM $MgCl_2$; 150 mM KCl and 0.1% Triton X-100), 0.2 mM of each dNTP (Promega), 0.4 µM primer (Operon Technologies), 20 ng of genomic DNA and 0.5 unit of Taq DNA

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Table 1. Identification primers and markers for grapevine varieties registered in the Czech Republic

	present marker
	absent marker
variety	it is posible to identify the variety by the present markers

Abbreviations	Name of the primer/ size of the band / Name of the variety	A7/750	A7/850	A7/1300	B2/500	B2/950	B4/600	B4/680	B17/750	B17/950	E9/520	M4/550	M4/700	M4/1000	O3/380	O3/1200	O3/1400	O4/700	O4/800	O4/1050	O5/1150	O7/780	O7/850	O7/1000	X3/650	X3/800	X3/900	X6/700	X6/800	X6/1500
AU	Aurelius						■				■									■							■		■	
DV	Děvín	■							▦																		■			
CHR	Chardonnay						■													■								■		
IO	Irsai Oliver Muscotaly															■			■							■				■
KR	Kerner										■									▦						■			■	▦
LN	Lena										■													■		■				■
ML	Malverina		■									■			■				■								■			
MM	Muscat Moravia												■						■						■					■
MO	Muscat Ottonel					■	▦												■				■							■
MT	Müller Thurgau									■	■																			■
NEU	Neuburger blanc							■	■																		■			
PA	Pálava										▦				■													■		■
RB	Pinot blanc						■													■								■		
RŠ	Pinot gris																								■			■		■
RR	Riesling blanc														▦				▦		■				■					■
RV	Riesling Italico	■						■		■	■																			
SA	Sauvignon blanc													■	■				■									■		
SZ	Gruner Silvaner						■				▦				▦					■								■		
TČ	Traminer rot										▦				▦													■		■
VČR	Frühroter Veltliner																													
VZ	Gruner Veltliner										■				■												■			■
VER	Veritas				■						■				■				▦											
AG	Agni							■			■								■							■				■
AL	Alibernet										■								■						■		■			
AN	André							■			■				■				■								■			
ARN	Ariana										■				■	■			■										■	
CM	Cabernet Moravia							■											■						■					■
CS	Petit Cabernet																■		■									■		■
FR	Blaufrankish							■			■								■			■					■		■	
MR	Merlot noir	■													■					■										
MP	Portugais Bleu					■													■				■		■			▦		▦
NR	Neronet	■									■															■		■		
RM	Pinot noir																									■		■		■
SV	St.Laurent										■			■	■				■							■				
ZW	Zweigelt blau							■							■				■							■				
AR	Arkadia										■			■						■						■			■	
DA	Diamant						■												■					■						■
CHB	Chasselas blanc					■																	■		■					■
CHČ	Chasselas rose					■																	■		■					■
JB	Julski biser						■												■					■						■
OL	Olšava	■									■								■				■		■					■
PK	Panonia Kincse						■																		■	■				■
PO	Pola																													
VT	Vitra	■									■								■				■		■					■
AM	Amos				■			■										■				■								
5BB	Kober 5BB			■			■											■				■								
SO4	SO - 4			■			■											■				■								
CR	Craciunel			■			■											■				■								
5C	Teleki 5C						■				■							■				■								
AA	125 AA			■			■											■				■								
LE	LE - K/1				■													■				■					■			

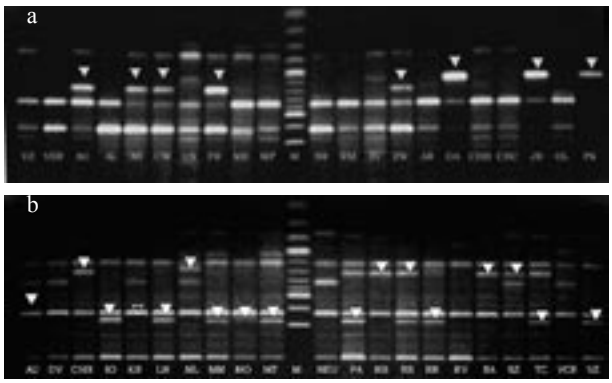

Fig. 1. RAPD profiles separated on agarose gels and stained with ethidium bromide. Samples were amplified with primers OPX 6 (a) and OPB 4 (b). Abbreviations of samples correspond with abbreviations in Table 1. M – 100 bp length marker. Arrows above the bands designate markers selected for identification (full – present bands, blanc – absent)

polymerase (DyNAzyme II). Amplification was performed on a T-gradient thermocycler (Biometra) for 45 cycles. The program started with initial denaturation at 94°C for 3 min. Each cycle consisted of: denaturing (1 min at 94°C), annealing (1 min at 36°C) and extension (1.5 min at 72°C). The last cycle of extension was prolonged to 9 min and the amplification products were then stored at 4°C. Amplification products were separated by electrophoresis on 1.5% agarose gels (1x TAE buffer), stained with ethidium bromide and photographed on a transilluminator using digital camera (DC 120 ZOOM). A 100 bp ladder (New England Biolab) was used as a molecular size standard.

Data analysis: The Cross Checker software was used to evaluate the results. Intense and reproducible bands separated on agarose gels were recorded as present (1) or absent (0) and the results were assembled in a data matrix table. Acquired data were subjected to analysis using POP-GENE 32 software (YEH, BOYLE 1997).

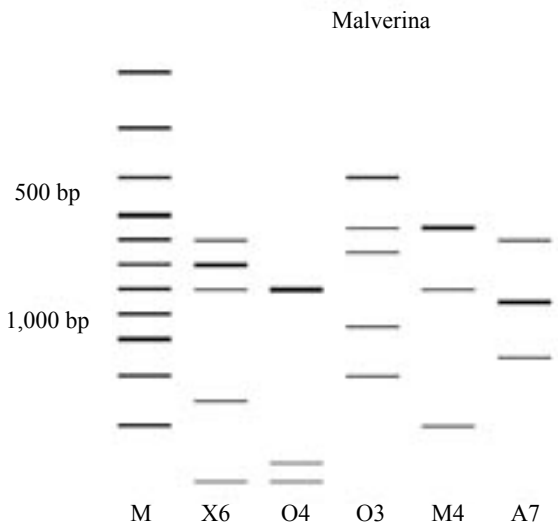

Fig. 2. An example of a typical RAPD fingerprint. Bold lines are characteristic markers for the variety, thin lines are the additional bands. M – 100 bp length marker

Amplified products were analysed by comparison of genotypes based on the percentage of common fragments and similarity matrix (NEI 1978). Dendrogram was constructed by means of the unweighted pair group method using arithmetic averages (UPGMA method, SNEATH, SOKAL 1973). The tree was generated by the TREE – VIEW software (PAGE 1996).

RESULTS

Screening

Nine varieties – Muscat Moravia, Pinot Blanc, Riesling Blanc, Cabernet Moravia, Pinot Noir, Chasselas Blanc, Olšava and rootstocks – Amos and SO4, were used for screening of RAPD reaction. Out of 120 screened primers 80 gave interpretable results, 54 (45%) were found to be polymorphic and 26 primers (21%) giving reproducible results had monomorphic spectra. Only 8 primers were not repeatable. Other primers gave poor, hardly interpreted spectra.

Out of all reproducible primers: 277 amplified fragments were scored and 138 of them (almost 50%) were evaluated to be polymorphic. Finally, 16 highly informative primers were chosen for testing all registered varieties.

Creation of dendrogram

Only reproducible bands (300–1,500 bp) were used for analysis. Additional bands in the patterns, even if they are less intense can be useful, especially for distinguishing small divergences between close relative varieties. However, it was not possible to use these additional bands for identification.

Eighty-seven bands from 16 primers were analysed and a dendrogram of genetic relationships was created (Fig. 3). Each cluster of the tree can be associated to types according to the origin and morphology of the varieties.

Identification by RAPD markers

Only strong and stable markers were selected for the identification of the varieties. All gels were prepared at least in two replications and only reproducible bands were used for the analysis. The analysis of acquired data was done primer by primer instead of marker by marker. The key for the variety identification was designed. The aim of this work was to find as few markers as possible to discriminate all the varieties from each other. Only 29 bands amplified by 12 primers were identified as markers. It was possible to identify 26 out of the 51 varieties registered in the Czech Republic only by positive discrimination. Nine varieties were identified only on the basis of present and absent bands. Some varieties, which are very close relatives, formed small groups:

- Pinot noir + Pinot gris (berry colour mutation),
- Pinot blanc + Pinot Chardonnay,
- Diamant (Julski biser × Panonia Kincse) + Julski Biser,

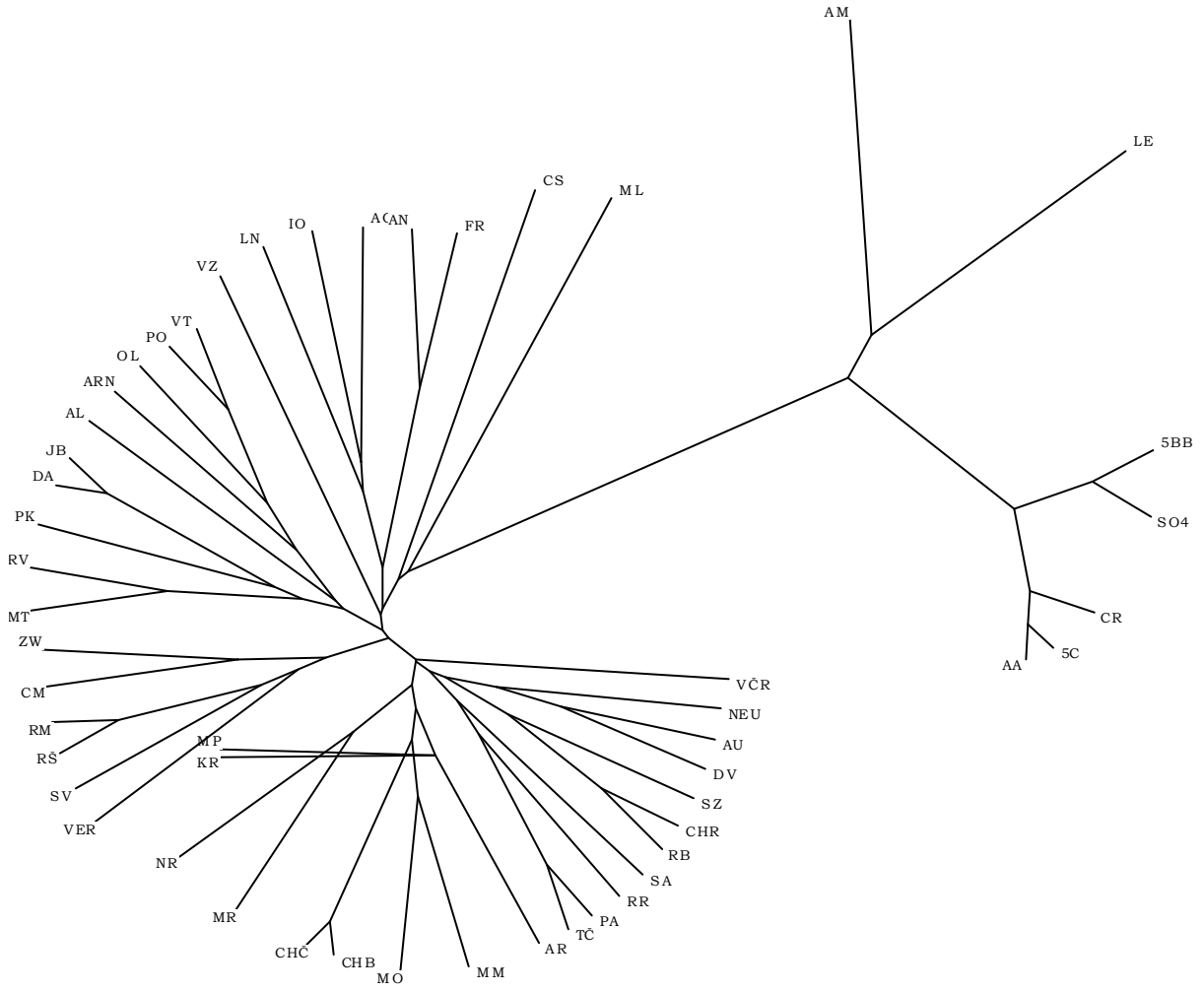

Fig. 3. The dendrogram of genetic relationships. Abbreviations of the varieties correspond with abbreviations in Table 1

– Chasselas blanc + Chasselas rose (berry colour mutation),
– Olšava (Košutův hrozen × Boskolena) + Vitra (Pobě-da × Košutův hrozen),
– Kober 5BB + SO-4 + Craucinel + 125 AA (all of them are rootstocks originating from the same crossing – *V. berlandieri* × *V. riparia*).

It was possible to identify these varieties only as whole clusters. Two varieties – Pola and Frühroter Veltliner (Malvoise rose) were not identifiable by the designed key.

It was necessary for the identification of each variety to use a combination of markers – usually more than 2 and less than 6. Unique markers were found only for Malverina and Cabernet Moravia. In this case, as few as one amplification might be enough to confirm or to disprove the difference from other varieties. For differentiation of the table grape varieties Lena, Diamant and Julski Biser, it was sufficient to use 2 markers.

DISCUSSION

Location of the varieties in the dendrogram mostly corresponds with generally accepted information about the origin and genetic relationships of the varieties. It is evident from the dendrogram that all 51 varieties are separated into two main clusters – traditional grapevine varieties and rootstocks.

– The rootstocks from crossing *V. berlandieri* × *V. riparia* formed one cluster. Amos and the Czech rootstock LE – K/1, which is derived from a different crossing, are separated.
– Malverina – the first and still the only one interspecific cultivar registered in the Czech Republic is located almost outside the main cluster:
– Influence of Muscat de Samur – (the forefather of the Muscats) is apparent in the Muscat family (Muscat Ottonel and Muscat Moravia). Chasselas blanc (the mother of Muscat Ottonel) is located on the neighbouring branch of the dendrogram together with Chasselas rose.

The table grape varieties form three small clusters.

1. Pola, Vitra and Olšava are derived from a research institute in Polešovice and they have the same parent – Košutův hrozen.
2. Diamant is a product of crossing – Julski Biser and Panonia Kincse.
3. Irsai Oliver is a parent of the table grape variety Lena and from its crossing with Andre the red va-

riety Agni is derived – all these varieties are in one cluster.

– The successful Czech variety Pálava (Traminer rot × Müller Thurgau) is located on the same branch with Traminer rot and very close to Riesling blanc – a parent of Müller Thurgau. The location of Müller Thurgau – out of all its supposed parents (Riesling blanc, Gruner Silvaner, Chasselas…) is interesting.
– The position of the Pinot group evokes many questions. For a long time, Chardonnay was considered to be Pinot Blanc. Both varieties were mixed and grown together as one variety. In 1896 Chardonnay was separated as an independent variety at the viticultural congress in Chalon (POSPÍŠILOVÁ 1981). REGNER et al. (2001) discovered by his SSR study that Chardonnay (together with several cultivars with international priority) is probably a product of crossing Pinot × Heunisch. Moreover, no allelic difference was detected within the Pinot family (P. noir, P. gris, P. blanc) and Chardonnay was identified outside this cluster (SEFC et al. 1998). Conversely, it was not possible to distinguish between Pinot blanc and Chardonnay in this study. Pinot noir and Pinot gris formed another group. The divergence between these two clusters is evident from the dendrogram and four strong markers that are able to distinguish these groups were found.

These results suggest that the supposed Pinot blanc, which was an object of the analysis, is probably wrongly named Chardonnay.

Pinot is a synonym of Sant Laurent and this variety is really located on the same branch with Pinot Noir and Pinot gris – this fact can be confirmed by REGNER et al. (2001) idea that Pinot is one of the supposed parents of Saint Laurent.

DNA of all varieties and their clones of the Pinot family which are grown in Oblekovice was already isolated and the samples are an object of DNA analysis to elucidate the situation.

CONCLUSION

The aim of the study was to create a catalogue of discriminating fingerprints of all varieties registered in the Czech Republic (Table 1). We were fully successful with 26 varieties – it is possible to identify them by present markers without any problems. This method was tested by various independent workers, different samples – various clones from different vineyards and DNA was isolated from various tissues (the results are not shown). All the results were satisfactory. The problem arises with a group of varieties where the absent marker is necessary for identification. The presence or absence classification of a band on the gel is always arguable and the validity of the results can decrease. All the varieties will be tested by SSR technique as well and the results of both methods will be compared. Finally, an optimal method for identification of each variety will be recommended.

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Použití RAPD markerů pro identifikaci odrůd révy vinné registrovaných v České republice

ABSTRAKT: Padesát jedna odrůd révy vinné registrovaných v ČR bylo předmětem RAPD analýzy. Devět odrůd, geneticky si pokud možno vzdálených, bylo použito pro skríning RAPD reakce, v jehož rámci bylo otestováno 120 primerů (firma Operon). Šestnáct primerů poskytujících reprodukovatelná a polymorfní spektra bylo posléze použito pro amplifikaci všech registrovaných odrůd. Byl sestrojen dendrogram genetické příbuznosti a metodika identifikace jednotlivých odrůd. Výsledný dendrogram převážně odpovídá všeobecně respektovaným poznatkům o původu a genetické příbuznosti odrůd. Pro identifikaci odrůd je nezbytné použít kombinaci RAPD markerů. Dvacet šest odrůd bylo možné jednoznačně identifikovat bez větších problémů. Pro identifikaci devíti odrůd bylo nutné požít kromě přítomných i nepřítomné markery. Některé odrůdy si byly geneticky natolik úzce příbuzné, že je nebylo možné pomocí RAPD vzájemně odlišit. Vytvořené malé skupiny odrůd byly proto identifikovány jako celek. Pouze dvě odrůdy nebylo možné pomocí navrženého klíče identifikovat vůbec.

Klíčová slova: réva vinná; registrované odrůdy; fingerprinting; RAPD

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