Effects of Exogenous Absciscic Acid on Phenolic Characteristics of Red *Vitis vinifera* Grapes and Wines

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Abstract The effects of exogenous abscisic acid (ABA) on phenolic characteristics of grapes and wines were investigated in *Vitis vinifera* cvs. Merlot and Cabernet Sauvignon. Exogenous ABA treatment at veraison significantly improved phenolic contents, mainly anthocyanins and flavonols, and antioxidant properties of the grape skins, but had no effects on total phenolics and antioxidant activities in the seeds or on basic fruit qualities. The wines made from ABA-treated grapes were also consequently enhanced in total phenolics, anthocyanins, flavonols and antioxidant activities. The proportions of methylated anthocyanins in the skins and acylated anthocyanins and derived pigments in the wines were decreased to some degree by exogenous ABA treatment. No distinct relationships were observed between ABA concentrations and phenolic characteristics, and the effects were observed even with 200 mg/L ABA. The results revealed that exogenous ABA applied at veraison offered opportunities to improve phenolic contents and nutritional values of grape skins and wines.

Keywords: ABA, grape, wine, phenolic compound, antioxidant activity

Introduction

Phenolic compounds are some of the most important secondary metabolites in grapes, and various species exist in different parts of the grape berries. Anthocyanins, flavonols, stilbenes and some flavan-3-ols existed in the skins, while most flavan-3-ols and phenolic acids (hydroxybenzoic and hydroxycinnamic acids) are mainly found in the seeds and pulps, respectively (1). These compounds can be extracted into wines during maceration and fermentation. Their contents and compositions play a crucial role in the organoleptic properties and quality of grape berries and wines. Anthocyanins are directly responsible for the color, while flavan-3-ols, secondarily flavonols and phenolic acids support and stabilize the color, acting as anthocyanin copigments in wines (2,3). Flavan-3-ols, especially polymers (proanthocyanins), also affect the astringency, bitterness and structure of wines, while flavonols are the major factor in bitterness (4). Many studies have indicated that these phenolic compounds might attenuate degenerative processes such as cancer and cardiovascular disease by their antioxidant and anti-inflammatory activities (5–7).

Phenolic contents and compositions in grapes and wines are regulated by internal and external factors, such as genotype, climate, viticulture practices and vinification processes. The application of elicitors and stimulating agents has proved to be an effective measure

in improving the biosynthesis of phenolic compounds. Absciscic acid (ABA) is a plant growth hormone naturally synthesized by plants. In grapes, endogenous ABA is believed to be an important regulation phytohormone of berry maturation and ripening, because its concentration peaks in berries at veraison, the onset of maturation (8). In addition, endogenous ABA serves as a signaling chemical to defend against adverse environmental conditions such as drought, cold and microbial infections (9). Recently, many researchers have reconsidered the potential application of ABA in viticulture as its cost has greatly decreased. For example, the application of exogenous ABA at veraison of grapes has been demonstrated to improve berry coloring (10) and increase anthocyanin accumulation (11). However, the effects of exogenous ABA on the phenolic characteristics of grape berries and wines have not been systematically researched. The objective of this study was to comprehensively investigate the effect of exogenous ABA application on the phenolic contents and compositions and antioxidant capacities in berries and wines of *Vitis vinifera* cvs. Merlot and Cabernet Sauvignon.

Materials and Methods

Materials This study was conducted during 2009 and 2010 on own-rooted 8-year-old ‘Merlot’ vines and 10-year-old ‘Cabernet

Sauvignon' vines planted in the commercial vineyard of Ji county, located at 117°39' E and 24°3' N. The vines were spaced 1 m within rows and 2.5 m between rows, bilateral-cordon trained and grown with no soil or water restrictions.

The vines were randomly assigned to receive one of four treatments: 0, 200, 400 or 600 mg/L ABA. All the treatment solutions were in water with 0.5% (v/v) cosolvent (30% ethanol) and 0.5% (v/v) adhesive and applied at veraison when approximately 50% of the berries started to turn color. The 0 mg/L ABA group was the control. Each experimental group contained all the grape clusters of 10 vines. The treatment time was determined after preliminary experiments (unpublished data). The treatment solutions were applied directly to the clusters with a handheld sprayer until runoff (approximately 10 mL solution per cluster). All the treatments were sprayed at dusk (sunset) to minimize ABA photodestruction.

All the treated grapes were harvested at technological ripeness. Six-clusters from two vines (3 clusters from each vine) were regarded as one replicate sample. We randomly selected three replications for the determination of grape berries, which were immediately taken to the laboratory in refrigerated bags. The skins and seeds were separated manually, then freeze-dried (LGJ-12; Songyuan Huaxing Corporation, Beijing, China). The dried materials were ground with a stainless-steel grinder (FW-135; Taister Corporation, Tianjin, China). The grounded seeds were defatted twice with petroleum ether for 3 h. The defatted seed powder and the skin powder were screened with 60 sieve size (0.25 mm), then stored in vacuum-packaged polyethylene pouches at 20°C for subsequent analysis.

The remaining grapes were used for small-scale vinification according to the previous method in our lab (12). Each wine sample from one fermented container (5 L) was considered as one replicate resulting in two replications for each treatment group.

Chemicals and standards The chemicals, ($\pm$)-ABA (99%), Folin and Ciocalteu's phenol reagent (2 N), 2,4,6-tripyridyl-s-triazine (TPTZ, 99%) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, 99%), and the standards, caffeic acid, gallic acid, resveratrol, malvidin-3-O-glucoside, quercetin and catechin were obtained from Sigma-Aldrich (St. Louis, MO, USA). The HPLC grade reagents, acetonitrile, formic acid, acetic acid and methanol were obtained from Fisher Co. (Fairlawn, NJ, USA). The adhesive of ABA solution was obtained from Kaixinyu Co. (Shanxi, China). Other chemicals were analytical grade and obtained from Lanyi Co. (Beijing, China).

Determination of berry weight, total soluble solids, titratable acidity and pH The grape berries from each replicate sample were selected randomly for the determinations of berry weight and titratable acidity. The grapes were weighed and recorded. The titratable acidity was determined by a titrimetric method (13). A volume of 20 mL was pipetted from must for making each wine sample to determine total soluble solids and pH with a hand-held refractometer (Wancheng Co., Beijing, China) and a pH meter (pH

211, HANNA, Padova, Italy), respectively.

Extraction of phenolic compounds The extraction of the total phenolics and anthocyanins was based on the slightly modified method of Xu *et al.* (14). Briefly, the extraction solvents for the skins and seeds were methanol/water/acetic acid (70:29:1, v/v/v) and methanol containing 0.1% acetic acid, respectively. The skin/seed powder (0.5 g) was placed in a shaker (SHZ-88A; Taicang Experiment Equipment Factory, Jiangsu, China) with 20 mL extraction solvent at 300 rpm for 2 h at 25°C in a dark environment. The extracts were then separated from the solids by centrifuge (Beckman Coulter Ltd., Palo Alto, CA, USA). The process was repeated three times and all the extracts for each sample were collected together. For the skin extracts, a volume of 1 mL for each replicate sample was retained to determine its total phenolic content and antioxidant capacity, and the remaining was dried (RE-52A; Yarong Biochemistry Instrument Factory, Shanghai, China) at 30°C and re-dissolved in the extraction solvent to a unified volume of 5 mL for anthocyanin analysis. The wine samples were used directly to analyze their total phenolic contents, antioxidant capacities and anthocyanins.

The non-anthocyanin phenolic compounds were extracted by the method of Jin *et al.* (15). All the collected extracts for each sample were dried under 30°C and re-dissolved with methanol to a unified volume of 2 mL.

Determination of total phenolic content and antioxidant capacity

The content of total phenolics was assayed by the Folin-Ciocalteu colorimetric method (16) modified by our lab (14). Briefly, all extracts were diluted with distilled water to make readings among the standard curve ranges. The phenolics in solutions were oxidized by Folin-Ciocalteu reagent, and then neutralized by sodium carbonate. The reaction was incubated in a 40°C water bath for 30 min, and absorbance was measured at 760 nm by UNICO UV-2800 spectrometer (UNICO, New York, NY, USA). The absorbances were converted to total phenolic contents expressed as mg gallic acid equivalents (GAE)/g of dry weight (DW) for grape skins/seeds and per liter for wines, respectively.

The antioxidant capability was assayed using the method of ferric reducing antioxidant power (FRAP) according to Benzie and Strain's method (17). The reaction mechanism is that Fe^{3+} could be reduced to Fe^{2+} by antioxidants, and TPTZ could chelate Fe^{2+} to form a Fe^{2+} -TPTZ complex absorbing at 595 nm (17). Antioxidant activity was expressed as μmol Trolox equivalents (TE)/g DW skins/seeds and μmol TE/L wine.

Analysis of phenolic compounds The skin anthocyanins were analyzed by the reported method (15), and the wine anthocyanin analysis followed Li *et al.* (18). These determinations were performed on an Agilent 1100 series LC-MSD trap (Agilent Technologies, Ltd., Santa Clara, CA, USA) and a Kromasil-C18 column (250×4 mm, 6.5 μm). Non-anthocyanins analysis was performed on a Waters

ACQUITY UPLC combined with tandem XEVO TQ mass (Waters Corp., Milford, MA, USA) and a ZORBAX SB-C18 column (3×50 mm, 1.8 µm). The non-anthocyanin compounds in the skin extracts and wines were analyzed according to the methods of Jin *et al.* (15) and Li *et al.* (18), respectively. For the quantitative evaluations of different phenolic groups, anthocyanins, flavonols, flavan-3-ols, hydroxycinnamic acids, hydroxybenzoic acids and stilbenes were expressed as micrograms of malvidin-3-glucoside equivalence (MGE), quercetin equivalence (QE), catechin equivalence (CE), caffeic acid equivalence (CAE), gallic acid equivalence (GAE) and resveratrol equivalence (RE) per gram of DM for grape skins and per liter for wines, respectively.

Statistical analysis Samples for phenolic compounds determination were analyzed in triplicate, total soluble solids, titratable acidity and pH were measured in duplicate, and berry weight were recorded 30 berries. All results were expressed as mean±standard deviations (SD). Data were subjected to one-way analysis of variance (ANOVA) and differences among treatment groups were tested tukey post-hoc test with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) at the 95% confidence level.

Results and Discussion

Berry weights, soluble solids, titratable acidities and pH of grapes

The grapes belonging to each cultivar were harvested at the same date of each year. Among all the ABA-treated grapes, only the average berry weight of ‘Cabernet Sauvignon’ grapes treated with 400 mg/L ABA was obviously higher than that of the control grapes. In general, the ABA treatments had no effect on berry weight, soluble solids, titratable acidity or pH of both cultivars (Table 1).

Effects of exogenous ABA on total phenolic contents and antioxidant capacities The total phenolic contents (TPCs) and antioxidant capacities of ‘Merlot’ and ‘Cabernet Sauvignon’ grapes and wines are shown in Table 2. The berry skin TPCs of the grapes

treated with different ABA concentrations were significantly higher than those of the control grapes in both years, except for 400 mg/L ABA group in 2009 and 600 mg/L ABA group in 2010 for ‘Merlot’. However, the changes in skin TPCs had no obvious correlation with ABA concentrations, cultivars or years. The increase in skin TPCs due to ABA in ‘Cabernet Sauvignon’ was greater than in ‘Merlot’. Interestingly, the TPCs of the control samples were obviously lower in 2009 than 2010 for both grape cultivars, but the ABA-treated samples in 2009 had a larger increase in total phenolics than those in 2010.

The skin antioxidant activities determined by FRAP in ABA-treated samples were stronger than those in the control samples for both grape cultivars in both years. Similarly, the skin FRAP values of ABA-treated samples in 2009 (approximately 2 times higher than control) showed a greater increase than those in 2010 (20-50% higher than control) for both grapes.

There were no differences in total phenolic content and antioxidant activity in the seeds of both cultivars between the ABA treated and the control groups. Overall, the TPCs and FRAPs of the ‘Cabernet Sauvignon’ seeds were substantially higher than those of the ‘Merlot’ seeds. The ‘Merlot’ samples in 2009 had greater values of both indicators than the samples in 2010, while there was no difference in the ‘Cabernet Sauvignon’ seeds between both years.

Genotype is the important internal factor which decide total phenolic contents and nutritional values of grapes. Xu *et al.* (14) found there were significantly different in phenolic contents and antioxidant capacities of skins and seeds of 18 grape cultivars belonging to *V. vinifera*, muscadine, East Asian and North American species/hybrids. For the seeds, *V. vinifera* ‘Cabernet Sauvignon’ had the highest total phenolics and antioxidant properties, followed by Muscadines. While the highest values of the skins appeared in most East Asian grape species. However, the same grape cultivar might also possess various phenolic contents and antioxidant activities under different viticulture environment and practice. For example, the seeds and skins of winter berries had significantly higher total phenolics and antioxidant activities than summer berries in two-

Table 1. Berry weight, soluble solids, titratable acidity and pH of grapes

Cultivar	Treatments	Berry weight (g) ¹⁾		Soluble solids (°Brix) ²⁾		Titratable acidity (%) ²⁾		pH ²⁾	
		2009	2010	2009	2010	2009	2010	2009	2010
Merlot	Control	1.28±0.29	1.33±0.31	20.40±0.39	21.67±0.51	0.50±0.02	0.49±0.02	3.19±0.04	3.12±0.08
	200 mg/L ABA	1.34±0.19	1.29±0.25	20.00±0.96	22.02±0.65	0.52±0.01	0.51±0.02	3.12±0.09	3.10±0.07
	400 mg/L ABA	1.31±0.41	1.40±0.11	21.04±0.76	21.91±0.71	0.54±0.03	0.46±0.03	3.15±0.06	3.13±0.05
	600 mg/L ABA	1.29±0.33	1.31±0.19	21.10±0.35	22.33±0.89	0.51±0.02	0.52±0.03	3.08±0.03	3.11±0.05
Cabernet Sauvignon	Control	1.45±0.35	1.41±0.34	21.09±0.50	21.81±0.45	0.58±0.03	0.52±0.02	3.10±0.04	3.09±0.02
	200 mg/L ABA	1.38±0.38	1.39±0.22	20.66±1.35	22.10±0.38	0.58±0.11	0.53±0.02	3.12±0.07	3.10±0.04
	400 mg/L ABA	1.31±0.26	1.45±0.56	22.49±0.66 ³⁾	21.47±0.65	0.51±0.05	0.49±0.03	3.10±0.09	3.12±0.08
	600 mg/L ABA	1.54±0.45	1.47±0.36	21.73±1.28	21.93±0.62	0.50±0.04	0.51±0.03	3.10±0.07	3.04±0.07

¹⁾Data are expressed as mean±standard deviation (n=30).

²⁾Data are expressed as mean±standard deviation (n=2).

³⁾The asterisk represented significant differentiation existed between treatment group and control group at 0.05 level from ANOVA.

Table 2. Effects of different exogenous ABA concentrations on the total phenolic contents and antioxidant activities in the grapes and wines

ABA Treatments		Total phenolic content (mg GAE/g DW or mg GAE/L) ^{1,2)}						Ferric reducing antioxidant power (μmol TE/g DW or μmol TE/L) ^{1,2)}					
		Merlot			Cabernet Sauvignon			Merlot			Cabernet Sauvignon		
		2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Skin	Control	28.44±0.41 ^{A/*}	33.16±0.94 ^{A/*}	29.12±0.85 ^{A/*}	37.72±2.52 ^{A/*}	144.49±29.45 ^{A/*}	252.39±27.68 ^{A/*}	187.39±9.08 ^{A/*}	276.15±40.67 ^{A/*}				
	200 mg/L	34.57±0.61 ^{B/*}	37.82±1.88 ^{B/*}	48.49±2.82 ^{B/*}	43.59±2.82 ^{C/*}	345.91±20.18 ^B	358.96±16.97 ^B	369.81±21.82 ^B	384.81±30.45 ^B				
	400 mg/L	30.71±2.25 ^{A/*}	38.09±0.99 ^{B/*}	49.74±5.23 ^B	44.34±3.97 ^C	292.20±17.71 ^{B/*}	361.60±5.35 ^{B/*}	385.89±36.06 ^B	415.96±33.83 ^B				
	600 mg/L	37.19±1.16 ^C	35.17±0.61 ^{AB}	46.69±2.71 ^B	44.89±3.56 ^B	358.61±16.38 ^{C/*}	299.60±12.02 ^{AB/*}	426.10±13.53 ^{B/*}	368.53±30.90 ^{B/*}				
	Control	105.49±4.61 [*]	86.76±7.89 [*]	159.76±7.21	151.21±7.53	554.49±31.08 [*]	454.87±16.74 [*]	785.91±26.41	745.76±33.74				
Seed	200 mg/L	112.36±2.09 [*]	87.21±9.32 [*]	154.76±10.02	155.96±9.56	539.06±50.00 [*]	454.43±25.84 [*]	771.63±32.19	744.86±39.23				
	400 mg/L	102.03±3.59 [*]	87.93±10.45 [*]	162.96±9.14	152.51±11.54	530.49±21.56 [*]	456.66±19.89 [*]	802.77±36.91	745.42±41.11				
	600 mg/L	109.43±6.90	89.34±8.65	154.29±12.83	156.44±8.64	509.91±25.67	457.23±21.63	773.63±23.23	742.48±29.67				
	Control	398.00±4.71 ^A	453.00±47.14 ^A	434.67±11.79 ^{A/*}	527.17±10.61 ^{A/*}	2906.43±141.42 ^{A/*}	3406.43±20.20 ^{A/*}	3299.29±131.32 ^{A/*}	3920.71±10.31 ^{A/*}				
Wine	200 mg/L	498.00±9.43 ^{B/*}	619.67±25.93 ^{B/*}	588.00±11.79 ^B	584.67±9.43 ^{AB}	4088.57±32.83 ^B	5017.14±378.81 ^B	4410.00±75.76 ^B	4310.00±35.36 ^B				
	400 mg/L	470.50±25.32 ^{B/*}	613.83±12.96 ^{B/*}	588.83±34.18 ^B	633.00±23.57 ^{BC}	3810.00±65.66 ^{B/*}	4770.71±121.21 ^{B/*}	4524.29±65.66 ^B	4717.14±65.66 ^C				
	600 mg/L	581.33±18.86 ^C	543.00±37.71 ^{AB}	554.67±2.36 ^{B/*}	672.17±24.75 ^{C/*}	4152.86±75.76 ^B	4224.29±35.36 ^B	4152.86±75.76 ^{B/*}	4910.00±35.36 ^{D/*}				

¹⁾Data are expressed as mean±standard deviation (n=3).²⁾Capital letters in each column of skin / seed / wine are significantly different among different ABA concentrations in the same year at 0.05 level from ANOVA. Asterisks (*) in each line are significantly different between two years for the same ABA concentration at 0.05 level from independent-samples t test.

crop-a-year viticulture because of dramatically contrasting climatic conditions between summer and winter growing seasons (19). The application of elicitors and stimulating agents belong to a method of viticulture practice to improve the quality of grape berries. In this study, the total phenolic contents and antioxidant activities in the skins of the both *V. vinifera* wine grapes were significantly enhanced by ABA treatment at veraison. The result was similar to a previous research for a muscadine grape 'Noble' (20). While the treatment of exogenous ABA had no effect on the both indicators in the seeds of the both grapes. This likely because the exogenous ABA sprayed on the berry surface couldn't reach the interior tissue to play a role.

The effects of exogenous ABA treatments observed in 'Merlot' and 'Cabernet Sauvignon' wine samples were similar to those observed in the grape skins. The values of TPC and FRAP in the wines were closely related to those in the grape skins. The wines of the ABA-treated groups had a significantly higher total phenolic content and antioxidant activity than the control wines, except for TPC of 600 mg/L ABA group in 2010 for 'Merlot' and TPC of 200 mg/L ABA group in 2010 and FRAP of 600 mg/L ABA group in 2009 for 'Cabernet Sauvignon'. Overall, 'Cabernet Sauvignon' wines had higher TPCs and FRAPs than 'Merlot' wines, and both indicators were greater in the 2010 wines than the 2009 wines.

Effects of exogenous ABA on anthocyanins and flavonols

Anthocyanins and flavonols of grape skins: Treatment with exogenous ABA markedly increased the accumulation of anthocyanins and flavonols in grape skins in both years (Table 3). However, no obvious relationships were observed between ABA concentration and the anthocyanin and flavonol contents.

The anthocyanins in *V. vinifera* grapes are mainly composed of the monoglucoside (glc) of five anthocyanidins, including delphinidin (dp), cyanidin (cy), petunidin (pt), peonidin (pn) and malvidin (mv). The five anthocyanins have different numbers of B-ring substituents, allowing them to be divided into two groups: 3',4'-substituents (cy- and pn-derivatives) and 3',4',5'-substituents (dp-, pt-, and mv-derivatives). The latter are the main type of anthocyanins in the grape skins of both cultivars. Anthocyanins can also be divided into non-methylated (dp- and cy-derivatives) and methylated varieties (pt-, pn-, and mv-derivatives). In general, the proportions of non-methylated anthocyanins in the skins of ABA-treated grapes tended to be increased, but only the treated 2009 'Merlot' and the treated 2010 'Cabernet Sauvignon' samples had significant differences compared to the control. *P*-coumaroyl and acetyl derivatives were the main types of acylated anthocyanins. There were no obvious trends found in the ABA-treated and control grapes for the two cultivars (Table 3).

There were six flavonol aglycones found in the 'Merlot' and 'Cabernet Sauvignon' skins and wines: quercetin (Q), kaempferol (K), myricetin (M), isorhamnetin (I), syringetin (S) and laricitrin (L). Similar to anthocyanins, flavonols can be divided into three groups: 4'-substituents (K-derivatives), 3',4'-substituents (Q- and I-derivatives)

and 3',4',5'-substituents (M-, L- and S-derivatives), according to their B-ring substituent numbers. In general, exogenous ABA treatment had no effect on flavonol composition in grape skins (Table 3).

The exogenous ABA treatment has been regarded as a viable new method to improve grape quality. ABA is an important signaling molecule in the development and ripening of grape berries. ABA concentration was found to peak twice: once during the early stages of berry development and once at late veraison, followed by a decline as the berries ripened (21,22). Veraison is a crucial stage of phytochemical synthesis and accumulation in grape berries, and the anthocyanins in particular start to accumulate at this time. We found that exogenous ABA treatment at veraison significantly enhanced anthocyanin levels in 'Merlot' and 'Cabernet Sauvignon' skins, consistent with several previous studies of other grape cultivars (23-26). This may be due to an increase in the activities of key enzymes involved in the anthocyanin biosynthetic pathway (27). We also found skin flavonol levels were increased by exogenous ABA. The anthocyanins and flavonols of grapes share the same upstream biosynthetic pathway in which naringenin converts into flavonoids by flavonoid 3'-hydroxylase, flavonoid 3',5'-hydroxylase and flavanone 3-hydroxylase (28). In their downstream pathway, the O-methylation reactions of the anthocyanins and the flavonols are the same, even if the enzymes involved in the reactions have not been proved to be the same (29). As a result, any factors affecting anthocyanin biosynthesis might have a similar influence on flavonol biosynthesis (30). A recent study concluded the structural genes and their transcriptional regulators in the phenylpropanoid and flavonoid biosynthetic pathways were upregulated by treatment with exogenous ABA (31). We also found each anthocyanin and flavonol compound was basically higher in ABA-treated grape skins than in the control skins. As a result, the effects of exogenous ABA on the anthocyanin and flavonol compositions were not as remarkable as on their concentrations.

Anthocyanins and flavonols of wines: As expected, the anthocyanin and flavonol concentrations in the wines made from the ABA-treated grapes were obviously higher than those in the wines made from the control grapes (Table 4).

The treatment of veraison grapes with exogenous ABA had some impact on the anthocyanin composition of their wines (Table 4). The 3',4'-substituent proportions in the wines made from ABA-treated 'Cabernet Sauvignon' berries decreased significantly compared to the control; conversely, the 3',4',5'-substituent proportions increased. This trend was also observed in 'Merlot' wines, but to a lesser extent. The level of acylated anthocyanins and derived pigments (mainly anthocyanin - flavanol dimers) in the wines made from treated grapes decreased compared to the control wines. While exogenous ABA treatment had no effect on flavonol composition of the wines (Table 4).

Wines are the final product of wine grapes, and whether the wine content of phenolic compounds with high bioactivities can be improved by grape treatment with exogenous ABA is an important

Table 3. Effects of different exogenous ABA concentrations on the contents and compositions of phenolic compounds in the skins of mature grapes

	Control ^{1,2}			200 mg/L ABA ^{1,2}			400 mg/L ABA ^{1,2}			600 mg/L ABA ^{1,2}		
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Merlot												
Total anthocyanins (mg MGE/g DW) ³	3.42±0.26 ^A *	5.16±0.11 ^A *	8.32±0.13 ^C *	9.19±0.28 ^B *	7.20±0.35 ^B *	9.98±0.16 ^B *	7.08±0.42 ^B	9.98±0.16 ^B *	7.08±0.42 ^B	9.98±0.16 ^B *	7.08±0.42 ^B	8.09±0.91 ^B
3',4'-substituted %	14.10±0.29 ^A *	19.20±2.87 ^A *	14.92±0.22 ^A *	18.21±0.79 ^A *	17.41±0.28 ^B *	29.03±0.96 ^B *	21.72±0.48 ^C	29.03±0.96 ^B *	21.72±0.48 ^C	29.03±0.96 ^B *	21.72±0.48 ^C	24.57±2.14 ^B
3',4',5'-substituted %	85.91±0.29 ^C *	80.80±2.87 ^B /a	85.08±0.22 ^C *	81.79±0.79 ^B *	82.59±0.28 ^B *	70.97±0.96 ^A *	78.28±0.48 ^A	70.97±0.96 ^A *	78.28±0.48 ^A	70.97±0.96 ^A *	78.28±0.48 ^A	75.43±2.14 ^A
Non-Methoxylated %	6.25±0.21 ^A	8.75±2.40 ^{AB}	5.59±0.24 ^A *	7.54±0.42 ^A *	7.35±0.51 ^B /b	11.04±1.23 ^B /b	7.52±0.36 ^B	11.04±1.23 ^B /b	7.52±0.36 ^B	11.04±1.23 ^B /b	7.52±0.36 ^B	9.32±1.47 ^{AB}
Methoxylated %	93.75±0.21 ^B	91.25±2.40 ^{AB}	94.41±0.24 ^B /a	92.46±0.42 ^B *	92.65±0.51 ^A *	88.96±1.23 ^A *	92.48±0.36 ^A	88.96±1.23 ^A *	92.48±0.36 ^A	88.96±1.23 ^A *	92.48±0.36 ^A	90.68±1.47 ^{AB}
Non-acetylated %	60.60±1.86 ^{AB} *	77.40±2.32 [*]	58.30±0.40 ^A *	76.13±1.73 [*]	66.53±1.95 ^B *	82.87±0.72 [*]	63.82±1.83 ^{BC}	82.87±0.72 [*]	63.82±1.83 ^{BC}	82.87±0.72 [*]	63.82±1.83 ^{BC}	75.65±1.76 ^B
Acetylated %	15.12±1.39 ^A *	2.78±0.17 [*]	19.14±0.66 ^B /a	2.25±0.29 [*]	14.84±1.15 ^A *	2.61±0.34 [*]	18.01±0.76 ^B *	2.61±0.34 [*]	18.01±0.76 ^B *	2.61±0.34 [*]	18.01±0.76 ^B *	3.82±0.75 [*]
Cinnamylated %	24.28±1.02 ^B /a	16.33±0.45 ^{AB} /a	22.56±0.53 ^B /a	19.17±0.80 ^B *	18.63±1.04 ^A *	13.19±0.39 ^A *	18.17±1.86 ^A	13.19±0.39 ^A *	18.17±1.86 ^A	13.19±0.39 ^A *	18.17±1.86 ^A	18.18±2.29 ^{AB}
Caffeiclylated %	nd	3.49±0.43	nd	2.45±0.19	nd	1.33±0.18	nd	1.33±0.18	nd	1.33±0.18	nd	2.35±0.58
Total flavonols (mg QE/g DW) ³	0.21±0.04 ^A *	0.33±0.00 ^A *	0.36±0.02 ^B /a	0.45±0.01 ^B /a	0.31±0.02 ^{AB} /a	0.46±0.02 ^B /a	0.30±0.00 ^{AB} /a	0.46±0.02 ^B /a	0.30±0.00 ^{AB} /a	0.46±0.02 ^B /a	0.30±0.00 ^{AB} /a	0.43±0.03 ^B /a
4'-substituted %	nd	6.08±0.53	nd	7.82±0.37	nd	7.75±1.22	nd	7.75±1.22	nd	7.75±1.22	nd	7.28±0.74
3',4'-substituted %	47.69±3.42	51.90±3.21	50.46±2.11	53.06±4.21	50.56±4.18	54.31±3.42	52.11±3.97	54.31±3.42	52.11±3.97	54.31±3.42	52.11±3.97	54.87±3.96
3',4',5'-substituted %	52.31±4.23	42.02±3.28	49.54±2.65 [*]	39.12±3.54 [*]	49.44±2.47 [*]	37.94±2.35 [*]	47.89±3.11 [*]	37.94±2.35 [*]	47.89±3.11 [*]	37.94±2.35 [*]	47.89±3.11 [*]	37.85±2.33 [*]
Total flavan-3-ols (mg CE/g DW) ³	0.21±0.01	0.17±0.01	0.23±0.02	0.19±0.01	0.24±0.02	0.20±0.01	0.23±0.02	0.20±0.01	0.23±0.02	0.20±0.01	0.23±0.02	0.19±0.00
Monomers %	27.34±0.53 [*]	25.17±0.24 [*]	24.27±0.82	22.43±1.11	24.16±1.75	22.29±1.06	24.68±0.56	22.29±1.06	24.68±0.56	22.29±1.06	24.68±0.56	23.60±0.19
Polymers %	72.66±0.53 [*]	74.83±0.24 [*]	75.73±0.82	77.57±1.11	75.84±1.75	77.71±1.06	75.32±0.56	77.71±1.06	75.32±0.56	77.71±1.06	75.32±0.56	76.40±0.19
Total stilbenes (mg RE/g DW) ³	0.16±0.01	0.17±0.01	0.18±0.01	0.18±0.01	0.14±0.01 [*]	0.18±0.01 [*]	0.17±0.01	0.18±0.01 [*]	0.17±0.01	0.18±0.01 [*]	0.17±0.01	0.18±0.01
Cabernet Sauvignon												
Total anthocyanins (mg MGE/g DW) ³	4.43±0.70 ^A *	7.54±0.20 ^A *	8.58±0.01 ^B /a	10.88±0.14 ^B *	13.27±0.39 ^C *	18.08±0.20 ^C *	8.22±1.57 ^B *	18.08±0.20 ^C *	8.22±1.57 ^B *	18.08±0.20 ^C *	8.22±1.57 ^B *	18.44±0.19 ^C *
3',4'-substituted %	13.07±1.03 ^B	13.57±2.04 ^B	9.46±0.67 ^A *	14.59±0.75 ^B *	10.34±1.10 ^A	11.32±0.43 ^A	9.89±0.30 ^A	11.32±0.43 ^A	9.89±0.30 ^A	11.32±0.43 ^A	9.89±0.30 ^A	11.93±0.61 ^{AB}
3',4',5'-substituted %	86.93±1.03 ^A	86.43±2.04 ^A	90.54±0.67 ^B /a	85.41±0.75 ^A *	89.66±1.10 ^B	88.68±0.43 ^B	90.11±0.30 ^B	88.68±0.43 ^B	90.11±0.30 ^B	88.68±0.43 ^B	90.11±0.30 ^B	88.07±0.61 ^{AB}
Non-Methoxylated %	6.68±1.62	9.22±2.52 ^A	6.70±0.11 [*]	11.26±1.03 ^B /a	7.77±1.09 [*]	13.13±0.84 ^C /a	6.83±1.31 [*]	13.13±0.84 ^C /a	6.83±1.31 [*]	13.13±0.84 ^C /a	6.83±1.31 [*]	11.68±0.78 ^B /a
Methoxylated %	93.32±1.62	90.78±2.52 ^C	93.30±0.11 [*]	88.74±1.03 ^B /a	92.23±1.09 [*]	86.87±0.84 ^A *	93.17±1.31 [*]	86.87±0.84 ^A *	93.17±1.31 [*]	86.87±0.84 ^A *	93.17±1.31 [*]	88.32±0.78 ^B /a
Non-acetylated %	71.66±4.58 [*]	81.25±1.73 [*]	75.73±1.57 [*]	80.57±0.47 [*]	76.00±3.37 [*]	82.38±0.44 [*]	71.03±2.30 [*]	82.38±0.44 [*]	71.03±2.30 [*]	82.38±0.44 [*]	71.03±2.30 [*]	81.21±0.46 [*]
Acetylated %	8.67±1.81	9.06±0.34 ^B	9.31±0.85	7.84±0.39 ^{AB}	8.37±0.53	6.66±0.23 ^A	10.53±1.20 [*]	6.66±0.23 ^A	10.53±1.20 [*]	6.66±0.23 ^A	10.53±1.20 [*]	7.99±0.23 ^B /a
Cinnamylated %	19.67±2.91 [*]	8.51±0.49 ^A /a	14.96±0.71 [*]	10.07±0.24 ^B /a	15.63±2.93 [*]	9.75±0.28 ^{AB} /a	18.44±2.05 [*]	9.75±0.28 ^{AB} /a	18.44±2.05 [*]	9.75±0.28 ^{AB} /a	18.44±2.05 [*]	9.58±0.23 ^{AB} /a
Caffeiclylated %	nd	1.18±0.12	nd	1.52±0.23	nd	1.21±0.14	nd	1.21±0.14	nd	1.21±0.14	nd	1.22±0.11
Total flavonols (mg QE/g DW) ³	0.58±0.04 ^A *	0.76±0.00 ^A *	0.73±0.00 ^B /a	0.85±0.02 ^B /a	0.84±0.01 ^B /a	0.92±0.02 ^C /a	0.78±0.05 ^B /a	0.92±0.02 ^C /a	0.78±0.05 ^B /a	0.92±0.02 ^C /a	0.78±0.05 ^B /a	0.93±0.06 ^C /a
4'-substituted %	19.19±1.98	20.07±1.77	21.17±1.22	20.28±1.78	18.63±1.53	18.58±1.84	19.71±2.44	18.58±1.84	19.71±2.44	18.58±1.84	19.71±2.44	18.50±1.95
3',4'-substituted %	60.72±4.42	57.26±3.98	56.64±3.21	57.63±4.56	53.85±2.87	58.93±3.87	52.42±2.41	58.93±3.87	52.42±2.41	58.93±3.87	52.42±2.41	55.91±3.16
3',4',5'-substituted %	20.08±2.23	22.66±2.64	22.20±1.14	22.09±2.11	27.52±3.41	22.49±2.97	27.87±3.56	22.49±2.97	27.87±3.56	22.49±2.97	27.87±3.56	25.59±3.12
Total flavan-3-ols (mg CE/g DW) ³	0.06±0.01	0.06±0.00 ^A	0.09±0.01	0.07±0.00 ^{AB}	0.08±0.00	0.08±0.01 ^{BC}	0.09±0.00	0.08±0.01 ^{BC}	0.09±0.00	0.08±0.01 ^{BC}	0.09±0.00	0.09±0.01 ^C
Monomers %	6.99±0.81 [*]	23.83±1.06 [*]	5.17±0.01 [*]	21.82±1.02 [*]	5.42±0.42 [*]	18.26±3.82 [*]	4.96±1.36 [*]	18.26±3.82 [*]	4.96±1.36 [*]	18.26±3.82 [*]	4.96±1.36 [*]	15.89±0.12 [*]
Polymers %	93.01±0.81 [*]	76.17±1.06 [*]	94.83±0.01 [*]	78.18±1.02 [*]	94.58±0.42 [*]	81.74±3.82 [*]	95.04±1.36 [*]	81.74±3.82 [*]	95.04±1.36 [*]	81.74±3.82 [*]	95.04±1.36 [*]	84.11±0.12 [*]
Total stilbenes (mg RE/g DW) ³	0.03±0.00 ^{AB} /a	0.04±0.00 [*]	0.02±0.00 ^B /a	0.04±0.00 [*]	0.03±0.00 ^A	0.03±0.01	0.04±0.01 ^{AB}	0.03±0.01	0.04±0.01 ^{AB}	0.03±0.01	0.04±0.01 ^{AB}	0.05±0.00

¹Data are expressed as mean±standard deviation ($n=3$). The content of each phenolic compound are shown in the supplementary file.²nd means not detected. tr means trace. Capital letters in each line are significantly different among different ABA concentrations in the same year at 0.05 level from ANOVA. Asterisks (*) in each line are significantly different between two years for the same ABA concentration at 0.05 level from independent-samples t test.³The total contents of anthocyanin, flavonol, flavan-3-ol, hydroxycinnamic acid, hydroxybenzoic acid and stilbene are expressed as micrograms of malvidin-3-glucoside equivalence (MGE), quercetin equivalence (QE), catechin equivalence (CE) and resveratrol equivalence (RE) per gram of DM grape skins, respectively.

question. As expected, there were remarkable correlations of total phenolic contents ($r=0.553$), total anthocyanin contents ($r=0.834$) and antioxidant activities ($r=0.760$), and a moderate correlation of total flavonol contents ($r=0.447$), between the grape skins and wines. In wines made from ABA-treated grapes, the total phenolic, anthocyanin and flavonol contents, as well as antioxidant activities were significantly enhanced, but no significant differences were found in the concentrations of flavan-3-ols, phenolic acids or stilbenes compared with the control wines. Luan *et al.* (32) indicated that 200 mg/L ABA, applied to the grape berries of 'Yan 73' and 'Cabernet Sauvignon' at pre-veraison, not only increase anthocyanin and flavonol concentrations but also raise flavan-3-ol and stilbene concentrations in their wines. In another research, the skin flavan-3-ols of 'Cabernet Sauvignon' only increased transiently during the veraison period by ABA solution sprayed at fruit set, and the flavan-3-ol contents in treated group were similar to the control group from ripening to harvest (21). These results maybe suggest both the different times applied ABA and the different environments for grape growth have diverse impacts on the phenolic contents of skins and wines, especially flavan-3-ols. In this study, the effects of exogenous ABA treatments of grape berries did improve the color and nutritional value of wines, however, exogenous ABA also decreased the proportion of acylated anthocyanins and anthocyanin-flavanol dimers to some extent. The influences on wine anthocyanin composition were similar to the research of Luan *et al.* (32). Acylated anthocyanins may increase their stability in water solution, and anthocyanin-flavanol dimers are derived pigments important to the color stability of aging red wines, suggesting that small changes in anthocyanin composition would have some impact on color stability and therefore needs further investigation.

Effects of exogenous ABA on other phenolic compounds The total flavan-3-ol contents of the ABA treated grape skins increased numerically compared with the control grape skins. However, the 400 and 600 mg/L ABA groups of 'Cabernet Sauvignon' had a statistically significant difference from the control group in 2010, which mainly attributed to the rise of polymers. The exogenous ABA treatments did not statistically change the proportions of flavan-3-ol monomers and polymers. The ABA treatments also had no significant effect on the flavan-3-ol content of the wines.

Because the phenolic acid (hydroxybenzoic and hydroxycinnamic acids) contents in all the skin samples were very low, those in the ABA treated and control skins were not compared. In general, there were no obvious influences on the phenolic acids contents of the wines or stilbene contents in the skins and wines for either 'Merlot' or 'Cabernet Sauvignon' in both years (Table 3 and 4).

As phenolic acids mainly come from grape pulps, and there was no influence by exogenous ABA on the phenolic acid contents in wines, we conclude that the differences in wine phenolic contents between the ABA-treated and control groups resulted from the enhancement of anthocyanin and flavonol accumulation in the skins caused by the

ABA treatment. In grape berries, the significant differences of the gene expression patterns in different organs diversify the phenolic content and composition (33). For red cultivars, all the genes of the phenolic synthesis pathway are expressed in skin with different temporal patterns during the process of berry development. In particular, phenylalanine ammonia lyase (PAL) and UDP-glucose: flavonoid 3-O-glucosyl transferase (UFGT) are not expressed in pulp where other gene expression levels are low (34). UFGT is an important enzyme involved in the last step of the anthocyanin pathway (35). The gene codified for UFGT is also not expressed in the seeds (36). The expression levels of the leucoanthocyanidin reductases (LAR) or anthocyanidin reductases (ANR) genes, which are involved in the proanthocyanin pathway, are higher in the seed than the skin (37). The phenolic compounds are likely synthesized in the skin, pulp and seed, respectively, during the process of grape ripening. As a result, anthocyanins only exist in skins (except dyer grapes), proanthocyanins were most in seeds, and phenolic content is low in pulps. Owen *et al.* (22) investigated ABA and its catabolites in 'Merlot' grape berries after application of exogenous ABA at veraison and concluded that little exogenous ABA could be taken into the pulps and metabolized. Most remained on the skin with a half-life of one week and resulted in a significant increase in the concentration of the ABA catabolite dihydrophaseic acid. In this study, exogenous ABA treatments of grape berries at veraison had important effects on anthocyanin and flavonol contents in skins, but no effect on phenolic contents in seeds and pulps. While there were no distinct relationships observed between ABA concentration and phenolic content in grape skins, it was proved that exogenous ABA applied at veraison on grape berries was taken up and metabolized in the skins to trigger an increase of anthocyanin and flavonol accumulation.

In this study, the influences of exogenous ABA on phenolic characteristics were systematically and comprehensively researched using with two *V. vinifera* grape cultivars, 'Merlot' and 'Cabernet Sauvignon', in two consecutive growing seasons. The research materials covered the skins and seeds of grape berries as well as the wines. The phenolic characteristics included the content and composition of phenolic compounds (total phenolics, anthocyanins, flavonols, flavan-3-ols, phenolic acids, and stilbenes) and antioxidant capacity, which were important to the organoleptic properties and nutritional values of grape berries and wines. In conclusion, exogenous ABA applied at veraison could enhance phenolic contents (mainly anthocyanins and flavonols) and antioxidant activities of grape skins and wines. The result had practical significance for the application of exogenous ABA in viticulture of wine grapes. However, we didn't find distinct dose-response relationship of ABA on the phenolic contents and antioxidant activities, and consistent effect of ABA on phenolic compositions for the both grape cultivars in two years. It could because the phenolic contents and compositions of grapes and wines are influenced many environmental factors in field, such as light, temperature, rainfall and biotic stress. Furthermore, more researches are needed into the molecular mechanisms of exogenous ABA

Table 4. Effects of different exogenous ABA concentrations on the contents and compositions of phenolic compounds in the wines made from the grapes

	Control ^(1,2)			200 mg/L ABA ^(1,2)			400 mg/L ABA ^(1,2)			600 mg/L ABA ^(1,2)		
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Merlot												
Total anthocyanins (mg MGE/L) ⁽³⁾	18.72±0.83 ^A	25.32±3.65 ^A	28.99±2.83 ^{B/*}	79.20±5.50 ^{C/*}	25.46±0.14 ^{B/*}	89.96±5.69 ^{C/*}	24.39±2.43 ^{B/*}	52.38±11.24 ^{B/*}				
3',4'-substituted %	11.73±0.34 [*]	16.49±0.14 ^{B/*}	9.07±0.22 [*]	15.16±0.72 ^{B/*}	11.97±3.09	15.32±0.66 ^B	10.07±0.74	11.92±0.61 ^A				
3',4',5'-substituted %	88.27±0.34 [*]	83.51±0.14 ^{A/*}	90.93±0.22 [*]	84.84±0.72 ^{A/*}	88.03±3.09	84.68±0.66 ^A	89.93±0.74	88.08±0.61 ^B				
Non-Methoxylated %	nd	nd	nd	1.44±0.01	nd	nd	nd	nd				
Methoxylated %	100.00±0.00	100.00±0.00	100.00±0.00	98.56±0.01	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00				
Non-acylated %	54.40±0.28 [*]	50.84±0.40 ^{A/*}	62.04±6.17 [*]	54.56±0.41 ^{B/*}	57.02±2.04	55.12±0.75 ^B	66.20±2.29 [*]	57.95±0.93 ^{C/*}				
Acetylated %	27.83±0.32 [*]	32.38±0.64 ^{C/b}	23.60±5.87 [*]	31.14±0.29 ^{C/*}	27.84±1.67 [*]	20.89±0.91 ^{A/*}	20.29±1.15 [*]	26.52±0.74 ^{B/*}				
Cinnamylated %	17.77±0.05	16.78±0.24 ^C	14.36±0.29 [*]	12.92±0.16 ^{A/*}	15.13±0.37 [*]	22.24±0.23 ^{D/*}	13.52±3.44	15.53±0.19 ^B				
Caffeiclylated %	nd	nd	nd	1.39±0.04	nd	1.75±0.08	nd	nd				
Anthocyanidin-3-glucosides %	37.51±0.62 [*]	42.82±1.15 ^{A/*}	41.78±8.29 [*]	55.64±0.74 ^{C/*}	39.41±0.88 [*]	49.45±0.10 ^{B/*}	34.41±2.00 [*]	59.96±1.63 ^{D/*}				
Pyrananthocyanins %	37.76±0.50 [*]	33.69±0.06 ^{A/*}	33.81±1.88 [*]	23.80±1.15 ^{B/*}	39.05±0.83 [*]	32.84±0.62 ^{A/*}	36.69±0.99 [*]	25.01±0.64 ^{B/*}				
Hydroxyphenyl-pyranoanthocyanins %	5.69±0.09 [*]	9.37±0.54 ^{A/*}	5.30±0.48 [*]	13.15±0.37 ^{B/*}	4.60±0.15 [*]	9.52±0.33 ^{A/*}	5.29±1.45 [*]	12.65±0.67 ^{B/*}				
Anthocyanin-flavanol dimers %	19.03±0.02 [*]	14.12±0.67 ^{C/*}	19.11±4.95 [*]	7.42±0.05 ^{B/*}	16.95±0.19 [*]	8.19±0.19 ^{B/*}	23.71±0.53 [*]	2.38±0.32 ^{A/*}				
Total flavonols (mg QE/L) ⁽³⁾	12.18±0.32 ^{A/*}	18.13±1.43 ^{A/*}	21.06±2.83 ^B	23.40±0.79 ^B	16.75±0.94 ^{AB/*}	23.87±1.56 ^{B/*}	20.48±1.55 ^{B/*}	25.42±1.78 ^{B/*}				
4'-substituted %	nd	14.86±0.47 ^B	3.62±0.78 ^{B/*}	12.21±0.05 ^{AB/*}	1.79±0.24 ^{A/*}	12.10±0.45 ^{AB/*}	3.50±0.10 ^{B/*}	8.81±1.67 ^{A/*}				
3',4'-substituted %	56.34±2.27 [*]	42.88±2.80 [*]	55.32±3.81	48.38±3.89	58.22±0.39 [*]	47.55±3.81 [*]	62.51±0.97 [*]	54.31±0.04 [*]				
3',4',5'-substituted %	43.66±2.27	42.26±3.27	41.06±4.59	39.41±3.94	39.99±0.63	40.35±4.26	33.99±1.07	36.88±1.71				
Total flavan-3-ols (mg CE/L) ⁽³⁾	38.96±5.01	34.11±2.08	41.80±1.39	39.55±2.26	40.57±3.23	39.06±4.09	39.85±1.98	41.91±0.85				
Monomers	50.00±2.22	51.12±5.21 ^B	45.88±0.71	48.82±2.83 ^{AB}	47.41±3.05	44.83±2.43 ^A	48.78±0.41 [*]	42.94±5.23 ^{AB/*}				
Polymers	50.00±2.22	48.88±5.21 ^A	54.12±0.71	51.18±2.83 ^{AB}	52.59±3.05	55.17±2.43 ^B	51.22±0.41 [*]	57.06±5.23 ^{AB/*}				
Total hydroxybenzoic acids (mg GAE/L) ⁽³⁾	10.06±1.14 [*]	21.72±2.83 [*]	10.86±0.36 [*]	23.29±1.42 [*]	10.63±1.62 [*]	22.16±2.72 [*]	10.47±0.74 [*]	21.78±1.22 [*]				
Total hydroxycinnamic acids (mg CAE/L) ⁽³⁾	6.00±0.46	4.29±0.61 ^A	5.89±0.70 [*]	3.73±0.23 ^{A/*}	5.60±1.91	5.19±0.73 ^B	5.77±0.91	5.49±0.20 ^B				
Total stilbenes (mg RE/L) ⁽³⁾	1.41±0.10 ^{A/*}	2.97±1.17 [*]	1.67±0.10 ^{AB/*}	2.78±0.59 [*]	1.58±0.11 ^{A/*}	2.36±0.12 [*]	2.01±0.13 ^B	2.07±1.24				

Table 4. Continued

	Control ^{1,2}			200 mg/L ABA ^{1,2}			400 mg/L ABA ^{1,2}			600 mg/L ABA ^{1,2}		
	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010	2009	2010
Cabernet Sauvignon												
Total anthocyanins (mg MGE/L) ³	24.37±0.73 ^{A/*}	88.56±4.7 ^{A/*}	29.41±0.76 ^{B/*}	101.89±6.55 ^{AB/*}	41.32±1.03 ^{D/*}	154.29±4.28 ^{B/*}	32.56±1.24 ^{C/*}	156.18±7.91 ^{B/*}				
3',4'-substituted %	5.04±0.87 ^D	6.32±0.76 ^C	4.32±0.26 ^C	3.17±1.09 ^A	3.03±0.47 ^B	4.64±1.47 ^B	3.77±1.34 ^A	5.14±0.40 ^B				
3',4',5'-substituted %	94.96±0.87 ^A	93.68±0.76 ^A	95.68±0.26 ^B	96.83±1.09 ^C	96.97±0.47 ^C	95.36±1.47 ^B	96.23±1.34 ^D	94.86±0.40 ^B				
Non-Methoxylated %	nd	nd	nd	1.36±0.09	nd	nd	nd	1.73±0.09				
Methoxylated %	100.00±0.00	100.00±0.00	100.00±0.00 [*]	98.63±0.09 [*]	100.00±0.00	100.00±0.00	100.00±0.00 [*]	98.27±0.09 [*]				
Non-acylated %	46.45±1.28 ^{A/*}	55.90±0.89 ^{A/b}	54.85±0.83 ^{C/*}	63.09±0.90 ^{B/*}	51.83±0.21 ^{B/*}	57.58±1.20 ^{A/*}	50.01±1.36 ^{B/*}	60.82±0.33 ^{B/*}				
Acetylated %	34.76±1.71 ^B	35.25±0.51 ^C	28.91±0.45 ^A	30.00±0.61 ^A	34.20±0.22 ^B	34.63±1.17 ^C	34.49±1.48 ^B	31.73±0.10 ^B				
Cinnamylated %	18.79±0.42 ^{C/*}	7.29±0.31 ^{B/*}	16.24±0.39 ^{B/*}	5.71±0.32 ^{A/*}	13.97±0.92 ^{A/*}	7.01±0.33 ^{B/*}	15.50±1.26 ^{B/*}	6.56±0.15 ^{AB/*}				
Caffeoylated %	nd	1.56±0.06	nd	1.21±0.02	nd	0.78±0.35	nd	0.89±0.08				
Anthocyanidin-3-glucosides %	43.43±0.86 ^{A/*}	84.52±0.20 ^{B/*}	48.90±1.83 ^{B/*}	78.85±1.32 ^{A/*}	53.36±0.60 ^{B/*}	83.68±0.90 ^{B/*}	46.18±0.93 ^{AB/*}	84.26±0.80 ^{B/*}				
Pyrananthocyanins %	32.82±0.50 ^{AB/*}	11.06±0.11 ^{A/*}	30.48±1.78 ^{A/*}	14.18±1.14 ^{B/*}	30.55±0.41 ^{A/*}	10.59±0.67 ^{A/*}	35.15±0.33 ^{B/*}	10.34±0.57 ^{A/*}				
Hydroxyphenyl-pyranoanthocyanins %	8.93±0.07 ^{D/*}	3.03±0.09 ^{A/*}	7.59±0.12 ^{C/*}	5.66±0.15 ^{C/*}	5.89±0.09 ^{A/*}	4.90±0.16 ^{B/*}	6.82±0.01 ^{B/*}	4.59±0.20 ^{B/*}				
Anthocyanin-flavanol dimers %	14.82±0.29 ^{D/*}	1.39±0.01 ^{B/*}	13.03±0.77 ^{C/*}	1.31±0.03 ^{B/*}	10.20±0.10 ^{A/*}	0.83±0.07 ^{A/*}	11.85±0.58 ^{B/*}	0.81±0.02 ^{A/*}				
Total flavonols (mg QE/L) ³	12.28±0.54 ^{A/*}	22.06±1.13 ^{A/*}	15.46±0.87 ^{B/*}	26.90±1.92 ^{B/*}	20.36±1.30 ^{C/*}	30.49±1.53 ^{B/*}	15.48±0.89 ^{B/*}	30.53±1.20 ^{B/*}				
4'-substituted %	12.71±4.56	14.15±1.31	16.30±0.33	14.53±0.83	16.26±1.80	14.97±1.14	18.10±0.76	16.19±1.84				
3',4'-substituted %	50.80±1.66 ^{B/*}	57.01±0.59 [*]	48.00±1.08 ^{B/*}	58.82±0.47 [*]	45.17±2.01 ^{AB/*}	56.75±0.90 [*]	40.05±2.42 ^{A/*}	54.95±2.32 [*]				
3',4',5'-substituted %	36.49±6.22	28.84±0.72	35.70±0.76 [*]	26.65±0.37 [*]	38.57±0.21 [*]	28.28±0.24 [*]	41.85±1.66 [*]	28.86±0.49 [*]				
Total flavan-3-ols (mg CE/L) ³	20.55±1.91 [*]	11.62±0.18 [*]	24.16±0.42 [*]	13.98±5.21 [*]	26.40±1.87 [*]	15.33±4.44 [*]	27.75±2.78 [*]	14.15±2.30 [*]				
Monomers	51.31±1.65 ^{B/*}	26.12±0.64 ^{B/*}	42.11±2.89 ^{A/*}	15.21±5.93 ^{A/*}	41.22±6.86 ^{A/*}	19.65±10.43 ^{A/*}	39.58±5.91 ^{A/*}	20.12±7.66 ^{A/*}				
Polymers	48.69±1.65 ^{A/*}	73.88±0.64 ^{A/*}	57.89±2.89 ^{B/*}	84.79±5.93 ^{B/*}	58.78±6.86 ^{B/*}	80.35±10.43 ^{B/*}	60.42±5.91 ^{B/*}	79.88±7.66 ^{B/*}				
Total hydroxybenzoic acids (mg GAE/L) ³	12.02±1.23 [*]	16.85±1.00 [*]	10.79±1.50 [*]	17.04±2.58 [*]	11.03±1.14 [*]	18.45±0.69 [*]	10.23±2.05 [*]	18.74±2.87 [*]				
Total hydroxycinnamic acids (mg CAE/L) ³	7.46±0.52 [*]	4.34±0.02 [*]	7.31±0.47 [*]	3.88±0.26 [*]	7.85±1.10 [*]	4.46±0.99 [*]	6.77±0.45 [*]	4.39±0.14 [*]				
Total stilbenes (mg RE/L) ³	0.36±0.10 [*]	1.37±0.49 [*]	0.66±0.13	1.29±0.57	0.31±0.07 [*]	1.24±0.13 [*]	0.35±0.11 [*]	1.60±0.11 [*]				

¹Data are expressed as mean±standard deviation (n=2). The content of each phenolic compound are shown in the supplementary file.

²nd means not detected. tr means trace. Capital letters in each line are significantly different among different ABA concentrations in the same year at 0.05 level from ANOVA. Asterisks (*) in each line are significantly different between two years for the same ABA concentration at 0.05 level from independent-samples t test.

³The total contents of anthocyanin, flavonol, flavan-3-ol, hydroxycinnamic acid and stilbene are expressed as micrograms of malvidin-3-glucoside equivalence (MGE), quercetin equivalence (QE), catechin equivalence (CE), caffeic acid equivalence (CAE), gallic acid equivalence (GAE) and resveratrol equivalence (RE) per liter wines, respectively.

increasing the phenolic content of grape skins, and also into the effect of the changes in anthocyanin composition on color stability of wines.

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