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ORIGINAL ARTICLE

Genome-wide identification, evolution and expression analysis of the *FtsH* gene during fruit development in pear (*Pyrus bretschneideri*)

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Abstract

Filtration temperature-sensitive H (FtsH) is an ATP-dependent protease family that plays important roles in many aspects of cellular processes and environmental adaption. However, little information is available about the *FtsH* genes in pear (*Pyrus bretschneideri*). In this study, 19 *PbrFtsH* genes were identified and classified into eight groups with several subgroups. The *FtsH* genes in pear show high sequence and structural conservation in the coding regions, and the majority of the *FtsH* members were located on the chromosome 3 (three members), chromosome 9 (four members), and chromosome 15 (three members). The WGD/segmenta, dispersed duplication played a major driving force in pear FtsH evolution. A Synteny analysis provided deep insight into the evolutionary relationships of *PbrFtsH* genes. The subcellular localization of the *PbrFtsH* genes showed them in the mitochondrion or chloroplast. Expression profiles derived from transcriptome data and RT-qPCR analysis revealed distinct expression patterns of *PbrFtsH* genes in different fruit developmental stages. These results provided valuable information that extends our understanding of the function, evolution and expression profile of the FtsH family during fruit development in pear, and a foundation for the further functional characterization of *PbrFtsH* genes, which can be applied to pear crop improvement.

Keywords Pear · FtsH · Identification · Evolution · Subcellular localization · Expression

Introduction

Filamentation temperature-sensitive H (FtsH) is an ATP-dependent zinc metalloprotease and chaperone protein family that belongs to the AAA (ATPases associated with diverse cellular activities) protein superfamily. It was originally identified in *Escherichia coli* and classified among the AAA proteases, which consist of ClpXP, Clpap, Hsuv, Lon, and FtsH (Santos et al. 1975; Tomoyasu et al. 1993; Confalonieri et al. 1995). Of these, FtsH is the only protease that plays an essential role in the life of *Escherichia coli* (Langer. 2000; Langklotz et al. 2012).

FtsH proteins are found ubiquitously in prokaryotes and eukaryotes. (Jayasekera et al. 2000; Bourdineaud et al. 2003; Kato et al. 2019). *FtsH* genes have been found in diverse plants, including *Arabidopsis* (Wagner et al. 2012), tobacco (Seo et al. 2000), alfalfa (Ivashuta et al. 2002), tomatoes (Sun et al. 2006), rice (Zhang et al. 2009), soybean (Yin et al. 2011), wheat (Li et al. 2015), and peanut (Zheng et al. 2016). Almost all the FtsH reported in higher plants have been localized in the mitochondria and chloroplast. The

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N-terminal transmembrane domain of the FtsH protein anchored membranes of mitochondria or thylakoid, whereas the C-terminal proteolytic domain contains a zinc-binding motif (Zaltsman et al. 2005).

In plants, FtsH protein not only functions as proteases, but also participates in protein assembly and folding as molecular chaperones, and plays roles in the responses to stress and plant development. For example, in Arabidopsis, inhibition of the expression of the *DS9* gene accelerated the hypersensitive reaction (Seo et al. 2000). AtFtsH1 and AtFtsH2 were related to the degradation of the 23 kDa fragment of the D1 protein (Zaltsman et al. 2005; Yin et al. 2011). AtFtsH1, AtFtsH2, AtFtsH5, and AtFtsH8 were divided into two types (type A, FtsH1 and FtsH5; type B, FtsH2 and FtsH8) which form a hexameric ring to regulate the thylakoid structure and remove photodamaged D1 protein from photosystem II (PS II) under light stress (Yoshioka-Nishimura et al. 2014; Sedaghatmehr et al. 2016; Zhang et al. 2018). The expression of type A (*AtFtsH1, AtFtsH5)* and type B (*AtFtsH2, AtFtsH8)* were significantly altered in senescing and aging leaves (Sakamoto et al. 2003). AtFtsH6, AtFtsH11, and other FtsH proteins of Arabidopsis were involved in senescence, the responses to light, heat, and other functions (Sedaghatmehr et al. 2016; Wagner et al. 2016; Chen et al. 2006). These proteins also play important roles in other plants. For example, one *FtsH* gene was independently regulated either by either low temperature or light in alfalfa (Ivashuta et al. 2002). *LeFstH6* was a typical heat shock gene and can interact with other heat shock factors (Nishimura et al.2016). GmFtsH9, an AtFtsH2-like protein, could be involved in regulating PSII function (Yin et al.2011). In peanut, *FtsH* genes may play an important role in resistance to salt stress (Zheng et al. 2016).

Previous studies have shown that FtsH is a relatively small and conserved family in many plants (Zhang et al. 2009) and the function of reported *FtsH* genes were predominantly related to stress. Several studies have reported the importance of FtsH proteases in development of plants, including the AtFtsH12 protease, which was an essential factor in the early development of the chloroplast. Arabidopsis FtsH4 mutant bloomed one week later than the wild type and flower development was also affected (Gibala et al. 2009; Zikhali et al. 2016). The interaction between WYMV CP (*Wheat yellow mosaic virus* coat protein) and FtsH2 in wheat may interfere with the greening of leaves of during plants' development and their function (Liang et al. 2019). However, research specialized specific to the FtsH proteins of pear (*Pyrus bretschneideri*) has been limited to rare reports. To study the function of FtsH family in pear fruit development, we performed a comprehensive bioinformatic-based analysis of the *FtsH* genes in pear. The completion of pear genome sequence made it possible to identify 19 pear *FtsH* genes, and we made the comprehensive analysis including the exon–intron organization, motif compositions, gene duplications, chromosome distribution, and phylogenetic and synteny. The expression analysis was performed to identify the expression of specific *FtsH* genes during 'Dangshansuli' different fruit development. Our findings contributed to a basic understanding of the FtsH family and provided a foundation for further clarification the *FtsH* gene function during fruit development of in pear.

Materials and methods

Plant materials

The 'Dangshansuli' (P. *bretschneideri Rehd*.) pear cultivar was maintained at the Jiangpu orchard of Nanjing Agricultural University, Nanjing, China. Pear fruits were collected at fruitlet (30 days after full blooming, DAFB30), first enlargement (98 days after full blooming, DAFB98), interval enlargement (126 days after full blooming, DAFB126), second enlargement (140 days after full blooming, DAFB140), and maturation (168 days after full blooming, DAFB168), respectively. The collected pear fruits were frozen in liquid nitrogen and stored at -80 °C for analysis.

Gene identification

To identify the members of *FtsH gene* family in pear, multiple database searches were performed. First, the amino acid sequences of *Arabidopsis FtsH* genes were downloaded from *Arabidopsis* Information Resource (TAIR) (http://www.Arabidopsis.org) (Swarbreck et al., 2008) and used to perform BLAST searches in the pear genome database (http://www.peargenome.njau.edu.cn) (Wu et al. 2013) and together with those of other fruit trees, including apple, peach, orange, papaya, grape, and strawberry (https://phytozome.jgi.doe.gov/). The isolated genes were further analyzed by checking the core sequences of *FtsH* genes family using Pfam. The pear genes were then manually examined to ensure the conserved sequence using the domain analysis program Inter ProScan. The acquired web results with the output row format reported. The isoelectric points, molecular weights, and lengths of the FtsH proteins were obtained with the ExPasy website (https://web.expasy.org/protparam/). Prediction of transmembrane regions and orientation was obtained using TMHMM software (http://www.cbs.dtu.dk/services-/TMHMM).

Sequence and phylogenetic analyses of FtsH genes

The characterized FtsH protein sequences were aligned using program Clustal W with default parameters. After the null members were removed, the phylogenetic trees were

constructed in MEGA version 7.0, based on the equally weighted neighbor-joining method, Bootstrap values of the phylogenetic tree were calculated with 1000 replicate analyses.

Gene structure and conserved motifs of FtsH genes.

To determine the gene intron/exon structure, pear *FtsH* genes information was implemented from the genome annotations using the online program Gene Structure Display Server (GSDS: http://gsds.cbi.pku.edu.cn) (Hu et al. 2015). MEME online program (http://meme.nbcr.net/meme/intro.html) was used to identify conserved motifs (Bailey et al., 2009). The gene structures and conserved motifs patterns were then drawn and visualized with the TBtools (Chen et al. 2006).

Chromosomal localization and gene duplication

All *FtsH* genes were mapped to the pear chromosomes based on the physical location information from the database of pear genome, and visualized using the TBtools. Gene family expansion models for the *FtsH* family were investigated based on the diverse chromosomal location of the genes, and included whole genome (WGD), and segmental, tandem, proximal, and dispersed duplications (Cannon et al. 2004). Multiple Collinearity Scan toolkit X (MCScanX) was used to analyze the gene duplication events, with the default parameters (Wang et al. 2012). To display the synteny analysis of the orthologous *FtsH* genes of pear, *Arabidopsis*, and other fruit trees, Syntenic analysis maps were constructed using the Dual Synteny Plotter software. Non-synonymous (Ka) and synonymous (Ks) substitution of each duplicated *FtsH* genes was calculated using TBtools.

Prediction and subcellular localization

Protein subcellular localization was predicted at the WOLF PSORT (http://wolfpsort.cbrc.jp/) (Horton et al. 2007) and BUSCA (http://busca.biocomp.unibo.it/) (Savojardo et al. 2018). We isolated three *PbrFtsH* genes which were cloned from the cDNA of flesh in different development stages. The open-reading frames (ORFs) of the three *PbrFtsH* genes were fused to the pCAMBIA1300-35S-GFP vector (Takara, Beijing, China) to produce the plasmids *PbrFtsH7-GFP*, *PbrFtsH12-GFP*, and *PbrFtsH13-GFP*. Tobacco leaves were infiltrated by *Agrobacterium tumefaciens* strain GV3101 harboring the recombinant constructs and then incubated for 3–4 days at 20–25℃. GFP fluorescence in the tobacco leaves was imaged using a Zeiss Axio LSM 700 inverted confocal laser scanning microscope (CLSM). All transient expression assays were repeated three times. Primers used for cloning genes were listed in additional file 8.

Expression analysis of FtsH genes in pear fruit

In a previous study, RNA-Seq was performed on fruit samples of pear (Pyrus *bretschneideri*) collected at five fruit developmental stages including the fruitlet (DAFB30), first enlargement (DAFB98), interval enlargement (DAFB126), second enlargement (DAFB140), and maturation (DAFB168) (Pei et al., 20,020). Based on the RNA-Seq data, the expression levels of *FtsH* genes were evaluated by calculating the Reads Per Kilobase per Million mapped reads (RPKM), and the expression patterns were visualized using heatmaps drafted in Cluster version 3.0 software (Stanford University, Palo Alto, CA).

Total RNA was extracted using the RNAprep Pure Plant Kit (Polysaccharides & Polyphenolics-rich) (Tiangen, Beijing, China). The first-strand cDNA was synthesized with TransScript One-Step gDNA Removal and cDNA synthesis Supermix (TransGen, Beijing, China) according to the manufacturer's instructions. qRT-PCR was carried out in the LightCycler 480® II/96 Thermal Cycler (Roche, USA), and the reaction mixture and cycling conditions were identical to a previous report (Hao et al. 2018) All analyses were performed with three biological replicates. The actin gene (*Pbr038418.1*) was used as the constitutive control in pear fruit. All of the primer sequences are listed in additional file 8.

Results

Phylogenetic analysis and classification of PbrFtsH genes

A total of 19 candidate *FtsH* genes were isolated from the pear genome (*Pyrus bretschneideri*) and designated *PbrFtsH1 – PbrFtsH19* (Table 1). The CDS and protein sequences of these genes were available in Additional file 1. The proteins lengths ranged from 582 (*PbrFtsH14*) to 1265 (*PbrFtsH5*) amino acid (aa). The pI of the proteins ranged from 5.76 (*PbrFtsH19*) to 9.89 (*PbrFtsH16*, and their molecular weight ranged from 63,966.67 (*PbrFtsH14*) to 138,773.43 (*PbrFtsH5*).

To examine the evolutionary relationships among the *FtsH* genes of pear, *Arabidopsis* and other fruit species, the various *FtsH* genes were isolated from strawberry (10), peach (12), orange (12), papaya (8), apple (13), grape (6) and in *Arabidopsis* (12) (Fig. 1). The *FtsH* genes were used to construct a phylogenetic tree (neighbor-joining method). A total of 92 genes were clearly divided into eight group, A → H (Fig. 1). Group B was the smallest clade with 4 members and Group D is constituted with 5 members, whereas Groups E and F were both larger. Based on reliable bootstrap values (> 90%), Group C, Group E, Group F, Group

Table 1 Basic information of the FtsH in pear

Gene name	Gene ID	start	Stop	CDS length (bp)	Protein length (aa)	pI	Molecular weigh	Gene family
PbrFtsH1	Pbr029305.1	104,813	109,190	2448	816	8.74	89,984.97	Group E
PbrFtsH2	Pbr038992.1	29,236	33,990	2448	816	8.87	90,130.17	Group E
PbrFtsH3	Pbr018930.1	27,231,140	27,236,113	2439	813	6.53	89,256.50	Group E
PbrFtsH4	Pbr029306.1	145,787	150,164	2448	816	8.74	89,984.97	Group E
PbrFtsH5	Pbr003862.1	26,150,277	26,165,306	3795	1265	6.05	138,773.43	Group E
PbrFtsH6	Pbr005758.2	2,034,158	2,037,680	2166	722	6.22	77,033.84	Group F
PbrFtsH7	Pbr005387.1	5,645,603	5,648,950	2085	695	5.89	73,919.55	Group H
PbrFtsH8	Pbr022822.1	1,733,382	1,736,994	2085	695	5.83	73,968.66	Group H
PbrFtsH9	Pbr019270.1	21,018,901	21,021,967	2055	685	6.06	73,927.66	Group H
PbrFtsH10	Pbr019852.2	6,463,703	6,467,820	2151	717	8.82	77,283.21	Group G
PbrFtsH11	Pbr013848.1	19,167,492	19,184,033	2163	721	6.42	78,584.96	Group G
PbrFtsH12	Pbr042309.1	26,858,556	26,863,831	2472	824	8.89	89,161.56	Group F
PbrFtsH13	Pbr041367.1	26,169,809	26,175,661	2496	832	8.41	90,597.13	Group F
PbrFtsH14	Pbr042755.1	6,447,619	6,452,408	1746	582	9.44	63,966.67	Group G
PbrFtsH15	Pbr022238.1	18,970,831	18,974,709	1941	647	9.82	72,093.23	Group C
PbrFtsH16	Pbr016552.1	17,981,761	17,987,152	1938	646	9.89	71,795.27	Group C
PbrFtsH17	Pbr019141.1	11,201,945	11,207,985	2658	886	9.00	100,228.58	Group C
PbrFtsH18	Pbr035075.1	10,580,724	10,586,147	2847	949	8.37	106,558.84	Group D
PbrFtsH19	Pbr004949.1	9,347,660	9,356,965	2550	850	5.76	95,639.12	

G, and Group H were further divided into two subclusters, respectively (C1and C2; E1and E2; F1and F2; G1andG2; H1and H2, respectively). *PbrFtsH15, 16, 17* were included in Group C*, PbrFtsH1, 2, 3, 4, 5* in Group E, *PbrFtsH6, 12, 13* were in Group F, *PbrFtsH10, 11, 14* were in Group G, *and PbrFtsH7, 8, 9* were in Group H. Only *PbrFtsH18* was exhibited in group D and *PbrFtsH19* was not exhibited to any group.

To identify the PbrFtsH family members more clearly, another phylogenetic tree was constructed with protein sequences of pear and *Arabidopsis*. Five pairs of FtsH genes *PbrFtsH1, 2*and *4, PbrFtsH3*and *5, PbrFtsH7*and *8, PbrFtsH12*and *13, PbrFtsH15*and *16* showed high degrees of similarity (Fig. 2). Compared with *Arabidopsis* FtsH genes, *AtFtsH2/8* with *PbrFtsH7/8*, *AtFtsH7/9* with *PbrFtsH12/13*, and *AtFtsH3/10* with *PbrFtsH3/5* have more than 99% bootstrap values inferring they have more larger homology, respectively.

Sequence alignment and transmembrane region of PbrFtsH genes

Multiple sequence alignment and visualization by Jalview revealed the five conserved domains: Walker A can be represented as GX1X2X3PX4GX5 LLX6GX7PPGTGKT and Walker B was represented as PX1X2X3FIDEIDA (where X is an uncharged residue,). The second region of homology (SRH), a characteristic domain of FtsH, can be represented as TNX1X2X3X4LDX5X6X7X8RX9GRX10DR. The SRH can be used to distinguish the FtsH proteins from other AAA proteases. The Zinc-binding motif HEX1X2H was the active site of the protease, and the only variant detected was at residue X1 of the PbrFtsH1-14 proteins, in which residue X2 is conserved as 'G'. No zinc-binding site presented in the sequence of PbrFtsH15-19 (Fig. 3). In addition, the non-named region that occurred between the SRH and zinc-binding motifs was described as TX1GFX2GAX3X4X5NX6X7NX8AA, and the only the variant detected was a residue in the PbrFtsH17and 19 proteins. These conserved sequences provided the criterion for the isolation and identification of the *FtsH* genes from other plants.

FtsH family is an independent member of AAA proteases super family and its members have their own sequence specificity and characteristics. The transmembrane regions of PbrFtsH proteins were predicted sing TMHMM. Most of them contained two transmembrane domains; a few of them (PbrFtsH1, PbrFtsH10, PbrFtsH14, and PbrFtsH19) have one transmembrane; and only PbrFtsH9 has no transmembrane (Additional file 4), suggesting that the functions of their transmembrane domains differ.

Conserved motifs and gene structure analysis of the PbrFtsH genes

To further identify the conserved motifs of the PbrFtsH, the MEME program and TBtools were used in this study. As

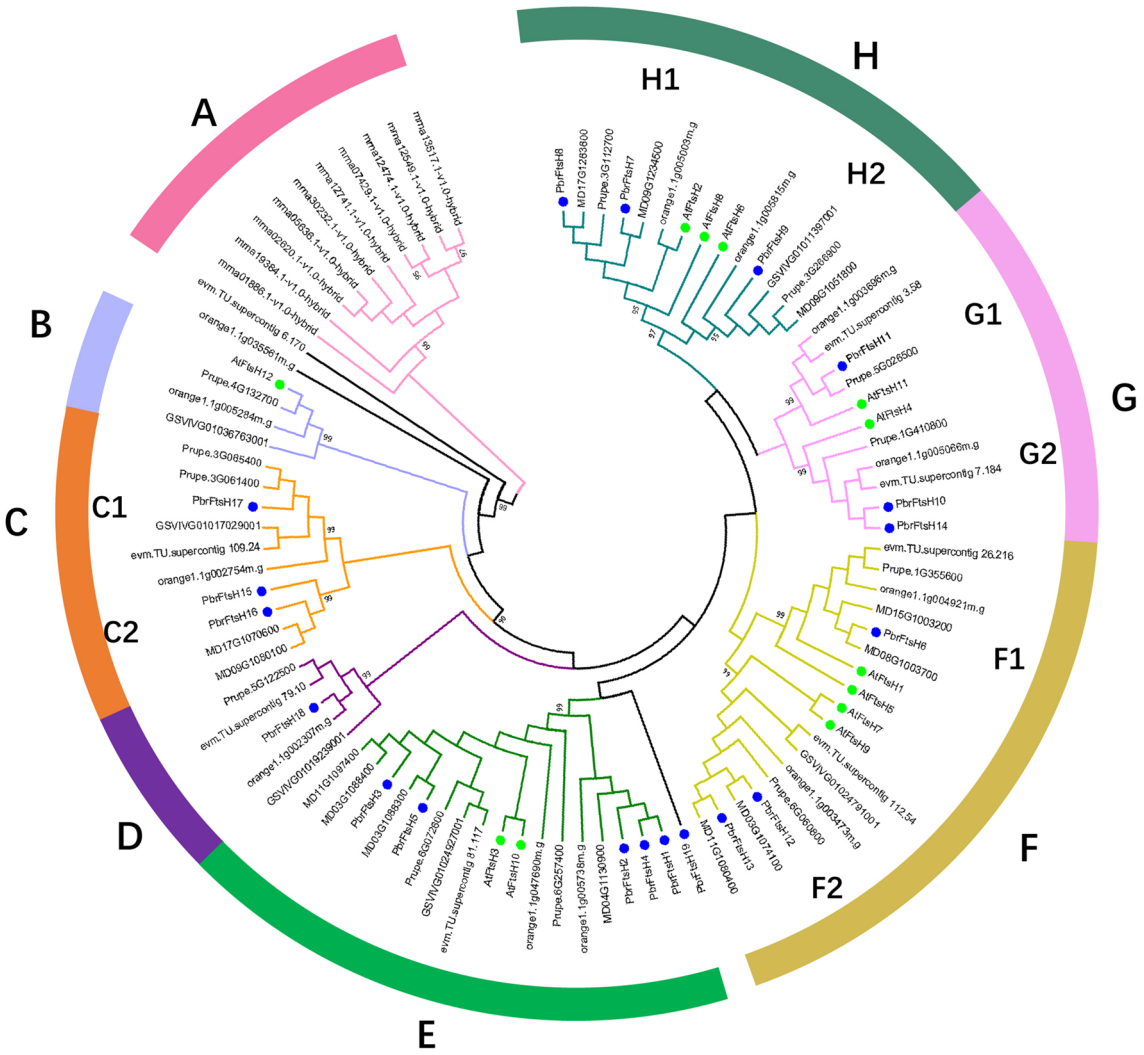

Fig. 1 A phylogenetic tree of FtsH genes isolated from pear, peach, apple, strawberry, papaya, orange, and grape, Arabidopsis. The tree was generated using MEGA 7.0 with the Neighbor Joining method. The proteins were clustered into eight different groups and marked with different colors. Different species be distinguished: "●" represented strawberry; "●" represented papaya; "●" represented orange; "●" represented Arabidopsis; "●" represented grape; "●" represented peach; "●" represented pear and "●" represented apple

shown in Fig. 4b, 10 conserved motifs with were found. The number of motifs in the individual FtsH sequences were vary (Additional file 5). Motifs 3,1,4,6,2,5,7 and 8 were commonly distributed in the *PbrFtsH* genes, except in *PbrFtsH15-19*. Motif 10 only existed in *PbrFtsH1-5*, which encoded FtsH-ext domain (Fig. 4d)., Pbr*FtsH5* was clearly longer than *PbrFtsH3*, and motif 10,3,6,2,5,7,8 and 9 were repeated in its N-terminal, t meaning two FtsH domains, which may indicate functional redundancy. Motif 9 was only existed in *FtsH1-6*, *FtsH9,* and *FtsH10*, which indicated different FtsH functional motifs arose during the evolution of the *FtsH* genes.

The schematic gene structures of *FtsH* genes were analyzed using the GSDS (Fig. 4b). The number of exons ranged from 3 to 18 (Table 2 and Fig. 4c, Fig.S1). *PbrFtsH11* had 18 exons with the most numbers. In Group C, *PbrFtsH15* and *PbrFtsH16* had four exons, and *PbrFtsH17* had eleven exons. *PbrFtsH12* and *PbrFtsH13* in Group F, respectively, have 5, 14, and 13 exons. *PbrFtsH7*, *PbrFtsH8,* and *PbrFtsH9* had 3, 3, and 5 exons in the subgroup H1, respectively. All Group E members contained 8 exons. *PbrFtsH18* was distinguished from the other clades in having 15 exons, suggesting an independent evolutionary line. *PbrFtsH5* was an exception with 14 exons, because it encoded one domain more than *PbrFtsH3*.

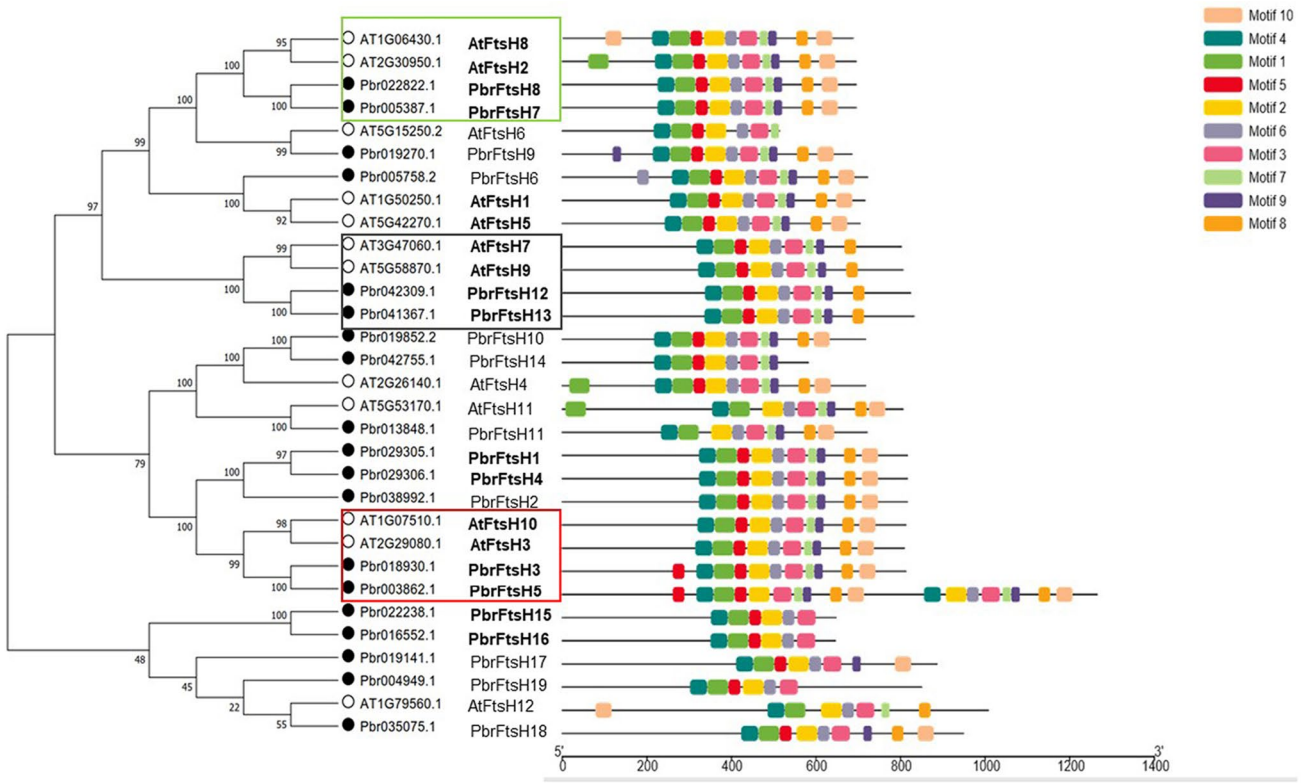

Fig. 2 The phylogenetic analysis and schematic diagram for motif of FtsH genes in pear and *Arabidopsis*. The phylogenetic tree was constructed from a complete alignment of 31 FtsH proteins by maximum-likelihood method with bootstrapping analysis (1000 iterations). The numbers beside the branches indicate the bootstrap values that support the adjacent node. The gene pairs are bolded and different color boxes marked three similar gene pairs of pear and *Arabidopsis* are by different color boxes exons, introns, and UTR, respectively. Motif models are drawn to scale as indicated on the bottom

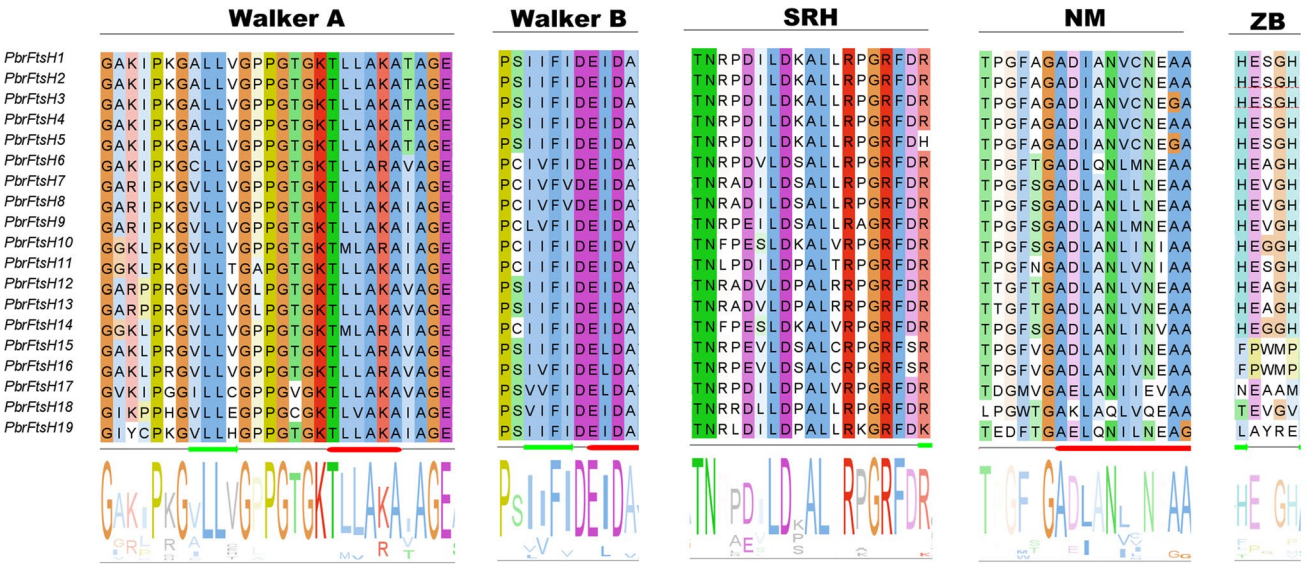

Fig. 3 Multiple sequence alignment of the FtsH from pear. *SRH* the second region of homology; *NM* non-named region of homology. *ZB* Zn binding. JNetPRED is a prediction, the α-helices are represented by red tubes, and the β-folds are represented by green

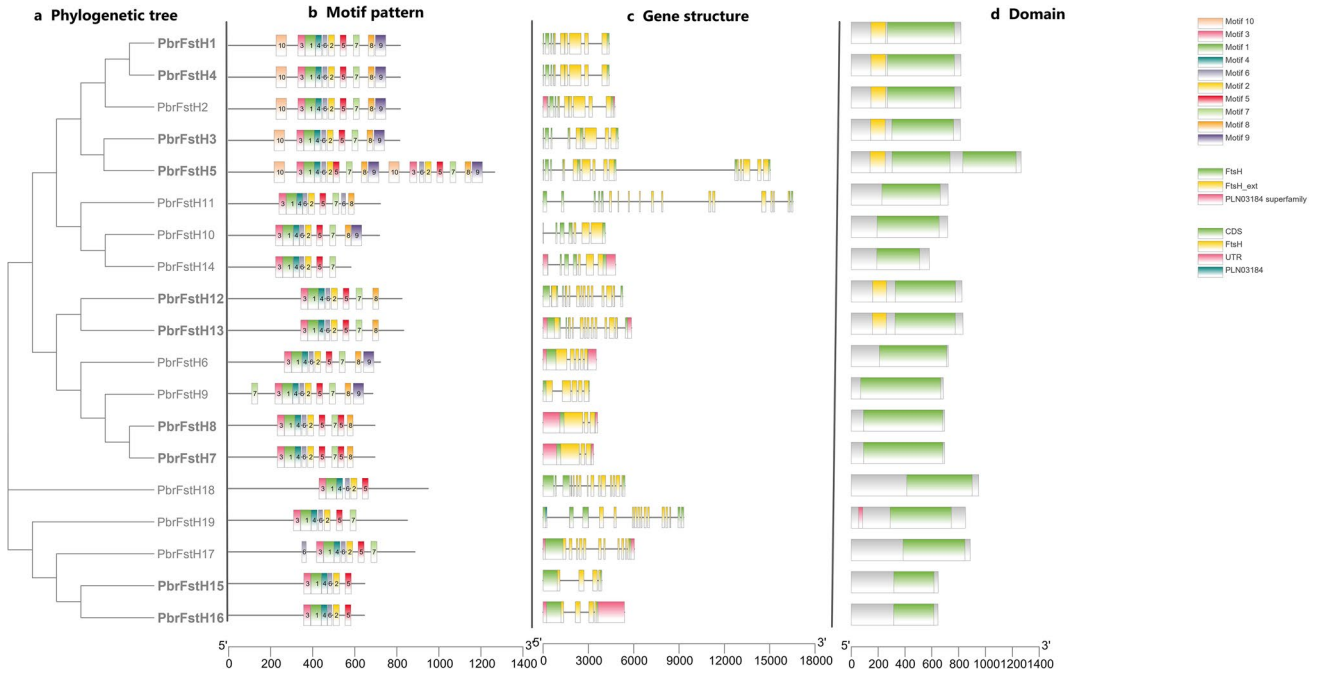

Fig. 4 Phylogenetic relationships, motif pattern, gene structure, and architecture of domains in FtsH genes from pear. **a** The phylogenetic tree was constructed based on the full-length sequences of pear FtsH proteins using MEGA7 software. Details of clusters are shown in different colors. **b** The motif composition of pineapple FtsH proteins. The motifs, numbers 1–10, are displayed in different colored boxes. The sequence information for each motif is provided in Additional file 2. The length of protein can be estimated using the scale at the bottom. **c** Exon–intron structure of pear FtsH genes. Pink boxes indicate untranslated 5′- and 3′-regions; yellow boxes indicated exons; black lines indicated introns. **d** The FtsH domains are highlighted by green boxes. The number indicates the phases of corresponding introns

Table 2 Groups classification and predicted subcellular localization of FtsH proteins of pear and *Arabidopsis*

Group number	Gene name	Number of exon	Predicted subcellular localization
A			
B	AtFtsH12	19	C
C	PbrFtsH15, PbrFtsH16, PbrFtsH17	4,4,11	M
D	PbrFtsH18	15	C
E	PbrFtsH1, PbrFtsH2, PbrFtsH3, PbrFtsH4, PbrFtsH5, AtFtsH3, AtFtsH10	8,8,8,8,14,8,8	M
F	PbrFtsH6, PbrFtsH12, PbrFtsH13, AtFtsH1, AtFtsH5, AtFtsH7, AtFtsH9	5,14,13,5,5,13,7	C
G	PbrFtsH10, PbrFtsH11, PbrFtsH14, AtFtsH4, AtFtsH11	7,18,7,7,17	M, C
H	PbrFtsH7, PbrFtsH8, PbrFtsH9, AtFtsH2, AtFtsH6, AtFtsH8,	3,3,5,4,5,4	C
	PbrFtsH19	16	C

C chloroplast, *M* mitochondrion

Chromosomal distribution and synteny analysis of FtsH genes

The *PbrFtsH* genes were unevenly distributed on the chromosomes (Fig. 5). Among the 17 chromosomes of pear genome, most of the FtsH members were located on chromosome 3 (three members), chromosome 9 (four members), and chromosome 15 (three members). Some chromosomes possessed only one gene. Chromosome 9 contained most members, indicating a hot spot in *FtsH* gene distribution. No *FtsH* gene was distributed on chromosome 1,5,7,8,10,12,13,14, or 16. These results indicated that the chromosomal locations of the *PbrFtsH* genes were preferential (Fig. 5). Furthermore, *PbrFtsH1* and *PbrFtsH4* were

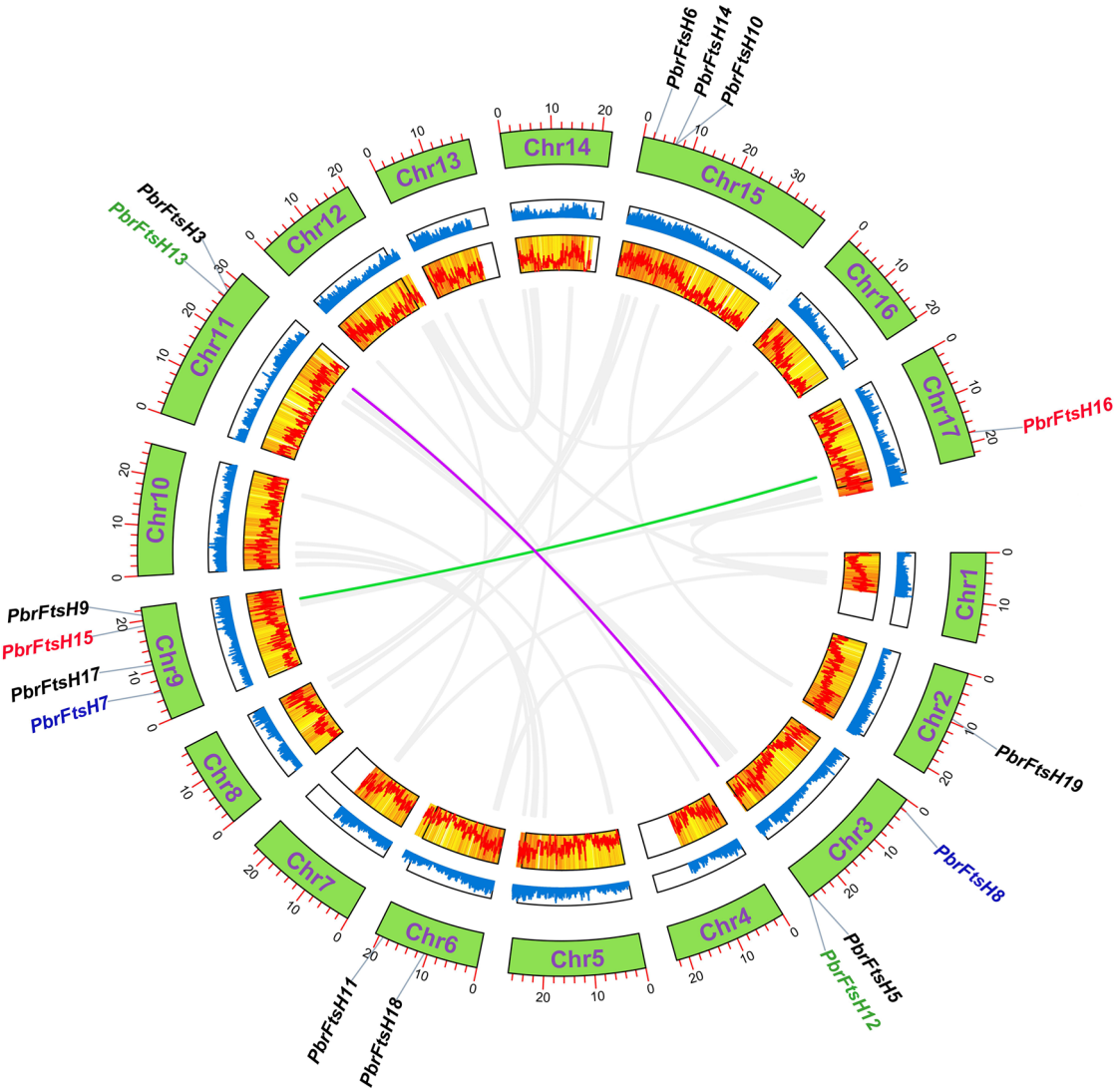

Fig. 5 Schematic representations for the chromosomal distribution and inter chromosomal relationships of FtsH genes. The circular forms of chromosomes are shown in green color. Gray lines indicated all synteny blocks in the pear genome, and the green and purple lines indicate syntenic relationships gene pairs. Bar in the blue and Heatmap displayed gene density. The positions of the PbrFtsH genes are marked with short lines on the circles

tandemly arrayed on the scaffold 490.0, and *PbrFtsH2* was located on unassembled scaffold823.0 (Fig.S2). No positive correlation could be detected between the chromosomal length and the number of *FtsH* genes.

We analyzed the mode of duplication for the FtsH genes using MCScanX. As a result, we detected two syntenic gene pairs, *PbrFtsH3* and *PbrFtsH5*, *PbrFtsH15* and *PbrFtsH16*, which probably derived from recent WGD or Segmental events (Fig. 5). It was noteworthy that *PbrFtsH* genes arose from singleton events (except *PbrFtsH1*, *PbrFtsH2*, and *PbrFtsH4*), six *PbrFtsH* genes were attributed to dispersed events, only two genes, *PbrFtsH10* and *PbrFtsH14*, were attributed to proximal events (Table 3), and no tandem duplication had not been occurred in pear FtsH family.

To further explore the phylogenetic evolutionary mechanisms of Pbr*FtsH* genes, comparative syntenic maps were constructed with five representative species *Arabidopsis*, strawberry, grape, peach, and apple (Fig. 6). A total of 19 *PbrFtsH* genes showed syntenic relationships with strawberry (2), grape (1), peach (2), apple (14), and *Arabidopsis* genes (1) (Additional file 6). The numbers of orthologous pairs between the pear genes and those of the other five species r were: strawberry 16, grape 12, peach 16, apple 28, and *Arabidopsis* 12. *PbrFtsH3* and *PbrFtsH5* genes were found to be associated with at least three syntenic gene pairs (particularly between pear and apple *FtsH* genes). Significantly, the results showed that some collinear *FtsH* gene pairs shared by pear and apple were anchored to the

Table 3 The duplication events in pear

Gene name	Gene ID	The duplicate event
PbrFtsH1	Pbr029305.1	Singleton
PbrFtsH2	Pbr038992.1	Singleton
PbrFtsH3	Pbr018930.1	WGD or segmental
PbrFtsH4	Pbr029306.1	Singleton
PbrFtsH5	Pbr003862.1	WGD or segmental
PbrFtsH6	Pbr005758.2	Dispersed
PbrFtsH7	Pbr005387.1	Dispersed
PbrFtsH8	Pbr022822.1	Singleton
PbrFtsH9	Pbr019270.1	Dispersed
PbrFtsH10	Pbr019852.2	Proximal
PbrFtsH11	Pbr013848.1	Dispersed
PbrFtsH12	Pbr042309.1	Singleton
PbrFtsH13	Pbr041367.1	Singleton
PbrFtsH14	Pbr042755.1	Proximal
PbrFtsH15	Pbr022238.1	WGD or segmental
PbrFtsH16	Pbr016552.1	WGD or segmental
PbrFtsH17	Pbr019141.1	Dispersed
PbrFtsH18	Pbr035075.1	Singleton
PbrFtsH19	Pbr004949.1	Dispersed

highly conserved syntenic blocks (Fig. 6), which spanned more than 20 genes. In contrast, pear and grape had only 12 orthologous gene pairs, as in *Arabidopsis* (Additional file 6). Similar patterns were also observed between pear-peach with pear-strawberry. Interestingly, *PbrFtsH18* and corresponding collinear pairs were showed between pear and strawberry, peach, and apple, respectively, but between pear and grape (Additional file 6). The syntenic *PbrFtsH11* and *MD06G1038500* identified only between pear and apple. However, orthologues of *PbrFtsH7* and *PbrFtsH8* were identified in pear and strawberry, peach, apple, and grape, but were not in *Arabidopsis*.

To better understand the evolutionary constraints that have acted on FtsH family, the classical measure nonsynonymous (Ka)/synonymous substitution (Ks) ratios of the FtsH gene pairs were calculated (Additional file 7). The ratio is associated with the magnitude and direction of selective constraints (Ka/Ks > 1, = 1, and < 1) and indicated neutral evolution, purifying selection, and positive diversifying selection, respectively. The results showed all *PbrFtsH* genes, and those of the other five species, had Ka/Ks < 1, suggesting that the pear FtsH family might have experienced strong purifying selective pressure during evolution.

Subcellular localization of FtsH proteins for pear

The predicted subcellular location of *PbrFtsH* genes showed that seven *PbrFtsH* genes were located in the mitochondrion or plasma membrane (*PbrFtsH1-5*, *PbrFtsH15*, and *PbrFtsH16*), whereas 10 genes were located in the chloroplast (*PbrFtsH6-10*, *PbrFtsH12-14*, *PbrFtsH18*, and *PbrFtsH19*) in Table 2.

To further verify the location of FtsH, we performed three *PbrFtsH* genes which were cloned from the fruit flesh in different developmental stages. The ORFs of three *PbrFtsH* were fused to the pCAMBIA1300 vector to express fusion proteins with green fluorescent protein (GFP) under the control of the CaMV 35S promoter. The fusion proteins (PbrFtsH-GFP) and control (35S-GFP alone) were separately transformed into Tobacco leaves. As shown in Fig. 7, PbrFtsH7, PbrFtsH12, and PbrFtsH13 were successfully expressed as PbrFtsH–GFP fusion proteins in chloroplasts. These observation were consistent with the predictions, indicating roles for these proteins in chloroplast or mitochondrion.

Expression patterns of FtsH genes in different development stages of pear fruit

To understand the functions of *FtsH* genes during different developmental stages of pear fruit, available transcriptome data and the RT-qPCR analysis were used to analyze their expression patterns (Fig. 8). As shown in Fig. 8, the expression patterns of the *FtsH* genes were investigated in five stages and varied greatly across them, which indicated that they may have different functions in the diverse growth stages of the pear fruit. On the basis of transcriptome data, the expression of *PbrFtsH11*, *PbrFtsH16*, *PbrFtsH18*, and *PbrFtsH19* were high in fruitlet, but decreased gradually in the subsequent developmental stages. The expression of *PbrFtsH2* and *PbrFtsH4* increased gradually and these genes exhibited preferential expression in the later stages, implying their roles in later fruit development and ripening. The expression levels of other genes (except *PbrFtsH11/16/18/19*) tended to increase early and decline (Fig. 8a). For instance, *PbrFtsH8*, *PbrFtsH9*, *PbrFtsH13*, and *PbrFtsH14* displayed relatively strong expression at DAFB98, indicating that they participated in the initial stages of fruit enlargement (Fig. 8a, b). *PbrFtsH1* and *PbrFtsH15* were observed relatively highest expression level during the middle stages of fruit enlargement, suggesting that they might play a role during this stage (Fig. 8a). *PbrFtsH7*, *PbrFtsH11*, *PbrFtsH12*, *PbrFtsH13*, *PbrFtsH14*, *PbrFtsH18*, and *PbrFtsH19* were relatively highly or moderately expressed during fruit development (Fig. 8b). These analyses showed that *PbrFtsH1*, *PbrFtsH3*, *PbrFtsH5*-14, and *PbrFtsH17* were more strongly expressed during fruit enlargement (including first enlargement, interval enlargement, and second enlargement) than the other stages, indicating that these genes were involved in pear fruit development, especially enlargement.

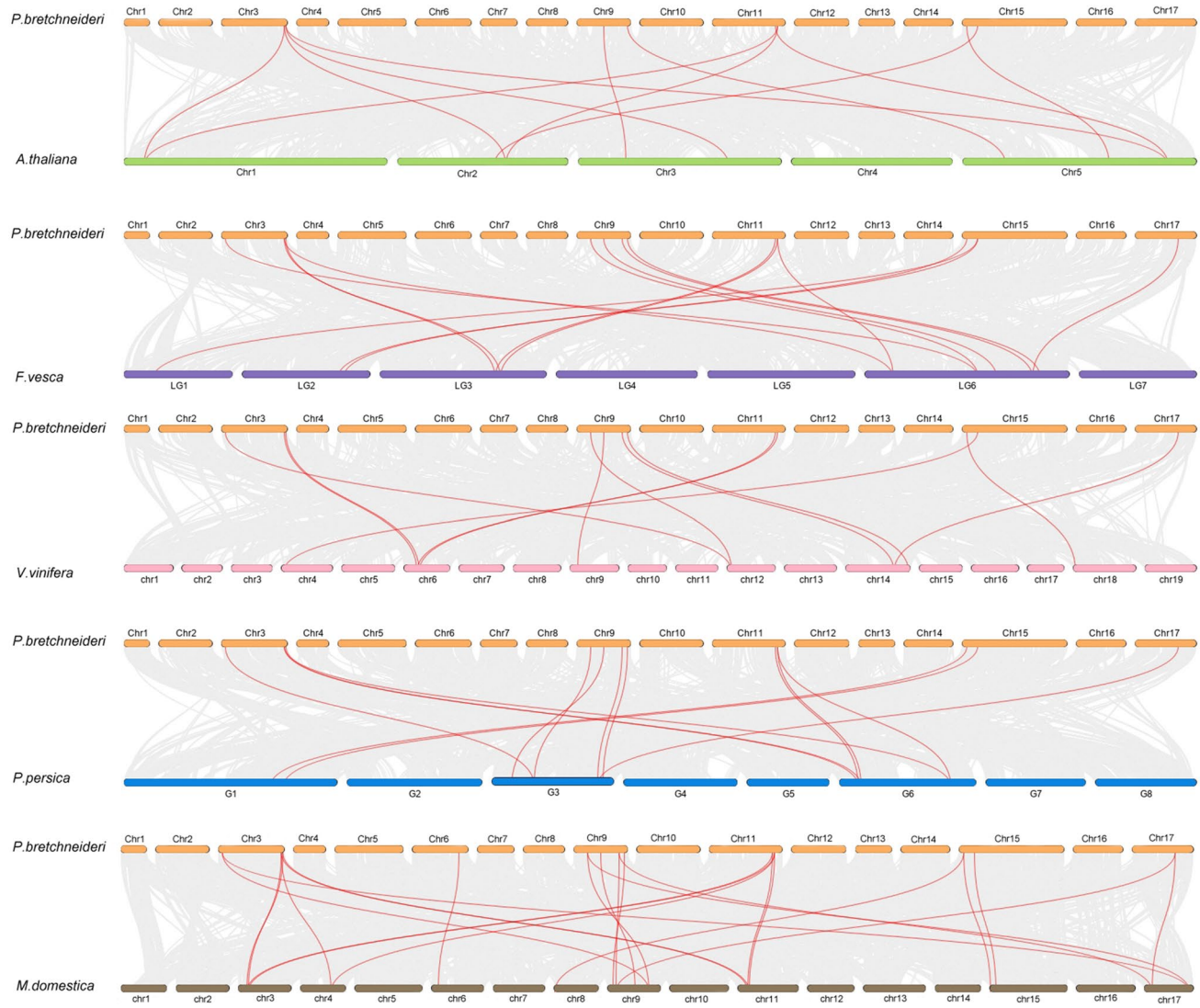

Fig. 6 Synteny analysis of FtsH genes between pear and five representative plant species. Gray lines in the background indicate the collinear blocks within pear and other plant genomes, while the red lines highlight the syntenic FtsH gene pairs. The specie names with the prefixes 'P.*bretchneideri*', 'A.*thaliana*', 'F.*vesca*', 'V.*vinifera*', 'P.*persica*', and 'M.*domestica*' indicated Pyrus bretschneideri, *Arabidopsis* thaliana, Fragaria vecsa, Vitis vinifera, Prunus persica, and Malus domestica, respectively

Discussion

Discovery of FtsH family

In recent years, genome-wide identification and functions of FtsH family have been widely carried out in many plants (Sun et al. 2006; Yin et al. 2011; Li et al. 2015,). However, research on the FtsH family in pear were not covered. The release of the whole-genome sequence of pear allowed us to perform a genome-wide investigation and evolution of pear *FtsH* genes. In this study, we identified 19 members of pear FtsH family, which designated *PbrFtsH1-19*. Phylogenetic analysis of *FtsH* genes was performed between the pear and *Arabidopsis*, strawberry, peach, orange, papaya, apple, and grape. These *FtsH* genes were classified into eight subfamilies which is consisted with the classification of *Arabidopsis FtsH* genes (Zhang et al. 2009). However, Group A members were all strawberry genes, implying that these genes had been amplified in a different direction. *PbrFtsH19* was not assigned to any group, implying that different characteristics have arisen within the FtsH family during evolution. Additionally nor could *evm.TU.supercontig 6.170, PbrFtsH19*, or *orange1.1g035561m.g* be classified into any of the groups. A comparative analysis of *FtsH* genes of pear and *Arabidopsis* identified highly similar gene structure and functional domains within the same group. Three sets of genes, *AtFtsH7/9* and *PbrFtsH12/13*, *AtFtsH3/10* and *PbrFtsH3/5*, *AtFtsH2/8* and *PbrFtsH7/8* were assigned to the

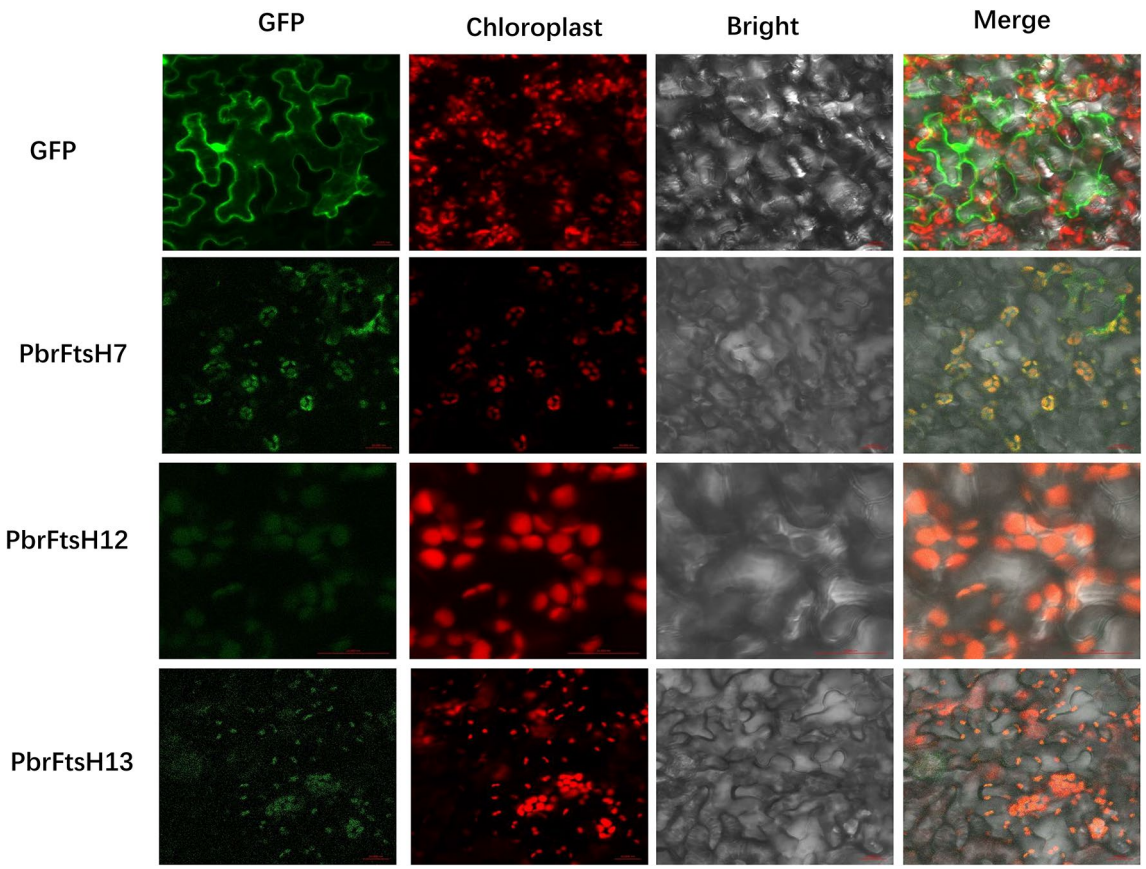

Fig. 7 Subcellular localization of PbrFtsH proteins. Selected *PbrFtsH* genes were cloned from pear fruit (*Pyrus bretchneideri Rehd.*). The PbrFtsH-GFP fusion proteins (PbrFtsH7-GFP, PbrFtsH12-GFP and PbrFtsH13-GFP) and the GFP control were transiently expressed in transformed tobacco leaf epidermal cells and were then observed using confocal laser scanning microscopy. Merged pictures include the green fluorescence channel (first panels), Chloroplast (second panels), and the bright channel (third panels); Bar = 20 μm

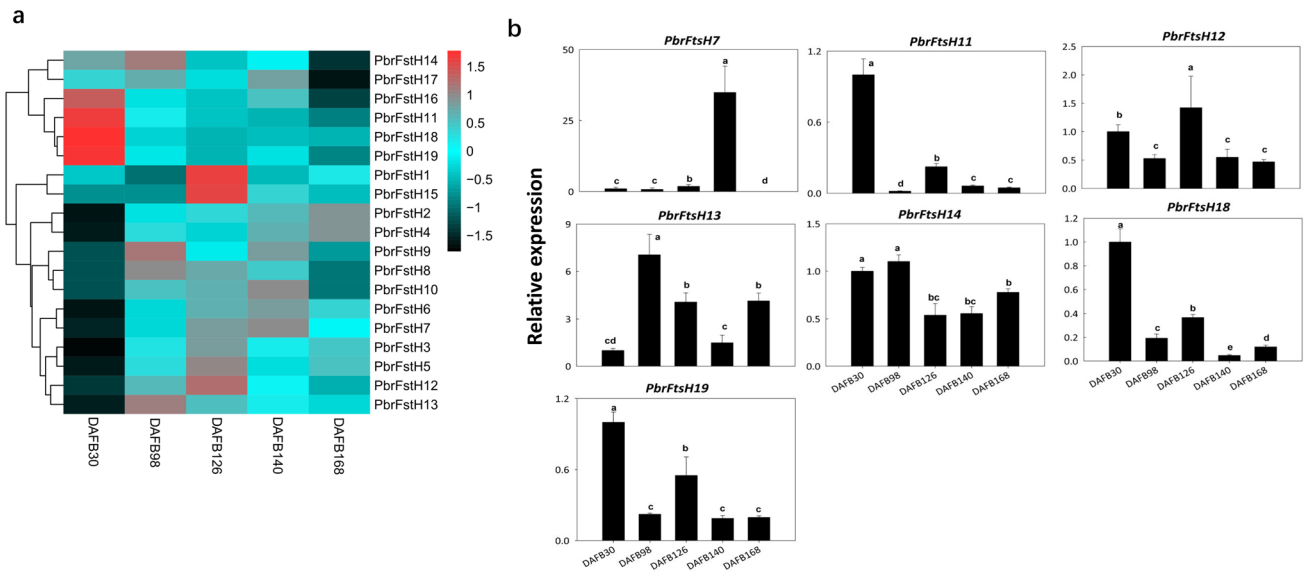

Fig. 8 Expression pattern analysis of PbrFtsH genes during 'Dangshansuli' pear fruit development stages. **a** Heat maps showed the hierarchical clustering of 19 PbrFtsHs based on their expression patterns by Transcriptome analysis. **b** 7 selected PbrFtsH genes expression patterns determined by qPCR assay

same cluster, and shared high similarity in motif and functional domain, respectively (Fig. 2 and Fig. 4).

It has been reported that most of AtFtsH proteins had two transmembrane domains at the N-terminus anchoring the FtsH proteins to the thylakoids or mitochondria membranes. The exception is AtFtsH11, which contained only one transmembrane domain (Chen et al. 2006). The transmembrane region and subcellular location of the PbrFtsH proteins were assessed in this study. One or two transmembrane regions were identified at the N-terminus in the majority of pear *FtsH* genes, anchoring the proteins to the membranes of the chloroplast or mitochondrion. PbrFtsH9 had no transmembrane region and the loss of a transmembrane region may lead to the loss of enzyme activity. Multiple sequence alignments revealed that five PbrFtsH proteins (PbrFtsH15, PbrFtsH16, PbrFtsH17, PbrFtsH18, and PbrFtsH19) had no zinc-binding domain, presumably indicating that they were inactive in proteolysis. The non-named region of homology was very similar to those in the rice and *Arabidopsis*, but the function remained unclear. Furthermore, the subcellular localization of PbrFtsH7, PbrFtsH12, PbrFtsH13 proteins in chloroplasts was consistent with published studies of *Arabidopsis* FtsHs. Therefore, the *PbrFtsH* genes have a similar subcellular localization pattern as the Arabidopsis *AtFtsH* genes, so the proteins may play important role in regulating chloroplast and mitochondria development, or photosystem II (PSII) reaction. (Zaltsman et al. 2005; Kato et al. 2009; Zhang et al. 2010).

Expression patterns of FtsHs in different development of pear fruit

We aimed to study its role in fruit developmental different stages. Based on the transcriptome data and RT-qPCR analysis, the expression of *PbrFtsH* genes during fruit development and enlargement were obtained. In this study, *PbrFtsH11*, *PbrFtsH16, PbrFtsH18*, and *PbrFtsH19* were strongly expressed in the fruitlet (28 DAFB), implying that the encoded proteins function in the fruitlet (Fig. 8a). The high expression of *PbrFtsH7*, *PbrFtsH12*, *PbrFtsH13*, and *PbrFtsH14* implied their key roles in enlargement stages. *PbrFtsH8* also showed the high expression in early (98 DAFB) and middle enlargement (126 DAFB) (Fig. 8a), which was consistent with the previous studies, in which *PbrFtsH8* was located within the Model I of the quantitative trait locus (QTL) (Pei et al. 2020). Pei et al. reported that pear fruit showed a single sigmoid pattern during development and single sigmoid patterns are coordinately mediated by Model I and II genes. It was indicated that *PbrFtsH8*, an *AtFtsH2-like* gene, was not only involved in chloroplast development, but also in fruit enlargement. Overall, these findings provided insight into the potential functions roles of *PbrFtsH* genes. However, the detailed biological functions of pear *FtsH* genes are still unclear. More in-depth studies about the *PbrFtsH* genes are required to better understand the roles in pear development.

Conclusions

A comprehensive analysis of FtsH family in pear was carried out in the present study. Nineteen full-length *FtsH* genes were characterized and further classified into eight main groups, with highly similar exon–intron structures and motif compositions within the same groups and subgroups. Distribution of *PbrFtsH* genes on chromosome displayed a clear preference. Synteny analysis and phylogenetic comparison of the *FtsH* genes from several different plant species, including *Arabidopsis,* provided valuable clues to the evolutionary history of pear *FtsH* genes. Subcellular localization showed that PbrFtsH were located in the mitochondrion or chloroplast. *PbrFtsH* genes played important roles in pear fruit growth and development as indicated by their expression patterns in different development stages of pear fruit. These results provide a valuable resource for better understanding the biological roles of individual *FtsH* genes in pear.

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Author contributions SLZ conceived this study and revised this manuscript; ZHG drafted the manuscript and contributed to most experiments with help from XG, HQC, and LY. CG conducted to transcriptome-based data analyses.

Declarations

Conflicts of interest The authors declare no conflict of interest.

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