

Genome wide comparative analysis of the BAHD superfamily in seven Rosaceae species and expression analysis in pear (*Pyrus bretschneideri*)

Chunxin Liu

Nanjing Agricultural University

Xin Qiao

Nanjing Agricultural University

Qionghou Li

Nanjing Agricultural University

Weiwei Zeng

Nanjing Agricultural University

Shuwei Wei

Nanjing Agricultural University

Xin Wang

Nanjing Agricultural University

Yangyang Chen

Nanjing Agricultural University

Xiao Wu

Nanjing Agricultural University

Jun Wu

Nanjing Agricultural University

Hao Yin (✉ yinhao@njau.edu.cn)

Nanjing Agricultural University

Shaoling Zhang

Nanjing Agricultural University

Research article

Keywords: BAHD, pear, evolution, Rosaceae, transcriptome, volatile esters

Posted Date: August 21st, 2019

DOI: <https://doi.org/10.21203/rs.2.13258/v1>

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Version of Record: A version of this preprint was published on January 8th, 2020. See the published version at <https://doi.org/10.1186/s12870-019-2230-z>.

Abstract

Background: The BAHD acyltransferase superfamily exhibits various biological roles in plants, including regulation the fruit quality; catalytic synthesis of terpene, phenolic and esters; improvement of stress resistance. However, the copy number, evolutionary history and potential functions of the BAHD superfamily genes in the genome sequenced Rosaceae species remains unclear. **Results:** Totally, 755 BAHD genes were obtained from the genomes of seven Rosaceae fruit species (*Pyrus bretschneideri*, *Malus domestica*, *Prunus avium*, *Prunus persica*, *Fragaria vesca*, *Pyrus communis* and *Rubus occidentalis*). Based on the classification results from model plants, we divided the BAHD family genes into seven subgroups (I-a, I-b, II-a, II-b, III-a, IV, V). Based on intra-species synteny analysis, 61 syntenic gene pairs were detected from the six Rosaceae species. Dispersed gene duplication occurred frequently in all investigated species. Different modes of duplicated gene pairs identified in each investigated species show that the K_a/K_s is less than one, indicating they evolved through purifying selection. Based on the correlation analysis between ester content and expression level of BAHD genes at different development stages, we selected five genes to perform qRT-PCR verification, and the results showed that Pbr020016.1, Pbr019034.1, Pbr014028.1 and Pbr029551.1 are the important candidate genes involved in aroma formation during pear fruit development. **Conclusion:** We have thoroughly annotated the BAHD superfamily genes and made a comprehensive comparative analysis of their colinearity, phylogenetic relationships and gene duplication patterns in the seven Rosaceae species, and also obtained four candidate genes might be involved in the aroma synthesis in the pear fruit. These presented results provide a theoretical basis for the future studies of the specific biological functions of BAHD superfamily members and the improvement of pear fruit quality. **Keywords:** BAHD, pear, evolution, Rosaceae, transcriptome, volatile esters

Background

In the process of plants growing, they can produce not only common carbohydrates, fats and proteins, but also a multitude of secondary metabolites, such as phenolics, terpenoids, alkaloids, and miscellaneous compounds [1]. The diversity of these compounds is catalyzed hydroxylation, decarboxylation, glycosylation, oxidation/reduction, acylation and methylation by expounding the basic skeleton and modifying enzymes [2]. These secondary metabolites have many significant effects. Phenolic compounds can be recognized as the most important dietary antioxidants, they have achieved remarkable results in preventing various oxidative stress associated diseases [3]. The volatile chemicals disperse at ambient temperatures playing different roles in organisms [4]. These secondary metabolites have long been used as flavor compounds in spices (fragrance) and preservatives in foods [5]. The *BAHD* (first letter of benzylalcohol O-acetyl transferase (BEAT), anthocyanin O-hydroxycinnamoyl transferases (AHCTs), N-hydroxycinnamoyl anthranilate benzoyl transferase (HCBT) and deacetylvinadoline 4-O-acetyltransferase (DAT) [8]) superfamily is composed of a large number of enzymes with two common domains (HXXXD and DFGWG) and similar amino acid sequences [6]. The previous discovery of a *BAHD* superfamily member showed that the HXXXD motif region was located in the reaction channel center and participating in catalysis. Meanwhile, as an indispensable motif for the reaction, the DFGWG motif is far from the active site [7]. Studies showed that BEAT from the California wildflower (*Clarkia breweri*) can produce the floral volatile benzylacetate [9, 10], DAT from *Catharanthus roseus* is related to the alkaloid vindoline synthesis [9, 11], HCBT from *Dianthus caryophyllus* is responsible for producing a class of phytoalexins known as anthramides [12], and AHCTs from *Gentiana triflora* can catalyze anthocyanin synthesis [13, 14]. *BAHD* superfamily genes have been reported play various roles during plant growth, such as regulation of fruit quality, improvement of stress resistance, catalytic synthesis of plant phenolic compounds, anthocyanin, lignin and volatile esters [15–17]. In addition, several genes of this superfamily play vital roles in the synthesis of morphine, taxol and vindoline, which are precursors to anti-cancer drugs (vincleucoblastine and vincristine) [18]. For example, Alcohol acyltransferase (*AAT*) genes of *BAHD* superfamily play variety roles in plant secondary metabolites synthesis, such as volatile esters, anthocyanins and terpenes [19]. Furthermore, *AAT* has been widely reported to be associated with the production of plant aroma compounds in apple, kiwi, and strawberry [20–23].

Pear is an important temperate Rosaceae fruit tree worldwide. According to historical records, pears originated from 55 to 65 million years ago, and their cultivation history can be traced back to more than 30,000 years [24]. In the year 2012, the

genome sequence of pear ('Dangshan suli', *Pyrusbretschneideri*) was released, providing a wealth of data resource for study in omics and molecular biology in pear. At present, genome sequences of six other Rosaceae fruit species are also available, leveraging the study in comparative genomics among Rosaceae species. In this study, we aim to identity all the *BAHD* superfamily members in genomes of *P. bretschneideri* and six other Rosaceae species. To comprehensively identify and elaborate the *BAHD* superfamily, and unravel their functional roles in plant development, especially in fruit aroma biosynthesis, we investigated the patterns of evolution and expression of *BAHD* superfamily genes using genome and transcriptome resources of seven species. Gene structure, conserved motifs, phylogeny, selection pressure, spatiotemporal expression profiles of *BAHD* genes were analyzed in this study. Furthermore, we verified gene expression patterns by qRT-PCR analysis, and several candidate *BAHD* genes which are closely related to pear volatile esters content and belong to Clade I and Clade IV were identified. Above all, these results will provide insight into the evolution and expansion of *BAHD* genes and further studying molecular function of *BAHD* superfamily in these species.

Results

Identification of *BAHD* genes in seven Rosaceae species

The *BAHD* superfamily characteristic domain (Pfam: PF02458) and the *BAHD* HMM configuration file (PF02458) were used as candidate genes to identify the *BAHD* members by the Hidden Markov model. The online site SMART (<http://smart.embl-heidelberg.de/>) was used to analysis protein sequences of candidate genes, and to determine the domain PF02458 of *BAHD*. We obtained 822 *BAHD* family candidate genes based on above analysis. Furthermore, multiple sequence alignment was conducted to verify the presence of two characteristic conserved domains (HXXXD and DFGWG) of *BAHD* family genes [6]. Six genes were removed from Chinese white pear due to the absence of two domains, while 17 in European pear, seven in woodland strawberry, eight in apple, 20 in sweet cherry, three in peach, and six in black raspberry. Finally, 114 *BAHD* genes were identified in *Pyrusbretschneideri* (the Chinese white pear), while 135 in *Pyrus communis* (European pear), 141 in *Malus domestica* (apple), 89 in *Fragaria vesca* (woodland strawberry), 125 in *Prunus avium*sweet cherry), 82 in *Prunus persica*peach), 69 in *Rubus occidentalis*black raspberry) (Table 1). Detailed information of gene features of *BAHD* genes can be retrieved (Additional file 2: Table S1).

Table 1 Genome information and *BAHD* genes number identified in Rosaceae species

Common name	Scientific name	Chromosome number	Release version	Genome gene number	Identified <i>BAHD</i> genes	Gene name prefix
Chinese white pear	<i>Pyrus bretschneideri</i>	34	NJAU v1.1	42,341	114(120)	Pbr
Apple	<i>Malus domestica</i>	34	JGI, v1.1	63,541	141(149)	Mdo
Strawberry	<i>Fragaria vesca</i>	14	GDR, v4.0	32,831	89(96)	Fve
European pear	<i>Pyrus communis</i>	34	GDR, v1.1	43,419	135(152)	pcp
Sweet cherry	<i>Prunus avium</i>	16	GDR, v1.0	43,679	125(145)	Pav
Peach	<i>Prunus persica</i>	16	JGI, v1.1	27,864	82(85)	Ppa
Black raspberry	<i>Rubus occidentalis</i>	14	GDR, v3.0	33,286	69(75)	Roc

The database address were listed below: NJAU (<http://peargenome.njau.edu.cn/>); GDR (<http://www.rosaceae.org/>); JGI (<http://www.jgi.doe.gov/>); The numbers in parentheses show the count of genes before filtering for unanchored and missing conserved domain genes.

Phylogenetic analysis and conserved motifs analysis of BAHD genes in Chinese white pear

BAHD amino acid sequences from Chinese white pear, European pear, *Arabidopsis* and *Populus* were used to construct a phylogenetic tree. The neighbor-joining phylogeny of *BAHD* from European pear was also constructed. (Additional file 1: Figure S1). The Chinese white pear *BAHD* family protein genes were classified as five clades (I, II, III-a, IV, V) according to the classification results from model plants (Fig.1). Clade I consist of two subclades (Clade I-a and Clade I-b). This result is consistent with that reported by Yu et al (2009). The *Arabidopsis* genes belong to Clade I-a have been found to be involved in modifying aromatic- and aliphatic alcohols in *Arabidopsis* and *Populus* Yu et al (2009) [25, 26], mirroring the function of *BAHD* genes in Chinese white pear. The members of Clade I-b are related to lignin monomeric intermediates biosynthesis [27, 28], such as tobacco and *Arabidopsis* shikimate hydroxycinnamoyltransferases [29]. Clade II consist of two subclades (Clade II-a and Clade II-b). The function of *Arabidopsis* genes belong to Clade II-a is unknown. Clade II-b contains 2 *Arabidopsis* genes, *AT3G29590.1* (*At5MAT*) and *AT1G03940.1* (*At3AT1*), which are associated with anthocyanin biosynthesis [30, 31]. Clade III-a contains less members, including four genes from Chinese white pear, three genes from *Arabidopsis*, and one gene from poplar. Clade V contains nine members, including one well-studied *Arabidopsis* genes *AT4G24510.1* (CER2) involved in regulating the cuticular wax biosynthesis [32]. Clade IV contains many members which are involved in catalyzing the acetylation of aromatic alcohols and acetylating small- or medium-chain alcohols (Yu et al). Above all, only three and four members were clustered in Clade III-a and Clade V. However, about 28.1% (32 of 114) *BAHD* genes were involved in Clade I and 36.0% (41 of 114) were involved in Clade IV, respectively. *BAHD* genes closely related to pear volatile esters content, such as alcohol acyltransferase (*AAT*), may be belong to Clade I and Clade IV. We detected 20 conserved motifs of the Chinese white pear using the online software MEME (Fig.2). All *BAHD* family members have motif1 or motif3 and about 65.8% (75 of 114) members contain both of them. Based on gene structure analysis, we found that motif1 and motif3 correspond to domains HXXXD and DFGWG, respectively. And motif sequences were CGGFAIGLSMSHKVADGSSLSTFINSWAE and FYEADFGWGKP, respectively. Members of the I-a, I-b, II-a, II-b, III-a, and V subfamilies do not contain motif17 except for *Pbr005916.1*; members of the I-a, I-b, II-a, II-b, III-a, and V subfamilies do not contain motif19 except for *Pbr010925.1*; Except for *Pbr005746.1*, *Pbr014025.1*, *Pbr035166.1* and *Pbr036245.1*, members of IV subfamilies do not contain motif10. Motif14 and motif16 were only detected in the Clade II-b. The type and distribution of the conservative motifs of the same subfamily are similar, further supporting the evolutionary tree classification (Fig.2). Related information of conservative motifs is shown in Additional file 3: Table S2.

Gene duplication events identified in pear *BAHD* superfamily and *BAHD* collinearity analysis in the seven Rosaceae species

Different patterns of gene replication have jointly promoted the evolution of the *BAHD* family, including Whole-genome duplication (WGD) or segmental duplication, tandem duplication (TD), proximal duplication (PD), transposed duplication (TRD), dispersed duplication (DSD) [33, 34]. We use DupGen_finder software [35] to detect duplicated gene pairs among *BAHD* family genes for seven Rosaceae genomes respectively. All the member of *BAHD* gene family was assigned to relative types: WGD, PD, TD, TRD or DSD. The number of WGD duplications in Chinese white pear and apple were 29 and 59, but only three in strawberry and peach, and four in black raspberry and sweet cherry, and nine in European pear. The number of dispersed duplications in Chinese white pear and sweet cherry were 113 and 142, and 91 of *BAHDs* in strawberry and 81 of *BAHDs* in apple; the number is higher than those in European Pear (29) and peach (76) and black raspberry (70). Genomic rearrangements and gene loss may lead to the large proportion of dispersed duplicates in these species, moreover, the RNA- and DNA-based transposable gene duplication can also result in this [33]. WGD and dispersed duplication made a crucial impact in the evolution of the *BAHD* superfamily in Chinese white pear, apple and European pear (Additional file 4: Table S3). In peach and strawberry tandem and dispersed duplication is the main force; proximal and dispersed duplication played major roles in black raspberry and sweet cherry. In pear, about 57.1% (113 of 198) *BAHD* genes were involved in dispersed gene duplication, while 66.9% (91 of 136) in woodland strawberry, 44.0% (81 of 184) in apple, 60.3% (76 of 126) in peach,

67.3% (70 of 104) in black raspberry, 66.4% (142 of 214) in sweet cherry and 52.7% (29 of 55) in European Pear, respectively (Fig.3). The result indicated that the dispersed duplication is ubiquitous in all investigated species.

In addition, we identified intra-genomic synteny blocks for each species [33]. As shown in Fig.4a, *BAHD* genes of Chinese white pear were distributed on 17 chromosomes with a random distribution; there is only one gene on chromosome 13. Similarly to that in Chinese white pear, the *BAHD* genes were detected randomly distributed in the other species. We found a total of 61 syntenic gene pairs from six Rosaceae species. Among them, 21 syntenic pairs were identified in Chinese white pear (Fig.4a), 29 pairs in apple (Fig.4b), compared only three in strawberry (Fig.4f), peach (Fig.4d), black raspberry (Fig.4e) and sweet cherry (Fig.4c) (Additional file 5: Table S4).

Ka, Ks, and Ka/Ks analysis for *BAHD* family genes

The stage of evolution for the WGD is usually estimated using Ks (synonymous substitutions per site) [36–38]. In addition to the original WGD ($Ks \sim 1.5-1.8$, ~ 140 Mya) (denoted asypaleohexaploidization event) that shared by core eudicots [39], a more recent WGD was detected in pear, and dated to 30–45 Mya ($Ks \sim 0.15$ to 0.3) [24]. As shown in Supplementary file S5, Ks values of WGD-derived gene pairs in Chinese white pear ranged from 0.006 to 3.909, the range of Ks values for gene pairs derived from TD, PD, TRD and DSD are 0.001~4.247, 0.07~3.670, 0.029~4.381 and 0.005~5.066, respectively. Similar results were found in apple. In Chinese white pear, there are nine WGD-derived genes pairs with Ks values ranged from 0.15 to 0.30, demonstrating that they may be derived from the current WGD (30–45 MYA) [24]. Some other duplicated gene pairs possessed higher Ks values (1.992~3.909), implying that they have probably originated from more ancient duplication events. We also found that the Ks values of the WGD-derived gene pairs in black raspberry is 1.356~2.965, in European pear is 0.153~4.362, in peach is 1.416~4.357, and in sweet cherry is 1.469~4.210. The higher Ks values of WGD-derived gene pairs in peach, black raspberry and sweet cherry suggest that they were duplicated and retained from more ancient genome duplication events, supporting the absence of more recent WGD events in these species.

Deleterious mutations can be removed by negative selection (purifying selection). Conversely, new favorable mutations could be accumulated by positive selection (Darwinian selection) and spread through the Population [40]. To detect the selection pressure acting on *BAHD* genes, we analyzed the nonsynonymous substitutions value (Ka) and nonsynonymous/synonymous substitution ratio (Ka/Ks) in the seven Rosaceae species (Additional file 6: Table S5). The direction and magnitude of selection pressure were inferred based on Ka/Ks ratio ($Ka/Ks > 1$: positive selection; $Ka/Ks = 1$: neutral evolution; and $Ka/Ks < 1$: purifying selection) [41]. The results showed that Ka/Ks values of all *BAHD* gene pairs of strawberry (Fig.5d), peach (Fig.5c) and European pear (Fig.5b) were less than one, indicating that these genes evolved through dramatic purifying selection (Fig.5). Similar results in the other four Rosaceae species (the Chinese pear (Fig.5f), sweet cherry (Fig.5a), black raspberry (Fig.5g) and apple (Fig.5e)), except for a few gene pairs with Ka/Ks values greater than one. It also can be seen from the box plot that these data distributions are more concentrated, especially in Chinese white pear, sweet cherry and apple.

Expression pattern of *BAHD* genes in Chinese white pear

Based on transcriptome data (Additional file 7: Table S6) from different pear tissues, we found that most genes in Chinese white pear expressed higher in root (Fig.6) and we discovered that 37 of the *BAHD* genes were expressed in all four stages of fruit development; *Pbr014238.1* only expressed in the four periods of pollen tube; *Pbr020016.1*, *Pbr027303.1*, *Pbr029551.1*, *Pbr014028.1* and *Pbr006821.1* were highly expressed in the late stage of fruit development (Fruit_S4); Most members of the *BAHD* superfamily showed no expression during the four periods of the pollen tube.

Gene expression analyses with qRT-PCR

Reference to transcriptome expression profiles and the ester content analysis, we selected five potential Chinese white pear genes (*Pbr020016.1*, *Pbr019034.1*, *Pbr014028.1*, *Pbr006821.1* and *Pbr029551.1*) that show strong correlation with content change of ester during fruit development. We used qRT-PCR to examine aforementioned candidate genes. In our study, we found that the individual genes' expression patterns were highly correlated with the ester content changes during pear fruit development (Fig.7). The expression pattern of *Pbr020016.1* (Fig.7a) rised first and then decreased and attained the maximum at the last stage. *Pbr006821.1* (Fig.7e), *Pbr014028.1* (Fig.7b), *Pbr019034.1* (Fig.7c) and *Pbr029551.1* (Fig.7d) showed similar expression pattern, they all reached the lowest value in the second period and then began to increase. *Pbr006821.1* had a slow increase in regulation from S2 (75DAF) to S4 (145DAF). Conversely, *Pbr014028.1*, *Pbr019034.1* and *Pbr029551.1* had a sharp increase from S2 to S4. The expression peak of *Pbr006821.1* emerged in the first stage; and the overall trend of *Pbr020016.1*, *Pbr029551.1* and *Pbr019034.1* is increased, so we speculate that they may play a role during fruit ripening.

Discussion

The *BAHD* superfamily is combined with various ester Synthetic pathways. Various reports have demonstrated the functions of several *BAHD* gene members [42–44]. When conducting this study, by detecting their HXXXD and DFGWG motifs through HMMER search, we identified 755 *BAHD* genes from these seven species. Similar to the results of *BAHD* genes classification in *Arabidopsis* [26], *BAHD* family protein genes of pear were classified as five clades (I, II, III-a, IV, V). In addition, conserved motif analysis with these *BAHD* amino acid sequences showed that the type and distribution of the conservative motifs of the same subfamily are similar, indicating that the classification results are authentic. All *BAHD* family members have motif1 (HXXXD) or motif3 (DFGWG), about 65.8% (75 of 114) members contain both of them. Hence, we conjectured that the motifs (1 and 3) may be related to the function of catalytic activity. A previous study proved that the HXXXD catalytic motif is located at the solvent channel, and it can also deprotonize oxygen or nitrogen atoms on the specific receptor substrate [6, 9], and the DFGWG motif is essential to the reaction but located far from the active site [6, 9, 18, 45, 46]. Combining the phylogenetic tree classification with the results of previous studies, we speculate that the *BAHD* genes which are closely related to pear volatile esters content, such as alcohol acyltransferase (*AAT*) were belong to Clade I and Clade IV and *AAT* are involved in the synthesis of esters and it closely related to the aroma of flowers and fruits [47]. At present, many *AAT* genes in fruit trees have been identified with clear functions. Such as *PpAAT1* in peach[48]; *FaAAT2* and *SAAT* in cultivated strawberry[22, 23]; *MpAAT1* in apple[21]; *CmAAT1,3,4* in melon [49, 50] et al.

Gene duplication patterns can be generally divided into five types, including WGD, PD, TD, DSD, TRD [33, 34]. Each pattern of gene duplication (tandem, tetraploid, segmental, transpositional) contributes differentially to gene family expansion [51]. It is estimated that 90% of the gene increase in *Arabidopsis thaliana* pedigree is due to genome duplications [52]. And the gene duplication type of WGD, TD and DSD, are main feature of eukaryotic genome evolution and mainly driving the development of new functions in the genome and genetic evolution systems [53, 54]. Gene families, such as *SWEET* and *HCT* (Hydroxycinnamoyl Transferase) families expand primarily through the type of WGD and DSD [55, 56]. TD was the major force to expand *AP2/ERF* and *WRKY* gene family [57, 58]. Two gene families, *ALMT* (Aluminum-activated malate transporters) and *Hsf* (Heat shock transcription factors) may be more easily amplified by WGD and DSD [38, 33]. *BAHD* number of genes identified in seven kinds of plants in the rose family is different because the recent WGD events and the ancient polyploid events. In this study, the results indicated that DSD and WGD were the main force that extend the *BAHD* genes in apple, European Pear and Chinese white pear, and the number of *BAHD* genes that have undergone WGD and DSD was the largest but the number of genes undergoing other replication modes is relatively small. Besides, because peach, sweet cherry, strawberry and sweet cherry have not undergo the current WGD replication events so that the number of DSD accounts for a large proportion. Accordingly, WGD replication patterns account for a large proportion in apples and pears, perhaps because they have experienced two rounds of genome replication [40]. In addition, according to the *Ka*, *Ks*, *Ka/Ks* analysis, we discovered that in peach, black raspberry and sweet cherry the *Ks* values of WGD-derived gene pairs were bigger than 1.3 suggest that they were duplicated and retained from more ancient genome duplication events, further supporting the

absence of more recent WGD events in these species. We also found that all *BAHD* gene pairs of strawberry, peach and European pear had $Ka/Ks < 1$, indicating that they have experienced strong purifying selection.

The diversity of the biochemical function of the *BAHD* genes has been determined in many species. For example, *BAHD* acyltransferase gene can enhance saccharification [43]; *BAHD* acyltransferase catalyzes the last step in cocaine biosynthesis [59]; *BAHD* acyltransferase protein plays an important role in plant innate immunity [60] et al. Furthermore, *AAT* genes' functions have been determined in several species. For instance, Dapeng Li et al. [61] discovered the *AAT* gene in apple, *MdAAT2*, may have a certain resistance to stress and can change the profile of the volatile compound. *VpAAT1* were reported involved in ester biosynthesis in Mountain papaya (*Vasconcellea pubescens*) [62]. Despite the diverse functions of the *BAHD* gene family, we were more concerned with its role in ester synthesis. Volatile esters produced by these alcohol acyltransferase (*AAT*) usually promote the recognition between plants and food because they contribute to the “fruity” aroma of edible fruits and some esters are also the key flavor or smell of a particular plant [20]. In this study, our RNA-seq data revealed that 37 *BAHD* genes were detected with various expression levels in four stages of pear fruit development. Surprisingly, though lacking motif3 (DFGWG), the *Pbr019034.1* and *Pbr020016.1* genes were both still expressed at all stages. Based on transcriptome expression profiles combined with the ester content analysis, five potential genes (*Pbr020016.1*, *Pbr019034.1*, *Pbr014028.1*, *Pbr006821.1* and *Pbr029551.1*) belong to Clade I and Clade IV that show strong correlation with content change of ester during fruit ripening for further qRT-PCR analysis. These results showed that the four genes of *Pbr020016.1*, *Pbr029551.1*, *Pbr019034.1*, and *Pbr014028.1* were basically consistent with the RNA-seq data and the changes in transcription levels. The peak of *Pbr006821.1* appeared in the first stage, and increased smoothly from S2 (75DAF) to S4 (145DAF), we speculate that *Pbr006821.1* may be related to the early ester synthesis of the fruit.

Conclusions

After analysis of this article, we identified 755 *BAHD* genes from seven species, among them, 114 were belong to Chinese white pear. And we divided the *BAHD* superfamily into five large clades according to the classification results from model plants. Dispersed gene duplication occurred frequently in all investigated species. All *BAHD* members of strawberry, peach and European pear were experience Purifying selection. Similar results in the other four Rosaceae species (the Chinese pear, sweet cherry, black raspberry and apple), except for a few gene pairs with Ka/Ks values greater than one. Finally, by analyzing the results of qRT-PCR, we speculate that *Pbr020016.1*, *Pbr029551.1*, *Pbr019034.1*, *Pbr014028.1* may be candidate genes related to ester synthesis. These results will provide a foundation for further study the molecular mechanism underlying aroma biosynthesis and release.

Methods

Source of Plants

Plant materials (the ‘Dangshan suli’ [24] fruit at four different developmental stages) were picked from the pear germplasm orchard of Center of Pear Engineering Technology Research located at Jiangpu of Nanjing. The fruit samples were collected on 26th May, 27th June, 28th July and 6th Sep in 2017. RNA-seq data as published previously was used to analyze the expression patterns of ‘Dangshan suli’ *BAHD* [63]. And all samples were ground with liquid nitrogen and stored at -80°C . The genome annotation files and genome sequences of pear were collected from our pear genome project (<http://peargenome.njau.edu.cn>), and the other four Rosaceae species sequences (*Fragaria vesca*, *Pyrus communis*, *Prunus avium* and *Rubus occidentalis*) were downloaded from the GDR (<http://www.rosaceae.org>). The sequence of apple (*Malus domestica*) and peach (*Prunus persica*) were downloaded from JGI (<http://www.jgi.doe.gov/>).

Sequence identification and collection

To identify putative *BAHD* genes from pear and the six other species, namely peach, apple, woodland strawberry, european pear, sweet cherry and black raspberry, several approaches were employed. Using *BAHD* family characteristic domain (Pfam: PF02458) as a query sequence, then, according to the HMM configuration file (PF02458) of *BAHD*, searching for candidate genes by the Hidden Markov model. We can download the HMM profile (PF02458) from the Pfam website (<https://pfam.xfam.org/>). Ultimately, we found 822 putative *BAHD* genes in the seven Species with an E-value < $1e^{-10}$. And then using the online site SMART (<http://smart.embl-heidelberg.de/>) to determine the domain PF02458 of *BAHD*, and then inspecting their HXXXD and DFGWG motifs, we use the online website MAFFT (<https://mafft.cbrc.jp/alignment/server/>) for multiple sequence alignments and download the comparison results to software GeneDoc, all members have both domains or have one of them, indicating that the results of screening and identification are reliable, 66 sequences were removed due to the lack of both domains. Ultimately, 755 *BAHD* genes were identified and analyzed.

Phylogenetic analysis

Amino acid sequence alignment was performed using Multiple Sequence Comparison with default parameters in MEGA7 [64]. The neighbor joining trees were constructed with bootstrap 1000 using MEGA7.

Conservative motif analysis of BAHD family members

The conservative motif was analyzed by the online software MEME (http://meme.sdsc.edu/meme4_3_0/intro.html), the maximum value of the motif was set to 20, and the motif length was set between 6 and 200.

Identification of gene duplication modes and collinearity analysis

Gene duplication analysis between seven Rosaceae genomes has been performed, and the method is carried out locally with reference to the method developed by PGDD (<http://chibba.agtec.uga.edu/duplication/>) [65]. First, the chromosomal location information of the *BAHD* gene family and related gene pairs information are obtained from the genome annotation files of each species, and then the localization and synteny of the *BAHD* genes were drawn using TB-tools software. MCScanX was further used to identify different duplication patterns in the *BAHD* superfamily [66, 67].

Calculating Ka and Ks

First, the MCScanX downstream analysis tool was used to calculate the Ka and Ks substitution rates of five different types of duplication gene pairs, and then the Ka and Ks values were calculated by the GMYN method. [68, 69].

Expression Analysis of BAHD in 15 samples of *Pyrus bretschneideri* and qRT-PCR.

RNA-seq data as published previously [63] was used to analyze the expression patterns of 'Dangshan suli' *BAHD*. These data include reads from 15 samples of 'Dangshan suli' including Fruit_S1, Fruit_S2, Fruit_S3, Fruit_S4, Root, Stem, Leaf, Bud, Petal, Sepal, Ovary, Mature pollen, Hydrated pollen, Pollen tube, Stop growth pollen. Then draw with TB-Tools.

Based on the correlation analysis of transcriptome data and ester content data of pear fruit during four developmental stages, we selected four possible *AAT* genes for qRT-PCR. The primers of all four possible genes were designed using Primer Premier 6.0; Total RNA was extracted by the Plant Total RNA Isolation Kit Plus (Fuji, China), and cDNA was synthesized using TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen, China). The composition PCR mixture under the following: 1ul of sense and anti-sense primer (10uM), 5ul fluorescent dye, 1ul template, and 3ul Sterilized water. qRT-PCR

was managed by LightCycler 480 SYBRGREEN I Master (Roche,USA). We ran the PCR reaction under the following conditions: 10 min at 95 °C, then 45 cycles of 95 °C for 3s, 60 °C for 10s, and 72 °C for 30 s. then using $2^{-\Delta\Delta ct}$ method to determine the relative expression and the reference gene is tubulin (Additional file 8: Table S7).

List Of Abbreviations

WGD: Whole-genome duplication; TD: Tandem duplication; PD: Proximal duplication; TRD: Transposed duplication; DSD: Dispersed duplication; AAT: Alcohol acyltransferases; MYA: Million years ago; HMM: Hidden Markov Model; NJ: Neighbor-joining. GDR: Genome Database for Rosaceae; JGI: Joint Genome Institute; NJAU: Nanjing Agricultural University.

Declarations

Acknowledgments

The authors would like to thank everyone who contributed to this article.

Funding

This study was supported by the National Key Research and Development Program [2018YFD1000200], the National Natural Science Foundation of China (31701890, 31601708), Fundamental Research Funds for the Central Universities (KJQN201818). These fundings provided the financial support to the research projects, but did not involved in project design, data collection, analysis, or preparation of the manuscript.

Authors' contributions

Conceived and designed the experiments: HY and SZ. Performed the experiments: CL, QL, XW, YC and JW. Analyzed the data: CL, QL, WZ, SW, HY and XQ. Wrote the paper: CL, XQ, HY and SZ. And all authors have read and approved the manuscript.

Ethics approval and consent to participate

All the pear materials used and analyzed for this study were collected from the Center of Pear Engineering Technology Research, Nanjing Agricultural University, which were public and available for non-commercial purpose. This article did not contain any studies with human participants or animals performed by any of the authors.

Availability of data and materials

The pear genome datasets used during the current study are available in our pear center website (<http://peargenome.njau.edu.cn/>), the other four Rosaceae species sequences (*Fragaria vesca*, *Pyrus communis*, *Prunus avium* and *Rubus occidentalis*) were downloaded from the GDR (<http://www.rosaceae.org>). The sequence of apple (*Malus domestica*) and peach (*Prunus persica*) were collected from JGI (<http://www.jgi.doe.gov/>).

Consent for publication

Not applicable.

Competing interests

References

1. Song CK, Härtl K, McGraphery K, Hoffmann T, et al. Attractive but toxic: Emerging roles of glycosidically bound volatiles and glycosyltransferases involved in their formation. *Mol Plant*. 2018.
2. Pichersky E, Noel JP, Dudareva N. Biosynthesis of plant volatiles: nature's diversity and ingenuity, *Science*. 2006, 311(5762): 808–811.
3. Legrand G, Delporte M, Khelifi C, Harant A, Vuylsteker C, et al. Identification and characterization of five BAHD acyltransferases involved in hydroxycinnamoyl ester metabolism in chicory. *Front Plant Sci*. 2016, 7: 741.
4. Dudareva N, Pichersky E, Gershenzon J. Biochemistry of plant volatiles. *Plant Physiol*. 2004, 135:1893–1902.
5. Schwab W, Davidovich-Rikanati R, Lewinsohn E. Biosynthesis of plant-derived flavor compounds. *Plant J*. 2008, 54:712–732.
6. Molina I, Kosma D. Role of HXXXD-motif/BAHD acyltransferases in the biosynthesis of extracellular lipids. *Plant Cell Rep*. 2015, 34(4): 587–601.
7. El-Sharkawy I, Manríquez D, Flores FB, Regad F, et al. Functional characterization of a melon alcohol acyl-transferase gene family involved in the biosynthesis of ester volatiles. Identification of the crucial role of a threonine residue for enzyme activity. *Plant Mol. Biol*. 2005, 59(2): 345–362.
8. St-Pierre B, Luca VD. Evolution of acyltransferase genes: origin and diversification of the BAHD superfamily of acyltransferases involved in secondary metabolism. *Phytochemistry*. Vol 3, 2000:285–315.
9. D'Auria JC. Acyltransferases in plants: a good time to be BAHD. *Curr. Opin. Plant Biol*. 2006, 9(3): 331–340.
10. Dudareva N, D'auria JC, Nam KH, Raguso RA, et al. Acetyl-CoA: benzylalcohol acetyltransferase—an enzyme involved in floral scent production in *Clarkia breweri*. *Plant J*. 1998, 14(3): 297–304.
11. St-Pierre B, Laflamme P, Alarco AM, Luca VD. The terminal O-acetyltransferase involved in vindoline biosynthesis defines a new class of proteins responsible for coenzyme A-dependent acyl transfer. *Plant J*. 1998, 14(6): 703–713.
12. Yang Q, Reinhard K, Schiltz E, Matern U. Characterization and heterologous expression of hydroxycinnamoyl/benzoyl-CoA: anthranilate N-hydroxycinnamoyl/benzoyltransferase from elicited cell cultures of carnation, *Dianthus caryophyllus* L. *Plant Mol. Biol*. 1997, 35(6): 777–789.
13. Fujiwara H, Tanaka Y, Yonekura-Sakakibara K, Fukuchi-Mizutani M, Nakao M, et al. cDNA cloning, gene expression and subcellular localization of anthocyanin 5-aromatic acyltransferase from *Gentiana triflora*. *Plant J*. 1998, 16(4): 421–431.
14. Fujiwara H, Tanaka Y, Fukui Y, Ashikari T, Yamaguchi M, Kusumi T. Purification and characterization of anthocyanin 3-aromatic acyltransferase from *Perilla frutescens*. *Plant Sci*. 1998, 137(1): 87–94.
15. Nevarez DM, Mengistu YA, Nawarathne IN, Walker KD. An N-aroylesterase of the BAHD superfamily has broad aroyl CoA specificity in vitro with analogues of N-dearoylpaclitaxel. *J. Am. Chem. Soc*. 2009, 131(16): 5994–6002.
16. Bontpart T, Cheynier V, Ageorges A, Terrier N. BAHD or SCPL acyltransferase? What a dilemma for acylation in the world of plant phenolic compounds. *New Phytol*. 2015, 208(3): 695–707.
17. Gonzalez M, Gaete-Eastman C, Valdenegro M, Figueroa CR, Fuentes L, Herrera R, et al. Aroma development during ripening of *Fragaria chiloensis* fruit and participation of an alcohol acyltransferase (FcAAT1) gene. *J. Agric. Food Chem*. 2009, 57(19): 9123–9132.
18. Ma X, Koepke J, Panjikar S, Fritzsche G, et al. Crystal structure of vinorine synthase, the first representative of the BAHD superfamily. *J. Biol. Chem*. 2005, 280(14): 13576–13583.
19. S. Srivastava, R. S. Sangwan, Analysis of *Artemisia annua* transcriptome for BAHD alcohol acyltransferase genes: Identification and diversity of expression in leaf, stem and root. *J. Plant Biochem. Biotechnol*. 2012, 21(1): 108–118.
20. Günther CS, Chervin C, Marsh KB, Newcomba RD, et al. Characterisation of two alcohol acyltransferases from kiwifruit (*Actinidia* spp.) reveals distinct substrate preferences. *Phytochemistry*. 2011, 72(8): 700–710.

21. Souleyre EJF, Greenwood DR, Friel EN, Karunairetnam S, et al. An alcohol acyl transferase from apple (cv. Royal Gala), MpAAT1, produces esters involved in apple fruit flavor. *FEBS J.* 2005, 272(12): 3132–3144.
22. Cumplido-Laso G, Medina-Puche L, Moyano E, Hoffmann T, et al. The fruit ripening-related gene FaAAT2 encodes an acyl transferase involved in strawberry aroma biogenesis. *J. Exp. Bot.* 2012, 63(11): 4275–4290.
23. Aharoni A, Keizer LCP, Bouwmeester HJ, Sun Z, Alvarez-Huerta M, et al. Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. *The Plant Cell.* 2000, 12(5): 647–661.
24. Wu J, Wang Z, Shi Z, et al. The genome of the pear (*Pyrus bretschneideri* Rehd.). *Genome Res.* 2013, 23(2): 396–408.
25. D'auria JC, Pichersky E, Schaub A, Hansel A, et al. Characterization of a BAHD acyltransferase responsible for producing the green leaf volatile (Z)-3-hexen-1-yl acetate in *Arabidopsis thaliana*. *Plant J.* 2007, 49(2): 194–207.
26. Yu X, Gou JY, Liu C. BAHD superfamily of acyl-CoA dependent acyltransferases in *Populus* and *Arabidopsis*: bioinformatics and gene expression. *Plant Mol.Biol.* 2009, 70(4): 421–442.
27. Hoffmann L, Maury S, Martz F, Geoffroy P, et al. Purification, cloning, and properties of an acyltransferase controlling shikimate and quinate ester intermediates in phenylpropanoid metabolism, *J. Biol. Chem.* 2003, 278(1): 95–103.
28. Niggeweg R, Michael AJ, Martin C. Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nat. Biotechnol.* 2004, 22(6): 746.
29. Grienenberger E, Besseau S, Geoffroy P, Debayle D et al. A BAHD acyltransferase is expressed in the tapetum of *Arabidopsis* anthers and is involved in the synthesis of hydroxycinnamoyl spermidines. *Plant J.* 2009, 58(2): 246–259.
30. Luo J, Nishiyama Y, Fuell C, Taguchi G, Elliott K, et al. Convergent evolution in the BAHD family of acyl transferases: identification and characterization of anthocyanin acyl transferases from *Arabidopsis thaliana*. *The Plant Journal. Plant J.* 2007, 50(4): 678–695.
31. D'Auria JC, Reichelt M, Luck K, Svatos A, Gershenzon J. Identification and characterization of the BAHD acyltransferase malonyl CoA: Anthocyanidin 5-O-glucoside-6 "-O-malonyltransferase (At5MAT) in *Arabidopsis thaliana*. *FEBS Lett.* 2007, 581(5): 872–878.
32. Xia Y, Nikolau BJ, Schnable PS. Developmental and hormonal regulation of the *Arabidopsis* CER2 gene that codes for a nuclear-localized protein required for the normal accumulation of cuticular waxes. *Plant Physiol.* 1997, 115(3): 925–937.
33. Qiao X, Li M, Li L, Yin H, Wu J, et al. Genome-wide identification and comparative analysis of the heat shock transcription factor family in Chinese white pear (*Pyrus bretschneideri*) and five other Rosaceae species. *BMC Plant Biol.* 2015, 15(1): 12.
34. Maher C, Stein L, Ware D. Evolution of *Arabidopsis* microRNA families through duplication events. *Genome Res.* 2006, 16(4): 510–519.
35. Qiao X, Li Q, Yin H, Qi K, Li L, et al. Gene duplication and evolution in recurring polyploidization–diploidization cycles in plants. *Genome Biol.* 2019, 20(1): 38.
36. Lemoine F, Lespinet O, Labedan B. Assessing the evolutionary rate of positional orthologous genes in prokaryotes using synteny data. *BMC Evol. Biol.* 2007, 7(1): 237.
37. Jun J, Mandoiu II, Nelson CE. Identification of mammalian orthologs using local synteny. *BMC Genomics.* 2009, 10(1): 630.
38. Xu L, Qiao X, Zhang M, et al. Genome-wide analysis of aluminum-activated malate transporter family genes in six rosaceae species, and expression analysis and functional characterization on malate accumulation in chinese white pear. *Plant Sci.* 2018, 274: 451–465.
39. Fawcett JA, Maere S, Van de Peer Y. Plants with double genomes might have had a better chance to survive the Cretaceous–Tertiary extinction event. *Proc. Natl. Acad. Sci.* 2009, 106(14): 5737–5742.
40. Starr TK, Jameson SC, Hogquist KA. Positive and negative selection of T cells. *Annu. Rev. Immunol.* 2003, 21(1): 139–176.
41. Yang Z. PAML 4: phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* 2007, 24(8): 1586–1591.

42. Li G, Jones KC, Eudes A, Pidatala VR, Sun J, et al. Overexpression of a rice BAHD acyltransferase gene in switchgrass (*Panicum virgatum* L.) enhances saccharification. *BMC Biotechnol.* 2018, 18(1): 54.
43. Zhang Z, Xu L. Arabidopsis brassinosteroid inactivator2 is a typical BAHD acyltransferase involved in brassinosteroid homeostasis. *J. Exp. Bot.* 2018, 69(8): 1925–1941.
44. de Souza WR, Pacheco TF, Duarte KE, Sampaio BL, et al. Silencing of a BAHD acyltransferase in sugarcane increases biomass digestibility. *Biotechnol. Biofuels.* 2019, 12(1): 111.
45. Unno H, Ichimaida F, Suzuki H, Takahashiet S, et al. Structural and mutational studies of anthocyanin malonyltransferases establish the features of BAHD enzyme catalysis. *J. Biol. Chem.* 2007, 282(21): 15812–15822.
46. Morales-Quintana L, Moya-León MA, Herrera R. Computational study enlightens the structural role of the alcohol acyltransferase DFGWG motif. *J. Mol. Model.* 2015, 21(8): 216.
47. Defilippi BG, Kader AA, Dandekar AM. Apple aroma: alcohol acyltransferase, a rate limiting step for ester biosynthesis, is regulated by ethylene. *Plant Sci.* 2005, 168(5): 1199–1210.
48. Cao X, Xie K, Duan W, Zhu Y, Liu M, et al. Peach carboxylesterase PpCXE1 is associated with catabolism of volatile esters. *J. Agric. Food Chem.* 2019.
49. Iijima Y. Metabolic Factory for Flavors in Fruits and Vegetables. *Handb. Fruit Veg. Flavors.* 2010: 705.
50. Lucchetta L, Manriquez D, El-Sharkawy I, et al. Biochemical and catalytic properties of three recombinant alcohol acyltransferases of melon. Sulfur-containing ester formation, regulatory role of CoA-SH in activity, and sequence elements conferring substrate preference. *J. Agric. Food Chem.* 2007, 55(13): 5213–5220.
51. Freeling M. Bias in plant gene content following different sorts of duplication: tandem, whole-genome, segmental, or by transposition. *Annu. Rev. Plant Biol.* 2009, 60: 433–453.
52. Maere S, De Bodt S, Raes J, Casneuf T, et al. Modeling gene and genome duplications in eukaryotes. *Proc. Natl. Acad. Sci.* 2005, 102(15): 5454–5459.
53. Friedman R, Hughes AL. Pattern and timing of gene duplication in animal genomes. *Genome Res.* 2001, 11(11): 1842–1847.
54. Moore RC, Purugganan MD. The early stages of duplicate gene evolution. *Proc. Natl. Acad. Sci.* 2003, 100(26): 15682–15687.
55. Li J, Qin M, Qiao X, Cheng Y, Li X, et al. A new insight into the evolution and functional divergence of SWEET transporters in Chinese white pear (*Pyrus bretschneideri*). *Plant Cell Physiol.* 2017, 58(4): 839–850.
56. Ma C, Zhang H, Li J, Tao S, Qiao X, et al. Genome-wide analysis and characterization of molecular evolution of the HCT gene family in pear (*Pyrus bretschneideri*). *Plant Syst. Evol.* 2017, 303(1): 71–90.
57. Du D, Hao R, Cheng T, Pan H, Yang W, Wang J, et al. Genome-wide analysis of the AP2/ERF gene family in *Prunus mume*. *Plant Mol. Biol. Rep.* 2013, 31(3): 741–750.
58. Guo C, Guo R, Xu X, Gao M, Li X, et al. Evolution and expression analysis of the grape (*Vitis vinifera* L.) WRKY gene family. *J. Exp. Bot.* 2014, 65(6): 1513–1528.
59. Schmidt GW, Jirschitzka J, Porta T, Porta T, Reichelt M, et al. The last step in cocaine biosynthesis is catalyzed by a BAHD acyltransferase. *Plant Physiol.* 2015, 167(1): 89–101.
60. Zheng Z, Qualley A, Fan B, Dudareva N, Chen Z. An important role of a BAHD acyl transferase-like protein in plant innate immunity. *Plant J.* 2009, 57(6): 1040–1053.
61. Li D, Shen J, Wu T, Xu Y, Zong X, et al. Overexpression of the apple alcohol acyltransferase gene alters the profile of volatile blends in transgenic tobacco leaves. *Physiol. Plant.* 2008, 134(3): 394–402.
62. Balbontin C, Gaete-Eastman C, Fuentes L, Figueroa CR, Herrera R, Manriquez D, et al. Moya-León, VpAAT1, a gene encoding an alcohol acyltransferase, is involved in ester biosynthesis during ripening of mountain papaya fruit, *J. Agric. Food Chem.* 2010, 58(8): 5114–5121.

63. Li Q, Qiao X, Yin H, et al. Unbiased subgenome evolution following a recent whole-genome duplication in pear (*Pyrus bretschneideri* Rehd.). *Hortic. Res.-England*. 2019, 6(1): 34.
64. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 2016, 33(7): 1870–1874.
65. Lee TH, Tang H, Wang X, Paterson AH. PGDD: a database of gene and genome duplication in plants. *Nucleic Acids Res.* 2012, 41(D1): D1152-D1158.
66. Tang H, Wang X, Bowers JE, Ming R, et al. Unraveling ancient hexaploidy through multiply-aligned angiosperm gene maps. *Genome Res.* 2008, 18(12): 1944–1954.
67. Wang F, Dong Q, Jiang H, Zhu S, Chen B. Genome-wide analysis of the heat shock transcription factors in *Populus trichocarpa* and *Medicago truncatula*. *Mol. Biol. Rep.* 2012, 39(2): 1877–1886.
68. Nei M, T. Gojobori, Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions, *Mol. Biol. Evol.* 1986, 3(5): 418–426.
69. Wang D, Zhang Y, Zhang Z, Zhu J, et al. KaKs_Calculator 2.0: a toolkit incorporating gamma-series methods and sliding window strategies. *Genomics, Proteomics Bioinf.* 2010, 8(1): 77–80.

Figures

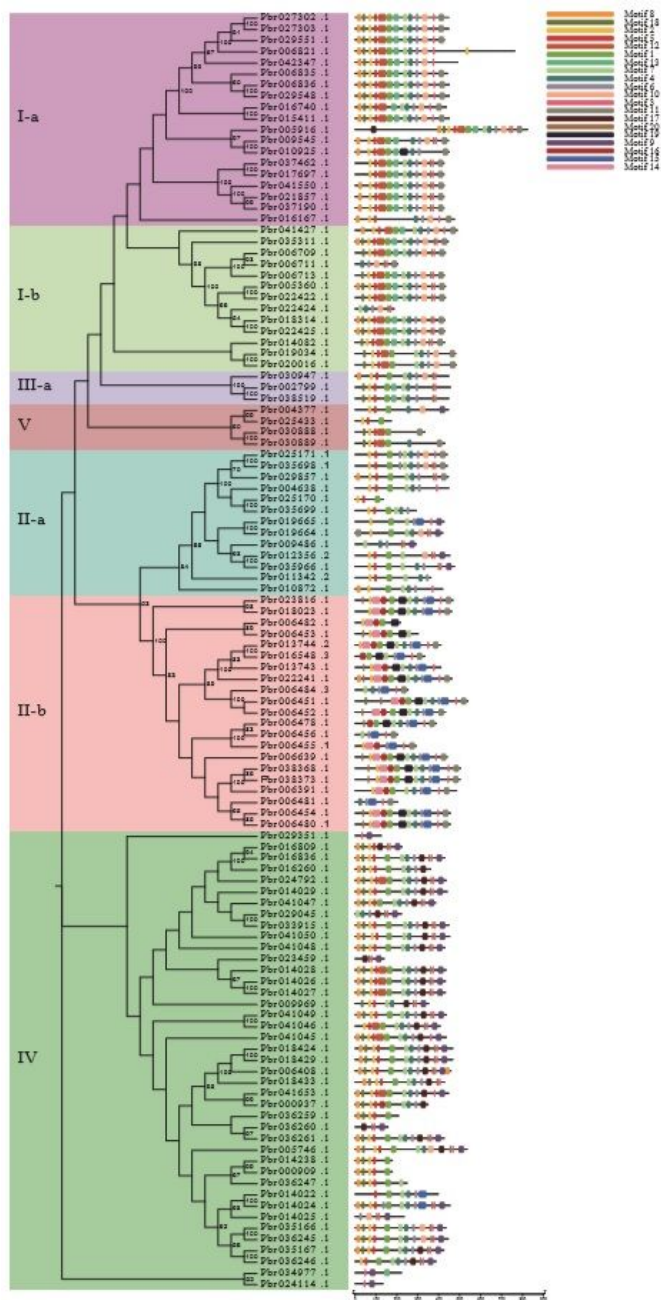


Figure 2

Fig. 2 Phylogeny and conserved motifs of BAHD genes in pear. Using MEME tools to found motifs, different colors were represent different motifs.

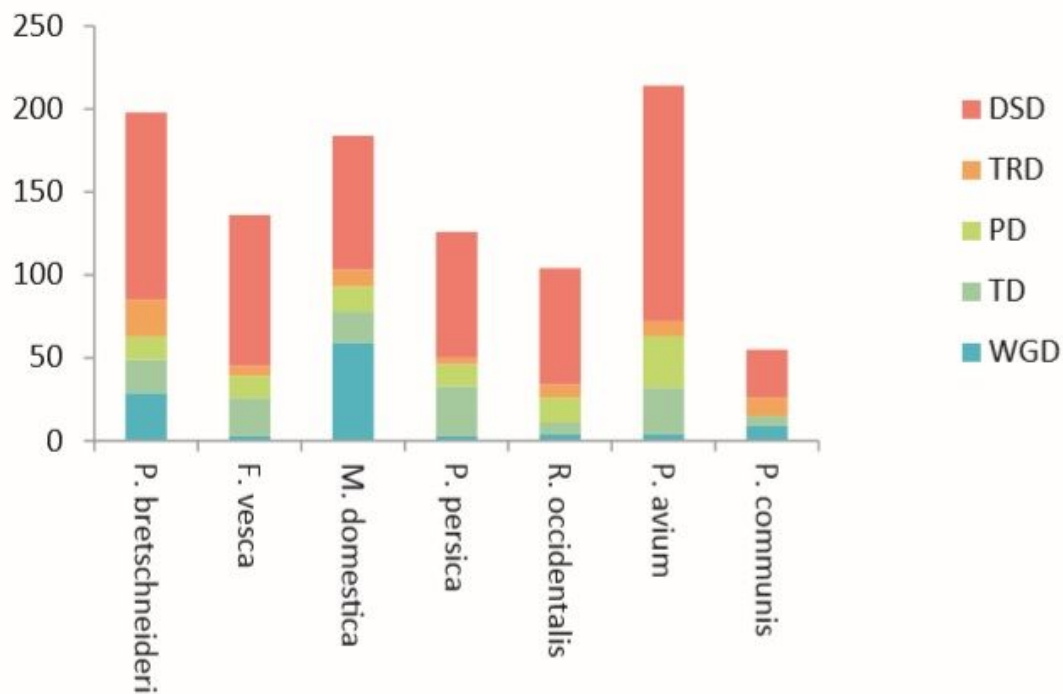


Figure 3

Fig. 3 Different modes of gene duplication in BAHD families. The x-axis represents the species. The y-axis represents the number of duplicated gene pairs.

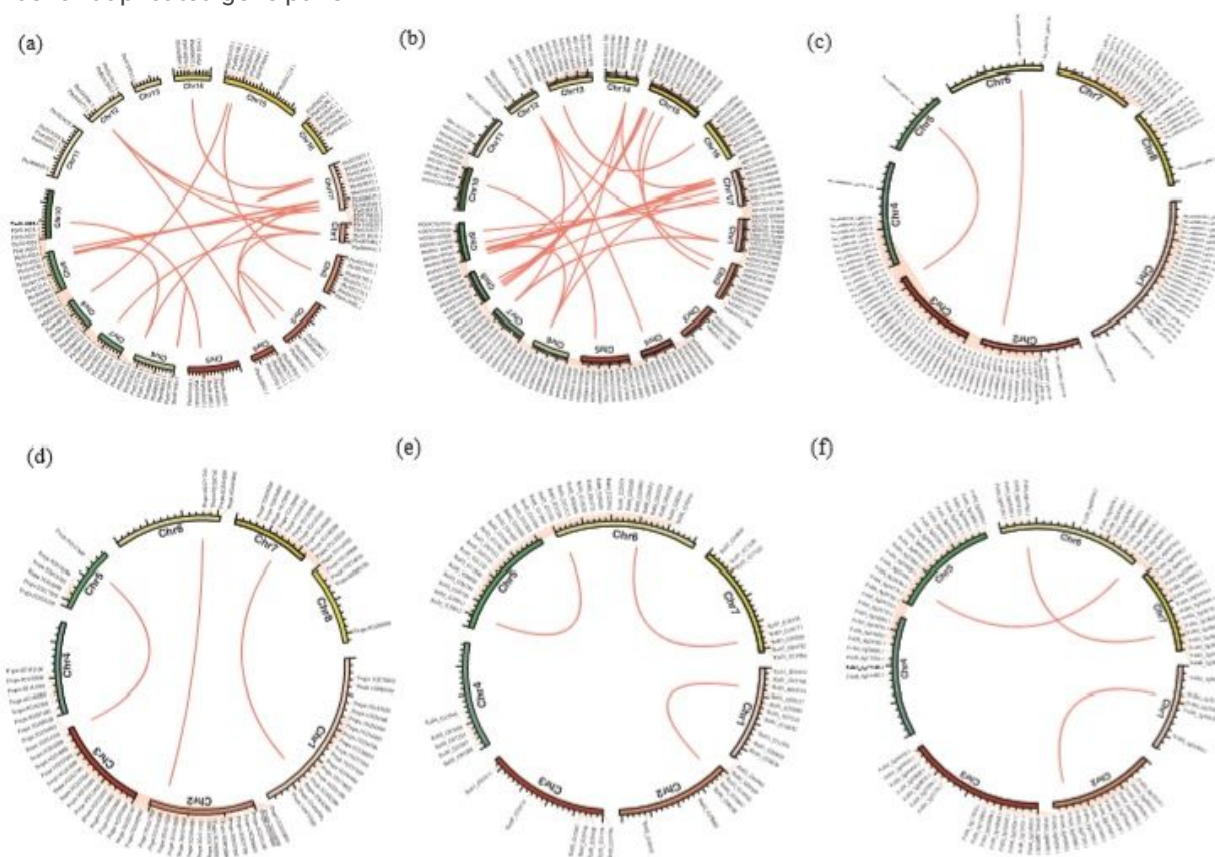


Figure 4

Fig. 4 Gene Localization and collinearity analysis of BAHD gene family . (a): Chinese white pear; (b): apple; (c): sweet cherry; (d): peach; (e): black raspberry and (f): strawberry. And the genes were located on the different chromosomes, except European pear. Red line represents the syntenic gene pairs.

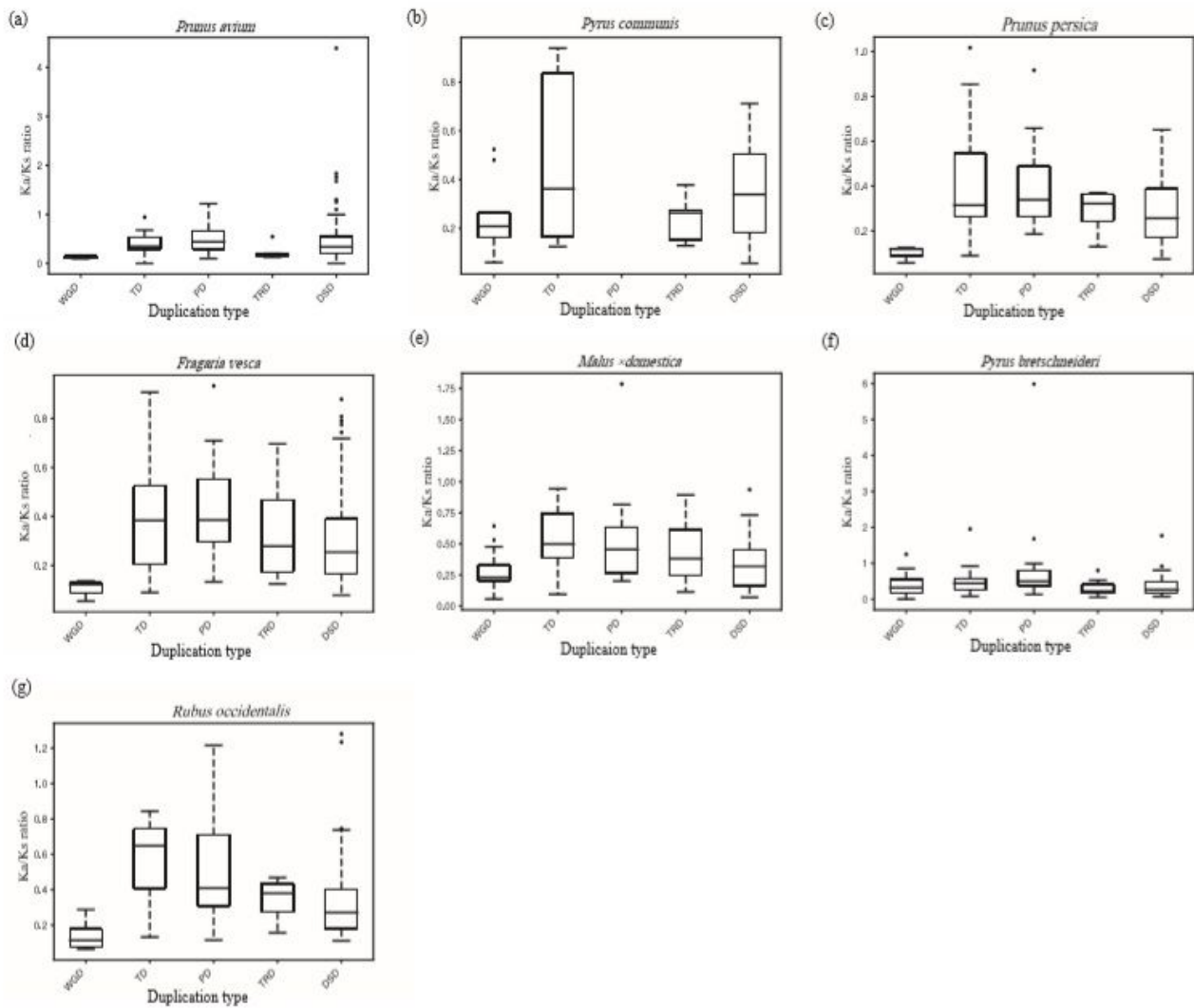


Figure 5

Fig. 5 Ka/Ks ratio of Seven Rosaceae species.We analyzed the Ka/Ks value using coding sequences (CDS). The x-axis represents five different duplication categories, the y-axis indicates the ratio of Ka/Ks .

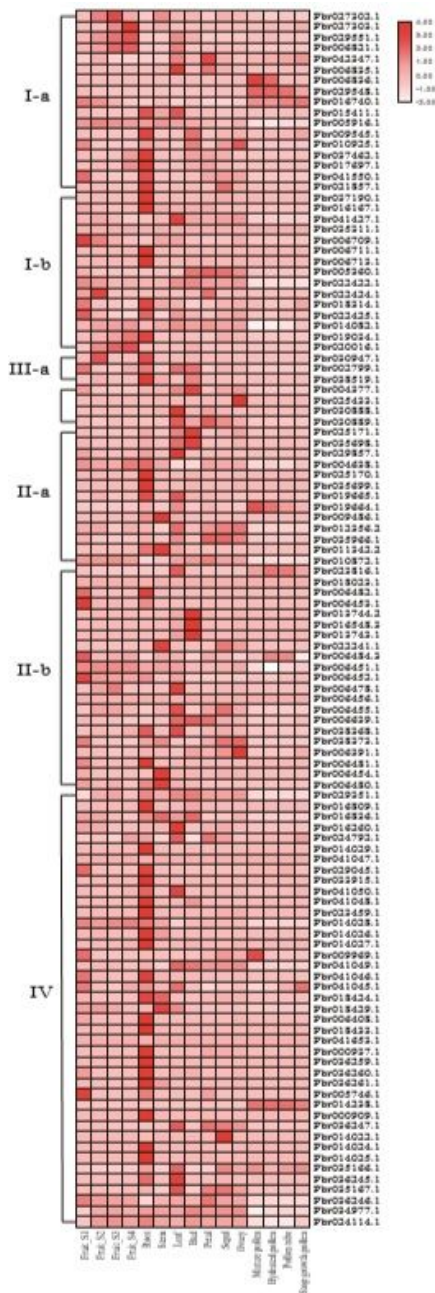


Figure 6

Fig. 6 Heat map analysis of BAHD genes in pear. Using RNA-seq data to determine the expression pattern of BAHD gene. The classes I-a~IV on the left imply the different clades. Fruit_S1~Fruit_S4 represents four distinct periods: on 16th May, 2015 (Fruit_S1); 1st July, 2015 (Fruit_S2); 31st July, 2015 (Fruit_S3); and 29th August, 2015 (Fruit_S4). Dark red indicates high expression and light pink denotes low expression.

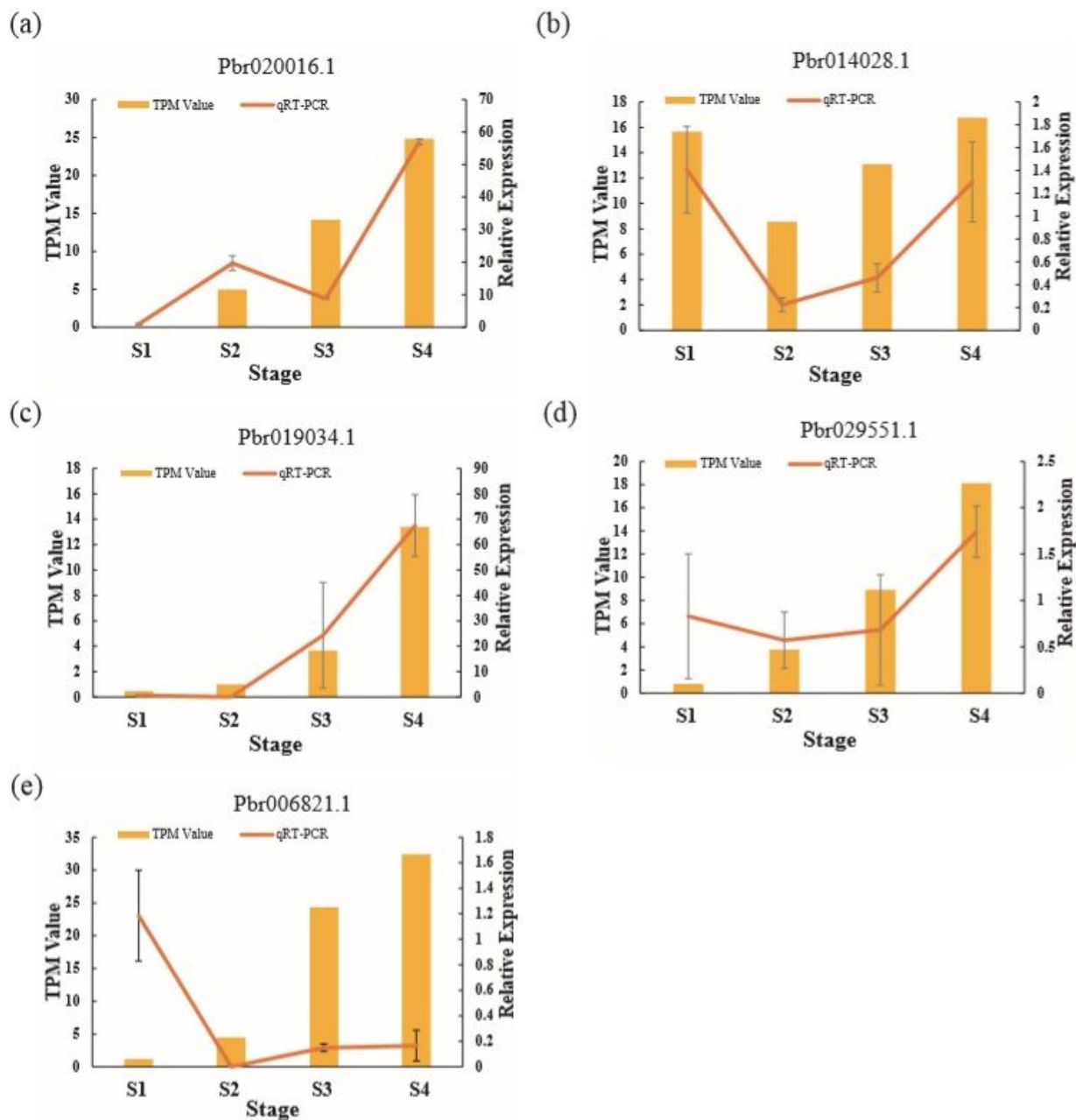


Figure 7

Fig. 7 The relative expression of several BAHD genes among different periods. Tubulin as a reference gene to measure expression levels in each period. X-axis indicate four distinct periods (45DAF, 75DAF, 105DAF and 145DAF), the y-axis represents the relative expression and TPM Value. Data represent the means $\pm$ SD (n = 3).

Supplementary Files

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