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An Identification and Expression Analysis of the *ABCG* Genes Related to Benzaldehyde Transportation among Three *Prunus* Species

Ruijie Hao [1,*,†], Jun Chang [1,†], Chen Qiu [2] and Shuting Yang [1]

[1] College of Horticulture, Shanxi Agricultural University, Taigu 030801, China; junchang1111@163.com (J.C.); amyyst@163.com (S.Y.)
[2] College of Urban and Rural Construction, Shanxi Agricultural University, Taigu 030801, China; q1269573600@163.com
* Correspondence: hrj000@126.com
† These authors contributed equally to this work.

Abstract: The plants of *Prunus* mostly bloom in early spring, and the flowers of various species possess their individual floral scent characteristics; *Prunus mume*, especially, can volatilize a large amount of benzenoid compounds into the air during the flowering phase. In order to elucidate the molecular basis of the differences in the volatile capacity of aromatic substances among *Prunus* flowers, the endogenous and the headspace volatile components and the expression of *ABCG* genes were studied among *P. mume*, *P. armeniaca*, and *P. persica*. We detected the floral components in the three species by gas chromatography-mass spectrometry (GC-MS), and we found that benzaldehyde was the key component. Meanwhile, the volatilization efficiency of benzaldehyde in *P. mume* and *P. armeniaca* were much higher than that in *P. persica*. Furthermore, 130, 135, and 133 ABC family members from *P. mume*, *P. armeniaca*, and *P. persica* were identified, respectively. WGCNA analysis demonstrated that candidate *ABCG* genes were positively correlated with benzaldehyde volatilization efficiency. Moreover, quantitative Real-time PCR indicated that *ABCG17* was more likely to be involved in the transmembrane transport of benzaldehyde. This study aimed to provide a theoretical basis for elucidating the transmembrane transport of benzaldehyde and to further the valuable information for fragrant flower breeding in *Prunus*.

Keywords: *P. mume*; *P. armeniaca*; *P. persica*; floral scent; benzaldehyde; *ABCG* subfamily genes

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1. Introduction

The genus *Prunus*, a large genus of the Rose family, are widely cultivated in the world, including many economically important fruit and ornamental trees [1]. The evolutionary relationship of *Prunus* was deeply studied by many researchers [2,3]. Phylogenetic analysis of the *Prunus* indicates that *P. mume* has a closer genetic relationship with *P. armeniaca*, and the divergence of *P. persica* evolution occurred earlier [2]. *P. mume* is the particular woody plant with aromatic characteristics, and it is mainly cultivated in southern China, while *P. armeniaca* and *P. persica* are cultivated as common fruit trees in northern China. They are highly ornamental in the early spring. However, only *P. mume* produces a strong floral fragrance, while the other two species lack a floral scent but possess stronger cold resistance. Why *P. mume* differed in the aromatic mechanism from other species in *Prunus*, such as *P. armeniaca* and *P. persica*, is still unclear.

Volatile organic compounds (VOCs) play critical roles in attracting pollinators; defending against pathogens, parasites, and herbivores; facilitating interactions with the environment; and protecting against abiotic stresses during plant growth and development [4]. The biosynthesis of aromatic component have been studied extensively [5], but regardless of whether VOCs biosynthesize in the cytoplasm or various specific organelle,

at the subcellular level, they usually move from their biosynthetic site through the cytosol and then subsequently traverse the plasma membrane, cross the hydrophilic cell wall, and finally release into the air or special glands [6]. Niinemets et al. (2010) believed that the release of volatile compounds was independent of concentration gradient [7], and we speculate that some transporters are involved in this process. Therefore, it is necessary to deeply explore the transmembrane transport mechanism of VOCs in plants.

ATP-binding cassette (ABC) transporters are essential membrane proteins that transport a wide variety of substrates across cellular membranes, and they are conserved in plants [8]. They consist of hydrophobic transmembrane domains (TMDs) and hydrophilic nucleotide-binding domains (NBDs) [9,10]. Most of these proteins transport secondary metabolites via TMDs using energy released by ATP hydrolysis at NBDs. ABC transporters are divided phylogenetically into eight subfamilies ABCA-ABCG and ABCI [11], and the largest ABCG subfamily is very significant among them [12]. Based on the sequence similarity and domain organization, ABCG transporters have been subdivided into either full-molecule transporter with NBD-TMD-NBD-TMD or half-molecule transporter with NBD-TMD, which are also known as pleiotropic drug resistance (PDR) and white-brown complex (WBC), respectively [13]. WBC has attracted many researchers' attention due to transporting many substances by forming homologous or heterologous dimers [14]. *P. mume* possesses diverse volatile components, however, the mechanism of substrate recognition and transmembrane transport has not been elucidated, and whether they are transported by WBC remains an enigma.

Benzaldehyde is abundant in plants, and it is mainly related to plant resistance. [15]. Benzaldehyde is an aromatic aldehyde with a bitter almond odor, a low molecular weight, but a large diffusion coefficient [16]. Our previous study demonstrated that benzaldehyde, benzyl alcohol, and benzyl acetate were the main volatile components in the full flowering stage of *P. mume*, and benzaldehyde was abundant in *Prunus* during this period [17], which provided an opportunity for us to study the difference in volatilization ability and to reveal the volatilization regularity of benzaldehyde in *Prunus*. Like other aldehydes, it is probably a nonspecific irritant [18] to defend against environmental stress [19]. Up to now, little is known about the relationship between the transport of benzaldehyde and ABC transporters.

Here, we identified floral scent components and endogenous contents using GC-MS analysis, and we compared floral volatilization specificity among three species with a close genetic relationship, *P. mume*, *P. armeniaca*, and *P. persica*. Based on the difference in the volatilization efficiency of benzaldehyde, the transcriptome sequencing on the blooming flowers of *P. mume*, *P. armeniaca*, and *P. persica* was performed, and the ABC family members were characterized in detail by bioinformatics methods. Furthermore, the expression pattern of the candidate genes was validated by quantitative RT-PCR assays. In conclusion, the current study provided a theoretical basis for revealing the transmembrane transport mechanism of benzaldehyde and further promoting fragrant flower breeding in *Prunus*.

2. Materials and Methods

2.1. Plant Materials

Plant materials were collected from the campus of Shanxi Agricultural University (Long. 112°587 E, lat. 37°424 N) during March–April 2021, including *P. mume*, *P. armeniaca*, and *P. persica*. When the branches on the outer canopy were ready to bloom, they were cut and then cultivated in a beaker with ultrapure water at 20 °C, 60 μmol·m^{-2}·s^{-1} of the light intensity and 12 h light and 12 h dark of the photoperiod in an incubator.

2.2. Collection and Analysis of Volatile Components

Flowers at the full-bloom stage were collected and weighed. Flowers from different species were then placed in 20 mL headspace bottles and volatile components were obtained and analyzed by the SPME method using a 50/30 μm DVB/CAR/PDMS, Stableflex fiber (Supelco, Bellefonte, PA, USA) [20]. The specific GC-MS conditions refer to Hao et al. [21].

Individual peaks were identified by matching the mass spectra with the NIST library, by comparing their retention indexes with Pherobase (http://www.pherobase.com/) (accessed on 16 May 2021), and by comparing their retention times and mass spectra with authentic standards.

2.3. Extraction and Analysis of the Endogenous Compounds in Flowers

The endogenous compounds were extracted using the solvent extraction method. First, the flowers at the full-bloom stage were immediately ground into powder in liquid nitrogen. Then, approximately 0.2 g powder and 1.5 mL ethyl acetate (Sigma-Aldrich, St. Louis, MO, USA) were put into a 2 mL centrifuge tube. This extract was dehydrated with anhydrous sodium sulphate [22]. After centrifugation at 12,000 rpm at 4 °C for 10 min, the supernatant was transferred to a new centrifuge tube. The 10 µL methyl benzoate standard solution (Sigma-Aldrich, St. Louis, MO, USA) was added as an internal standard to quantification. The steps were repeated three times to obtain biological triplicates for each sample. The components of the extracts were analyzed by the GC-MS method described above.

2.4. Identification of ABC Gene Family Members

The whole genome data of *P. mume*, *P. armeniaca*, and *P. persica* were obtained from the *P. mume* genome database (http://Prunusmumegenome.bjfu.edu.cn (accessed on 16 May 2021)) [23] and the GDR (https://www. rosaceae.org/ (accessed on 16 May 2021)) [24,25]. The ABC transporters domain ABC_tran (PF00005) file was from the Pfam protein structure database (http://pfam.xfam.org/ (accessed on 16 May 2021)) [26]. The ABC hidden Markov model was constructed using HMMER3.0, and the sequence on the *P. mume*, *P. armeniaca*, and *P. persica* genomic protein database was aligned with the *Arabidopsis thaliana* ABC transporters sequence by BLASTP to obtain ABC family members of *P. mume*, *P. armeniaca*, and *P. persica* (PmABC, PpABC and PaABC). The *Arabidopsis thaliana* database (https://www.arabidopsis.org/ (accessed on 16 May 2021)) was used as the reference sequence and the parameters were set to default.

2.5. Physical and Chemical Properties and Subcellular Location of PmABC, PpABC, and PaABC Members

The physical and the chemical properties of ABC members were evaluated on the ExPASY (https://web.expasy.org/protparam/ (accessed on 16 May 2021)) [27]. The subcellular location was performed by the PSORT Prediction tool (http://psort1.hgc.jp/form.html (accessed on 16 May 2021)).

2.6. Collinearity Analysis of ABC Genes and Phylogenetic Tree Construction

All *ABC* genes were mapped on chromosomes by Circos [28]. The collinear regions of the obtained homologous gene pairs were analyzed using the MCScanX tool [29]. TBtools was used to visualize the intraspecific collinearity of the *ABC* gene family. Multiple sequence alignments between the obtained ABCG sequences of *Prunus* and the phylogenetic tree were constructed by MEGA7 with 1000 times of bootstrap value and the Poisson model [30].

2.7. ABCG Genes Expression Patterns Analysis

Transcriptome sequencing of blooming flowers of the three species, *P. mume*, *P. armeniaca*, and *P. persica*, was performed at the Beijing Biomarker Company (Beijing, China). The heat map of gene expression was constructed using TBtools, according to the reads per kilobase per million reads (RPKM) of the transcriptome data of *Prunus* in order to evaluate the difference in expression profiles of *ABCG* subfamily genes among the three species.

2.8. Weighted Gene Co-Expression Network Analysis (WGCNA)

In previous research, we obtained the transcriptome of different parts and the volatilization and endogenous content of benzaldehyde in *P. mume* [21]. In this study, WGCNA was employed to identify significant gene modules correlated to benzaldehyde based on the

dynamic gene expression patterns of five different flower organs in *P. mume*. Differentially expressed genes (DEGs) with a differential multiple greater than 5 and a false discovery rate $\leq$ 0.001 were filtered by R package 'DeSeq2' and then used to construct a weight co-expression network [31]. According to the topological overlap matrix value in each pair of genes, the gene cluster tree was constructed by the hierarchical clustering method and further cut into modules via the dynamic tree cut method.

2.9. Quantitative Expression Analysis of PmABC Members

Total RNA was extracted using the hexadecyltrimethyl ammonium bromide (CTAB) method [32], and reverse transcription was performed using the First Strand cDNA Synthesis Kit (KP211, Tiangen, Beijing, China). The qRT-PCR was run on Roche Light Cycler480 instrument with SYBR Green (FP411) Kit. The primers were designed using the Primer Quest Tool (http://sg.idtdna.com/Primerquest/Home/Index (accessed on 16 May 2021)), and it is listed in Table S1. The *UBC* gene was used as a reference gene [33]. The reaction was as follows: 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s, and 60 °C for 1 min. In addition, the gene expression levels were evaluated according to the $2^{-\triangle\triangle}$Ct method [34], with *P. mume* as the control.

3. Results

3.1. Floral Scent Components Analysis in P. mume, P. armeniaca, and P. persica

To explore the differences of the main aromatic components among *P. mume*, *P. armeniaca*, and *P. persica*, the floral components were identified in three *Prunus* species (Figure S1), including 30 volatile compounds (Table S2). As shown in Figure 1a, the volatile components and the relative contents displayed obvious differences among the three species. Benzyl acetate accounted for more than 60% of the total volatile content, it was the main volatile component of *P. mume*, and it only existed in *P. mume*. In addition, it was noted that the highest content in *P. persica* was 3,5-dimethoxytoluene, reaching 86.19%. A principal aromatic compound, benzaldehyde, was detected in the flowers from all three *Prumus* species. Meanwhile, the relative amounts of benzaldehyde in *P. armeniaca* and *P. mume* were higher, both accounting for approximately 10% of their total volatilization amounts. Interspecies comparisons showed that the relative total peak areas of benzaldehyde in *P. armeniaca* and *P. mume* were very close, and significantly higher than that in *P. persica* (Figure 1b).

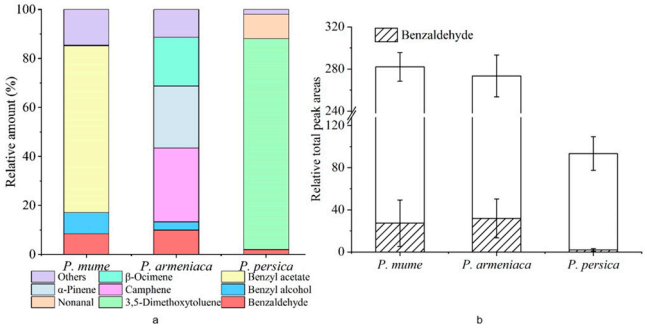

Figure 1. Floral scent components analysis in *P. mume*, *P. armeniaca*, and *P. persica*. (**a**) The relative amounts of major components in the headspace volatiles from the flowers in *P. mume*, *P. armeniaca*, and *P. persica*; (**b**) Relative total peak area of benzaldehyde volatilization in *P. mume*, *P. armeniaca*, and *P. persica*.

Meanwhile, twenty endogenous contents were identified in three *Prunus* species (Table S3), among which benzaldehyde was common to all endogenous components of the three flowers, and the relative content was the highest in *P. armeniaca*. Figure 2 shows that the endogenous benzaldehyde content was higher than the volatile in all three species, especially in *P. persica*, reaching 757.58 $\mu g \cdot g^{-1}$. In *P. mume*, the headspace of benzaldehyde was 17.87 $ng \cdot g^{-1} \cdot h^{-1}$, which was seven times that of *P. persica*.

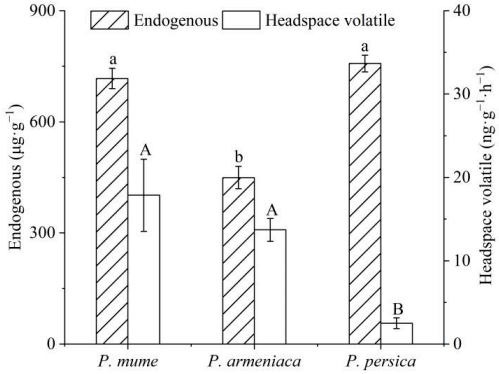

Figure 2. Quantitative analysis of benzaldehyde in flowers among the three species. The different lowercase and uppercase letters in the same column indicated a significant difference in Duncan's multiple range test ($p < 0.05$).

The volatilization efficiency of benzaldehyde was calculated based on the ratio of its volatile and endogenous amounts. In Figure 3, the volatilization efficiency of benzaldehyde varied greatly among different species, and the volatilization efficiency of *P. mume* and *P. armeniaca* was six times as high as that of *P. persica*, indicating that the volatilization efficiency of benzaldehyde did not depend on its endogenous content.

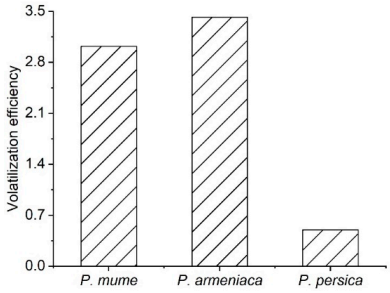

Figure 3. Volatilization efficiency of benzaldehyde in different species. The vertical axis represented the ratio of the volatilization amount to the endogenous content normalized with the natural logarithm.

3.2. Identification of ABC Family Members in P. mume, P. armeniaca, and P. persica

In order to comprehensively identify the ABC gene family members in the genome of *P. mume*, *P. armeniaca*, and *P. persica*, ABC protein sequences were aligned with *Arabidopsis thaliana*. A total of 130 *PmABCs*, 135 *PaABCs*, and 133 *PpABCs* family members were identified via the Pfam structure model of ABC transporters and systematically numbered according to eight subfamilies, respectively. Meanwhile, the physical and the chemical properties of the *ABC* genes from the three identified relatives were analyzed (Tables S4–S6). The numbers of amino acids encoded by the *ABCs* genes from the three closely related species ranged from 121 to 3148, and the molecular weight of protein were from 13.0 to 352.4 KD. The subcellular localization revealed that 79, 84, and 85 ABC proteins were located on the plasma membrane in *P. mume*, *P. armeniaca*, and *P. persica*, respectively. Notably, 38, 36, and 37 of them belonged to the ABCG subfamily, respectively.

3.3. Interspecific Collinearity Analysis of PmABC, PaABC, and PpABC

In order to further elucidate the association of the *ABC* family among the three species, the interspecific collinear relationships of *ABC* genes in *P. mume*, *P. armeniaca*, and *P. persica* were constructed. It was found in Figure 4 that *PmABC* displayed 105 collinear relationships with *PaABC* and 94 collinear relationships with *PpABC*, suggesting that *P. mume* and *P. armeniaca* had a closer genetic relationship. *PmABCC8*, *11*, *14* and *PmABCG5*, *11*, *14*, *20* were collinear only with *PpABC*, but not with *PaABC*, suggesting that these collinear

gene pairs arose after *P. persica* differentiated from the common ancestor of *P. mume* and *P. armeniaca*. Some *PmABCs* shared three pairs of collinearity with *P. persica* or *P. armeniaca*, for example, *PmABCG15* and *PmABCG29* had three pairs of collinearity with *PaABC* and *PmABCG6* had three pairs of collinearity with *PpABC*, inferring that these *ABC* genes may play an important role in the evolution process.

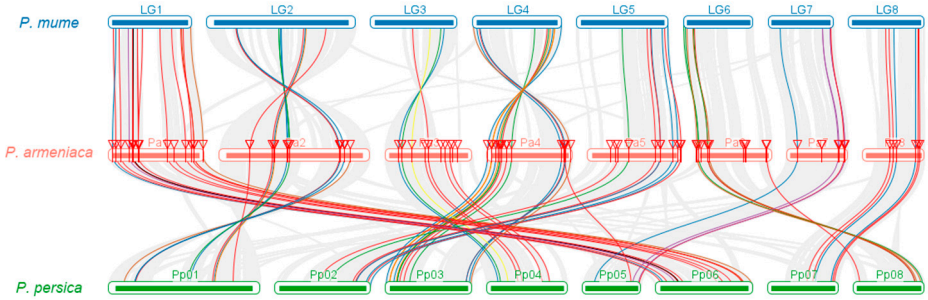

Figure 4. Analysis of interspecific collinearity among *P. mume*, *P. armeniaca*, and *P. persica*. Gray lines represent the collinearity of all genes among species, colored lines represent the collinearity of *ABC* gene family among species, and different colors represent different subfamilies.

3.4. Phylogenetic Analysis of the ABCG Genes in P. mume, P. armeniaca, and P. persica

The *ABCG* subfamily, as the biggest subfamily of ABC transporters, included 55, 53, and 58 family members in *P. mume*, *P. armeniaca*, and *P. persica*, respectively. A phylogenetic tree of *ABCGs* was constructed using the NJ method of MEGA7 (Figure 5). Among these *ABCG* family genes, *ABCG1-29* belonged to the WBC type and the others were PDRs [21]. As shown in Figure 5, the *ABCG* genes of the three species on the same clade were closely related, most of which were *WBC* genes.

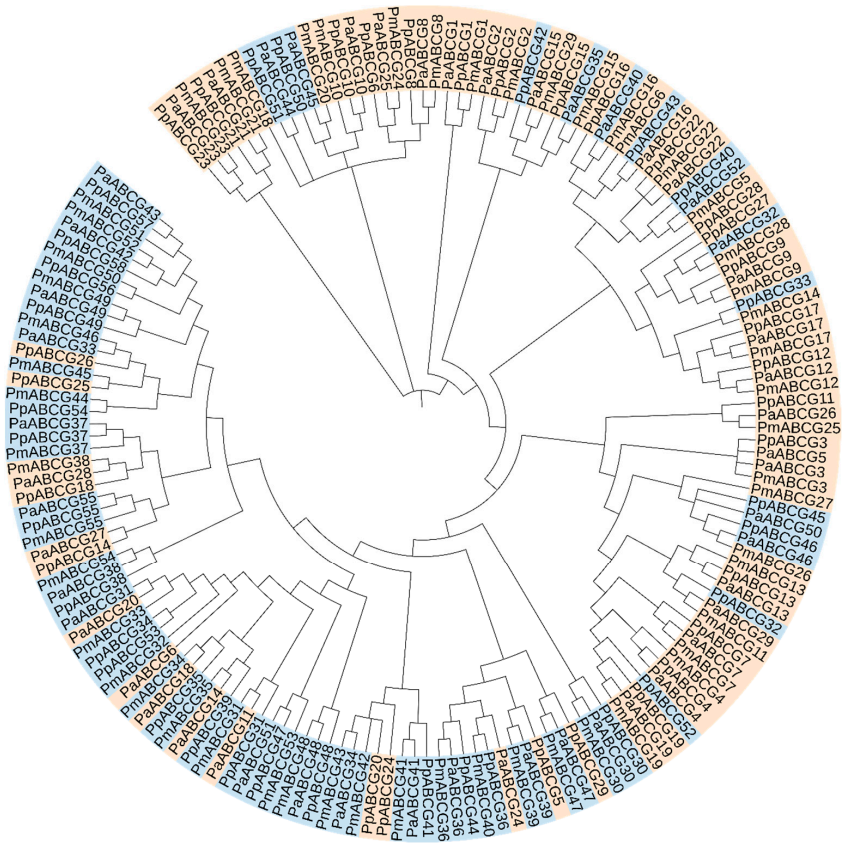

Figure 5. Phylogenetic tree of *PmABCGs*, *PaABCGs*, and *PpABCGs.* The *WBC* genes were in pink, and the *PDR* genes were in blue.

3.5. Transcription Expression Analysis of PmABCG, PaABCG and PpABCG Family Members

In order to further study the expression patterns of *ABCG* genes in the flowering stage, we carried out transcriptome sequencing of the blooming flowers from the three closely related species. Combined with interspecific collinearity and clustering relationships, 23 *ABCG* genes were obtained in the present study (Figure 6). The results showed that 23 *ABCG*s displayed the different expression levels in *P. mume*, *P. armeniaca*, and *P. persica*, and the first 17 *ABCG* genes (*ABCG1-23* in Figure 6) belonged to the WBC type. It can be seen from Figure 6 that the *ABCG23* gene was the highest expressed in *P. mume* and *P. persica*, while the *ABCG16* gene was highly expressed in *P. armeniaca*. Interestingly, the *ABCG* genes with a high expression mainly belonged to the WBC type. Moreover, some genes are expressed higher in *P. mume* and *P. armeniaca* than in *P. persica*, such as *ABCG9*, *13*, *19*, etc.

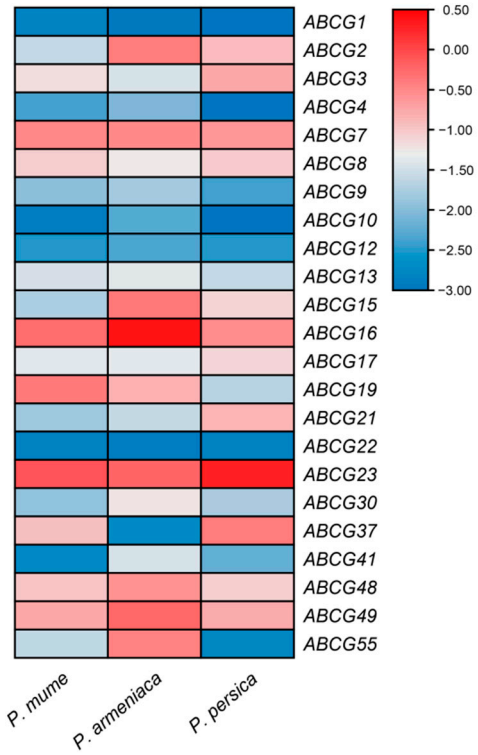

Figure 6. Expression pattern analysis of ABCG genes in *P. mume*, *P. armeniaca*, and *P. persica* by transcriptome sequencing analysis. The gene expression levels normalized by natural logarithms were shown from blue to red according to the scale.

3.6. WGCNA Analysis between Benzaldehyde Volatilization and PmABCGs Gene Expression

A co-expression network was constructed using WGCNA to identify the association between *PmABCG* gene expression from five different floral organs (petals, filaments, anthers, calyx, and other) and the volatilization efficiency of benzaldehyde in *P. mume* (Figure 7). Five co-expression modules were obtained by WGCNA analysis, and the correlation coefficients of brown, green, pink and turquoise were negative, indicating that the genes of these four modules were negatively correlated with the volatilization efficiency of benzaldehyde. The black module with a correlation coefficient of 0.88 was positively correlated with benzaldehyde volatilization, including *ABCG2*, *7*, *15*, *17*, *23*, *29*, *30*, *35*, and *ABCG44*. According to the expression patterns of *ABCG* genes in different species and WGCNA analysis, *ABCG2*, *7*, *9*, *15*, *17*, and *23* belonging to the WBC type were most likely related to the volatilization of benzaldehyde.

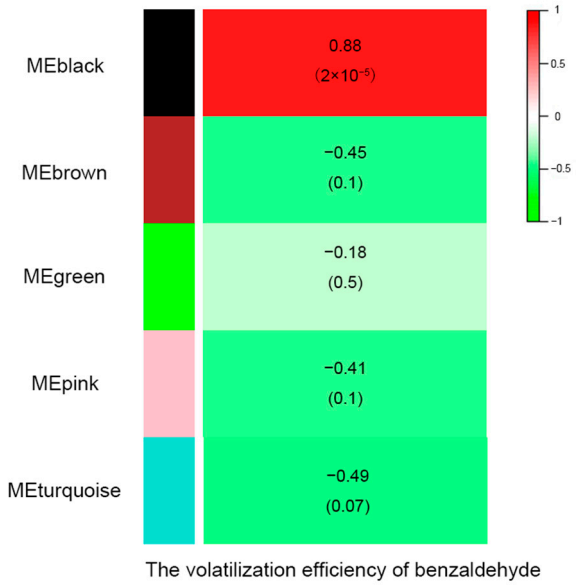

Figure 7. WGCNA for ABCG genes expression in different floral organs of *P. mume* and the volatilization efficiency of benzaldehyde.

3.7. qRT-RCR Analysis of the Candidate ABCG Genes

To further verify the expression profiles of the candidate *ABCG* genes in flowers from the three species, qRT-PCR testing was conducted (Figure 8). We found that the expression level of the candidate ABCG genes showed significant difference in the three species. The *ABCG17* gene expression was significantly higher in *P. mume* and *P. armeniaca* than in *P. persica*, which was consistent with the volatilization efficiency of benzaldehyde. *ABCG2* expressed significantly higher in *P. armeniaca* than in the other two species, 18-fold higher than in *P. mume*. Additionally, the gene expression of *ABCG7* was different among the three species, the highest in *P. mume* followed by *P. persica*. *ABCG9*, *15*, and *23* expressed significantly higher in *P. persica* than in the other species, especially, the expression level of *ABCG15* was 313-fold higher than that in *P. armeniaca*. In summary, *ABCG17* may participate in benzaldehyde transport.

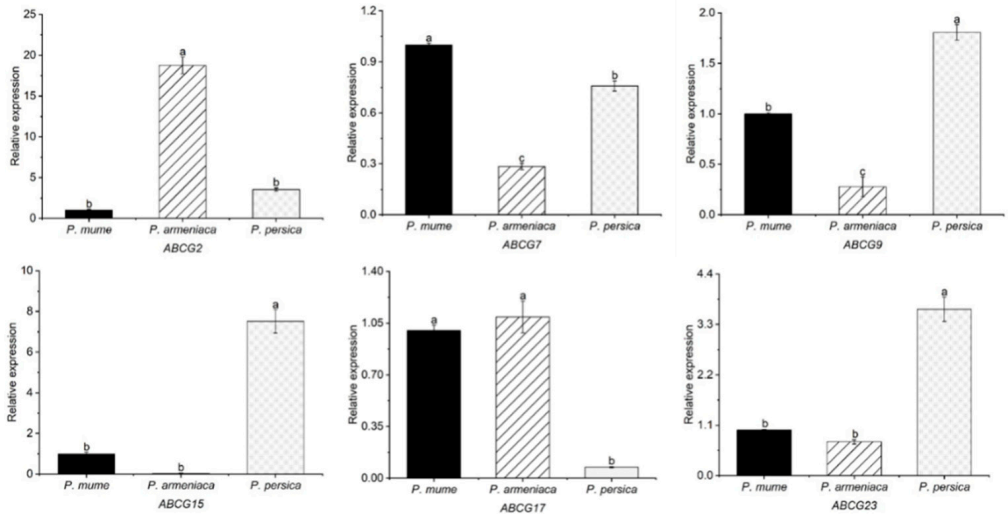

Figure 8. The expression analysis of the candidate *ABCG* genes in *P. mume*, *P. armeniaca*, and *P. persica* using qRT-PCR. Different lowercase letters indicate significant differences ($p < 0.05$) by Duncan's multiple range.

4. Discussion

Plant floral scents play important roles during growth and reproduction [35]. Plant floral scent diversity mainly depends on the number, composition, relative content, and spatiotemporal volatilization patterns of different VOCs, and they have significant interspecies variation [36]. The previous study found that there were significant differences in the floral scent profiles between *Buddleja fallowiana* and *B. officinalis*, and the main reasons for the inter-specific difference were seven floral scent compounds including benzaldehyde [37]. The current study showed that the considerable variation of the volatile components appeared in *P. mume*, *P. armeniaca*, and *P. persica*. Notably, benzaldehyde was detected in the flowers from all of the three species.

Benzaldehyde has a relatively primitive status in volatiles. Schiestl (2010) found that benzaldehyde presented in flowers from as many as 57 angiosperms, and its volatilization was strongly correlated with the pollination of insects in angiosperms [15]. Benzaldehyde is a characteristic component of the genus *Capsella*, but some species such as *C. rubella* lacked the volatilization ability of benzaldehyde due to evolution driven by geographical distribution differences [38]. Similarly, based on our findings, benzaldehyde was the common volatile component of *P. mume*, *P. armeniaca*, and *P. persica*, but benzyl acetate volatilized greatly in *P. mume*, while 3,5-dimethoxytoluene was the main volatile component in *P. persica*. El-Sayed (2018) reported that 3,5-dimethoxytoluene had a higher attracting efficiency to insects compared with benzaldehyde [39]. *P. armeniaca* and *P. persica* were mainly distributed in the north of China, and the flowering period of the three *Prunus* species was different, which indicated that they had adopted different evolutionary strategies based on the volatilization of benzaldehyde in response to environmental pollinators.

The endogenous contents of benzaldehyde were high in all tree species. Benzaldehyde was considered the best repellant for bees [40], and it was easily detected by hawk moths [41]. Floral scent component analysis demonstrated that the endogenous content of benzaldehyde was much higher than the volatilization content in the three species, indicating that the volatilized content of benzaldehyde was partly affected by the concentration of endogenous benzaldehyde. From similar research in the *Petunia*, ectopically expressed *PhABCG1* affected the internal concentration of the volatile benzenoid compound but not its release [42]. In addition, the *PhABCG1* transporter was involved in the transmembrane transport of benzenoid volatiles in *Petunia* [6]. It is speculated that the various volatilization efficiency of benzaldehyde in *P. mume*, *P. armeniaca*, and *P. persica* may be related to active transmembrane transport mediated by ABC transporters.

The evolutionary conserved ABC transporters are widely presented in plants, and they are important for plant development as well as interactions with the environment [13]. They are related to the transport of secondary metabolites in plants, especially ABCG transporters, which play an important role in the environmental adaptation for the terrestrial plants [43]. Creatures are able to increase the number of transporters through gene amplification events to meet the needs of substrate transport [44]. In our study, however, the number of *ABCG* subfamily genes of *P. mume*, *P. armeniaca*, and *P. persica* were 55, 53, and 58, respectively, indicating that they may not transport too many different substrates in a one-to-one relationship due to such a similar number of transporters. In the eukaryotic, ABCG transporters include full-molecule and half-molecule types [45], also the half-molecule *PhABCG1* from the *Petunia* effectively transported benzyl benzoate [6]. Furthermore, half-molecule transporters accommodate more substrates by binding into heterodimers [46]. In the present study, 17 half-molecular *WBC* genes were specifically expressed in flowers from three species, suggesting that these half-molecular transporters have adaptive changes in the types and the amounts of transported substrates in their respective species. In addition, the qRT-PCR results for *ABCG9* and *ABCG17* were inconsistent with transcriptome sequencing results, we speculate that it may be due to the nonspecific co-amplification of highly similar family genes. A similar problem was also reported in a study of poplars infected with leaf spot [47].

Regarding the key domains of ABC transporters, NBD evolution was relatively conservative, while TMD evolution showed a certain activity [48]. The structural changes of TMD were more related to the diversity of transport functions and the lineage expansion of transporter types [45]. Point mutations at TMD had additive effects, which reduced the restriction of substrate selection by transporters, thereby increasing their transport capacity to multiple substrates [49]. The WGCNA analysis in our study showed that *ABCG2*, *7*, *9*, *15*, and *17* were the key candidate genes for benzaldehyde volatilization; furthermore, based on the sequence analysis (Figure 5), the sequence similarity was closer between *P. mume* and *P. armeniaca*, which was farther with *P. persica*. Accordingly, compared with *P. mume* and *P. armeniaca*, the sequence of the corresponding *ABCG* gene in *P. persica* may be changed, leading to transporting other substances. For example, *P. persica* had stronger volatile ability of 3, 5-dimethoxy. *P. mume* and *P. armeniaca* were closely related [25], but we also noticed that *ABCG9* and *23* (Figure 5) showed higher sequence similarity between *P. persica* and *P. armeniaca*, partly because the corresponding *ABCG* of *P. mume* mutated after being separated from *P. armeniaca*. These findings provided valuable information for elucidating the special aromatic characteristics in *P. mume*.

5. Conclusions

P. mume, *P. armeniaca*, and *P. persica* are all famous woody flowers in *Prunus*, but their aromas were distinctly different. The analysis of floral scent components showed that the endogenous content of benzaldehyde was much higher than the volatile amount. Meanwhile, the benzaldehyde volatilization efficiency in *P. mume* and *P. armeniaca* was much higher than that of *P. persica*, which was consistent with the results of the collinear analysis that *P. mume* had a closer genetic relationship with *P. armeniaca* than with *P. persica*. We identified 130, 135, and 133 *ABC* family members in *P. mume*, *P. armeniaca*, and *P. persica*. Additionally, these *ABC* family genes were divided into 8 subfamilies, with 55, 53, and 58 belonging to the *ABCG* subfamily, respectively. Combined with the expression of *ABCG* genes in the three species and WGCNA analysis, our results indicated that *ABCG2*, *7*, *9*, *15*, *17*, and *23* were the key candidate genes. The expression profile of *ABCG17* was extremely consistent with the volatilization efficiency of benzaldehyde and it was subcellularly localized on the plasma membrane in all three flowers, indicating that it was largely involved in benzaldehyde transport. This study provides a perspective for elucidating the transmembrane transport of benzaldehyde.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8060475/s1, Table S1: Gene primer sequence, Table S2: Relative amounts of volatile compounds detected from *P.mume*, *P. armeniaca*, and *P. persica*, Table S3: Relative amounts of endogenous compounds extracted from *P.mume*, *P. armeniaca*, and *P. persica*, Table S4: Physicochemical properties and prediction of subcellular location in *P. mume*, Table S5: Physicochemical properties and prediction of subcellular location in *P. armeniaca*, Table S6: Physicochemical properties and prediction of subcellular location in *P. persica*; Figure S1: Chromatogram of volatile compounds from *P.mume*, *P. armeniaca*, and *P. persica*.

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