

THE SEPARATION OF GRAPEVINE (*VITIS VINIFERA* L.) CONCVLTA MEMBERS WITH MOLECULAR MARKERS

ROZLIŠENÍ RÉVY VINNÉ (*VITIS VINIFERA* L.) MOLEKULÁRNÍMI MARKERY

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ABSTRACT

The berry color is one of the most characteristic in case of grapevine cultivars (*Vitis vinifera* L.). This morphological property is important to make difference between the cultivars in the time of the full maturity. Complemented with the patterns of the leaf, bunch, vine, shoot-tip could be enough to identify a cultivar. Two main groups can be made according to the color of the berry, white and red wine cultivars. The red wine cultivars contain anthocyanin in the epidermal section of the berry. The amount and concentration of the coloring matter define the typical color of the cultivars. *VvmybA1* (a *Myb*-related transcription factor) regulate the anthocyanin synthesis, consequently the function of this pathway responsible among others to the berry skin. The presence of the retrotransposon *Gret1* near to the coding sequence of *VvmybA1* causes the white berry skin with the blocking of the expression of the gene. In this study we demonstrate the separation of the grapevine cultivars which different from each other visibly only in the color of the berry. Two main methods used: SSR (Simple Sequence Repeat) and the *Gret1*-based separation of the cultivars. Such as Pinot, Chasselas, Bajor, Gohér group of cultivars can be distinguishes according to the presence or absence of the *Gret1* in their promoter region or with SSR analysis.

Key words: *VvMYBA1*, retroelement, SSR, group of cultivars

INTRODUCTION

In case of the concultas (color variations/bud sports) where more members are grown, for example 'Pinot', it is important to differentiate the members at any phenological stages (winter), not only at full maturation. During the period of wood propagation when the plants not give the most characteristic patterns also necessary to find differences. Pinot (noir, blanc, grey) and Chasselas (blanc, rouge) origin from different geographical areas. Pinot conculta is origin in the west part of Europe (convar. *occidentalis*) while the Chasselas conculta spread from the east (Central-Asia, Iran) (convar. *orientalis*). These groups came into being in the same way, all of the conculta members are origin from one ancient cultivar (which is member of the conculta) with bud-mutation and later human selection ('Pinot' conculta hypothetically origin from the Pinot noir). Inside of each concultas the members are differentiate only in a few visible pattern, which is the color of the berry, the autumn coloration of the leaf (Németh 1973). This character is under the control of the anthocyanin biosynthesis pathway. It is proved that such cultivars which evolutes with mutations (Ruby okuyama, Flame muscat, Pinot noir) different from the original cultivar (Italia, Muscat of Alexandria, Pinot blanc) mainly only in the color of the berry. These changes happened by the reason of the mutation in the anthocyanin pathway (Kobayashi et al. 2001, Yakushiji et al. 2006).

Regner et al. (2000) could not found any different between the Pinot conculta with 34 microsatellites. It means that the mutations have no affect to the non-coding regions where the investigated SSR regions are.

The anthocyanin pathway described in detail by Kobayashi et al. (2001) and Ageorges et al. (2006). According to these results, the gene expression of the genes in this pathway; in the above mentioned cultivars; are the same in white and red cultivars also. The only experienced different are in the expression of the UFGT gene (UDP-glucose: flavonoid 3-*O*-glucosyltransferase). This gene has no expression in case of the cultivars with white berry color. The sequences of UFGT gene and the promoter region were the same in case of all investigated cultivars. Results proved that the transcription factor of this gene which regulates the expression of the UFGT and the anthocyanin synthesis is blocked (Kobayashi et al. 2001).

The *VvMYBA1* transcription factor described by (Kobayashi et al. 2005) regulates the expression of the UFGT. In case of red skinned cultivars this transcription factor works suitably. However in case of white skinned cultivars has no anthocyanin synthesis because the *gypsy-type* retroelement (*Gret1*) is blocking the expression of the *VvMYBA1*. This retroelement translocated into the promoter region of the MYB gene. Hypothetically this movement is the reason of the different between the white and red cultivars.

The "original" Pinot cultivar probably was the Pinot noir. The Pinot blanc and Pinot gris are bud mutations of this cultivar. As it described before (Hocquigny et al. 2004) the Pinot gris is a periclinal chimera which expresses anthocyanin in the L1 layer, in the epidermis, but no expression in L2, under the epidermal cell layer. The Pinot blanc also result of a bud mutation of Pinot noir. Yakushiji et. al (2006) proved that a deletion in the region of *VvMYBA1* causes that, the red allele of the MYB gene is missing. On the other locus the *Gret1* blocked *VvMYBA1* (white allele) controlled expression of the UFGT. This is the reason

of the color changes. As it seems the taxonomic definition of the conculta is not else but the mutation of the promoter of the *VvMybA1* gene.

No any *Vitis vinifera* L. were found which no contains the *Gret1* element on each allele of the promoter of *VvMYBA1*. It means that the method of separation of the separation of conculta members can base on the presence of the *Gret1* in the promoter region of the *VvMYBA1*. The red berried cultivars are heterozygote for the *Gret1*. Only on one allele is detectable the *Gret1*, but on the other allele is no absence of the element. Because of it, there is expression of the *VvMYBA1* and the UGFT can express the anthocyanin. The presence or absence of the *Gret1* retroelement can be used as a molecular marker for the separation of the conculta members. Our aim was to separate group of cultivars from each other which separation is questionable or not possible with microsatellites.

MATERIALS AND METHODS

Plant material (Table), young leafs were collected in the cultivar collections of Nagyréde (N) (Szőlőskert Ltd.), Helvécia (H), and Ministry of Agriculture and Rural Development (MARD) Research Institute for Viticulture and Oenology Pécs (P).

DNA extraction was made with the Qiagen DNeasy Plant Mini Kit according to the manufactures protocol (Qiagen- Biomarker Ltd. Gödöllő, Hungary). The successfully of the extraction was checked on agarose gel electrophoresis.

Seven fluorescent-labeled microsatellite primer pairs (VvS2, VvMD5, VvMD7, VvMD27, VrZAG21, VrZAG62 and VrZAG79) were used in this study (Biomi Ltd.-Gödöllő, Hungary). The PCR conditions for the primers are described before by Regner et al 2000. Three oligonucleotide primers: promoF', Cr', LTR5f' were applied, described before by This et al. 2004. These primers are designed for the detection of the *Gret1* retrotransposon according to the presence or the absence of the LTR5 region of that (Figure 1.).

The PCR program for the oligonucleotide primers (detection of the *Gret1*) was: denaturation on 94 °C for 3 min, additional 94 °C 30 s / 55 °C 30 s / 72 °C 1:30 min. repeated for 35 cycles, and the final step is 72 °C 10 min. The PCR products were checked on 1% agarose with ethidium-bromide.

RESULTS

The SSR results show that there is no any difference within the Chasselas and Pinot samples. All the seven loci show homology. Earlier investigations made by Regner (2000) proved that the Pinot samples could not be separated with 34 SSR markers. Our data show the same result. By this time only the 'Pinot' conculta (noir and blanc) could be separated with the retrotransposon based selection (Figure 2). As it described before, the genes of the anthocyanine pathway have the same sequences in each of the conculta members. The only differ is the presence or the absence of the *Gret1* in either of the alleles. As it proved before the Pinot noir are contains the *Gret1* only in one allele of the MYB gene, these is the reason of the red berry color. The Pinot blanc as a white berried cultivar not produce anthocyanine in the skin of the berry.

Investigations with the Chasselas blanc and rouge show that the genetic differences can not detectable on these loci. Conculta members of ‘Gohér’ show homology at all loci except the ‘Gohér altering’ which was homozygote at the locus VVMD27. Interestingly the ‘Bajor blue’ – member of the ‘Bajor’ conculta – is distinct from the other conculta members at the same locus (Table). These results proved that these conculta members are differ from each other not only on the promoter region of *VvMybA1* but in non-coding regions also, as is seems on the microsatellite data.

As it can be seen on the Table ‘Sárfehér’ and ‘Sárpiros’; ‘Járdovány black’ and ‘Járdovány white’ are not conculta members because there are several differences. They are probably homonyms with different berry color.

This work was the first step to describe the promoter region of *VvMybA1* gene of the world known conculta members and separate those with Gret1 based molecular markers.

CONCLUSION

The most used grapevine identification method these days are the SSR (simple sequence repeat) or microsatellite analysis. This method is suitable for identification and separation of cultivars. Although this method is widely used for *Vitis vinifera* L. investigation, not well for separate the conculta members, such as Pinot and Chasselas. These cultivars are widely used in Hungary and the separation of them is not as easy in winter and the propagation period as in the full maturing. The plants give the most characteristic pattern in the time of maturing, when bunches have the characteristic color, shape and size. With the help of the genetic background of the berry color as a genetic marker it will be possible to separate conculta members.

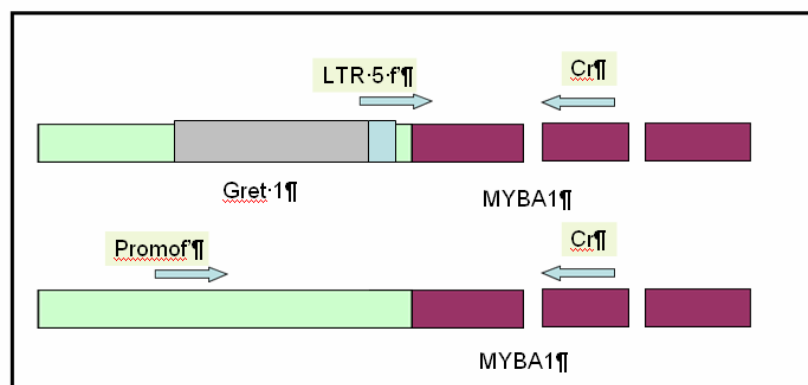


Figure 1: The schematic picture of the *VvMYBA1*, *Gret1*, and the place of the oligonucleotide primers

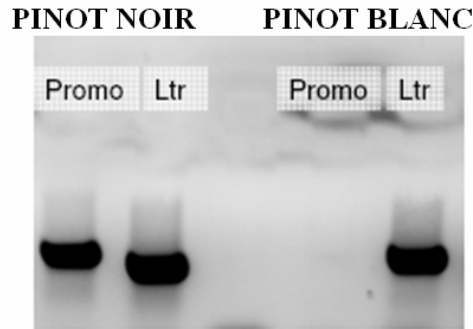


Figure 2.: The gel picture of Pinot noir and Pinot blanc

Table : The collected cultivars and the fragment length data of the investigated loci

| Place of collecting | Cultivar | Locus | Fragment Length Data (bp) | | | | | | | | | | | | | |
|---------------------|-------------------|-------|---------------------------|-------|-------|---------|--------|---------|---------|------|-------|-------|---------|--------|---------|---------|
| | | | VvS2 | VvMD5 | VvMD7 | VrZAG21 | VvMD27 | VrZAG62 | VrZAG79 | VvS2 | VvMD5 | VvMD7 | VrZAG21 | VvMD27 | VrZAG62 | VrZAG79 |
| N | Chasselas rouge | | 133 | 143 | 228 | 236 | 239 | 247 | 199 | 205 | 185 | 189 | 195 | 205 | 251 | 259 |
| N | Chasselas blanc | | 133 | 143 | 228 | 236 | 239 | 247 | 199 | 205 | 185 | 189 | 195 | 205 | 251 | 259 |
| N | Pinot noir | | 137 | 151 | 228 | 238 | 239 | 243 | 203 | 209 | 185 | 189 | 189 | 195 | 239 | 245 |
| N | Pinot gris | | 137 | 151 | 228 | 238 | 239 | 243 | 203 | 209 | 185 | 189 | 189 | 195 | 239 | 245 |
| N | Pinot blanc | | 137 | 151 | 228 | 238 | 239 | 243 | 203 | 209 | 185 | 189 | 189 | 195 | 239 | 245 |
| P | Gohér white | | 131 | 151 | 236 | 236 | 238 | 248 | 201 | 205 | 179 | 191 | 188 | 204 | 248 | 258 |
| P | Gohér red | | 131 | 151 | 236 | 236 | 238 | 248 | 201 | 205 | 179 | 191 | 188 | 204 | 248 | 258 |
| P | Gohér altering | | 131 | 151 | 236 | 236 | 238 | 248 | 201 | 205 | 179 | 179 | 188 | 204 | 248 | 258 |
| P | Bajor blackwooden | | 131 | 151 | 226 | 236 | 238 | 238 | 199 | 205 | 179 | 191 | 188 | 196 | 248 | 258 |
| P | Bajor blue | | 131 | 151 | 226 | 236 | 238 | 238 | 199 | 205 | 179 | 179 | 188 | 196 | 248 | 258 |
| P | Bajor grey | | 131 | 151 | 226 | 236 | 238 | 238 | 199 | 205 | 179 | 191 | 188 | 196 | 248 | 258 |
| H | Sárfehér | | 131 | 151 | 226 | 230 | 236 | 244 | 199 | 205 | 187 | 191 | 190 | 198 | 246 | 248 |
| H | Sárpiros | | 131 | 151 | 222 | 222 | 236 | 244 | 199 | 199 | 187 | 191 | 186 | 202 | 246 | 248 |
| H | Járdovány black | | 131 | 151 | 222 | 232 | 238 | 248 | 199 | 205 | 177 | 191 | 168 | 200 | 246 | 248 |
| H | Járdovány white | | 141 | 144 | 228 | 236 | 244 | 244 | 199 | 205 | 179 | 191 | 202 | 202 | 240 | 246 |

LITERATURE

- Ageorges A., Fernandez L., Vialet S., Merdinoglu D., Terrier N., Romieu C. (2006): Four specific isogenes of the anthocyanin metabolic pathway are systematically co-expressed with the red colour of grape berries. *Plant Sci*, 170: 372-383.
- Azuma, A. (2006): A skin color mutation of grapevine, from black-skinned Pinot noir to white-skinned Pinot blanc, is caused by deletion of the functional *VvmybA1* allele. *Biosci. Biotechnol. Biochem*, 70 (6), 1506-1508.
- Hocquigny S., Pelsy F., Dumas V., Kindt S., Heloir MC., Merdinoglu D. (2004): Diversification within grapevine cultivars goes through chimeric states. *Genome* 47:579–589
- Kobayashi S., Ishimaru M., Ding CK., Yakushiji H., Goto N (2001): Comparison of UDP-glucose:flavonoid 3-O-glucosyltransferase (UFGT) gene sequences between white grapes (*Vitis vinifera*) and their sports with red skin. *Plant Sci.*, 160: 543–550.
- Kobayashi S., Yamamoto NG., Hirochika H. (2005): Association of *VvmybA1* gene expression with anthocyanin production in grape (*Vitis vinifera*) skin-color mutants. *J Jpn Soc Hort Sci* 74: 196–203
- Németh M (1973): Régi magyar borszőlőfajták. *Agrobotanika*. 15, 37-55.
- Yakushiji, H., Kobayashi, S., Goto- Yamamoto, N., Jeong, S.T., Sueta, T., Mitani, N., Azuma, A. (2006) A skin color mutation of grapevine, from black-skinned Pinot noir to white-skinned Pinot blanc, is caused by deletion of the functional *VvmybA1* allele. *Biosci. Biotechnol. Biochem*, 70 (6), 1506-1508.
- Regner, F.; Stadlbauer, A.; Eisenheld, C.; Kaserer, H. (2000): Genetic relationship among Pinot and related cultivars. *Am. J. Enol. Vitic.* 51, 7-14.
- This P., Cadle-Davidson M., Lacombe T., Owens CL. (2007): Wine grape (*Vitis vinifera* L.) color associates with allelic variation in the domestication gene *VvmybA1*. *Theor. Appl. Genet.* 114:723–730.