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# GENETIC ANALYSIS AND QTL MAPPING FOR FRUIT SKIN ANTHOCYANIDIN IN GRAPE (*VITIS VINIFERA*)

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**Abstract**

In this study, an F1 population was created by the cross '87-1'×'9-22'. The female parent '87-1' was a black purple cultivar and the male parent was an excellent breeding line with green pericarp. the skin color separation of population and distribution, and determined the content of each individual fruit peel pigment. On the basis of the genetic map of Vitis vinifera L., We carried out the grape skin pigment content quantitative trait locus (QTL) analyses. The results show that the fruit color performance for continuous variation and the inheritance of fruit skin anthocyanidin content was a quantitative inheritance. The color of offspring ranges from green and black-blue and existing distribution. Using SSR and SRAP molecular markers to construct 188 female parent maps,175 male parent maps and 251 consensus maps, and the total map distance is 1047.5 cM,1100.2 cM and 1264.2 cM respectively. The result of QTL showed that there were more QTLs exist in the linkage group of 1, 2, 3, 4, 9, 13, 14, 16 and 19 and in the linkage group of 3, 4, 13 and 14, we detected QTLs in the similar position with the result of the study in the year of 2011 and 2012, and based on this we will conduct the fine QTL location in the future, this result will lay a good foundation for the grape in the department of molecular assistant breeding in the future.

**Key words:** *Grapes; Anthocyanidin; Genetic*; QTL.

## Introduction

Grapevine is an ancient economic crop of great importance, and is almost cultivated through out the world. Coloring of grape berry is vitally important for commercial production in both table grape and wine making (Li *et al*., 2004). Clarification on genetics of berry color is convenient for obtaining grape cultivars of various colors. With respect to the inheritance of grape berry color, most of the researchers believed that it was controlled by 2 pairs of genes. Black and red are dominant over white, and black is dominant over red. White berry is controlled by two recessive genes (Shen *et al*., 1985; Zhao *et al*., 1988). Others considered that grape berry color was controlled by one pair of genes. White is controlled by two recessive genes, and other colors are dominant over white (Milutinovic *et al*., 2000). Li *et al*. (2004) found that inheritance of anthocyanidin content in pericarp was a quantitative trait showing continuous variation, so as to infer that the inheritance of grape berry color might be controlled by major gene as well as minorgene-the major gene controlled the existence or inexistence of color, and the minorgene controlled the shade of color. The inheritance of grape berry color is complicated. Along with the traditional quantitative genetics, molecular marker technique and QTL detection study for anthocyanidin would be convenient for revealing the molecular mechanism of grape berry genetics.

Great progress has been made in genomics, transcriptomics and metabonomics (Di Gaspero *et al*., 2010; Vidal *et al*., 2010; Yu *et al*., 2012), which lays a solid groundwork for grape molecular genetic study and for molecular breeding for grape anthocyanidin. Since the release of the first Vitis genetic map (Lodhi *et al*., 1995), lots of genetic maps have been constructed by grape researchers according to their goal (Riaz *et al*., 2004; Adam-Blondon *et al*., 2004; Fischer *et al*., 2004; Lowe & Walker 2006; Moreira *et al*., 2011; Blasi *et al*., 2011; Liu *et al*., 2013; Guo *et al*., 2015). Huang *et al*. (2012) firstly located markers related to grape pericarp synthesis on chromosome 1, 11, 13 and 15. Seeking for molecular markers close to anthocyanidin content is of great significance in fine mapping of genes and map-based cloning, and also in marker assisted selection so as to improve breeding efficiency. In this study, we applied two types of molecular marker--SSR and SRAP to construct genetic maps, based on which we carried out QTL analysis for pericarp anthocyanidin content. Findings of molecular markers closely linked to pericarp anthocyanidin content would provide some evidence for further molecular assisted breeding.

## Materials and Methods

**Plant materials:** F1 population derived from '87-1'× '9-22' was created in 2007. The maternal parent '87-1' produces black purple berries, while the paternal parent '9-22' produces yellow green berries. One hundred forty-nine progenies were randomly selected as the mapping population. Along with the 2 parents, molecular marker analysis and genetic map construction were carried out. Experiment was done in Molecular Biology Lab, Pomology Department, Shenyang Agriculture University. Plant materials were all kept in Grape Breeding Nursery, Shenyang Agriculture University. the mean annual precipitation here is 714 mm, mean temperature is 8.0$^{o}$C， the mean annual sunshine time is 2372.5 hours, the mean annual accumulated temperature is 3908$^{o}$C, the frost-free season is between 146 to 163 days.

Description of pericarp color and measurement of anthocyanidin content: According to Grape germplasm resource description specification and data standards (Liu *et al*., 2006), berries reaching commercial ripening were picked from well developed vines for pericarp color description. 1 g of pericarp was used for anthocyanidin extraction. Samples were dried by filter paper, then were grinded in 1% HCl-75% C2H5OH extract (solid-liquid ratio=1:5). Then the liquid was transferred into 10 ml centrifuge tube, going through 20 min of 40 Hz ultrasound under 60$^{o}$C (Miao *et al*., 2009). The content of anthocyanidin was determined by pH differential analysis.

DNA extraction: Genomic DNA was isolated by modified CTAB method (Hanania *et al*., 2004). The concentration and quality were determined by ultraviolet spectrophotometer and agarose gel electrophoresis. And the samples were diluted into 10 ng/ul, and were stored at -20°C. Genetic map construction and QTL analysis: SSR primers were from http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=2976. SSR primers of VLG series were designed by us on the basis of Vitis genome sequences (http://www.genoscope.cns.fr/externe/Genome-Browser/Vitis/)(Table 1). SRAP primers were from Li and Quiros (2001). All primers were synthesized in Dingguo Biotechnology Company, Beijing.

The total volume of SSR amplification reaction system was 16 ul, including 10 ng of template DNA, 0.3 umol/L primer, 0.1 mmol/L dNTPs, 2.0 mmol/L Mg2+ and 0.8U Taq DNA polymerase. The PCR protocols was as follows: 4 min at 94°C followed by 24 cycles of 1 min at 94°C, 1 min at annealing temperature, 1 min at 72°C followed by a final step of 10 min at 72°V.

The total volume of SRAP amplification reaction system was 16 ul, including 10 ng of template DNA, 0.5 umol/L primer, 0.1 mmol/L dNTPs, 2.0 mmol/L Mg2+ and 1.5U Taq DNA polymerase. The PCR protocols was as follows: 5 min at 94°C followed by 5 cycles of 1 min at 94°C, 1 min at 35°C, 1.5 min at 72°V followed by 35 cycles of 1 min at 94°C, 1 min at 50°C, 1.5 min at 72°C followed by a final step of 10 min at 72°C. All amplification products were separated by 5% polyacrylamide gel electrophoresis, then were detected by silver stain.

Molecular marker data was analyzed using software Joinmap 3.0 (LOD=4.0-6.0, the maximum recombination value=0.4). Maternal parent segregating data and common data for both parents were joint into one data set. Paternal segregating data and common data for both parents were also jointed into one data set. Recombination rate was transferred into map distance (cM, Kosambi function). Maps for both parents were drawn by software Mapchart 2.2. Linkage groups were named following IGGP (http://www.vitaceae.org) and international reference map (Doligez *et al*., 2006). QTL analysis was done by MapQTL 5.0. through Interval Mapping (IM). Parameters such as contribution rate of site to phenotype were evaluated. The existence of QTL was assured when LOD ≥3.0.

## Results

**Genetic analysis of pericarp color:** The cross between '87-1' with purple black pericarp and '9-22' with green pericarp generated a widely separated F1 population, among which the pericarp color ranged from light yellow to black blue, showing many intermediate color. Among the 149 progenies used for genetic mapping, 9 generated yellow pericarp, 67 green, 16 pink, 15 red, 35 purplish red and 7 blue black (Fig. 1). The separation of pericarp color indicated that it was a quantitative trait with continuous variant. However, it didn't match the model of classic quantitative genetics, because the ratio of color to non-color (73:76) was 1:1, which may be caused by some major gene.

**Genetic map construction:** From the tested 468 SSR primer pairs and 30 SRAP primer pairs, we chose those that can produce clear and polymorphic bands for population amplification. Totally, we got 97 markers specific to female parent, 81 markers specific for male parent and 141 common markers for both parents. 238 markers were used for the construction of female parent '87-1' map in which 188 markers covered a total length of 1074.5 cM. The average linkage group length was 56.6 cM. The longest linkage group (111cM) is LG19 containing 19 markers. 222 markers were used for the construction of male parent '9-22' map in which 175 markers covered a total length of 1100.2 cM. The average linkage group length was 57.9 cM. The longest linkage group (107.4cM) is LG12 containing 14 markers. Using all of the 319 markers to construct the genetic map shared by the two parents, at last 251 markers were added into the genetic map which has the total length of 1264.2cM, these markers constituted 19 linkage groups, the average length of the groups was 66.5cM, and the average distance between each two markers was 5.0 cM (Table 2).

The order of most co-dominant markers in our map was the same with that of the international reference map, except for several marker inversions and absences. According to the information of Vitis genome sequence, SSR markers of VLG series designed by us appeared on LG1 in our map.

**Table 1. SSR primers sequences.**

| Primer name | Forward primer sequence( 5'-3') | Reverse primer sequence( 5'-3') |
|---|---|---|
| VLG101 | TGGACACACACACACACACA | GCATGTGCTCACTGATGCTT |
| VLG102 | TATCAGGGCTTTGCGTAACC | TGTGCAACACTGCAAACAAA |
| VLG103 | GTTACCAAACAGGGCTAGGG | CATGAAGAAGGGTTGCCAGT |
| VLG104 | TGCTTCTCGAGTTCCCTTTT | CCTGTTAGAACCAAAGAAGACCA |
| VLG105 | TTCATTTGAGAACCGGAATC | CTCCAAAGTCCCAATTTTCA |
| VLG106 | TGGAATACGAGGGGAGTCTG | CTGATGGTGGGAAGAAAAGC |
| VLG107 | CCAGAGTGCCATCAGAATCC | CATTGAAGTTTGGGGAGGAA |
| VLG108 | CCCCTCAAAGAATCAATAGACC | AGTGCAGTGACACCAGCAAC |
| VLG109 | CATCAAAATATGCCCCAGCTA | CCTGTCCACAGACCGTGTTT |
| VLG110 | TAGACGGTCAGTGTGCAAGC | CCGCAATTATGAAGCGTTCT |
| VLG111 | CCCAGAAATATCTTAAGGGATGG | ATGTGTGCGCCTGTACCATA |
| VLG112 | ATTGCTTTTGTGTGGAGGAA | CAGGGAGCCCTTTGCATTAT |
| VLG113 | TCTGACTGACATTACACCGATTC | TCTGTTCACATCACACCCAAT |
| VLG114 | CCCATGGAGATTGATTGAGG | TTCAAGTGGACAATGAAGCAAC |
| VLG115 | CAAGTTGCAGAAGTGGCTGA | CCTCTTCTTCCCCATCAACA |
| VLG116 | TCAAGAACAGACGGAAACCA | AGGGCCTTCAATGCTCTACA |
| VLG117 | CCTGCCAATAAAGAACCCATT | TCAAGTGCCAAATCATCAGG |

Fig. 1. The fruit color performance of parents and F1 generation.

**Table 2. Main characteristics of linkage groups in the integrated, the maternal '87-1' and the paternal '9-22' maps.**

| LGs | Map of female parent '87-1' | | Consensus map | | Map of male parent '9-22' | |
|---|---|---|---|---|---|---|
| | Covered length (cM) | No. of markers | Covered length (cM) | No. of markers | Covered length (cM) | No. of markers |
| 1 | 42 | 11 | 51.8 | 17 | 43 | 15 |
| 2 | 20.4 | 5 | 20.3 | 5 | 14.9 | 4 |
| 3 | 58.2 | 9 | 64.2 | 19 | 53.3 | 13 |
| 4 | 80.6 | 14 | 83.5 | 14 | 71 | 10 |
| 5 | 78 | 13 | 74.6 | 14 | 45 | 9 |
| 6 | 37.9 | 13 | 84.7 | 19 | 100.8 | 8 |
| 7 | 33 | 7 | 40.7 | 14 | 35.8 | 5 |
| 8 | 70.2 | 8 | 88.8 | 12 | 88.6 | 10 |
| 9 | 92.4 | 13 | 130.1 | 13 | 72 | 10 |
| 10 | 99 | 13 | 107.8 | 17 | 88.9 | 12 |
| 11 | 8.9 | 3 | 33.6 | 7 | 24.8 | 4 |
| 12 | 48.8 | 8 | 70 | 16 | 107.4 | 14 |
| 13 | 84.2 | 6 | 112 | 12 | 75.7 | 9 |
| 14 | 85.9 | 17 | 85.7 | 18 | 51.5 | 12 |
| 15 | 15 | 4 | 42.3 | 7 | 42.3 | 6 |
| 16 | 33.7 | 11 | 33.1 | 12 | 33 | 11 |
| 17 | 27.5 | 5 | 29.1 | 6 | 28.8 | 5 |
| 18 | 47.8 | 9 | 47.7 | 9 | 48.4 | 4 |
| 19 | 111 | 19 | 80.2 | 20 | 75 | 14 |
| Total | 1074.5 | 188 | 1264.2 | 251 | 1100.2 | 175 |
| Average | 56.6 | 9.9 | 66.5 | 13.2 | 57.9 | 9.2 |

**QTL detection analysis:** The content of anthocyanidin in pericarp for the mapping population has been measured for 2 successive years (Table 3). Thus, QTL detection has been done based on consensus map data and anthocyanidin phenotype data from 2 years. In 2011, 13 QTLs related to anthocyanidin content had been found. They were allocated into 10 linkage groups, and the phenotypic variation a single QTL could explain ranged from 19.3% to 60.4%. In 2012, we found 17 QTLs allocating into 12 linkage groups. The phenotypic variation a single QTL could explain was from 52.2%to 75.8%. More QTLs had been found on LG 1, 2, 3, 4, 9, 13, 14, 16 and 19 (Figs. 2 and 3). QTLs have been detected at similar locations on LG3, 4, 13 and 14 in both 2011 and 2012.

## Discussion

Referring to the inheritance of grape pericarp color, some researchers believe that it was controlled by two pairs of genes (Shen, 1985; Zhao, 1988), while others believed it was controlled by one pair (Milutinovic *et al*., 2000). The study of Li *et al*. (2004) showed that grape pericarp color was quantitative trait. By the analysis of large amount of hybridization progenies used in this study, the grape pericarp color expressed a continuous variation, with the color ranging from yellowish green to blue-dark. And also, we've obtained many QTLs of pericarp anthocyanidin content. So we take grape pericarp color for quantitative trait controlled by not only major genes.

With the help of genetic maps and QTLs, researchers could confirm the numbers and locations of factors which control the quantitative traits (Staub *et al*., 1996). So they are of great importance in analyzing genetic regularity, carrying out marker assisted selection and enhancing breeding efficiency. Up to now, numbers of grapevine genetic maps have been published including both intraspecific (Riaz *et al*., 2004; Adam-Blondon *et al*., 2004) and interspecific (Fischer *et al*., 2004; Lowe *et al*., 2006; Moreira *et al*., 2011) maps of vinifera as well as other Vitis species (Blasi *et al*., 2011; Liu *et al*., 2013). Some QTLs of important traits have been located. In this study, an intraspecific mapping population was created by the cross of '87-1' (a special cultivar in China, extremely early ripening, with strong flavor, purple pericarp) and '9-22' (a breeding line, mid-late ripening, with no flavor, green pericarp). Genetic maps of high density were constructed. Some linkage groups were splited into two parts, and some gaps still existed, but the map covered 19 chromosomes of vitis genome, so it can be used in QTL detection. The VLG markers designed by us were included in the map, meaning that more EST-SSR and SNP markers should be developed to supplement the shortcomings of the map. More over, 119 SRAP markers were added into the SSR-based vinifera map (Table 2). These SRAP markers lengthened the linkage groups, increased the marker density and filled up some gaps between SSR markers.

Grape pericarp color was mainly controlled by ingredients and contents of anthocyanidin (Zohary *et al*., 1975; He, 1999). Researchers have focused on biosynthetic pathway and regulation mechanism of anthocyanidin (Goto-Yamamoto *et al*., 2002; Bogs *et al*., 2006; Ageorges *et al*., 2006; Waters *et al*., 2005; Petit *et al*., 2007). Some structural and regulator genes have also been cloned from different vitis species and cultivars. These genes are valuable for further SNP association of relative traits so as to uncover the genetic mechanism of pericarp color.

Huang *et al*. (2012) reported 40 QTLs relating to grape pericarp anthocyanidin synthesis, and they mainly located in LG1, 11, 13 and 15. Here we consistently detected some QTLs on LG1, 11 and 13. But the genes are to be mutually verified due to the different marker types used. Other QTLs were found in LG3 and LG4 in our study but in LG15 in Huang's study, that might be because of the population difference (wine cultivars in Huang's study but table grapes in our study). To illuminate the function of these loci, fine mapping is needed. Along with transcriptom and association analysis, genes relating to pericarp anthocyanidin synthesis were to be found precisely and comprehensively.

**Table 3. The results of QTL mapping for fruit skin anthocyanidin in Grape (*Vitis vinifera*).**

| | Linkage group | QTL | LOD | Flanks markers | Peak (cM) | Nearest marker (cM) | R2 (%) |
|---|---|---|---|---|---|---|---|
| 2011 | LG1 | *sesu1* | 4.81 | m1e19M-491,VMCNG2G7 | 16.6 | 3 | 54.7 |
| | LG2 | *sesu 2* | 4.94 | VMC8C2 | 3.2 | 0 | 19.3 |
| | LG3 | *sesu 3* | 4.76 | VMC1A5,UDV043 | 22.4 | 2.4 | 58.5 |
| | LG4 | *sesu 4* | 5.83 | UDV034,VMCNG1F1-A | 70.3 | 6 | 58.4 |
| | LG9 | *sesu 5* | 6.11 | m10e17M-190, VMC3G8-2 | 7 | 7 | 53 |
| | LG13 | *sesu 6* | 7.9 | VMC2C7-A1-A, m20e17F-432 | 67.4 | 2.5 | 51.8 |
| | LG13 | *sesu 7* | 5.53 | m16e15F-190, m16e15C-180 | 102 | 5 | 56.4 |
| | LG14 | *sesu 8* | 5.87 | VRZAG112, VMC5B3 | 10.2 | 2 | 58.6 |
| | LG14 | *sesu 9* | 4.74 | VMC5B3, UDV033 | 16.1 | 1.2 | 60.4 |
| | LG16 | *sesu 10* | 5.67 | m16e15M-270, VMC1E11 | 18.7 | 1 | 58.8 |
| | LG16 | *sesu 11* | 6.25 | VMC1E11-2, UDV116 | 23 | 1 | 58.7 |
| | LG17 | *sesu 12* | 3.57 | VVIB09, UDV092 | 20.5 | 7 | 55.3 |
| | LG19 | *sesu 13* | 4.92 | VMC5E9, UDV023 | 61.4 | 3 | 58.5 |
| 2012 | LG1 | *sesu 1* | 3.14 | VLG107, VLG102 | 32.5 | 1 | 59.8 |
| | LG2 | *sesu 2* | 3.99 | VMC5A7, VMC8E8 | 19 | 1.3 | 58.4 |
| | LG3 | *sesu 3* | 3.48 | VMC1A5, UDV043 | 20.4 | 2 | 75.8 |
| | LG4 | *sesu 4* | 3.28 | UDV034, VMCNG1F1-A | 71.3 | 5.3 | 63 |
| | LG7 | *sesu 5* | 5.22 | m10e23M-236, m10e21M-155 | 9.2 | 3.8 | 52.2 |
| | LG9 | *sesu 6* | 3.25 | VMC3G8-2, VVIU37 | 28.6 | 7 | 62.1 |
| | LG9-1 | *sesu 7* | 4.35 | VMC4D3, m8e15C-272 | 17 | 3.1 | 57.1 |
| | LG10 | *sesu 8* | 6.87 | m17e12C-308, UDV073 | 18.9 | 1.2 | 65 |
| | LG10 | *sesu 9* | 7.64 | VVIR21, VrZAG25 | 28.9 | 1 | 64.2 |
| | LG12 | *sesu 10* | 4.77 | VMC7F1, m3e19M-500 | 14.2 | 1 | 55.7 |
| | LG13 | *sesu 11* | 5.4 | m16e15F-190, m16e15C-180 | 109 | 2.5 | 59.2 |
| | LG14 | *sesu 12* | 3.59 | VRZAG112, VMC5B3 | 10.2 | 2.0 | 63.3 |
| | LG14 | *sesu 13* | 3.47 | UDV095, VVIV69 | 30 | 3 | 60.3 |
| | LG16 | *sesu 14* | 4.28 | m16e15C-411, UDV052 | 8 | 8 | 58.1 |
| | LG19 | *sesu 15* | 4.47 | m20e17C-118 | 1.6 | 0 | 59.6 |
| | LG19 | *sesu 16* | 3.86 | UDV031, m1e15F-87 | 12.7 | 1 | 61.9 |
| | LG19 | *sesu 17* | 4.1 | m6e19C-520 | 19.6 | 0 | 64.6 |

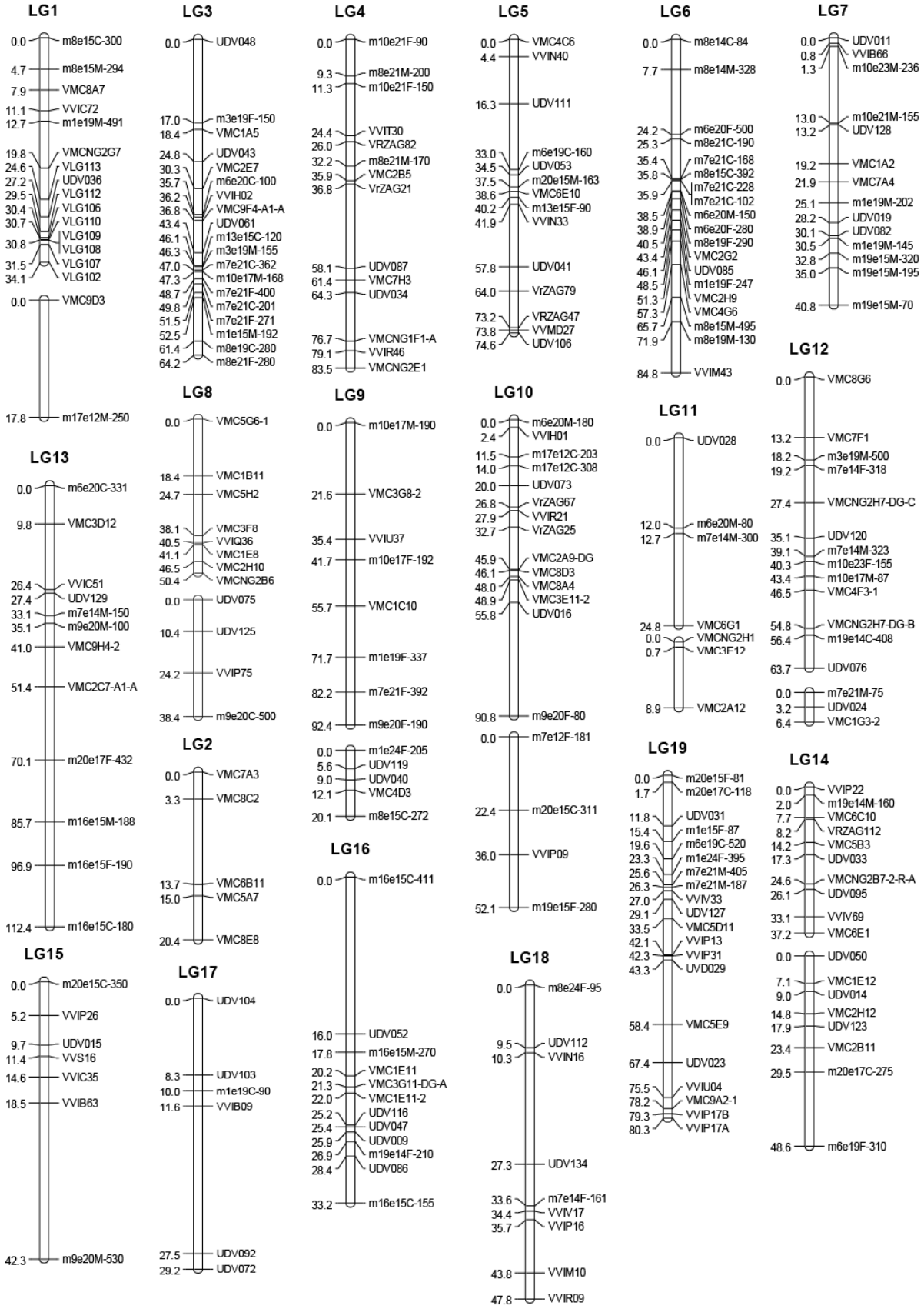


Fig. 2. Consensus map of *Vitis vinifera* 87-1 × 9-22. Linkage groups are numbered according to [Doligez *et al*., 2006].

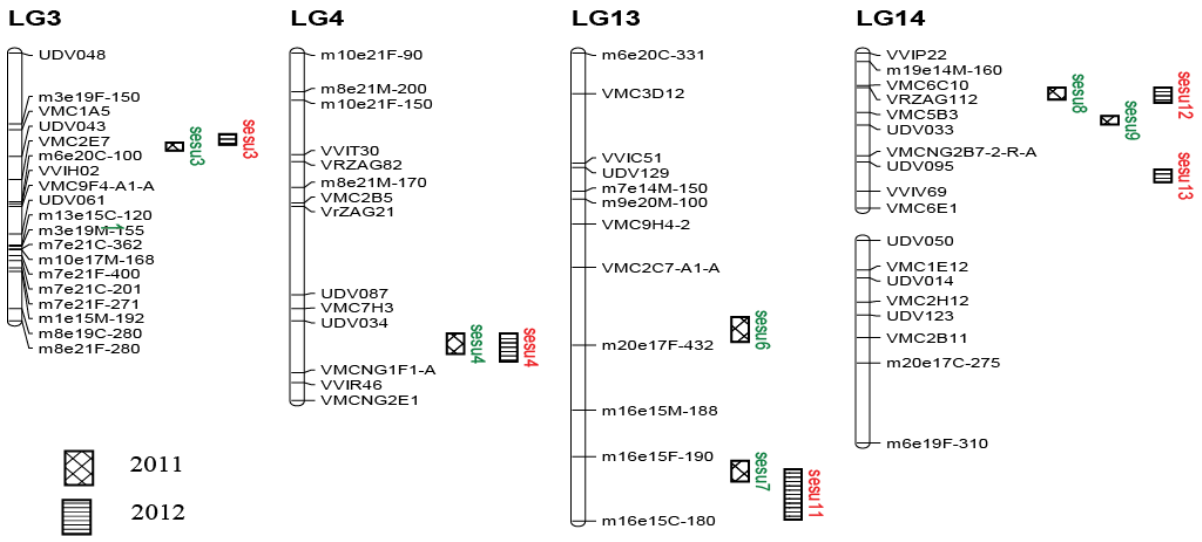


Fig. 3. The linkages group 3、4、13 and 14 with the QTL for fruit skin anthocyanidin in Grape.


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## References


Adam-Blondon, A.F., C. Roux, D. Claux, G. Butterlin, D. Merdinoglu and P. This. 2004. Mapping 245 SSR markers on the Vitis vinifera genome: a tool for grape genetics. *Theor. Appl. Genet*., 109(5): 1017-1027.

Ageorges, A., L. Fernandez, S. Vialet, D. Merdinoglu, N. Terrier and C. Romieu. 2006. Four specific isogenes of the anthocyanin metabolic pathway are systematically co-expressed with the red colour of grape berries. *Plant Science*, 170(2): 372-383.

Blasi, P., S. Blanc, S. Wiedemann-Merdinoglu, E. Prado, E.H. Rühl, P. Mestre and D. Merdinoglu. 2011. Construction of a reference linkage map of Vitis amurensis and genetic mapping of Rpv8, a locus conferring resistance to grapevine downy mildew. *Theor. Appl. Genet*., 123(1): 43-53.

Bogs, J., A. Ebadi, D. McDavid and S.P. Robinson. 2006. Identification of the flavonoid hydroxylases from grapevine and their regulation during fruit development. *Plant Physiology*, 140(1): 279-291.

Di Gaspero, G. and F. Cattonaro. 2010. Application of genomics to grapevine improvement. *Aus. J. Grape Wine Res*., 16:122-130.

Doligez, A., A.F. Adam-Blondon, G. Cipriani, G. Di Gaspero, V. Laucou, D. Merdinoglu, C.P. Meredith, S. Riaz, C. Roux, P. This. 2006. An integrated SSR map of grapevine based on five mapping populations. *Theor. Appl. Genet*., 113:369-382.

Fischer, B., I. Salakhutdinov, M. Akkurt, R. Eibach, K.J. Edwards, R. Toepfer and E.M. Zyprian. 2004. Quantitative trait locus analysis of fungal disease resistance factor on a molecular map of grapevine. *Theor. Appl. Genet*., 108: 505-515.

Goto-Yamamoto, N., G.H. Wan, K. Masaki and S. Kobayashi. 2002. Structure and transcription of three chalcone synthase genes of grapevine(Vitis vinifera). *Plant Science*, 162(6): 867-872.

Guo, Y.S., G.L. Shi, Z.D. Liu, Y.H. Zhao, X.X. Yang, J.C. Zhu, K. Li and X.W. Guo. 2015. Using specific length amplified fragment sequencing to construct the high-density genetic map for Vitis (*Vitis vinifera* L. × *Vitis amurensis* Rupr.). *Front. Plant Sci*., 6:393.

Hanania, U., M. Velcheva, N. Sahar and A. Pert. 2004. An improved method for isolating high-quality DNA from *Vitis vinifera* nuclei. *Plant Mol. Biol. Rep*., 22: 173-177.

He, P.C. 1999. Grape. Beijing、China Agriculture Press、8-32.

Huang, Y.F., A. Doligez, A. Fournier-Level, L. Le Cunff, Y. Bertrand, A. Canaguier and P. This. 2012. Dissecting genetic architecture of grape proanthocyanidin composition through quantitative trait locus mapping. *BMC Plant Biol*., 12(1): 30.

Li, G. and C.F. Quiros. 2001. Sequence-related amplified polymorphism (SRAP), A new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in Brassica. *Theor. Appl. Genet*., 103: 455-461.

Li, K., X.W. Guo, H.G. Xie, Y.S. Guo, C.X. Li and Y.H. Li. 2004. Inheritance of fruit skin Anthocyanidin in Grape Self-bred and crossed progenies. *J. Fruit Sci*., 2l(5): 406-408.

Liu, C.H., Y.J. Chen, J. Shen and J.N. Guo. 2006. Descriptot's and Data Standard for Grape (*Vitis* L.). Beijing: Agriculture Press.

Liu, Z.D., X.W. Guo, Y.S. Guo, H. Lin, P.X. Zhang, Y.H. Zhao, K.Li and C.X. Li. 2013. SSR and SRAP marker based linkage map of *Vitis Amurensis* Rupr. *Pak. J. Bot*., 45(1): 191-195.

Lodhi, M.A., M.J. Daly, G.N. Ye, N.F. Weeden and B.I. Reisch. 1995. A molecular marker based linkage map of *Vitis*. *Genome*, 38: 786-794.

Lowe, K.M. and M.A. Walker. 2006. Genetic linkage map of the interspecific grape rootstock cross Ramsey (*Vitis champinii*) × *Riparia Gloire* (*Vitis riparia*). *Theor. Appl. Genet*., 112:1582-1592.

Miao, Y. and S.J. Wu. 2009. Extraction of pigment from grape skin by ultrasonic wave. *Food Research and Development*, 30(3):190-192.

Milutinovic, M., D. Nikolic, L. Avramov and Rakonjac V. 2000. Recombination of some characteristics in F1 generation of grapevine. *Acta Hort*., 528: 64l-644.

Moreira, F.M., A. Madini, R. Marino, L. Zulini, M. Stefanini, R. Velasco, P. Kozma and M.S. Grando. 2011. Genetic linkage maps of two interspecific grape crosses (*Vitis* spp.) used to localize quantitative trait loci for downy mildew resistance. *Tree Genetics & Genomes*, 7: 153-167.

Petit, P., T. Granier, B.L. d'Estaintot, C. Manigand, K. Bathany, J.M. Schmitter and B. Gallois. 2007. Crystal structure of grape dihydroflavonol 4-reductase, a key enzyme in flavonoid biosynthesis. *J. Mol. Biol*., 368(5): 1345-1357.

Riaz, S., G.S. Dangl, K.J. Edwards and C.P. Meredith. 2004. A Microsatellite based framework linkage map of *Vitis vinifera* L. *Theor. Appl. Genet*., 108: 864-872.

Shen, D.X. 1985. *Fruit Tree Thrematology*. Beijing: Agriculture Press.

Staub, J.E. and F.C. Serquen. 1996. Genetic markers, map construction, and their application in plant breeding. *Hort. Sci*., 31(5): 729-741.

Vidal, J.R., C. Gomez, M.C. Cutanda, B.R. Shrestha, A. Bouquet, M.R. Thomas and L. Torregrosa. 2010. Use of gene transfer technology for functional studies in grapevine. *Aus. J. Grape Wine Res*., 16: 138-151.

Waters, D.L., T.A. Holton, E.M. Ablett, L.S. Lee and R.J. Henry. 2005. cDNA microarray analysis of developing grape (*Vitis vinifera* cv. Shiraz) berry skin. *Functional & Integrative Genomics*, 5(1): 40-58.

Yu, Y., Z.X. Lin and X.L. Zhang. 2012. Genome-wide identification of recombination rates of male versus female gametes in interspecific population of cotton. *Pak. J. Bot*., 44(2): 521-529.

Zhao, F.P. 1988. Deduce grape fruit color genotype. *Sino-Overseas Grapevine and Wine*, 2:1-4.

Zohary, D. and P.S. Roy. 1975. Beginnings of fruit growing in the old world. *Science*, 187: 319-327.

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