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Cloning and expression of class I chitinases in Hami melon after *Penicillium* infection

F.Y. Jiang, B.B. Shui, F.X. Tang and C.H. Shan

Food College of Shihezi University, Shihezi, China

Corresponding author: C.H. Shan
E-mail: sch_0909@163.com

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ABSTRACT. Chitinases are important disease-related proteins that play critical roles in plant defense against disease. To investigate the function of chitinases in the resistance of Hami melon to *Penicillium* infection, the gene encoding chitinases, *HmCHT-2*, was cloned and RT-PCR was used to measure expression levels of *HmCHT-2*. When the Hami melon was infected by *Penicillium* sp after 0, 12, 36, 48, 60, and 72 h. The results showed that comparing to the control group, the time of expression levels reaching to the peak delayed and the expression levels maintained at a significantly high level for a longer time. These results suggest that *HmCHT-2* may contribute to the defense of Hami melon against fungal infection.

Key words: Chitinases; RT-PCR; *Penicillium* infection; *HmCHT-2*

INTRODUCTION

Hami melon, a cultivar of muskmelon and important food crop in China, is susceptible to infection by pathogenic fungi during postharvest storage, which may lead to spoilage. *Penicillium* is one of the major genera of pathogenic fungi involved in the deterioration of the fruit (Chen et al., 2012; Liu et al., 2013). Prolonging the limited post-harvest lifespan of Hami melon has been a significant research topic in China, where a number of studies have been aimed at the control of pathogenic infections (Dangl and Jones, 1998; Guo et al., 2007). Systemic acquired resistance is an important component of disease resistance that contributes significantly to plant health (Ryals et al., 1994). Some treatments have been found to improve disease resistance in Hami melon. For example, the application of natamycin in combination with bilayer films consisting of chitosan and polyethylene wax have been shown to inhibit the growth of *Alternaria alternate* and *Fusarium semitectum* on the melon surface (Cong et al., 2007), while sodium silicate treatment has been found to inhibit the growth of *Trichothecium roseum* (Bi et al., 2006).

Chitin is a structural component of the cell wall of many phytopathogenic fungi, which indicates that plant chitinases are typical pathogenesis-related proteins related to the innate defense against plant diseases (Kombrink et al., 1988; Stintzi et al., 1993). Recent researches have proven that chitinases contribute to pathogen resistance in plants (Taira, 2010; Graça et al., 2016). In plants, chitinases are hydrolytic enzymes that are involved in many aspects of plant growth and development, including cell wall metabolism and disease resistance (Sharma et al., 2011). Some of them are not affected by pathogenic fungus or stress and related to plant growth and development. For example, BC15/OsCTL1, a class II chitinase-like protein, mediated cellulose biosynthesis and cell wall remodeling in rice (Wu et al., 2012), while some chitinases can be induced by phytopathogenic fungi and contribute to disease resistance and stress. For example, when upland rice was exposed to various abiotic stresses (including drought, salt and low temperature), the expression levels of DIP3 encoding a chitinase III protein accumulated rapidly (Guo et al., 2013). Furthermore, the overexpression of the *RCH10* chitinases gene via agrobacterium-mediated transformation conferred resistance to fungal infection by *Botrytis cinerea* in *Lilium* (González et al., 2015).

However, research on chitinases in Hami melon is limited. An understanding of the structure and biological function of chitinases in this fruit will provide a theoretical basis for its response to phytopathogenic fungi, and may also provide an alternative solution for prolonging its post-harvest storage life.

MATERIAL AND METHODS

Sample collection

The *Penicillium* strain used in this study was isolated from Hami melon surface in previous study by our lab. The *Penicillium* strain was grown in the potato medium (2% glucose, 2% agar, 20% potato) and then cultivated at 28°C until a large quantity of spores were produced. The *Penicillium* spores were harvested by using sterile water flushing and the spores suspensions were diluted with sterile water to obtain 1 x 10^5 spores/mL dilutions.

Plant material

The Hami melons used in this study were late-maturing, in which reducing sugar content was 11-12% and no obvious damage was discovered. Six melons, about 3 kg per one, were divided into two groups containing 3 parallels. Six and eight incisions were made on every fruit using a sterile hole-puncher (1 cm deep by 1 cm in diameter) in the first and the second groups, respectively (Figure 1). Then 10 µL *Penicillium* spore suspensions (1 x 10^5 spores/mL) was added into three of six wounds in each melon in the first group and four of eight wounds in each melon in the second group, while 10 µL sterile water was added into other wounds in each melon in the first group and the second group as control. The melons were then covered with plastic wrap and maintained at room temperature (about 25°C). After inoculation for 0, 12, 24, 36, 48, 60, and 72 h (Figure 2), 3 parallel samples around the incision points from the first and the second groups were drawn and immediately frozen in liquid nitrogen.

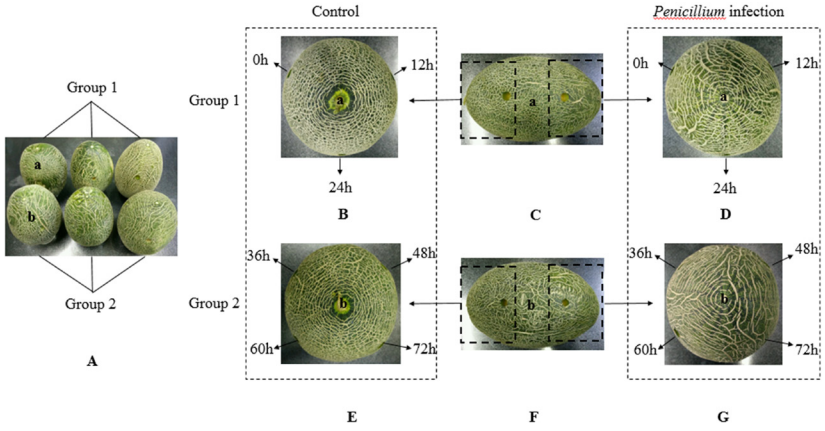

Figure 1. Incisions of Hami melon. **A.** photographs of two groups containing 3 parallels; **B. C. D.** incisions on one Hami melon in Group 1; **E. F. G.** incisions on one Hami melon in Group 2.

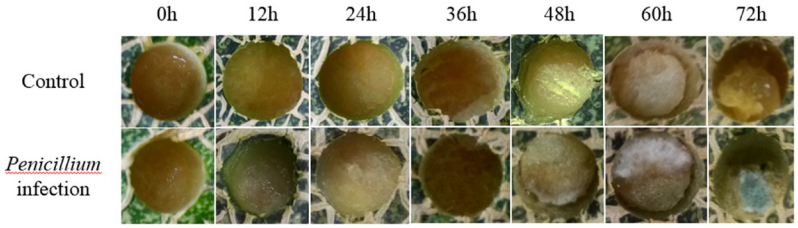

Figure 2. Effects of *Penicillium* infection on Hami melon.

Screening of chitinase genes

We had previously established the transcriptome of Hami melon prior to and after infection with the *Penicillium* strain using an Illumina Hiseq 2000 sequencing system (ABlife Inc., Wuhan, China). We also analyzed differentially expressed genes at different time

points after inoculation (0, 48 and 60 h) using RNA-Seq technique (Shan, 2015). Sixteen differentially expressed chitinase genes were identified (Table 1). Among them, the CL2231. Contig2_QHMG chitinase gene displayed an observably higher expression level then others and was selected in this study.

Table 1. Differentially expressed genes of chitinase in Hami melon.

GeneID	0 h RPKM	48 h RPKM	60 h RPKM	BLASTnr
Unigene8343_QHMG	-	76.15	76.15	gi|312191345|gb|ADQ43720.1| class I chitinase [*Casuarina equisetifolia*]
Unigene8344_QHMG	-	148.62	148.62	gi|449508755|ref|XP_004163402.1| endochitinase-like [*Cucumis sativus*]
Unigene22455_QHMG	-	5.71	1.49	gi|8272386|dbj|BAA96445.1| endo-chitinase class III [*Pyrus pyrifolia*]
CL4824.Contig1_QHMG	4.39	826.96	1,607.41	gi|449456357|ref|XP_004145916.1| acidic endochitinase-like [*Cucumis sativus*]
CL2231.Contig2_QHMG	107.54	14,493.83	25,398.46	gi|23496435|dbj|BAB40817.2| endochitinase MCHT-2 [*Cucumis melo*]
CL2231.Contig1_QHMG	2.45	324.35	608.04	gi|449508755|ref|XP_004163402.1| endochitinase-like [*Cucumis sativus*]
CL4824.Contig2_QHMG	0.83	73.74	95.46	gi|7595839|gb|AAF64474.1| chitinase 1 [*Cucumis melo*]
Unigene17882_QHMG	2.61	34.10	46.15	gi|449454971|ref|XP_004145227.1| endochitinase PR4-like [*Cucumis sativus*]
Unigene17906_QHMG	3.29	34.60	33.09	gi|449457929|ref|XP_004146700.1| acidic mammalian chitinase [*Cucumis sativus*]
Unigene13259_QHMG	302.10	1,266.18	1,799.97	gi|5919201|gb|AAD56239.1| class III chitinase [*Benincasa hispida*]
Unigene19961_QHMG	17.67	5.10	4.65	gi|449433057|ref|XP_004134314.1| acidic endochitinase-like [*Cucumis sativus*]
Unigene29851_QHMG	7.47	1.47	2.65	gi|449503856|ref|XP_004162209.1| chitinase-like protein 2-like [*Cucumis sativus*]
Unigene16405_QHMG	8.92	1.45	0.58	gi|13548699|dbj|BAB40818.1| endochitinase MCHT-3 [*Cucumis melo*]
Unigene18657_QHMG	26.43	3.82	3.60	gi|449503856|ref|XP_004162209.1| chitinase-like protein 2-like [*Cucumis sativus*]
CL726.Contig4_QHMG	6.28	0.75	0.21	gi|13548699|dbj|BAB40818.1| endochitinase MCHT-3 [*Cucumis melo*]
Unigene19548_QHMG	59.74	3.25	0.65	gi|449456861|ref|XP_004146167.1| basic endochitinase-like [*Cucumis sativus*]

Extraction and quantification of total RNA

RNA extraction was performed with the Trizol protocol according to the Qiagen Plant RNA Extraction Kit 74903 manual. The quantity and quality of RNA were determined by ultraviolet light absorbance and confirmed by gel electrophoresis, which contained 1.2% agarose.

Synthesis of first strand of cDNA

First-strand cDNA was synthesized by using 2 µg RNA, random primers and RNase inhibitors, and so forth, which had a final volume of 10.5 µL.

Cloning and sequencing of gene encoding chitinases

Based on our previously obtained Hami melon transcriptome library, the following primers were designed and synthesized to amplify the chitinase gene: GAAACAAACACAACAAAATCACAAAGG(forwardprimer)andGGGAGGGTATATATTA AAAAATATG (reverse primer). The 50-µL reaction mixtures consisted of: 34.8 µL H_2O, 10 µL buffer, 1 µL dNTP, 1 µL forward primer, 1 µL backward primer, 2 µL template, and 0.2 µL Taq polymerase. The thermal profile consisted of: 94°C denaturation for 3 min, 39 cycles of 94°C for 30 s, 51°C for 40 s, 72°C for 45 s and a final extension at 72°C for 7 min.

The PCR product was analyzed using 1% agarose gel electrophoresis and then cloned into a pMD 18T vector (TaKaRa) for DNA sequencing. A BLAST search on the NCBI Genbank was performed using the DNA sequences.

Bioinformatic analysis

The open reading frame (ORF) and the encoded amino acid sequence were

analyzed using ORF Finder on NCBI (http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi). The physiochemical properties, hydrophilicity/hydrophobicity, signal peptide, secondary structure and transmembrane structure of the resultant protein were analyzed by using ProtParam (http://web.expasy.org/protparam/), ProtScale (http://web.expasy.org/protscale/), SignalP V.3.0 Server (http://www.cbs.dtu.dk/services/SignalP/), SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) and TMHMM Server V. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/), respectively. Protein tertiary structure were predicted by using SWISS-MODEL. The subcellular localization of the protein was predicted using ProtComp V. 9.0 (http://linux1.softberry.com/berry.phtml?group=programs&subgroup=proloc&topic=protcompan) and the PredictProtein service (https://www.predictprotein.org/). The amino acid sequence of the protein encoded by the longest ORF was analyzed using BLASTp on NCBI. The sequences with high similarity were used to construct a phylogenetic tree by using MEGA 5.0.

Analysis of gene expression

Based on the sequence of the target gene, the following primers were designed for real-time quantitative PCR analysis using Primer Express Software V. 2.0: GAAGAAACAATGGCTGCCCA (forward primer) and TTGTCCCCAATAGATGGCGA (reverse primer). The melon GAPDH gene was used as an internal reference (forward primer: CTTTCCGTGTTCCTACCGTT; reverse primer: CAGTGTACCCCAAAATTCCC). Real-time quantitative PCR was performed using cDNA obtained from RT-PCR and the amplification product was detected using fluorescent dye staining. The reaction mixture consisted of: 5 μL H_2O, 8 μL 2X SYBR GREEN PCR mix, 1 μL forward primer, 1 μL backward primer, and 1 μL cDNA. The reaction was mixed well and placed in an ABI ViiA7 Real Time PCR System (Applied Biosystems) for amplification. The thermocycle consisted of 95°C pre-denaturation for 2 min, 40 cycles of 94°C for 10 s, 60°C for 10 s and 72°C for 40 s. The relative changes in gene expression ports was analyzed by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The results were compared using variance analysis in SPSS V. 17.0.

RESULTS

Cloning and sequencing of gene encoding chitinases

A 1011-bp gene fragment was amplified and the agarose gel electrophoresis result was shown in Figure 3. The BLASTp results indicated that the cloned sequence belonged to family 19 chitinases, and had 98% similarity with the chitinases of *Cucumis melo* (muskmelon: LOC103485181). The gene was named *HmCHT-2* and submitted to NCBI with the accession No. KX083346.

Sequence analysis of gene encoding Hami melon chitinases HmCHT-2

To investigate the features of the chitinases, number of amino acids, molecular weight, theoretical isoelectric point, fat index, and grand average of hydropathy were predicted by using ProtParam and ProtScale. Results were shown in Table 2. The prediction of protein tertiary structure is shown in Figure 4.

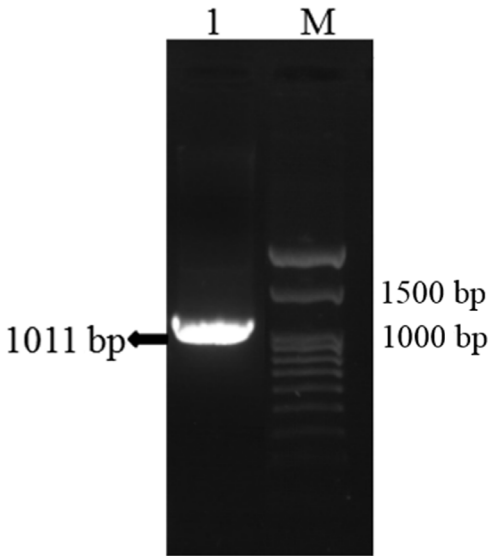

Figure 3. Electrophoresis of PCR products.

Table 2. Physical and chemical properties prediction of Hami melon chitinases HmCHT-2.

Analysis index	Value	Prediction software
Number of amino acids	312	ProtParam (http://web.expasy.org/protparam/)
Molecular weight	33.81 kU	ProtParam (http://web.expasy.org/protparam/)
Theoretical isoelectric point	8.42	ProtParam (http://web.expasy.org/protparam/)
Fat index	51.70	ProtParam (http://web.expasy.org/protparam/)
Grand average of hydropathy	0.435	ProtScale (http://web.expasy.org/protscale/)

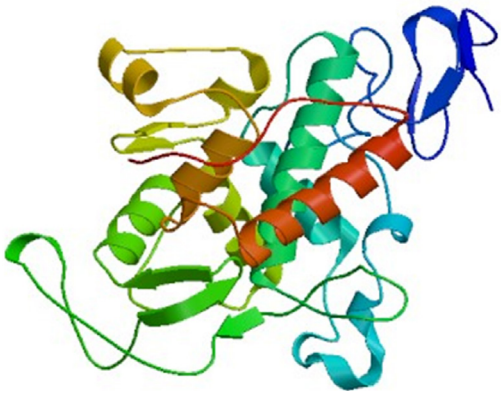

Figure 4. Tertiary structure model of HmCHT-2 protein.

The *HmCHT-2* gene included a complete ORF that encoded a protein product of 312 amino acids (Figure 5). The protein, encoded by the *HmCHT-2*, consisted of an N-terminal signal peptide, chitin-binding domain (CBD) and catalytic domain, and belonged to class I chitinases. The bioinformatic analysis predicted that the molecular weight and theoretical

isoelectric point of the *HmCHT-2*-encoded protein was 33.81 kU and 8.42, respectively. The instability index of this protein was predicted to be 34.13, which indicated that this protein was stable. The total average hydrophilicity was -0.435, suggesting the protein is hydrophilic. The analysis also predicted a signal peptide with a cleavage site between the 20th and 21st amino acids. Predictions of the transmembrane domains indicated that there was no transmembrane domain in the *HmCHT-2*-encoded protein and that the entire peptide chain was extra-membranous. The prediction of the subcellular localization indicated that HmCHT-2 mainly accumulates in the vacuole, suggesting that HmCHT-2 may contain a C-terminal extension and could thus be characterized as a class Ia chitinase. The function of the C-terminal extension within class Ia chitinases is to guide the transportation of mature chitinases from the Golgi apparatus to the vacuole. The chitinases that do not contain the C-terminal extension will be secreted to the outside of the cell plasma membrane (Flach et al., 1992). The prediction of the secondary protein structure of HmCHT-2 indicated that 55.45% of the amino acids participated in the formation of random coils, 22.76% participated the formation of the α-helix, 15.06% participated in the formation of extended structures and 6.73% participated in the formation of β-turns.

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1      CTTGCATGCCTGCAGGTCGACGATTATGAAGACATATTCACTTATAATCCTATCCTTTGC
1                                M  K  T  Y  S  L  I  I  L  S  F  A
61     CTTTCTTTTGGGAGCTGCCTCGGCCGAGCAATGTGGGCGGCAGGCCAATGGTGCTCTATG
13       F  L  L  G  A  A  S  A  E  Q  C  G  R  Q  A  N  G  A  L  C
121    CCCCAATAACCTCTGCTGCAGCCAGTTTGGGTTCTGCGGTGACACTGACGACTATTGTAA
33       P  N  N  L  C  C  S  Q  F  G  F  C  G  D  T  D  D  Y  C  K
181    AAATGGTTGTCAGAGCCAGTGTCGTGGCTCTAGTACCCCTACGCCCTCCGGCGGCAGTGG
53       N  G  C  Q  S  Q  C  R  G  S  S  T  P  T  P  S  G  G  S  G
241    TGTGGGAAGTATCATAAGCGAAAGCCTTTACAATCAAATGCTCAAATATAGTAGGGATCC
73       V  G  S  I  I  S  E  S  L  Y  N  Q  M  L  K  Y  S  R  D  P
301    TCGATGTCCTAGTAACGGATTCTATACCTATAATGCTTTTATTACTGCTGCGCGATCCTT
93       R  C  P  S  N  G  F  Y  T  Y  N  A  F  I  T  A  A  R  S  F
361    CCCAACCTTTGGTACCACAGGAGATGCAACTACTCGTAAGAGGGAGATTGCAGCTTTTTT
113      P  T  F  G  T  T  G  D  A  T  T  R  K  R  E  I  A  A  F  F
421    CGGTCAAACTTCTCACGAAACTACAGGAGGATRGTCCACTGCACCAGATGGCCCATATGC
133      G  Q  T  S  H  E  T  T  G  G  X  S  T  A  P  D  G  P  Y  A
481    ATGGGGATATTGTTTCATACGAGAGAGAAATCAACAAAYATATTGCACACCTAGTCAACA
153      W  G  Y  C  F  I  R  E  R  N  Q  Q  X  Y  C  T  P  S  Q  Q
541    ATGGCCGTGTGCCCCTGGTCAACAATATTACGGTCGTGGACCAATCCAACTAACCCACAA
173      W  P  C  A  P  G  Q  Q  Y  Y  G  R  G  P  I  Q  L  T  H  N
601    CTACAACTATGGACCAGCAGGGAAAGCAATAGGAGCGCCGTTGCTAACCAACCCCRATAC
193      Y  N  Y  G  P  A  G  K  A  I  G  A  P  L  L  T  N  P  X  T
661    AGTGGCCACAGATCCAGTTACATCCTTCAAGACAGCCCTATGGTTTTGGATGACAGCRCA
213      V  A  T  D  P  V  T  S  F  K  T  A  L  W  F  W  M  T  A  Q
721    AGGAAATAAACCTTCTTGTCATAATGTTATTACCGGCAATTGGCAACCTTCGAGCGCTGA
233      G  N  K  P  S  C  H  N  V  I  T  G  N  W  Q  P  S  S  A  D
781    CAATGCTGCAGGAAGAGTCCCTGGTTATGGTGTCATCACCAACATCATTAACGGTGGACT
253      N  A  A  G  R  V  P  G  Y  G  V  I  T  N  I  I  N  G  G  L
841    CGAGTGTGGACATGGCCCAGATGATAGAGTTAAAGACAGAATTGGATTTTACAAACGATA
273      E  C  G  H  G  P  D  D  R  V  K  D  R  I  G  F  Y  K  R  Y
901    CTGCGACATGCTGGGTATTGGTTATGGCAACAATTTAGATTGCTACAATCAAAGGTCTTT
293      C  D  M  L  G  I  G  Y  G  N  N  L  D  C  Y  N  Q  R  S  F
961    CTAAAATCTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAATCCATGT
         *
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Figure 5. Nucleotide acid sequence and deduced amino acid sequence of HmCHT-2 (*termination codon).

BLAST analysis showed that the *HmCHT-2*-encoded protein shared the highest similarity with endochitinase MCHT-2 of *C. melo* (NP_001284473.1). Phylogenetic analysis of the amino acids indicated that class I chitinases of different species have evolved from a common ancestor. The class I chitinase *HmCHT-2* that we identified from the Hami melon belongs to same branch as *C. melo*, and is also closely related to cucumber (Figure 6).

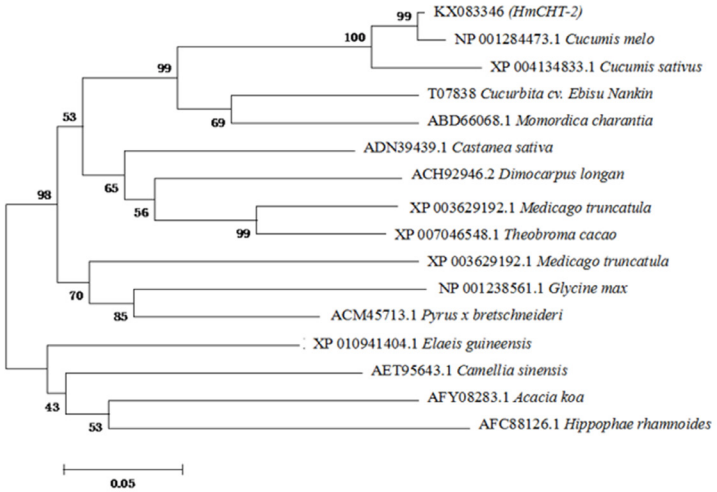

Figure 6. Neighbor-joining tree based on *HmCHT-2* of Hami melon and other species.

Gene expression analysis of *HmCHT-2*

The *HmCHT-2*-encoded class I chitinase-like enzyme may contribute to the defense against *Penicillium* infection in Hami melon. A preliminary real-time PCR study was performed to investigate the changes in expression levels of *HmCHT-2* subsequent to infection with the *Penicillium* strain which we isolated from Hami melon. The results showed that in the control group (absence of *Penicillium* inoculation), the transcription level of *HmCHT-2* was upregulated continuously during the first 24 h, peaked at 24 h (which was 62-fold higher than 0 h), and then dropped rapidly, reaching baseline level (0 h) at 48 h. Thereafter, the transcription level increased only slightly. In contrast, in the experiments where Hami melon was infected with *Penicillium*, the transcription level of *HmCHT-2* showed an overall increasing trend. While during the first 48 h the increase in transcription level of *HmCHT-2* was slow, 60 h post-infection the transcription level of *HmCHT-2* started to increase dramatically and peaked at 72 h post-infection; 545-fold higher than at 0 h. Thus, the time taken to reach peak level was delayed in the group infected with *Penicillium*. The incremental increases in the transcription level of *HmCHT-2* of the infected group was less than that of control group during the first 36 h, but increased significantly thereafter. Furthermore, the period of time for which high transcription levels were observed was also significantly greater than that of the control group ($P < 0.05$; Figure 7). Therefore, we conclude that chitinases are rapid and effective enzymes involved in the response of Hami melon to *Penicillium* infection.

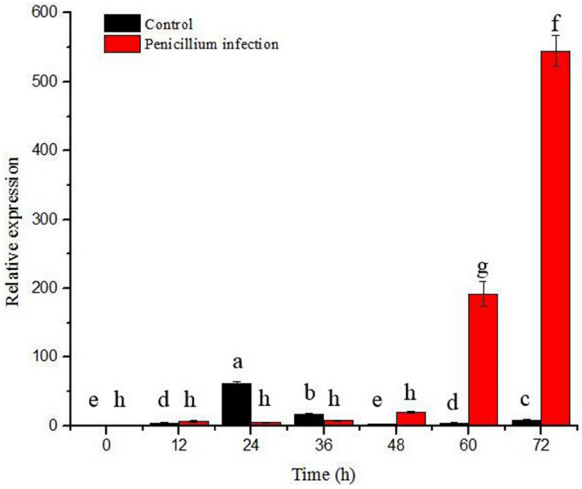

Figure 7. Relative expression of *HmCHT-2* gene at different time points. Values are reported as means ± SE (N = 3). Different letters indicate significant difference (P value < 0.05).

DISCUSSION

Based on amino acid sequence homology, three-dimensional protein structure and the hydrolysis mechanism of the catalytic reaction, chitinases can be characterized into 7 classes (Grover, 2012). Of these, class I chitinases are only found in plants and have a structure consisting of an N-terminal signal peptide domain, CBD and catalytic domain (Lu et al., 2012). Class I chitinases can be further divided into basic class Ia and acidic class Ib. Class Ia contains a C-terminal extension domain which generally guides the protein to the vacuole (Flach et al., 1992), while class Ib is mainly located extracellularly. Protein structure prediction results in this study indicated that *HmCHT-2* possesses all the typical structural features of a class I chitinase; including a 20-amino acid signal peptide followed by a 40-amino acid CBD, a catalytic domain after 140th amino acid, and six glycosylation-binding sites. ProtComp9.0 forecasts suggest that HmCHT-2 and AC=19171 in the database have a high similarity, the score of HmCHT-2 located in vacuoles (Vacuolar) is 5.56, while the score of HmCHT-2 located in Extracellular is 4.43. Again, we use PredictProtein online prediction, the result shows that it is located in the vacuole, with a signal score of 65. The specific location still need further experiments.

Chitinases play dual roles in the defense against fungal diseases. Exochitinases mainly function at the early stages of the infection process. When mycelium passes through plant cell walls, the interaction between exochitinases and the chitin in the mycelial cell wall leads to the release of inducers and the transduction of invasive signals. At a later stage, these particles bind to specific receptors, converting the initial innate immune response to an adaptive immune response. This response includes an increased expression level and synthesis rate of exochitinases, as well as increased induction of the synthesis of vacuole chitinases. The dramatic increase in exochitinases accelerates the production of inducers, therefore indirectly enhancing the invasive signal transduction (de A Gerhardt et al., 1997). When mycelium penetrates into intracellular spaces and damages the cells, the protoplast is disrupted and vacuole chitinases begin to exert anti-fungal functions by degrading the newly synthesized

chitin chain and inhibiting fungal proliferation. In the response of plants to pathogenic attack, exochitinases might be the main mediator for the invasive signal transduction, while vacuole chitinases might be the main executor to inhibit fungal growth. Vacuole chitinases catalyze chitin more effectively than exochitinases, while exochitinases hydrolyze water-soluble chitin more effectively than vacuole chitinases. In the coevolution of plants and fungi, vacuole chitinases (especially Class I) have played important roles in plant defense mechanisms (Bishop et al., 2000).

Plants have developed a remarkable array of structural, chemical and protein-based defenses system (Simmons, 1994). Extensive research on the interaction between plant hosts and pathogens has revealed that chitinase-mediated defense, which is able to inhibit the growth of pathogens, belongs to the first type of four major biochemical reactions in plant defense against fungal invasion (Ebrahim et al., 2011; Keen, 1990). In this study, we investigated the expression level of one chitinase. The results indicated that the HmCHT-2 may related to Hami melon in defense against disease. To further understand how Hami melon responds to *Penicillium*, we aim to systematically elucidate the structural characteristics, precise subcellular localization and change in expression levels of additional chitinases.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Bi Y, Tian SP, Guo YR, Ge YH, et al. (2006). Sodium silicate reduces postharvest decay on Hami melons: induce resistance and fungistatic effects. *Plant Dis.* 90: 279-283. http://dx.doi.org/10.1094/PD-90-0279

Bishop JG, Dean AM and Mitchell-Olds T (2000). Rapid evolution in plant chitinases: molecular targets of selection in plant-pathogen coevolution. *Proc. Natl. Acad. Sci. USA* 97: 5322-5327. http://dx.doi.org/10.1073/pnas.97.10.5322

Chen CK, Wang WS, Ning J and Gao YH (2012). Isolation and identification of the main pathogenic fungi from postharvest Hami melon in later period of storage. *Adv. Mat. Res.* 343: 1212-1216.

Cong F, Zhang Y and Dong W (2007). Use of surface coatings with natamycin to improve the storability of Hami melon at ambient temperature. *Postharvest Biol. Technol.* 46: 71-75. http://dx.doi.org/10.1016/j.postharvbio.2007.04.005

Dangl J and Jones JD (1998). Plant-microbe interactions. Affairs of the plant: colonization, intolerance, exploitation and co-operation in plant-microbe interactions. *Curr. Opin. Plant Biol.* 1: 285-287. http://dx.doi.org/10.1016/1369-5266(88)80047-5

de A Gerhardt LB, Sachetto-Martins G, Contarini MG, Sandroni M, et al. (1997). *Arabidopsis thaliana* class IV chitinase is early induced during the interaction with *Xanthomonas campestris*. *FEBS Lett.* 419: 69-75. http://dx.doi.org/10.1016/S0014-5793(97)01332-X

Ebrahim S, Usha K and Singh B (2011). Pathogenesis-related (PR)-proteins: Chitinase and β-1,3-glucanase in defense mechanism against malformation in mango (*Mangifera indica*, L.). *Sci. Hortic. (Amsterdam)* 130: 847-852. http://dx.doi.org/10.1016/j.scienta.2011.09.014

Flach J, Pilet PE and Jollès P (1992). What's new in chitinase research? *Experientia* 48: 701-716. http://dx.doi.org/10.1007/BF02124285

González FFNDC, Davey MR, Sanchez EC and Wilson ZA (2015). Conferred resistance to *Botrytis cinerea* in *Lilium* by overexpression of the RCH10 chitinase gene. *Plant Cell Rep.* 34: 1-9.

Graça I, Liang J, Guilherme M, Tavares P, et al. (2016). Cloning, overexpression and functional characterization of a class III chitinase from *Casuarina glauca* nodules. *Symbiosis* 70: 139-148. http://dx.doi.org/10.1007/s13199-016-0403-1

Grover A (2012). Plant Chitinases: Genetic diversity and physiological roles. *Crit. Rev. Plant Sci.* 31: 57-73. http://dx.doi.org/10.1080/07352689.2011.616043

Guo XL, Bai LR, Su CQ, Shi LR, et al. (2013). Molecular cloning and expression of drought-induced protein 3 (DIP3) encoding a class III chitinase in upland rice. *Genet. Mol. Res.* 12: 6860-6870. http://dx.doi.org/10.4238/2013.December.19.5

Guo Y, Liu L, Zhao J and Yang B (2007). Use of silicon oxide and sodium silicate for controlling *Trichothecium roseum*, postharvest rot in Chinese cantaloupe (*Cucumis melo*, L.). *Int. J. Food Sci. Technol.* 42: 1012-1018. http://dx.doi.org/10.1111/j.1365-2621.2006.01464.x

Keen NT (1990). Phytoalexins and their elicitors. *ACS Symp.* 439: 114-129.

Kombrink E, Schröder M and Hahlbrock K (1988). Several "pathogenesis-related" proteins in potato are 1,3-β-glucanases and chitinases. *Proc. Natl. Acad. Sci. USA* 85: 782-786. http://dx.doi.org/10.1073/pnas.85.3.782

Liu T, Tong JM, Ma WR, Wang J, et al. (2013). Separation and identification of main pathogen in Hami melon during cold storage. *Mod. Food Sci. Technol.* 8: 2030-2034.

Livak KJ and Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCt method. *Methods* 25: 402-408. http://dx.doi.org/10.1006/meth.2001.1262

Lu HC, Lin JH, Chua ACN, Chung TY, et al. (2012). Cloning and expression of pathogenesis-related protein 4 from jelly fig (*Ficus awkeotsang* Makino) achenes associated with ribonuclease, chitinase and anti-fungal activities. *Plant Physiol. Biochem.* 56: 1-13. http://dx.doi.org/10.1016/j.plaphy.2012.04.004

Ryals J, Uknes S and Ward E (1994). Systemic acquired resistance. *Plant Physiol.* 104: 1109-1112. http://dx.doi.org/10.1104/pp.104.4.1109

Shan CH (2015). Transcriptomics and Proteomics of Hami melon responding to *Penicillium* infection and change analysis of resistance-related enzymes. Jiangnan University, Wuxi.

Sharma N, Sharma KP, Gaur RK and Gupta VK (2011). Role of chitinase in plant defense. *Asian J. Biochem.* 6: 29-37. http://dx.doi.org/10.3923/ajb.2011.29.37

Simmons CR (1994). The physiology and molecular biology of plant 1,3-β-D-glucanases and 1,3;1,4-β-D-glucanases. *Crit. Rev. Plant Sci.* 13: 325-387.

Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinoglu S, et al. (1993). Plant 'pathogenesis-related' proteins and their role in defense against pathogens. *Biochimie* 75: 687-706. http://dx.doi.org/10.1016/0300-9084(93)90100-7

Taira T (2010). Structures and antifungal activity of plant chitinases. *J. Appl. Glycosci.* 57: 167-176. http://dx.doi.org/10.5458/jag.57.167

Wu B, Zhang B, Dai Y, Zhang L, et al. (2012). Brittle culm15 encodes a membrane-associated chitinase-like protein required for cellulose biosynthesis in rice. *Plant Physiol.* 159: 1440-1452. http://dx.doi.org/10.1104/pp.112.195529