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GENOMIC DEOXYRIBONUCLEIC ACID (DNA) OF THE DISTANT HYBRIDS OF VINE (*VITIS VINIFERA* L. x *MUSCADINIA ROTUNDIFOLIA* MICHX.)

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Abstract

For the isolation of the genomic deoxyribonucleic acid (DNA) from vine leaves of distant hybrids (Vitis vinifera L. x Muscadinia rotundifolia Michx.), the DNA isolation protocol based on CTAB method was used. The grouping of distant hybrids in the obtained dendrogram shows that at the DNA level there are some differences between them, differences sometimes unnoticeable at the level of ampelographic characterization. As a result, in the characterization of varieties and hybrids of vines, the ampelographic analysis needs to be supplemented by an analysis at the molecular level, based on DNA amplification techniques. As a result of distant hybrids grouping based on the size of alleles, it was found that there are two distinct main groups denoted by A and B, each having secondary branches. The hybrid F_4 BC_3 DRX-M_4-541 1 is closely akin to the variety Chasellas dóre. Also, the two samples of Vitis vylvestris Gmel. have been found to be genetically different, being placed in different subgroups. The hybrid F_4 BC_3 DRX-M_4-536 is genetically close to the male specimen of Vitis sylvestris Gmel. The distant hybrid DRX-M_4-660, which proved to have larger differences at the molecular level, isn't grouped in a cluster with any other hybrid.

Keywords: *alleles, distant hybrids, DNA, leaves, primers*

INTRODUCTION

SSR (Simple Sequence Repeats) genetic fingerprinting technique can be used successfully in the determination of phylogeny relationships in the biological material analyzed. The representation of the number and size of alleles using the barcode technique gives a clear view of the molecular similarities and differences that occur between hybrids and reference varieties analyzed.

MATERIALS AND METHODS

The distant hybrids of vine (*Vitis vinifera* L. x *Muscadinia rotundifolia* Michx.): BC1 - DRX-55 (prob. 1); BC4 - DRX-M4-536 (prob. 2), DRX-M4-578 (prob. 3), DRX-M4-545 (prob. 4), DRX-M4-604 (prob. 5), DRX-M4-508 (prob. 6), DRX-M4-660 (prob. 7); BC2 – DRX-M3-3-1 (prob. 8); - Vitis sylvestris Gmel. (♀) (prob. 9); - *Vitis sylvestris* Gmel. (♂) (prob. 10); BC3 - DRX-M4-580 (prob. 11), DRX-M4-541 (prob. 12), DRX-M4-507 (prob. 13), DRX-M4-537 (prob. 14) served as study material [1, 2, 3,15].

For the isolation of genomic deoxyribonucleic acid (DNA) from vine leaves of distant hybrids (*Vitis vinifera* L. x *Muscadinia rotundifolia* Michx.), the specimens of *Vitis sylvestris* Gmel. and the two international varieties taken as reference, it was used the DNA isolation protocol, based on CTAB method (the protocol of Lodhi et al., 1997, modified by Rodica Pop et al., 2003). The quantification of the quality and quantity of deoxyribonucleic acid (DNA) was performed using Nanodrop ND-1000 Spectropho - tometer (Thermo Scientific) [4, 5, 6, 7, 8, 10]. Each sample has been subjected to three readings using Nanodrop with the aim of obtaining an average value used for the dilutions required for PCR amplification. It was used a concentration of DNA of 20 ng/μL. [11, 12, 13, 14, 15, 16]

PCR amplification was performed in thermocycler type Palm Cycler (Corbett Research) under the conditions of touch down. The primers used were VVS2, MD5, MD7, MD27, ZAG 62 and ZAG 79,

synthesized by the company IDT (USA). The selection of primers was done taking into consideration the recommendations of the gene bank European Vitis Database [17, 18]. The characteristics of the used primers are shown in Table 1.

Table 1. Characteristics of the used primers

No crt	Name of the primer	Nucleotide sequence	Tm (melting temperature)	Type of fluorochrome for marking
1	vvs2 forward	5'-CAGCCCGTAAATGTATCCATC-3'	53.3	5' Well Red D2
2	vvs2 reverse	5'-AAATTCAAAATTCTAATTCAACTGG-3'	48.9	-
3	MD5 forward	5'-CTAGAGCTACGCCAATCCA-3'	53.9	5' Well Red D3
4	MD 5 reverse	5'- TATACCAAAAATCATATTCCTAAA-3'	45.9	-
5	MD7 forward	5'-AGAGTTGCGGAGAACAGGAT-3'	56	5' Well Red D4
6	MD 7 reverse	5'-CGAACCTTCACACGCTTGAT-3'	55.6	
7	MD27 forward	5'- CCCCAAGGCTCTGAAAACAAT-3'	55.8	5' Well Red D4
8	MD 27 reverse	5'-ACGGGTATAGAGCAAACGGTGT-3'	58.3	-
9	ZAG 62 forward	5'-ACGGTGTGCCTCTCATTGTCATTGAC-3'	64.7	5' Well Red D4
10	ZAG 62 reverse	5'- CCATGTCTCTCCTCAGTTCTCAGT-3'	57.7	-
11	ZAG 79 forward	5'-AGATTGTGGAGGAGGGAACAAACCG-3'	60.8	5' Well Red D2
12	ZAG 79 reverse	5'- TGCCCATTTTCAAACTCCCTTCC-3'	58.0	-

Improving the amplification protocol consisted in using Touchdown PCR amplification so that the truthfulness of the final results was consistent with the specialized literature. It is worth mentioning that after the optimization of all amplification protocols, all the used primers generated amplification products, which were studied with the help of the genetic analyzer CEQ 8800^{TM} capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA) in the next stage of experimentation, in order to determine the number of alleles and their size.
In order to identify the optimum temperature of attaching primers, there was performed a heat shock that exceeded by about five degrees Celsius the melting temperature of the forward primer, then the temperature gradually decreased with about one degree Celsius at each amplification cycle until it was reached the temperature at which primers attachment could be more specific.
The optimization of the amplification protocol is important because it helps to avoid obtaining non-specific amplification products. It was also found that the attachment optimum temperature depends on the melting temperature of the most unstable primer, from thermal point of view, of the primer pair.

In Table 2 there are presented the PCR amplification programs which were optimized and used in order to study the migration of the reaction products in the genetic analyzer.

Table 2. Amplification protocol of vine samples analyzed with the primers vvs2, MD5, MD7, MD27, ZAG 62, ZAG 79

No. crt.	Name of the primer	PCR condition	The composition and the volume (µL) of the PCR reaction mixture	DNA quantity used\sample (µL)
1	vvs2	1. 95 ^{0}C - 0:30 min (1 cycle of amplification) 2. 95 ^{0}C – 0:30 min (1 cycle of amplification) 57 ^{0}C - 51 ^{0}C – 1:00 min (by one cycle of amplification touchdown) 72 ^{0}C – 1:00 min 3. 95 ^{0}C – 0:30 min 50 ^{0}C – 1:00 min 72 ^{0}C – 1:00 min (25 cycles of amplification) 4. 72 ^{0}C – 5 min 4 ^{0}C – 99 min	$H_2$0- 4 $MgCl_2$- 1.2 dNTP mix – 0.6 Buffer – 4 Primer R – 1 Primer F – 1 Taq Pol. 0.2	3
2	MD5	1. 95 ^{0}C – 0 :30 min (1 cycle of amplification) 2. 95 ^{0}C – 0:30 min (1 ciclu de amplificare) 58 ^{0}C - 56 ^{0}C – 1 :00 min (by one cycle of amplification touchdown) 72 ^{0}C – 1:00 min 3. 95 ^{0}C – 0:30 min 52 ^{0}C – 1:00 min 72 ^{0}C – 1:00 min (25 cycles of amplification) 4. 72 ^{0}C – 5 min 4 ^{0}C – 99 min	$H_2$0- 4 $MgCl_2$- 1.2 dNTP mix – 0.6 Buffer – 4 Primer R – 1 Primer F – 1 Taq Pol. 0.2	3
3	MD7	1. 95 ^{0}C – 0 :30 min (1 cycle of amplification) 2. 95 ^{0}C – 0:30 min (1 cycle of amplification) 60 ^{0}C - 56 ^{0}C – 1 :00 min (by one cycle of amplification touchdown) 3. 95 ^{0}C – 0:30 min 55 ^{0}C – 1:00 min 72 ^{0}C – 1:00 min (25 cycles of amplification) 4. 72 ^{0}C – 5 min 4 ^{0}C – 99 min	$H_2$0- 4 $MgCl_2$- 1.2 dNTP mix – 0.6 Buffer – 4 Primer R – 1 Primer F – 1 Taq Pol. 0.2	3
4	MD27	1. 95 ^{0}C – 0 :30 min (1 cycle of amplification) 2. 95 ^{0}C – 0:30 min (1 cycle of amplification) 59 ^{0}C - 56 ^{0}C – 1 :00 min (by one cycle of amplification touchdown) 72 ^{0}C – 1:00 min 3. 95 ^{0}C – 0:30 min 55 ^{0}C – 1:00 min 72 ^{0}C – 1:00 min (25 cycle of amplification) 4. 72 ^{0}C – 5 min 4 ^{0}C – 99 min	$H_2$0- 4 $MgCl_2$- 1.2 dNTP mix – 0.6 Buffer – 4 Primer R – 1 Primer F – 1 Taq Pol. 0.2	3
5	ZAG 62	1. 95 ^{0}C – 0 :30 min (1 cycle of amplification) 2. 95 ^{0}C – 0:30 min (1 cycle of amplification) 65, 64, 63, 60, 57, 55, 53^{0}C - 1 :00 min (by one cycle of amplification touchdown) 72 ^{0}C – 1:00 min 3. 95 ^{0}C – 0:30 min 55 ^{0}C – 1:00 min 72 ^{0}C – 1:00 min (25 cycles of amplification) 4. 72 ^{0}C – 5 min 4 ^{0}C – 99 min	$H_2$0- 4 $MgCl_2$- 1.2 dNTP mix – 0.6 Buffer – 4 Primer R – 1 Primer F – 1 Taq Pol. 0.2	3
6	ZAG 79	1. 95 ^{0}C - 0:30 min (1 cycle of amplification) 2. 95 ^{0}C - 0:30 min (1 cycle of amplification) 62 ^{0}C - 56 ^{0}C - 1 :00 min (by one cycle of amplification touchdown) 72 ^{0}C – 1:00 min 3. 95 ^{0}C – 0:30 min 55 ^{0}C – 1:00 min 72 ^{0}C – 1:00 min (25 cycle of amplification) 4. 72 ^{0}C – 5 min 4 ^{0}C – 99 min	$H_2$0- 4 $MgCl_2$- 1.2 dNTP mix – 0.6 Buffer – 4 Primer R – 1 Primer F – 1 Taq Pol. 0.2	3

PCR amplification products obtained after using the 6 SSR primers mentioned above were verified by migration in agarose gel 1.4 % (1.4 g agarose LE Analytical Grade, Promega in 100 ml solution TAE). In Figure 1 there are shown the PCR amplification products obtained with primers pair MD5 and migrated in agarose gel and the ladder of 100 bp used [19, 20, 21].
Optimal dilutions of PCR products were obtained by probing and we found that satisfactory results concerning the migration

conditions were recorded at the following dilutions:
- PCR products amplified with the primer ss2 were diluted at a ratio of 1:5 and then a volume of 1μL was used for migration;

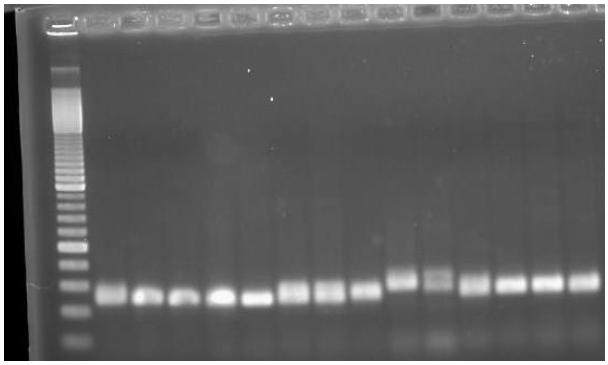

Fig. 1. The image of agarose gel with the PCR products resulting from amplification with the pair of primers MD5 and visualized with the help of the picture-taking system UPV. L-ladder Mass Ruler 100 bp (Promega)-molecular weight marker.

- PCR products amplified with the primer md5 were diluted at a ratio of 1:20 and then a volume of 1μL was used for migration;
- PCR products amplified with the primer md7 were diluted at a ratio of 1:40 and then a volume of 1μL was used for migration;
- PCR products amplified with the primer md27 were diluted at a ratio of 1:40 and then a volume of 1μL was used for migration;
- PCR products amplified with the primer ZAG 62 were diluted at a ratio of 1:40 and then a volume of 1μL was used for migration;
- PCR products amplified with the primer ZAG 79 were diluted at a ratio of 1:5 and then a volume of 1μL was used for migration;

The PCR products obtained with the help of the six primers were migrated in the genetic analyzer Ceq TM 8800 (Beckman Coulter), using a volume of 0,25 μL standard 400 bp from Beckman Coulter and solution for migration -38,3 μL SLS (sample loading solution).
In order to analyze more accurately the results, there were used for comparison two international vine varieties, Sauvignon Blanc and Chasellas Dóre, whose size and number of alleles are given in the literature.
The determination of the number and size of alleles at the analyzed varieties was performed automatically with the help of the software used for data interpretation included in the genetic analyzer CEQ 8800$^{TM\ from}$ Beckman Coulter Company.
The dendrogram on the way of grouping of hybrids was done with the help of the programs PAST and FIG. TREE using the EUCLIDEAN method.

RESULTS AND DISCUSSIONS

Results on DNA isolation
The amount of DNA (ng/μL) and its purity (expressed through the values of the ratio 260/280) obtained from the analyzed vines samples are shown in the images from below:

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
v 1.2	Default	3/4/2013	1:26 PM	520.64	10.413	5.780	1.80	1.93	50.00	230	5.395	0.824
v 1.2	Default	3/4/2013	1:26 PM	518.40	10.368	5.773	1.80	1.91	50.00	230	5.422	0.842
v 1.2	Default	3/4/2013	1:26 PM	534.74	10.695	5.981	1.79	1.88	50.00	230	5.700	0.906
v 2.1	Default	3/4/2013	1:28 PM	624.02	12.480	6.063	2.06	1.95	50.00	230	6.388	0.474
v 2.1	Default	3/4/2013	1:28 PM	615.58	12.312	5.920	2.08	1.95	50.00	230	6.325	0.539
v 2.1	Default	3/4/2013	1:28 PM	609.27	12.185	5.871	2.08	1.93	50.00	230	6.309	0.637
v 3.1	Default	3/4/2013	1:29 PM	2325.09	46.502	22.112	2.10	2.08	50.00	230	22.318	0.882
v 3.1	Default	3/4/2013	1:29 PM	2282.99	45.660	21.767	2.10	2.07	50.00	230	22.010	1.038
v 3.1	Default	3/4/2013	1:29 PM	2241.85	44.837	21.268	2.11	2.07	50.00	230	21.649	0.932
v 4.1	Default	3/4/2013	1:30 PM	2261.95	45.239	21.194	2.13	2.16	50.00	230	20.975	0.784
v 4.1	Default	3/4/2013	1:30 PM	2240.66	44.813	21.002	2.13	2.15	50.00	230	20.808	0.881
v 4.1	Default	3/4/2013	1:31 PM	2956.71	59.134	28.177	2.10	2.12	50.00	230	27.834	3.209
v 5.1	Default	3/4/2013	1:31 PM	2142.19	42.844	20.260	2.11	2.06	50.00	230	20.802	1.020
v 5.1	Default	3/4/2013	1:32 PM	2082.33	41.647	19.688	2.12	2.05	50.00	230	20.315	1.061
v 5.1	Default	3/4/2013	1:32 PM	2769.71	55.394	26.483	2.09	2.04	50.00	230	27.206	1.488
v 6.1	Default	3/4/2013	1:34 PM	1918.78	38.376	18.366	2.09	2.05	50.00	230	18.749	0.504
v 6.1	Default	3/4/2013	1:34 PM	1892.10	37.842	18.118	2.09	2.04	50.00	230	18.513	0.534
v 6.1	Default	3/4/2013	1:34 PM	1868.42	37.368	17.870	2.09	2.04	50.00	230	18.315	0.613
v 7.2	Default	3/4/2013	1:35 PM	2030.90	40.618	19.420	2.09	2.01	50.00	230	20.181	0.837
v 7.2	Default	3/4/2013	1:35 PM	2013.89	40.278	19.261	2.09	2.01	50.00	230	20.054	0.865
v 7.2	Default	3/4/2013	1:36 PM	2179.73	43.595	20.923	2.08	2.01	50.00	230	21.673	0.967
v 8.1	Default	3/4/2013	1:36 PM	1555.29	31.106	15.152	2.05	2.00	50.00	230	15.559	0.885
v 8.1	Default	3/4/2013	1:37 PM	1541.12	30.822	15.003	2.05	1.99	50.00	230	15.505	0.980
v 8.1	Default	3/4/2013	1:37 PM	1577.18	31.544	15.353	2.05	1.99	50.00	230	15.813	1.040
v 9.1	Default	3/4/2013	1:38 PM	4532.00	90.640	46.849	1.93	1.96	50.00	230	46.170	1.143

Fig. 2. Centralizing table generated by Nanodrop on the results of DNA quantification at the analyzed vine hybrids (9 samples)

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
v 6.1	Default	3/4/2013	1:34 PM	1868.42	37.368	17.870	2.09	2.04	50.00	230	18.315	0.613
v 7.2	Default	3/4/2013	1:35 PM	2030.90	40.618	19.420	2.09	2.01	50.00	230	20.181	0.837
v 7.2	Default	3/4/2013	1:35 PM	2013.89	40.278	19.261	2.09	2.01	50.00	230	20.054	0.865
v 7.2	Default	3/4/2013	1:36 PM	2179.73	43.595	20.923	2.08	2.01	50.00	230	21.673	0.967
v 8.1	Default	3/4/2013	1:36 PM	1555.29	31.106	15.152	2.05	2.00	50.00	230	15.559	0.885
v 8.1	Default	3/4/2013	1:37 PM	1541.12	30.822	15.003	2.05	1.99	50.00	230	15.505	0.980
v 8.1	Default	3/4/2013	1:37 PM	1577.18	31.544	15.353	2.05	1.99	50.00	230	15.813	1.040
v 9.1	Default	3/4/2013	1:38 PM	4532.00	90.640	46.849	1.93	1.96	50.00	230	46.170	1.143
v 9.1	Default	3/4/2013	1:38 PM	4481.90	89.638	46.307	1.94	1.96	50.00	230	45.819	1.276
v 9.1	Default	3/4/2013	1:39 PM	4477.20	89.544	46.064	1.94	1.97	50.00	230	45.491	1.316
v 10.2	Default	3/4/2013	1:39 PM	4659.17	93.184	49.161	1.90	1.95	50.00	230	47.892	1.574
v 10.2	Default	3/4/2013	1:40 PM	4686.44	93.729	49.168	1.91	1.95	50.00	230	48.011	1.670
v 10.2	Default	3/4/2013	1:40 PM	4657.66	93.153	49.039	1.90	1.95	50.00	230	47.846	1.626
v 11.2	Default	3/4/2013	1:41 PM	1486.59	29.732	14.317	2.08	1.98	50.00	230	15.012	0.697
v 11.2	Default	3/4/2013	1:41 PM	1477.64	29.553	14.260	2.07	1.97	50.00	230	14.964	0.741
v 11.2	Default	3/4/2013	1:41 PM	1467.66	29.353	14.187	2.07	1.97	50.00	230	14.874	0.711
v 12.1	Default	3/4/2013	1:42 PM	2589.12	51.782	24.903	2.08	2.09	50.00	230	24.749	0.662
v 12.1	Default	3/4/2013	1:42 PM	2827.31	56.546	27.354	2.07	2.09	50.00	230	27.096	0.818
v 12.1	Default	3/4/2013	1:43 PM	3139.08	62.782	30.655	2.05	2.07	50.00	230	30.266	0.843
v 13.1	Default	3/4/2013	1:43 PM	3724.60	74.492	36.437	2.04	2.06	50.00	230	36.145	0.715
v 13.1	Default	3/4/2013	1:44 PM	3705.13	74.103	36.205	2.05	2.06	50.00	230	36.001	0.733
v 13.1	Default	3/4/2013	1:44 PM	3688.85	73.777	36.051	2.05	2.06	50.00	230	35.895	0.724
v 14.1	Default	3/4/2013	1:45 PM	3604.78	72.096	34.926	2.06	2.08	50.00	230	34.658	0.725
v 14.1	Default	3/4/2013	1:45 PM	3626.94	72.539	35.253	2.06	2.08	50.00	230	34.875	0.725
v 14.1	Default	3/4/2013	1:46 PM	3611.90	72.220	35.037	2.06	2.08	50.00	230	34.754	0.772

Fig. 3. Centralizing table generated by Nanodrop on the results of DNA quantification at the analyzed vine hybrids (9 samples)

After quantification of the samples, DNA dilutions were made so that all the samples used for migration to have a concentration of 20 ng/μL. In Table 4 there are shown the average values of the samples of DNA, and the values of the dilution factor and the volumes of DNA stock and those of sterile double-distilled

water used for samples dilution.

Table 3. Summarizing table on stock samples of DNA dilutions in order to achieve PCR amplification

Proba	cantitate ng/µL	Puritate 260/280	Suma	Media	Fdilutie	DNA	Apa	
1	520,64	1,8	1573,78	524,59	26,23	3,8	96,2	
	518,4							
	534,74							
2	624,02	2,08	1848,87	616,29	30,81	3,2	96,8	
	615,58							
	609,27							
3	2325,09	2,1	6849,93	2283,31	114,17	0,9	99,1	
	2282,99							
	2241,85							
4	2261,95	2,13	7459,32	2486,44	124,32	0,8	99,2	
	2240,66							
	2956,71							
5	2142,19	2,1	6994,23	2331,41	116,57	0,9	99,1	
	2082,33							
	2769,71							
6	1918,78	2,09	5679,3	1893,10	94,66	1,1	98,9	
	1892,1							
	1868,42							
7	2030,9	2,09	6224,52	2074,84	103,74	1,0	99,0	
	2013,89							
	2179,73							
8	1555,29	1,98	4673,59	1557,86	77,89	1,3	98,7	
	1541,12							
	1577,18							
9	4532	1,94	13491,1	4497,03	224,85	0,4	99,6	
	4481,9							
	4477,2							
10	4659,17	1,9	14003,27	4667,76	233,39	0,4	99,6	
	4686,44							
	4657,66							
11	1486,59	2,07	4431,89	1477,30	73,86	1,4	98,6	
	1477,64							
	1467,66							
12	2589,12	2,07	8555,51	2851,84	142,59	0,7	99,3	
	2827,31							
	3139,08							
13	3724,6	2,05	11118,58	3706,19	185,31	0,5	99,5	
	3705,13							
	3688,85							
14	3604,78	2,08	10842,72	3614,24	180,71	0,6	99,4	
	3626,94							
	3611							

The results obtained concerning the number and size of alleles and are shown in Table 5:

Table 4. The analyzed number and size of the obtained alleles of the local and newly created varieties (the red colour indicates the international varieties used as reference in this study)

Denumirea probei	ss2		md5		md27		md7		zag 62		zag 79	
	129-155 bp		226-246 bp		173-194 bp		233-263 bp		185-203 bp		236-260 bp	
F2 BC1 DRX 55	137	149	233	239	184	190	244	244	188	204	251	261
F3 BC2 DRX M3 31	137	137	239	239	184	184	244	260	186	194	255	261
F4 BC3 DRX M4 536	139	139	239	239	190	190	226	244	186	204	241	261
F4 BC3 DRX M4 578	149	149	239	239	180	190	252	252	194	204	261	261
F4 BC3 DRX M4 545	139	139	239	239	180	190	244	252	188	204	255	261
F4 BC3 DRX M4 604	137	153	229	239	180	190	240	248	188	204	255	261
F4 BC3 DRX M4 508	137	137	233	233	180	190	248	248	188	204	261	261
F4 BC3 DRX M4 660	139	149	233	239	180	180	268	268	188	194	261	261
F4 BC3 DRX M4 580	137	153	227	237	180	190	244	244	192	204	255	261
F4 BC3 DRX M4 541	137	149	239	239	180	190	244	252	194	204	247	255
F4 BC3 DRX M4 507	149	149	239	265	180	190	252	252	188	194	261	261
F4 BC3 DRX M4 537	137	137	233	263	180	190	250	250	188	204	255	261
Vitis sylvestris female	139	149	233	233	190	206	240	240	190	204	255	255
Vitis sylvestris male	147	147	233	239	196	196	226	260	198	204	255	255
Sauvignon blanc	137	155	233	237	180	190	240	248	194	204	245	247
Chasellas dóre	137	147	229	239	176	190	240	258	194	204	251	257

Data grouping was done using the program "Excel" (Table 5), establishing the time of identification of alleles' size so that it could include all the values obtained after the migration of the samples analyzed in the genetic analyzer Beckman Coulter Ceq 8800 TM.

The migration of PCR products was performed in the genetic Analyzer Beckman Coulter Ceq 8800 TM in order to identify the number and size of alleles of vine varieties using the SSR technique. In the figures 4 and 5 are shown some migrated samples so that the heterozygous (at the same locus, allele, Figure 4.) or homozygous state (Fig. 5, sample 2 - F3 BC2 - DRX-M3-3-1) may be highlighted.

Table 5. The representation of the numberand size of alleles of the distant hybridsanalyzed by DNA barcode

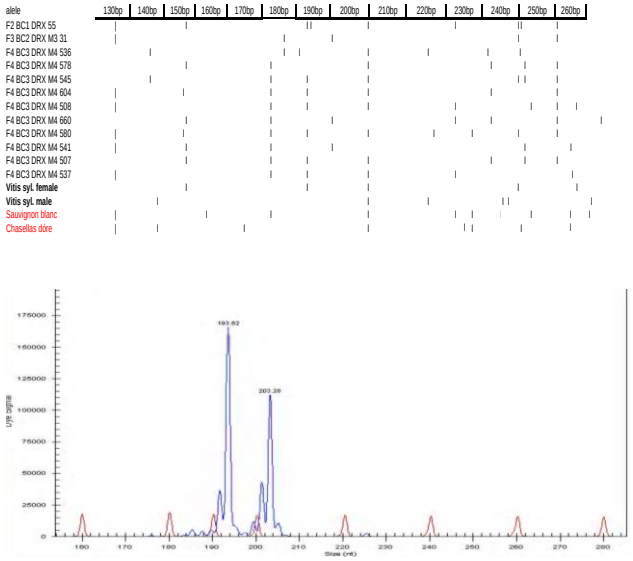

Fig. 4. Migration in the genetic Analyzer Beckman Coulter Ceq 8800 TM in order to identifythe number and size of alleles of vine varieties using the technique SSR with the primer ZAG 62.

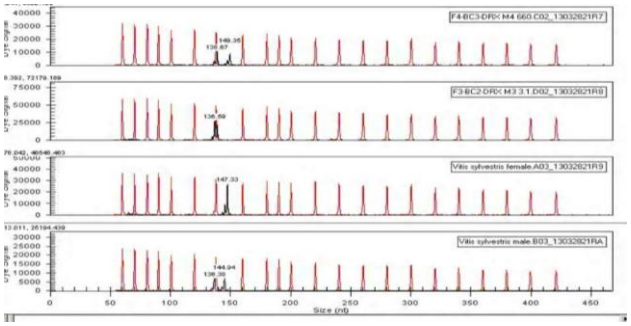

Fig. 5. Migration in the genetic Analyzer Beckman Coulter Ceq 8800 TM in order to identify the number and size of alleles of vine varieties using the technique SSR with the primer VVS2

Grouping distant hybrids based on the size of the alleles identified using SSR technique was performed in order to determine their type of genetic similarity/difference (Fig. 6).

Thus, it can be seen that there were formed two different main groups denoted by A and B, each of them having some secondary ramifications. It is worth mentioning the fact that the hybrid F4 BC3 DRX-M4-541 is very akin to the variety Chasellas dóre and it is possible that the latter may have contributed to the formation of the hybrid mentioned

above.

Also, the two samples of *Vitis sylvestris* Gmel. were found to be genetically different, being placed in separate subgroups. The hybrid F4 BC3 DRX-M4-536 is genetically close to the male specimen of *Vitis sylvestris* Gmel., and it may have contributed to the formation of the hybrid.

Among the hybrids F4 BC3, DRX-M4-660 stands out, because it has proved to have more differences at the molecular level, being unable to be grouped in a cluster with any other hybrid.

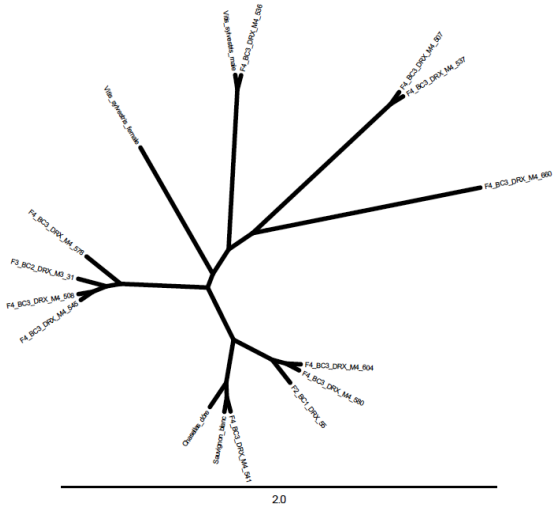

Fig. 6. The dendrogram, drawn up according to the Euclidean method, on the backcross hybrids and the reference varieties analyzed.

CONCLUSIONS

1. SSR (Simple Sequence Repeats) genetic fingerprinting technique can be used successfully in the determination of phylogeny relationships in the biological material analyzed.

2. The representation of the number and size of alleles using the barcode technique gives a clear view of the molecular similarities and differences that occur between the hybrids and the reference varieties analyzed.

3. The grouping of hybrids in the generated dendrogram shows that there are some differences between them at DNA level, differences sometimes unnoticeable at the level of ampelographic characterization. As a result, the characterization of varieties and hybrids of vine requires the ampelographic analysis to be completed by an analysis at the molecular level, based on DNA amplification techniques.

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