Plant Biotechnology 32, 267–272 (2015)
DOI: 10.5511/plantbiotechnology.15.0729b

Note

The metabolic profile of grape berry skin and a comparison of metabolomes before veraison and at harvest

Mami Suzuki[1,2], Ryo Nakabayashi[3], Tetsuya Mori[3], Kazuki Saito[3,4], Katsuhiro Shiratake[1,*]

[1]Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi 464-8601, Japan; [2]Research Institute of Environment, Agriculture and Fisheries, Habikino, Osaka 583-0862, Japan; [3]RIKEN Center for Sustainable Resource Science, Yokohama, Kanagawa 230-0045, Japan; [4]Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Chiba 260-8675, Japan
*E-mail: shira@agr.nagoya-u.ac.jp Tel & Fax: +81-52-789-4026

Received January 10, 2015; accepted July 29, 2015 (Edited by K. Aoki)

Abstract Grape (*Vitis vinifera* L.) berry skin accumulates high amounts of secondary metabolites, such as catechin, resveratrol, and anthocyanin. Metabolome analysis using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) was performed to profile and compare metabolites of grape berry skin before veraison and at harvest. Anthocyanines, such as marvidine 3-glucoside, were the most abundant phenolic compounds found in grape skins at harvest, whereas the amount of catechin was higher before veraison. Principal component analysis revealed seven stage-specific peaks of metabolites in grape berry skin. Two unidentified peaks were speculated by MS/MS databases searches, annotated or characterized as amino acids, with one annotated as arginine and the other characterized as neutral loss ion of glutamate and glutamine. Amino acids, including arginine, are important for grape berry taste and wine quality. Our metabolome analysis showed not only well known metabolites related to ripening of grape berry skin, including anthocyanines, but also speculated some amino acids, are important for grape berry quality.

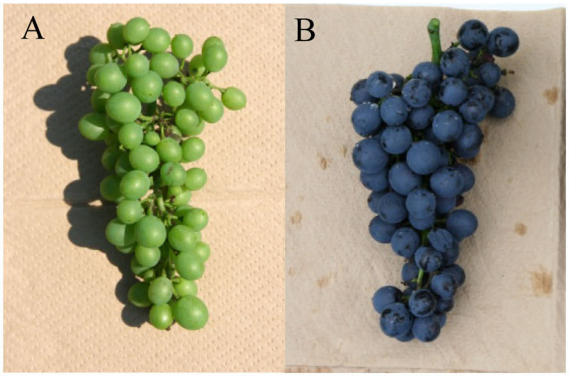
Key words: Grape berry, maturation, metabolome analysis, MS/MS fragment searching.

Grape (*Vitis vinifera* L.) accumulates many polyphenolic compounds, such as catechin, resveratrol, and anthocyanin in the berry skin. These metabolites are important not only for resistance to abiotic and biotic stresses but also for the determination of fruit qualities, such as the color of berry, astringency, and consumer health benefits (Kader 2002). After veraison, which is the turning point of ripening of grape berry with the increase of abscisic acid (Coombe and Hale 1973), the characteristics of the grape berry change drastically. For example, fruit softening, sugar accumulation, acid reduction, and coloring of the berry begin after veraison (Kanellis and Roubelakis-Angelakis 1993). Studies measuring polyphenolic compounds during grape berry ripening are well represented. However, most of these studies focused only on major metabolites, which could be determined with a reference standard (Dai et al. 2014). Recently, metabolome analyzes of various plants have been performed (Saito and Matsuda 2010). Some metabolome analyzes of grape detected >1,000 metabolite peaks (Degu et al. 2014; Marti et al. 2014; Sternad et al. 2013; Suzuki et al. 2015; Zamboni et al. 2010). However, only a few studies discuss unidentified metabolite peaks (Zamboni et al. 2010). Recently, large data sets of MS/MS fragments of plant metabolites have been collected and mass spectrum databases have been developed and made publicly available (Horai et al. 2010; Saito and Matsuda 2010; Sawada et al. 2012; Smith et al. 2005). Using these databases, we can speculate the characteristics of unidentified plant metabolites without a reference standard and identify novel metabolites related to physiological events in plants, including fruit ripening.

In the current study, we performed metabolome analysis of grape berry skin to compare two ripening stages, namely Before Veraison (BV, Fig. 1A) and at Harvest time (H, Fig. 1B), using liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) focusing on phenolic compounds. The scheme of this study is depicted in Fig. 1C. Berry clusters of grape (*Vitis vinifera* L. 'Pinot Noir') before veraison (BV) and at harvest time (H) were harvested at the vineyard of the AZUMI Apple Corporation (Nagano, Japan) in 23 July and 16 September 2010. Three clusters, derived from three different plants, were collected as

Abbreviations: MS, mass spectrometry; MS/MS, tandem mass spectrometry; LC-QTOF-MS, liquid chromatography-quadrupole time-of-flight mass spectrometry; PCA, principal component analysis; ESI, electrospray ionization; BV, Before Veraison; H, at Harvest.

This article can be found at http://www.jspcmb.jp/
Published online August 29, 2015



C

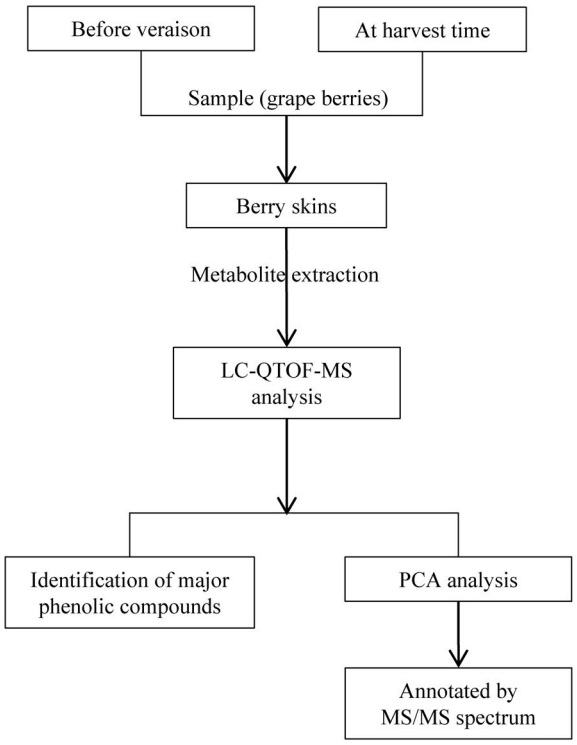


Figure 1. A grape cluster before veraison (A) and at harvest time (B). Overview of the experimental scheme used in this study (C).

biological replicates. Berry skins were collected randomly after harvest. All samples were frozen immediately in liquid nitrogen and stored at −80°C. Extraction of metabolites and their analysis using an LC-Q-TOF-MS system equipped with an ESI interface (LC: Waters Acquity UPLC system; MS: Waters Xevo G2 Q-Tof, Waters, Germany) were performed according to Suzuki et al. (2015). Identification and semi-quantification were compared retention time and m/z with a 100 μM chemical reference standard. Semi-quantification were compared the intensities with them. Lidocaine ($[M+H]^+$, m/z 235.1809) was used as the internal control. To find key metabolites involved in grape ripening, the processed data were used for PCA using SIMCA-P 11.5 (Umetrics, Sweden). Three samples of biological replicates were divided into two aliquots and a total of six samples were analyzed for each treatment. Quantities of major metabolites in the phenylpropanoid pathway were determined with a reference standard. Standard chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), ChromaDex (California, United States of America), Cayman Chemical Company (Michigan, United States of America), Enzo Life Sciences (New York, United States of America), Polyphenols Laboratories AS (Sandnes, Norway), EXTRASYNTHESE S.A. (Genay, France), Nacalai Tesque (Kyoto, Japan), and Tokyo Chemical Industry (Tokyo, Japan).

Some unidentified peaks were annotated or characterized using the MS/MS databases. The best hit fragment of each MS/MS fragment was found by searching MS/MS databases (METLIN; http://metlin.scripps.edu/index.php, MassBank; http://www.massbank.jp/, ReSpect; http://spectra.psc.riken.jp/menta.cgi/respect/index,). Annotation of each peak was assigned according to Sumner et al. (2007). A metabolite matched to a same compound by more than two databases was classified as "annotated". A metabolite matched to two or more compounds was classified as "characterized". A metabolite matched to no data set was classified as "unknown".

In the LC-QTOF-MS analysis, 1,197 metabolite peaks, including unidentified peaks, were detected in the positive ion mode (Supplemental data 1). The data were normalized before analyzes. Table 1 shows the metabolites identified with a reference standard. Therefore, 12 peaks could be identified in grape berry skins. Most of their intensities were very low (<500 counts) in BV, except phenylalanine and catechin. In contrast, peak intensities in H were much higher.

Amounts of anthocyanins increased drastically in H (Table 1). Malvidin 3-glucoside (Metabolite ID 1050, CAS 7228-78-6), which is known as a major anthocyanin in red grape (Quintieri et al. 2013), was most abundant (7.44 mg g^{-1} FW) in the identified metabolites. Other anthocyanins, such as peonidin 3-glucoside (Metabolite ID 1008, CAS 68795-37-9) and petunidin 3-glucoside (Metabolite ID 1035, CAS 6988-81-4), also increased in H. The color of berry skin changes from green to purple (Fig. 1A, B), which can be correlated with the accumulation of anthocyanins shown by the metabolome data.

On the other hand, a decrease of catechin (Metabolite ID 0668, CAS 154-23-4) was observed in H (Table 1); the concentration in BV was 2.03 mg g^{-1} FW, while 0.18 mg g^{-1} FW in H. Bogs et al. (2005) reported that the most abundant flavanol in grape berries was catechin. High accumulation of flavanol and proanthocyanidine, which is a polymer of flavanol, was observed in young berries (Bogs et al. 2005; Fujita et al. 2007; Sternad Lemut et al. 2013). Proanthocyanidine, also called condensed

Table 1. List of metabolites identified by one-point calibration before veraison and at harvest time.

Category	Metabolite ID	CAS	Compound	Ret. Time	*m/z*	UV maxima (nm)		Intensity [a,b]		H/BV Ratio	Conc. (mg g^{-1} FW)	
						Obs.	Authentic sample	BV	H		BV	H
	0264	63-91-2	L-Phenylalanine	2.32	166.0866	—	—	0.204	0.632	3.1	0.05	0.16
Resveratrol	0472	501-36-0	Resveratrol, 3,4′,5-Trihydroxystilbene	5.00	229.0861	304	305	*0.021	0.185	8.9	—	0.11
	0887	27208-80-6	Piceid	4.00	391.1389	321 [c]	318	*0.021	0.033	1.6	—	0.29
	0988	62218-08-0	(−)-epsilon-Viniferin	5.76	455.149	326 [c]	322	*0.021	0.037	1.8	—	0.02
Anthocyanin	1014	6906-38-3	Delphinidin 3-glucoside	2.65	465.1026	277, 523	275, 522	*0.021	0.306	14.8	—	1.33
	1050	7228-78-6	Malvidin 3-glucoside	3.19	493.1338	277, 522	276, 526	*0.021	5.184	250.8	—	7.44
	1035	6988-81-4	Petunidin 3-glucoside	2.90	479.1181	274, 524	275, 522	*0.021	0.776	37.6	—	5.07
	0963	7084-24-4	Cyanidin 3-glucoside	2.85	449.1073	278, 519	279, 515	*0.021	0.530	25.7	—	0.54
	1008	68795-37-9	Peonidin 3-glucoside	3.11	463.123	278, 520	278, 519	*0.021	4.444	215.0	—	5.62
Proanthocyanidin	0668	154-23-4	(+)-Catechin	3.09	291.0867	278	278	1.476	0.134	0.1	2.03	0.18
	0666	490-46-0	(−)-Epicatechin	3.42	291.0864	278	278	0.027	0.194	7.3	—	0.22
Flavonol	1012	482-35-9	Quercetin 3-glucoside	4.02	465.1017	321 [c]	255, 353	*0.021	0.166	8.0	—	0.54

[a] Values represent the means of six intensities. [b]*: Low intensities (≤0.021) were detected at background noise levels. [c] UV maxima derived from other overlapping metabolite.

tannin, contributes to astringency and bitterness of grape or wine (Jaakola 2013). Our observation of a decrease of catechin during ripening might be caused by the polymerization of catechin and its deposition.

Although stilbenoids, including resveratrol (Metabolite ID 0472, CAS 501-36-0), were detected in H, those concentrations did not change drastically such as with anthocyanins (Table 1). Stilbenoids, such as resveratrol and its dimer, viniferin (Metabolite ID 0988, CAS 62218-08-0), are known as phytoalexins (Langcake and Pryce 1977) and are induced by abiotic and biotic stresses (Adrian et al. 2000; Aziz et al. 2003; Belchí-Navarro et al. 2012; Pezet et al. 2003; Suzuki et al. 2015; Takayanagi et al. 2004). Takayanagi et al. (2004) reported that stilbenoids are induced more easily in young grape berry skin as compared to that in mature grapes. The results of the present study agree with that of Takayanagi et al. (2004), that stilbenoids are induced by stresses rather than by fruit ripening.

As a summary of the trends observed for phenolic compounds, most phenolic compounds did not accumulate in BV, except for catechin. After veraison, catechin decreased and the other phenolic compounds, especially anthocyanines increased.

PCA analysis was performed to determine characteristic metabolites of H or BV. A score scatter plot, which reflects the sample relationships from the data matrix by PCA (Supplemental data 1), showed the groups of BV and H (Fig. 2A). A loading scatter plot, which shows all peaks related to score the scatter plot (Fig. 2A), tended to bias towards H (Fig. 2B). Although many metabolite peaks were plotted as increased peaks in H, the loading scatter plot shown in Fig. 2B reveals four metabolite peaks as more specific peaks of H and three metabolite peaks as more specific peaks of BV (Fig. 2B). Details of these seven peaks are listed in Table 2. Their

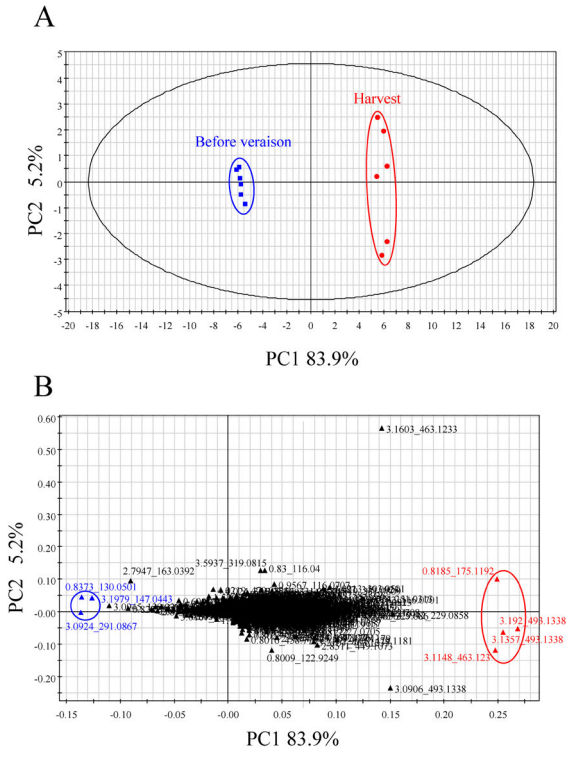


Figure 2. Principal component analysis (PCA) of grape berry skin metabolome (A); Score scatter plot of a PCA model on the berry skin metabolome before veraison (■) and at harvest time (●) (n=6 for each samples). (B). Loading scatter plot from the PCA analysis of berry skin before veraison and at harvest time (n=1,197 metabolite peaks). Each annotation showed the retention time and m/z.

intensities were significantly different between the BV and H groups (Table 2). Three peaks (No. 0668, 1008, and 1050) were identified with a reference standard (Table 1), but the other peaks could not been identified. We then attempted to annotate the four unidentified peaks using exact mass (m/z), MS/MS spectra (Supplemental data 2), and three MS/MS databases.

Table 2. List of metabolites picked up by PCA with speculated annotations.

No.[a]	Sample[b]	Ret. Time	*m/z*	Intensity (normalized)[c]			Metabolite name	Identification level[e]	MS/MS fragments [f]	MSMS Fragment search[g]			Chemical Search			Formura	CAS
				BV	H	*t*-test [d]				METLIN	ReSpect	MassBank	KEGG	KNApSAcK	PubChem		
0102	BV	0.84	130.0501	2.18 ±0.17	0.83 ±0.15	**	Amino acid (Glutamate and Glutamine)	Characterized	130.0499, 84.0438, 147.0761, 102.0565, 131.0483, 101.0652, 148.0681,	(*R*)-(+)-2-Pyrolidone-5-carboxylic acid (D-Pyroglutamic acid),	L-Glutamic acid, *N*-acetyl-L-Glutamic acid, *S*-Lactoylglutathione, L-Glutamine, L-lysine, L-Lysine monohydrochloride, L-Pyroglutamic acid	5,6-Dimethylbenzimidazol Glutamate Glutamine L-(+)-Lysine Lysine Beta Ala-Lys	C00025(Glu), C00064(Gln)	C00001358(Glu), C00001359(Gln)	3327(Glu), 3364(Gln)	C5H9NO4(Glu), C5H10N2O3(Gln)	56-86-0(Glu), 56-85-9(Gln)
0188	BV	3.20	147.0443	1.61 ±0.18	0.45 ±0.06	**	—	Unknown	147.0450, 119.0519, 91.0547, 148.0398, 120.0394, 92.0587, 149.1320, 125.4183, 101.0375, 65.0399, 189.3775,	Coumarin, *p*-coumaric acid	4-Hydroxy-3-methoxycinnamaldehyde, Hinokitol, 4-coumaric acid	No candidate compound	—	—	—	—	—
0300	H	0.82	175.1192	0.06 ±0.01	4.56 ±0.39	**	L-Arginine	Annotated	175.1162, 116.0716, 158.0942, 70.0664, 176.0591, 112.0876, 130.0993, 60.0532, 71.0610, 159.0900, 157.1024, 177.5481, 143.1008, 117.0753, 98.0651,	L-Arginine	L-Arginine, L-Arginine monohydrochloride, *N*-alpha-Acetyl L-ornithine,	L-Arginine, L-Arginine monohydrochloride, *N*-alpha-Acetyl L-ornithine, Octopine, Phosphoarginine,	C00062	C00001340	6322	C6H14N4O2	74-79-3
0668	BV	3.09	291.0867	1.48 ±0.13	0.13 ±0.01	**	Catechin	Identified	139.0394, 291.0867, 147.0431, 165.0542, 292.0917, 293.0883, 1235.2754	Epicatechin	(+)-Epicatechin, (−)-Epicatechin	(+)-Catechin, (+)-Epicatechin, (−)-Epicatechin	C06562	C00000947		C15H14O6	154-23-4
1008	H	3.11	463.1230	0.02 ±0.00[h]	4.44 ±0.52	**	Peonidin 3-glucoside	Identified	301.0703, 463.1241, 302.0737, 286.0466, 464.1260, 258.0474, 465.1449, 303.0702	No candidate compound	Peonidin-3-*O*-beta-galactopyranoside, peonidin-3-*o*-beta-D-glucopyranoside	Peonidin-3-*O*-beta-D-glucoside, Peonidin-3-*O*-beta-galactopyranoside	C12141	—		C22H23O11	68795-37-9
1049	H	3.14	493.1338	0.02 ±0.00[h]	4.68 ±0.30	**	—	No peak top	—	—	—	—	—	—	—	—	—
1050	H	3.19	493.1338	0.02 ±0.00[h]	5.18 ±0.28	**	Malvidin 3-glucoside	Identified	331.0812, 493.1340, 332.0858, 494.1374, 315.0506	Malvidin 3-*O*-glucoside	Malvidin-3-galactoside chloride, Oenin	Malvidin 3-*O*-beta-galactoside, Oenin	C12140	C00006735		C23H25O12	7228-78-6

[a] The number of compounds were showed in Supplemental Data 1. [b] BV: before veraison, H: at harvest. [c] Values represent the means of six intensities±standard deviation. [d]**: Significant difference by *t*-test between BV and H at $p<0.01$. [e] Annotation levels were proposed by Sumner et al. (2007). [f] MS/MS spectrum were showed in Supplemental Data 2. [g] Databases were introduced in material and method. [h] Low intensities (≤0.02) were detected at background noise levels.

One peak (No. 1049) was not a peak top ion (Table 2). It speculates that it was a part of ion from next peak (No. 1050) because their m/z was equal. The other unidentified peak was associated with MS/MS fragments and thus, a MS/MS database search was performed and annotated according to Sumner et al. (2007). A specific peak in H (No 0300) was annotated as arginine (Table 2). Arginine is one of the major amino acids in grape, accumulates in berry flesh and skin (Lamikanra and Kassa 1999), and its content increases during berry ripening (Lamikanra and Kassa 1999). Arginine is a substrate for polyamine (Agdelo-Romero 2013), which relates to berry ripening (Aziz 2005; Mattoo and Handa 2008) and leaf senescence (Pandey et al. 2000). It is interesting that arginine was found as a ripening specific metabolite in the current study, because arginine increases the sweetness of grape juice (Hirano et al. 1998).

A specific peak in BV (No 0102) was characterized by some amino acids (Table 2). When the retention time of the peak was confirmed, it was similar to that of two peaks (No. 0195 and 0191), speculated as glutamate and glutamine (data not shown). MS/MS fragments of them suggest that a specific peak (No 0102) is possible to be a neutral loss ion of glutamate (No. 0195) and glutamine (No. 0191), because they have a same fragment (m/z=130). The annotation level of it was assign as characterized. Glutamate was reported as the fifth major amino acid in grape berry 'Pinot Noir,' although the contents of amino acids are different among cultivars (Sato et al. 1994). The concentration of glutamine is less than that of glutamate (Sato et al. 1994). Glutamate plays a central role in ammonia metabolism (Forde and Lea 2007) and is an intermediate of arginine and proline syntheses (Forde and Lea 2007). Glutamate is also important for alcohol fermentation of wine yeast (Castor 1953). On the other hand, some databases suggested that the MS/MS fragment of one of the specific peaks in BV (No 0188) is similar to *p*-coumaric acid (Table 2). However, the retention time and exact mass (3.1979_147.0443, Table 2) was quite different from that of *p*-coumaric acid registered in databases (3.932_164.05). Therefore it remains unknown (Table 2).

In conclusion, we found characteristic metabolites of BV and H by metabolome analysis. Anthocyanines, such as marvidine 3-glucoside, were identified as major phenolic compounds in grape berry skin in H, whereas catechin was identified as a major phenolic compound in BV. In addition, we speculated the annotation of characteristic metabolites of BV or H by searching the MS/MS spectrum data sets. One specific peak of BV was characterized as glutamate and glutamine, whereas one specific peak of H was annotated as arginine. These metabolites are closely related to the taste of berry and quality of wine. Therefore, the results obtained in the current study may be important for improving the qualities of grape berry and wine. As far as we know, no previous metabolome analysis has revealed this kind of ripening specific metabolic change in grape berry skin. The current study demonstrates the effectiveness of metabolome analysis to clarify metabolic change in fruit, and the obtained results may be useful within the horticultural and wine production industry.

Acknowledgements

We thank Mr. Hiroya Saito and Mr. Chiharu Uchikata at AZUMI Apple Corporation for supplying grape berries. We also thank Mr. Makoto Suzuki at RIKEN CSRS for technical assistance. This work was technically supported by the Programme for Japan Advanced Plant Science Network and financially supported by the Programme for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry from Bio-oriented Technology Research Advancement Institution (BRAIN) and by Grants-in-Aid for Scientific Research from The Japan Society for the Promotion of Science (JSPS).

References

Adrian M, Jeandet P, Douillet-Breuil AC, Tesson L, Bessis R (2000) Stilbene content of mature *Vitis vinifera* berries in response to UV-C elicitation. *J Agric Food Chem* 48: 6103–6105

Agudelo-Romero P, Bortolloti C, Paos MS, Tiburcio AF, Fortes AM (2013) Study of polyamines during grape ripening indicate an important role of polyamine catabolism. *Plant Physiol Biochem* 67: 105–119

Aziz A (2005) Spermidine and related-metabolic inhibitors modulate sugar and amino acid levels in *Vitis vinifera* L.: possible relationships with initial fruitlet abscission. *J Exp Bot* 54: 355–363

Aziz A, Poinssot B, Daire X, Adrian M, Bézier A, Lambert B, Joubert JM, Pugin A (2003) Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. *Mol Plant Microbe Interact* 16: 1118–1128

Belchí-Navarro S, Almagro L, Lijavetzky D, Bru R, Pedreño MA (2012) Enhanced extracellular production of *trans*-resveratrol in *Vitis vinifera* suspension cultured cells by using cyclodextrins and methyljasmonate. *Plant Cell Rep* 31: 81–89

Bogs J, Downey MO, Harvey JS, Ashton AR, Tanner GJ, Robinson SP (2005) Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. *Plant Physiol* 139: 652–663

Castor JGB (1953) The free amino acids of musts and wines. ii. the fate of amino acids of must during alcoholic fermentation. *J Food Sci* 18: 146–151

Coombe BG, Hale CR (1973) The hormone content of ripening grape berries and the effects of growth substance treatments. *Plant Physiol* 51: 629–634

Dai ZW, Meddar M, Renaud C, Merlin I, Hilbert G, Delrot S, Gomès E (2014) Long-term in vitro culture of grape berries and its application to assess the effects of sugar supply on anthocyanin accumulation. *J Exp Bot* 65: 4665–4677

Degu A, Hochberg U, Sikron N, Venturini L, Buson G, Ghan R, Plaschkes I, Batushansky A, Chalifa-Caspi V, Mattivi F, et al. (2014) Metabolite and transcript profiling of berry skin during

fruit development elucidates differential regulation between Cabernet Sauvignon and Shiraz cultivars at branching points in the polyphenol pathway. *BMC Plant Biol* 14: 188

Forde BG, Lea PJ (2007) Glutamate in plants: metabolism, regulation, and signaling. *J Exp Bot* 58: 2339–2358

Fujita A, Soma N, Goto-Yamamoto N, Mizuno A, Kiso K, Hashizume K (2007) Effect of shading on proanthocyanidin biosynthesis in the grape berry. *J Jpn Soc Hortic Sci* 76: 112–119

Hirano K, Kubota S, Nishi T, Okamoto G (1998) Effect of amino acid composition on grape juice taste. *J ASEV Jpn* 9: 89–96, in Japanese

Horai H, Arita M, Kanaya S, Nihei Y, Ikeda T, Suwa K, Ojima Y, Tanaka K, Tanaka S, Aoshima K, et al. (2010) MassBank: A public repository for sharing mass spectral data for life sciences. *J Mass Spectrom* 45: 703–714

Jaakola L (2013) Phenylpropanoid metabolism and biosynthesis of anthocyanins. In: Seymour GB, Poole M, Giovannoni J, Tucker GA (eds) *The Molecular Biology and Biochemistry of Fruit Ripening*. Blackwell Publishing, Oxford, pp 117–134

Kader AA (2002) Fruits in the global market. In: Knee M (ed) *Fruit Quality And Its Biological Basis*. Sheffield Academic Press, Sheffield, pp 1-16

Kanellis AK, Roubelakis-Angelakis KA (1993) Grape. In: Seymour GB, Taylor JE, Tucker GA (eds) *Biochemistry of Fruit Ripening*. Springer Netherlands Press, London, pp 189–234

Lamikanra O, Kassa AK (1999) Changes in the free amino acid composition with maturity of the noble cultivar of *vitis rotundifolia* michx. grape. *J Agric Food Chem* 47: 4837–4841

Langcake P, Pryce RJ (1977) A new class of phytoalexins from grapevines. *Experientia* 33: 151–152

Marti G, Schnee S, Andrey Y, Simoes-Pires C, Carrupt PA, Wolfender JL, Gindro K (2014) Study of leaf metabolome modifications induced by UV-C radiations in representative Vitis, Cissus and Cannabis species by LC-MS based metabolomics and antioxidant assays. *Molecules* 19: 14004–14021

Mattoo AK, Handa AK (2008) Higher polyamines restore and enhance metabolic memory in ripening fruit. *Plant Sci* 174: 386–393

Pandey S, Ranade SA, Nagar PK, Kumar N (2000) Role of polyamines and ethylene as modulators of plant senescence. *J Biosci* 25: 291–299

Pezet R, Perret C, Jean-Denis JB, Tabacchi R, Gindro K, Viret O (2003) δ-viniferin, a resveratrol dehydrodimer: One of the major stilbenes synthesized by stressed grapevine leaves. *J Agric Food Chem* 51: 5488–5492

Quintieri AM, Baldino N, Filice E, Seta L, Vitetti A, Tota B, De Cindio B, Cerra MC, Angelone T (2013) Malvidin, a red wine polyphenol, modulates mammalian myocardial and coronary performance and protects the heart against ischemia/reperfusion injury. *J Nutr Biochem* 24: 1221–1231

Saito K, Matsuda F (2010) Metabolomics for functional genomics, systems biology, and biotechnology. *Annu Rev Plant Biol* 61: 463–489

Sato M, Nagao A, Nishi Y, Ariizumi K, Yagi Y, Otsuka K (1994) Varietal similarity in amino acid profiles of grapw juice and correlation with classification by ampelograohy. *ASEV Jpn. Rep* 5: 202–214, in Japanese

Sawada Y, Nakabayashi R, Yamada Y, Suzuki M, Sato M, Sakata A, Akiyama K, Sakurai T, Matsuda F, Aoki T, et al. (2012) RIKEN tandem mass spectral database (ReSpect) for phytochemicals: A plant-specific MS/MS-based data resource and database. *Phytochemistry* 82: 38–45

Smith CA, O'Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R, Siuzdak G (2005) METLIN: A metabolite mass spectral database. *Ther Drug Monit* 27: 747–751

Sternad Lemut M, Sivilotti P, Franceschi P, Wehrens R, Vrhovsek U (2013) Use of metabolic profiling to study grape skin polyphenol behavior as a result of canopy microclimate manipulation in a 'Pinot Noir' vineyard. *J Agric Food Chem* 61: 8976–8986

Sumner LW, Amberg A, Barrett D, Beale MH, Beger R, Daykin CA, Fan TW, Fiehn O, Goodacre R, Griffin JL, et al. (2007) Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics* 3: 211–221

Suzuki M, Nakabayashi R, Ogata Y, Sakurai N, Tokimatsu T, Goto S, Suzuki M, Jasinski M, Martinoia E, Otagaki S, et al. (2015) Multiomics in grape berry skin revealed specific induction of the stilbene synthetic pathway by ultraviolet-C irradiation. *Plant Physiol* 168: 47–59

Takayanagi T, Okuda T, Mine Y, Yokotsuka K (2004) Induction of resveratrol biosynthesis in skins of three grape cultivars by ultraviolet irradiation. *J Jpn Soc Hortic Sci* 73: 193–199

Zamboni A, Di Carli M, Guzzo F, Stocchero M, Zenoni S, Ferrarini A, Tononi P, Toffali K, Desiderio A, Lilley KS, et al. (2010) Identification of putative stage-specific grapevine berry biomarkers and omics data integration into networks. *Plant Physiol* 154: 1439–1459