Genome-wide identification and expression analysis of the \textit{ftsH} protein family and its response to abiotic stress in \textit{Nicotiana tabacum} L

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Abstract

**Background:** The filamentous temperature-sensitive H protease (\textit{ftsH}) gene family plays an important role in plant growth and development. \textit{FtsH} proteins belong to the AAA protease family. Studies have shown that it is a key gene for plant chloroplast development and photosynthesis regulation. In addition, the \textit{ftsH} gene is also involved in plant response to stress. At present, the research and analysis of the \textit{ftsH} gene family are conducted in microorganisms such as \textit{Escherichia coli} and \textit{Oenococcus} and various plants such as \textit{Arabidopsis}, pear, rice, and corn. However, analysis reports on \textit{ftsH} genes from tobacco (\textit{Nicotiana tabacum} L.), an important model plant, are still lacking. Since \textit{ftsH} genes regulate plant growth and development, it has become necessary to systematically study this gene in an economically important plant like tobacco.

**Results:** This is the first study to analyze the \textit{ftsH} gene from \textit{Nicotiana tabacum} L. K326 (\textit{NtftsH}). We identified 20 \textit{ftsH} genes from the whole genome sequence, renamed them according to their chromosomal locations, and divided them into eight subfamilies. These 20 \textit{NtftsH} genes were unevenly distributed across the 24 chromosomes. We found four pairs of fragment duplications. We further investigated the collinearity between these genes and related genes in five other species. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis identified differential expression patterns of \textit{NtftsH} in different tissues and under various abiotic stress conditions.

**Conclusions:** This study provides a comprehensive analysis of the \textit{NtftsH} gene family. The exon–intron structure and motif composition are highly similar in \textit{NtftsH} genes that belong to the same evolutionary tree branch. Homology analysis and phylogenetic comparison of \textit{ftsH} genes from several different plants provide valuable clues for studying the evolutionary characteristics of \textit{NtftsH} genes. The \textit{NtftsH} genes play important roles in plant growth and development, revealed by their expression levels in different tissues as well as under different stress conditions. Gene expression and phylogenetic analyses will provide the basis for the functional analysis of \textit{NtftsH} genes. These results provide a valuable resource for a better understanding of the biological role of the \textit{ftsH} genes in the tobacco plant.

**Keywords:** \textit{FtsH} family, Gene expression patterns, Abiotic stress

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**Background**

Chloroplasts are the site of photosynthesis in plants. The processing, maturation, and degradation of proteins in chloroplasts are important for normal plant growth and development and a common regulatory mechanism towards environmental changes [1]. Chloroplast proteases play an important role in this process, which...
maintains the stability of the protein system in the chloroplast and ensures the orderly progress of the normal life activities of plants. The proteases in plant chloroplasts are divided into three categories: metalloproteases, serine proteases, and aspartic proteases [2]. FtsH, a type of metalloprotease originally discovered in *Escherichia coli*, belongs to the AAA family of proteins. It is an ATP-dependent metalloproteinase encoded by the *ftsH* gene, with ATPase, proteolysis, and chaperone activity [3, 4]. The members of the *FtsH* family aggregate to form functional homohexamers or heterohexamers. Each *ftsH* protease contains an N-terminal transmembrane segment and a C-terminal region composed of the AAA-ATPase domain that belongs to the M41 peptidase family of protease domains [5, 6].

The FtsH protease plays an important role in eukaryotes and is generally found in mitochondria and chloroplasts. Several studies have shown that *ftsH* protease participates in the initial cleavage of D1-binding protein in the reaction center of photosystem II, thereby reducing the damage to the photosynthetic system from high-intensity light [7–10]. In addition, under the combined stress condition of high temperature and strong light, Deg1 and *ftsH* proteases participate in the regulation of D1 protein turnover. Recent studies have found that *ftsH* protease is essential for chloroplast development. An *ftsH* rice mutant plant show ed albino traits [11, 12]. In addition, *ftsH* protease plays an important role during stress conditions such as drought, high temperature, long exposure to ultraviolet light, and high salt concentration [10, 13–16].

As a model organism for studying the basic biological processes of plants, tobacco is also a source of plant BY-2 cell lines and an important tool for plant molecular research. Like other nightshade plants, including potatoes, tomatoes, and peppers, it is also used as a model for plant disease susceptibility, and tobacco is an important commercial crop. The growth and development of tobacco are affected by the environment showing varied growth rates under different environmental conditions. An unsuitable environment can inhibit tobacco growth and even lead to plant death.

At present, the analytical research on the *ftsH* gene family is carried out on a genome-wide scale that involves many organisms, including microorganisms such as *Lactobacillus plantarum*, *Escherichia coli*, *Bacillus subtilis*, cyanobacteria, *Acidovorax citrulli* [4, 9, 17–20], and plants such as Arabidopsis, pear, rice, corn, and peanut [14, 21–25]. However, no report on the *ftsH* gene family has been found in tobacco. Because of the importance of the *ftsH* gene family in the process of plant growth and development, it is also to screen out new resistance genes and provide a basis for the molecular mechanism of tobacco resistance to stress. Therefore, it is important to systematically study the tobacco *ftsH* gene family. This study performed a comprehensive analysis of the *NtftsH* gene family, including gene structure, motif composition, chromosomal location, and phylogenetic tree. The evolutionary relationship of tobacco with multiple species was established by sequence comparison, and the expression profile of *FtsH* under different stress conditions was analyzed. Therefore, this study puts across an in-depth understanding of the function of the *ftsH* gene family, specifically its role in stress resistance in tobacco.

**Results**

**Identification of tobacco *ftsH* gene**

From tobacco’s whole genome sequence data, we identified 20 members of the *ftsH* gene family (Additional file 1: Table S1). The genes were renamed according to their order and position on the chromosome, from *NtftsH*1 to *NtftsH*20 [26]. At the same time, the physicochemical properties and subcellular localization of gene sequences were analyzed. *NtftsH*9 is the smallest protein with 456 amino acids (aa), while *NtftsH*3 is the largest protein with 1239 amino acids (aa); the molecular weights of proteins range from 51.15 (*NtftsH*9) to 135.53 kDa (*NtftsH*3), the Pi ranged from 5.61 (*NtftsH*13) to 11.17 (*NtftsH*18).

The results of subcellular localization showed that except few (*NtftsH*3, *NtftsH*7, *NtftsH*13, and *NtftsH*17), the *ftsH* genes are expressed in the chloroplast, many of which were specific to the chloroplast (*NtftsH*5, *NtftsH*10, *NtftsH*14, and *NtftsH*16). In addition, except for the three genes *NtftsH*5, *NtftsH*10, and *NtftsH*14, 17 other *ftsH* gene products were localized in the nucleus, out of which, three were exclusively localized in the nucleus (*NtftsH*3, *NtftsH*7, and *NtftsH*13). Finally, *NtftsH*17 is exclusively expressed in the mitochondria.

**Multiple sequence alignment and phylogenetic analysis of *NtftsH* gene**

The multiple sequence alignment of tobacco *ftsH* protein sequences from tobacco (*NtftsH*) and *Arabidopsis thaliana* (*AtftsH*) showed the highly conserved regions of these proteins had specific amino-acid sequences, PGT-GKT and RPRG (Additional file 2: Figure S1). *FtsH* proteins sequences from Arabidopsis (dicotyledonous), rice (monocotyledonous), and tobacco were aligned to construct a phylogenetic tree to understand the evolutionary relationship. In addition, the 41 *ftsH* genes were divided into 8 groups according to the percentage of homology between these genes. The results showed that 8 genes from rice and 9 *NtftsH* genes belonged to group I and group VIII, respectively; others were distributed from group II to group VII (Fig. 1). Compared with rice, the
homology between tobacco and Arabidopsis ftsH genes is higher. This phenomenon may be due to the difference in leaf structure between tobacco, Arabidopsis and rice, resulting in differences in homology. The subcellular localization results revealed a higher homology between genes expressed in the same site either in tobacco or in Arabidopsis, which may be due to their similar function. It is logical to conclude that the NtftsH and AtftsH proteins may perform the same biological function in the cell.

**Structure and motif composition of NtftsH gene family**
Based on the gene annotation (GFF format), the gene structure of the NtftsH gene was analyzed. The results showed that all NtftsH genes contained multiple exons and introns (Fig. 2A,B). Also, the number and lengths of introns/exons of the genes grouped into the same branch of the evolutionary tree were roughly similar. Of these, 9 NtftsH genes had untranslated regions (UTRs).

All 20 NtftsH genes contained FtsH-related conserved domains — a hallmark feature of the ftsH protein family (Fig. 2A, C). In addition, the ftsH gene is not only highly conserved in chloroplasts and mitochondria but also homologous to the membrane-bound ATP-dependent protease in prokaryotes.

Using MEME online software, the composition of NtftsH conservative motif and the number of conservative motifs were analyzed, and a total of 10 conservative motifs were identified, which were named motif1-motif10 in turn (Fig. 2D). All tobacco ftsH genes contain conservative motifs 2 and 3. In addition to NtftsH19, the remaining 19 members of the family have developed conserved motifs6, suggesting that these motifs can serve as markers for identifying the NtftsH gene. At the same time, we
found that genes in the same branch contain basically the same number and type of motif, and in general, the sequences of closely related members are similar, and the motif is similar. The existence of the same type of conserved motif may indicate similar functions among members of the NtftsH gene family, and also confirm the correct construction of the evolutionary tree.

Chromosomal location of NtftsH gene and gene segment duplication events

Chromosomal location analysis was performed to further understand the distribution of NtftsH genes in chromosomes (Additional file 3 Figure S2). Out of 24 chromosomes (Nt1-Nt24), Nt1, Nt3, Nt4, Nt7, Nt9, Nt10, Nt11, Nt13, Nt15, Nt20, and Nt21 did not contain the ftsH gene; the 20 ftsH genes are unevenly distributed on the remaining chromosomes. Three NtftsH genes were mapped to chromosomes Nt17 and Nt24 each, and two NtftsH genes were located on each of the chromosomes Nt12, Nt16, and Nt23. The reason for this phenomenon may be due to the ftsH gene duplication or gene loss during the evolution of the tobacco plant, resulting in the absence of the ftsH gene on some chromosomes and the appearance of multiple genes on others. In addition, the frequency of gene occurrence was not related to chromosome length and gene density [26].

Some studies have shown that the expansion of plant gene families is mainly facilitated by segmental and tandem duplication events [27, 28]. Segmental duplication frequently occurs during polyploidization and chromosomal rearrangements, resulting in the presence of repeated chromosomal blocks in the genome; tandem duplications are clusters of multiple adjacent homologous gene family members (two or more members) on a chromosome [29]. We explored and screened gene pairs showing segmental and tandem duplication throughout the tobacco genome to better understand gene duplication events in tobacco. According to the definition of segmental and tandem duplications, the points on the diagonal line correspond to tandem duplication gene pairs, and the rest of the points indicate segment duplication gene pairs. Our findings support that tobacco has a highly repetitive genome that contains gene pairs with a large number of segmental duplications (Fig. 3;
Supplementary file 4 Table S2). In the Circos plot (Fig. 3), the four gene pairs with segmental duplications are connected by red curves. Taken together, we suggest that the expansion of the \( \text{NtftsH} \) gene family is related to tandem and segment duplication events, the latter being the main source of adding new members to the \( \text{NtftsH} \) gene family.

**Analysis of the cis-acting element of the NtftsH gene**

Using the 2000 bp upstream sequence from the \( \text{NtftsH} \) gene promoter, cis-acting elements were analyzed for 20 \( \text{NtftsH} \) genes. According to the results (Fig. 4; Additional file 5 Table S3), cis-acting elements are regulated by hormones and abiotic stress conditions (drought, cold, and light). Light-responsive cis-acting elements were the most abundant, followed by hormone-related response elements (RE), such as abscisic acid-RE, gibberellin-RE, auxin-RE, methyl jasmonate-RE, and salicylic acid-RE. The results showed that these cis-acting elements also contain regions associated with the cell cycle, endosperm development, and circadian rhythm. Nociceptive RE (a

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**Fig. 3** Schematic diagram of the chromosomal distribution and segmental duplication of the \( \text{NtftsH} \) genes. Grey lines represent all colinear blocks in the tobacco genome, and red lines represent duplicated \( \text{ftsH} \) gene pairs.
cis-acting element that responds to biotic stress) was also identified in the tobacco \( ftsH \) gene promoter. It indicates that the \( NtftsH \) genes are closely related to plant resistance to stress conditions, including drought, low temperature, and intense light.

**Synteny analysis of \( FtsH \) genes**

To find the collinear relationship between members of the \( NtftsH \) gene family and \( ftsH \) genes of other species, we selected a dicotyledonous plant (Arabidopsis), a monocotyledonous plant (rice), and three Solanaceae plants (tomato, potato, and pepper). Collinear blocks of 5, 4, 15, 15, and 12 were identified in Arabidopsis, rice, tomato, potato, and pepper, respectively. Therefore, based on the collinearity between tobacco and these 5 plants, we have drawn 4 collinear diagrams representing homologous gene pairs of tobacco and other species, connected by blue lines (Fig. 5; Additional file 6 Table S4). There are five pairs of \( ftsH \) genes between tobacco and Arabidopsis, and 4 pairs of genes between tobacco and rice collinear. About 11 genes among the 20 identified \( ftsH \) genes showed no collinearity with either Arabidopsis or rice, suggesting that these homologous gene pairs may have formed after the divergence of dicots and monocots or after the divergence of the two species. In addition, a collinear pairing of the \( ftsH \) genes was found between tobacco and two other species, suggesting that this gene (\( NtftsH6 \)) may have existed before evolutionary differentiation. We found that the \( ftsH \) genes in tobacco and three Solanaceae plants have high homology among the same family members; few genes without many homologies may have happened due to the independent expansion of the \( ftsH \) gene family in these plants.

**GO annotation analysis of \( ftsH \) gene**

The Gene Ontology (GO) analysis was performed and the \( AtftsH \) protein sequence was used as a reference. The results showed that \( NtftsH \) protein might be involved in various biological, cellular, and molecular processes (Fig. 6; Additional file 7 Table S5). Analysis of the biological processes mediated by \( NtftsH \) protein showed that \( NtftsH \) protein is mainly involved in catabolic processes, precursor metabolites and energy production, macromolecular catabolic processes, nitrogen compound metabolism, organelle organization, protein breakdown, and responding to light stimuli, radiation, and cellularity. In addition, analysis of cellular components revealed that \( NtftsH \) protein is localized in intracellular organelles, including chloroplasts, mitochondria, and symplasts. The \( NtftsH \) protein also has various molecular functions, including ATP-dependent peptidase activity, catalytic enzyme activity, hydrolase activity, and metalloprotease activity.
Fig. 5  Syntenic analysis of *ftsH* genes between tobacco and five representative plant species. Gray lines in the background indicate the collinear blocks within the tobacco and other plant genomes, while the blue lines highlight the syntenic *ftsH* gene pairs.
**Gene tissue expression specific analysis**

To study the physiological role of the *NtftsH* gene, we selected 9 out of 20 *NtftsH* genes to study their expression patterns. The expression levels of these 9 genes in 7 tissues (calyx, petals, stigma, leaves, stems, fruits, and roots) were detected by qRT-PCR (Fig. 7). The expression patterns of these genes in different organs of tobacco varied, indicating that *NtftsH* genes have diverse functions in tobacco. The selected 9 genes were expressed in all organs. The expression levels of *NtftsH*3, *NtftsH*9, *NtftsH*12, and *NtftsH*20 were higher in leaves than in other organs, and the amount of *NtftsH*5 in leaves was second only to calyx. In the calyx tissue, *NtftsH*5, *NtftsH*11, and *NtftsH*14 genes were highly expressed, and the expression levels of other genes were relatively low; *NtftsH*18 was highly expressed in the petal tissue. The expression of *NtftsH*10 was the highest in the stigma tissue, followed by *NtftsH*3, *NtftsH*5, *NtftsH*9, and *NtftsH*20. All nine genes were expressed in roots; *NtftsH*20 had the highest expression, followed by *NtftsH*12.

**Gene expression analysis under different stresses**

To study the expression of *NtftsH* under abiotic stress conditions, we used qRT-PCR to evaluate the expression of 3 *NtftsH* genes in roots, stems, and leaves. Around 9 abiotic stress situation was created with the following: polyethylene glycol (PEG), high-temperature, low temperature, strong light, high salt (NaCl), ultraviolet (UV) light, salicylic acid, auxin, and gibberellin treatments (Fig. 8). The expression patterns of each *NtftsH* gene were different under the 9 abiotic stress conditions; some genes were significantly up-regulated, and some genes were inhibited. The expression patterns of most genes changed significantly during the early stages of the stress response (Fig. 8); the expression of some *NtftsH* genes also changed over time or in different tissues.
under several stress treatments. For example, in response to 15% PEG6000, the expression of NtftsH3 in leaves reached a peak post 12 h of treatment and then gradually decreased; both NtftsH5 and NtftsH12 were up-regulated with prolonged treatment time, but the expression level of NtftsH12 was not as high as that of NtftsH5. The expression levels of all NtftsH in tissues showed a trend of initial up-regulation and then down-regulation; NtftsH5 and NtftsH12 also demonstrated this expression pattern in stem and leaf tissues. The low-temperature stress induced the expression of the three genes in roots, stems, and leaves, which also showed a trend of high production initially and reached a very significant level in leaves. Also, under the stress of high temperature, the expression of NtftsH12 was high in the stem at 24 h of treatment. Around 24 h after hormone treatment with IAA or SA, the expressions of NtftsH3 in various tissues were significantly down-regulated, NtftsH5 was up-regulated in leaves, and NtftsH12 was up-regulated in roots and stems.

**Discussion**

FtsH gene is commonly present in all plants and plays an important role in plant growth and development, especially in the development of plant chloroplasts. This gene has been intensively studied in many plants, including tomato, potato, and rice plants. The genetic background of common tobacco is special, it is allotetraploid, and it is divided into ss and tt. The gene mutation in the evolution process and the gene recombination in the process of tobacco cross-breeding make the ss and tt blend, making the tobacco genetic background more complex, so the analysis of the tobacco FtsH gene family is very important [30]. The ftsH gene family has been widely studied in many species based on genome-wide analysis. In this study, 20 genes were identified as members of the

![Fig. 7](image_url)
ftsH gene family in the tobacco genome; they were named according to their distribution and location on the chromosome, from NtftsH1 to NtftsH20. Similar numbers of ftsH genes were found in other plants even though genome size varies between species. For example, rice has 9 and Arabidopsis has 12 ftsH genes. This indicates that the number of ftsH family members is relatively stable and has no absolute correlation with genome size.

In the process of plant evolution, the appearance and expansion of introns are related to gene family expansion and subsequent acquisition of new gene functions; introns usually appear in the early stage of gene family expansion but gradually disappear over time [31–33]. We found that introns are widespread in the ftsH gene through the study of gene structure. The number of introns in the 20 NtftsH genes ranged from 3 to 27. The structure of the NtftsH gene is similar to that of Arabidopsis, pear, and other plants, and this indicates that the FtsH gene is highly conserved among different species. Some of these genes contain large-span introns, which may be recently evolved NtftsH genes. NtftsH genes clustered on the same phylogenetic branch exhibited similar intron–exon composition and conserved domains, suggesting that they perform similar functions in plants. Notably, NtftsH shows similarity in structure and conserved domains with members of the ftsH gene reported in rice, pear, and apple [23]. At the same time, some NtftsH gene members contain the FtsH_ext domain at the N-terminus, and this change in the structure could be due to the altered function of the NtftsH gene. In addition, the conservation of the ftsH domain maintains the basic functions of the gene family, as diversity reduces the selection pressure during evolution [34].

Gene duplication plays an important role in biological evolution and the expansion of gene families. This study found that the NtftsH gene had 4 pairs of segmental duplications. Similar to a previous study on the pear tree [23], no tandem duplication of the NtftsH gene was found in the tobacco genome. These results suggest that segment duplication may contribute more to the expansion of the NtftsH family than tandem duplication. This is consistent with previous studies on other plant species, and segment duplication events may have contributed to the formation and evolution of some NtftsH genes. Out of 24, only 13 chromosomes harbor NtftsH genes, and there was no correlation between the distribution of the NtftsH gene and the density of genes on the chromosomes. This suggests that the NtftsH gene family was affected by gene deletion during the evolutionary process. Therefore, some ftsH genes may have arisen by duplication events, suggesting that duplication events played an important role in the rapid expansion of ftsH family members in plants.

Fig. 8 Expression of 3 Nicotiana tabacum L. ftsH in plants subjected to abiotic stresses (NaCl, strong light, PEG6000, UV, 38 °C, 4 °C, and hormones (100 μmol/L IAA, SA, GA3)) at the seedling stage. ftsH: filamentous temperature-sensitive H; qRT-PCR: Real-time quantitative PCR. Expression patterns of 3 NtftsH in root, stem, and leaf organs were examined by qRT-PCR. Error bars represent standard error. Lowercase letters above bars indicate a significant difference (P < 0.05, LSD) among the treatments.
Due to the complexity of biological research and the increasing scale of biological research, Gene Ontology provides a comprehensive description method for gene function and its products in order to describe the gene products uniformly. We performed GO analysis on the NtftsH gene based on the Arabidopsis protein sequence. NtftsH is structurally similar to rice, Arabidopsis and other plants, which may lead to their similarities in molecular functions. FtsH proteins are ATP-dependent active enzymes and possess hydrolase activities as well. According to the GO annotation results, the ftsH gene is a component of important cellular organelles such as chloroplasts and mitochondria, and is also involved in the catabolism of PSII-related light-harvesting complexes, which is similar to the studies of Arabidopsis, rice, bayberry and other plants [16, 35–37]. Deg1 protein in photosynthesis photosystem II was degraded to prevent damage to leaves under strong light conditions, and knockout of the rice ftsH gene resulted in an albino phenotype of the F2 generation, indicating that the ftsH gene is involved in the synthesis of chloroplasts [7, 8, 11]. The GO annotation results showed that the ftsH is also involved in the development of plant embryos and leaves similar to the function of AtftsH reported in Arabidopsis plants [38, 39], further confirming the accuracy of the GO annotation results. Many elements related to chloroplast and photosynthesis are enriched in the annotation of NtftsH molecular functions, indicating that the ftsH gene is closely related to photosynthesis functions. And it has been confirmed in cyanobacteria, Arabidopsis, bayberry, rice, corn and other organisms that perform photosynthesis [7, 9–11, 24, 40].

Analysis of gene expression profiles can provide a better understanding of the potential biological functions of genes. To understand the role of NtftsH, we selected 9 NtftsH genes with significant structural differences to study their expression patterns in different tissues. These 9 NtftsH genes were expressed in all organs tested, with relatively high expression levels in calyx, leaves, and roots indicating its role in growth and development — this result corroborates with the findings in Arabidopsis and rice.

Several studies have shown that the NtftsH gene plays an important role in plant stress response [41–44]. We also investigated the gene expression patterns of three NtftsH genes under nine abiotic stress conditions. The results showed that the expression of most NtftsH genes varies under different abiotic stress situations. For example, under drought stress, the expression of NtftsH5 was up-regulated in roots, stems, and leaves; the expression of NtftsH12 was up-regulated first and then down-regulated in roots, stems, and leaves. Interestingly, the expression of the same genes showed an opposite profile under other stress conditions. In stems, the expression of the NtftsH3 gene was up-regulated under high-temperature stress but remained unchanged under cold stress. These results suggest that NtftsH genes are involved in the plant’s response to abiotic stress and participate in a complex cross-regulatory network in plants. When the plants were subjected to cumulative abiotic stressors such as PEG, high temperature, low temperature, strong light, and NaCl, the expression levels of these three genes changed significantly in different tissues. This indicated that they may be co-expressed under various stress conditions. This is confirmed with the results of the cis-acting element analysis, which shows that the NtftsH gene contains a large number of cis-acting elements related to light and temperature stress. It may be that these cis-acting elements and their corresponding transcription factors are activated under stress, thereby regulating the expression of the NtftsH gene. Previous studies have shown that the ftsH gene is an important regulatory protein in plant response to high light stress. Therefore, the NtftsH gene may have functions similar to the ftsH genes of other plants [7, 8, 44]. Follow-up studies can verify the function of the NtftsH gene and assess its ability to resist stress conditions in different varieties of tobacco plants.

In conclusion, this study provides a comprehensive survey of the NtftsH genes as a reference to studying the ftsH genes’ function. In this study, we verified the potential role of the NtftsH gene in the process of the plant facing adversity in the form of various stress by analyzing gene expression profiles. However, the role of the NtftsH gene in response to stress and other biological processes requires further studies.

**Conclusion**

A comprehensive analysis of the tobacco ftsH gene family was performed in this study. The identified 20 ftsH genes were renamed and divided into different groups according to the classification of AtftsH in Arabidopsis and OsftsH in rice and the evolutionary relationship between different members. The exon–intron structure and motif composition of the NtftsH genes in the same evolutionary branch show high similarity. Homology analysis and phylogenetic comparison with ftsH genes from several other plants provided valuable clues for studying the evolutionary characteristics of NtftsH genes. The NtftsH gene plays an important role in plant growth and development, as indicated by its expression patterns in different tissues and under different treatment conditions. Phylogenetic and gene expression analyses will provide the basis for the functional analysis of the NtftsH gene in the future. The results of this study offer a valuable resource for a better understanding of the biological role of the ftsH gene in tobacco.
Methods

FtsH gene identification
We downloaded the Hidden Markov Model (HMM) file corresponding to the FtsH domain (PF06480) from the Pfam protein family database (http://pfam.xfam.org). The ftsH gene was searched from the tobacco genome database using HMMER 3.0 (http://hmmer.org/), with default parameters, and the cut-off was set to 0.01 [45]. All candidate genes that may contain the ftsH domain were shortlisted based on HMMER 3.0 analysis; Pfam and SMART programs were then used to confirm the ftsH core sequence [46–48]. Each potential gene was then manually inspected to ensure that conserved sequences in the N-terminal region of the ftsH domain conformed to the predicted sequences. The wrongly predicted genes were manually screened out, some genes were verified by PCR amplification and sequencing, and redundant sequences were manually discarded. After the comprehensive screening, 20 NtftsH genes were finally identified in the tobacco genome, and then they were renamed according to the order and position of the genes on the chromosome [49]. The physicochemical properties such as molecular weight (MW) and isoelectric point (pI) of NtftsH family members were predicted and analyzed using the online analysis software ExPASy (https://web.expasy.org/) [50]. Subcellular localization prediction was performed using Cell-Ploc2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/) [51].

Sequence and phylogenetic analyses of ftsH genes
We used the ClustalW default parameters to align the protein sequences of ftsH from tobacco and Arabidopsis thaliana. After the null members were removed, the phylogenetic trees were constructed in MEGA 11.0, based on the equally weighted neighbor-joining method. Bootstrap values of the phylogenetic tree were calculated with 1000 replicate analyses. The amino acid sequence of the deduced ftsH domain was then manually adjusted using GeneDoc software. We used the online website Gene Structure Display Server (GSDS: http://gsds.cbi.pku.edu.cn) to compare the predicted coding sequence with the corresponding full-length sequence to determine the exon–intron of the NtftsH gene structure [52]. Conserved domain analysis was performed by MEME (Multiple Expectation maximizations for Motif Elicitation; http://meme-suite.org/tools/meme) online software, the number of motifs was set to 10, and the range of motif width was set to 50–100 amino acids [53].

Chromosomal distribution and analysis of promoter cis-acting elements
The tobacco annotation information (https://solgenomics.net/), present in the Solanaceae database, helped to identify the beginning of the ftsH gene on the chromosome. The duplication events of the genes were assessed by comparing the two genes. The NtftsH genes were mapped to chromosomes using the online tool MapGene2Chrom web v2 (http://mg2c.iask.in/mg2c_v2.0/) after determining their chromosomal positions from the tobacco genomic database. The 2000 bp upstream gene sequence of NtftsH was intercepted from the Solanaceae database, and the cis-acting elements were analyzed by screening the PlantCARE database (to identify plant cis-acting regulatory elements).

Gene duplication and gene collinearity analysis
Gene duplication events were analyzed using the multicollinear scanning tool (MCScanX) [54]. The ftsH collinear analysis map was constructed by Dual Syteny Plotter software, and the ftsH collinear relationship between tobacco and pepper, tomato, potato, rice, and Arabidopsis were determined separately.

Plant material and its handling
Nicotiana tabacum L. (K326) seeds, provided by the Key Laboratory for Tobacco Quality Research Guizhou Province, were used as the experimental material. Tobacco seeds were sown on a special substrate for flue-cured tobacco with the following conditions: 75% relative humidity, temperature 28 °C during the day (16 h) and 20 °C at night (8 h), and floating seedlings. About 80 days after transplanting, the calyx, petals, anthers, stigmas, leaves, stems, fruits, and roots of 3 similar healthy plants were collected; this sampling was repeated three times. We selected 3 NtftsH genes to study the expression patterns of NtftsH genes under different abiotic stress conditions. With the emergence of the sixth leaf, the plants showing similar growth vigor were selected and subjected to stress conditions as follows: salt (5% NaCl), strong light (1000 μmol m−2 s−1), drought (15% PEG6000), UV, high temperature (38 °C), low temperature (4 °C), and hormones (100 μmol/L IAA, SA, GA3). Three replicates were used for each abiotic stress treatment; samples were collected after 0 h, 2 h, 12 h, and 24 h of treatment. The samples were either immediately frozen in liquid nitrogen or stored in an ultra-low temperature freezer at −80 °C for subsequent qRT-PCR analysis.

Extraction of the total RNA from the plant material, reverse transcription of cDNA, and analysis by qRT-PCR
Total RNA was extracted from the sample with RNAprep Pure Plant Kit (TIANGEN, DP432), and its quality and concentration were detected by agarose gel electrophoresis and concentration detector, respectively. cDNA was obtained using FastKing gDNA Dispelling RT SuperMix kit (TIANGEN, KR118) following the manufacturer's
instructions. We selected nine NtftsH genes to study gene expression patterns. qRT-PCR primers were designed by Primer Premier, version 6 (Additional file 5: Table S5) to analyze gene expression. We used the Actin gene as an internal PCR control because it is stably expressed in almost all tissues at every growth phase. We performed the qRT-PCR analysis with Talent qPCR PreMix (SYBR Green; TIANGEN, FP209). Each reaction was performed in three biological replicates, and the final detection result was calculated by the $2^{-\Delta\Delta Ct}$ method [55].

Statistical analysis

SPSS (Statistical Product and Service Solutions) software was used to analyze the variance of the data, and data were compared with the least significant difference test at the 0.05 and 0.01 levels. OriginPro 2021 software was used for the generation of graphs.

Abbreviations

ftsH: Filamentous temperature-sensitive H; CDS: Coding sequence length; SPSS: Statistical Product and Service Solutions; MEGA: Molecular Evolutionary Genetics Analysis; MEME: Multiple Expectation maximizations for Motif Elcita-
tion; Mw: Protein molecular weight; PEG: Polyethylene glycol; pI: Isoelectric point; qRT-PCR: Real-time quantitative PCR; SMART: Simple Modular Architecture Research Tool; UV: Ultraviolet.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08719-x.

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Authors’ contributions

TP and YL planned and designed the research, analyzed the data, and wrote the manuscript. MZ and TP studied the gene expression by qRT-PCR. TP and LS identified the gene family and analyzed gene structure, chromosome distribution, and gene duplication, and performed syntenic analysis. LW and KW analyzed the evolutionary relationship of ftsH in several different species. YL supervised the research. JY revised the manuscript. RL provides the seeds for the experiments. All authors read and approved the final manuscript.

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Availability of data and materials

The whole Nicotiana tabacum L. genome sequence information was obtained from the Sol Genomics website (https://solgenomics.net/organism/Nicotiana_tabacum/genome) and the website is open to all researchers. Nicotiana tabacum L. (K326) seeds, provided by the Key Laboratory for Tobacco Quality Research Guizhou Province, were used as the experimental material. The datasets supporting the conclusions of this article are included in the article and its Additional files.

Declarations

Ethics approval and consent to participate

We guarantee that the collection of plant material and experimental research and field studies on plants comply with relevant institutional, national, and international guidelines and legislation.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Liu X, Zhang X, Cao R, Jiao G, Hu S, Shao G, Sheng Z, Xie L, Tang S, Wei X, et al. CDE4 encodes a pentatricopeptide repeat protein involved in chloroplast RNA splicing and affects chloroplast development under low-temperature conditions in rice. J Integr Plant Biol. 2021;63(10):1724–39.
2. Nishimura K, Kato Y, Sakamoto W. Chloroplast Proteases: Updates on Proteolysis within and across Suborganellar Compartments. Plant Physiol. 2016;171(4):2280–93.
3. Schumann W. FtsH – a single-chain charonin? FEBS Microbio Rev. 1999;23(1):1–11.
4. Tomoyasu T, Taki T, Morimura S, Morii H, Yamanaka K, Niki H, Hiraga S, Ogura T. The Escherichia coli FtsH protein is a prokaryotic member of a protein family of putative ATPases involved in membrane functions, cell cycle control, and gene expression. J Bacteriol. 1993;175(5):1344–51.
5. Langklotz S, Baumann U, Narberhaus F. Structure and function of the bacterial AAA protease FtsH. Biochimica et Biophysica Acta (BBA) Mol Cell Res. 2012;1823(1):40–8.
6. Bieniossek C, Schalch T, Bumann M, Meister M, Meier R, Baumann U. The molecular architecture of the metalloprotease FtsH. Proc Natl Acad Sci U S A. 2006;103(9):3066–71.
7. Miho Y, Yasui Y. Quality control of Photosystem II: Where and how does the degradation of the D1 protein by FtsH proteases start under light stress? – Facts and hypotheses. J Photochem Photobiol B Biol. 2011;104(1):229-35.
8. Yoshida K, Uchida S, Morii H, Komayama K, Ohira S, Morita N, Nakano-Tsukada Y. Quality control of photosystem II. J Biol Chem. 2006;281(31):21660–9.
9. Komenda J, Barker M, Kuvikova S, de Vries R, Mullineaux CW, Tchyi M, Nixon PJ. The FtsH-Protease slt0228 Is Important for Quality Control of Photosystem II in the Thylakoid Membrane of Synechocystis sp. PCC 6803. J Biol Chem. 2006;281(12):1145–51.
10. Cheregi O, Sirosa C, Kös PB, Barker M, Nixon PJ, Vass I. The role of the FtsH and Deg proteases in the repair of UV-B radiation-damaged Photosys-
tem II in the cyanobacterium Synechocystis PCC 6803. Biochimica et Biophysica Acta (BBA) Bioenergetics. 2007;1767(6):820–8.
30. Yu J, Tang T, Yang L, Wang Q, Zhao Y, Jiang D, Ruan X. The essential roles of OsFtsH2 in developing the chloroplast of rice. BMC Plant Biol. 2021;21(445):1-14.

12. Yu F, Park S, Rodermel SR. The Arabidopsis FtsH metalloprotease gene family: interchangeability of subunits in chloroplast oligomeric complexes. Plant J. 2004;37(6):864–76.

13. Yuan L, Huijun J, Jun L, Yunguo L, Xiaojun H. Characteristics of TaFtsH6 Gene and Expression Analysis under Dry and Heat Stress in Wheat. Mol Plant Breed. 2020;6(10):1-12.

14. Zheng C, Kong X, Su J, Shu C, Zhao C. Identification, classification and expression analysis of metalloprotease family gene FtsH in peanut under salt stress. Jiangsu Agric Sci. 2016;44(12):74–7.

15. Xu T. The molecular mechanism study about photoinhibition of Bayberry (Myrica rubra Siebert Zucc.) leaves under the combined stress of light and low temperature. Zhejiang A&F University; 2015.

16. Wang L. The Study of the Gas Exchange and FtsH Gene Expression in Bayberry (Myrica rubra Siebert Zucc.) under High Temperature and Strong Light. Zhejiang A&F University; 2014.

17. Fiocco D, Collins M, Muscariello L, Hols P, Kleerebezem M, Msaedek T, Spano G. The Lactobacillus plantarum ftsH gene is a novel member of the ctsr stress response regulon. J Bacteriol. 2009;191(5):1688–94.

18. Deuering L, Mogk A, Richter C, Purucker M, Schumann W. The FtsH gene of Bacillus subtilis is involved in major cellular processes such as sporulation, stress adaptation and secretion. Mol Microbiol. 1997;23(5):921–33.

19. Bove P, Capozzi V, Gorafalo C, Rieu A, Spano G, Fiocco D. Inactivation of the ftsH gene of Lactobacillus plantarum W25F1. Effects on growth, stress tolerance, cell surface properties and biofilm formation. Microbiol Res. 2012;167(4):187–93.

20. Wei-qin J, Jian-pei Y, Xue B, Pei Q, Zhi-peng L, Yi-wen Y, Wei G, Tian-chang L. Functional analysis of the gene ftsH in Acidovorax citrulli. Acta Phytopathologica Sinica. 2019;49(04):488–99.

21. Zhang J, AS,. Genome-wide comparative analysis of the metalloprotease ftsH gene families between Arabidopsis thaliana and rice. BMC Plant Biol. 2021;21(561):1-21.

22. Wei-qin J, Jian-pei Y, Xue B, Pei Q, Zhi-peng L, Yi-wen Y, Wei G, Tian-chang L. Two members of the FtsH gene family in maize (Zea mays L.). Mol Biol Rep. 2010;37(2):855–63.

23. Guo Z, Gao X, Cai H, Yu L, Gu C, Zhang SL. Genome-wide identification, classification and expression profiling during development and abiotic stresses. BMC Plant Biol. 2021;21(578):1-20.

24. Cannon SB, Mitra A, Baumgarten A, Young ND, May G. The roles of segmental and tandem gene duplication in the evolution of large gene families in Arabidopsis thaliana. BMC Plant Biol. 2004;4:10.

25. Zhu Y, Wu H, Song W, Yin Q, Qin X, Yan Y, Hu Y. Soybean (Glycine max) expansin gene superfamily origins: segmental and tandem duplication events followed by divergent selection among subfamilies. BMC Plant Biol. 2014;14:93.

26. Yu J, Wang J, Lin W, Li S, Li H, Zhou J, Ni P, Dong W, Hu S, Zeng C, et al. The Genomes of Oryza sativa: a history of duplications. PLoS Biol. 2009;7(3):202–8.

27. Guo An-Yuan ZQCX. GSDS: a gene structure display server. Hereditas. 2009;153(3):157–61.

28. Letunic I, Bork P. 20 years of the SMART protein domain annotation resource. Nucleic Acids Res. 2018;46(W1):W200–4.

29. Roy SW, Penny D. A Very High Fraction of Unique Intron Positions in the Intron-Rich Diatom Thalassiosira pseudonana Indicates Widespread Intron Gain. Mol Biol Evol. 2007;24(7):1447–57.

30. Iwamoto M, Maekawa M, Saito A, Higo H, Higo K. Evolutionary relationship of plant catalase genes inferred from exon-intron structures: isozyme divergence after the separation of monocots and dicots. Theor Appl Genet. 1998;97(1):9–19.

31. William Roy S, Gilbert W. The evolution of spliceosomal introns: patterns, puzzles and progress. Nat Rev Genet. 2006;7(3):211–21.

32. Li W, Shang H, Ge Q, Zou C, Cai J, Wang D, Fan S, Zhang Z, Deng X, Tan Y, et al. Genomic-wide identification, phylogeny, and expression analysis of pectin methylesterases reveal their major role in cotton fiber development. BMC Genomics. 2016;17(1):1-13.

33. Zaltsman A, Or N, Adam Z. Two Types of FtsH protease gene blocks are required for chloroplast biogenesis and photosystem II repair in Arabidopsis. Plant Cell. 2005;17(10):2782–90.

34. Wang R, Zhao J, Jia M, Xu N, Liang S, Shao J, Qi Y, Liu X, An L, Yu F. Balance between cytosolic and chloroplast translation affects leaf variegation. Plant Physiol. 2018;176(1):804–18.

35. Smakowska E, Skibior-Blazyczyk R, Czarna M, Kolodziejczak M, Kwasniak-Owczezak M, Parys K, Funk C, Janska H. Lack of FTSH4 Protease affects protein carbonylation, mitochondrial morphology, and phospholipid content in mitochondria of arabidopsis; new insights into a complex interplay. Plant Physiol. 2016;171(4):2516–35.

36. Su Y. The Function of two Chloroplast-targeted Proteins-FtsH3 and FtsH5 in Embryogenesis in Arabidopsis. Soochow University; 2011.

37. Yu F, Liu X, Alsheikh M, Park S, Rodermel S. Mutations in SUPPRESSOR OF VARIATION1, a factor required for normal chloroplast translation, suppress var2-mediated leaf variegation in Arabidopsis. Plant Cell. 2008;20(7):1786–804.

38. Adam Z, Rudella A, van Wijk K. Recent advances in the study of Clp, FtsH and other proteases located in chloroplasts. Curr Opin Plant Biol. 2006;9(3):234–40.

39. Liu Q, Galli M, Liu X, Federici S, Buck A, Cody J, Labra M, Gallavotti A. NEEDLE1 encodes a mitochondria localized ATP-dependent metalloprotease required for thermotolerant maize growth. Proc Natl Acad Sci. 2019;116(39):19736–42.

40. Kamal SM, Rybakte ML, Nimtz M, Sperlein S, Giske C, Trček J, Deschamps J, Briandet R, Dini L, Jansh L, et al. Two FtsH Proteases Contribute to Fitness and Adaptation of Pseudomonas aeruginosa Clone C Strains. Front Microbiol. 2019;10(1372):1-18.

41. Fiocco D, Collins M, Muscariello L, Hols P, Kleerebezem M, Msaedek T, Spano G. The Lactobacillus plantarum ftsH gene is a novel member of the ctsr stress response regulon. J Bacteriol. 2009;191(5):1688–94.

42. Mistry J, Chou KC, Shen HB. Large-scale plant protein subcellular location prediction. Nucleic Acids Res. 2005;33(1):W100–7.

43. Kamal SM, Rybakte ML, Nimtz M, Sperlein S, Giske C, Trček J, Deschamps J, Briandet R, Dini L, Jansh L, et al. Two FtsH Proteases Contribute to Fitness and Adaptation of Pseudomonas aeruginosa Clone C Strains. Front Microbiol. 2019;10(1372):1-18.

44. Pieter SC, Luciani A, Eddy SR, Park Y, Lopez R, Finn RD. HMMER web server: 2018 update. Nucleic Acids Res. 2018;46(W1):W202–4.

45. Letunic I, Bork P. SMART: recent updates, new developments and Adaptation of Pseudomonas aeruginosa Clone C Strains. Front Microbiol. 2019;10(1372):1-18.

46. Iwamoto M, Maekawa M, Saito A, Higo H, Higo K. Evolutionary relationship of plant catalase genes inferred from exon-intron structures: isozyme divergence after the separation of monocots and dicots. Theor Appl Genet. 1998;97(1):9–19.

47. Letunic I, Khedkar S, Bork P. The evolution of spliceosomal introns: patterns, puzzles and progress. Nat Rev Genet. 2006;7(3):211–21.

48. Li W, Shang H, Ge Q, Zou C, Cai J, Wang D, Fan S, Zhang Z, Deng X, Tan Y, et al. Genomic-wide identification, phylogeny, and expression analysis of pectin methylesterases reveal their major role in cotton fiber development. BMC Genomics. 2016;17(1):1-13.

49. Zaltsman A, Or N, Adam Z. Two Types of FtsH protease gene blocks are required for chloroplast biogenesis and photosystem II repair in Arabidopsis. Plant Cell. 2005;17(10):2782–90.
54. Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13(8):1194–202.

55. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods. 2001;25(4):402–8.

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