AGRICULTURAL BIOLOGY,
2020, V. 55, № 3, pp. 523-532
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA)

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 634.8:577.22

doi: 10.15389/agrobiology.2020.3.523eng
doi: 10.15389/agrobiology.2020.3.523rus

COMPARATIVE ANALYSIS OF THE *VvMybA1* LOCUS ALLEAL STATE IN SOME INDIGENOUS AND INTRODUCENT GRAPEVINE VARIETIES

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The authors declare no conflict of interests
Acknowledgements:
The authors thank the JSC Evrogen company for the help in sequencing.
Supported financially by Russian Foundation for Basic Research and the Administration of the Krasnodar Territory, grant No. № 19-44-233003
Received October 10, 2019

Abstract

European cultivated grape *Vitis vinifira* L. is one of the most common agricultural crops grown by man since ancient times. Of course, the usual companion of the cultivation of this culture is a traditional hybridization and clonal selection, which allows you to select spontaneous mutants. Nevertheless, the study of such an important agrobiological trait as anthocyanin coloration and, in particular, the genes that determine it, is one of the most important problems both in modern grape genetics and in studies of plant metabolic pathways in general. The study of this problem can not only provide the basis for subsequent fundamental research on the functioning of both individual genes and the genome as a whole, but also create a basis for the selection of varieties for economically valuable traits. The *VvMybA* family of grape transcription factors is responsible for anthocyanin accumulation in berries of cultivated and wild grapes. In the present work, alleles of the *VvMybA1* gene were first identified in native Russian grape varieties. Theses alleles in the colored and uncolored grape varieties were found out to be the same in size. Sequence alignment showed the characteristic features of alleles for each of the studied genotypes. The purpose of our study, we set a description of the four alleles of the *VvMybA1* gene in the Chardonnay, Sibirkovskii, Cabernet Cortis and Sypun cherny varieties. Two introduced and well-known varieties and two indigenous varieties also cultivated in the Krasnodar Territory were investigated. The leaves for DNA extraction was collected at the Anapa zonal experimental station. DNA was isolated by CTAB method with mercaptoethanol. PCR was performed using primers and amplification parameters published in the literature. For PCR amplification and isolation of *VvMybA1* gene alleles we used markers which allow simultaneous identification of two alleles, *VvMybA1b* and *VvMybA1c*. We sequenced and compared with each other and with the GenBank NCBI database the sequences of the *VvMybA1* gene alleles of these varieties. Alignment of sequences in the ClustalO program revealed structural features of the allele nucleotide sequences. In particular, a single nucleotide insert was found in white-berry varieties and nucleotide substitutions in different places in varieties with colored and uncolored berries. Further, comparing with the GenBank NCBI database found that the alleles of colored varieties have a structure characteristic for varieties with a pronounced color of berries, while uncolored varieties apparently have a specific reason for the loss of color. Thus, it was found that the Chardonnay variety has an allele with the insertion of *Gret-1* transposon which blocks the normal expression of *VvMybA1* gene. It was also revealed that the Sibirkovky variety also has the allele of *VvMybA1* gene which is not functional due to the blocking by *Gret-1* transposon. As shown in previous studies by other authors, this allele is also present in other uncolored varieties, and, therefore, this is the reason for blocking gene expression. A study of the amino acid sequence also revealed differences between the groups of colored and uncolored varieties. These differences can be obviously divided into those characteristic of colored and uncolored varieties. However, a

mutation was detected in Sypun cherny variety, which affected the replacement of the amino acid isoleucine with valine, but did not affect the overall color of the berries. When searching for the amino acid sequence in the GenBank NCBI database, it was revealed that this mutation is not unique in nature, as it was found in the Alphonse Lavallée variety, as well as in interspecific hybrids.

Keywords: *Vitis vinifera* L., indigenous varieties, introduced varieties, allele, gene, *VvMybA1*, sequencing, anthocyanin, mutations, amino acids, transposons

European wine grape (*Vitis vinifera* L.) is one of the most widespread and economically important agricultural crops. The color of its berries is one of the main characteristics when describing existing varieties and creating new forms. The trait depends on the amount and composition of anthocyanins, which the colored varieties accumulate in the berries, while the uncolored ones do not synthesize [1].

Methods of cultivating grapes and processing the grape vine harvest depend on which hybrid, clone and stock are used in production, that is, on the ampelographic properties [2]. Vegetative reproduction preserves the desired traits, but significantly influenced the frequency of spontaneous somatic mutations observed in vineyards [3, 4]. Thence, many traits of grape plants were acquired not only by hybridization, but also due to clonal selection, for example, yield per bush, shape and compactness of a bunch, size and color of berries [5-7]. That is, the varieties intermediate in berry color (pink, red, yellow, etc.) appeared as a result of hybridization and mutations. The ripening and berry formation are also influenced by the environment [8-10], however, berry color is determined by genes. The metabolic pathways of anthocyanin color are primary regulated by the group of *MYB* genes [11-13].

VvMybA family of grape transcription factors is responsible for the content of anthocyanins in the berries of cultivated and wild grapes. Previous studies have shown that uncolored grapes arose due to the insertion of a retroelement in *VvMybA1* [14-16] and a single nucleotide polymorphism mutation in *VvMybA2* [17, 18]. That is, a gene cluster located on chromosome 2 is responsible for most of the color change, and the phenotype is due to the joint work of the *VvMybA* genes [19]. This locus consists of three *MYB* genes, among which *VvMybA1* and *VvMybA2* are functionally involved in berry pigmentation [13, 14]. It was shown that the appearance of a genotype characteristic of white berry varieties depends exactly on *VvMybA1* and *VvMybA2* [11, 13, 15]. Loss of *VvMybA1* gene function occurs due to the insertion of the *Gret1* transposon [16, 20], while *VvMybA2* can have a single nucleotide polymorphism (SNP) K980 in the coding sequence, which modifies the putative α-helix of the recognition domain R2R3 and leads to the loss of the allele functionality [15, 21].

It should be noted that in our country, well-known western introduced varieties have earlier been studied intensively, while the native varieties of the Black Sea basin, which have a huge potential for breeding new hybrids and selecting clones, remain unexplored. In particular, the structures of their genes, for example, *VvMybA1*, remains unknown, information about which is important for identifying the unique genetic structure of alleles, understanding particular cases of phenotypic diversity, and studying *Vitaceae* family evolution.

In this work is the first to identify *VvMybA1* gene alleles in native Russian grape varieties. The size of the alleles in colored and uncolored varieties is the same, but alignment of the sequences showed characteristic features of the allele structure for each of the studied genotypes

Our investigation aimed to reveal and identify structural features and to compare the *VvMybA1* gene alleles in two native and two introduced grape varieties.

Materials and methods. Two colored (Cabernet Cortis and Sypun black) and two uncolored (Chardonnay and Sibirkovy) grape varieties (respectively, native and introduced) were compared. Plant apical leaves were collected in Anapa zonal ampelographic collection SKFNTSSVV (AZOSViV). DNA was extracted by a modified CTAB method with mercaptoethanol [22]. For PCR optimization, the DNA concentration was measured (an Implen NP80 nanophotometer, Implen GmbH, Germany) and adjusted to 20 ng/μl with deionized water.

To perform classical PCR and identify target alleles of *VvMybA1* gene, we used markers described by Azuma et al. [23], which allow identification of two alleles, *VvMybA1b* and *VvMybA1c*, at once in a single PCR run. A 25 μl PCR mixture contained 20 ng of a template DNA, 200 mM dNTP, 0.2 mM of each primer [23] synthesized by JSC Evrogen (Russia), and one unit Taq-DNA polymerase (JSC Evrogen, Russia). PCR was performed in the following mode: 3 min at 95 °C (initial denaturation); 30 s at 94 °C, 30 s at 65 °C, 30 s at 72 °C (35 cycles); 10 min at 72 °C (DT-322, DNA-Technology LLC, Russia). The PCR results were checked for compliance with the expected fragment lengths in 6% PAGE with 1× TBE buffer; amplification products were separated in a VE-20 vertical electrophoresis chamber (Helicon, Russia) for 3 h (7 V/cm) (molecular weight marker M12, NPO SibEnzyme, Russia).

The amplicons were separated in 2% agarose gel with 0.5× TAE buffer at 5 V/cm, then excised from the gel plate and extracted using Cleanup Standard kit (Evrogen, Russia). The DNA concentration in the sample was measured (an Implen NP80 nanophotometer), and the volume was adjusted to 6 μl with deionized water (the purified amplified fragment concentration was 30 ng/μl).

Sequencing was performed with forward and reverse PCR primers [23] to ensure greater reliability (equipment of ZAO Evrogen, Russia).

A compliance with the expected amplicon size was assessed by searching the sequence in the GenBank NCBI database (https://www.ncbi.nlm.nih.gov/genbank) with BLAST, blastx, and CD-search web tools [24]. The obtained DNA and protein sequences were aligned according to standard parameters of the ClustalO program using the VIENNA (for fasta alignment) and ClustalW (for subsequent analysis) formats [25]. MView interface as applied to analyze the aligned sequences [26]. Phylogenetic relationships between the studied amino acid sequences were established using MEGA7 program [27] by the Maximum Likelihood method [28] with the Jones-Taylor-Thornton model [29] (999 bootstraps).

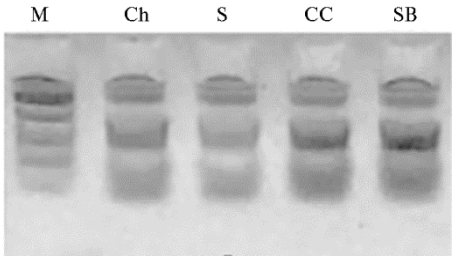

Fig. 1. Separation of amplified DNA fragments of *VvMybA1* alleles in colored (Cabernet Cortis CC and Sypun black SB) and uncolored (Chardonnay Ch and Sibirkovy S) **varieties of European wine grape (*Vitis vinifera* L.)** (M — molecular weight marker M12, NPO SibEnzim, Russia; Anapa zonal ampelographic collection SKANCSVV).

Results. After separation of the amplification products in agarose gel (Fig. 1), it was found that all four grape samples had alleles of the *VvMybA1* gene of the same size (approximately 850 bp). Similar works on grapes have already demonstrated the similarity of DNA sequences obtained by PCR not only in size, but also in primary structure [14, 16, 30]. However, in addition to this, it was shown that areas of the same size can be polymorphic and differ in structure, which also affects the color trait [16, 21]. We sequenced the amplified DNA regions to establish their nucleotide sequences in alleles of the same size and obtained the following results (Fig. 2):

Fig. 2. Sequencing of 850 bp amplicons of *VvMybA1* alleles in colored (Cabernet Cortis and Sypun black) **and uncolored** (Chardonnay and Sibirkovy) **varieties of European wine grape (*Vitis vinifera* L.)** (Anapa zonal ampelographic collection SKANCSVV) (for the figure, see http://www.agrobiology.ru).

The obtained nucleotide sequences were compared with each other using the ClustalO program (Fig. 3):

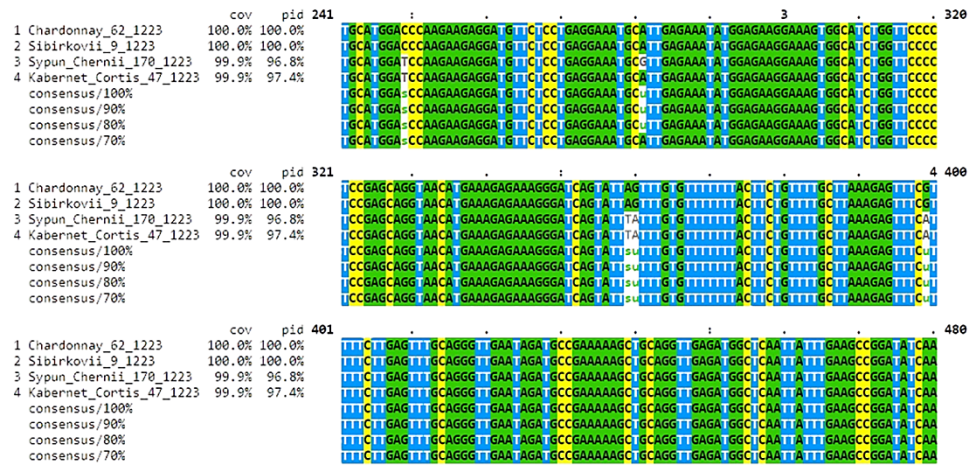

Fig. 3. Analysis of *VvMybA1* gene allele regions in four native and introduced varieties of European wine grape (*Vitis vinifera* L.) (colored Cabernet Cortis and Sypun black and uncolored Chardonnay and Sibirkovy) (ClustalO program; Anapa zonal ampelographic collection SKANCSVV). Nucleotide polymorphisms identified by alignment of the analyzed sequences in four genotypes are highlighted as in white. A unique nucleotide substitution at nucleotide position 281 in Sipun black variety is marked by white background. For the figure, see http://www.agrobiology.ru.

Sequence alignment revealed nucleotide substitutions that made it possible to distinguish between white and red berry varieties, for example, nucleotides 4-10. These differences were revealed in both pairs of the varieties we studied, the colored (Sypun black and Cabernet Cortis) and uncolored (Chardonnay and Sibirkovy). The same was confirmed by a search among the corresponding sequences deposited in the GenBank NCBI databases where the 100% similarity was found (except for the Sipun black variety).

The nucleotide sequences of the analyzed alleles in the Chardonnay and Sibirkovy cultivars were identical and coincided with that of the allele characteristic of the white berry cultivars with the *Gret-1* insert. This retrotransposon, as previously reported [16, 31], causes loss of the *VvMybA1* allele function. Consequently, it is the presence of such an insert in the specified gene that suppresses the synthesis of anthocyanins which provide berry coloration in the Sibirkovy variety. Despite the fact that we identified in the Sipun black cultivar a unique mutation in nucleotide 281 (see Fig. 3), in general, the nucleotide sequence of this allele (as in the Cabernet

Cortis cultivar) was characteristic of the colored cultivars (in particular KY406230.1, GU145121.1 and GU145120.1 accession numbers). Interestingly, a similarity appeared with the *VvMybA1*^{SUB} allele which was present but not expressed in uncolored varieties Sultanina, Pirovano 166A, and others [16], that is, did not play a decisive role in berry coloration.

Cabernet Cortis

Score	Expect	Method	Identities	Positives	Gaps
164 bits(415)	2e-50	Composition-based stats.	81/111(73%)	81/111(72%)	29/111(26%)

```
Query  1    MESLGVRKGAWIQEEDVLLRKCIEKYGEGKWHLVPLRAGNMKEKGISIYLCFFTSVLLKE  60
            MESLGVRKGAW QEEDVLLRKCIEKYGEGKWHLVPLRAG
Sbjct  1    MESLGVRKGAWTQEEDVLLRKCIEKYGEGKWHLVPLRAG----------------------  39

Query  61   FHFLEFAGLNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR  111
                    LNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR
Sbjct  40   --------LNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR  82
```

Sypun black

Score	Expect	Method	Identities	Positives	Gaps
163 bits(413)	4e-50	Composition-based stats.	80/111(72%)	81/111(72%)	29/111(26%)

```
Query  1    MESLGVRKGAWIQEEDVLLRKCVEKYGEGKWHLVPLRAGNMKEKGISIYLCFFTSVLLKE  60
            MESLGVRKGAW QEEDVLLRKC+EKYGEGKWHLVPLRAG
Sbjct  1    MESLGVRKGAWTQEEDVLLRKCIEKYGEGKWHLVPLRAG----------------------  39

Query  61   FHFLEFAGLNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR  111
                    LNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR
Sbjct  40   --------LNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR  82
```

Chardonnay

Score	Expect	Method	Identities	Positives	Gaps
166 bits(419)	6e-51	Composition-based stats.	82/111(74%)	82/111(73%)	29/111(26%)

```
Query  1    MESLGVRKGAWTQEEDVLLRKCIEKYGEGKWHLVPLRAGNMKEKGISISLCFFTSVLLKE  60
            MESLGVRKGAWTQEEDVLLRKCIEKYGEGKWHLVPLRAG
Sbjct  1    MESLGVRKGAWTQEEDVLLRKCIEKYGEGKWHLVPLRAG----------------------  39

Query  61   FRFLEFAGLNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR  111
                    LNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR
Sbjct  40   --------LNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR  82
```

Sibirkovy

Score	Expect	Method	Identities	Positives	Gaps
166 bits(419)	6e-51	Composition-based stats.	82/111(74%)	82/111(73%)	29/111(26%)

```
Query  1    MESLGVRKGAWTQEEDVLLRKCIEKYGEGKWHLVPLRAGNMKEKGISISLCFFTSVLLKE  60
            MESLGVRKGAWTQEEDVLLRKCIEKYGEGKWHLVPLRAG
Sbjct  1    MESLGVRKGAWTQEEDVLLRKCIEKYGEGKWHLVPLRAG----------------------  39

Query  61   FRFLEFAGLNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR  111
                    LNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR
Sbjct  40   --------LNRCRKSCRLRWLNYLKPDIKRGEFALDEVDLMIRLHNLLGNR  82
```

Fig. 4. A search for amino acid sequences translated from *VvMybA1* gene alleles in four native and introduced varieties of European wine grape (*Vitis vinifera* L.) (colored Cabernet Cortis and Sypun black and uncolored Chardonnay and Sibirkovy) (blastx algorithms; Anapa zonal ampelographic collection SKANCSVV). When compared with the reference sequences in NCBI Protein (https://www.ncbi.nlm.nih.gov/protein/), an insert was identified which is highlighted by dashes (most likely, it is the intron sequence not deleted by blastx tool during translation).

After assignment of the sequences to the sought alleles, we used blastx tool to search among the corresponding amino acid sequences. For all four sequences, the presumptive structure of the amino acid chain was established (Fig. 4). The results of the search showed 100% coincidence in all analyzed samples, with the exception of a large insert in the middle of the sequences which were turned out to be characteristic of all genotypes and resulted from translation of the intron region not excluded by the algorithm for some reason. This statement is also supported by a search in EnsemblPlants [32] and CD-search [33].

Having shown single nucleotide polymorphisms, we aligned the amino acid sequences in ClustalO program (Fig. 5). Differences between the white berry and

red berry varieties were found for amino acids 12, 49, and 62. A unique substitution of isoleucine for valine was found in Sypun black variety (amino acid 23). In addition, two of the four polymorphisms were located in the coding part of the sequence. One of them was typical for white and red berry genotypes, and amino acid 23 in Sipun black variety was unique. As per CD-search, this substitution was located in a conservative region. It is noteworthy that no differences were found in this position from the variety Alphonse Lavallée [16]. However, both varieties are brightly colored. Therefore, the change in amino acid sequence did not affect the expression of this trait in any way. The search for the amino acid sequence of Cabernet Cortis in NCBI Protein confirmed that it is often found among interspecific hybrids that have a similar sequence of amino acids encoded by *VvMybA1* [11, 13], but differ from the Sipun black genotype by replacing valine to isoleucine.

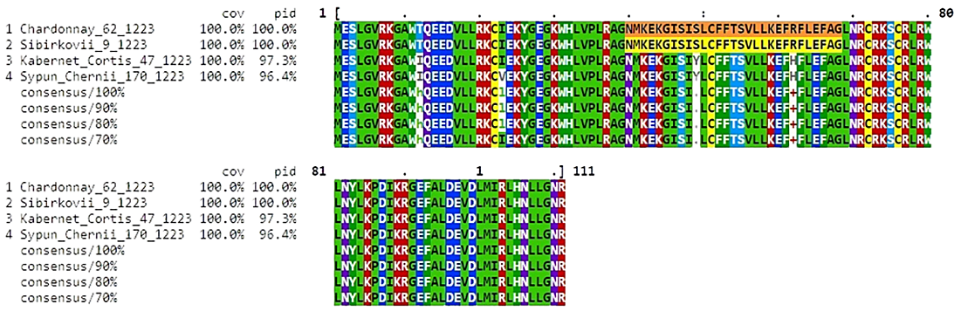

Fig. 5. Alignment of amino acid sequences (with an intron insertion marked) **translated from *VvMybA1* gene alleles in four native and introduced varieties of European wine grape (*Vitis vinifera* L.)** (colored Cabernet Cortis and Sypun black and uncolored Chardonnay and Sibirkovy) (ClustalO program; Anapa zonal ampelographic collection SKANCSVV). In Sypun black variety, in the position 23 (highlighted in white background) there is an amino acid substitution (isoleucine characteristic of the other three varieties is replaced to valine). For the figure, see http://www.agrobiology.ru.

As a whole, our results were in line with the expected ones, since it was revealed that the studied varieties are similar in nucleotide sequences of *VvMybA1* gene alleles to the known genotypes. An unexpected result was the similarity of the amino acid sequence encoded by the *VvMybA1* gene allele in the Sibirkovy variety and the colored varieties Benitaka, Cabernet Sauvignon, and Roditis [16, 34]. This can be explained by the fact that Benitaka variety is a spontaneous mutant of the white-berry variety Italia [31], whereas varieties Cabernet Sauvignon and Roditis are hybrids harboring both *VvMybA1b* and *VvMybA1c* alleles [16, 34, 35].

To compare the sequences of genes that determine colored and uncolored berries, we clustered them based on amino acid sequences (Fig. 6). The genotype *Rosa chinensis* (XP_024179665.1) of *Rosaceae* family was involved as an outgroup.

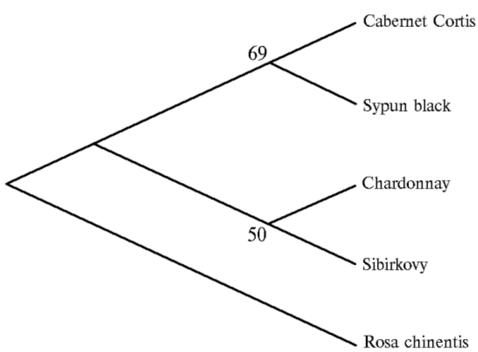

Fig. 6. Clustering of four native and introduced varieties of European wine grape (*Vitis vinifera* L.) (colored Cabernet Cortis and Sypun black and uncolored Chardonnay and Sibirkovy) **based on amino acid sequences translated from the sequenced *VvMybA1* gene alleles** (Anapa zonal ampelographic collection SKANCSVV). Bootstrap values over 50 are indicated.

In clustering, genotypes formed three groups (the outgroup, colored and uncolored varieties). Interestingly, on the generated tree the white-berry varieties were located separately from the main

branch between the colored varieties and the outgroup. These data are confirmed by other studies. Indeed, some uncolored varieties, such as Pinot blanc and Pinot Gris, appeared as a result of mutation [36], while the reverse process was also observed, i.e. the appearance of berry coloration in the Benitaka variety, that is, a transition from white berry color to pink [31].

Thus, in the introduced and native Russian grape varieties, the *VvMybA1* gene alleles are identified, the size of which does not differ in colored and uncolored varieties. The alleles reveal unique single nucleotide substitutions inherent in the specific genotypes we studied, including a unique nucleotide substitution in the Sypun black variety, as well as an insert in the uncolored Chardonnay and Sibirkovy varieties. A search for these sequences in the GenBank NCBI database using BLAST algorithms showed that three genotypes (varieties Cabernet Cortis, Sypun black, and Chardonnay) have alleles characteristic of white and black berry forms. In the Siberian variety, the *VvMybA1* gene allele is similar to the *VvMybA1a* allele with *Gret-1* transposon insertion. Obviously, the *VvMybA1* allele expression in the Sibirkovy variety is turned off by this very factor. A similar result was obtained for the Chardonnay variety (the presence of the *Gret-1* transposon which suppresses functional activity of the *VvMybA1* allele).

REFERENCES

1. Boss P.K., Davies C., Robinson S.P. Expression of anthocyanin biosynthesis pathway genes in red and white grapes. *Plant Molecular Biology*, 1996, 32(3): 565-569 (doi: 10.1007/BF00019111).
2. Swanepoel J.J., Southey J.M. The influence of rootstock on the rooting pattern of the grapevine. *South African Journal of Enology and Viticulture*, 1989, 10(1): 23-28 (doi: 10.21548/10-1-2295).
3. Forneck A. Plant breeding: clonality — a concept for stability and variability during vegetative propagation. In: *Progress in botany, vol. 66*. K. Esser, U. Lüttge, W. Beyschlag, J. Murata (eds.). Springer, Berlin, Heidelberg, 2005: 164-183 (doi: 10.1007/3-540-27043-4_8).
4. Pelsy F., Hocquigny S., Moncada X., Barbeau G., Forget D., Hinrichsen P., Merdinoglu D. An extensive study of the genetic diversity within seven French wine grape variety collections. *Theoretical and Applied Genetics*, 2010, 120(6): 1219-1231 (doi: 10.1007/s00122-009-1250-8).
5. Rühl E., Konrad H., Lindner B., Bleser E. Quality criteria and targets for clonal selection in grapevine. *Acta Horticulturae*, 2004, 652: 29-33 (doi: 10.17660/ActaHortic.2004.652.1).
6. Torres N., Goicoechea N., Morales F., Antolín M.C. Berry quality and antioxidant properties in *Vitis vinifera* cv. Tempranillo as affected by clonal variability, mycorrhizal inoculation and temperature. *Crop and Pasture Science*, 2016, 67(9): 961-977 (doi: 10.1071/CP16038).
7. Van Leeuwen C., Roby J.P., Alonso-Villaverde V., Gindro K. Impact of clonal variability in *Vitis vinifera* Cabernet franc on grape composition, wine quality, leaf blade stilbene content, and downy mildew resistance. *Journal of Agricultural and Food Chemistry*, 2013, 61(1): 19-24 (doi: 10.1021/jf304687c).
8. Anesi A., Stocchero M., Dal Santo S., Commisso M., Zenoni S., Ceoldo S., Tornielli G.B., Siebert T.E., Herderich M., Pezzotti M., Guzzo F. Towards a scientific interpretation of the terroir concept: plasticity of the grape berry metabolome. *BMC Plant Biology*, 2015, 15(1): 191 (doi: 10.1186/s12870-015-0584-4).
9. Dal Santo S., Zenoni S., Sandri M., De Lorenzis G., Magris G., De Paoli E., Di Gaspero G., Del Fabbro C., Morgante M., Brancadoro L., Grossi D., Fasoli M., Zuccolotto P., Tornielli G.B., Pezzotti M. Grapevine field experiments reveal the contribution of genotype, the influence of environment and the effect of their interaction (G×E) on the berry transcriptome. *The Plant Journal*, 2018, 93(6): 1143-1159 (doi: 10.1111/tpj.13834).
10. Guidoni S., Ferrandino A., Novello V. Effects of seasonal and agronomical practices on skin anthocyanin profile of Nebbiolo grapes. *American Journal of Enology and Viticulture*, 2008, 59(1): 22-29.
11. Azuma A., Kobayashi S., Mitani N., Shiraishi M., Yamada M., Ueno T., Kono A., Yakushiji H., Koshita Y. Genomic and genetic analysis of *Myb*-related genes that regulate anthocyanin biosynthesis in grape berry skin. *Theoretical and Applied Genetics*, 2008, 117(6): 1009-1019 (doi: 10.1007/s00122-008-0840-1).
12. Azuma A., Udo Y., Sato A., Mitani N., Kono A., Ban Y., Yakushiji H., Koshita Y., Kobayashi S. Haplotype composition at the color locus is a major genetic determinant of skin color variation

in Vitis×labruscana grapes. *Theoretical and Applied Genetics*, 2011, 122(7): 1427-1438 (doi: 10.1007/s00122-011-1542-7).
13. Kobayashi S., Ishimaru M., Hiraoka K., Honda C. *Myb*-related genes of the Kyoho grape (*Vitis labruscana*) regulate anthocyanin biosynthesis. *Planta*, 2002, 215(6): 924-933 (doi: 10.1007/s00425-002-0830-5).
14. Kobayashi S., Goto-Yamamoto N., Hirochika H. Retrotransposon-induced mutations in grape skin color. *Science*, 2004, 304(5673): 982 (doi: 10.1126/science.1095011).
15. Walker A.R., Lee E., Bogs J., McDavid D.A., Thomas M.R., Robinson S.P. White grapes arose through the mutation of two similar and adjacent regulatory genes. *The Plant Journal*, 2007, 49(5): 772-785 (doi: 10.1111/j.1365-313X.2006.02997.x).
16. Lijavetzky D., Ruiz-García L., Cabezas J.A., De Andrés M.T., Bravo G., Ibáñez A., Carreño J., Cabello F., Ibáñez J., Martínez-Zapater J.M. Molecular genetics of berry colour variation in table grape. *Molecular Genetics and Genomics*, 2006, 276(5): 427-435 (doi: 10.1007/s00438-006-0149-1).
17. Giannetto S., Velasco R., Troggio M., Malacarne G., Storchi P., Cancellier S., De Nardi B., Crespan M. A PCR-based diagnostic tool for distinguishing grape skin color mutants. *Plant Science*, 2008, 175(3): 402-409 (doi: 10.1016/j.plantsci.2008.05.010).
18. De Lorenzis G. Investigation of *VvMybA1* and *VvMybA2* berry color genes in 'Aglianico' biotypes. In: *Progress in Vitis vinfera diversity evaluation and use, vol. 54*. G. De Lorenzis, D. Carrasco, R. Arroyo Garcia, M. Rossoni, G.S. Di Lorenzo, O. Failla (eds.). Bundesanstalt fur Zuchtungsforschung an Kulturpflanzen, Geilweilerhof, 2015: 43-44.
19. Fournier-Level A., Le Cunff L., Gomez C., Doligez A., Ageorges A., Roux C., Bertrand Y., Souquet J., Cheynier V., This P. Quantitative genetic bases of anthocyanin variation in grape (*Vitis vinifera* L. ssp. *sativa*) berry: a quantitative trait locus to quantitative trait nucleotide integrated study. *Genetics*, 2009, 183(3): 1127-1139 (doi: 10.1534/genetics.109.103929).
20. This P., Lacombe T., Cadle-Davidson M., Owens C.L. Wine grape (*Vitis vinifera* L.) color associates with allelic variation in the domestication gene *VvmybA1*. *Theoretical and Applied Genetics*, 2007, 114(4): 723-730 (doi: 10.1007/s00122-006-0472-2).
21. Fournier-Level A., Lacombe T., Le Cunff L., Boursiquot J.M., This P. Evolution of the *VvMybA* gene family, the major determinant of berry colour in cultivated grapevine (*Vitis vinifera* L.). *Heredity*, 2010, 104(4): 351-362 (doi: 10.1038/hdy.2009.148).
22. Porebski S., Bailey L.G., Baum B.R. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter*, 1997, 15(1): 8-15 (doi: 10.1007/BF02772108).
23. Azuma A., Kobayashi S., Yakushui H., Yamada M., Mitani N., Sato A. *VvmybA1* genotype determines grape skin color. *Vitis*, 2007, 46(3): 154-155.
24. McGinnis S., Madden T.L. BLAST: at the core of a powerful and diverse set of sequence analysis tools. *Nucleic Acids Research*, 2004, 32(suppl_2): 20-25 (doi: 10.1093/nar/gkh435).
25. Sievers F., Higgins D.G. Clustal Omega, accurate alignment of very large numbers of sequences. In: *Multiple sequence alignment methods, vol. 1079*. D. Russel (ed.). Humana Press, Totowa, NJ, 2014: 105-116 (doi: 10.1007/978-1-62703-646-7_6).
26. Brown N.P., Leroy C., Sander C. MView: a web-compatible database search or multiple alignment viewer. *Bioinformatics,* 1998, 14(4): 380-381 (doi: 10.1093/bioinformatics/14.4.380).
27. Kumar S., Stecher G., Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 2016, 33(7): 1870-1874 (doi: 10.1093/molbev/msw054).
28. Murshudov G.N., Vagin A.A., Dodson E.J. Refinement of macromolecular structures by the maximum-likelihood method. *Acta Crystallographica*, 1997, 53(3): 240-255 (doi: 10.1107/S0907444996012255).
29. Jones D.T., Taylor W.R., Thornton J.M. A model recognition approach to the prediction of all-helical membrane protein structure and topology. *Biochemistry*, 1994, 33(10): 3038-3049 (doi: 10.1021/bi00176a037).
30. Ferreira V., Pinto-Carnide O., Arroyo-García R., Castro I. Berry color variation in grapevine as a source of diversity. *Plant Physiology and Biochemistry*, 2018, 132: 696-707 (doi: 10.1016/j.plaphy.2018.08.021).
31. Azuma A., Kobayashi S., Goto-Yamamoto N., Shiraishi M., Mitani N., Yakushiji H., Koshita Y. Color recovery in berries of grape (*Vitis vinifera* L.) 'Benitaka', a bud sport of 'Italia', is caused by a novel allele at the *VvmybA1* locus. *Plant Science*, 2009, 176(4): 470-478 (doi: 10.1016/j.plantsci.2008.12.015).
32. Bolser D.M., Staines D.M., Perry E., Kersey P.J. Ensembl plants: integrating tools for visualizing, mining, and analyzing plant genomic data. In: *Plant genomics databases. Methods in Molecular Biology, V. 1533*. A. van Dijk (ed.). Humana Press, New York, NY, 2017: 1-31 (doi: 10.1007/978-1-4939-6658-5_1).
33. Marchler-Bauer A., Bryant S.H. CD-Search: protein domain annotations on the fly. *Nucleic Acids Research*, 2004, 32(2): 327-331 (doi: 10.1093/nar/gkh454).
34. Walker A.R., Lee E., Robinson S.P. Two new grape cultivars, bud sports of Cabernet Sauvignon

bearing pale-coloured berries, are the result of deletion of two regulatory genes of the berry colour locus. *Plant Molecular Biology*, 2006, 62(4): 623-635 (doi: 10.1007/s11103-006-9043-9).
35. Bowers J.E., Meredith C.P. The parentage of a classic wine grape, Cabernet Sauvignon. *Nature Genetics*, 1997, 16(1): 84-87 (doi: 10.1038/ng0597-84).
36. Vezzulli S., Leonardelli L., Malossini U., Stefanini M., Velasco R., Moser C. Pinot blanc and Pinot gris arose as independent somatic mutations of Pinot noir. *Journal of Experimental Botany*, 2012, 63(18): 6359-6369 (doi: 10.1093/jxb/ers290).