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Molecular cloning and characterization of ABCG/PDR-type ABC transporter in grape berry skin

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Abstract: Grape (*Vitis vinifera* L.) skin contains the phenolic compound resveratrol which is important not only for resistance to biotic and abiotic stresses but also for human health. However, little is known about resveratrol transport in plant cells. ABC (ATP binding cassette) transporters are well-known transporters responsible for secondary metabolite accumulation in plants. Previous reports speculated that the full-size ABCG transporter pleiotropic drug-resistant (PDR) is involved in resveratrol transport in fungi and plants. In this paper, all full-size ABCG transporters found in the grape genome database are listed and focus is placed on *VvABCG44/VvPDR14* as a candidate resveratrol transporter. The full-length cDNA of *VvABCG44* was cloned by RT-PCR using mRNAs extracted from grape berry skin. *VvABCG44* expression was induced by UV irradiation, and the expression pattern of *VvABCG44* in various grape organs was similar to that of stilbene synthase (STS), a key enzyme in resveratrol synthesis. Resveratrol content in grape berry skin increased after UV irradiation. These results suggest that VvABCG44 functions as a resveratrol transporter in grape.

1. Introduction

Grape (*Vitis vinifera* L.) is an economically important fruit crop, served fresh and used for wine production. Grape is one of the most studied fruit crops, given that the grape genome sequence is available (Jaillon *et al.,* 2007; Velasco *et al.,* 2007). Grape skin contains several phenolic compounds, such as anthocyanin, resveratrol, and catechin, which are important not only for resistance to biotic and abiotic stresses but also for berry qualities such as color, astringency, and human health benefits (Kader, 2002; Steyn, 2009). Resveratrol, a stilbenoid accumulating in the grape berry, is a key compound in the "French paradox" (Renaud and De Lorgeril, 1992) and is attracting attention in medicine and food science.

ATP binding cassette (ABC) transporters are well-known transporters responsible for secondary metabolite accumulation in plants (Yazaki, 2006). They form a large gene family and are found in all living organisms (Rea, 2007). Plants have much larger numbers of ABC transporters than animals or microorganisms: *Arabidopsis* and rice have more than 120 ABC proteins (Rea, 2007; Yazaki *et al*., 2009; Kretzschmar *et al*., 2011).

ABC transporters have a transmembrane domain (TMD) and a nucleotide-binding domain (NBD) comprising ATP-binding Walker A and B motifs (Martinoia *et al*., 2002). ABC transporters are classified into eight subfamilies (ABCA-H) according to their structure and sequence similarity. Half-size ABC transporters contain one TMD and one NBD, whereas full-size ABC transporters contain two repeats of the structure of half-size ABC transporters, two TMDs and two NBDs (Verrier *et al*., 2008).

The substrate specificity of ABC transporters is broad, and plant ABC transporters have been reported to transport various compounds, such as secondary metabolites, heavy metals, lipids, chlorophyll catabolites, xenobiotics, and plant hormones (Rea, 2007; Yazaki *et al*., 2009). ABC transporters show different localizations, such as the plasma membrane, vacuole, ER, Golgi apparatus, mitochondrion, and peroxisome; subcellular localizations of ABC transporters in the same subfamily are not always the same (Yazaki *et al*., 2009; Kretzschmar *et al*., 2011).

The ABCG subfamily is a major ABC transporter subfamily. It contains both half-size transporters, called the

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white-brown complex (WBC) subfamily, and full-size transporters, called the pleiotropic drug-resistant (PDR) subfamily. One of the best studied full-size ABCG subfamily members is yeast PDR5 (Lamping *et al*., 2010; Prasad and Goffeau, 2012). PDR5 is an exporter in yeast plasma membrane and is associated with multidrug resistance (Decottignies and Goffeau, 1997; Golin *et al*., 2007). In plants, full-size ABCG transporters have been reported to transport phytoalexins (Banasiak *et al*., 2013), abscisic acid (ABA) (Kang *et al*., 2010), strigolactone (Kretzschmar *et al*., 2012), and other compounds.

A strain of the plant pathogenic fungus *Botrytis cinerea*, lacking a full-size ABCG transporter BcatrB, is sensitive to resveratrol (Schoonbeek *et al*., 2001). On the other hand, after treatment with an elicitor, cyclodextrin, grape culture cells accumulated resveratrol and full-size ABCG transporter genes were induced in the cells (Zamboni *et al*., 2009). These results suggest that full-size ABCG transporters are associated with resveratrol transport in fungi and plants.

In this study, we listed all full-size ABCG transporters (PDRs) found in the grape genome database and focused on *VvABCG44/VvPDR14* as a candidate resveratrol transporter. We cloned the full-length cDNA of *VvABCG44* and determined its gene expression in various organs and after UV irradiation. *VvABCG44* expression was induced by UV irradiation and the expression pattern of *VvABCG44* in various grape organs was similar to that of stilbene synthase (STS), a key enzyme in resveratrol synthesis. To the best of our knowledge, this is the first report of an ABCG transporter in grape.

2. Materials and Methods

Plant material and treatments

Vitis vinifera "Pinot Noir" was harvested in the vineyards of the AZUMI Apple Corporation in Nagano Prefecture and of Nagoya University in Aichi Prefecture, Japan. For molecular cloning and gene expression analysis, young leaves, mature leaves, tendrils, stems, seeds, pulp, and berry skin were harvested in June and July. For molecular cloning, the skin of the berries after UV irradiation was used. For UV irradiation and ABA treatment analyses, the grape berry clusters were harvested in June and July, before the veraison stage. UV irradiation and ABA treatment were performed as described below.

Berry clusters were irradiated using a UV-C lamp (253.7 nm, GL-15, TOSHIBA, Japan) at a 50-cm distance for 1 h. Control samples (dark) were covered with a box and placed beside the sample receiving UV irradiation. For RNA extraction, the skin of the berries was collected immediately after UV irradiation. For measurement of resveratrol content, after UV irradiation, berry clusters were maintained for 23 h in the dark at room temperature and then the skins of the berries were collected.

Berry clusters were sprayed with 960 mM ABA containing 0.05% (v/v) Tween 20 and maintained in the dark at room temperature for 48 h. Control samples (water) were sprayed with water containing 0.05% (v/v) Tween 20 and placed beside the ABA-treated samples. After treatment, the skins of the berries were collected.

Three biological replicates were assayed for each treatment.

Identification of full-size ABCG transporter genes in the 12× version 1 of Vitis vinifera *genome*

Full-size ABCG transporters in grape were searched with BLAST (Basic Local Alignment Search Tool) at NCBI (http://www.ncbi.nlm.nih.gov/) against the predicted protein sequence dataset of the 12× version 1 (v1) of CRIBI (http://genomes.cribi.unipd.it/grape/) using the NpPDR1 protein sequence (CAC40990) as a query. Because the average full-size ABCG protein comprises 1,400 amino acids (Rea, 2007), only sequences comprising more than 400 amino acids were taken into account. These nomenclatures were represented according to Çakır and Kılıçkaya (2013). The sequences corresponding to full-size ABCG transporters confirmed that there was at least one PDR motif.

Molecular cloning of VvABCG44

The genome sequence corresponding to the partial cDNA sequence of a grape full-size ABCG transporter [tentative consensus sequence- TC76318, the grape gene index database (http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=grape)], induced by cyclodextrin (Zamboni *et al*., 2009), was searched in the NCBI database (http://www.ncbi.nlm.nih.gov/) by BLAST. A genome sequence (accession number AM449250.2), provided by the IASMA Research Center (http://genomics.research.iasma.it/), was matched. The open reading frame (ORF) of the gene was predicted by Softberry (http://linux1.softberry.com/berry.phtml) and primers to amplify the entire ORF were designed (Table 1).

Total RNA was extracted from the berry skin by hot borate method (Wan and Wilkins, 1994). The full-length cDNA of *VvABCG44* was amplified using the PrimeScript High Fidelity RT-PCR kit (TaKaRa, Japan) according to the manufacturer's instructions. Three motifs (Walker A, Walker B, and ABC signature) were confirmed according to van den Brûle and Smart (2002). TMD was predicted by PHD (NPS@) (Rost and Sander, 1993, 1994). Sequence data of *VvABCG44* have been deposited in DDBJ under accession number AB910387.

Gene expression analysis

Total RNA from grape tissues was extracted by the technique described above. Total RNA was reverse-transcribed using a PrimeScript RT reagent Kit with gDNA eraser (perfect real-time) (TaKaRa) according to the manufacturer's recommendations.

Transcript levels were determined by quantitative RT-PCR using SYBER Premix EX Taq II (perfect real-time) (TaKaRa) and Thermal Cycler Dice Real Time System TP800 (TaKaRa) software ver. 3.00D. Primers for *VvABCG44*, *STS*, and *actin* are shown in Table 1. Reaction conditions for thermal cycling were as follows: after enzyme

Table 1 - Primers used in this study

Primer name	Purpose	Primer sequence(5'-3')
Take2_Forward	Cloning	CAC CAT GGC GAC GGC TGA AAT TTA TAR AG
Take2_Reverse	Cloning	TCG CCT TTG GAA GTT CAA TGC
VvPDR14_exp_Fw	Gene expression	TAG GAG TGG TTG CAG CTG TG
VvPDR14_exp_Rv	Gene expression	TTT TGC TCC GTG TGA CTT CTT
VvSTS_exp_Fw	Gene expression	GGG TCA CTA AGA GCG AGC AC
VvSTS_exp_Rv	Gene expression	GCT CCT CAA GCA TTT CTT CG
VvACT_Fw	Gene expression	TCC TGT GGA CAA TGG ATG GA
VvACT_Rv	Gene expression	CTTGCA TCC CTC AGC ACC TT

activation at 95°C for 10 s, amplification was performed in a two-step PCR with 40 cycles of 5 s at 95°C for denaturation and 30 s at 60°C for annealing/extension. Transcript levels were calculated using a standard curve, and normalized against *actin* as described by Reid *et al*. (2006). All reactions were performed in triplicate with three biological replicates.

Measurement of resveratrol content

Extraction of resveratrol (CAS number 501-36-0) and its analysis using an LC-Q-TOF/MS system equipped with an ESI interface (HPLC: Waters Acquity UPLC system; MS: Waters Xevo G2 Q-Tof , Waters, Germany) were performed according to Tamura *et al*. (2014). Identification, determination, and semi-quantification were compared with a 100 µM chemical reference standard. 10-camphorsulfonic acid was used as the internal control. Three samples of biological replicates were divided into two aliquots and a total of six samples were analyzed for each treatment.

3. Results

Zamboni *et al*. *(*2009*)* reported the partial sequence of a grape full-size ABCG gene that was induced by cyclodextrin. To obtain the full-length cDNA clone of the gene, we searched the genome sequence corresponding to the gene and successfully amplified a full-length cDNA using primers designed from the genome sequence data. The gene was designated *VvABCG44 or VvPDR14*. *VvABCG44* had a 4,350 bp coding region and was predicted to encode a protein of 1,450 amino acids (Fig. 1A) with two TMDs and two NBDs (Fig. 1B).

A phylogenetic tree of plant full-size ABCG transporters including VvABCG44 and all full-size ABCG transporters in *Arabidopsis* (Fig. 2) shows that NtPDR1 (BAD07483), NpPDR1 (CAC40990), and MtABCG10 (AES68070) are the closest homologues to VvABCG44. NtPDR1 (Crouzet *et al*., 2013) and NpPDR1 (Jasiński *et al*., 2001) were reported to transport diterpenes including sclareol, whereas MtABCG10 (Banasiak *et al*., 2013) transported isoflavonoids. A close homologue of VvABCG44, SpTUR2 (O24367) (van den Brûle and Smart, 2002), transported sclareol. Other close homologues transport different compounds; AtABCG40 (AAF71978) (Kang *et al*., 2010) and PaPDR1 (JQ292812) (Kretzschmar *et al*., 2012) transport ABA and strigolactone, respectively. The substrate range of VvABCG44 homologues is very broad, and it is not easy to identify the substrate of VvABCG44.

To determine the tissues in which *VvABCG44* is expressed, quantitative RT-PCR analyses were performed

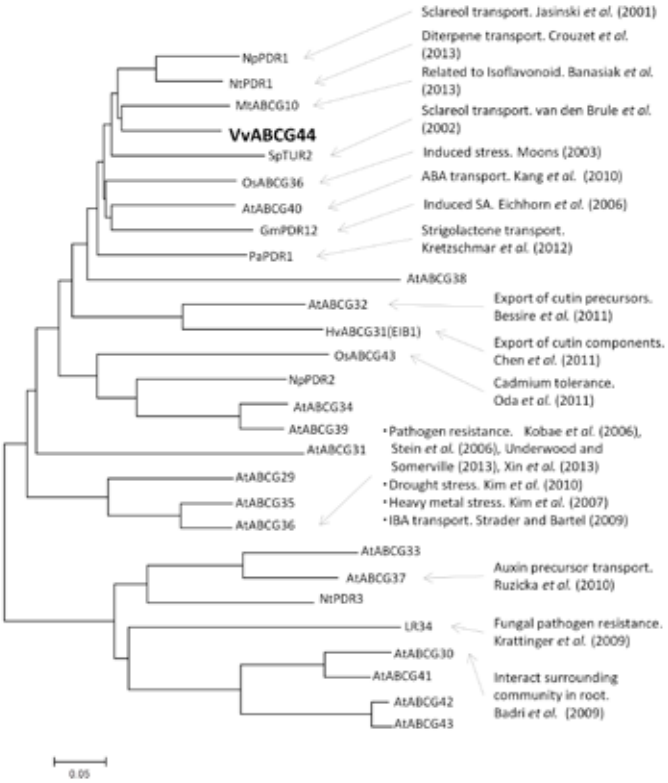
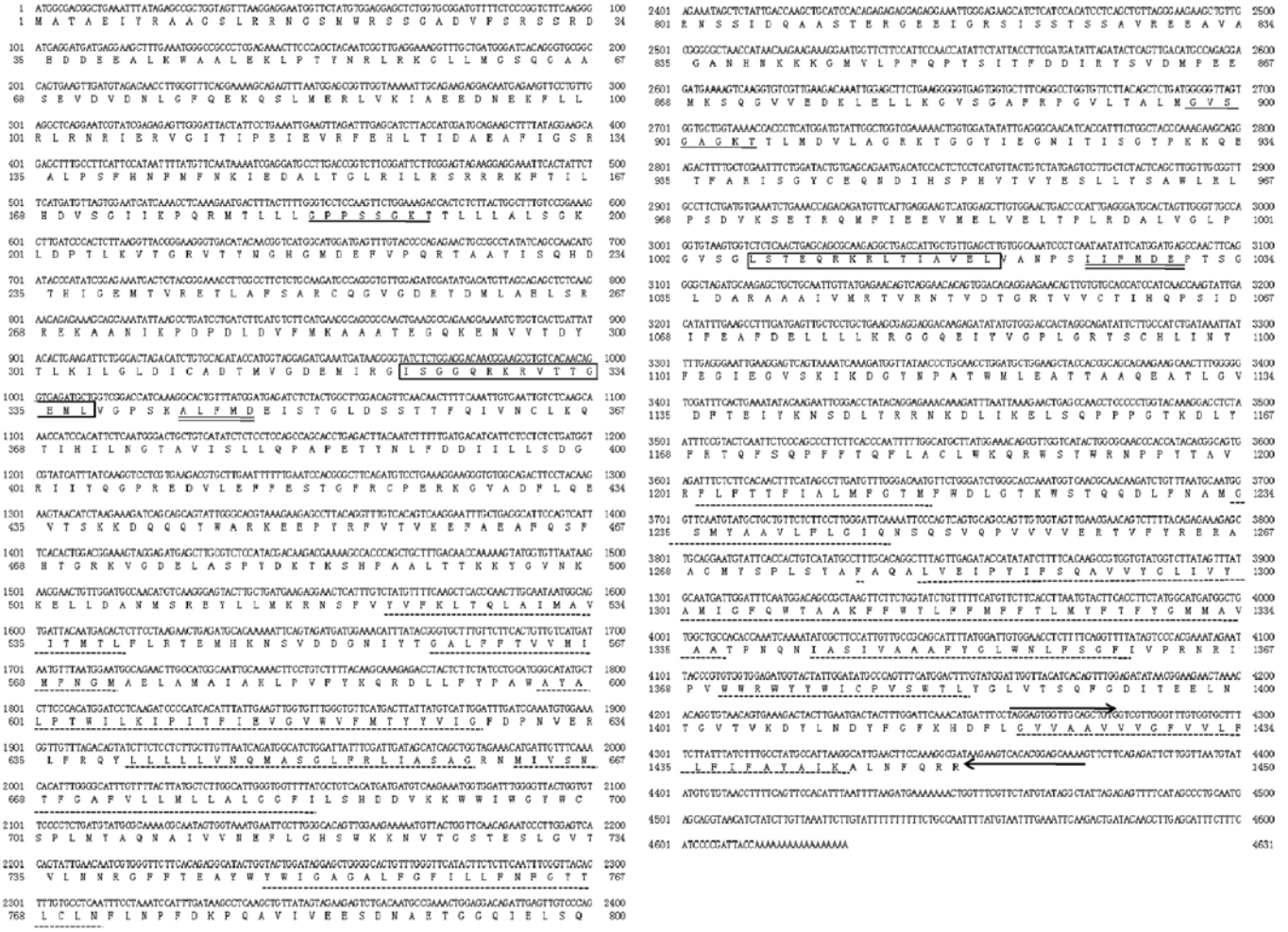


Fig. 2 - Phylogenetic tree of all full-size ABCGs in *Arabidopsis*, *VvABCG44*, and characterized full-size ABCGs from various plant species. NpPDR1 and NpPDR2 from *Nicotiana plumbaginifolia*, NtPDR1 and NtPDR3 from tobacco, SpTUR2 from *Spirodella polyrhiza*, OsABCG36 and OsABCG43 from rice, HvABCG31 from barley, PaPDR1 from Petunia, Lr34 from wheat, and GmPDR12 from soybean. The neighbor-joining tree was constructed with MEGA5 (Tamura *et al*., 2011).

A



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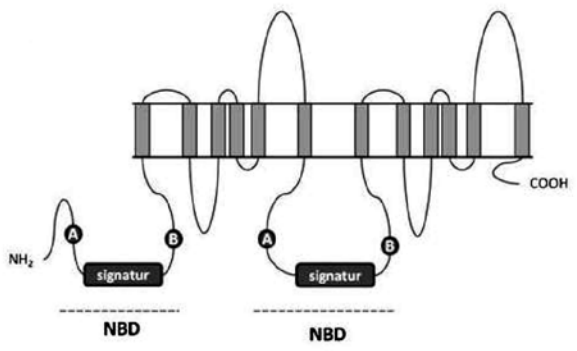

Fig. 1 - Nucleotide sequence, amino acid sequence, and topology of VvABCG44 A: Nucleotide sequence of *VvABCG44* and the deduced amino acid sequence. Walker A motifs are underlined. ABC signature motifs are boxed. Walker B motifs are double underlined. Transmembrane domains are dotted-lined. Arrows indicate the primers for quantitative PCR analysis. B: Putative topology of VvABCG44. The protein is composed of two halves and each half harbors TMD (gray boxes) and NBD (dashed lines), which contains an ABC signature and Walker A and B motifs.

(Fig. 3). The highest expression of *VvABCG44* was observed in mature leaves, which was 9.6 times higher than that in young leaves. *VvABCG44* expression in tendril and stem was higher than that in young leaves, but was not as high as that in mature leaves. *VvABCG44* expression was relatively low in the grape berry*VvABCG44*, where it was highest in the skin and lowest in seeds. We also determined the gene expression of stilbene synthase (STS), a key enzyme in resveratrol synthesis. The expression pattern of *STS* in various grape organs is similar to that of *VvABCG44* (Fig. 3), suggesting a relationship between VvABCG44 and resveratrol synthesis.

Later, we determined the induction of *VvABCG44* in grape berry skin by UV irradiation and by ABA treatment. Expression of *VvABCG44* was upregulated 2.7 times by UV irradiation, and the STS gene was strongly induced by UV irradiation. Furthermore, resveratrol content in the grape berry skin increased 159 times after 23 h of incubation following UV irradiation (Fig. 4). These results suggest a relationship between VvABCG44 and resveratrol accumulation. On the other hand, the expression of *VvABCG44* was not induced by ABA treatment in the grape berry skin (Fig. 5).

4. Discussion and Conclusions

There are few reports of grape ABC transporters, although comprehensive analyses, such as transcriptomics and proteomics, report the expression of ABC transporters in grape. Recently, Çakır and Kılıçkaya (2013) identified all ABC proteins using whole genome sequencing with 12× coverage and Francisco *et al*. (2013) identified an ABCC transporter of grape as a vacuolar anthocyanin

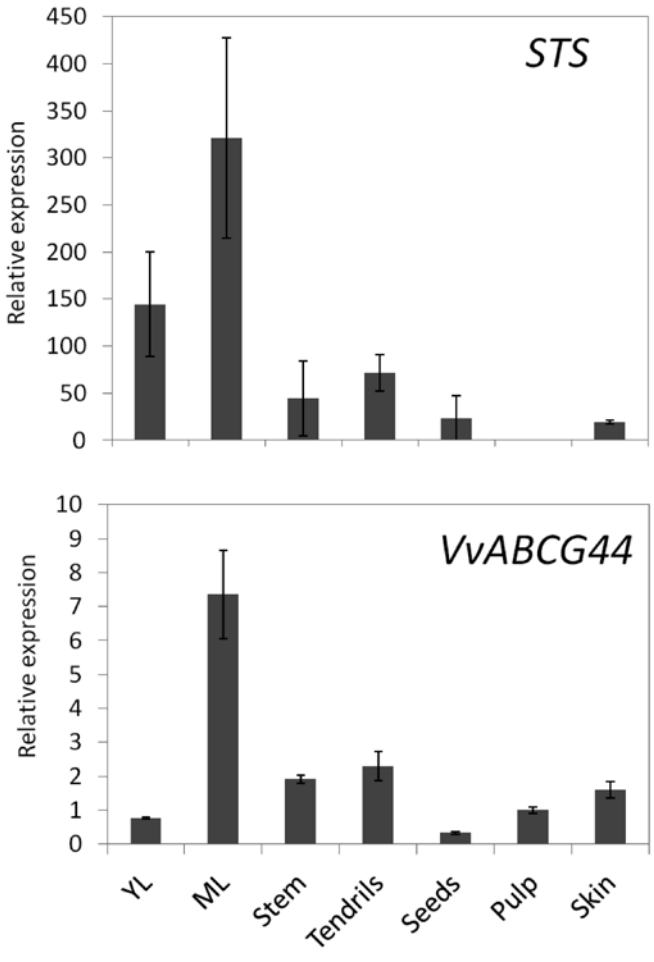

Fig. 3 - Gene expression of STS andVvABCG44 in various grape organs [YL (young leaves), ML; (mature leaves), stem, tendrils, seeds, pulp, and skin]. mRNA levels of STS and VvABCG44 were detected by quantitative PCR. Actin was used as an internal control. Each value represents mean ± SE of three independent experiments.

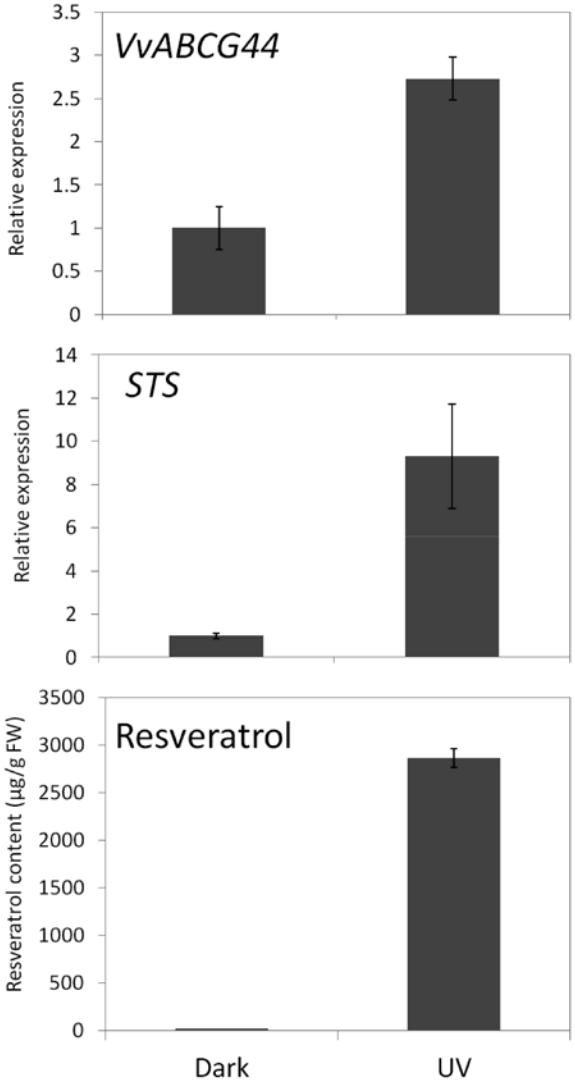

Fig. 4 - Expression of STS,VvABCG44 and resveratrol content in the grape berry skin after UV irradiation. mRNA levels of STS and VvABCG44 were detected by quantitative PCR. Actin was used as an internal control. Each value represents mean ± SE of three independent experiments. Resveratrol content was assayed by LC-ESI-Q-TOF/MS system in negative ion mode. Each value represents mean ± SE of six independent measurements.

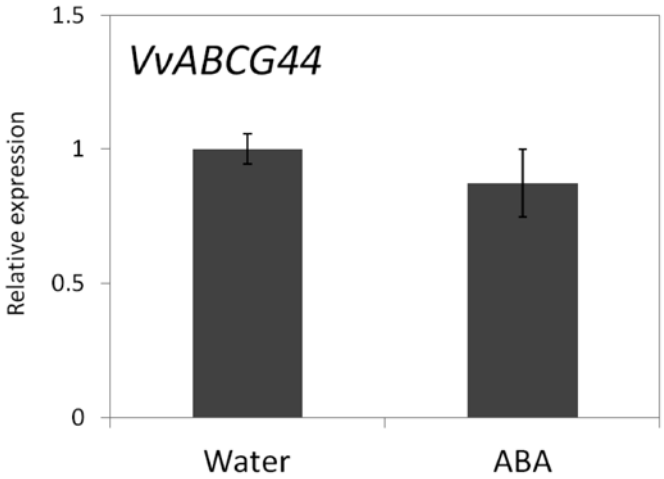

Fig. 5 - Expression of VvABCG44 in the grape berry skin after ABA treatment. mRNA level of VvABCG44 was detected by quantitative PCR. Actin was used as an internal control. Each value represents mean ± SE of three independent experiments.

transporter. To the best of our knowledge, this is the only characterized full-size ABC transporter in grape.

Why have such few full-size ABC transporters been studied? This is because of the difficulty in cloning full-length cDNA encoding full-size ABC transporters, particularly full-size ABCG transporters. One of the reasons for this difficulty is the very large size (ca. 4,000 bp) of full-size ABCG transporter cDNAs. Another reason is the frequently observed low growth rate of *Escherichia coli* harboring full-size ABCG transporter cDNA. The reason for this low growth rate of *E. coli* is unclear. Therefore, few or no full-length cDNA clones encoding full-size ABCG transporters are found in public cDNA databases or resources, and should be cloned.

Although 15 full-size ABCG transporters are present in *Arabidopsis* (van den Brûle and Smart, 2002), only five of them, AtABCG40, AtABCG37, AtABCG36, AtABCG32, and AtABCG30, have been characterized (Campbell *et al*., 2003; Lee *et al*., 2005; Ito and Gray, 2006; Kobae *et al*., 2006; Stein *et al*., 2006; Kim *et al*., 2007; Badri *et al*., 2009; Strader and Bartel, 2009; Kang *et al*., 2010; Kim *et al*., 2010; Růžička *et al*., 2010; Bessire *et al*., 2011; Underwood and Somerville, 2013; Xin *et al*., 2013). In other plant species, only two full-size ABCG transporters (OsABCG36, OsABCG43) in rice (Moons, 2003; Oda *et al*., 2011) and five full-size ABCG transporters (NpPDR1, NpPDR2, NtPDR1, NtPDR3, and ABCG5/PDR5) in tobacco family plants have been studied (Jasinski *et al*., 2001; Sasabe *et al*., 2002; Schenke *et al*., 2003; Ducos *et al*., 2005; Stukkens *et al*., 2005; Trombik *et al*., 2008; Bultreys *et al*., 2009; Navarre *et al*., 2011; Bienert *et al*., 2012; Seo *et al*., 2012; Crouzet *et al*., 2013).

In this study, we successfully cloned the full-length cDNA of *VvABCG44* using the primers designed from the grape genome sequence data, using a high-grade enzyme for PCR reactions and optimized *E. coli* culture conditions (culture at lower temperature and in higher volume). This appears to be the first report of a grape full-size ABCG transporter.

Two different data sets of grape genome sequences have been disclosed to the public. First, the Pinot Noir clone ENTAV115 was released by an Italian group, IASMA Research Center (http://genomics.research.iasma.it/) (Velasco *et al*., 2007). We used this information for cDNA cloning of *VvABCG44*. Second, the Pinot Noir-derived inbred PN40024 was sequenced by the French-Italian public consortium (Jaillon *et al*., 2007) (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/). The latter data set was updated from 8× to 12× and is now widely used. Recently the 12× version1(v1) has been made available by an Italian group, CRIBI (Grimplet *et al.*, 2012) (http://genomes.cribi.unipd.it/grape/). Therefore, we used v1 to find all full-size ABCG transporters in the grape genome (Table 2).

Fifteen and 23 full-size ABCG transporters were found in *Arabidopsis* (van den Brûle and Smart, 2002) and rice (Moons, 2008), respectively. In the grape genome data, we found 34 full-size ABCG transporters (Table 2, Fig. 6). This number is much larger than that in *Arabidopsis* and rice, suggesting a diversity of roles of full-size ABCG transporters in grape. As mentioned above, substrate and subcellular localization of full-size ABCG transporter cannot be determined from sequence similarity. However, full-size ABCG transporters are responsible for transport of secondary metabolites, plant hormones, cutins, and heavy metals (Fig. 2) and should have an important role in grape berry.

Recent reports showed that plant full-size ABCG transporters, transport plant hormones or their precursors, such

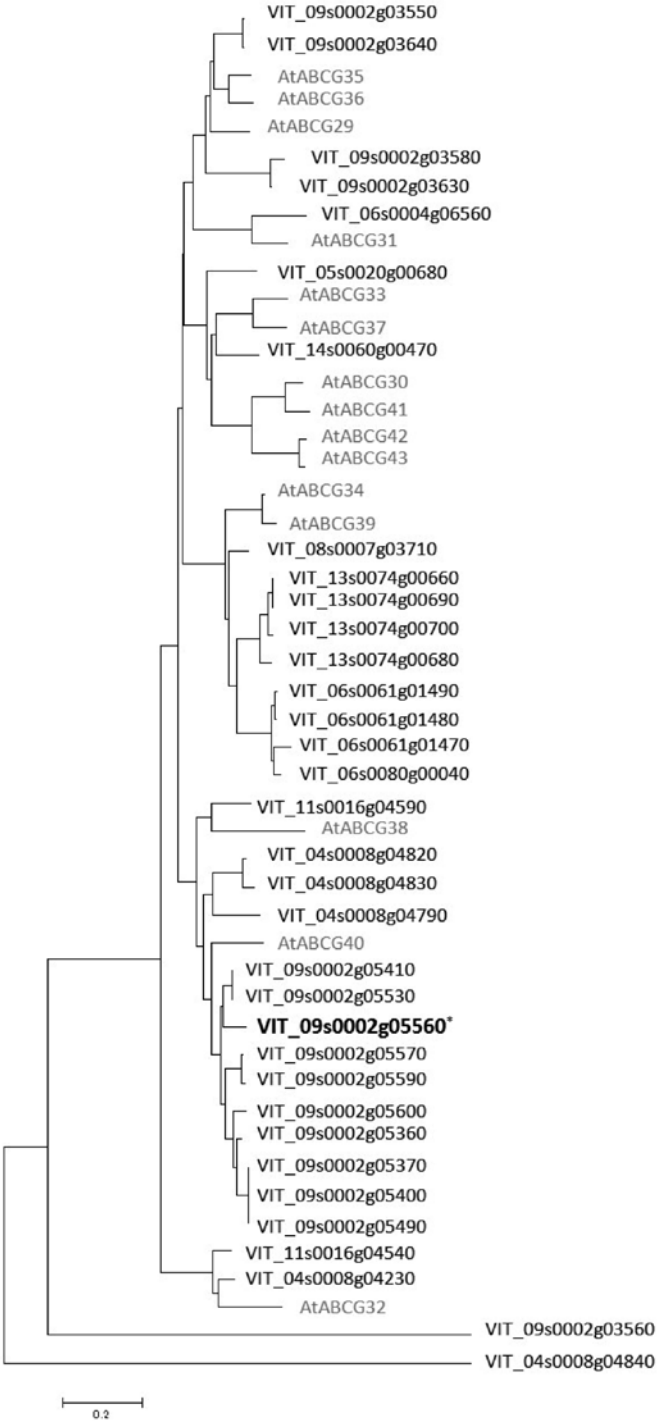

Fig. 6 - Phylogenetic tree of all full-size ABCGs in Arabidopsis and VvABCG44. A neighbor-joining tree was constructed with MEGA5 (Tamura *et al*., 2011).

Table 2 - Full-size ABCG transporters in grape (Vitis vinifera). Columns contain the *Vitis Vinifera* 12× V1 ID, chromosome location, protein length, PDR signatures, annotated description by Tair10, protein acronym (Name) and *Vitis Vinifera* 12× V0 ID for each gene are given

12X V1 ID	Chromosome location				Protein	PDR signatures**			Description of Tair10		Sanchez-	HGNC***	12X V0 ID
	Chr	Strand	Start	End		LLLGPP	GLDSST	GLDARA-	AGI code	Short description	Subfamily name	Subfamily name	
VIT_11s0016g04540	11	+	3825506	3837079	1422	+	+	+	AT2G26910.1	pleiotropic drug resistance 4	VvPDR1	VvABCG31	GSVIVT01015456001
VIT_11s0016g04590	11	-	3891367	3898727	1478	+	+	-	AT1G15520.1	pleiotropic drug resistance 12	VvPDR2	VvABCG32	GSVIVT01015461001
VIT_09s0002g03550	9	+	3229012	3242582	649	+	+	-	AT1G15210.1	pleiotropic drug resistance 7	VvPDR3	VvABCG33	GSVIVT01016991001
VIT_09s0002g03560	9	+	3242583	3244574	427	-	-	+	AT3G16340.1	pleiotropic drug resistance 1	VvPDR4	VvABCG34	GSVIVT01016992001
VIT_09s0002g03580	9	+	3246544	3252734	691	+	+	-	AT3G16340.1	pleiotropic drug resistance 1	VvPDR5	VvABCG35	GSVIVT01016993001
VIT_09s0002g03630	9	-	3318732	3327354	1411	+	+	+	AT1G59870.1	ABC-2 and Plant PDR ABC-type	VvPDR6	VvABCG36	GSVIVT01016998001
VIT_09s0002g03640	9	-	3328212	3336626	1494	+	+	+	AT3G16340.1	pleiotropic drug resistance 1	VvPDR7	VvABCG37	GSVIVT01016999001
VIT_09s0002g05360	9	-	5099146	5114849	1490	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR8	VvABCG38	GSVIVT01017184001
VIT_09s0002g05370	9	-	5115505	5122760	1422	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR9	VvABCG39	GSVIVT01017185001
VIT_09s0002g05400	9	-	5146167	5160090	1565	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR10	VvABCG40	GSVIVT01017187001
VIT_09s0002g05410	9	-	5169125	5176189	1438	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR11	VvABCG41	GSVIVT01017188001
VIT_09s0002g05490	9	-	5216536	5223507	1280	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR12	VvABCG42	GSVIVT01017196001
VIT_09s0002g05530	9	-	5259175	5266314	1460	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR13	VvABCG43	GSVIVT01017198001
VIT_09s0002g05560*	9	-	5281296	5288255	1455	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR14	VvABCG44	GSVIVT01017201001
VIT_09s0002g05570	9	-	5294437	5301677	1455	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR15	VvABCG45	GSVIVT01017202001
VIT_09s0002g05590	9	-	5316144	5323420	1455	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR16	VvABCG46	GSVIVT01017204001
VIT_09s0002g05600	9	-	5336090	5343699	1451	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR16	VvABCG46	GSVIVT01017204001
VIT_05s0020g00680	5	+	2548762	2557921	1438	+	+	+	AT3G53480.1	pleiotropic drug resistance 9	VvPDR17	VvABCG47	GSVIVT01017676001
VIT_06s0004g06560	6	+	7284901	7297724	1274	+	+	+	AT2G29940.1	pleiotropic drug resistance 3	VvPDR18	VvABCG48	GSVIVT01024743001
VIT_14s0060g00470	14	+	439701	448696	1449	+	+	-	AT3G53480.1	pleiotropic drug resistance 9	VvPDR19	VvABCG49	GSVIVT01031314001
VIT_06s0061g01490	6	-	1,9E+07	1,9E+07	1455	+	+	+	AT2G36380.1	pleiotropic drug resistance 6	VvPDR20	VvABCG50	GSVIVT01031377001
VIT_06s0061g01480	6	-	1,9E+07	1,9E+07	1461	+	+	+	AT2G36380.1	pleiotropic drug resistance 6	VvPDR21	VvABCG51	GSVIVT01031378001
VIT_06s0061g01470	6	-	1,9E+07	1,9E+07	1123	+	+	+	AT1G66950.1	pleiotropic drug resistance 11	VvPDR22	VvABCG52	GSVIVT01031380001
VIT_08s0007g03710	8	+	1,8E+07	1,8E+07	1452	+	+	+	AT2G36380.1	pleiotropic drug resistance 6	VvPDR23	VvABCG53	GSVIVT01033804001
VIT_13s0074g00660	13	-	8818113	8827874	1473	+	+	-	AT2G36380.1	pleiotropic drug resistance 6	VvPDR24	VvABCG54	GSVIVT01034741001
VIT_13s0074g00680	13	-	8859786	8867047	1477	+	+	-	AT1G66950.1	pleiotropic drug resistance 11	VvPDR25	VvABCG55	GSVIVT01034745001
VIT_13s0074g00690	13	-	8876000	8883078	1379	+	+	-	AT2G36380.1	pleiotropic drug resistance 6	VvPDR26	VvABCG56	GSVIVT01034746001
VIT_13s0074g00700	13	-	8897688	8904965	1481	+	+	-	AT2G36380.1	pleiotropic drug resistance 6	VvPDR27	VvABCG57	GSVIVT01034748001
VIT_04s0008g04230	4	-	3596683	3605452	1422	+	+	-	AT2G26910.1	pleiotropic drug resistance 4	VvPDR28	VvABCG58	GSVIVT01035715001
VIT_04s0008g04790	4	-	4227017	4234518	1437	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR29	VvABCG59	GSVIVT01035780001
VIT_04s0008g04820	4	+	4258541	4265241	1420	+	+	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR30	VvABCG60	GSVIVT01035784001
VIT_04s0008g04830	4	+	4282425	4286094	764	+	+	-	AT1G15520.1	pleiotropic drug resistance 12	VvPDR31	VvABCG61	GSVIVT01035785001
VIT_04s0008g04840	4	+	4286954	4295631	1120	-	-	+	AT1G15520.1	pleiotropic drug resistance 12	VvPDR32	VvABCG62	GSVIVT01035786001
VIT_06s0080g00040	6	+	2E+07	2E+07	1507	+	+	-	AT2G36380.1	pleiotropic drug resistance 6	VvPDR33	VvABCG63	GSVIVT01036184001

*VIT_09s0002g05560 shown with bold letters is corresponded to VvABCG44.

**PDR signatures were reported by van den Brûle and Smart (2002).

***Sanchez-Fernandez and HGNC subfamily names were reported by Çakır and Kılıçkaya (2013).

as ABA (Kang *et al*., 2010), strigolactone (Kretzschmar *et al*., 2012), and auxin (Ruzicka *et al*., 2010). One of the closest homologues of VvABCG44, AtABCG40, was reported to transport ABA (Kang *et al*., 2010) (Fig. 2). In grape berry, ABA accumulates just before maturation, called "veraison", and ABA works as a trigger of berry maturation (Coombe and Hale, 1973; Davies *et al*., 1997). After veraison, both sugar and anthocyanin accumulate considerably in the grape berry (Coombe, 1992; Davies *et al*., 1997; Deluc *et al*., 2007). In this study, we determined *VvABCG44* induction by ABA in the berry skin before veraison. However, no induction was observed (Fig. 5).

Many plant full-size ABCG transporters have been suggested to be associated with biotic and abiotic stress resistance, particularly resistance against pathogens, and some have been observed to transport secondary metabolites that function as phytoalexins (Fig. 2). Therefore, *VvABCG44* is considered to be associated with biotic and abiotic stress resistance and transports phytoalexins. *VvABCG44* was first found as an elicitor-induced gene in grape culture cells and the induction of *VvABCG44* corresponded to resveratrol accumulation in the cells (Zamboni *et al*., 2009).

It is known that UV irradiation induces resveratrol accumulation in the grape berry skin (Douillet-Breuil *et al*., 1999; Adrian *et al*., 2000; Versari *et al*., 2001; Takayanagi *et al*., 2004). Therefore, we determined the effect of UV irradiation on *VvABCG44* expression together with STS expression, a key enzyme for resveratrol synthesis and resveratrol accumulation. A clear induction of *VvABCG44* by UV irradiation, though not large compared with that of *STS* expression and resveratrol accumulation, *VvABCG44* was observed (Fig. 4). A similar pattern in the gene expression of *VvABCG44* and *STS* in various grape organs was also observed (Fig. 3). These results suggest a relationship between *VvABCG44* and resveratrol accumulation.

Close homologues of *VvABCG44*, NtPDR1 (Crouzet *et al*., 2013), NpPDR1 (Jasiński *et al*., 2001), MtABCG10 (Banasiak *et al*., 2013), SpTUR2 (van den Brûle and Smart, 2002), AtABCG40 (Kang *et al*., 2010), and PaPDR1 (Kretzschmar *et al*., 2012), transport diterpenoids, isoflavonoids, ABA, and strigolactones (Fig. 2). These functions are surprising because their molecular structures are completely different. Resveratrol is a compound belonging to the stilbenoids and both stilbenoids and flavonoids belong to the phenylpropanoids. The closest homologue of *VvABCG44*, MtABCG10, transports isoflavonoids (Banasiak *et al*., 2013). Although no direct evidence of stilbenoid transport activity of full-size ABCG transporter has been reported, it was observed that *B. cinerea*, lacking a full-size ABCG transporter, BcatrB, was more sensitive to resveratrol than the wild-type strain (Schoonbeek *et al*., 2001). This result suggests that BcatrB is an exporter of resveratrol in *B. cinerea.* It can be concluded that *VvABCG44* may work as a resveratrol transporter in grape.

We attempted to express *VvABCG44* in yeast lacking eight ABC transporters (Kang *et al*., 2010) and measure resveratrol transport activity. However, this attempt was unsuccessful because heterologous expression of plant full-size ABCG transporters is difficult not only in *E. coli* but also in yeast.

We identified 34 full-size ABCG transporters in the grape genome, including *VvABCG44*. It is assumed they transport key compounds for plant growth and stress resistance, including secondary metabolites, plant hormones, cutins, and heavy metals, and have important roles in grape. Further study on full-size ABCG transporters in grape is warranted.

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