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Anthocyanin composition and content in grape berry skin in *Vitis* germplasm

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ABSTRACT

The composition and content of anthocyanins were surveyed by HPLC–MS for assessing genotypic variation in berry skin of 110 grape cultivars, including 3 species and 5 interspecific hybrids. Twenty-nine anthocyanins were identified. For total anthocyanin content, *Vitis vinifera* and hybrids of *Vitis labrusca* and *V. vinifera* were low, and in general, wild species and rootstock were higher than interspecific hybrids, and wine grapes were higher than table grapes in the same species. As regards the composition of anthocyanins, malvidin-derivatives were the most abundant anthocyanins in the majority of germplasms. All anthocyanins were monoglucoside derivatives in *V. vinifera*, but all the other *Vitis* germplasms had both mono- and di-glucoside derivatives. Moreover, peonidin-derivatives and malvidin 3-*O*-glucoside were, respectively, main anthocyanins in table and wine grapes of *V. vinifera*. Via principal component analysis, the distribution of the cultivars in a scatter plot depended upon their total anthocyanins content, mono- and di-glucoside derivatives.

1. Introduction

Grape is one of the most important fruit crops in the world, and its production was more than 6.64×10^7 ton in 2005 (FAO, 2005). About 23% of the total grapes harvested are table grapes for fresh consumption, while 86.6% of the crop is processed, especially for wine-making (Liu, Wu, Fan, Li, & Li, 2006).

The quality of grape berries greatly depends on skin colour. The skin colour determines the market value of table grapes, and also the quality of red wine and juice. Skin colour varies mainly due to the composition and the content of anthocyanins (Carreño & Martínez, 1995; Cooper-Driver, 2001). The composition of anthocyanins is primarily decided by genetic factors, and the relative content of any one anthocyanin is stable in grape skin for a given cultivar, i.e. the percentage of one anthocyanin does not differ from one year to the next (Pomar, Novo, & Masa, 2005). Therefore, skin colour is an important factor within grape germplasm resources.

There is a long history of studying the composition and the content of anthocyanins. Paper chromatography (PC) and thin layer chromatography (TLC) were utilised first for separating and identifying anthocyanins, especially for the flower corolla (Fong, Webb, & Kepner, 1974; Hrazdina & Franzese, 1974; Koeppen & Basson, 1965). Then, high pressure liquid chromatography (HPLC) became the most popular and frequently-used technique for analysing anthocyanins (Goldy, Maness, Stiles, Clark, & Wilson, 1989; Morais, Ramos, Forgács, Cserháti, & Oliiera, 2002; Pomar et al., 2005; Wulf & Nagel, 1978). The virtues of HPLC are the strong and rapid separation capability, and immediate and highly sensitive detection. In recent years, mass spectrometry coupled to HPLC has become an important tool for anthocyanin identification (Alcalde-Eon, Saavedra, Pascual-Teresa, & Rivas-Gonzalo, 2004; Bakker et al., 1997; Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Mateus, Pascual-Teresa, Rivas-Gonzalo, Santos-Buelga, & Freitas, 2002; Revilla, Pérez-Magariño, González-SanJosé, & Beltrán, 1999). The anthocyanins are comprised of cyanidin, delphinidin, petunidin, peonidin and malvidin 3-monoglucosides (or 3,5-diglucosides) along with the corresponding acetyl, *p*-coumaroyl, and caffeoyl derivatives in cultivars with red, blue and violet skin. There are only 3-monoglucoside derivatives of anthocyanins in *Vitis vinifera*, while there are also 3,5-diglucosides derivatives in other grapes species (Goldy et al., 1989; Hrazdina & Franzese, 1974; Koeppen & Basson, 1965; Wulf & Nagel, 1978). Previous studies (González-San José, Diez, Santa-María, & Garrido, 1988; Roggero, Ragonnet, & Coen, 1984; Sugui, Wood, Yang, Boham, & Nicholson, 1999; Tsanova-Savova, Dimov, & Ribarova, 2002) have reported several primary anthocyanins of a few cultivars. However, there are few studies on the characteristics of anthocyanin composition and content across the broader germplasm of *Vitis* species.

Vitis germplasm resources are very rich, with at least 70 species (Jing, 1999). With a long history of cultivation, extensive geographical distribution and the selection of new cultivars from hybrids

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between species, there is an abundance of grape cultivars throughout the world. However, grapes cultivated throughout the world today mainly belong to four types, the European type (*V. vinifera* L.), the American bunch type (*Vitis labrusca* L. and its derivatives, especially the hybrids obtained from *V. labrusca* and *V. vinifera*), the Muscadine type (*Vitis. rotundifolia* Michx.), and Amurensis type (*Vitis amurensis* and its derivatives, especially hybrids between *V. amurensis* and *V. vinifera*). It would be valuable to determine the composition and content of anthocyanins that influence skin colour across the germplasm material that currently exists.

Although there are many reports about grape anthocyanins (Fernández-López, Hidalgo, Almela, & López-Roca, 1992; Gao & Cahoon, 1995; Goldy et al., 1989; Roggero, Coen, & Ragonnet, 1986), there are few reports comparing germplasm. The objective of the present report was to explore the characteristics of anthocyanin composition and content in 110 grape cultivars, in order to acquire information for future breeding efforts aimed at improvement of fruit quality in grapes via effects on anthocyanins.

2. Experiment

2.1. Plant materials

One hundred and ten grape cultivars (Table 1) were harvested in 2005, including 52 table grapes, 1 juice grape and 1 wine grape from hybrids of *V. labrusca* and *V. vinifera* (LV), 37 table grapes and 11 wine grapes from *V. vinifera* (V), 2 juice grape hybrids of *Vitis thunbergii* and *V. vinifera* (TV), 1 juice grape hybrid of *V. labrusca* and *V. amurensis* (LA), 2 wine grape hybrids of *V. vinifera* and *V. amurensis* (VA), 1 grape of *V. labrusca* (L), 1 Chinese wild grape species (*V. amurensis* var. *dissecta*), and 1 rootstock grape hybrid of *Vitis berlandier* and *Vitis riparia* (BR). Samples were collected from the experimental vineyard of the germplasm repository for grapes in the Institute of Botany, Chinese Academy of Sciences in Beijing. All the cultivars were planted in spring of 1993. The grapevines were trained to cordons, 1.5 m apart within the row and 2.5 m apart between rows with a north–south row orientation. The entire vineyard was managed the same regarding fertilization, irrigation, pruning and controlling disease.

Grape berries were sampled at ripening, determined based on the former years' ripening dates and as judged from seed colour change to dark brown without senescence of berry tissue. Three clusters were chosen at random from three grapevines for each cultivar. About 50 g of berries from different positions within each cluster were considered as one replication resulting in three replications for every cultivar.

The berry samples were taken to the laboratory immediately after harvest, washed in deionized water and dried with gauze. Berries were then peeled with forceps and skins were bagged in aluminium foil. The skins were frozen in liquid N_2, and stored at −40 °C for later analysis.

2.2. Chemicals

The anthocyanin standards (malvidin 3-*O*-glucoside chloride and malvidin 3-*O*-glucoside-5-*O*-glucoside chloride) were purchased from Extrasynthese (Genay, France). HPLC gradient acetonitrile was obtained from Sigma (Sigma, USA). Formic acid and the other analytical-reagent grade chemicals were from Beijing Chemistry Factory (Beijing, China). HPLC grade water was obtained from an Auto Ultrapure Water Evaporator (SZ-93, Yarong, Shanghai, China).

2.3. Extraction of anthocyanins

The extraction of anthocyanins was performed according to Morais et al. (2002). The samples were ground in liquid N_2 using

Table 1
Grape cultivars used in this study

Genotype group	Cultivar number	Cultivars
Table grape of LV[a]	52	Venus (1), Jingya (2), Jingxing Seedless (3), Violet Vehara (4), Bronx Seedless (5), Red Niagara (6), Jiangshang (7), Pondicherry (8), Mars Seedless (9), Hongboduo (10), Jifeng (11), Swenson Red (12), Harata 314 (13), Hyuga (14), Yigawa 1015 (15), Vehava 540 (16), Guixiangyi (17), Vehava 180 (18), Black Olympia (19), Beniyamabiko (20), Jingyou (21), Fujiminori (22), Red Queen (23), Pione (24), Beini Zawa (25), Yigawa 1055 (26), Beniizu (27), Benizuiho (28), Bennifuji (29), Urbana (30), Tano Red (31), Hanazawa Kyoho (32), Kyoho (33), Beni Saiku (34), Izunishiki (35), Rhodo Berry (36), Fenghou (37), Yigawa 1050 (38), Yigawa 1060 (39), Catawba (40), Jingchao (41), Takatsuma (42), Takasumi (43), 86-11 (44), Takao (45), 96-5 (46), Libier (47), Jasmine (48), Dongwuhe (49), Yigawa 1011 (50), Hongdajiubao (51), Wase Takasumi (52)
Juice grape of LV	1	Honey Juice (53)
Wine grape of LV	1	Super Hamburg (54)
Table grape of V[b]	38	Jingxiu (55), Misket Dounvaski (56), Buffalo (57), Red Globe (58), Zaomanao (59), Otilia (60), Zhengzhouzaohong (61), Early Muscat (62), Fenghuang No. 51 (63), Jingzijing (64), Christmas Rose (65), Jingkejing (66), Ghibao (67), Flame tokay∗Niunai (68), Shiyaira (69), Jingdajing (70), Rizamat (71), Hiro Hamburg (72), Muscat Hamburg (73), Bimeijia (74), Ruby Seedless (75), Kuratsufuri (76), Cardinal (77), Beauty Seedless (78), Dadujiaoshou (79), Heibala (80), Blush Seedless (81), Maduona (82), 96-2 (83), Jingfeng (84), Meirenzhi (85), Flame Seedless (86), Hongjiu (87), 96-10 (88), 97-4 (89), Shu-118 (90), Red Alexandria (91)
Wine grape of V	11	Semillon (92), Blue French (93), Italian Riesling (94), Meichun (95), Wuyuezi (96), Merlot (97), Cabernet Gernischet (98), Mulent Black (99), Carigane (100), Cinsaut (101), Shu-162 (102)
Juice grape of TV[c]	2	Beifeng (103), Beixiang (104)
Juice grape of LA[d]	1	Russia Concord (105)
Wine grape of VA[e]	2	Beihong (106), Beimei (107)
V. labrusca	1	Concord (108)
V. amurensis var. *dissecta*	1	Yanshan (109)
Rootstock grape of BR[f]	1	Hairless (110)

The number in parenthesis following the cultivar indicates the accession number.
[a] LV, hybrids between *V. labrusca* and *V. vinifera*.
[b] V, *V. vinifera*.
[c] TV, *V. thunbergii* × *V. vinifera*.
[d] LA, *V. lubrusca* × *V. amurensis*.
[e] VA, *V. vinifera* × *V. amurensis*.
[f] BR, *V. berlandier* × *V. riparia*.

a mortar and pestle, and then put into 100-ml Erlenmeyer flasks with 50 ml 2% formic acid in methanol. The flasks were placed in a shaker (THZ-C-1, Jiangshu, China) at 4 °C in a dark environment. Anthocyanins were extracted for 24 h and the extract solutions were separated from the solid matrix with a funnel. Fresh extraction solutions were added to the flasks once a day until the pomace became colourless. The extractions were pooled, filtered and concentrated under vacuum at 35 °C using a rotary evaporator (RE-52AA, Yarong, Shanghai, China), then evaporated to dryness and finally resolubilized in 25 ml with distilled water. About 5 ml of each solution was filtered through a 0.45 μm Millipore filter.

2.4. High performance liquid chromatography–mass spectrometry analysis of anthocyanins

High performance liquid chromatography/hybrid quadrupole time of flight mass spctrometer (HPLC/Q-ToF MS/MS) (Micromass Q-ToF micro, Waters, USA) was employed for identifying anthocyanins. It was equipped with a Waters Alliance 2695 HPLC Pump, Waters Alliance 2695 Autosampler and Waters 996 photodiode array detector which were coupled directly to the sprayer needle where ions were generated by electrospray ionization (ESI) in both positive and negative ionization modes. At the same time, anthocyanins were quantified using a Dionex P680 HPLC system (Dionex Corporation, CA, USA) fitted with a Dionex PDA-100 detector.

The same method was used by the two HPLC systems. A reverse-phase C18 column Kromasil-100 (4 μm particle sizes, 250 mm × 4 mm I.D.) from Tracer Analitica (Barcelona, Spain) and a C18 Nova Pack guard precolumn (Waters, USA) was used to analyse all the samples. The HPLC method was according to Pomar et al. (2005) with some modification in total elution time. The mobile phase was; water:formic acid (90:10) as solvent A, and acetronitrile:water:formic acid (45:45:10) as solvent B. The gradient profile began at 15–35% A at 25 min, 50% A at 35 min, 55% A at 40 min, and then returned to initial conditions at 45 min and for 5 min. The flow rate was 1.0 ml min^{-1} and the column temperature was set at 30 °C. The injection volume was 20 μl in the Waters system and 10 μl in the Dionex system. Anthocyanins were detected by UV absorbance at 313 nm and 546 nm in order to obtain chromatograms. Moreover, all peaks were recorded from 210 nm to 600 nm.

For MS analyses, nitrogen was used as drying and nebulizing gas and nebulizer pressure was 380 Pa. Gas flowed at 10 l min^{-1} and 350 °C. The capillary voltage was 3000 V and the spectra were recorded in positive and negative ionization modes between m/z 200 and 1000.

Anthocyanins were quantified using malvidin 3-*O*-glucoside and malvidin 3-*O*-glucoside-5-*O*-glucoside chloride as a standard according to Cacho, Fernandez, Ferreira, and Castells (1992) and Yokotsuka and Singleton (1997) with some modification for using malvidin 3-*O*-glucoside-5-*O*-glucoside chloride instead of malvidin 3-*O*-glucoside chloride.

The equation was: Concentration (mg/ml) = 0.003 × Area, with an r^2 = 0.995. Molecular weights (MW) must be taken into account for calculating the concentration of each anthocyanin, so the concentration obtained was multiplied by the MW of the anthocyanin and dividing by the MW of malvidin 3-*O*-glucoside-5-*O*-glucoside chloride (MW = 691.5). The final equation was: Concentration (mg/ml^{-1}) = 0.003 × Area × MW/691.5.

2.5. Statistical analysis

Mean values of each grape cultivar were from three replications, and were used for further analysis. The cultivar variation in anthocyanin content was analysed by S-Plus (MathSoft Inc.). The boxplot was developed to display the anthocyanin range, median and distribution density of a variable in sample size (Becker, Chambers, & Wilks, 1988). The median of the data was indicated by the horizontal line in the interior of the box. The height of the box was equal to the interquartile distance, which was the difference between the third quartile of the data and the first quartile. The whiskers (the dotted lines extending from the top and bottom of the box) were extended to 1.5 × interquartile distance from the center. Almost all the data fell inside the whiskers. The data outside these whiskers was indicated by horizontal lines.

Principal component analysis was performed using the 'princomp' function of S-Plus (MathSoft Inc.). The graphical representations were performed using Sigmaplot 10.0 for Windows (SPSS, USA). The standardised data of other *Vitis* (103–110) were included in the PCA as supplementary cultivars, i.e. they were not used to determine PCs, but they were positioned on PCA planes determined by grape cultivars from LV and V. It was then possible to compare the position of each grape of LV and V on the PCA planes with other *Vitis*.

3. Results and discussion

3.1. Identification of anthocyanins

Table 2 indicates the identification results based on the data including the peak number, retention time, molecular ions, important fragment ions and UV–Vis spectra absorbance maxima, which were obtained from MS and HPLC profiles.

Thirty three peaks were detected in all cultivars, including 29 anthocyanins and 4 unknown components. All anthocyanins were monoglucoside or diglucoside derivatives of 5 anthocyanins: delphinidin (Dp), cyanidin (Cy), petunidin (Pt), peonidin (Pn) and malvidin (Mv). Derivatives included 6-*O*-acetyl, 6-*O*-coumaryl. In addition, malvidin 3-*O*-(6-*O*-caffeoyl)-glucoside was also detected. Four unknown components (peaks 9, 14, 15, 16) have not been identified due to the shortage of information.

3.2. Total content of anthocyanins

The frequency distribution and median of the total anthocyanins in berry skin are shown in Fig. 1. Total anthocyanins content ranged from 0.1 to 97.5 mg $(100\ g)^{-1}$ fresh weight (FW) in LV table grapes and the median was 6.48 mg $(100\ g)^{-1}$ FW. 'Black Olympia' (19) had the most anthocyanin (97.5 mg $(100\ g)^{-1}$ FW). 'Hyuga' (14), 'Takao' (45) and 'Vehava 180' (18) also produced high amounts of anthocyanins in berry skin (more than 30 mg $(100\ g)^{-1}$ FW).

As regards *V. vinifera*, the total anthocyanin content in table grapes was similar to that in LV table grapes and wine grapes had significantly higher total anthocyanin content than table grapes. The three cultivars of table grapes with the highest content of total anthocyanins were 'Otilia' (60), 'Zhengzhouzaohong' (61) and 'Jingkejing' (66), while 'Mulent Blanc' (99) had the highest total content (118.5 mg $(100\ g)^{-1}$ FW) in all V wine grapes. The V hybrids 'Super Hamburg' (54, LV), 'Beihong' and 'Beimei' (106 and 107, VA) also had high total anthocyanins content (79.7, 33.1 and 114.4 mg $(100\ g)^{-1}$ FW, respectively).

The total anthocyanins in berry skin varied widely with the genetic background of the other *Vitis* germplasm. Chinese wild grape 'Yanshan' (109) and rootstock 'Hairless' (hybrid of BR, 110) had a very high content of total anthocyanins, more than 200 mg $(100\ g)^{-1}$ FW, while the content of total anthocyanins was very low in the skin of 'Russia Concord' (105) and 'Honey Juice' (53, LV) (less than 5.5 mg $(100\ g)^{-1}$ FW). In contrast, total anthocyanins were relatively high for 'Beifeng' (103) and 'Beixiang' (104), both TV hybrids (99.9 mg $(100\ g)^{-1}$ FW and 130 mg $(100\ g)^{-1}$ FW,

Table 2
The characteristics of the anthocyanins of chromatography and mass spectrometry found in grapes

Peak number	t_R (min)	Molecular ion $M^+(m/z)$	Fragment ions M(m/z)	Absorbance maxima (nm)	Identity
1	5.2	627	303, 465	282, 520	Delphinidin 3-*O*-glucoside-5-*O*-glucoside
2	7.5	611	287, 449	282, 516	Cyanidin 3-*O*-glucoside-5-*O*-glucoside
3	8.3	465	303	280, 523	Delphinidin 3-*O*-glucoside
4	8.81	641	317, 479	274,523	Petunidin 3-*O*-glucoside-5-*O*-glucoside
5	10.8	449	287	279, 515	Cyanidin 3-*O*-glucoside
6	11.2	625	301, 463	278, 513	Peonidin 3-*O*-glucoside-5-*O*-glucoside
7	11.9	479	317	277, 526	Petunidin 3-*O*-glucoside
8	13.4	655	331, 493	275, 524	Malvidin 3-*O*-glucoside-5-*O*-glucoside
9	14.9	523	317	352, 528	Unknown
10	15.5	653	287, 449, 611	280, 516	Cyanidin 3-*O*-(6-*O*-acetyl)-glucoside-5-*O*-glucoside
11	16.2	463	301	279, 515	Peonidin 3-*O*-glucoside
12	17.3	683	317, 479, 641	280, 530	Petunidin 3-*O*-(6-*O*-acetyl)-glucoside-5-*O*-glucoside
13	18.4	493	331	278, 530	Malvidin 3-*O*-glucoside
14	19.5	1142	305	314	Unknown
15	19.9	1043	305, 609	278, 528	Unknown
16	20.6	1416	301, 571	351	Unknown
17	21.6	507	303, 465	280, 521	Delphinidin 3-*O*-(6-*O*-acetyl)-glucoside
18	23.2	697	331, 493, 655	278, 530	Malvidin 3-*O*-(6-*O*-acetyl)-glucoside-5-*O*-glucoside
19	25.6	491	287, 449	279, 514	Cyanidin 3-*O*-(6-*O*-acetyl)-glucoside
20	26.1	773	303, 465, 627	279, 530	Delphinidin 3-*O*-(6-*O*-coumaryl)-glucoside-5-*O*-glucoside
21	28.6	522	317, 479	280, 530	Petunidin 3-*O*-(6-*O*-acetyl)-glucoside
22	29.9	757	287, 449, 611	280, 524	Cyanidin 3-*O*-(6-*O*-coumaryl)-glucoside-5-*O*-glucoside
23	30.6	773	303, 465, 627	282, 530	Delphinidin 3-*O*-(6-*O*-coumaryl)-glucoside
24	31.4	783	317, 479, 641	280, 530	Petunidin 3-*O*-(6-*O*-coumaryl)-glucoside-5-*O*-glucoside
25	32.3	505	301, 463	280, 518	Peonidin 3-*O*-(6-*O*-acetyl)-glucoside
26	32.6	535	331, 493	280, 521	Malvidin 3-*O*-(6-*O*-acetyl)-glucoside
27	34.4	771	301, 463, 625	279, 520	Peonidin 3-*O*-(6-*O*-coumaryl)-glucoside-5-*O*-glucoside
28	35.1	801	331, 493,655	280, 530	Malvidin 3-*O*-(6-*O*-coumaryl)-glucoside-5-*O*-glucoside
29	35.5	595	287, 449	283, 522	Cyanidin 3-*O*-(6-*O*-coumaryl)-glucoside
30	36.1	625	317, 479	280, 531	Petunidin 3-*O*-(6-*O*-coumaryl)-glucoside
31	37.5	655	331, 493	283, 538	Malvidin 3-*O*-(6-*O*–caffeoyl)-glucoside
32	39.8	609	301, 463	279, 523	Peonidin 3-*O*-(6-*O*-coumaryl)-glucoside
33	40.5	639	331, 493	280, 521	Malvidin 3-*O*-(6-*O*-coumaryl)-glucoside

The peak numbers in the table correspond to the peak order of a typical HPLC chromatogram of anthocyanins extracts detected at 546 nm.

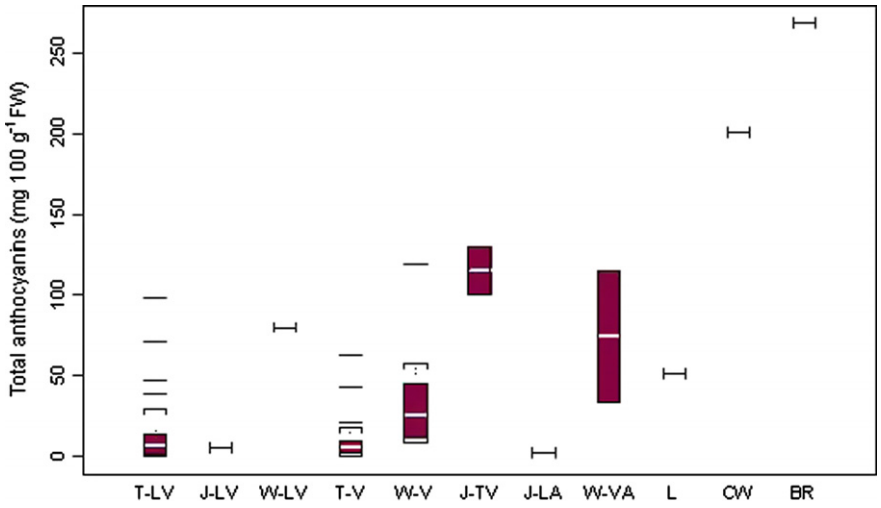

Fig. 1. Range and distribution of total content in anthocyanins of grape skin. The horizontal lines in the interior of the box are the median values. The high in a box is equal to the interquartile distance, indicating the distribution for 50% of the data. Approximately 99% of the data falls inside the whiskers (the dotted lines extending from the top and bottom of the box). The data outside these whiskers are indicated by horizontal lines. T-LV, table grape of *V. labrusca* and *V. vinifera*; J-LV, juice grape of *V. labrusca* and *V. vinifera*; W-LV, wine grape of *V. labrusca* and *V. vinifera*; T-V, table grape of *V. vinifera*; W-V, wine grape of *V. vinifera*; J-TV, juice grape of *V. thunbergii* and *V. vinifera*; J-LA, juice grape of *V. lubrusca* and *V. amurensis*; W-VA, wine grape of *V. vinifera* and *V. amurensis*; L, *V. labrusca*; CW, Chinese wild grape *V. amurensis* var. *dissecta*; BR, rootstock grape of *V. berlandier* and *V. riparia*.

respectively). 'Concord' (108), as a representative cultivar of *V. labrusca*, had an anthocyanin content of 51.4 mg $(100\ g)^{-1}$ FW.

Further statistical analysis (Table 3) showed that the content of anthocyanins of rootstock and wild species were significantly higher than the cultivars of V and LV. Moreover, wine grapes had significantly higher total anthocyanins than table grapes for the V cultivars.

3.3. Composition of anthocyanins

3.3.1. Hybrids of *V. labrusca* and *V. vinifera*

Table 3 and Table 4 show the ranges and average content of anthocyanins in the cultivars of different genotype groups and purpose of uses. In LV grapes, Mv-derivatives were the most abundant components, accounting for 69% of total anthocyanins (Table 3),

Table 3
Average content of different type anthocyanins in different kinds of grapes (mg (100 g)$^{-1}$ FW)

Type of anthocyanins	Grape of LV[a]	Table grape of V[b]	Wine grape of V[b]	Other *Vitis*
Cy[c]	1.019 ± 2.066b	0.718 ± 0.855b	0.845 ± 1.628b	4.376 ± 6.433a
Dp[c]	0.777 ± 0.969b	0.319 ± 0.484b	2.207 ± 2.988b	15.092 ± 19.813a
Pt[c]	0.576 ± 0.939b	0.361 ± 0.643b	8.842 ± 22.696a	9.098 ± 9.067a
Pn[c]	1.574 ± 2.146b	3.35 ± 3.959ab	4.987 ± 9.095a	4.804 ± 4.137a
Mv[c]	8.83 ± 16.961b	3.839 ± 9.175b	17.55 ± 10.113b	79.203 ± 61.983a
Mono-g[c]	6.031 ± 9.062b	8.586 ± 12.062b	34.43 ± 32.139a	31.351 ± 36.507a
Di-g[c]	6.744 ± 13.598b	0	0	81.222 ± 59.86a
No[c]	4.748 ± 7.305c	6.92 ± 9.057c	23.715 ± 30.945b	73.203 ± 72.096a
Acetyl[c]	0.429 ± 0.69c	0.159 ± 0.372c	2.586 ± 2.648b	5.147 ± 7.294a
Coumaryl[c]	7.519 ± 14.756b	1.468 ± 4.099b	7.843 ± 4.57b	34.146 ± 26.841a
Caffeoyl[c]	0.079 ± 0.121b	0.038 ± 0.124b	0.286 ± 0.151a	0.077 ± 0.079b
Sum[d]	12.776 ± 19.926c	8.586 ± 12.062c	34.43 ± 32.139b	112.573 ± 88.288a

Means followed by the different letters are significantly different between the cultivars of different genotype groups and purpose of uses at $P < 0.05$ level.
[a] LV, hybrid between *V. labrusca* and *V. vinifera*.
[b] V, *V. vinifera*.
[c] Cy, cyanidin; Dp, delphinidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; Mono-g, monoglucoside; Di-g, diglucoside; No, no combined organic acid; Acetyl, coumaryl and caffeoyl includes all their derivatives.
[d] Sum, summation of anthocyanins.

with 'Hyuga' (14), 'Black Olympia' (19) and 'Super Hamburg' (54) having higher Mv levels, 82.9%, 85.3% and 89.2%, respectively. Most Mv-derivatives were malvidin 3-*O*-(6-*O*-coumaryl)-glucoside-5-*O*-glucoside which accounted for more than 41.3% of the total Mv-derivatives of LV grapes (Table 4). Pn-derivatives accounted for 12% of total anthocyanins, the second most abundant group, with 'Harata 314' (13), 'Beniyamabiko' (20) and 'Fenghou' (37) having Pn at 52% to 62.5% of total anthocyanins. Cy- and Dp-derivatives accounted for 8.7% and 6.1% of total anthocyanins, respectively. However, 'Red Niagara' (6), 'Pondicherry' (8), 'Hongze' (25), 'Catawba' (40), and 'Hongdajiubao' (51) had Cy-derivatives more than 60% of total anthocyanins and more than a half of the total anthocyanins were Dp-derivatives in the skin of 'Yigawa 1055' (26), 'Bennifuji' (28), 'Tano Red' (31) and 'Yagawa 1011'. Pt-derivatives were very low for all LV grapes.

The number of glucosides per derivative depended upon anthocyanin content. Diglucoside derivatives were more abundant than monoglucoside derivatives across the cultivars with a high anthocyanin content (>5 mg (100 g)$^{-1}$ FW) except for 'Venus' (1), 'Jingxing Seedless' (3), 'Red Niagara' (6), 'Pondicherry' (8), 'Guixiangyi' (17), 'Vehava 180' (18), 'Hongze' (25), 'Catawba' (40), '86-11' (44), 'Libier' (47) and 'Honey Juice' (53). On the contrary, a higher content of monoglucoside derivatives than diglucoside derivatives were observed in the cultivars with a low anthocyanin content (<5 mg (100 g)$^{-1}$ FW) except for 'Tano Red' (31).

As regards organic acid derivatives, *p*-coumaroyl derivatives were the most abundant in cultivars with a high content except for 'Red Niagara' (6), 'Pondicherry' (8), 'Guixiangyi' (17), 'Vehava' (18), 'Catawba' (40), 'Takasumi' (43), '86-11' (44), 'Libier' (47), 'Jasmine' (48), and 'Honey Juice' (53). For cultivars with low anthocyanin content, there were few organic acid derivatives except for 'Pione' (24).

There were significant correlations amongst the types of anthocyanins in LV grapes. Mv-derivatives were highly and positively correlated with Dp ($r = 0.499$, $P < 0.01$), Pn ($r = 0.703$, $P < 0.01$) and Pt-derivatives ($r = 0.877$, $P < 0.01$), and Pn-derivatives were highly and positively correlated with Dp ($r = 0.539$, $P < 0.01$) and Pt-derivatives ($r = 0.595$, $P < 0.01$). Pt-derivatives were highly and positively correlated with Dp-derivatives ($r = 0.605$, $P < 0.01$). However, Cy was only positively correlated with Dp ($r = 0.483$, $P < 0.01$).

3.3.2. V. vinifera grapes

All anthocyanins were monoglucoside derivatives in V grapes (Table 3). In V table grapes, anthocyanins were mainly composed of Mv and Pn-derivatives, accounting for 44.7% and 37.7% of total anthocyanins, respectively. Malvidin 3-*O*-glucoside was the main Mv-derivatives in the most V table grapes (Table 4). Some cultivars had abundant Cy, Dp and Pt-derivatives although the average of those three derivatives was low in V table grapes. The content of Cy-derivatives was high in 'Shiyaira' (69), '96-2' (83), and 'Red Alexandria' (91), having 89.6%, 52.8% and 57.3%, respectively. Moreover, abundant Dp-derivatives (61.2%) and Pt-derivatives (20.4%) were found in 'Fenniu' (68).

Differing from V table grapes, Mv-derivatives were dominant anthocyanins in V wine grapes except for 'Italian Riesling' (94), 'Meichun' (95) and 'Mulent Black' (99) with content at less than 32% of total anthocyanins. Pt-derivatives were also important in V wine grapes, higher than LV grapes and V table grapes. Among all V grapes, 'Mulent Black' (99) had the highest content in Pt-derivatives (65.1% of total anthocyanins). Abundant Pn-derivatives were found in 'Italian Riesling' (94%, 62.2%) and 'Meichun' (95%, 55.1%). Dp-derivatives were less than 10% of total anthocyanins and Cy-derivatives were very low in V wine grapes.

Most anthocyanins did not combine with organic acids in *V. vinifera* table or wine grapes. For the rest that combined with organic acids, *p*-coumaroyl derivatives were the dominant.

Pn 3-*O*-glucoside and Mv 3-*O*-glucoside were dominant anthocyanins in table grapes (Table 4). As regards wine grapes, most anthocyanin was Mv 3-*O*-glucoside (26.9%) and followed by Pt 3-*O*-glucoside with 23.3%. The content of Pn 3-*O*-glucoside and Mv 3-*O*-(6-*O*-coumaryl)-glucoside were also important in wine grapes. So Pt 3-*O*-glucoside and Mv 3-*O*-(6-*O*-coumaryl)-glucoside should also play important roles in wine colour.

The correlations among anthocyanins in V grapes were different from those in LV grapes. In V grapes, Mv-derivatives were highly and positively correlated with Dp ($r = 0.639$, $P < 0.01$) and Pt-derivatives ($r = 0.374$, $P < 0.01$). The correlations were positively significant between Pn-derivatives and Cy-derivatives ($r = 0.536$, $P < 0.01$), and between Dp-derivatives and Pt-derivatives ($r = 0.910$, $P < 0.01$).

3.3.3. Other Vitis

In general, other *Vitis* were higher for both total anthocyanins and each kind anthocyanin than LV and V (Table 3). Mv-derivatives were the most abundant component, accounting for 80% of total anthocyanins except for 'Russia Concord' (105) and 'Concord' (108). Mv-derivatives accounted for less than 10% of total anthocyanins while 69.2% and 25% of Cy-derivatives were found in 'Russia Concord' and 'Concord', respectively. Moreover, 'Concord' was characterised by a very high content of Dp-derivatives, 44.6% of total anthocyanins. The content of Pt- and Pn-derivatives was low for

Table 4
Anthocyanin average content and range in parentheses in grape berry skin (mg (100 g)$^{-1}$ FW)

Peak number	T-LV[a]	J-LV[b]	W-LV[c]	T-V[d]	W-V[e]	J-TV[f]	J-LA[g]	W-VA[h]	L[i]	CW[j]	BR[k]
1	0.09 (0–2.61)	-[m]	-[m]	-[m]	-[m]	3.38 (1.85–4.9)	0.01	0.53 (0.3–0.76)	2.81	3.62	5.99
2	0.10 (0–2.11)	-[m]	-[m]	-[m]	-[m]	0.17 (0.09–0.25)	0.14	0.05 (0.03–0.07)	1.52	1.34	5.9
3	0.38 (0–3.56)	0.02	0.02	0.24 (0.01–1.66)	1.57 (0.11–8.7)	0.62 (0.22–1.02)	0.41	0.8 (0.19–1.41)	11.63	8.81	41.38
4	0.04 (0–1.15)	-[m]	0.01	-[m]	-[m]	4.42 (2.61–6.23)	0.01	0.85 (0.57–1.13)	0.57	4.02	14.76
5	0.69 (0.01–8.16)	0.01	-[m]	0.57 (0.03–2.19)	0.64 (0.02–5.25)	0.03 (0.01–0.05)	1.69	0.05 (0.03–0.08)	3.45	0.69	9.44
6	0.29 (0–2.62)	0.01	0.08	-[m]	-[m]	2.96 (2.17–3.76)	0.01	1.55 (0.99–2.12)	0.39	3.98	9.13
7	0.05 (0–0.36)	0.03	0.04	0.26 (0–2.45)	8.03 (0.18–75.16)	2.87 (2.51–3.23)	0.01	1.14 (0.53–1.76)	0.39	1.39	3.36
8	1.2 (0–16.5)	0.03	0.21	-[m]	-[m]	52.65 (41.97–63.33)	0.13	36.57 (19.14–54)	1.73	51.23	100.84
10	0.05	-[m]	-[m]	-[m]	-[m]	-[m]	-[m]	-[m]	0.17	0.13	0.03
11	0.58 (0–8.19)	0.02	0.02	3.01 (0.02–21.37)	4.22 (0.15–31.7)	0.13 (0.1–0.16)	0.13	0.12 (0.05–0.2)	0.39	0.78	3.22
12	0.04	-[m]	-[m]	-[m]	-[m]	0.05 (0.01–0.08)	-[m]	-[m]	0.1	1.07	0.15
13	1.29 (0–15.38)	0.16	0.44	2.84 (0.03–31.26)	9.26 (1.33–20.65)	3.83 (2.17–5.5)	0.08	4.07 (1.27–6.88)	0.95	16.88	38.83
17	0.03 (0–0.16)	-[m]	0.01	0.01 (0–0.1)	0.1 (0–0.38)	0.01 (0–0.02)	0.01	0.02 (0–0.04)	1.81	1.09	0.79
18	0.19 (0–1.92)	-[m]	0.11	-[m]	-[m]	1.29 (1.2–1.38)	-[m]	0.98 (0.55–1.41)	0.19	15.81	1.18
19	0.03 (0–0.27)	-[m]	0.01	0.01 (0–0.13)	0.03 (0–0.1)	0.02 (0.01–0.03)	-[m]	0.09 (0–0.19)	-[m]	0.21	0.04
20	0.09 (0–0.93)	-[m]	0.07	-[m]	-[m]	2.19 (1.74–2.65)	-[m]	0.21 (0.13–0.28)	5.43	4.79	4.52
21	0.02 (0–0.22)	-[m]	0.01	0.01 (0–0.12)	0.15 (0.02–0.43)	0.03 (0.02–0.04)	-[m]	0.02 (0–0.04)	0.38	0.67	0.95
22	0.08 (0–0.65)	-[m]	0.01	-[m]	-[m]	0.07 (0.03–0.11)	-[m]	0.01 (0–0.01)	3.1	0.66	0.86
23	0.19 (0–2.14)	0.01	0.04	0.07 (0–1.28)	0.54 (0.03–1.54)	0.77 (0.45–1.09)	-[m]	0.46 (0–0.0–91)	1.26	1.82	6.61
24	0.15 (0–1.83)	-[m]	0.17	-[m]	-[m]	2.5 (2.24–2.77)	-[m]	0.83 (0.44–1.21)	-[m]	4.77	4.12
25	0.05 (0–1.08)	-[m]	-[m]	0.04 (0–0.45)	0.19 (0.01–0.71)	-[m]	-[m]	0.02 (0–0.03)	7.24	0.09	-[m]
26	0.1 (0–1.03)	0.01	0.04	0.09 (0–1.92)	2.12 (0.02–7.39)	0.2 (0.15–0.24)	-[m]	0.06 (0.02–0.1)	0.35	2.25	0.91
27	0.34 (0–3.73)	0.01	0.24	-[m]	-[m]	0.21 (0.12–0.29)	-[m]	0.26 (0.18–0.33)	0.04	1.2	0.05
28	3.19 (0–44.16)	0.07	5.41	-[m]	-[m]	28.97 (25.87–32.07)	-[m]	21.29 (8.36–34.23)	0.81	61.63	6.88
29	0.16 (0–3.29)	-[m]	-[m]	0.14 (0–2.56)	0.17 (0.02–0.53)	-[m]	0.03	-[m]	4.62	0.03	-[m]
30	0.26 (0–2.86)	0.01	0.11	0.09 (0–1.73)	0.67 (0.05–1.69)	1.19 (0.88–1.51)	-[m]	0.55 (0.02–1.08)	1.3	1.51	4.35
31	0.08 (0–0.5)	0.01	0.02	0.04 (0–0.72)	0.29 (0.03–0.51)	0.14 (0.09–0.2)	-[m]	0.09 (0–0.17)	0.05	0.12	-[m]
32	0.29 (0–1.9)	0.04	0.03	0.30 (0.01–1.62)	0.58 (0–1.06)	0.13 (0.09–0.16)	0.01	0.07 (0.01–0.13)	0.33	0.39	0.16
33	1.68 (0–22.81)	0.11	0.87	0.87 (0–18.91)	5.88 (0.42–10.38)	6.21 (6.2–6.21)	0.01	3.1 (0.34–5.86)	0.44	9.77	3.69
Sum[l]	11.63 (0.11–97.53)	0.55	7.97	8.59 (0.42–63.03)	34.43 (8.77–118.45)	115.02 (99.93–130.11)	2.7	73.77 (33.13–114.42)	51.43	200.73	268.14

The peak numbers in this table correspond to the same number as in Table 2.
[a] T-LV, table grape of *V. labrusca* and *V. vinifera*.
[b] J-LV, juice grape of *V. labrusca* and *V. vinifera*.
[c] W-LV, wine grape of *V. labrusca* and *V. vinifera*.
[d] T-V, table grape of *V. vinifera*.
[e] W-V, wine grape of *V. vinifera*.
[f] J-TV, juice grape of *V. thunbergii* and *V. vinifera*.
[g] J-LA, juice grape of *V. lubrusca* and *V. amurensis*.
[h] W-VA, wine grape of *V. vinifera* and *V. amurensis*.
[i] L, *V. labrusca*.
[j] CW, Chinese wild grape *V. amurensis* var. *dissecta*.
[k] BR, rootstock grape of *V. berlandier* and *V. riparia*.
[l] Sum, summation of anthocyanins.
[m] -, is not detected.

the other *Vitis* germplasm, less than 10% of total anthocyanins in general, except for 'Concord' and 'Hairless' (110). All the other *Vitis* germplasms had both monoglucoside and diglucoside derivatives. However, diglucoside derivatives were much more abundant, almost 3-fold, than monoglucosides except for 'Russia Concord' and 'Concord' in which higher monoglucoside derivatives were observed. The contents of *p*-coumaroyl derivatives were greater than acetyl and caffeoyl derivatives.

The composition of the anthocyanins in the skin varied widely with the genetic background of the other *Vitis* species (Table 4). Dp 3-*O*-glucoside was the most abundant anthocyanin, and Pn 3-*O*-(6-*O*-acetyl)-glucoside, Dp 3-*O*-(6-*O*-coumaryl)-glucoside-5-*O*-glucoside, Cy 3-*O*-(6-*O*-coumaryl)-glucoside, Cy 3-*O*-glucoside and Cy 3-*O*-(6-*O*-coumaryl)-glucoside-5-*O*-glucoside were also important composition of anthocyanins in 'Concord' (108, *V. labrusca*). Chinese wild grape (*V. amurensis* var. *dissecta*) was characterised by Mv-derivatives, and Mv 3-*O*-(6-*O*-coumaryl)-glucoside-5-*O*-glucoside and Mv 3-*O*-glucoside-5-*O*-glucoside accounted for 30.7% and 25.6% of total anthocyanins, respectively. Mv 3-*O*-glucoside-5-*O*-glucoside was dominant anthocyanins (37.6% of total anthocyanins), and Dp 3-*O*-glucoside and Mv 3-*O*-glucoside were also important anthocyanins (about 15%) in 'Hairless' (110), a hybrid of BR.

3.4. Principal component analysis

Principal component analysis was used to analyse the data for 29 anthocyanins in 102 red grapes. The first 10 PCs accounted for 85% of total variance. PC 1, PC 2 and PC 3 explained a relatively high percentage (28% for PC 1; 17.2% for PC 2; 12.7% for PC 3) of total variance. PC 1 was clearly connected with the content of total anthocyanins, exhibiting a positive value, except for Cy 3-O-gluco-

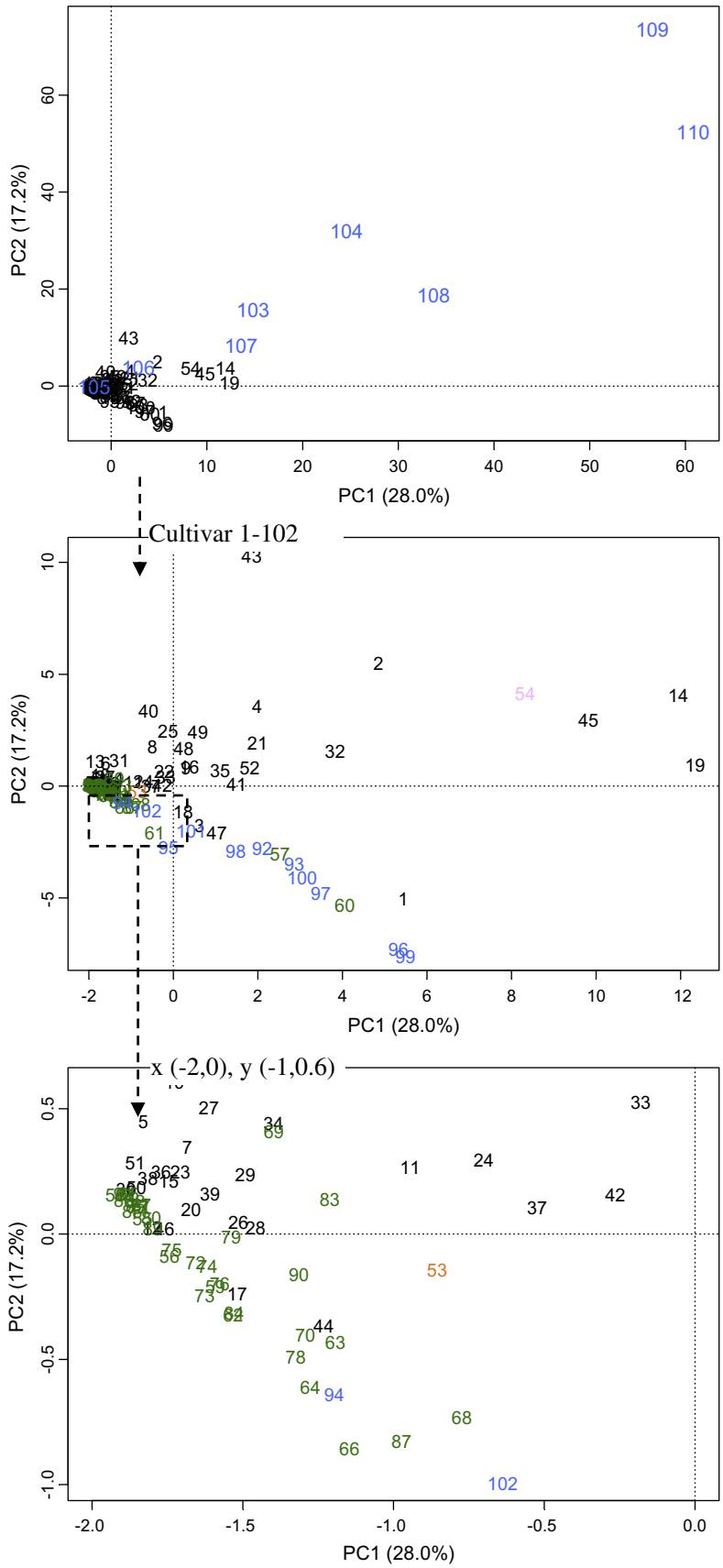

Fig. 2. Positions of PC scores of 110 grape cultivars (indicated in numbers) according PC1 and PC2. Percentages in parenthesis represent the variance of each component. The numbers in the figure represent the sample numbers, which correspond to the same cultivars as in Table 1. Cultivars number 1 to number 54 (black) are hybrids between *V. labrusca* and *V. vinifera*; cultivars from 55 to 91 are table grape of *V. vinifera*; 92 to 102 are wine grape of *V. vinifera*; 103 to 110 are the other interspecific hybrid grapes or wild species.

side and Pn 3-O-glucoside. PC 2 was decided by monoglucoside and diglucoside derivatives. Monoglucoside derivatives except for Cy-derivatives exhibited a negative value. In contrast, all diglucosides and Cy-derivatives exhibited a positive value. PC 3 was represented by diglucoside, diglucoside-acetyl, and diglucoside-*p*-coumaryl derivatives. Diglucoside-*p*-coumaryl derivatives exhibited a negative value while the others exhibited a positive value.

Fig. 2 is a scatter plot showing the distribution of 110 grape cultivars according to PC 1 and PC 2. In the scatter plot, the content of total anthocyanins for the cultivars tended to increase proceeding from negative to positive values of PC1. From negative to positive values of PC2, cultivar means generally increased in their content of diglucoside derivatives while the content of monoglucoside derivatives generally decreased.

The position of all genotypes could be divided into three groups. LV grapes except for six cultivars were located in the upper part of the PC 1 axis, explained by the presence of diglucoside derivatives. The positions in the lower part of the scatter plot for the other six cultivars, 'Venus' (1), 'Libier' (47), 'Jingxing Seedless' (3), 'Vehava' (18), '86-11' (44) and 'Guixiangyi' (17), was due to a low ratio of diglucosides to monoglucoside derivatives. Most V table grapes lay down at left and on the PC 1 axis. But several cultivars, 'Shiyaira' (69), '96-2' (83), 'Red Globe' (58), were located upper part of the PC 1 axis due to high Cy-derivatives, and 'Buffalo' (57) and 'Otilia' (60) were situated the right of the PC 2 axis due to their high Mv-derivatives. Wine grapes of V (from 92 to 102) were located in general down at the right due to their high content of total anthocyanins and monoglucoside derivatives; however, 'Italian Riesling' (94) and 'Shu-162' (102) were situated to the left of the group on the PC 1 axis due to high Pn 3-*O*-glucoside. For the other *Vitis*, most were found in the positive area of both the PC 1 and PC 2 axes of the scatter plot except for 'Russia Concord' (105) which was negative on the PC 1 axis due to high Cy 3-*O*-glucoside.

4. Conclusions

Anthocyanin content largely varied with genetic background as well as their purpose of use. Sixteen monoglucoside anthocyanins were detected in V grape and twenty-nine anthocyanins were detected in LV grape and other *Vitis*. For total anthocyanin content, wild species and rootstock cultivars were higher than interspecific hybrids, cultivars were low in general, and wine grapes were higher than table grapes in same species. Mv-derivatives were the most abundant anthocyanins in cultivars with a high content, and Cy-derivatives were the most abundant in low content cultivars of LV grape. Pn-derivatives were the most abundant anthocyanins in the majority of V table grapes, and Mv-derivatives were the greatest in V wine grapes.

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