GENETIC DIVERSITY EVALUATION OF SOME AUTOCHTHONOUS GRAPEVINE VARIETIES BY RAPD MARKERS

EVALUAREA DIVERSITATII GENETICE A UNOR SOIURI AUTOHTONE DE VITA DE VIE CU AJUTORUL MARKERILOR RAPD

GHEORGHE RALUCA NICOLETA [1], CARMEN FLORENTINA POPESCU[1], PAMFIL D.[2], PAUCHNECHT AL. ED.[1]

[1]National Research and Development Institute for Biotechnology in Horticulture, Stefanesti

[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca

Abstract. *The present study was designed to establish the genetic relationships among 11 grapevine varieties grown in two different regions by using RAPD markers. Nine highly polymorphic markers were selected to characterize the native Vitis vinifera L. cultivars, listed in the Romanian Official Catalog of plant varieties. The random primers yielded 111 amplified fragments ranging between 230 and 4041 bp in size. A general characteristic for all obtained RAPD patterns with DNA amplicons was the high degree of variation among cultivars revealed by proportion of polymorphic bands ranging from 78% to 100%. Genetic relationships were established by statistic analysis of RAPD markers using LabImage software and the dendrogram was constructed based on Jaccard's similarity coefficient and UPGMA clustering. The selected RAPD markers proved to be very efficient to discriminate the tested varieties and provide valuable genetic information about Romanian grapevine germplasm. The high level of genetic variation among tested varieties could be attributable to differences among cultivars within geographical areas and also to specificity of Romanian viticulture tradition.*

Rezumat. *Obiectivul acest studiu a fost acela de a utiliza markeri moleculari RAPD în scopul stabilirii relației genetice între 11 soiuri de viță de vie listate în Catalogul Oficial al soirilor de plante şi care provin din două areale de cultură diferite. Cu primerii selectați pentru caracterizarea soiurilor locale de Vitis vinifera L., s-au obținut 111 fragmente de amplificare a căror mărime a variat între 230 şi 4041 pb. Caracteristica comună pentru toți markerii testați a fost distribuția foarte diferită a fragmentelor de amplificare în gel, proporția de benzi polimorfice fiind cuprinsă între 78% şi 100%. Analiza statistică a markerilor RAPD cu programul LabImage şi dendrograma obținută pe baza coeficientului de similaritate Jaccard, au stat la baza aprecierii relațiilor genetice între soiurile luate în studiu. Metoda folosită s-a dovedit a fi relativ uşoară şi foarte eficientă pentru evidențierea diferențelor dintre soiuri, furnizând informații valoroase pentru caracterizarea genetică a germoplasmei viticole româneşti. Variația genetică mare între genotipurile testate poate fi atribuită pe de o parte zestrei genetice specifice în arealele geografice din care provin, iar pe de altă parte păstrării în timp a tradiției viticole locale.*

Key words: romanian grapevine varieties, RAPD markers, genetic diversity, *Vitis vinifera* sp.

Grapevine is the most important perennial crop worldwide and is today grown throughout the temperate and tropical regions of the world for fresh and dried fruit, juice, and wine production (Oprea and Moldovan. 2007; Sestras. 2004) . The world's collections of grape plant material are estimated to contain about 5000–15.000 cultivars. For the management of germplasm collections one essential aspect is to use only certain and complete characterized cultivars and to avoid any problem arising from synonymous and homonymous cultivar designations. Cultivar identification in grapevine can be very difficult when relying only upon ampelography and botanical characteristics. The use of molecular markers for grapevine identification proved to be a viable alternative or supplement to ampelography (Thomas et al. 1993). PCR-based DNA markers provide a more reliable alternative for cultivar identification, for similarities detection, and for defining genetic relationships among grapevine varieties (Bowers et al. 1993; Sefc et al. 1999). Random Amplified Polymorphic DNA is considered to be a reasonably low-price method and proves to be successful in distinguishing grape varieties (Gogorcena et al. 1993; Grando et al. 1995). Moreover, RAPD technique is fast and easy, since it does not require any prior knowledge of the markers sequences, is capable of detecting high levels of genetic variation and is very efficient in assessments of genetic diversity of grape as well as other taxa.

The present work investigates the genetic relationships among eleven native Romanian varieties with the goal to obtain a genotype-specific profile. In this study RAPD markers were used to detect and evaluate the polymorphism degree of the analyzed grapevine cultivars originated in Podisul Transilvania.

MATERIAL AND METHODS

Plant material: The cultivars (*Vitis vinifera* L.) used in this study are listed in table 1. The eleven varieties analysed were provided by the Research and Development Station for Viticulture and Oenology Blaj and by the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

DNA extraction: Total genomic DNA was extracted from young leaves frozen in liquid nitrogen and grounded to a fine powder, following the protocol of Doyle and Doyle (1990), modified by Cipriani and Morgante (1993).

PCR conditions: RAPD amplification was performed in a reaction volume of 25 µl containing 4 mM $MgCl_2$, 0.4 mM of each dNTP, 0.6 units Taq DNA polymerase, 0.8 µM primer and 100-200 ng genomic DNA. The amplification reactions were carried out in an Abi Prism 7900 HT Real Time PCR (Applied Biosystem) programmed as following: preliminary denaturation of DNA at 94°C for 30 s, 45 cycles of 92°C for 30 s, 36°C for 25 s and 72°C for 74 s, and a final extension step at 72°C for 8 min. The amplification of each genotype-primer combination was repeated twice. The PCR products were separated by gel electrophoresis on a 0.8 % agarose gel, in 1 x TBE buffer during 1 h at 90 V and stained with ethidium bromide (10 mg/ml). The 100 bp DNA Ladder (Promega) was used as a molecular size standard. Photographs of the gels were obtained with a Gene Flash Syngene Bio Imaging.

Scoring and data analysis: The PCR fragments were scored for the presence (1) or absence (0) of equally sized bands revealed in the photographs of gel profiles

(Figure 1) using LabImage software. Only reproducible bands were considered and than the binary character matrix was used for the FreeTree computer program. These data assured the calculation of pairwise genetic distances among cultivars with Jaccard's coefficient. The distance similarity matrix was subjected to a cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA). The dendrogam was generated using TreeView software.

Table 1

Grapevine cultivars investigated in this study

No	Cultivar	Utility	Berry colour
1	Timpuriu de Cluj	Table grape	White
2	Napoca	Table grape	Black
3	Transilvania	Table grape	Black
4	Cetățuia	Table grape	Black
5	Splendid	Table grape	Red-black
6	Blasius	Wine grape	White
7	Selena	Wine grape	Pink
8	Amurg	Wine grape	Black
9	Brumariu	Wine grape	White
10	Astra	Wine grape	White
11	Radames	Wine grape	Red-grey

RESULTS AND DISCUSSIONS

The eleven Romanian cultivars were subjected to RAPD marker analysis in order to evaluate their degree of genetic variation. As an initial step, a total of 30 arbitrary 10-mer primers (Operon Technologies, Alameda, California) were screened for their ability to amplify the extracted DNA. Only 10 informative primers were selected, on the basis of their ability to produce unambiguous and stable RAPD markers.

These random primers yielded a total of 111 reproducible bands ranging from 230 bp (OPA05) to 4041 bp (OPA19). The number of bands per primer varied from 5 (OPA20) to 23 (OPA05) with an average of 11 bands per primer (table 2). One hundred and six bands (95%) out of the 111 reproducible bands were polymorphic. A general characteristic for all obtained RAPD patterns with DNA amplicons was the high degree of variation among cultivars revealed by proportion of polymorphic bands ranging from 78% to 100%.

According to each template banding patterns, no single primer proved to distinguish all studied varieties. So, primers OPA 02 and OPA 01 could distinguish 9 cultivars out of 11, while with OPA 07 generated specific patterns for 8 cultivars (Fig. 1). The banding patterns obtained with primers OPA04, OPA05, OPA19, OPA20 and OPB04 produced stable RAPD markers, but the level of polymorphism was lower.

Table 2

Amplification products obtained with RAPD primers

Primer	Sequence	No of bands	No of polymorphic bands
OPA01	CAGGCCCTTC	15	15
OPA02	TGCCGAGCTG	13	12
OPA04	AATCGGGCTG	9	7
OPA05	AGGGGTCTTG	23	22
OPA07	GAAACGGGTG	17	17
OPA08	GTGACGTAGG	10	10
OPA10	GTGATCGCAG	12	12
OPA19	CAAACGTCGG	7	7
OPA20	GTTGCGATCC	5	4
OPB04	GGACTGGACT	4	3

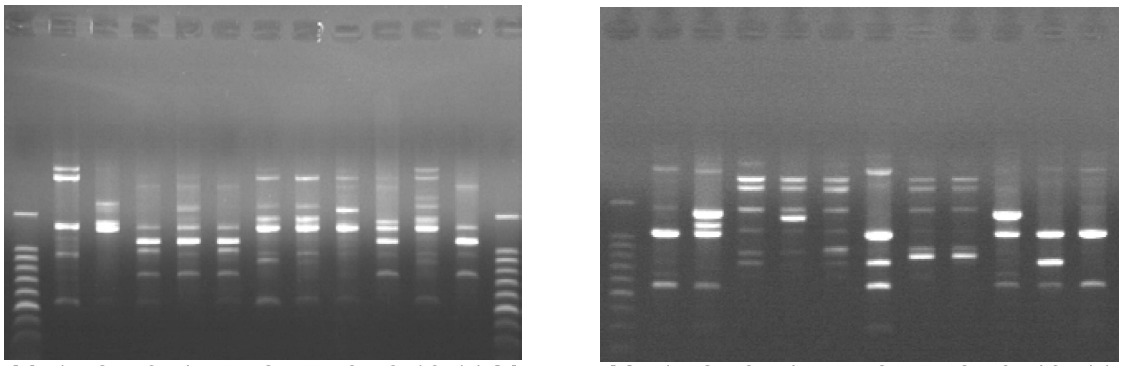

Figure 1. Representative RAPD profiles of the 11 genotypes generated with primer OPA02 (a) and OPA07 (b) The numbers represent: 1-Timpuriu de Cluj; 2-Napoca; 3-Transilvania; 4-Cetatuia; 5-Splendid; 6-Blasius; 7-Selena; 8-Amurg; 9-Brumariu; 10-Astra; 11-Radames

Based on Jaccard's coefficient and UPGMA clustering program, was constructed the dendogram (figure 2), which revealed also the genetic relationship between studied cultivars. The genetic diversity ranged from 0.17 (Napoca and Radames) to 0.71 (Transilvania and Splendid). This dendrogram strongly supports also the relationship among cultivars belonging to a certain region and also illustrates the level of similarity in agreement with at least one common parents used as ascendant (e.g. Amurg and Selena; Splendid and Transilvania). Consistent with other results, our RAPD analysis allowed discrimination among grape cultivars (Ye et al. 1998, Vidal et al. 1999). On the basis of the RAPD profiles, the resulting distance values and the dendrogram, it can be concluded that all the cultivars of our analysis are different to a relatively high degree.

The stability of the dendrogram was also analyzed by the bootstrap method, which provided confidence for the different nodes. A value higher than 50% indicates a most reliable branch of the tree and, in the same time, indicates a certain degree of relatedness or similarity between studied genotypes. Thus, our results proved to be in accordance with cultivars pedigree and demonstrated once again the usefulness of RAPD analysis in assessing the genetic relationships in grape germplasm.

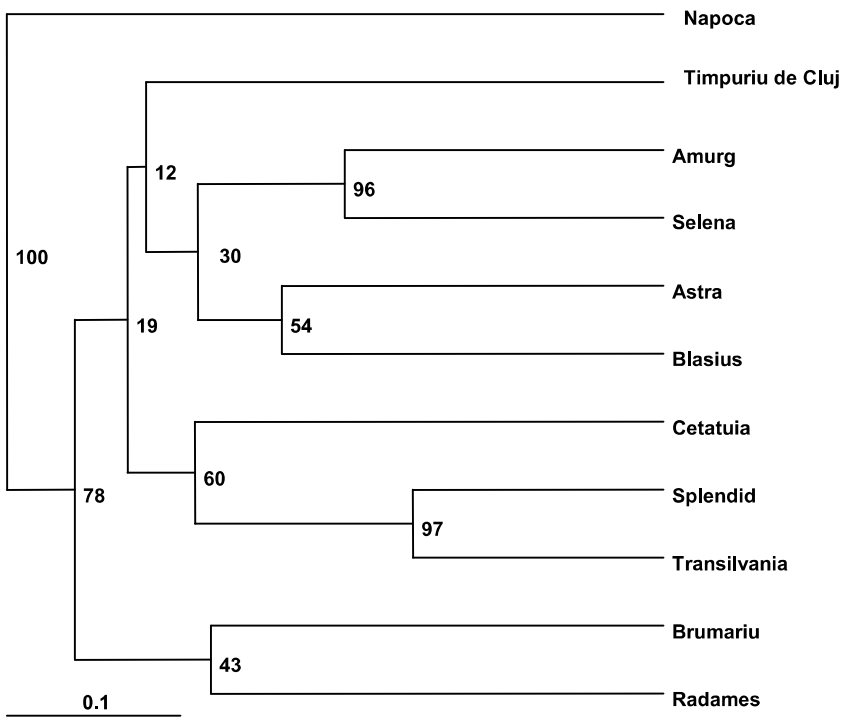

Figure 2. - Neighbor-joining tree of 11 Romanian genotypes. Numbers at the nodes indicate the number of times the grouping occured from 100 bootstrap replication

The RAPD approach, for its high capacity of generating DNA markers, allows a genome scanning limited only by the number of primers involved. Therefore, it seems to be helpful to use these type of markers to evaluate the genetic diversity present in our *Vitis* germplasm collections, and especially to the Romanian cultivars. The ability to differentiate the tested cultivars by RAPD markers suggested that this technique may provide a rapid and inexpensive method for the identification of cultivars indigenous in Romania, even between phenotipically similar cultivars (Ye et al. 1998).

The informative primers identified and tested in our studies will be useful in genetic analysis of grapevine accessions in germplasm holdings. Further, putative species-specific RAPD markers could be converted to sequence characterized amplification regions (SCARs) after sequencing and designing primer pairs to develop robust species specific markers.

CONCLUSIONS

The data obtained in this study provide valuable genetic information, important to reveal the uniqueness of Romanian grapevine varieties and their high degree of polymorphism detected by RAPD markers.

The high level of genetic variation among tested varieties could be attributable to differences among cultivars within geographical areas, significant inter-population distance and the Romanian viticulture tradition.

According to these results, each of the investigated cultivars constitutes an independent source of genetic variation, and a valuable resource of genetic traits for grapevine breeding programs.

Taking into consideration the invaluable importance of national grapevine varieties and of knowledge on their genetic relationships as a basis for biodiversity protection and efficient exploitation, our group will continue working on Romanian grapevine cultivars with primers providing the most polymorphic patterns.

REFERENCES

1. **Aradhya M.K., Dang G.S., Prins B.H. et al, 2003** - *Genetic structure and differentiation in cultivated grape Vitis vinifera L*. Genetical Research, 81, 179–192.
2. **Bowers J.E., E.B. Bandman, C.P. Meredith, 1993 –** *DNA fingerprint characterization of some wine grape cultivars*. Am J Enol Viticult 44: 266–274.
3. **Cipriani G**., **Morgante M.,** 1993 – *Evidence of chloroplast variation DNA variation in the genus Actinidia revealed by restriction analysis of PCR - amplified fragments*. Journal of Genetics and Breeding 47: 319-326
4. **Doyle J. J., Doyle J.L., 1990** - *Isolation of plant DNA from fresh tissue*. Focus 12:13–15
5. **Gogorcena Y., S. Arulsekar, A.M. Dandekar, D.E. Parfitt, 1993** - *Molecular markers for grape characterization*. Vitis 32: 183–185.
6. **Grando M. S., De Micheli L., Biasetto L., Scienza A., 1995** - *RAPD markers in wild and cultivated Vitis vinifera*. Vitis 34:37–39
7. **Lopes M. S., Sefc K. M., Eiras Dias E., Steinkellner H., Laimer da Câmara Machado, M., da Câmara Machado A., 1999 -** *The use of microsatellites for germplasm management in a Portuguese grapevine collection.* Theor Appl Genet 99:733–739
8. **Luo S., He P., 2001 -** *Discrimination of wild grapes native to China by RAPD markers*. Vitis 40 (3), 163-168.
9. **Oprea St**., **Moldovan S.D., 2007** - *Ameliorarea vitei de vie in Romania.* Editura Poliam Cluj Napoca, 336 pag
10. **Sefc K. M., Regner F., Turetschek E., Glossl J., Steinkellner H.,** 1999 - *Identification of microsatellite sequences in Vitis riparia and their applicability for genotyping of different Vitis species.* Genome 42(3):367–373
11. **Sestraş R**., **2004** - *Ameliorarea speciilor horticole*, Ed. AcademicPres Cluj-Napoca, 334 pag.
12. **Thomas M.R., Matsumoto S., Cain P., Scott N.S., 1993** - *Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification*. Theor. Appl. Genet. 86: 286–289.
13. **Vidal J.R., Coarer M., Defontaine A., 1999** - *Genetic relationships among grapevine varieties grown in different French and Spanish regions based on RAPD markers.* Euphytica 109 (3):161-172
14. **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 37:33–38