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Research Note

Molecular marker screening of new promising wine grape clones

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Introduction: Clonal selection is a well assessed method to improve the performance of wine grapevine (*Vitis vinifera*) cultivars. It gives the possibility to differentiate clonal lines within the same cultivar for improved propagation material and sanitary certification. For this aim reliable and precise methods of clonal characterization are requested. Identification of clones has been traditionally based on ampelographic and ampelometric traits, but their expression can be affected by developmental and environmental factors and may cause unsure attributions, while DNA-based methods are not influenced by environmental factors. At present a lot of potentially polymorphic sequence are available in literature as markers. Among them SSRs are largely used for the differentiation of wine grape cultivars or accessions (This *et al.* 2004), while contrasting results about the usefulness of molecular markers to assess genetic differences among clones have been reported (Cretazzo *et al.* 2010, Moncada and Hinrichsen 2007).

In the present research, the possibility of differentiating putative clones of 'Aligote', 'Cabernet Sauvignon', 'Merlot', 'Riesling', 'Pinot' and 'Sauvignon' derived from a clonal selection program by analyzing SSR markers was investigated. The purpose of this project was to study the utility of this marker type in genotypes (clones) discrimination and about the quantification of molecular variability of these important grapevine cultivars.

Material and Methods: Leaves of fifteen grapevine putative clones of 'Aligote', 'Cabernet Sauvignon', 'Merlot', 'Riesling', 'Pinot' and 'Sauvignon' varieties were collected for the analyses. Collection of leaf samples for molecular genetic analysis was performed in the “Kuban” didactic farm of the Kuban State Agricultural University and in vineyards of "Fanagoria" and "South" agricultural farms in Temryuksky district of Krasnodarsky region. After the sampling, leaves were maintained in at -20 °C.

DNA extraction from leaves by modified CTAB method and PCR were carried out as described in Zvyagin (2010). For the analysis of genetic diversity 6 microsatellite markers have been used: VrZag62, VrZag79, VVMD5, VVMD7, VVMD27 and VVS2. To establish differences between clones, acrylamide gel was used. Differences lower than 10 b.p. were not considered significant.

Results and Discussion: Between the two putative clones of 'Aligoté', differences in 16 nucleotides at the locus VrZag79 and 20 nucleotides in the locus VVMD5 were put in evidence. Among the three putative clones of 'Cabernet Sauvignon' a number of differences were detected in the loci VrZag79, VVS2, VVMD5. Among the three pu-

Table

Results of genetic analysis (SSR markers) of grapevine cultivars and putative clones

Sample	VrZag62	VrZag62	VrZag79	VrZag79	VVS2	VVS2	VVMD5	VVMD5	VVMD7	VVMD7	VVMD27	VVMD27
Aligoté 7-7 (Control)	192	202	244	250	120	128	225	243	247	264	172	172
Aligoté 7-10	182	190	228	234	120	126	255	265	262	262	N/A*	N/A
Cabernet Sauvignon 217 (Control)	196	200	256	262	137	149	229	238	239	239	172	172
Cabernet Sauvignon 210-4	184	188	284	290	126	145	229	229	247	247	172	172
Cabernet Sauvignon 210-8	186	186	276	282	145	155	227	242	262	262	172	182
Cabernet Myskhako (KM15)	190	190	289	295	120	128	250	261	253	253	172	172
Merlot 348 (Control)	182	186	256	282	139	155	225	243	247	247	N/A	N/A
Merlot 10-8	184	184	291	297	124	131	224	234	251	251	172	172
Merlot 10-9	190	190	233	256	131	149	227	250	255	255	172	172
Riesling 245-5 (Control)	192	200	233	262	135	151	225	234	264	264	172	172
Riesling 245-7	188	192	233	268	128	143	223	223	264	264	172	182
Pinot Noir 50-11 (Control)	190	194	230	236	126	147	224	232	247	264	174	184
Pinot Noir 50-8	192	192	236	254	124	145	225	225	257	257	172	172
Sauvignon Blanc 23-11 (Control)	186	186	246	252	128	151	227	242	247	264	172	172
Sauvignon Blanc 23-8	184	192	282	288	124	145	225	232	247	264	172	172

* - where N/A means that no data about the allele

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tative clones of Merlot in locus VrZag79 differences were detected on loci VVS2 and VVMD5. In the group of Riesling putative clones, Riesling 245-5 differed from Riesling 245-7 in locus VVMD5 in 11 nucleotides. In the group of Pinot Noir, in locus VrZag79 Pinot Noir 50-8 differed from Pinot Noir 50-11 in 18 nucleotides. In the group of Sauvignon Blanc in locus VrZag79 Sauvignon Blanc 23-8 differed on 36 nucleotides from Sauvignon Blanc 23-11.

Conclusions: The analysis revealed differences, enabling to recognize the putative clones as separate varieties. In accordance with molecular markers results and repeated ampelographic observations (data not shown), putative 'Aligote', 'Cabernet Sauvignon', 'Merlot', 'Pinot Noir', 'Riesling' and 'Sauvignon blanc' clones selected in commercial vineyards and transferred to the Russian State Department for Testing and Conservation of New Selection Achievements, have to be considered distinct varieties, For this reason they have been renamed as: 'Aligote Fanagoriis', 'Cabernet Miskhako', 'Cabernet Fanagoriis', 'Pinogrik', 'Riesling Fanagoriis' and 'Sauvignon Fanagoriis'. This resulted in the releasing by the Russian authorities of patents for the new varieties.

Cretazzo, E.; Meneghetti, S.; De Andrés, M. T.; Gaforio, L.; Frare, E.; Cifre, J.; 2010: Clone differentiation and varietal identification by means of SSR, AFLP, SAMPL and M-AFLP in order to assess the clonal selection of grapevine: the case study of Manto Negro, Callet and Moll, autochthonous cultivars of Majorca. Ann. Appl. Biol. **157**, 213-227.

Moncada, X.; Hinrichsen, P.; 2007: Limited genetic diversity among clones of red wine cultivar 'Carmenère' as revealed by microsatellite and AFLP markers. Vitis **46**, 174-180.

This, P.; Jung, A. ; Boccacci, P. ; Borrego, J. ; Botta, R.; Costantini, L.; Crespan, M.; Dangl, G. S.; Eisenheld, C.; Ferreira-Monteiro, F.; Grando, S.; Ibañez, J.; Lacombe, T.; Laucou, V.; Magalhães, R.; Meredith, C. P.; Milani, N.; Peterlunger, E.; Regner, F.; Zulini, L.; Maul, E.; 2004: Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor. Appl. Genet. **109**, 1448-1458.

Zviagin, A. S.; 2010: Extraction of DNA from herbarium leaves of *Vitis vinifera.* Scient. J. KubSAU **58**, 336-347.