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Use of Random Amplified Polymorphic DNA (RAPD) to Study Genetic Diversity among Romanian Local Vine (*Vitis vinifera* L.) Cultivars

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Abstract. To estimate genetic relationships among 36 local vine cultivars, RAPD analysis was performed with 24 decamer primers selected from a total of 40 primers. These primers generated polymorphic bands among the studied genotypes. The purity and amount (ng/µL) of total DNA extracted from each sample were sufficient for optimal RAPD analysis. UPGMA dendrogram was constructed based on genetic distances using the program Tree View. The genotypes analyzed clustered into three main groups and the values of genetic distances between data shows that there are differences at molecular level.

Keywords: vine, romanian cultivars, RAPD, genetic diversity

INTRODUCTION

Research on genetic diversity in *Vitis vinifera* Romanian cultivars is important not only in studies of the origin of various cultivars but also those on the improvement and preservation germplasm fund to this important species.

Classical methods of identification, based on botanical and ampelographic characterization not always were the most accurate way due to instability of morphological characteristics under the influence of the internal and external environment.

In plant breeding, the identification of cultivars in the first stages of development is extremely difficult as the elements characteristic to the cultivars are coming up gradually, during the following years. At first appearance some organs, such as tendrils and inflorescences are becoming poorly developed, then more typical as the transition years (Ardelean, 1986).

During these last years, with the coming out of molecular markers, the identification of cultivars has been carried out at protein level with the aid of isoenzymes as well as at DNA level assisted by RAPD (Random Amplified Polymorphic DNA); RFLP (Restriction Fragment Length Polymorphism); AFLP (Amplified Fragment Length Polymorphism); and SSR (Simple Sequence Repeats) methods.

Due to simplicity and low cost of RAPD method – Random Amplified Polymorphic DNA (Williams *et al.*, 1990), i.e., that requires very small quantities of DNA as well as its ability to reveal high degree of polymorphism, it has successfully been applied with various plant species, including *Vitis vinifera* (Bohm and Zyprian, 1998; Fanizza *et al.*, 1999; Pamfil, 1999 ; Ryan *et al.* 2001; Tessier *et al.*, 1999; Wolf *et al.*, 2001).

Researches in molecular genetics vine are oriented in general, to identify DNA polymorphism within *Vitis* species and also to establish phylogenetic origin of species or cultivars. Ampelographic and molecular characterization of autochthonous vine cultivars used to obtain white and red sorts of wine is an important aim for Romanian viticulture.

MATERIALS AND METHODS

The biological material used for DNA isolation was the young leaves from 36 local vine cultivars presented in Tab. 1.

Tab.1

Wine grape cultivars used for RAPD analysis

No.	Name of cultivars	Usage of cultivars
1.	Negru Căușani	Obtaining red table wine
2.	Negru vârtos	Obtaining red table wine
3.	Negru aromat	Obtaining flavouros wine
4.	Neagră moale	Obtaining red table wine
5.	Fetească neagră	Obtaining red quality wine
6.	Fetească regală	Obtaining white quality wine
7.	Fetească albă	Obtaining white quality wine
8.	Băbească neagră	Obtaining red table wine
9.	Bătută neagră	Obtaining red table wine
10.	Blasius	Obtaining white quality wine
11.	Selena	Obtaining white quality wine
12.	Balada	Obtaining red table wine
13.	Miorița	Obtaining white table wine
14.	Amurg	Obtaining red quality wine
15.	Roz de miniș	Obtaining white quality wine
16.	Tămâioasă românească	Obtaining flavouros wine
17.	Negru de Drăgășani	Obtaining red quality wine
18.	Mustoasă de Maderat	Obtaining white table wine
19.	Creața de Banat	Obtaining white table wine
20.	Băbească gri	Obtaining white table wine
21.	Busuioacă Bohotin	Obtaining flavouros wine
22.	Grasa Cotnari	Obtaining white quality wine
23.	Galbena de Odobeşti	Obtaining white table wine
24.	Gordin	Obtaining white table wine
25.	Gordan	Obtaining white table wine
26.	Cioinic	Obtaining white table wine
27.	Zghihara	Obtaining white table wine
28.	Frâncuşa	Obtaining white quality wine
29.	Novac	Obtaining red quality wine
30.	Cruciulița	Obtaining white table wine
31.	Arcaş	Obtaining red table wine
32.	Armaş	Obtaining white quality wine
33.	Codana	Obtaining red table wine
34.	Furmint de Miniş	Obtaining white quality wine
35.	Vulpe	Obtaining red table wine
36.	Șarba	Obtaining white quality wine

Prior to isolation, approximately 200 mg leaves provided from each sample were grind in liquid nitrogen into a fine powder. Total DNA was extracted using the protocol developed by Lodhi *et*

al. (1994) and modified by Pop *et al.* (2003). DNA concentration and the absorbance ratio at A_{250}: A_{280} was quantified with Nano Drop Nd-1000 Spectrophotometer (Nanodrop Technologies). Reaction mixture for PCR in 25 µL volume consisted of 50 ng DNA, 200 µM of each dNTP (Promega), 0,2 µM primer Mycrosynth (Tab. 2), 2.5 mM $MgCl_2$, 2.5 mM 10 X Buffer, 1 U Taq DNA Polymerase (Promega), 2 % PVP (Sigma) and bidistillated sterile water.

Tab. 2

The primers used for RAPD analysis

No.	Primer Name	Primer sequence 5'-3'	No. of polymorphic bands/primer
1.	OPA 02	TGCCGAGCTG	4
2.	OPA 17	GAC CGC TTG T	6
3.	OPA 20	GTT GCG ATC C	7
4.	OPB 8	GTCCACACGG	6
5.	OPB 9	TGGGGGACTC	7
6.	OPB 10	CTGCTGGGAC	4
7.	OPB 11	GTAGACCCGT	8
8.	OPB 12	CCTTGACGCA	5
9.	OPB 17	AGGGAACGAG	5
10.	OPB 18	CCACAGCAGT	4
11.	OPAB 11	GTG CGC AAT G	6
12.	OPAB 18	CTG GCG TGT C	6
13.	OPC 4	CCGCATCTAC	4
14.	OPC 8	TGGACCGGTG	5
15.	OPC 15	GACGGATCAG	5
16.	OPC 16	CACACTCCAG	6
17.	OPD 19	CTGGGGACTT	0
18.	OPD 20	ACCCGGTCAC	7
19.	OPF 02	GAGGATCCCT	0
20.	OPF 04	GGTGATCACC	0
21.	OPE 14	TGCGGCTGAG	6
22.	OPG 07	GAACCTGCGG	0
23.	OPH 02	TCGGACGTGA	0
24.	OPAL 20	AGGAGTCGGA	8

Amplification was performed in an Eppendorf Mastercycler Gradient programmed for this thermal cycling profile: denaturation step (3 min. at 95°C) followed by 45 cycles (1 min. at 93°C; 1 min. at 34°C and 1 min. at 72°C for each cycle) and final extension of 72 degree/10 minutes. The molecular marker used was 100 bp DNA Step Ladder (Promega Corp., Madison, WI, USA). Gels were visualized on a UV light Biospectrum AC Imaging System (UVP BioImaging Systems, Upland, CA) after 25 minute of staining with 0.5 µg/µl Ethidium Bromide (Promega).

TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) were used for gels images analysis and the resulting DNA bands after RAPD amplification were scored as present (1) or absent (0); data obtained were entered into a binary matrix. The genetic distance between analyzed cultivars was calculated using Nei and Li's coefficient of similarity. Cluster analysis was conducted with a Neighbor-Joining algorithm using FreeTree software and a dendrogram was constructed.

RESULTS AND DISCUSSION

Approaching genetic of 36 Romanian local vine cultivars analyzed, determined on the basis of genetic distance matrix and Neighbor Joining Tree algorithm, are shown in Fig. 1 as a dendrograme.

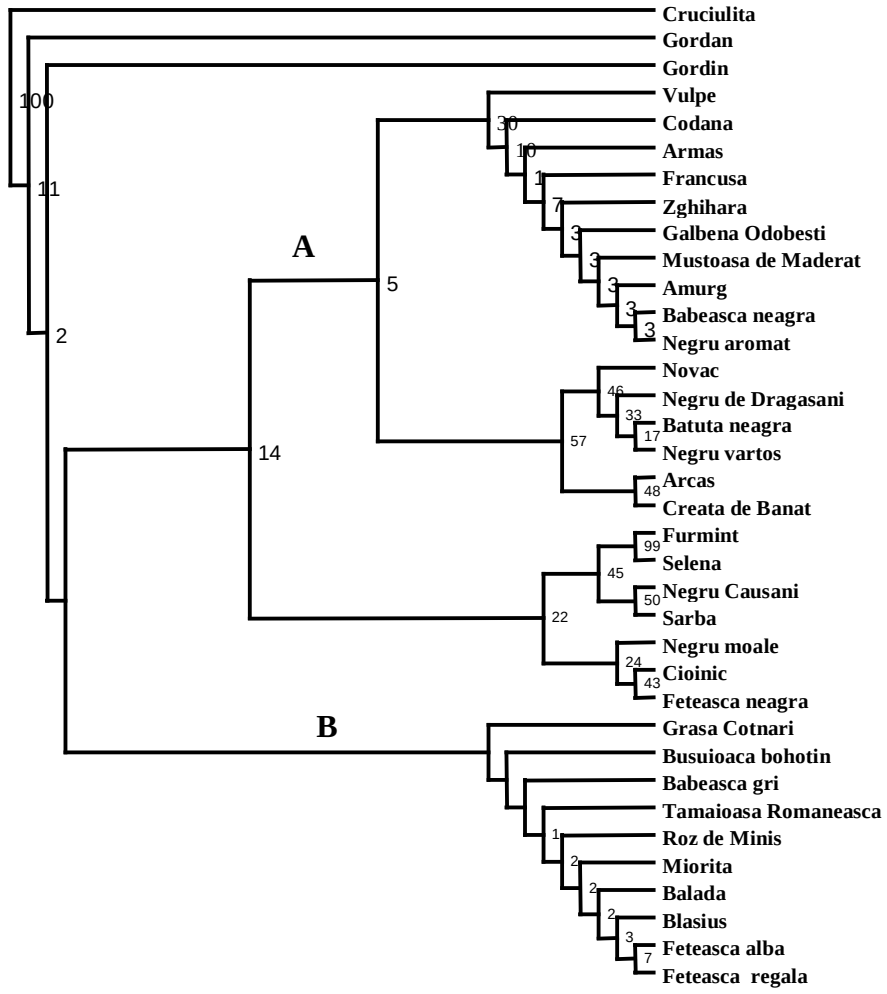

Fig.1. Dendrogram of the 36 cultivars of *Vitis vinifera* tested

This dendrogram reveals that Cruciulita cultivar constituted a distinct genotype seems related to Galbenă verde and Alb românesc (Alb de Cetatea Albă), all of them being old Romanian cultivars with unknown origin. Similar results are observed in the case of Gordan and Gordin cultivars also with uncertain origin. Dendrograme image shows to main groups (A and B) of cultivars. The first group comprises most of the analyzed cultivars (23) while group B contains ten cultivars (see Fig.1).

Noteworthy is that the cultivars Feteasca alba and Feteasca regala, forming part of the second group, were located in the same branch. The genetic closeness between the two cultivars,

resulting from RAPD-analysis, thus stands witness to the previous results confirming the common ancestry of the respective cultivars. From the ampelography the two cultivars are considered close because of their morphological particularities. Also, the literature shows that cultivar Feteasca regala would be a natural hybrid between Feteasca alba and Grasa de Cotnari, which shows the approximation of the two genetic cultivars.

Preliminary results obtained in our experiences will be confirmed or cancelled by using more sensitive molecular techniques (i.e. SSR markers) because ampelographic and molecular characterization of autochthonous vine cultivars used to obtain white and red sorts of wine is an important aim for Romanian viticulture.

Fig. 2. shows the image of amplification products obtained with primer OPAL 20.

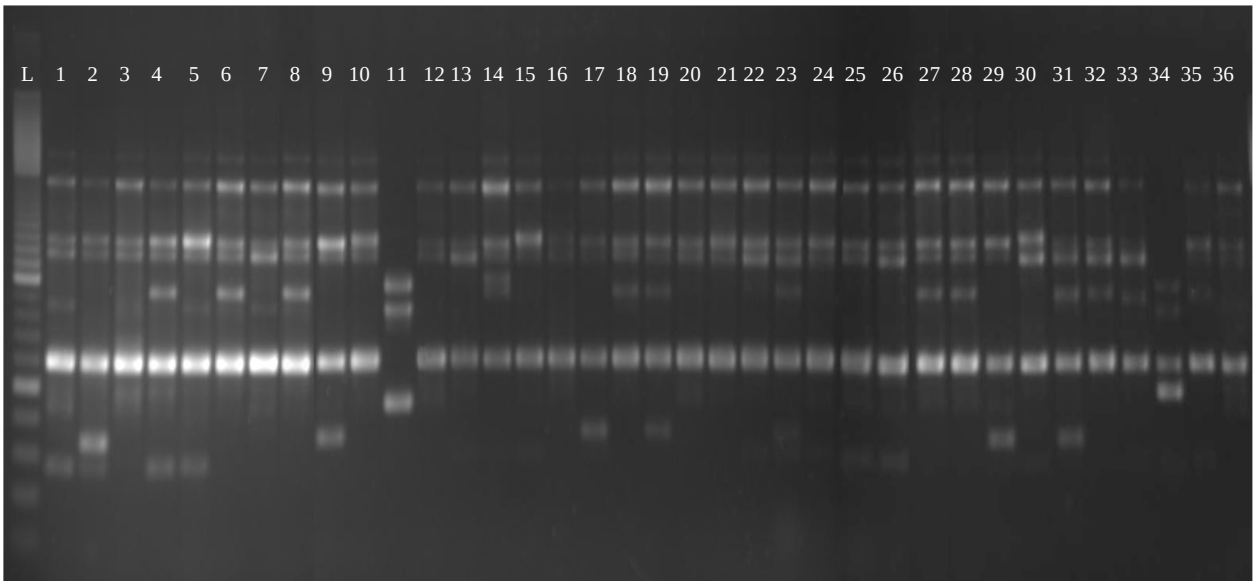

Fig. 2. RAPD profile obtained with primer OPAL 20.L-ladder; 1-Negru Căuşani; 2-Negru vârtos; 3-Negru aromat; 4-Neagră moale; 5-Fetească neagră; 6- Fetească regală; 7- Fetească albă; 8-Băbească neagră; 9-Bătută neagră; 10-Blasius; 11-Selena; 12-Balada; 13-Miorița; 14-Amurg; 15- Roz de Miniş; 16- Tămăioasă românească; 17-Negru de Drăgăşani; 18-Mustoasă de Măderat; 19- Creața de Banat, 20- Băbeasca gri; 21-Busuioacă bohotin; 22-Grasă de Cotnari; 23-Galbenă de Odobeşti; 24-Gordin; 25-Gordan; 26-Cionic; 27-Zghihară; 28-Frâncuşă; 29-Novac; 30-Cruciuliță; 31- Arcaş; 32-Armaş; 33-Codană; 34-Furmint de Miniş; 35-Vulpe; 36-Şarbă

CONCLUSIONS

The RAPD analysis reveals obvious genetic differences among the 36 vine Romanian autochthonous cultivars; the result can be considered a valuable tool for revealing molecular polymorphism. These preliminary results obtained in present experiment must be complete by using more sensitive molecular techniques (i.e. SSR markers).

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