

Research Note

Investigation of *VvMybA1* and *VvMybA2* berry color genes in ‘Aglianico’ biotypes

G. DE LORENZIS¹, D. CARRASCO², R. ARROYO-GARCIA²,
M. ROSSONI¹, G. S. DI LORENZO¹ and O. FAILLA¹

¹) Dipartimento di Scienze Agrarie ed Ambientali, Università degli Studi di Milano, Italy

²) Departamento de Biotecnología, CBGP-INIA, Campus de Montegancedo, Pozuelo de Alarcón, Madrid, Spain

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Introduction: ‘Aglianico’ is a red grape cultivar widespread in Southern Italy renowned for the quality of its wines. It is grown in several Italian regions, but it is mainly cultivated in Campania (Taburno and Taurasi areas) and Basilicata (Vulture area). This variety is well defined from the ampelographic point of view, however, differences in the grape winemaking potential among the traditional ‘Aglianico’ growing areas, possibly also due to different biotypes locally selected, are claimed. One of them is the level of anthocyanin accumulated in the berries during the ripening.

Anthocyanins are synthesised in grape berries following the same multi-branched phenylpropanoid pathway described in many plant species (BOSS *et al.* 1996). The key enzyme of this pathway, regulated by Myb-related genes, is the UDP glucose-flavonoid 3-O-glucosyl transferase (*UFGT*; BOSS *et al.* 1996, KOBAYASHI *et al.* 2002). It was shown that a retrotransposon-induced mutation in *VvMybA1* gene (*Gret1* retrotransposon) and a single nucleotide polymorphism (SNP) in *VvMybA2* gene are associated with the loss of pigmentation in white skinned cultivars of *Vitis vinifera* (KOBAYASHI *et al.* 2004, WALKER *et al.* 2007).

Since the anthocyanins are crucial compounds determining the quality of grapes and wine, the purpose of this work was to investigate the differences in anthocyanin level in three closely related ‘Aglianico’ biotypes (= putatively different clonal lines, belonging to the same cultivar, distinguishable by some morphological traits mainly referred to the bunch and leaf morphology) from the genetic point of view and to correlate the genetic profile to the anthocyanins profile.

Material and Methods: The analyses were carried on three biotypes of ‘Aglianico’ (Taurasi, Taburno and Vulture) located in an experimental vineyard (Galluccio municipality, Caserta province, Italy).

In order to determine the differences in the anthocyanin content among the ‘Aglianico’ biotypes, triplicate berry samples for each biotype at ripening time were collected during the 2008 season and the total anthocyanin content and the anthocyanin profile were determined (RUSTIONI *et al.* 2011). Statistical analysis was performed with the SPSS 19.0 statistical software.

From the genetic point of view, the detection of functional and non-functional alleles for *VvMybA1* (*Gret1* insertion and other length polymorphisms) and gene polymorphism (R44 and C22 SNP) for *VvMybA2* were performed, as well as the sequencing of *VvMybA1* promotor region and part of the coding sequence (CARRASCO *et al.* 2015). The *VvMybA1* sequences were aligned using the NCBI BLAST tool and the indexes of genetic diversity among them were investigated by DnaSP 5.10 software.

Results and Discussion: The total anthocyanin content in three ‘Aglianico’ biotypes (Taburno, Taurasi and Vulture) measured at ripening time showed statistically significant differences in ‘Aglianico’ Vulture biotype. The level of anthocyanins accumulated in ‘Aglianico’ Vulture berries reached 650 mg·kg⁻¹ of grapes and in Taburno and Taurasi biotypes 580 mg·kg⁻¹ of grapes.

The average anthocyanin profile of ‘Aglianico’ resulted to be mainly composed of 82 % malvidin, followed by 6 % delphinidin, 7 % petunidin, 5 % peonidin and less than 1 % cyanidin; acylated and *p*-coumarated anthocyanins reached about 21 % and 1 %, respectively, of total anthocyanins. The data are consistent with anthocyanin profiles reported in MATTIVI *et al.* (2006). The anthocyanin profile detected per biotype revealed that ‘Aglianico’ Taburno showed a very high proportion of malvidin (77 %), a low proportion of delphinidin, petunidin and peonidin (8 %, 9 % and 5 % respectively) and a very low proportion of cyanidin (1 %). Taurasi and Vulture biotypes showed a very similar anthocyanin profile, having even higher proportion of malvidin (84 %) in comparison to ‘Aglianico’ Taburno, a low proportion of delphinidin, petunidin and peonidin (4 %, 6 % and 6 % respectively) and a very low proportion of cyanidin (less than 1 %). The ‘Aglianico’ Taburno anthocyanin profile showed statistically significant differences from the other biotypes.

The profiles of functional and non-functional alleles of *VvMybA1* and *VvMybA2* in three ‘Aglianico’ biotypes are shown in the Table. Concerning *VvMybA1*, the analysis detected the non-functional allele, characterized by the insertion of *Gret1* retrotransposon in the promotor region of *VvMybA1* gene (*VvMybA1a* allele), for ‘Aglianico’ Taburno and Taurasi biotypes, and the functional allele (*VvMybA1c*) characterized by no insertion in the promotor region, for all the three biotypes. The SNaPshot analysis detected the functional allele G for the R44 *VvMybA2* locus over all the three biotypes, while for the C22 *VvMybA2* locus, the samples revealed different alleles. The locus was polymorphic for ‘Aglianico’ Taburno and Vulture biotypes (G and T nucleotides were detected) and monomorphic for ‘Aglianico’ Taurasi (only T nucleotide was detected). The allelic profiles of *VvMybA1* and *VvMybA2* genes were consistent with the berry color of ‘Aglianico’ cultivar,

according to the literature (KOBAYASHI *et al.* 2004, WALKER *et al.* 2007).

The sequencing of *VvMybA1* promotor region and part of coding sequence (*VvMybA1c* allele) was performed per each ‘Aglanico’ biotype. The amplicons produced an 846 bp sequence corresponding to GSVIVT000226590001 isogene. The comparison with other published sequences (FOURNIER-LEVEL *et al.* 2010), revealed that the *Gret1* insertion was at the same position, as well as introns and exons. The genetic diversity indexes, used to characterise the levels of diversity among the analysed biotypes, revealed five polymorphic sites arranged in two haplotypes, one for ‘Aglanico’ Taurasi and Taburno biotypes and the other one for ‘Aglanico’ Vulture biotype. All the polymorphic sites were synonymous substitutions.

The genetic results suggested that the number of functional alleles affects the capability of anthocyanin accumulation in grape berry skin, as demonstrated by other studies (FOURNIER-LEVEL *et al.* 2010, AZUMA *et al.* 2011). This could be one of the reasons whereby the ‘Aglanico’ Vulture biotype had the capability to accumulate higher anthocyanin level in berry skin.

Since the regulation of biosynthetic anthocyanin pathway in *V. vinifera* is coordinated by transcriptional control of structural genes and also post-transcriptional control was reported for same genes, it could be reasonably speculated that the differences of anthocyanin metabolism in the ‘Aglanico’ biotypes are not only due to the polymorphism of *Myb* genes, but some other mechanisms, including differential expression of transcriptional factors or transporters, could better help to comprehend the scenario.

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- AZUMA, A.; UDO, Y.; SATO, A.; MITANI, N.; KONO, A.; BAN, Y.; YAKUSHIJI, H.; KOSHITA, Y.; KOBAYASHI, S.; 2011: Haplotype composition at the color locus is a major Genetic determinant of skin color variation in *Vitis x labruscana* grapes. *Theor. Appl. Genet.* **122**, 1427-1438.
- BOSS, P. K.; DAVIES, C.; ROBINSON, S. P.; 1996: Anthocyanin composition and anthocyanin pathway gene expression in grapevine sports differing in berry skin colour. *Aust. J. Grape Wine Res.* **2**, 163-170.
- CARRASCO, D.; DE LORENZIS, G.; MAGHRADZE, D.; REVILLA, E.; BELLIDO, A.; FAILLA, O.; ARROYO-GARCÍA, R.; 2015: Allelic variation in the *VvMYBA1* and *VvMYBA2* domestication genes in natural grapevine populations (*Vitis vinifera* subsp. *sylvestris*). *Plant Syst. Evol.* **301**, 1613-1624.
- KOBAYASHI, S.; GOTO-YAMAMOTO, N.; HIROCHIKA, H.; 2004: Retrotransposon-induced mutations in grape skin color. *Science* **304**, 982-982.
- KOBAYASHI, S.; ISHIMARU, M.; HIRAOKA, K.; HONDA, C.; 2002: Myb-related genes of the Kyoho grape (*Vitis labruscana*) regulate anthocyanin biosynthesis. *Planta* **215**, 924-933.
- FOURNIER-LEVEL, A.; LACOMBE, T.; LE CUNFF, L.; BOURSQUOT, J.M.; THIS, P.; 2010: Evolution of the *VvMYBA* gene family, the major determinant of berry colour in cultivated grapevine (*Vitis vinifera* L.). *Heredity* **104**, 351-362.
- MATTIVI, F.; GUZZON, R.; VRHOVSEK, U.; STEFANINI, M.; VELASCO, R.; 2006: Metabolite Profiling of Grape: Flavonols and Anthocyanins. *J. Agric. Food Chem.* **54**, 7692-7702.
- RUSTIONI, L.; ROSSONI, M.; CALATRONI, M.; FAILLA, O.; 2011: Influence of bunch exposure on anthocyanins extractability from grapes skins (*Vitis vinifera* L.). *Vitis* **50**, 137-143.
- WALKER, A. R.; LEE, E.; BOGS, J.; McDAVID, D. A. J.; THOMAS, M. K.; ROBINSON, S. P.; 2007: White grapes arose through the mutation of two similar and adjacent regulatory genes. *Plant J.* **49**, 772-785.