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Characteristics of *TaFTSH6* Gene and Expression Analysis under Drought and Heat Stress in Wheat

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Abstract In order to study the characteristics and functions of wheat *FTSH* genes, *TaFTSH6* was cloned from wheat ‘Bobwhite’. Phylogenetic tree analysis showed that the amino acid sequence was very similar to *FTSH6* sequence of other species. SMART analysis showed that *TaFTSH6* had a conserved N-terminal transmembrane domain, ATP binding sites and hydrophilic Zn²⁺ metalloproteinase domain. The gene was located on chr7D chromosome of wheat, named as *TaFTSH6-7D*. *Cis*-elements analysis of *TaFTSH6* promoter sequence showed that there are several drought response sites CAACTG and a heat stress response site GACnnCTCnnGAA in the promoter, as well as many *cis*-elements related to hormone signal transduction. Quantitative expression analysis of *TaFTSH6* gene in wheat seedlings under drought and heat stress showed that both drought and heat stress could increase the expression level of *TaFTSH6* gene in root and leaf tissues, which indicated that *TaFTSH6* was induced by drought and heat stress. Further study on the function of *TaFTSH6* gene in wheat will partly support theoretical guidance to wheat breeding for drought and heat stress tolerance.

Keywords Wheat; *TaFTSH6*; Drought stress; Heat stress

FTSH (filamentation temperature-sensitive H) is a cytoplasmic membrane protein endopeptidase and belongs to the AAA (ATPase associated with diverse cellular activities) protease family (Ogura and Wilkinson, 2001). The family has an N-terminal transmembrane domain, ATP and Zn²⁺ binding sites, forms complexes with regulatory factor *HflK* or *HflC* oligomers in the form of cyclic homohexamer, and relies on ATP to degrade short-lived proteins and misassembled membrane proteins, so as to regulate the normal growth and development of organisms (Kihara et al., 1997; Kihara and Ito, 1998; Ito and Akiyama, 2005). *FTSH* is necessary for the growth of many organisms. The decrease of gene expression or gene mutation of this family will lead to serious growth defects and even death of *Bacillus subtilis* and *Escherichia coli* (Deuerling et al., 1997; Jayasekera et al., 2000). *FTSH* gene was first independently discovered by 4 different research groups when screening different phenotypes in *Escherichia coli* (Schumann, 1999). So far, it has been found and studied in a variety of organisms such as *Bacillus subtilis* (Deuerling et al., 1995), *Lactococcus lactis* (Duwat et al., 1995), *Arabidopsis thaliana* (Lindahl et al., 1996), *Nicotiana tabacum* (Seo et al., 2000), *Medicago sativa* (Ivashuta et al., 2002), *Citrullus lanatus* (Akashi et al., 2004), *Synechocystis sp* (Kamata et al., 2005), *Solanum lycopersicum* (Sun et al., 2006a), *Zea mays* (Andjelkovic and Thompson 2006), *Spinacia oleracea* (Yoshioka et al., 2006), *Brassica juncea* (Knight et al., 2006), *Solanum tuberosum* (Fan et al., 2007), *Xerophyta viscose* (Ingle et al., 2007), *Oryza sativa* (Zhang and Sun, 2009), *Glycine max* (Yin et al., 2011), *Lactobacillus plantarum* (Bove et al., 2012), *Arachis hypogaea* (Zheng et al., 2016, Jiangsu Agricultural Sciences, 44(12): 74-77) and *Acidovorax citrulli* (Ji et al., 2019).

FTSH was involved in the response of organisms to a variety of stress. Heat stress or light stress increased the expression of *FTSH* gene in *Synechocystis sp* cells (Kamata et al., 2005). *Lactobacillus plantarum* mutants lacking *FTSH* protease showed significant sensitivity to the increase of heat and salt concentration, while *FTSH* overexpression led to the increase of heat and salt stress tolerance (Bove et al., 2012). The growth ability of the

FTSH gene deletion mutant of *Acidovorax citrulli* was significantly reduced under heat stress and high salt stress (Ji et al., 2019). It was also found that *FTSH* was involved in resisting stress responses such as cold stress, heat stress, drought stress and salt stress. Stress could increase the expression of *FTSH* gene. For example, cold stress increased the expression of *MsFTSH* gene in *Medicago sativa* (Ivashuta et al., 2002); Heat stress increased the expression of *AtFTSH11* gene in *Arabidopsis thaliana* (Chen et al., 2006) and *LeFTSH6* gene in *Solanum lycopersicum* (Sun et al., 2006b); Drought stress significantly increased the expression of *SoFTSH4-like* gene in leaves and roots of *Solanum tuberosum* (Fan et al., 2007); Salt stress up-regulated the expression of 9 *FTSH* gene in *Arachis hypogaea*, including one *FTSH6-like* gene (Zheng et al., 2016). *FTSH* gene can improve the tolerance of plants to stress. Overexpression of *AtFTSH11* in *Arabidopsis thaliana* contributed to the overall tolerance to heat stress (Chen et al., 2006); *FTSHi5* can inhibit the expression of aging related genes and maintain cell redox balance (Wang et al., 2018b; Havé et al., 2018). However, there was no report on the application of *FTSH* gene in wheat.

Wheat is an important food crop in the world. In different regions of the world, wheat is often subjected to drought and heat stress throughout its growth period, and its yield and quality are seriously affected. Therefore, improving the drought and heat resistance of wheat is of great significance to the high and stable yield and high quality of wheat. In order to study the characteristics and functions of wheat *FTSH* genes, *TaFTSH6* was cloned from wheat ‘Bobwhite’, and its expression changes under drought and heat stress were analyzed by bioinformatics to provide a theoretical basis for wheat drought and heat resistance and provide a new idea for wheat breeding for drought and heat stress tolerance.

1 Results and Analysis

1.1 *TaFTSH6* gene cloning and evolutionary tree analysis of amino acid sequence

Using wheat leaf cDNA as template, *TaFTSH6F* and *TaFTSH6R* were used for PCR amplification. The amplification products were detected by 1% agarose gel electrophoresis. The gene fragment larger than 2000 bp was observed (Figure 1), which was consistent with the expectation. The target fragment was recovered, purified and connected with pMD19-T vector to transform *E. coli* DH5α competent cells, the transformed products were screened by colony PCR for positive clones, and the plasmids were extracted and sent to BGI for sequencing. The sequence has an ORF of 2 049 bp and encodes 683 amino acids. The gene sequence was compared on the wheat gene bank URGI, and it was found that the gene was located on the chr7D chromosome, so it was named *TaFTSH6-7D*.

Figure 1 Amplification products of *TaFTSH6-7D* PCR

Note: M: DL 2000 Marker; 1: PCR product of *TaFTSH6-7D* ORF

The evolutionary tree analysis of *FTSH* amino acid sequences of different species showed that *TaFTSH6* sequence was highly similar to *FTSH6* of other species, and *FTSH6* sequence also showed obvious differences between

monocotyledons and dicotyledons (Figure 2). *TaFTSH6-7D* had the highest homology with its diploid ancestor *Aegilops auschii*, up to 99%, and was slightly different from *TaFTSH6-7A* and *TaFTSH6-7B*. Moreover, *TaFTSH6-7D* was closely related to *Hordeum vulgare*, *Brachypodium distachyon*, *Oryza sativa*, *Sorghum bicolor* and *Zea mays*, and far from *FTSH6* of other species.

Figure 2 Evolutionary tree analysis of FTSH6

Note: the access number and *Latin* name of the proteins and their species in the figure are as follows: QlFSTH6(*Quercu lobatas*; XP_030954247.1); QsFSTH6 (*Quercus suber*; XP_023912746.1); JrFSTH6 (*Juglans regia*; XP_018813999.1); GmFSTH6 (*Glycine max*; XP_003552529.1); ApFSTH6 (*Abrus precatorius*; XP_027351682.1); VvFSTH6 (*Vitis vinifera*; XP_002283393.2); PaFSTH6 (*Prunus avium*; XP_021812467.1); MdFSTH6 (*Malus domestica*; XP_028963645.1); AtFSTH6 (*Arabidopsis thaliana*; NP_568311.2); CsFSTH6 (*Citrus sinensis*; XP_006482602.1); CmFSTH6 (*Cinnamomum micranthum*; RWR84016.1); DcFSTH6 (*Dendrobium catenatum*; XP_020697708.1); MaFSTH6 (*Musa acuminata*; XP_009381413.1); AoFSTH6 (*Asparagus officinalis*; XP_020255491.1); PdFSTH6 (*Phoenix dactylifera*; XP_008802626.1); EgFSTH6 (*Elaeis guineensis*; XP_010939484.1); ClFSTH6 (*Carex littledalei*; KAF3329191.1); AcFSTH6 (*Ananas comosus*; OAY79220.1); AeFSTH6 (*Aegilops tauschii*; XP_020164802.1); HvFSTH6 (*Hordeum vulgare*; KAE8798980.1); BdFSTH6 (*Brachypodium distachyon*; XP_003564049.1); OsFSTH6 (*Oryza sativa*; XP_015641788.1); SbFSTH6 (*Sorghum bicolor*; XP_002438106.1); ZmFSTH6 (*Zea mays*; ACG28886.1); DoFSTH6 (*Dichanthelium oligosanthos*; OEL32993.1); PhFSTH6 (*Panicum hallii*; XP_025812318.1); SiFSTH6 (*Setaria italica*; XP_004965045.1); OsFTSH2 (*Oryza sativa*; XP_015643053.1); AtFTSH8 (*Arabidopsis thaliana*; NP_563766.3); AtFTSH2 (*Arabidopsis thaliana*; XP_015643053.1); OsFTSH1 (*Oryza sativa*; XP_015643811.1); AtFTSH1 (*Arabidopsis thaliana*; NP_564563.1); AtFTSH5 (*Arabidopsis thaliana*; NP_568604.1); AtFTSH7 (*Arabidopsis thaliana*; NP_566889.1); AtFTSH9 (*Arabidopsis thaliana*; NP_568892.1); OsFTSH7 (*Oryza sativa*; XP_015625409.1); AtFTSH3 (*Arabidopsis thaliana*; NP_850129.1); AtFTSH10 (*Arabidopsis thaliana*; NP_172231.2); OsFTSH3 (*Oryza sativa*; XP_015626112.1); OsFTSH8 (*Oryza sativa*; XP_015639995.1); AtFTSH11 (*Arabidopsis thaliana*; NP_568787.1); OsFTSH9 (*Oryza sativa*; XP_015621895.1); AtFTSH4 (*Arabidopsis thaliana*; NP_565616.1); OsFTSH4 (*Oryza sativa*; XP_015621656.1); OsFTSH5 (*Arabidopsis thaliana*; XP_015615459.1); AtFTSH12 (*Arabidopsis thaliana*; NP_565212.1)

1.2 Structural analysis of TaFTSH6 protein

By comparing the TaFTSH6 protein sequence of wheat with that of *Oryza sativa*, *Hordeum vulgare* and *Zea mays*, it was found that the FTSH6 sequence was highly conserved and had the typical characteristics of FTSH family, especially between 90~670 aa. It showed a conserved N-terminal transmembrane domain at 164~182. There were typical ATP binding sites at 260~268, 315~320 and 360~366. The conserved WalkerA domain GXXGXXGK (S/T)

was located at 260~268, and the conserved WalkerB domain VFIDE was located at 315~320. Behind the ATP binding site, there was a typical LLRXGRX arginine ring structure (at 375~381) (Figure 3).

Figure 3 ClustalW analysis of FTSH6 among different species

Note: HvFSTH6, OsFSTH6 and ZmFSTH6 are amino acid sequences of FTSH6 protein of barley, rice and maize, and the access numbers are KAE8798980.1, and XP_015641788.1 and ACG28886.1 respectively

1.3 Cis-elements analysis of *TaFTSH6* promoters

The promoter sequence in the region about 2 kb upstream of the start codon of *TaFTSH6-7D* gene coding region was analyzed. The results showed that CAACTG drought response site was found at 56 bp, 339 bp, 1 101 bp and 1 833 bp upstream of ATG, and GACnnCTCnnGAA heat shock transcription factor binding site was found at 234 bp upstream of ATG. In addition, there were many cis-acting regulatory elements involved in growth and development, cis-acting regulatory elements involved in hormone signal transduction pathway and cis-acting regulatory elements involved in abiotic stress response in the promoter region of *TaFTSH6-7D* gene (Table 1).

1.4 The expression analysis of *TaFTSH6-7D* gene under drought and heat stress

In order to study the response of *TaFTSH6-7D* gene to drought and heat stress, the expression of *TaFTSH6* gene in leaves and roots of wheat at seedling stage was analyzed by RT-PCR. The results showed that under drought stress, the expression of *TaFTSH6-7D* gene in leaves and roots of wheat increased, especially after 12 hours of treatment, the expression of *TaFTSH6-7D* gene increased significantly and extremely significantly in leaves and roots, respectively. Under heat stress, the expression of *TaFTSH6-7D* gene in leaves and roots of wheat also increased.

At 6 h, 2 d (6 h/d) and 3 d (6 h/d), the expression of *TaFTSH6-7D* gene increased significantly in leaves and roots. The above results showed that *TaFTSH6-7D* gene was induced by drought and heat stress.

Table 1 Cis-elements analysis of *TaFTSH6-7D* promoters

Name	Position	Function	Sequence
AAGAA-motif	25-, 1409-	Cis-acting element involved in the abscisic acid responsiveness	GAAAGAA
ABRE	1595+, 95+, 230+, 190+, 231+	Cis-acting element involved in the abscisic acid responsiveness	ACGTG, CACGTG
ACE	796-	Cis-acting element involved in light responsiveness	CTAACGTATT
ARE	402+, 1777+, 564-	Cis-acting regulatory element essential for the anaerobic induction	AAACCA
Box 4	36+	Part of a conserved DNA module involved in light responsiveness	ATTAAT
C-box	1821+	Cis-acting regulatory element involved in light responsiveness	ACGAGCACCGCC
CAT-box	1471+, 1950+, 1500+	Cis-acting regulatory element related to meristem expression	GCCACT
CCAAT-box	857+	MYBHv1 binding site	CAACGG
CGTCA motif	956-, 1834+	Cis-acting regulatory element involved in the MeJA-responsiveness	CGTCA
G-Box	94-, 230+, 189-, 1594-, 1592+, 1750-	Cis-acting regulatory element involved in light responsiveness	CACGTT, CACGTC, GCCACGTGGA, CACGAC
GATA-motif	1491+	Part of a light responsive element	AAGGATAAGG
GC-motif	1617-, 1682+	Enhancer-like element involved in anoxic specific inducibility	CCCCCG
GT1-motif	909+	Light responsive element	GGTTAA
I-box	1492-, 1493+	Part of a light responsive element	CCTTATCCT , GTATAAGGCC
MBS	268-, 1662+, 900-, 1945-	MYB binding site involved in drought-inducibility	CAACTG
MYB	661-, 1300-, 908-, 1333-, 768-, 1081-	MYB response element	CAACCA, TAACCA
MYB recognition site	857-	MYB response element	CCGTTG
MYB-like sequence	908-, 1333-	MYB response element	TAACCA
MYC	615-, 1537+, 1205-, 693-, 1221+	MYC response element	CATGTG
Myb	248-, 1945-, 900-, 509-, 1662+	MYB response element	CAACTG
P-box	394	Gibberellin-responsive element	CCTTTTG
RY-element	726	Cis-acting regulatory element involved in seed-specific regulation	CATGCATG
Sp1	1674-, 1679-	Light responsive element	GGGCGG
TATC-box	610+	Cis-acting element involved in gibberellin-responsiveness	TATCCCA
TC-rich repeats	1044	Cis-acting element involved in defense and stress responsiveness	CTTTTCTTAC
TCA-element	1480+	Cis-acting element involved in salicylic acid responsiveness	CCATCTTTTT
TCT-motif	272+, 1048+, 375-	Part of a light responsive element	TCTTAC
TGA-element	1385+	Auxin-responsive element	AACGAC
TGACG-motif	956+, 1834-	Cis-acting regulatory element involved in the MeJA-responsiveness	TGACG

Figure 4 Expression profile of *TaFTSH6-7D* gene under drought and heat stress

Note: Panel A is the change of *TaFTSH6-7D* gene expression under drought treatment; Panel B is the change of *TaFTSH6-7D* gene expression under heat stress

2 Discussion

Plants face a variety of abiotic and biotic stresses throughout their life cycle, and protein degradation *in vivo* is one of the important ways for plants to respond to environmental stress and coordinate the relationship between growth and stress response (Chen et al., 2020). Organisms degrade end-of-life proteins and misassembled membrane proteins to regulate the normal growth and their response to the environment (Wang et al., 2018a). There are mainly ATP-independent lysosomal pathway and ATP-dependent ubiquitin proteasome pathway for protein degradation in organisms. Recent studies have also found the caspase pathway, the La protease pathway of mitochondria, the Kex2 hydrolase pathway in the Golgi body, and the hydrolase pathway on the cell membrane surface (Ling et al., 2019). Protein degradation by FTSH protease depends on ATP to provide energy. In *Escherichia coli*, FTSH forms complexes with the regulator *HflKC* oligomer in the form of cyclic homo-hexamers, and its activity is modified by phosphorylation (Kato and Sakamoto, 2019), and it degrades short-lived proteins and misassembled proteins (Nishimura et al., 2016). Its special feature is that it is membrane anchored, which can degrade wrong proteins and maintain membrane stability under stress conditions (Kato and Sakamoto, 2018). It is a hydrolase on the surface of cell membrane. Therefore, the FTSH protease family has an N-terminal transmembrane domain and a C-terminal region extending into the matrix. The C-terminal region contains ATPase and protease domain. ATPase domain plays the function of unfolding enzyme and transfers the substrate to the protease domain degradation chamber through a narrow pore. This study found that the FTSH6 protein sequences of different species were highly conserved in the N-terminal transmembrane domain, the C-terminal ATP binding site and the arginine ring.

At present, the mechanism of FTSH protease is still poorly understood. FTSH protease can extract and degrade the intact membrane protein from the membrane (Kato and Sakamoto, 2018). There are 12 *FTSH* coding genes in *Arabidopsis thaliana*, and 9 of them are located on the thylakoid membrane of the chloroplasts, 3 are located on the mitochondrial membrane (Sakamoto et al., 2003). *FTSH* is involved in the formation of thylakoid membrane during the early development of chloroplasts. The FTSH mutation in *Nicotiana tabacum* caused the collapse of thylakoid membrane in the later stages of leaf development (Kato et al., 2012). In addition, *FTSH* is also involved in the degradation and assembly of D1 protein in photosystem II repair cycle and several protein complexes in photosynthetic electron transport pathway. In *Arabidopsis thaliana* mutants lacking FTSH, light-damaged D1 protein accumulated, ROS (reactive oxygen species) signals were not effectively transmitted, and showed higher sensitivity to strong light stress (Zaltsman et al., 2005; Dogra et al., 2017; Wang et al., 2018a). However, the regulation mode of protease activity of FTSH, the pattern of FTSH and its complex recognizing damaged proteins, and the specific function and function mode of FTSH need to be further studied.

FTSH has been reported to be involved in the response to drought and heat stress. Under drought conditions, the increase of *FTSH* gene expression or a large amount of FTSH protein synthesis were found in plants such as *Xerophyta viscosa* (Ingle et al., 2007), *Citrullus lanatus* originated from African desert (Akashi et al., 2004), *Zea mays* (Andjelkovic and Thompson, 2006), *Brassica juncea* (Knight et al., 2006) and *Solanum tuberosum* (Fan min et al., 2007). The increase of *FTSH* gene expression in the roots of *Oryza sativa* can enhance drought stress tolerance (Zhang and sun, 2009). Heat stress can increase the expression of *LeFTSH6* gene in *Solanum lycopersicum* (Sun et al., 2006a; 2006b). Overexpression of *FTSH11* in *Arabidopsis thaliana* contributed to the overall tolerance to heat stress (Chen et al., 2006). In wheat, it was found that the expression of *FTSH2* in heat-resistant varieties was significantly higher than that in heat sensitive varieties under heat stress (Wang et al., 2015). In this study, it was found that the expression of *TaFTSH6-7D* gene in wheat increased significantly under PEG simulated drought or 36°C heat treatment at seedling stage, which was consistent with the reports about other plants. The results of this study showed that *TaFTSH6-7D* gene was induced by drought and heat stress. The further study on the function and regulation mode of *TaFTSH6* gene will provide a theoretical guidance to wheat molecular breeding for drought and heat stress tolerance.

3 Materials and Methods

3.1 Plant materials and treatment

In the light incubator, the seeds of wheat (*Triticum aestivum* L.) variety ‘Bobwhite’ were cultured in the medium at 4°C for 5 d, 12°C for 5 d and 25°C for 2 d, and then transferred to 1/2MS medium and cultured until three leaf stage under the conditions of 25°C (16 h)/20°C (8 h) (day/night) and 75% relative humidity. The seedlings were subjected to simulated drought (15% PEG, 6 h, 12 h, 24 h and 48 h), heat (36°C, 3 h, 6 h, 2 d (6 h/d) and 3 d (6 h/d)) stress, and compared with normal growing seedlings. The samples were frozen in liquid nitrogen and stored at -80°C.

3.2 RNA extraction and cDNA synthesis

The total RNA was extracted from wheat leaves and roots by Trizol method, and the plants extracted RNA were seedlings and in good growth condition. After removing DNA from RNA samples, cDNA was synthesized according to the instructions of one-step reverse transcription kit from Tiangen Company and stored at -20°C.

3.3 Primer design and PCR amplification

The primers used in this experiment were shown in Table 2. *TaFTSH6F* and *TaFTSH6R* were used for gene cloning. The PCR reaction procedure was as follows: pre-denaturation at 94°C for 5 min, denaturation at 94°C for 45 s, annealing at 60°C for 45 s, extension at 72°C for 120 s, as to the first five cycles, the annealing temperature of each cycle was 1°C lower than that of the previous one, and after reducing to 55°C, 30 cycles need to be performed, and then extended at 72°C for 10 min, and finally kept at 12°C. The ABI Stepone plus quantitative PCR instrument was used for fluorescence quantitative PCR, and the operation was carried out according to the instructions of SYBR Green PCR Master Mix (Applied biosystems) kit. In order to ensure the specificity of

primers, the 3'- end primers were designed in untranslated sequences, the specificity of primers was checked on the Wheat Gene Index database (<http://blast.jcvi.org/euk-blast/index.cgi?project=tae1>) website, and the melting curve presented a single peak. The expression of housekeeping gene TaRP15 did not change under drought and heat stress. Three replicates were set up.

Table 2 Primers sequences listed

Primer	Sequence
TaFTSH6F	5'-ATGTCGCCCACGGCCATGTCGC-3'
TaFTSH6R	5'-TCAAGCAGTGACCATGTCCGTC-3'
TaFTSH6-Q7DF	5'-ACGTGCTCATGGAGAAGGAGAC-3'
TaFTSH6-Q7DR	5'-CCTACCACAGTCTGCACTACACAA-3'
TaRP15-F	5'-CGGGATCGGAGTAATGA-3'
TaRP15-R	5'-TTCGCAGTTGTTCGTCTT-3'

3.4 Bioinformatics analysis

the URGI (https://urgi.versailles.inra.fr/blaST/?dbgroup=wheat_all&program=blaSTn) was used for obtaining the physical location of *TaFTSH6* gene based on the known sequence of wheat. According to the obtained positioning, cDNA sequences were obtained from (<http://202.194.139.32/>). The intron exon analysis of *TaFTSH6* gene was performed in FGENESH HMM based Gene Structure prediction of Softberry. The FTSH6 sequences of other species were searched through BLASTX (<http://www.ncbi.nlm.nih.gov/>) on NCBI website. In MEGA10.0, ClustalW method was used for multi sequence alignment analysis, and then neighbor-joining method (bootstrap=1000) was used to construct evolutionary tree. The transmembrane domain analysis was performed on (<http://www.cbs.dtu.dk/services/TMHMM-2.0/>). The analysis of cis-acting elements of promoter was performed on (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html>).

Authors' contributions

LY was the executor of this study, completing data analysis and writing the first draft of the manuscript; JHJ participated in designing the experiments and analyzing the results; LJ and LYG were the conceivers of the project. HXJ directed the experimental design, data analysis, manuscript writing and revision. All authors read and approved the final manuscript.

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References

- Akashi K., Nishimura N., Ishida Y., and Yokota, A., 2004, Potent hydroxyl radical-scavenging activity of drought-induced type-2 metallothionein in wild watermelon, *Biochemical & Biophysical Research Communications*, 323(1): 72-78
<https://doi.org/10.1016/j.bbrc.2004.08.056>
- Andjelkovic V., and Thompson R., 2006, Changes in gene expression in maize kernel in response to water and salt stress, *Plant Cell Reports*, 25(1): 71-79
<https://doi.org/10.1007/s00299-005-0037-x>
- Bove P., Capozzi V., Garofalo C., Rieu A., Spano G., and Fiocco D., 2012, Inactivation of the *FTSH* gene of *Lactobacillus plantarum* *WCFS1*: effects on growth, stress tolerance, cell surface properties and biofilm formation, *Microbiological Research*, 167(4): 187-193
<https://doi.org/10.1016/j.micres.2011.07.001>
- Chen J., Burke J.J., Velten J., and Xin Z., 2006, *FTSH11* protease plays a critical role in *Arabidopsis* thermotolerance, *Plant Journal for Cell & Molecular Biology*, 48(1): 73-84
<https://doi.org/10.1111/j.1365-313X.2006.02855.x>
- Chen Q., Yu F., and Xie Q., 2020, Insights into endoplasmic reticulum-associated degradation in plants, *New Phytologist*, 226(2): 345-350
<https://doi.org/10.1111/nph.16369>
- Deuerling E., Paeslack B., and Schumann W., 1995, The *FTSH* gene of *Bacillus subtilis* is transiently induced after osmotic and temperature up shock. *Journal of Bacteriology*, 177(14): 4105-4112
<https://doi.org/10.1128/jb.177.14.4105-4112.1995>
- Deuerling E., Mogk A., Richter C., Purucker M., and Schumann W., 1997, The *FTSH* gene of *Bacillus subtilis* is involved in major cellular processes such as sporulation, stress adaptation and secretion, *Molecular Microbiology*, 23(5): 921-933
<https://doi.org/10.1046/j.1365-2958.1997.2721636.x>

- Dogra V., Duan J., Lee K.P., Lv S., Liu R., and Kim C., 2017, *FTSH2*-dependent proteolysis of EXECUTER1 is essential in mediating singlet oxygen-triggered retrograde signaling in *Arabidopsis thaliana*, *Frontiers in Plant Science*, 8: 1145
<https://doi.org/10.3389/fpls.2017.01145>
- Duwat P., Ehrlich S.D., and Gruss A., 1995, The *recA* gene of *Lactococcus lactis*: characterization and involvement in oxidative and thermal stress, *Molecular Microbiology*, 17(6): 1121-1131
https://doi.org/10.1111/j.1365-2958.1995.mmi_17061121.x
- Fan M., Jin L.P., Huang S.W., Xie K. Y., Liu Q C., and Qun D Y, 2007, Cloning and expression of a full-length cDNA of *SoFTSH* gene in potato under drought stress, *Acta Agronomica Sinica*, 33 (11): 1748-1754
- Havé M., Balliau T., Cottyn-Boitte B., Déron E., Cueff G., Soulay F., Lornac A., Reichman P., Dissmeyer N., Avise J.C., Gallois P., Rajjou L., Zivy M., and Masclaux-Daubresse C., 2018 , Increases in activity of proteasome and papain-like cysteine protease in *Arabidopsis* autophagy mutants: back-up compensatory effect or cell-death promoting effect? *Journal of Experimental Botany*, 69(6): 1369-1385
<https://doi.org/10.1093/jxb/erx482>
- Ingle R.A., Schmidt U.G., Farrant J.M., Thomson J.A., and Mundree S.G., 2007, Proteomic analysis of leaf proteins during dehydration of the resurrection plant *Xerophyta viscosa*, *Plant Cell Environment*, 30(4): 435-446
<https://doi.org/10.1111/j.1365-3040.2006.01631.x>
- Ito K., and Akiyama Y., 2005, Cellular functions, mechanism of action, and regulation of *FTSH* protease, *Annual Review of Microbiology*, 59(1): 211-231
<https://doi.org/10.1146/annurev.micro.59.030804.121316>
- Ivashuta S., Imai R., Uchiyama K., Gau M., and Shimamoto Y., 2002, Changes in chloroplast *FTSH-like* gene during cold acclimation in alfalfa (*Medicago sativa*), *Journal of Plant Physiology*, 159(1): 85-90
<https://doi.org/10.1078/0176-1617-00661>
- Jayasekera M.M., Foltin S.K., Olson E.R., and Holler T.P., 2000, *Escherichia coli* requires the protease activity of *FTSH* for growth, *Archives of Biochemistry & Biophysics*, 380(1): 103-107
<https://doi.org/10.1006/abbi.2000.1903>
- Ji W.Q., Yan J.P., Bai X., Qiao P., Li Z.P., Yang Y.W., Guan W., and Zhao T.C., 2019, Functional analysis of the gene *FTSH* in *Acidovorax citrulli*, *Plant Pathology*, 49(4): 488-499
- Kato Y., Kouso T., and Sakamoto W., 2012, Variegated tobacco leaves generated by chloroplast *FTSH* suppression: Implication of *FTSH* function in the maintenance of thylakoid membranes, *Plant Cell Physiology*, 53: 391-404
<https://doi.org/10.1093/pcp/pcr189>
- Kato Y., and Sakamoto W., 2018, *FTSH* Protease in the thylakoid membrane: physiological functions and the regulation of protease activity, *Frontiers in Plant Science*, 9: 855
<https://doi.org/10.3389/fpls.2018.00855>
- Kato Y., and Sakamoto W., 2019, Phosphorylation of the chloroplastic metalloprotease *FTSH* in *Arabidopsis* characterized by Phos-Tag SDS-PAGE, *Frontiers in Plant Science*, 10: 1080
<https://doi.org/10.3389/fpls.2019.01080>
- Kamata T., Hiramoto H., Morita N., Shen J.R., Mann N.H., and Yamamoto Y., 2005, Quality control of Photosystem II: an *FTSH* protease plays an essential role in the turnover of the reaction center D1 protein in *Synechocystis* PCC 6803 under heat stress as well as light stress conditions, *Photochemical & Photobiological sciences*, 4(12), 983-990
<https://doi.org/10.1039/b506068k>
- Kihara A., Akiyama Y., and Ito K., 1997, Host regulation of lysogenic decision in bacteriophage lambda: transmembrane modulation of *FTSH* (*HflB*), the cII degrading protease, by *HflKC* (*HflA*), *Proceedings of the National Academy of Sciences of the United States of America*. USA, 94: 5544–5549
<https://doi.org/10.1073/pnas.94.11.5544>
- Kihara A., and Ito K., 1998, Translocation, folding, and stability of the *HflKC* complex with signal anchor topogenic sequences, *Journal of Biological Chemistry*, 273(45): 29770–29775
<https://doi.org/10.1074/jbc.273.45.29770>
- Knight C.A., Vogel H., Kroymann J., Shumate A., and Mitchell-Olds T., 2006, Expression profiling and local adaptation of *Boechera holboellii* populations for water use efficiency across a naturally occurring water stress gradient, *Molecular Ecology*, 15(5), 1229-1237
<https://doi.org/10.1111/j.1365-294X.2006.02818.x>
- Lindahl M., Tabak S., Cseke L., Pichersky E., Andersson B., and Adam Z., 1996, Identification, characterization, and molecular cloning of a homologue of the bacterial *FTSH* protease in chloroplasts of higher plants, *Journal of Biological Chemistry*, 15, 271(46): 29329-29334
<https://doi.org/10.1074/jbc.271.46.29329>
- Ling Q.H., Broad W., Trösch R., Töpel M., Sert T.D., Lymperopoulos P., Baldwin A., and Jarvis R.P., 2019, Ubiquitin-dependent chloroplast-associated protein degradation in plants, *Science*, 363(6429):eaav4467
<https://doi.org/10.1126/science.aav4467>
- Nishimura K., Kato Y., and Sakamoto W., 2016, Chloroplast Proteases: Updates on proteolysis within and across suborganellar compartments, *Plant Physiology*, 171(4): 2280-2293
<https://doi.org/10.1104/pp.16.00330>

- Ogura T., and Wilkinson A.J., 2001, AAA+ superfamily ATPases: common structure--diverse function, *Genes to Cells*, 6(7): 575-597
<https://doi.org/10.1046/j.1365-2443.2001.00447.x>
- Schumann W., 1999, *FTSH*—a single-chain charonin? *FEMS Microbiology Reviews*, 23(1): 1-11
[https://doi.org/10.1016/S0168-6445\(98\)00024-2](https://doi.org/10.1016/S0168-6445(98)00024-2)
- Seo S., Okamoto M., Iwai T., Iwano M., Fukui K., Isogai A., Nakajima N., and Ohashi Y., 2000, Reduced levels of chloroplast *FTSH* protein in tobacco mosaic virus-infected tobacco leaves accelerate the hypersensitive reaction, *Plant Cell*, 12(6): 917-932
<https://doi.org/10.1105/tpc.12.6.917>
- Sun A.Q., Yi S.Y., Yang J.Y., Zhao C.M., and Liu J., 2006a, Identification and characterization of a heat-inducible *FTSH* gene from tomato (*Lycopersicon esculentum* Mill.), *Plant Science*, 170: 551-562
<https://doi.org/10.1016/j.plantsci.2005.10.010>
- Sun A.Q., Yang J.Y., Yi S.Y., Zhao C.M., and Liu J., 2006b, Cloning and molecular characteristic of the metalloprotease gene *LeFTSH6* from tomato. *Journal of Plant Physiology & Molecular Biology*, 32(1): 64-72
- Wang Q. L., Chen J. H., He N. Y., and Guo F. Q., 2018a, Metabolic reprogramming in chloroplasts under heat stress in plants, *International journal of molecular sciences*, 19(3): 849
<https://doi.org/10.3390/ijms19030849>
- Wang T., Li S.H., Chen D., Xi Y., Xu X.Z., Ye N.H., Zhang J.H., Peng X.X., and Zhu G.H., 2018b, Impairment of *FTSHi5* function affects cellular redox balance and photorespiratory metabolism in *Arabidopsis*, *Plant & cell physiology*, 59(12): 2526-2535
<https://doi.org/10.1093/pcp/pcv174>
- Wang X., Dinler B.S., Vignjevic M., Jacobsen S., and Wollenweber B., 2015, Physiological and proteome studies of responses to heat stress during grain filling in contrasting wheat cultivars, *Plant Science*, 230: 33-50
<https://doi.org/10.1016/j.plantsci.2014.10.009>
- Yoshioka M., Uchida S., Mori H., Komayama K., Ohira S., Morita N., Nakanishi T., and Yamamoto Y., 2006, Quality control of photosystem II. leavage of reaction center D1 protein in spinach thylakoids by FtsH protease under moderate heat stress, *Journal of Biological Chemistry*, 281(31): 21660-21669
<https://doi.org/10.1074/jbc.M602896200>
- Yin Z.T., Meng F.F., Song H.N., Wang X.L., Chao M.N., Zhang G.Z., Xu X.M., Deng D.X., and Yu D.Y., 2011, *GmFTSH9* expression correlates with in vivo photosystem II function: chlorophyll a fluorescence transient analysis and eQTL mapping in soybean, *Planta*, 234(4): 815-827
<https://doi.org/10.1007/s00425-011-1445-5>
- Zaltsman A., Feder A., and Adam Z., 2005, Developmental and light effects on the accumulation of FTSH protease in *Arabidopsis chloroplasts* - implications for thylakoid formation and photosystem II maintenance, *Plant Journal*, 42: 609-617
<https://doi.org/10.1111/j.1365-313X.2005.02401.x>
- Zhang J.D., and Sun A.Q., 2009, Genome-wide comparative analysis of the metalloprotease *FTSH* gene families between *Arabidopsis thaliana* and rice, *Chinese Journal of Biotechnology*, 25(9): 1402-1408
- Zheng C.H., Kong X.Y., Sui J.M., Shu C., and Zhao C.M., 2016, Identification, classification and salt stress expression analysis of metalloproteinase *FTSH* family in peanut (*Arachis hypogaea* L.), *Journal of Jiangsu Agricultural Sciences*, 44(12):74-77