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ARTICLE

Anthocyanin Profiles in Grape Berry Skins of Different Species of Wine Grapes in Shanxi, China

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ABSTRACT

To understand the anthocyanin characteristics of wine grape varieties, the anthocyanin composition and content of 31 wine grape varieties were analyzed to explore the use of anthocyanins as chemical fingerprints to distinguish varieties. Results showed that a total of 21 anthocyanins were detected in the skins, including cyanidin, delphinidin, petunidin, peonidin and malvidin 3-monoglucosides (or 3,5-diglucosides) along with the corresponding acetyl and p-coumaroyl derivatives. The highest and lowest total amount of anthocyanins were detected in 'Ruby Cabernet' and 'Muscat Rouge', respectively. In the 21 *Vitis vinifera* grapes, there were 3~11 monoglucoside anthocyanins detected, however, there were 4 to 9 monoglucoside anthocyanins and 1~7 diglucoside anthocyanins detected in the 10 other species of grapes. Except for 'Zhesexiang' 'Seibel Noir', '44-6-7-1' and 'Beibinghong', the contents of diglucoside anthocyanins in the other six varieties accounted for more than 52% of the total anthocyanins. Except for 'Zhesexiang', 'Muscat Rouge' and 'Beibinghong', the content of methylated anthocyanins accounted for more than 75% of total anthocyanins. There were significant differences in the anthocyanin types and contents in the skins among *V. vinifera* and other grapes. The results of the principal component analysis and the cluster classification of 31 grape varieties (lines) were nearly consistent, which suggested that anthocyanins can be used as chemical fingerprints to distinguish wine grape varieties.

KEYWORDS

Anthocyanin; cluster analysis; principal component analysis; wine grape

1 Introduction

As the most important water-soluble pigments in plants, anthocyanins are widely found in the flowers and fruits of higher plants, which gives them different colors [1,2]. Grape is one of the most important fruit crops in the world, with a 2018 total surface of 7.4 million hectares and a production of 7.8 million tonnes, about 50% destined to wine production [3]. The anthocyanins in grape berry skin are one of the most important criteria to assess grape quality, especial the wine quality. The anthocyanins in grapes impart the color of red wines, which can also enter the wine from the peel through the process of pressing and fermentation [4]. The type and content of anthocyaniins not only play an important role in the color, flavor, taste but also contribute greatly to nutritional value of wine [5–7].

The anthocyanins in grapes are the glucoside forms of cyanidin (Cy), delphinidin (Dp), petunidin (Pt), peonidin (Pn), pelargonidin (Pg) and malvidin (Mv), along with the corresponding acetyl, p-coumaroyl, and caffeoyl derivatives in cultivars [8,9]. Varieties, environmental factors and agronomic measures all affect the anthocyanin content in grapes [5,10,11], but the proportions of individual anthocyanins are primarily determined by genotype, so the anthocyanin profiles can be used to distinguish red grapes and wines produced by different cultivars [12–14]. Liang et al. [5] analyzed the anthocyanin components of 110 grape varieties and found that the composition and content of anthocyanins in the grape skins of different species of the genus Vitis varies significantly. *Vitis vinifera* L. usually contains only mono-glucosides including Mv, Cy, Dp, Pn and Pt 3-monoglycosides with their corresponding acetyl, p-coumaroyl and caffeoyl derivates but no Pg anthocyanins [15]. Malvidin-3-O-glucoside is the chief anthocyanin in both grapes and wines of many European red vine varieties [16]. However, non- *Vitis vinifera* grape species, such as *V. labrusca*, *V. rupestris* and Chinese wild grape, contain 3,5-diglucosides and Pg-derived anthocyanins [5,17,18].

In this paper, 31 red wine grape varieties in Shanxi province of China were used as materials to determine the anthocyanin composition and content by high-performance liquid chromatography-mass spectrometry (HPLC-MS). The anthocyanin types in different grape varieties were compared. Cluster analysis and principal component analysis were carried out based on the anthocyanin content of each monomer to differentiate species by using anthocyanins as chemical fingerprints.

2 Materials and Methods

2.1 Materials and Sampling

The fruits of 31 wine grape varieties were collected from Pomology Institute, Shanxi Academy of Agricultural Science, Taigu (Taigu) and the National Grape Germplasm Resources (Taigu) during the fruit ripening period from August to October in 2017. Each variety chose the same growth plants, three plants were 1 plot and three replicates, a total of nine plants. The fruits were sampled from the middle of dragon, and 4 clusters were randomly collected from each plot (considering the shaded and sunny side of the ear, 2 clusters on both sides of the fence, a total 12 clusters). After sampling, the fruits were taken back to the laboratory, all berries were mixed, then the skins were quickly peeled off with tweezers under 4°C, frozen in liquid nitrogen and stored at −80°C. The anthocyanins were extracted and analyzed in the laboratory from November to December in 2017. The names of the test materials and the sampling times are shown in Supplementary Tab. 1.

2.2 Detection of Anthocyanins in Grape Skin

The anthocyanins in the skins were extracted and detected according to the method of Liu et al. [19]. The grape skins stored at −80°C were ground into powder using liquid nitrogen. Then 1.000 g powder were extracted with 5 mL of 1% hydrochloric acid-methanol under 30°C for 2 h in the dark. The liquid extract and powdered grape skin were separated by centrifugation at 9 000 r·min^{-1} for 10 min at 4°C. The grape powder was re-extracted for 3 times, and the liquid extract were combined, rotated and evaporated at 35°C. Then the residue was diluted to 5 mL using chromatographic grade methanol and stored at −40°C for testing.

The HPLC-MS analyses were carried out using a WATERS ACQUITY UPLC-PDA detector. Chromatographic separation was carried out using a C18 column (100 mm × 2.1 mm i.d. 1.7 μm) and thermostated at 45°C. The mobile phase was a linear gradient of water/formic acid (98:2) (solvent B) in acetonitrile (solvent A), at a flow rate of 0.3 mL·min^{-1}. The following elution gradient was used: 0–20 min, 6%–16% A; 20–28 min, 16%–23% A; 28–35 min, 23%–50% A; 35–37 min, 50% A;

37–40 min, 50%–60% A. Then the anthocyanins were identified using WATERS MALDI SYNAPT QTOF-MS. The operating parameters were: Capillary voltage, 3 000 V; cone voltage, 30.0 V; cone gas flow, 10 $L \cdot h^{-1}$; desolvation gas flow, 700 $L \cdot h^{-1}$; ion source temperature, 100°C; desolvation temperature, 400°C. The instrument was operated in a positive ion mode scanning from 50 to 2 000 $m \cdot z^{-1}$.

The anthocyanin contents were identified based on the HPLC-UV-MS fingerprint library of grape and wine anthocyanins [19]. The type of monomer anthocyanins was determined by using the full ion scanning spectrum of mass spectrometry through the quality spectrum analysis, retention time and comparative analysis of literature reports. The types of anthocyanin monomers detected in the grapes were shown in Supplementary Tab. 2. Anthocyanins were quantified using malvidin 3-O-glucoside as a standard according to Liu et al. [19].

2.3 Statistical Analysis

The data were reported as the mean ± standard error (S.E.) of three replicates. Analysis of significant differences was performed using SPSS 17.0 data analysis software at $p \leq 0.05$. Principal component analysis (PCA) and cluster analysis were performed to analyze the data for anthocyanins using SAS 8.0.

3 Results

3.1 Anthocyanin Contents in Grape Skins of 31 Varieties

The total anthocyanin contents in the skins of 31 grape varieties were significantly different, ranging from 36.793 to 3995.079 $mg \cdot kg^{-1}$ FW (Tab. 1). The highest contents of anthocyanins were observed in the skins of ‘Ruby Cabernet’ and ‘Seibel Noir’, while those in ‘Beibinghong’, ‘Beihong’ and ‘44-6-7-1’ were significantly lower than them. The contents in grape skins of ‘Petit Verdot’ and ‘Malbec’ were significantly lower than that of ‘Beibinghong’, but there were no significant differences between them and ‘Beihong’, ‘44-6-7-1’. The contents of ‘Mei Yu’, ‘Petit Verdot’ and ‘Malbec’ were not significantly different. But they were significantly higher than those of ‘Merlot181’ and ‘Cabernet Gernischt’. The total contents of anthocyanins in ‘Cabernet Sauvignon 169’, ‘Marselan c980’, ‘Syrah 100’, ‘Tempranillo’ and ‘Areni’ were 50.9%~55.5% of that in ‘Ruby Cabernet’ and ‘Seibel Noir’; while the ones in ‘Muscat Rouge’ and ‘Zhesexiang’ were significantly lower than those in other varieties, only 0.8% to 2.7% of the total contents in ‘Ruby Cabernet’ and ‘Seibel Noir’.

Table 1: The contents of different types of anthocyanins in the skins of 31 grape varieties ($mg \cdot kg^{-1}$FW)

Varieties	Delphinidins	Petunidins	Malvidins	Cyanidins	Peonidins	Total anthocyanins
Cabernet Franc327	102.381 ± 8.092ijk	95.329 ± 4.243hij	1119.966 ± 28.480hij	20.297 ± 1.917ijkl	167.030 ± 2.050fgh	1505.003 ± 12.177klm
Cabernet Sauvignon169	254.313 ± 6.143f	162.842 ± 2.054f	1503.547 ± 24.785ef	34.040 ± 1.584efg	153.927 ± 2.659ghi	2108.669 ± 28.738gh
Merlot181	324.339 ± 2.335e	258.314 ± 2.328de	1451.5719 ± 63.624fg	97.212 ± 1.066c	311.686 ± 16.003b	2443.123 ± 53.350e
Cabernet Gernischt	136.457 ± 3.829h	160.062 ± 0.361f	1934.229 ± 0.580c	11.577 ± 0.907lmn	109.292 ± 3.146j	2351.618 ± 1.808ef
Marselan c980	90.154 ± 7.046klm	104.119 ± 6.966ghi	1791.266 ± 6.045cd	–	48.514 ± 2.884klm	2034.053 ± 19.149ghi
Ruby Cabernet	608.144 ± 21.896b	430.195 ± 15.710bc	2804.578 ± 56.687a	41.116 ± 0.517e	111.046 ± 1.659j	3995.079 ± 96.469a

(Continued)

Table 1 (continued).

Varieties	Delphinidins	Petunidins	Malvidins	Cyanidins	Peonidins	Total anthocyanins
Meiyu	189.825 ± 30.291g	216.846 ± 27.358e	2252.252 ± 128.098b	11.673 ± 1.668lmn	126.689 ± 10.373ij	2797.284 ± 197.788d
Carinena	82.726 ± 37.376klm	101.257 ± 18.195ghij	643.695 ± 262.684k	4.706 ± 0.487n	227.942 ± 77.484de	1060.326 ± 266.380pq
Petit Verdot	275.275 ± 6.555f	257.098 ± 0.448de	2205.892 ± 118.928b	11.272 ± 2.271lmn	70.318 ± 8.265k	2819.855 ± 101.390cd
Malbec	195.080 ± 2.800g	259.387 ± 1.952de	2380.979 ± 92.490b	–	40.288 ± 1.844klmn	2875.734 ± 89.798cd
Blue French	36.963 ± 4.525op	52.104 ± 6.017jk	1105.866 ± 108.568hij	11.479 ± 1.920lmn	274.763 ± 29.284c	1481.174 ± 150.314lmn
Pinot Noir115	40.264 ± 1.913no	45.171 ± 0.824k	603.996 ± 26.921k	18.662 ± 0.563jklm	254.515 ± 12.351cd	962.607 ± 16.744qr
Syrah100	119.584 ± 19.154hij	154.332 ± 17.216f	1540.084 ± 129.568ef	28.796 ± 7.023fghi	197.372 ± 40.965ef	2040.168 ± 213.925ghi
Medoc Noir	92.018 ± 11.839kl	133.257 ± 20.635fgh	1054.045 ± 158.42j	16.897 ± 0.293klm	68.746 ± 1.923k	1364.963 ± 192.524mno
Muscat Hamburg	31.785 ± 3.515op	25.600 ± 3.165k	282.656 ± 26.735l	23.596 ± 1.851hijk	138.514 ± 0.920hij	502.151 ± 30.644s
Muscat Rouge	3.586 ± 0.261q	–	–	25.138 ± 1.286ghijk	8.069 ± 0.488n	36.793 ± 0.537t
Gamay Teinturier	27.770 ± 7.347opq	41.116 ± 10.230k	1087.36 ± 229.302ij	6.077 ± 0.891n	197.137 ± 14.458ef	1359.460 ± 262.227mno
Tempranillo	392.975 ± 25.500d	290.343 ± 17.351d	1271.470 ± 29.987ghi	65.013 ± 10.202d	140.700 ± 18.249hij	2160.501 ± 102.289fg
Canepabn	19.685 ± 1.689opq	19.933 ± 0.760k	632.775 ± 26.978k	10.183 ± 1.286mn	599.757 ± 42.269a	1282.333 ± 72.983no
Armenia	108.070 ± 3.270ijk	120.856 ± 2.083fgh	1419.053 ± 16.327fg	28.172 ± 3.009fghij	173.615 ± 11.457fgh	1849.765 ± 3.492ij
Areni	143.710 ± 0.248h	145.316 ± 1.867fg	1507.007 ± 31.363ef	36.339 ± 7.042ef	239.229 ± 37.929cd	2071.600 ± 11.493gh
Zhesexiang	10.795 ± 1.612pq	–	–	110.783 ± 12.536b	5.631 ± 0.018n	127.209 ± 14.166t
Seibel Noir	476.611 ± 18.826c	580.397 ± 1.253a	2650.154 ± 121.934a	–	181.793 ± 11.377fg	3888.955 ± 113.272a
Beibinghong	591.268 ± 6.383b	471.857 ± 21.681b	765.479 ± 48.349k	1239.924 ± 198.56a	25.909 ± 1.720opq	3094.438 ± 85.223b
Huapu 1	64.450 ± 11.150mn	57.865 ± 1.455ijk	711.513 ± 27.516k	–	10.235 ± 2.428n	844.063 ± 42.549r

(Continued)

Table 1 (continued).

Varieties	Delphinidins	Petunidins	Malvidins	Cyanidins	Peonidins	Total anthocyanins
Gongniang 2	67.956 ± 5.138lm	117.611 ± 6.638ghij	1509.068 ± 23.131ef	–	–	1694.635 ± 34.907jk
Beihong	695.520 ± 14.964a	394.714 ± 3.873c	1844.269 ± 34.960cd	32.804 ± 1.761efgh	55.902 ± 5.434kl	3023.210 ± 12.449bc
Beimei	69.683 ± 1.639lm	65.213 ± 0.595ijk	1081.135 ± 4.48ij	–	–	1216.031 ± 3.444op
2–1-3	98.751 ± 0.705jk	130.884 ± 5.909fgh	1696.674 ± 42.596de	–	–	1926.309 ± 49.210hi
44-6-7-1	199.199 ± 68.580g	412.150 ± 208.975c	2402.220 ± 533.103b	3.921 ± 0.130n	27.613 ± 2.092lmn	3045.103 ± 390.746bc
Moldova	129.155 ± 29.784hi	149.890 ± 31.790fg	1283.437 ± 132.042gh	–	16.336 ± 1.946mn	1578.819 ± 195.563kl

In this study, the anthocyanins were comprised of cyanidin (Cy), delphinidin (Dp), petunidin (Pt), peonidin (Pn) and malvidin (Mv) 3-monoglucosides (or 3,5-diglucosides) along with the corresponding acetyl and p-coumaroyl derivatives in the skins of grape. Pt and Mv were not detected in the skins of 'Muscat Rouge' and 'Zhesexiang', while in the other 29 varieties, the contents of Mv accounted for 24.7%~89.0% of total anthocyanins, the highest and lowest contents of Mv were observed in 'Seibel Noir' and 'Beibinghong', respectively (Tab. 1). In 'Cabernet Franc 327', 'Cabernet Sauvignon 169', 'Merlot 181', 'Ruby Cabernet', 'Petit Verdot', 'Muscat Hamburg', 'Tempranillo', 'Bei Binghong', 'Huapu 1', 'Beihong' and 'Beimei', the contents of Dp were 6.9%~76.2% higher than those of Pt; while in the other 18 varieties, the contents of Pt were 1.1%~73.1% higher than those of Dp. Cy was not detected in the skins of 'Gongniang 2', 'Beimei', '2-1-3', 'Marselan c980', 'Malbec', 'Seibel Noir', 'Huapu 1' and 'Moldova', while Pn was not detected in the skins of 'Gongniang 2', 'Beimei' and '2-1-3'. In 'Muscat Rouge', 'Zhesexiang' and 'Beibinghong', the contents of Cy were 3.1, 19.7, and 47.9 times those of Pn, while the contents of Pn in the other 20 varieties were 1.7 to 58.9 times those of Cy (Tab. 1). In 'Zhesexiang', 'Muscat Rouge' and 'Beibinghong', the methylated anthocyanins accounted for only 4.4%, 21.9%, 40.8% of the total anthocyanins, respectively; while in the other 28 varieties, the contents accounted for 75.9%~97.7% of the total anthocyanin contents, which showed that the methylated anthocyanins are the main anthocyanin type (Tab. 2). The highest and lowest proportion was observed in 'Saperavi' and 'Beihong', respectively.

Table 2: Anthocyanin profiles in grape skins of 31 varieties (mg·kg^{-1}FW)

Varieties	Diglucoside anthocyanins	Monoglucoside anthocyanins	Acylated anthocyanins	Coumarylated anthocyanins	Methylated anthocyanins
Cabernet Franc327	–	1505.003 ± 12.177g	28.420 ± 3.211ijk	251.156 ± 6.308lm	1382.325 ± 22.187lm
Cabernet Sauvignon169	–	2108.669 ± 28.738d	12.243 ± 0.479mn	167.349 ± 2.710no	1820.316 ± 24.179ghi

(Continued)

Table 2 (continued).

Varieties	Diglucoside anthocyanins	Monoglucoside anthocyanins	Acylated anthocyanins	Coumarylated anthocyanins	Methylated anthocyanins
Merlot181	–	2443.123 ± 53.350c	25.601 ± 2.608jk	313.497 ± 37.893kl	2021.572 ± 49.949ef
Cabernet Gernischt	–	2351.618 ± 1.808c	58.925 ± 20.307cd	602.204 ± 31.241efg	2203.583 ± 2.928de
Marselan c980	–	2034.053 ± 19.149de	30.245 ± 0.906ijk	590.346 ± 40.903fg	1943.899 ± 26.195fg
Ruby Cabernet	–	3995.079 ± 96.469a	63.557 ± 1.495bc	701.660 ± 25.123cd	3345.819 ± 74.056a
Meiyu	–	2797.284 ± 197.788b	50.718 ± 0.387de	754.627 ± 32.225bc	2595.786 ± 165.829c
Carinena	–	1060.326 ± 266.380i	46.636 ± 5.269ef	442.407 ± 56.555ij	972.894 ± 229.364op
Petit Verdot	–	2819.855 ± 101.390b	25.996 ± 2.069jk	411.976 ± 40.244j	2533.308 ± 110.216c
Malbec	–	2875.734 ± 89.798b	102.778 ± 7.115a	1087.759 ± 77.118a	2680.654 ± 92.598bc
Blue French	–	1481.174 ± 150.314g	3.794 ± 0.843n	134.733 ± 27.509o	1432.732 ± 143.869lm
Pinot Noir115	–	962.607 ± 16.744i	–	4.382 ± 2.889p	903.682 ± 15.394pq
Syrah100	–	2040.168 ± 213.925de	48.472 ± 3.617ef	644.407 ± 36.215def	1891.788 ± 187.749fgh
Medoc Noir	–	1364.963 ± 192.524gh	62.779 ± 9.827bc	553.812 ± 74.587gh	1256.048 ± 180.979mn
Muscat Hamburg	–	502.151 ± 30.644k	–	56.835 ± 3.418p	446.770 ± 28.980r
Muscat Rouge	–	36.793 ± 0.537n	–	–	8.069 ± 0.488s
Gamay Teinturier	–	1359.460 ± 262.227gh	13.573 ± 3.155lm	426.114 ± 91.775j	1325.613 ± 253.989lmn
Tempranillo	–	2160.501 ± 102.289d	40.254 ± 4.753fgh	328.566 ± 38.281k	1702.513 ± 65.587ij
Canepabn	–	1282.333 ± 72.983h	–	162.478 ± 21.191no	1252.465 ± 70.007mn
Armenia	–	1849.765 ± 3.492f	37.086 ± 1.737ghi	646.349 ± 26.388def	1713.524 ± 2.788hij
Areni	–	2071.600 ± 11.493de	44.666 ± 1.430efg	660.873 ± 12.354de	1891.551 ± 4.699fgh

(Continued)

Table 2 (continued).

Varieties	Diglucoside anthocyanins	Monoglucoside anthocyanins	Acylated anthocyanins	Coumarylated anthocyanins	Methylated anthocyanins
Zhesexiang	17.735 ± 3.049g	109.475 ± 11.117mn	–	7.032 ± 0.963p	5.631 ± 0.018s
Seibel Noir	925.157 ± 94.193d	2963.798 ± 19.079b	43.159 ± 1.297efg	791.740 ± 19.695b	3412.345 ± 132.098a
Beibinghong	239.441 ± 18.798f	2854.997 ± 66.425b	60.713 ± 2.748bc	337.266 ± 16.604k	1263.245 ± 71.750mn
Huapu 1	588.535 ± 15.292e	255.528 ± 27.257lm	11.554 ± 2.090mn	150.486 ± 27.222o	779.613 ± 31.399q
Gongniang 2	1422.021 ± 29.579b	272.613 ± 5.328lm	24.905 ± 0.478jk	409.384 ± 2.028j	1626.679 ± 29.769jk
Beihong	1571.513 ± 122.168ab	1451.697 ± 109.720gh	32.275 ± 1.716hij	256.825 ± 27.653lm	2294.886 ± 25.652d
Beimei	886.350 ± 11.452d	329.681 ± 8.008kl	21.933 ± 1.111kl	323.960 ± 18.984k	1146.348 ± 5.084no
2–1-3	1661.225 ± 42.915a	265.084 ± 6.294lm	23.840 ± 2.068jk	499.666 ± 48.464hi	1827.558 ± 48.505ghi
44-6-7-1	1146.129 ± 402.710c	1898.974 ± 11.964ef	69.926 ± 9.108b	743.065 ± 100.028bc	2841.983 ± 322.036b
Moldova	826.488 ± 57.197d	752.331 ± 138.366j	28.039 ± 1.295jk	217.432 ± 10.934mn	1449.664 ± 165.779kl

Note: Different small letters within the same column indicate significant difference at $p < 0.05$. The same below. – Means the type of anthocyanin was undetected in the variety.

As shown in Tab. 2, no 3-p-caffeylated glucoside derivatives were detected in the grape skins. The acetylated glucosides were not detected in 'Pinot Noir 115', 'Muscat Hamburg', 'Canepabn', 'Muscat Rouge' and 'Zhesexiang'; however, in the other 26 grape varieties, the contents accounted for 0.3% ~4.6% of the total anthocyanin contents, the lowest and highest proportion were respectively detected in the skins of 'Blue French' and 'Malbec'. Except for 'Muscat Rouge', 3-p-coumaroylated glucoside accounted for 0.5% to 41.7% of the total anthocyanin contents in the other 30 varieties; the lowest and highest proportion were observed in 'Pinot Noir 115' and 'Carinena', respectively. Twenty-one of the *V. vinifera* varieties have only 3-monoglucoside derivatives of anthocyanins in the skins (Tab. 2), while the contents of 3,5-diglucoside derivatives of anthocyanins in the remaining 10 grape varieties were 17.735~1661.225 mg·kg^{-1}FW, which accounted for 7.7%~86.2% of total anthocyanin contents; the lowest and highest proportion were detected in 'Beibinghong' and '2-1- 3' respectively.

3.2 Characteristics of the Anthocyanins in the 21 Eurasian Grape Varieties

As shown in Fig. 1, three to eleven anthocyanin monomers were detected in 21 *V. vinifera* L varieties. In 'Muscat Rouge', there were only three species of anthocyanin monomers detected, including delphinidin 3-O-monoglucoside (M1), cyanidin 3-O-monoglucoside (M2) and petunidin 3-O-monoglucoside (M3); the content of M2 was the highest, which accounted for 68.3% of the total anthocyanin contents. Eleven anthocyanin monomers were detected in 'Cabernet Franc 327', 'Armenia' and 'Areni', including 2 acetylated glucosides, 4 coumaric acylated glucosides and 5 basic mono-glucosides. Ten anthocyanin monomers were detected in ‘Cabernet 169’, ‘Merlot 181’, ‘Tempranillo’, ‘Cabernet Gernischt’, ‘Syrah 100’, ‘Medoc Noir’ and ‘Gamay Teinturier’; 9 anthocyanin monomers were detected in ‘Marselan c980’, ‘Ruby Cabernet’, ‘Petit Verdot’, ‘Mei Yu’, ‘Carinena’ and ‘French Blue’; 8 anthocyanin monomers were detected in ‘Malbec’, ‘Canepabn’ and ‘Muscat Hamburg’; and 5 anthocyanin monomers were detected in ‘Pinot Noir 115’.

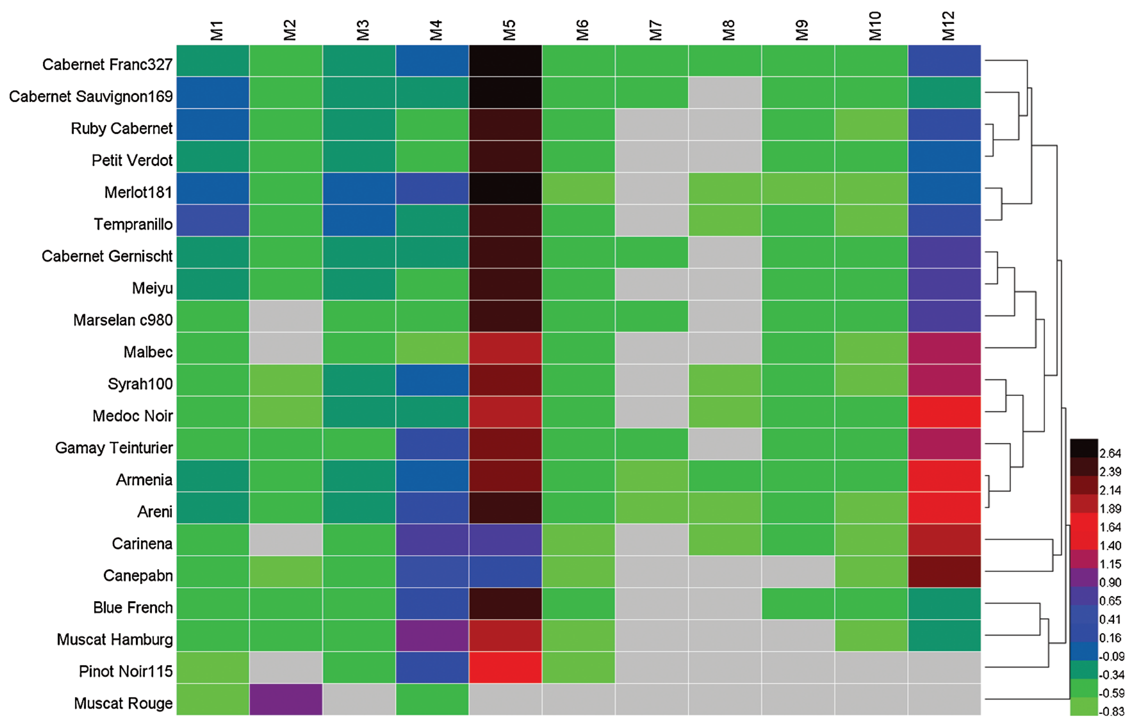

Figure 1: The hierarchically clustered heatmap based on the ratios of anthocyanin monomer content to total anthocyanin in 21 grape skins of *Vitis vinifera*. M1, delphinidin 3-O-monoglucoside; M2, cyanidin 3-O-monoglucoside; M3, petunidin 3-O-monoglucoside; M4, peonidin 3-O-monoglucoside; M5, malvidin-3-O-monoglucoside; M6, delphinidin-3-O-(trans-6-O-coumaroyl)-glucoside; M7, malvidin 3-O-(6-O-acetyl)-glucoside; M8, cyanidin 3-O-(6-O-coumaroyl)-glucoside; M9, petunidin 3-O-(6-O-acetyl)-glucoside; M10, malvidin 3-O-(cis-6-O-coumaroyl)-glucoside; M12, malvidin 3-O-(trans-6-O-coumaroyl)-glucoside

As shown in Fig. 1, the most abundant anthocyanin in ‘Carinena’ and ‘Canepabn’ were malvidin 3-O-(trans-6-O-coumaroyl)-glucoside (M12), which accounted for 37.4% and 57.7% of the total anthocyanin contents, respectively. The second and third abundant anthocyanin in ‘Carinena’ were malvidin-3-O-monoglucoside (M5), and peonidin 3-O-monoglucoside (M4), which respectively accounted for 21.8% and 21.5% of the total anthocyanin contents; while in ‘Canepabn’, M4 and M5 accounted for 22.4% and 17.8% of the total anthocyanin contents, respectively. Except for ‘Muscat Rouge’, ‘Carinena’ and ‘Canepabn’, M5 was the most abundant anthocyanin in the other 18 varieties, which accounted for 40.8% ~65.8% of the total anthocyanin contents; the lowest and highest proportion were observed in ‘Medoc Noir’ and ‘Blue French’ , respectively. In ‘Cabernet 169’, ‘Tempranillo’ M1 ranked second, which accounted for 11.7%, 15.9% of the total anthocyanin contents, while M3, M12, and M3 ranked third, respectively. In ‘Merlot 181’, ‘Blue French’, ‘Pinot Noir 115’ and ‘Muscat Hamburg’, M4 was the second abundant anthocyanin monomer, while M1, M12, M3 and M12 was the third abundant monomers, respectively. In the other 12 varieties, M12 was the second abundant anthocyanin, which accounted for 13.5%~34.6% of the total anthocyanin contents, the lowest and the highest proportion of M12 was detected in 'Petit Verdot' and 'Medoc Noir', respectively. M4 was the third abundant anthocyanin in 'Cabernet Franc327', 'Syrah 100', 'Gamay Teinturier', 'Armenia' and 'Areni', while M3 was the third abundant anthocyanin in 'Cabernet Gernischt', 'Marselan c980', 'Meiyu', 'Malbec' and 'Medoc Noir'. From the above, except for 'Malbec', 'Medoc Noir', 'Pinot Noir 115' and 'Muscat Rouge', the main anthocyanins in the other 17 varieties were M1, M3, M4, M5 and M12, which accounted for 91.2%~99.3% of the total anthocyanin contents.

The cluster analysis of anthocyanin monomer characteristics showed that 'Armenia' and 'Areni' were the most similar among the 21 varieties, followed by 'Ruby Cabernet' and 'Petit Verdot'. The monomer compositions of 'Cabernet Gernischt' and 'Mei Yu', 'Merlot 181' and 'Tempranillo' were also relatively similar, but 'Muscat Rouge' was very different from the other varieties.

3.3 Characteristics of the Anthocyanins in Ten Hybrid Grape Varieties

The ten hybrid grape varieties included two *Vitis vinifera* × *Vitis labrusca* hybrids, three *Vitis amurensis* Rupr. × *Vitis vinifera* hybrids, two *Vitis vinifera* × *Vitis amurensis* Rupr. hybrids, one *Vitis vinifera* × *Vitis heyneana* Roem. et Schult hybrid and two interspecific hybrids. There were five to fifteen anthocyanin monomers detected in the grape skins. As shown in Fig. 2, there were five anthocyanin monomers detected in 'Zhesexiang', including four mono-glucoside anthocyanins [M1, M2, M4, and M8 (cyanidin 3-O-(6-O-coumaroyl)-glucoside)] and one di-glucoside anthocyanin (cyanidin 3,5-O-diglucoside, D2); among them, the content of M2 was the highest, which accounted for 67.6% of the total anthocyanin contents. Fifteen kinds of anthocyanin monomers were detected in the grape skins of 'Seibel Noir', 'Beibinghong' and 'Beihong', including 8~9 kinds of mono-glucoside anthocyanins, 6~7 kinds of di-glucoside anthocyanin. Fourteen and thirteen anthocyanin monomers were detected in the grape skins of '44-6-7-1' and its female parent '2-1-3', including 5 and 7 di-glucoside anthocyanins, respectively. Twelve anthocyanin monomers were detected in the grape skins of 'Huapu 1' and 'Gongniang 2', including 5 or 6 di-glucoside anthocyanins, respectively; while nine anthocyanin monomers were detected in the grape skins of 'Beimei' and 'Moldova', including 3 and 2 di-glucoside anthocyanins, respectively.

In 'Zhesexiang' and 'Beibinghong', the most abundant anthocyanin was M2, which accounted for 67.6% and 38.5% of the total anthocyanin contents, respectively, the second abundant anthocyanin was D2 and M5, respectively, which accounted for 13.9% and 18.3%; while M1 ranked third, which accounted for 8.5% and 14.8%, respectively. The top four monomers in 'Seibel Noir' were M5, M3, M1, and D9, which accounted for 43.4%, 11.8%, 10.1% and 9.8% of the total anthocyanin contents, respectively. Except for 'Zhesexiang', 'Beibinghong' and 'Seibel Noir', malvidin 3,5-O-diglucoside (D5) was the most abundant anthocyanin in the other seven varieties, which accounted for 30.0%~63.6% of the total anthocyanin contents. The second abundant anthocyanin in 'Gongniang 2', 'Beimei' and '2-1-3' was malvidin-O-(trans-6-O-coumaroyl)-glucoside-5-glucoside (D9), which accounted for 14.6%, 15.9%, 16.5% of total anthocyanin content, respectively; however, M5 ranked second in 'Huapu 1', 'Moldova' and '44-6-7-1', which accounted for 11.7%, 21.7%, 27.3% of the total anthocyanin contents. M12 ranked third in 'Gongniang 2', '2-1-3' and '44-6-7-1', which accounted for 5.3%, 4.8% and 14.8% of the total anthocyanin contents, respectively; while in 'Huapu 1', 'Beimei' and 'Moldova', D9, M5 and M3 ranked third, respectively. In 'Beihong', the monomers ranked second and third were M1 and M5, which accounted for 17.8% and 14.1%, respectively. D5, D9, M5 and M12 were the main anthocyanins in 'Huapu 1', 'Gongniang 2', 'Beimei' and '2-1-3', the total contents of them accounted for 84.3%~88.9% of the total anthocyanin contents; however, in '44-6-7-1' and 'Moldova', the main anthocyanins included D5, M5, M12 and M3 , which accounted for 82.3% and 83.7% of the total anthocyanin content, respectively.

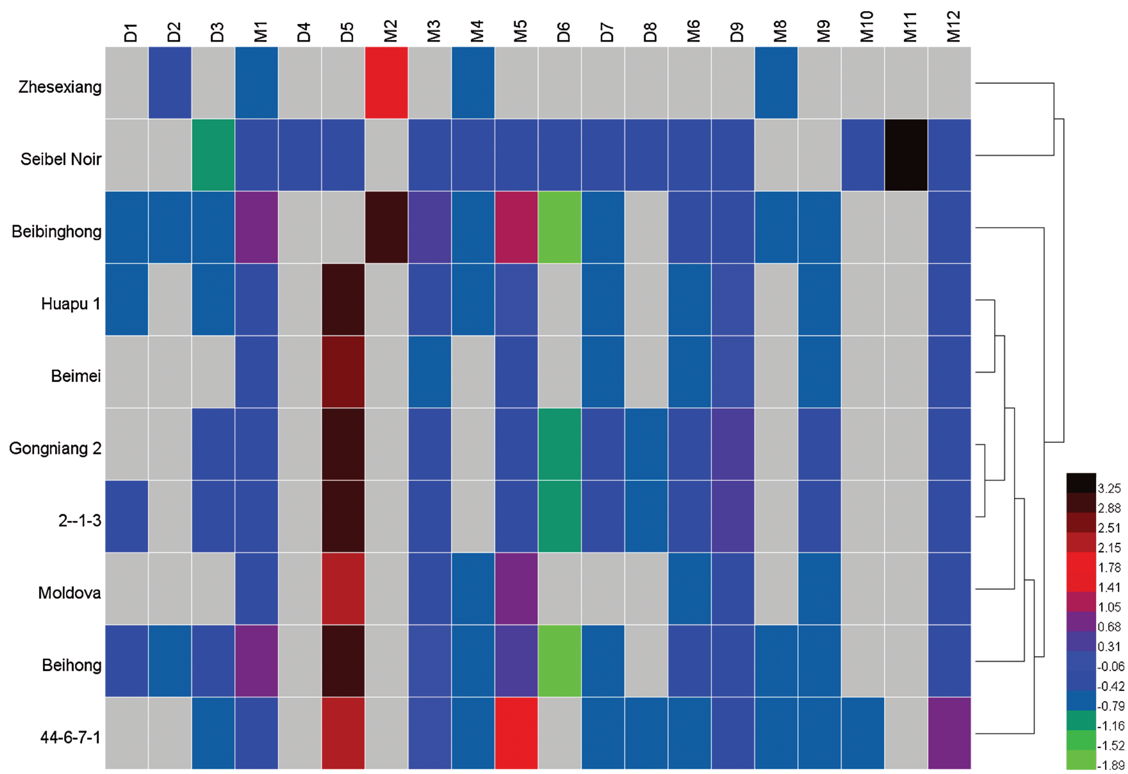

Figure 2: The hierarchically clustered heatmap based on the ratios of anthocyanin monomer content to total anthocyanins in ten grape varieties. M represents the mono-glucoside anthocyanin, D represents the diglucoside anthocyanin; the color scale representing average values is shown on the right side of the figure. M1, delphinidin 3-O-monoglucoside; M2, cyanidin 3-O-monoglucoside; M3, petunidin 3-O-monoglucoside; M4, peonidin 3-O-monoglucoside; M5, malvidin-3-O-monoglucoside; M6, delphinidin-3-O-(trans-6-O-coumaroyl)-glucoside; M8, cyanidin 3-O-(6-O-coumaroyl)-glucoside; M9, petunidin 3-O-(6-O-acetyl)-glucoside; M10, malvidin 3-O-(cis-6-O-coumaroyl)-glucoside; M11, petunidin 3-O-(trans-6-O-coumaroyl)-glucoside; M12, malvidin 3-O-(trans-6-O-coumaroyl)-glucoside; D1, delphinidin 3,5-O-diglucoside; D2, cyanidin 3,5-O-diglucoside; D3, petunidin 3,5-O-diglucoside; D4, peonidin 3,5-O-diglucoside; D5, malvidin 3,5-O-diglucoside; D6, delphinidin 3-O-(6-O-coumaroyl)-glucoside-5-glucoside; D7, petunidin 3-O-(6-O-coumaroyl)-glucoside-5-glucoside; D8, malvidin 3-O-(cis-6-O-coumaroyl)-glucoside-5-glucoside; D9, malvidin-O-(trans-6-O-coumaroyl)-glucoside-5-glucoside

According to the cluster analysis of anthocyanin monomer characteristics, ‘Gongniang 2’ and ‘2-1-3’ were the closest among the 10 varieties, followed by ‘Huapu 1’ and ‘Beimei’. ‘Zhesexiang’ and ‘Seibel Noir’ were very different from the other varieties.

3.4 Principal Component Analysis of Different Grape Varieties Based on Anthocyanin Content

The principal component analysis (PCA) was carried out based on the ratios of different anthocyanin monomer content to total anthocyanin and the total anthocyanin of 21 *Vitis vinifera* grape varieties. The first three principal components (PC) accounted for 73.16% of the total variance. PC1, PC2 and PC3 explained 33.56%, 24.17% and 15.43% of the total variance, respectively. Fig. 3 was a scatter plot showing the distribution of 21 grape varieties according to PC1 and PC2. ‘Ruby Cabernet’, ‘Petit Verdot’, ‘Tempranillo’, ‘Merlot 181’, ‘Cabernet Gernischt’, ‘Mei Yu’ and ‘Marselan c980’ lay down on right and upper part of the PC1 axis; ‘Cabernet Sauvignon169’, ‘Cabernet Franc327’, ‘Pinot Noir 115’ and 'Blue French' were located on left and upper part of the PC1 axis; 'Muscat Hamburg', 'Gamay Teinturier', 'Muscat Rouge' and 'Canepabn' were situated to left and bottom part of the PC1 axis; and the other varieties were located on right and bottom part of the PC1 axis.

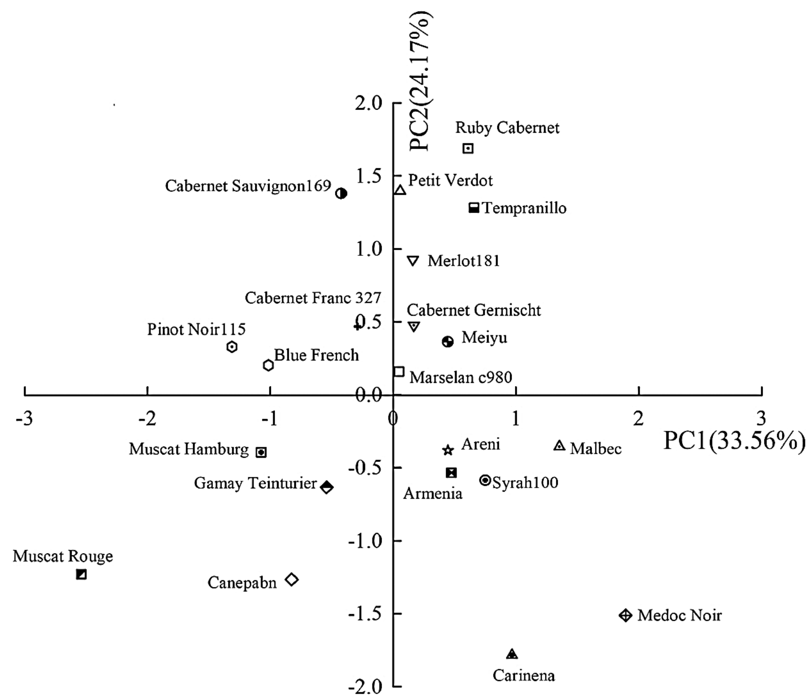

Figure 3: Principal component analysis of the anthocyanins in grape skins of 21 grape skins of *Vitis vinifera*. The analysis was based on the ratios of anthocyanin monomer content to total anthocyanin, and the total anthocyanin. The principal components 1, 2 and 3 explained 33.56%, 24.17% and 15.43% of the total variance, respectively. The principal component 1 and 2 were the horizontal and vertical coordinates, respectively

PCA was also carried out based on the ratios of different anthocyanin monomer content to total anthocyanin and the total anthocyanin of the 10 other grape varieties (Fig. 4). The first three PC accounted for 78.25% of the total variances. PC1, PC2, and PC3 explained 32.75%, 26.31%, and 19.19% of the total variance, respectively. Fig. 4 was a scatter plot showing the distribution of 10 grape varieties according to PC1 and PC2. 'Seibel Noir' and '44-6-7-1' were located on right and upper part of the PC1 axis; 'Beibinghong', 'Beihong' and 'Zhesexiang' were located on left and upper part of the PC1 axis; 'Moldova' and 'Huapu 1' were situated to left and bottom part of the PC1 axis, while 'Beimei', '2-1-3' and 'Gongniang 2' were situated to right and bottom part of the PC1 axis (Fig. 4).

3.5 Cluster Analysis of Different Grape Varieties Based on Anthocyanin Content

As shown in Fig. 5A, minimum variance analysis of 21 *Vitis vinifera* species was carried out according to the ratios of each anthocyanin monomer to total anthocyanin and total anthocyanin content. When the minimum variance was 0.150, the 21 varieties were divided into 3 major groups. Group I consisted of 11 varieties, which were divided into three categories at a variance of 0.050. The first category included 'Cabernet Franc 327' and 'Cabernet Gernischt'; the second category included 7 varieties, 'Armenia' and 'Areni' were the closest and clustered with 'Syrah 100', 'Marselan c980' and 'Meiyu' was also close; the third category included 'Carinena' and 'Medoc Noir'. Group II included 5 varieties, 'Cabernet Sauvignon

169’ and ‘Petit Verdot’ clustered first and then clustered with ‘Ruby Cabernet’, ‘Merlot 181’ and ‘Tempranillo’. Group III included 5 varieties, ‘Blue French’ and ‘Pinot Noir 115’ were the closest, they clustered first, and then clustered with ‘Muscat Hamburg’.

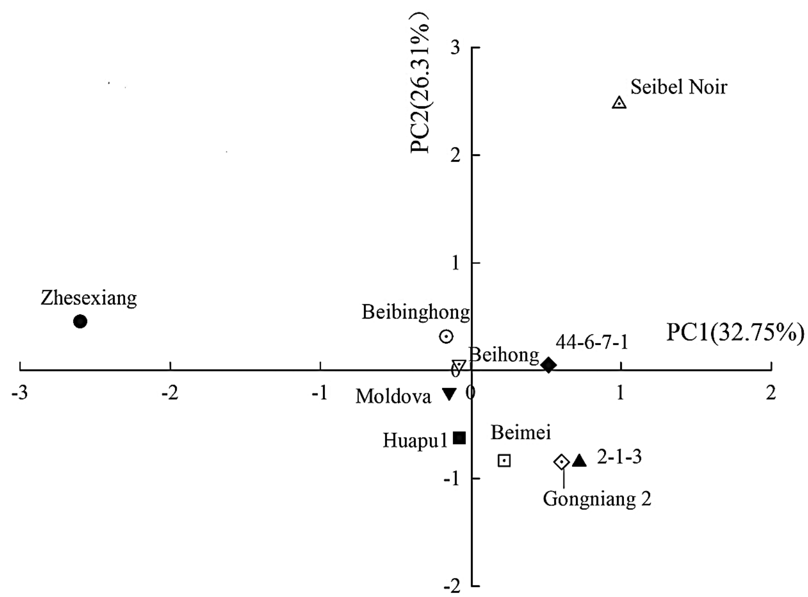

Figure 4: Principal component analysis of the anthocyanins in grape skins of 10 other grape skins. The analysis was based on the ratios of anthocyanin monomer content to total anthocyanin, and the total anthocyanin. The principal components 1, 2 and 3 explained 32.75%, 26.31%, and 19.19% of the total variance, respectively. The principal component 1 and 2 were the horizontal and vertical coordinates, respectively

As shown in Fig. 5B, when the minimum variance was 0.200, the 10 grape varieties were divided into 3 groups. ‘Zhesexiang’ and ‘Seibel Noir’ were each a group. The third group included eight varieties which divided into three categories at 0.100. The first category included ‘Beibinghong’ and ‘Beihong’; in the second category, ‘Huapu 1’ and ‘Beimei’ were the closest, and they clustered with ‘Moldova’; the third category included ‘Gongniang 2’ and ‘2-1-3’.

4 Discussion

The anthocyanins of grapes are mainly found in the skins of colored grape varieties. The composition and contents of anthocyanins vary greatly among different grape varieties [5,8,12]. All anthocyanins are mono-glucoside derivatives in *Vitis vinifera* [5,19,20], but there are also di-glucoside derivatives in other grape species [21–23]. Sun et al. [18] showed that except for *Vitis adenoclada* Hand., the main anthocyanins of *V. davidii*, *V. heyneana*, *V. bryoniaefolia*, *V. amurensis* and *V. pseudorticulata* are di-glucoside derivatives. However, the proportion of mono-glucoside derivatives in the late mature stage of ‘NW196’ (*Vitis quinquangularis* × *Vitis vinifera*) was higher than that of di-glucoside derivatives [24]. In our study, only mono-glucoside derivatives were detected in the 21 *Vitis vinifera* varieties, while both mono-glucoside and di-glucoside derivatives were detected in the other 10 varieties. Except for ‘Zhesexiang’ ‘Seibel Noir’, ‘44-6-7-1’ and ‘Beibinghong’, in the other six varieties, di-glucoside derivatives accounted for more than 52% of the total anthocyanins, which was consistent with Sun et al. [18].

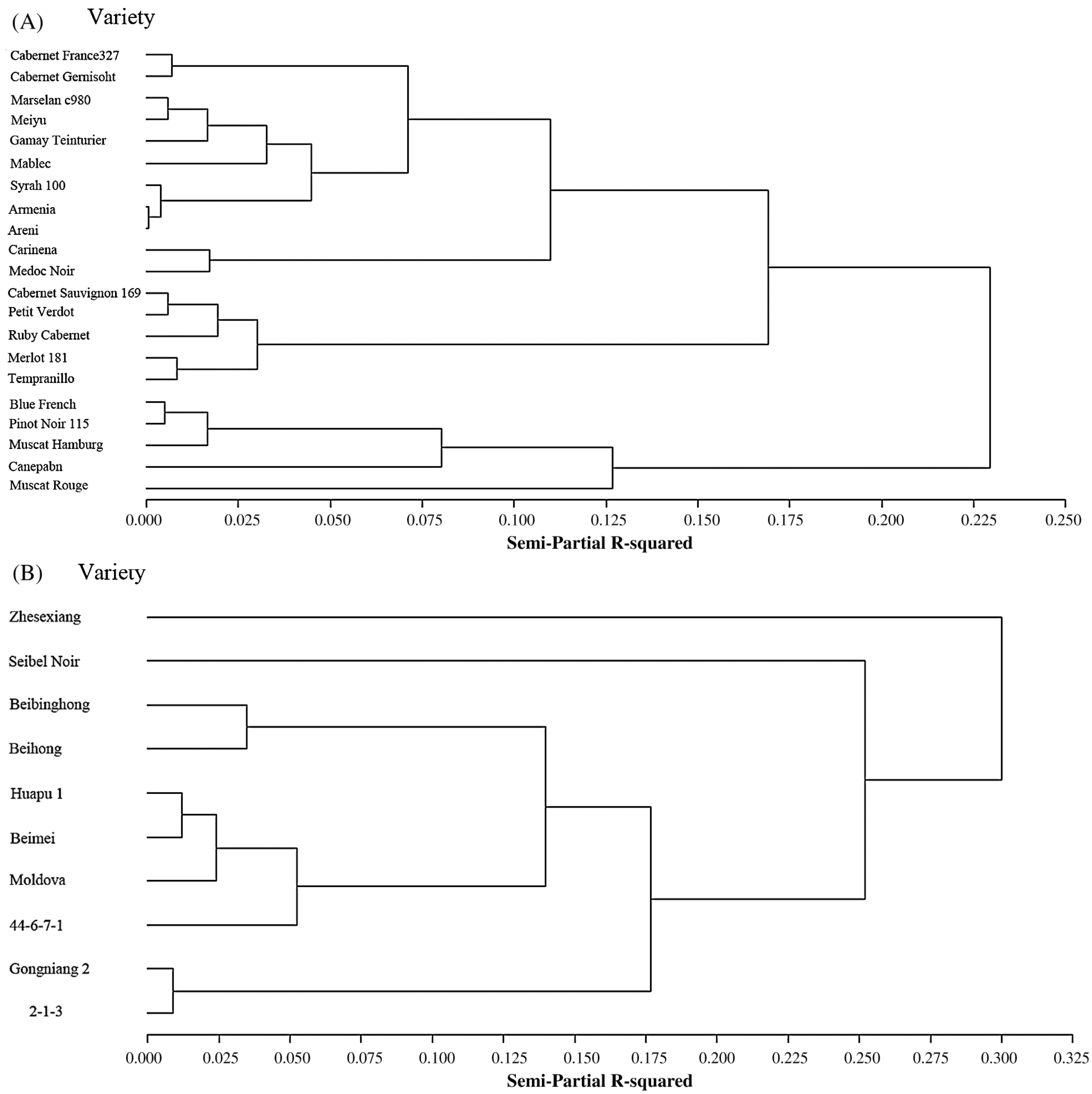

Figure 5: The cluster analysis of 31 grape species based on the ratios of each anthocyanin monomer to total anthocyanin and total anthocyanin content. The cluster analysis used the minimum variance method. (A) The cluster analysis of 21 *Vitis vinifera* species. (B) The cluster analysis of 10 other grape species

Many factors can influence the anthocyanin content of grape, such as variety, climate, soil and cultivation methods, and the composition and proportion of anthocyanins in different varieties and even different lines of the same variety are very different [5,22,24,25]; however, they are mainly determined by genetic factors. In this study, the total anthocyanin content in the skins of 'Ruby Cabernet' was the highest among the 21 *Vitis vinifera* grape varieties, followed by 'Petit Verdot' and 'Malbec'. The total anthocyanin content of 'Syrah 100' was lower than that of 'Merlot 181', 'Marselan c980' and 'Tempranillo', which was inconsistent with Xing et al. [26]. The content of 'Cabernet Franc 327' was lower than that of ‘Merlot 181’, ‘Marselan c980’, ‘Syrah 100’ and ‘Tempranillo’, which was consistent with Xing et al. [26]. The content of ‘Cabernet Sauvignon169’ was lower than that of the interspecific hybrid ‘Moldova’, which was inconsistent with Liu et al. [19]. Differences in climate, soil and cultivation management of the sampling area may result in differences in anthocyanin content.

Studies have found that anthocyanins found in *Vitis vinifera* L. include malvidin, cyanidin, delphinidin, peonidin and petunidin 3-monoglucosides with their corresponding acetyl, p-coumaric acylated and caffeoyl derivates [5]. In this study, there were 3–11 types of anthocyanins in the 21 *Vitis vinifera* varieties, including 3-monoglucosides of Cy, Dp, Pn, Pt, Mv, and their acetylated derivatives, *p*-coumaric acylated derivatives, but no 3-*p*-caffeylated derivatives, and the contents of anthocyanin monomer varied among varieties. Except for ‘Muscat Rouge’, in the other 20 varieties, the most abundant anthocyanins were malvidin-3-O-monoglucoside (M5) or malvidin 3-O-(trans-6-O-coumaroyl)-glucoside (M12), which was consistent with the literature [20,26]. According to the cluster analysis, ‘Armenia’ and ‘Areni’ were the closest, which because the two grapes are the same variety but have different names [27]. ‘Cabernet Franc 327’ and ‘Cabernet Gernischt’ were close to each other and were grouped with ‘Marselan c980’, ‘Mei Yu’, ‘Gamay Teinturier’, ‘Malbec’ and ‘Syrah 100’. After ‘Cabernet Sauvignon 169’, ‘Petit Verdot’ and ‘Ruby Cabernet’ clustered, they were combined with ‘Merlot 181’ and ‘Tempranillo’. ‘Cabernet Franc’ is the mother of ‘Merlot’ and the father of ‘Cabernet Sauvignon 169’, ‘Cabernet Gernischt’; ‘Cabernet Sauvignon’ is the mother of ‘Marselan’ and the father of ‘Ruby Cabernet’. There are parent-child relationships in all six varieties. The heritability of the parents may affect the clustering of anthocyanin contents.

A total of 5 to 15 anthocyanin monomers were detected in the skins of 10 hybrid grape varieties, all varieties contained di-glucoside anthocyanins. The most abundant monomer in ‘Zhesexiang’ and ‘Beibinghong’ was M2, while the ones in ‘Seibel Noir’ and other 7 varieties was M11 and D5, respectively. The anthocyanin ‘Moldova’ were consistent with Liu et al. [19], while the results for ‘2-1-3’ (*Vitis quinquangularis* × *Vitis vinifera*) were inconsistent with the study of ‘NW196’ [25]. From the cluster analysis, ‘Beihong’ and ‘Beibinghong’ were relatively closer and clustered into one category, while ‘Huapu 1’, ‘Beimei’, ‘Moldova’ and ‘44-6-7-1’ were clustered into one large category, ‘2-1-3’ and ‘Gongniang 2’ were close. ‘Huapu 1’, ‘Beimei’, ‘Beihong’, ‘Beibinghong’ and ‘Gongniang 2’ were all the hybrid offspring of *Vitis amurensis R* and *Vitis vinifera*, while ‘2-1-3’ is the female parent of ‘44-6-7-1’; thus, the genetic relationship may affect the anthocyanin content.

5 Conclusion

There were significant differences in the anthocyanin types and contents of the skins between *V. vinifera* and other grapes. In *V. vinifera*, all anthocyanins were mono-glucoside derivatives, but in the other Vitis germplasms, there are also di-glucoside derivatives. There were 21 anthocyanins in the grape skins. Mv-derivatives were the most abundant anthocyanins in *V. vinifera* and eight other grapes with a high content. The most abundant anthocyanins in different varieties may be different. Anthocyanins can be used as chemical fingerprints to distinguish wine grape varieties.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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Appendix

Supplementary Table 1: List of 31 grapevine varieties and collected date

Variety No.	Variety	Origin	Parents	Sampling date (day/month)
1	Cabernet Franc 327	*Vitis vinifera*	–	25/9
2	Cabernet Sauvignon 169	*Vitis vinifera*	Sauvignon Blanc × Cabernet Franc	26/9
3	Merlot 181	*Vitis vinifera*	Magdeleine Noire des Charentes × Cabernet Franc	19/9
4	Cabernet Gernischt	*Vitis vinifera*	Carménère (natürliche Kreuzung von Moural × Cabernet Franc)	26/9
5	Marselan c980	*Vitis vinifera*	Cabernet Sauvignon × Grenache	14/9
6	Ruby Cabernet	*Vitis vinifera*	Carinena × Cabernet Sauvignon	25/9
7	Meiyu	*Vitis vinifera*	Merlot × Petit Verdot	11/9
8	Carinena	*Vitis vinifera*	–	26/9

(Continued)

Supplementary Table 1 (continued).

Variety No.	Variety	Origin	Parents	Sampling date (day/month)
9	Petit Verdot	*Vitis vinifera*	–	26/9
10	Malbec	*Vitis vinifera*	Magdeleine Noire des Charentes × Prunelard	26/9
11	Blue French	*Vitis vinifera*	Gouais blanc × Blaue Zimmettraube	11/9
12	Pinot Noir 115	*Vitis vinifera*	–	31/8
13	Syrah 100	*Vitis vinifera*	Mondeuse Blanche × Dureza	14/9
14	Medoc Noir	*Vitis vinifera*	–	25/8
15	Muscat Hamburg	*Vitis vinifera*	Schiava Grossa × Muscat of Alexandria	26/9
16	Muscat Rouge	*Vitis vinifera*	Muscat Blanc a Petits Grains bud mutation	13/9
17	Gamay Teinturier	*Vitis vinifera*	Gamay Noir × Teinturier du Cher	25/8
18	Tempranillo	*Vitis vinifera*	Albillo Mayor × Benedicto	15/9
19	Canepabn	*Vitis vinifera*	–	11/9
20	Armenia	*Vitis vinifera*	–	25/8
21	Areni	*Vitis vinifera*	–	4/8
22	Zhesexiang	*Vitis vinifera* × *Vitis labrusca*	Delaware × Royal Rose	13/9
23	Seibel Noir	*Vitis vinifera* × *Vitis labrusca*	–	11/9
24	Beibinghong	*Vitis amurensis* Rupr. × *Vitis vinifera*	Zouyouhong × 84-26-53	18/9
25	Huapu 1	*Vitis amurensis* Rupr. × *Vitis vinifera*	Zuoshan 1 × White Malaga	13/10
26	Gongniang 2	*Vitis amurensis* Rupr. × *Vitis vinifera*	*Vitis amurensis* Rupr. × Muscat	11/9
27	Beihong	*Vitis vinifera* × *Vitis amurensis* Rupr.	Muscat × *Vitis amurensis* Rupr.	19/9
28	Beimei	*Vitis vinifera* × *Vitis amurensis* Rupr.	Muscat × *Vitis amurensis* Rupr.	19/9
29	2–1-3	*Vitis quinquangularis* × *Vitis vinifera*	Muscat Rouge × *Vitis heyneana* Roem. et Schult	18/9
30	44-6-7-1	Interspecific hybrids	2-1-3 × Ruby Cabernet	20/9
31	Moldova	Interspecific hybrids	Guzal ikala × SV.12-375	26/9

Note:–means that its parents are unknown.

Supplementary Table 2: Molecular and fragmentation ions of anthocyanins in grapes as determined using HPLC-MS

Code	Compounds detected in skins	MS and MS^2 ($m \cdot z^{-1}$)
D1	delphinidin 3,5-O-diglucoside	627(465,303)
D2	cyanidin 3,5-O-diglucoside	611(449,287)
D3	petunidin 3,5-O-diglucoside	641(479,317)
M1	delphinidin 3-O-monoglucoside	465(303)
D4	peonidin 3,5-O-diglucoside	625(463,301)
D5	malvidin 3,5-O-diglucoside	655(493,331)
M2	cyanidin 3-O-monoglucoside	449(287)
M3	petunidin 3-O-monoglucoside	479(317)
M4	peonidin 3-O-monoglucoside	463(301)
M5	malvidin-3-O-monoglucoside	493(331)
D6	delphinidin 3-O-(6-O-coumaroyl)-glucoside-5-glucoside	773(611,465,303)
D7	petunidin 3-O-(6-O-coumaroyl)-glucoside-5-glucoside	787(625,479,317)
D8	malvidin 3-O-(cis-6-O-coumaroyl)-glucoside-5-glucoside	801(639,493,331)
M6	delphinidin-3-O-(trans-6-O-coumaroyl)-glucoside	611(303)
M7	malvidin 3-O-(6-O-acetyl)-glucoside	535(331)
D9	malvidin-O-(trans-6-O-coumaroyl)-glucoside-5-glucoside	801(639,493,331)
M8	cyanidin 3-O-(6-O-coumaroyl)-glucoside	595(287)
M9	petunidin 3-O-(6-O-acetyl)-glucoside	625(317)
M10	malvidin 3-O-(cis-6-O-coumaroyl)-glucoside	639(331)
M11	petunidin 3-O-(trans-6-O-coumaroyl)-glucoside	609(301)
M12	malvidin 3-O-(trans-6-O-coumaroyl)-glucoside	639(331)