Genome-wide identification and expression analysis of chitin-binding gene family in Brassica oleracea L. reveals its role in different disease resistance

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Abstract

Background

Chitinase, a category of pathogenesis-related proteins, is thought to play an important role in defending external stress in plants. However, comprehensive analysis of chitin-binding gene family has not yet been reported in cabbage (*Brassica oleracea* L.), especially their roles in response to different diseases.

Result

in this study, A total of 20 chitinase genes were identified using a genome-wide search method. Phylogenetic analysis classified these genes into two groups. They were distributed unevenly across six chromosomes in cabbage, and all of them contained few introns (≤ 2). The results of colinear analysis showed that the cabbage genome contained 1–5 copies of each chitinase gene (excluding *Bol035470*) found in *Arabidopsis*. The heatmap of the chitinase gene family showed that these genes were expressed in various tissues and organs. In addition, under four different stresses of Fusarium wilt, powdery mildew, black spot and downy mildew, we detected 9, 5, 8 and 8 genes with different expression, respectively.

Conclusions

Our results provide insights for further understanding the role of chitinase in host plants response to different diseases.

Background

Chitin, a polymer of N-acetyl-β-D-glucosamine, is widely found in insect carapace and the cell walls of pathogenic bacteria [1]. The cell wall determines the shape and strength of the pathogen cells and is a key determinant of cell morphology development. As one of the main components of the cell wall, chitin plays a very important role in the growth and development of pathogenic bacteria as well as defense against external stress [2]. The degradation product of chitin is chitin oligosaccharide, which can induces the natural immunity of plants [3]. Previous study suggested that the perception of the chitin oligosaccharides contributes to enhance disease resistance against the rice blast fungus in rice [4].

Chitinase is an enzyme system that uses chitin as a substrate and hydrolyzes it to N-acetol oligosaccharide and glucose. Chitinase is widely present in various organs of higher plants and can be rapidly produced and accumulated after infection by pathogens [5]. The induction of pathogenic bacteria can enhance the activity of chitinase, which then inhibit spore germination and mycelial growth, and even directly degrade the chitin of the fungal cell wall [6]. Abiotic stresses such as heavy metals and high temperatures can also induce the expression of chitinase gene and promote their accumulation [7, 8]. Margispinheiro et al. (1994) examed the expression pattern of chitinases in bean in response to various

stresses and showed that salicylic acid (SA) and heat shock treatment can induce the expression of chitinase [9]. Liu et al. (2005) reported that a class IV chitinase gene was induced and chitinase was accumulated in both susceptible and slow-canker-growth resistant seedlings of western white pineafter infection by *Cronartium ribicola* [10]. Chitinase genes were also induced during the infection by *Botryosphaeria berengeriana* and *Aphis citricota* in *Malus hupehensis* [11].

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most important leafy vegetables belonging to *Brassica oleracea* species cultivated worldwide, with a yield of 28.4 tons per hectare [12].

Fusarium wilt (FW) is a fungal disease caused by *Fusarium oxysporum*, which can infect many different types of crops such as tomatoes, cucumbers, bananas, cotton, flax and chrysanthemums [13]. The specialization type of cabbage wilt pathogen is *Fusarium oxysporum f. sp. Conglutinans*. This type include two races No. 1 and No. 2, among which the race 1 is the main race in the world [14]. *F. oxysporum* can break through the barrier of the cell wall of the root of the crop by secreting cell wall degrading enzyme (CWDE) [15]. It then secretes toxins that destroy the structure of the host cell and impede the normal metabolic processes of the host plant [16]. In addition, pathogenic bacteria can also adjust their own metabolic reactions and secreted proteins to adapt to the surrounding environmental conditions, so that they can complete the infection more effectively [17].

black spot (BS) disease is one of the important diseases of cruciferous vegetables, mainly caused by *Alternaria*. The spores of *Alternaria brassicae* adhere to the host with the help of lipase and recognize the leaves with waxy matter. Then, with the help of several serine esterases, mycelium was able to penetrate the host epidermis [18]. The invasion of pathogenic bacteria will lead to the change of metabolite content in the crops. Doughty et al. (1991) reported that the contents of glucosinolate in rapeseed increased after being infected by *Alternaria brassicae* [19]. Chawla et al. (2001) found that the infection of pathogenic bacteria will induce an increase of polyphenol oxidase in *Brassica juncea* [20].

Plasmodiophora brassicae Wor. is a soil-borne biotrophic pathogen, leading to clubroot in *Brassica* crops and then cause severe crop yield loss [21]. The life cycle of *P. brassicae* contained two stages: primary infection stage and secondary infection stage. Firstly, resting spores release primary zoospores, which can penetrates through the cell wall of the root hairs and form primary plasmodia therein. Subsequently, primary plasmodia form secondary resting zoospores then infected cortical cells of the main roots, followed by gall formation in the tissues. The plasmodia finally develop into a new generation of resting spores, followed by their release back into soil as survival structures. The rotten swollen root finally releases the resting spores into the soil, thus the life history was completed [22].

Cabbage powdery mildew (PM), caused by *Erysiphe cruciferarum*, is also a common disease in cruciferous crops. The disease is transmitted mainly through the air. The mycelia of pathogenic bacteria can be attached to the epidermal tissue of the plant, and then insert a sucker into the tissue to absorbed nutrient. The typical symptoms of the disease are chlorosis and yellowing of the leaves, and even plant death.

downy mildew (DM) is another common fungal disease of cruciferous crops, mainly caused by *Hyaloperonospora brassicae* [23]. Pathogenic spores can overwinter in the soil and wait for suitable environmental conditions to germinate and infect new plants. It will cause a white mold layer on the back of the infected plant's leaves, where with more stomata and is conducive to the reproduction of pathogen [24]. which will then turn yellow-brown. In the later stages of the disease, the leaves gradually turn yellow-brown and die.

Chitinase can protect plants from a variety of pathogenic bacteria. Ntui et al. (2011) increased tobacco resistance to FW by transferring chitinase genes into tobacco [25]. The same results were also confirmed in tomatoes [26]. Similarly, transgenic grapes carrying the wheat chitinase gene have increased resistance to DM [27]. Marchant et al. (1998) reports that expression of the chitinase transgene reduced the severity of blackspot development by 13–43% in rose [28]. Ludwig-Müller et al. (1994) reports that the activity of chitinase in infected Chinese cabbage was significantly enhanced [29]. Similarly, Chen et al. (2018) found that the expression of chitinase reduced the symptoms of clubroot in Chinese cabbage [30]. In addition, exogenous application of chitinase to barley can also inhibit the proliferation of PM pathogens.

Although the function of chitinase has been analyzed in various plant species, such as tomato [31], potato [32], rice [33], *Brassica juncea* [34], wheat [35] and apple [36], the role of chitinase gene family in cabbage with FW, BS, clubroot, PM and DM are currently unavailable.

In this study, 20 chitinase genes were identified in cabbage and their chromosome location, gene structure, colinearity relationships, evolution and the *cis*-acting regulatory elements in promoters were further analyzed. The expression patterns of the chitinase family genes responses to FW, BS, clubroot, PM and DM were also investigated. Our results will promote to understand the chitinase functions associated with various diseases response, as well as inform the genetic improvement of cabbage.

Results

Genome-wide identification and phylogenetic analysis of chitinase genes in cabbage

To identify chitin-binding genes in cabbage genome (02–12), using hidden Markov model for predicting chitin recognition protein in cabbage protein sequences. A total of 20 chitinase genes were identified (Table 1). The length of these 20 chitinase proteins ranged from 117 (*Bol030012*) to 447 (*Bol007321*) amino acid (aa). Within the 20 chitinase proteins, 13 members shared the similar localization to vacuole, 1 to extracell and 6 members were located in more than one compartment.

Based on the amino acid sequence of cabbage (21), *Arabidopsis* (8), and *Brassica. rapa* (17) chitinase proteins, the chitinase proteins phylogenetic tree of the chitinase family genes was constructed using software MEGA 6.0. According to previous studies [37], The chitinase genes were classified into two groups (class I and IV; Fig. 1), which contained 14 (ten cabbage, one *Arabidopsis* and three *Brassica. rapa*

members), and 32 (eleven cabbage, seven *Arabidopsis* and fourteen *Brassica. rapa* members) members, respectively. All chitinases belong to the glycoside hydrolasis 19 (GH-19) family and they all have an N-terminal chitin. binding domain and a GH-19 catalytic domain [38].

Chromosomal distribution and collinear analysis

The 20 chitinase genes were assigned to six chromosomes of cabbage (Fig. 2). The distribution of the chitinase genes on each chromosome was uneven. The numbers of chitinase genes on each chromosome are as follows: 2 on C01, 8 on C03, 4 on C04, 3 on C05, 2 on C08, 1 on C09.

Gene duplication is a common phenomenon in the evolution of plants, which is the reason for the formation of homologous genes in different plants. Due to the importance of gene duplications on the evolution of gene families in plants, chitinase gene replication in cabbage and the colinearity between cabbage and *Arabidopsis* of chitinase genes was analyzed.

The cabbage genome contained 1–5 copies of each chitinase gene (excluding *Bol035470*) found in *Arabidopsis* (Fig. 3; Additional file 2: Table S2). For example, *AT1G56680.1* contained only one homologous gene (*Bol029467*) in cabbage, while *AT2G43590.1* contained up to five homologous genes (*Bol004604, Bol039802*, *Bol021626*, *Bol030012*, and *Bol025197*) in cabbage. In addition, 32 segmentally duplicated gene pairs were also identified among the 20 chitinase genes in the cabbage genome (Fig. 3; Additional file 1: Table S1).

Structure and conserve motifs analysis of chitinase genes

To further explore the structural diversity of chitinase genes, the gene structure among 20 chitinase genes were detected (Fig. 4b). Thirteen genes contain two exons, 4 genes contain 3 exons, and 3 genes contain only 1 exon. The length of exons in most genes was similar while the length of introns for some genes varied widely. For example, *Bol021627* and *Bol029469* contained one shorter introns, whereas *Bol029467* contained two extremely long introns.

To better understand the structural characteristics of the chitinase genes, the conversed domain and motifs were also detected (Fig. 4a and b). All members contained motifs 2 and 6. Motifs 3, 4 and 9 were uniquely present in members of Class I, while Motifs 7 and 8 were almost present in members of Class IV. Only one member of Class I contains motif 7. Among the 20 chitinase proteins, sixteen members contained motifs 1, 15 members contained motif 5 and 14 members contained motif 10. Motifs 2, 3, 6, 1, 4, 9 displayed in same order were found in Class I and motifs 5, 10, 2, 7, 6, 1, 8 displayed in same order were found in Class IV. In addition, *Bol011420*, *Bol040748*, *Bol041024, Bol023322* and *Bol035470* have the same motif composition.

As shown in Fig. 4b, most proteins contained chitinase bind domain and GH19. Only one protein contained K^+ transit domain. The chitinase like domain was present in 14 genes. Cystatin domain only existed on 1 gene (*Bol007321*). In addition, Most genes in Class I contained lysozyme like domain, which only exited on 1 (*Bol029467*) gene in Class IV.

The cis-elements in the promoter of B. oleracea chitinase genes

To further clarify the regulatory mechanism of chitinase genes in cabbage response to FW, clubroot, BS, PM and DM, we identified the *cis*-elements using PlantCARE database based on the promoter sequences and ten types of *cis*-acting regulatory elements were detected (Fig. 5). All 20 chitinase genes contained 3–17 light responsiveness *cis*-elements. Seven chitinase genes contained gibberellin-responsive *cis*-elements. Eight chitinase genes contained MYBHv1 binding *cis*-elements. Salicylic acid responsiveness *cis*-elements was detected in 10 chitinase genes while the *cis*-elements related to MeJA and auxin-responsiveness were detected in 15 and 11 chitinase genes, respectively. In addition, the *cis*-elements related to defense and stress responsiveness as well as low-temperature responsiveness existed in 8 and 9 genes, respectively. The distribution of drought-inducibility *cis*-elements were relatively small and was detected only in 3 genes. Anyhow, the *cis*-element analysis illustrated that chitinase genes could respond to different stimuli.

Expression patterns of chitinase genes and qRT-PCR verification

The RNA-Seq dataset (GSE42891) was examined to determine the expression levels of chitinase genes in the leaves, stem, flowers, siliques, buds, calli and roots of cabbage. Most of the chitinase genes exhibited different expression patterns (Fig. 6). Eighteen were expressed in all organs, while the expression of two (*Bol030015* and *Bol007323*) was almost not detected. *Bol023322* and *Bol041024* were highly expressed in all tissues, however some genes were expressed only in one or two organ types such as *Bol030012* in leaves and *Bol029469* in siliques and calluses. The multiple expression patterns of the chitinase genes indicate their extensive biological functions during the growth and development of cabbage.

In order to explore the role of chitinase in cabbage responding to the infection of different pathogens, we inoculated different cabbage materials with five pathogens and extracted plant tissue RNA at a specific period for transcriptome sequencing. Then two heatmaps was established according to the RNA-seq data (Fig. 6; Fig. 7).

The expression levels of chitinase genes in 01–20 and 96–100 infected by *F. oxysporum* were quite defferent. Four genes (*Bol010293*, *Bol007321*, *Bol021626* and *Bol025197*) were upregulated in 96–100 compared with 01–20, while five genes (*Bol035464*, *Bol021627*, *Bol035467*, *Bol029469* and *Bol040748*) were downregulated. In different stages, the expression patterns of chitinase genes are also different. Five (*Bol035464*, *Bol010293*, *Bol021627*, *Bol007321* and *Bol035467*) and two genes (*Bol023322* and *Bol041024*) were significantly up- and down-regulated, respectively, in both 01–20 and 96–100 after innoculated by *F. oxysporum*. The expression levels of the three genes (*Bol029469*, *Bol030015* and *Bol004604*) increased first and then decreased in both 01–20 and 96–100 after innoculated. In contrast, the expression levels of the two genes (*Bol011420* and *Bol040748*) decreased first and then increased in 96–100 after innoculated. Compared with 01–20, five (*Bol035464*, *Bol010293*, *Bol021627*, *Bol035467*

and *Bol040748*) and five (*Bol007321*, *Bol041024*, *Bol0304604*, *Bol021626* and *Bol025197*) genes were significantly down- and up-regulated, respectively, in 96–100 after innoculated. qRT-PCR was conducted to verify the chitinase gene expression patterns under stress of *F. oxysporum* in different inoculation periods of 01–20 and 96–100. As shown in Fig. 8, seven genes we detected by qRT-PCR are roughly consistent with the RNA-seq analysis except for *Bol004604*, which further confirmed their expression patterns.

The expression patterns of the chitinase genes in response to invasion of *Erysiphe cruciferarum*, *Alternaria*, and *Hyaloperonospora brassicae* is also different in diseased and normal leaves. Compared with PM leaves, three (*Bol023322*, *Bol041024* and *Bol035470*) and two genes (*Bol040748* and *Bol021626*) were up- and down-regulated, respectively, in normal leaves. Similarly, three (*Bol007321*, *Bol023322* and *Bol041024*) and five (*Bol035470*, *Bol011420*, *Bol040748*, *Bol021626* and *Bol025197*) genes were up- and down-regulated, respectively, in normal leaves compare to BS leaves. In addition, compared to the leaves with DM, two (*Bol041024* and *Bol035470*) and six (*Bol010293*, *Bol007321*, *Bol023322*, *Bol011420*, *Bol040748* and *Bol021626*) chitinases were up-regulated and down-regulated in normal leaves, respectively.

Under stress of *P. brassicae*, there was little change of the expression levels of all chitinase genes in 8 different treatments, which may suggest that chitinase genes were of little effect in resistanting clubroot. And the qRT-PCR results of six genes were also basically consistent with the RNA-seq analysis (Fig. 9).

Discussion

Induced resistance means that the stimulus of the pathogen increases the defense of the plant [39]. Chitinases, as a subgroup of pathogenesis-proteins, play an important role in plant defense against pathogen invasion [40]. Previously, chitinases have been identified and their role in defending against various pathogens in different crops has been discussed [41–43]. However, the expression patterns of chitinase genes in response to *F. oxysporum*, *P. brassicae*, *Alternaria*, *Erysiphe cruciferarum* and *Hyaloperonospora brassicae* in cabbage have remained uncertain until now. In this study, 20 chitinase genes were identified and their phylogenetic relationship, colinearity, structures, chromosomal locations, *cis*-elements and expression patterns in response to the invasion of different pathogens in cabbage were reported. This research provides comprehensive information for a better understanding of the chitinase gene family in cabbage.

Gene duplication, including segmental duplication, tandem duplication, transposition events and whole-genome duplication [44], have been considered of the potential drive for evolution and increase in biological diversity [45, 46]. 350 million years ago, a triploidization of the genome of *Arabidopsis thaliana* provided an opportunity for large-scale expansion of certain genes in *Brassica* crops [47]. In this study, we found that the number of chitinase genes in *B. rapa* (17) and *B. oleracea* (21) nearly doubled or tripled when compared with the number in *A. thaliana* (8) (Fig. 1; Fig. 3;). Every chitinase gene in *Arabidopsis* has at least one homologous gene in the cabbage genome. In addition, we also found 32 segmentally

duplicated gene pairs among the 20 chitinase genes in the cabbage genome. Therefore we speculate that the chitinase gene also undergoes differentiation and fractionation (loss) after undergoing a triploidization event [48]. In summary, both the segmental duplication and the large-scale wholegenome duplicated events contributed to the the functional diversity of cabbage chitinase family.

Differences in gene structure have important effects on gene function [49]. Genes with fewer introns can be transcribed faster to cope with various external stresses such as pathogenic and heat shock stress [30, 50]. Similar studies have been reported in the leucine-rich repeat family [51] and the GRF family [52]. In our study, only two genes (*Bol029467* and *Bol007321*) contained longer introns, whereas the other genes contained only one or no intron. This may these chitinase genes will be able to react faster for the infected of different pathogen in cabbage. Additionally, We have noticed similar motif arrangements and domain composition in the same subfamily members (Fig. 4). This correlation between motif arrangement and domain composition further confirmed the classifications of the chitinase genes.

Hormones also have a certain effect on the defense response of plants. SA or Methyl jasmonate (MeJA) inducers can triggered the more aggressive defense responses and provided better protection against FW, clubroot, PM, DM and BS [53–57]. Exogenous application of gibberellin can prevent persimmon fruit BS [58]; And the changes of activity and level of gibberellin in oil palm caused by Fusarium oxysporum will lead to stunting of host. In this study, salicylic acid responsiveness *cis*-elements was detected in 10 chitinase genes. The *cis*-elements related to MeJA and gibberellin were detected in 15 and 7 genes. In addition, In addition, we also detected eight genes containing the *cis*-elements related to defense and stress responsiveness. This may indicated the special role of these genes in defending against different diseases.

Transcriptome sequencing revealed that the expression of 20 chitinase genes in different tissues were quite different. *Bol023322* and *Bol041024* were highly expressed in all tissues, showing the expression characteristics of housekeeping genes [59]. For the chitinase gene members, we were particularly interested in those that might play crucial roles in disease resistance. In this study, we analyzed the expression patterns of 20 chitinase genes under 5 different disease stresses. Under the stress of FW, we detected four (*Bol010293*, *Bol007321*, *Bol021626* and *Bol025197*) and five genes (*Bol035464*, *Bol021627*, *Bol035467*, *Bol029469* and *Bol040748*) that up- and down-regulated significantly in 96–100 compared with 01–20. For PM, three (*Bol023322*, *Bol041024* and *Bol035470*) and two genes (*Bol040748* and *Bol021626*) were up- and down-regulated, respectively, in normal leaves compared with PM leaves. Similarly, three (*Bol007321*, *Bol023322* and *Bol041024*) and five (*Bol035470, Bol011420*, *Bol040748*, *Bol021626* and *Bol025197*) genes were up- and down-regulated, respectively, in normal leaves compare to BS leaves. Additionly, compared to the leaves with DM, two (*Bol041024* and *Bol035470*) and six (*Bol010293*, *Bol007321*, *Bol023322*, *Bol011420*, *Bol040748* and *Bol021626*) chitinases were up-regulated and down-regulated in normal leaves, respectively. The promoter region of all the above differentially expressed genes (except for *Bol025197*) contained at least one defense-related *cis*-elements such as SA, MeJA and defense and stress responsiveness *cis*-elements, which may indicated their crucial role in defending against different diseases. It is worth noting that several genes (such as *Bol007321* and

Bol040748) were differentially expressed in three or four different diseases, which further proves the role of chitinase in the defence of the organism against pathogen attack in plants [41].

Strangely, under the stress of rhizoma, almost all the chitinase genes did not show obvious differential expression, which may suggest that the defense effect of chitinase against clubroot disease was not significant in this study.

Conclusions

Here, a genome-wide analysis of *B. oleracea* chitinase genes was performed, and 20 chitinase genes were confirmed. Subsequently, analyses of chitinase genes on gene structures, phylogeny, chromosomal location, gene duplication and gene expression patterns were conducted based on bioinformatics and qRT-PCR methods. These genes were expressed in various tissues and organs. In addition, there were 9, 5, 8 and 8 genes with differential expression, respectively, under four different stresses of FW, PM, BS and DM, The study provides comprehensive information for further understanding the role of chitinase in host plants response to different diseases.

Methods

Genome-wide identification of the chitinase genes

The *Brassica oleracea* Genomics Database (www.ocri-genomics.org/bolbase/blast/blast.html) was used to download the cabbage whole-genome protein sequences. The Hidden Markov Model was download from the Pfam (http://www.sanger.ac.uk/Software/Pfam/) database (Pfam:PF00012). The conserved domains were analyzed by CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) databases. The subcellular locations were predicted using Cell-PLoc 2.0 (http://www. csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/).

Construction of the phylogenetic tree

Based on the amino acid sequences of chitinase derived from cabbage, *Arabidopsis thaliana* and *B. rapa*, we used MEGA6.0 [60] to construct an unrooted neighbor-joining (bootstrap = 1000) phylogenetic tree (bootstrap = 1000).

Localization analysis of the chitinase genes

We used The MapInspect software to draw the gene chromosome location diagrams based on the information of chitinase genes available at the cabbage genome database (http://plants.ensembl.org/Brassica_oleracea/Info/Index).

Collinearity analysis of chitinase genes

The microsyntenic relationships of the chitinase genes in cabbage and *Arabidopsis thaliana* were detected using BLAST searches of these genes against the whole genomes of these species. Then we

collected the physical location of the chitinase genes on each chromosome from the respective databases. The Circos tool [61] was used to visualize the relationships between two species.

Gene structure and conserved motif analyses

We used the MEME program (http://meme-suite.org/index.html) and NCBI-CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) to identify the conserved motif and protein sequences, respectively. TBtools was used to draw the gene exon-intron structure.

Analysis of cis-acting elements in chitinase genes

The *cis*-acting elements in the promoter of the chitinase genes were identified through submitting the upstream sequences (1.5 kb) of the initiation codon (ATG) of each chitinase gene to PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html).

Plant materials and treatments

The *F. oxysporum* used in this study belongs to race 1, which is the main race worldwide. Inbred lines 01–20 and 96–100 used for inoculation are susceptible and resistant to *F. oxysporum*, respectively. The roots of seedlings with three real leaves was soaked in a 1×10^6 cfu/ml spore suspension for 15 minutes, then transfer the seedlings to 32-well plugs. Two leaves from each plant of 01–20 and 96–100 at 0, 3, 6 and 9 dai (day after inoculation) were collected (18 individuals per treatment, 6 individuals per replicate), respectively, for RNA extraction.

The *P. brassicae* used in this study belongs to race 4 based on the differential sets of Williams [62]. A resting spore inoculum of 2×10^8 spores/ml was prepared before inoculation. Two commercial cabbage cultivars, Xiangan 336 and Jinfeng No. 1, which were resistant and susceptible to *Plasmodiophora brassicae*, respectively, were sown in 32-well (8 × 4) plugs. When the seedlings grown to two real leaves, we use a pipette to inject 2 ml of resting spore suspension in the soil around the roots of each seedling. Two kinds of treatment were performed for each cultivars. A treatment without inoculation was set as the control. Eight kinds of different root tissue samples, including Jingfeng No. 1 not inoculated at 7 days, Jingfeng No. 1 inoculated at 7 days, Xiangan 336 not inoculated at 7 days, Xiangan 336 inoculated at 7 days, Jingfeng No. 1 not inoculated at 28 days, Jingfeng No. 1 inoculated at 28 days, Xiangan 336 not inoculated at 28 days and Xiangan 336 inoculated at 28 days were collected (24 individuals per treatment, 8 individuals per replicate) for RNA extraction.

The cabbage material used for PM inoculation experiment was cabbage inbred line D157. When the seedlings grown to 4–5 real leaves, a resting spore suspension of 1×10^5 spores/ml was sprayed evenly onto the leaves of the plants in the treatment group. The plants of the control group was sprayed with equal amount of sterile water. During the pod-setting period, one diseased leaf of each plant in treatment group and one healthy leaf of each plant in control group were taken for RNA extraction. Three replicates was set up in the treatment group and the control group and each replicate contains 8 plants.

The cabbage material used for BS inoculation experiment was cabbage inbred line W18. When the seedlings grown to 2 real leaves, a resting spore suspension of 1×10^4 pfu/ml was sprayed evenly onto the leaves of the plants in the treatment group. The plants of the control group was sprayed with equal amount of sterile water. During the heading stage, one diseased leaf of each plant in treatment group and one healthy leaf of each plant in control group were taken for RNA extraction. Twenty-four plants were set up in this experiment and every eight plants was regarded as one biological repetition.

The cabbage material used for DM inoculation experiment was cabbage inbred line 01–20. When the seedlings grown to 2 real leaves, a resting spore suspension of 5×10^4 spores/ml was sprayed evenly to the back of the leaves of the plants in the treatment group. The plants of the control group was sprayed with equal amount of sterile water. During the heading stage, one diseased leaf of each plant in treatment group and one healthy leaf of each plant in control group were taken for RNA extraction. Twenty-four plants were set up in this experiment and every eight plants was regarded as one biological repetition.

All of the samples were quickly frozen in liquid nitrogen and stored at − 80℃ until RNA extraction.

The inbred line 01–20 was introduced to China from Canada in 1966 by the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences (IVF-CAAS). 96–100, D157 and W18 are backbone inbred lines cultivated by the cabbage-broccoli research group of IVF-CAAS for many years. Jinfeng No. 1 was developed by China Vegetable Seed Co.,Ltd and Xiangan 336 was developed by Syngenta Seeds. The resistance of 01–20 and 96–100 to FW has been identified by previous researchers [63]. Similarly, the resistance of Xiangan 336 and Jinfeng No. 1 to clubroot has been identified by predecessors [64]. The resistance of D157, W18 and 01–20 to PM, BS and DM, respectively, has been identified by researchers from the cabbage-broccoli research group of IVF-CAAS. The voucher specimens of all the above materials have been deposited in a public herbarium in IVF-CAAS.

Total RNA extraction

Total RNA was extracted from cabbage samples using TRIzol following the supplier's instructions (Transgen, Beijing, China). Then, the RNA quality was assessed by using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) and 1% formaldehyde gel electrophoresis. The cDNA was reverse transcribed with the HiScript® III 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China).

The specific primers for chitinase genes were designed with Premier 3.0 (Additional file 3: Table S3). qRT-PCR was carried out using 2 × RealStar Green Fast Mixture (GeneStar) in a Bio-Rad CFX96 Real Time PCR System. Each amplification reaction was conducted in a 20-μl reaction volume containing 10 μl KAPA SYBR, 0.5 μl of each primer, 2 μl diluted cDNA and 7 μl ddH2O. The PCR program was set as follows: 95℃ for 2 min, followed by 40 cycles of 95℃ for 15 s, 60℃ for 30 s and 72℃ for 30 s. Melting curve analysis was performed from 65 °C to 95 °C with increments of 0.5℃ every 5 s. Three independent biological and technical replicates were carried out for each reaction. The housekeeping gene actin was used as the internal reference gene.

Abbreviations

qRT-PCR: Quantitative real-time polymerase chain reaction; dai: Days after inoculation; MeJA: Methyl jasmonate; SA: salicylic acid; CDD: Conserved domain database; BLAST: Basic Local Alignment Search Tool; NCBI: National Center for Biotechnology Information; FW: Fusarium wilt, PM: powdery mildew, BS: black spot DM: downy mildew; aa: amino acid.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Availability of data and materials

All of the datasets supporting the results of this article are included within the article and its Additional files.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MZZ collected the public dataset, perform bioinformatics analysis and also drafted the manuscript. CCK and MZ contributed to bioinformatics analysis and the making of all the figures and tables. YYZ, HHL, JJL and XLH conceived this study and reviewed the manuscript. ZYF, YW and LMY reviewed the manuscript. All of the authors read and approved the final manuscript.

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Tables

Table 1 Information of cabbage chitinase genes

Gene ID	Chr	Genomic Location	Gene Length (bp)	Protein Length (aa)	Predicted Localization
Bol007321	C01	36002654-36007105	4452	447	Vacuole
Bol007323	C01	35993587-35994336	750	249	Vacuole
Bol004604	C03	8091984-8093153	1170	269	Extracell/Vacuole
Bol029467	C03	12670792-12678156	7365	408	Vacuole
Bol029469	C03	12681165-12682265	1101	273	Vacuole
Bol029470	C03	12701848-12702306	459	152	Cell wall/Vacuole
Bol035464	C03	20687053-20689765	2713	393	Vacuole
Bol035467	C03	20710123-20712350	2228	340	Vacuole
Bol035470	C03	20721329-20722591	1263	244	Vacuole
Bol039802	C03	8643798-8645535	1738	262	Extracell/Vacuole
Bol021626	C04	39859234-39859620	387	128	Cell wall/Vacuole
Bol021627	C04	39860449-39861636	1188	281	Vacuole
Bol030012	C04	1604570-1604999	430	117	Cell wall/Vacuole
Bol030015	C04	1617304-1618422	1119	274	Extracell/Vacuole
Bol010293	C05	30176713-30178044	1332	322	Vacuole
Bol040748	C05	494246-495037	792	231	Vacuole
Bol041024	C05	2018512-2020042	1531	321	Vacuole
Bol023322	C08	2077858-2079450	1593	322	Vacuole
Bol025197	C08	28963242-28964148	907	273	Vacuole
Bol011420	C09	680349-681621	1273	247	Extracell

Additional Files

Additional file 1: Table S1. Chitinase homologous genes in the genomes of *B. oleracea*.

Additional file 2: Table S2. Chitinase homologous genes in the genomes of *Arabidopsis thaliana* and *B. oleracea*.

Additional file 3: Table S3 The primer sequences of 9 chitinase genes used for qRT-PCR.

Figures

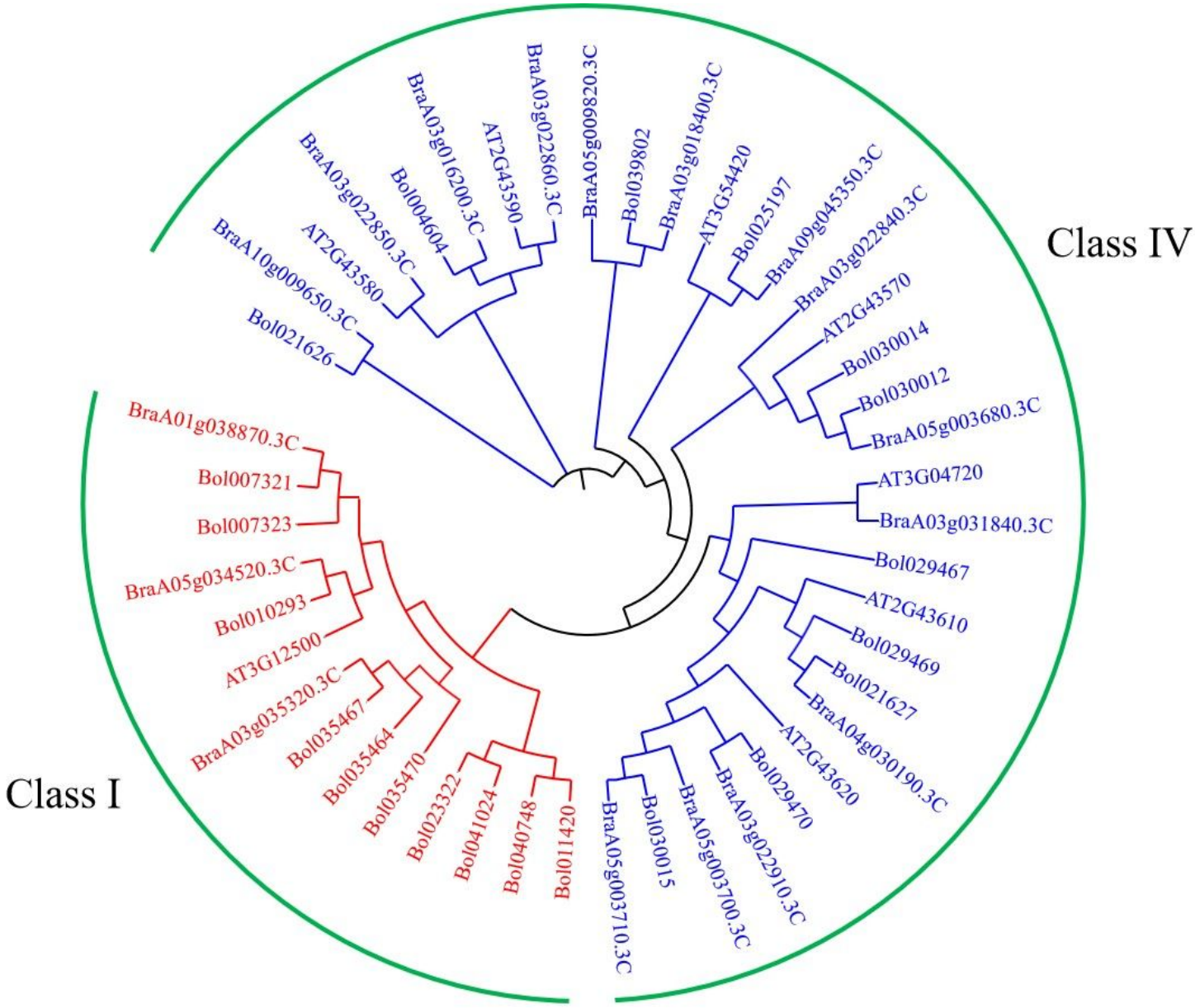

Figure 1

Phylogenetic tree of chitinase genes from cabbage, A. thaliana and B. rapa. The phylogenetic tree was built using the neighbor-joining (NJ) method with 1000 bootstrap replications. Roman numerals (I and IV) represent each gene cluster, which are labeled with different colors.

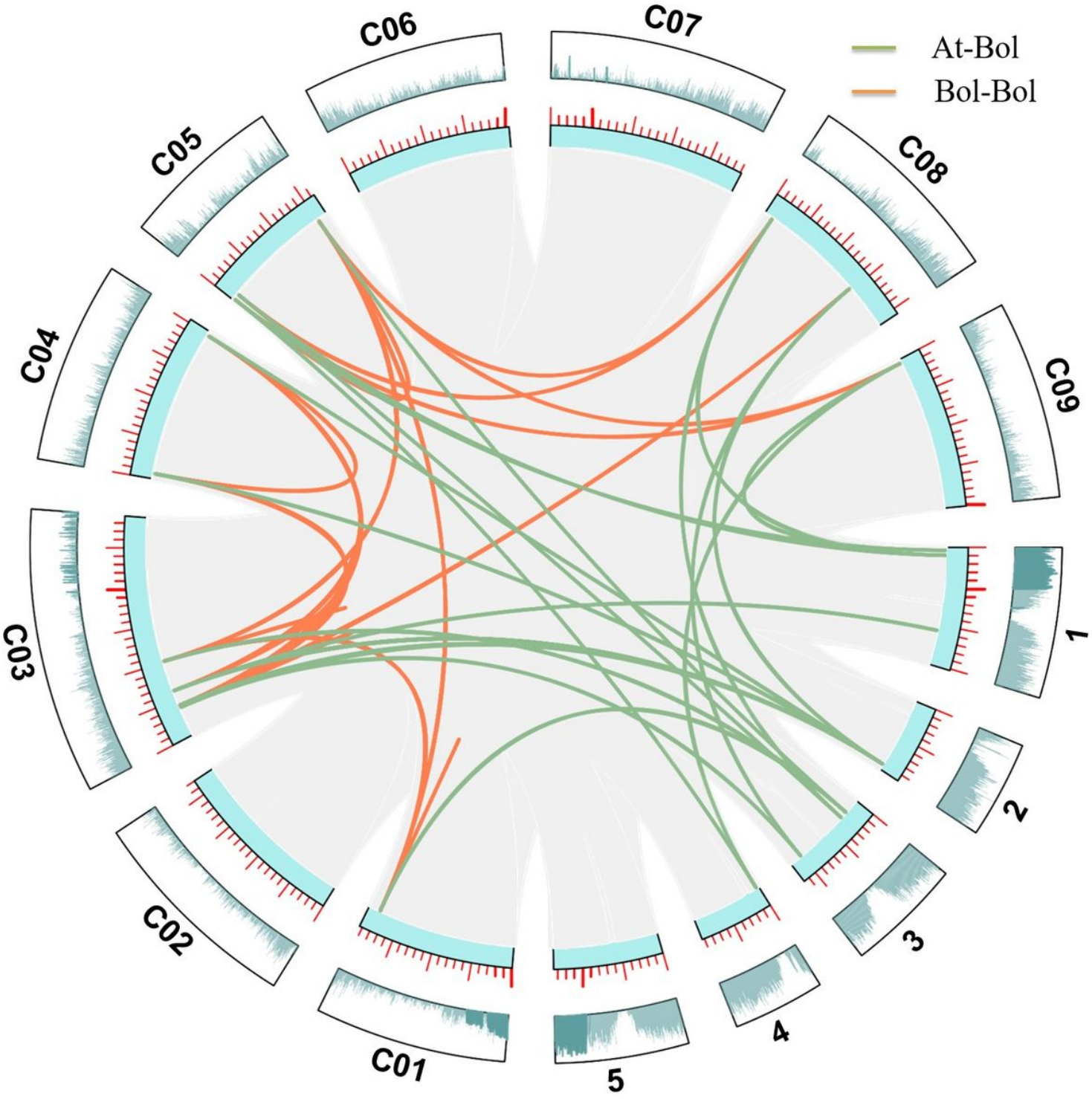

Figure 2

Syntenic relationship of cabbage and A. thaliana chitinase genes shown on the chromosome maps. C01-C09, nine chromosomes of cabbage. 1-5, five chromosomes of A. thaliana. Orange lines, homologous gene between cabbage chromosomes. Green lines, homologous genes between cabbage and A. thaliana chromosomes.

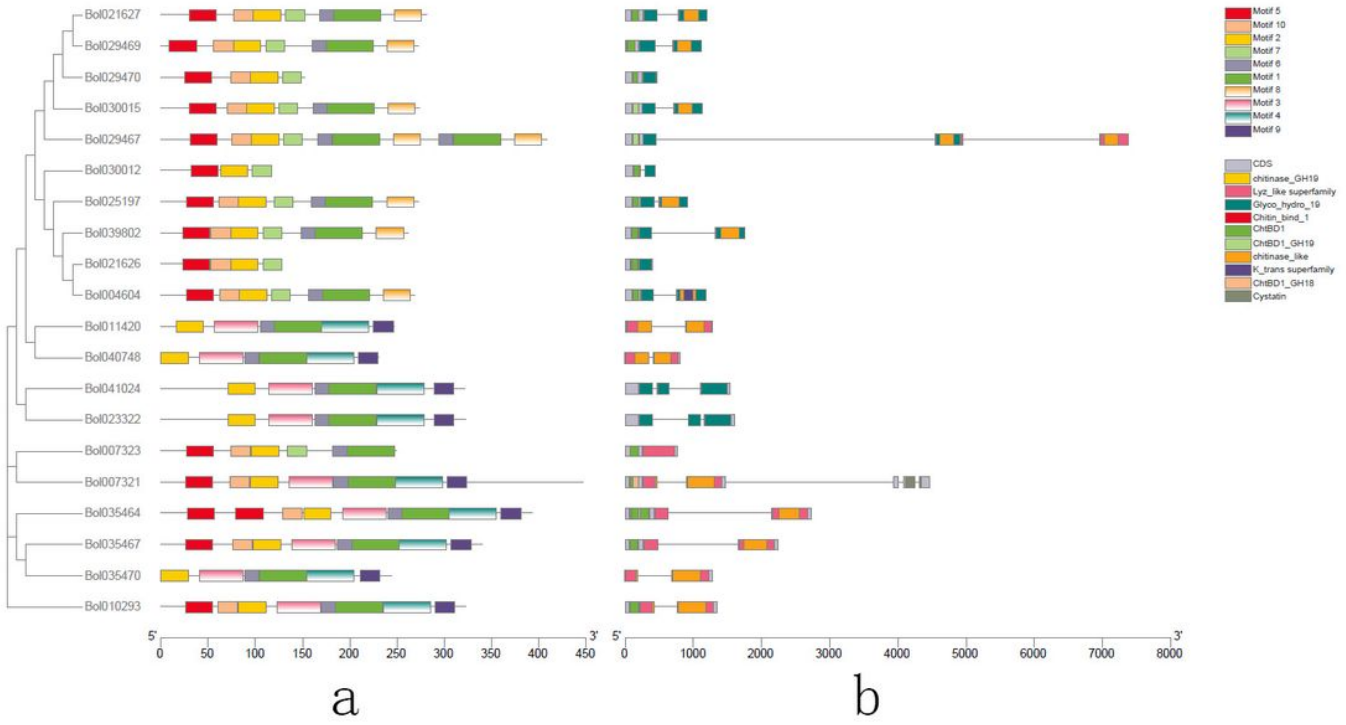

Figure 3

Conserved motifs (a) and gene structures (b) analysis of chitinase genes. The motifs are indicated in different colored boxes. Exons are represented by boxes, while introns are represented by gray lines.

Figure 4

Predicted cis-acting elements in chitinase gene promoters.

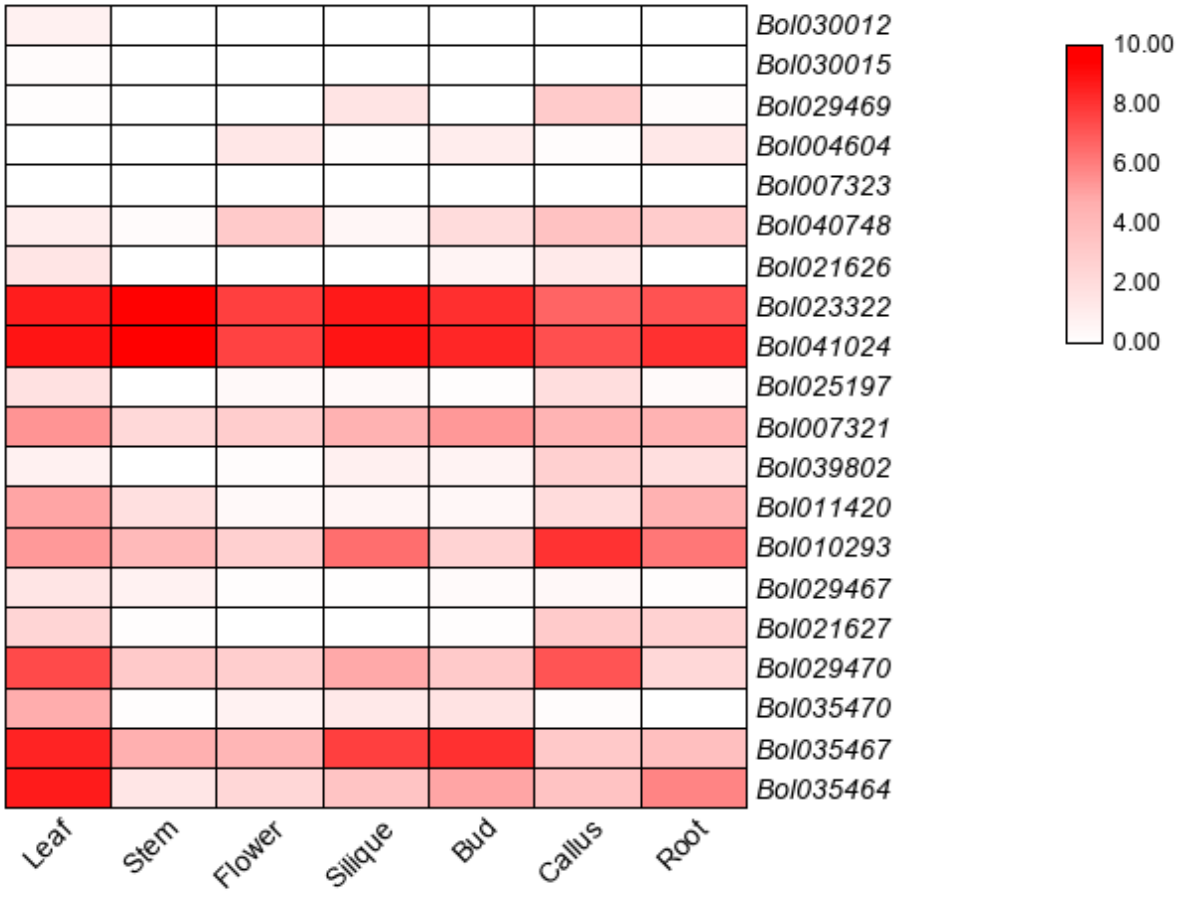

Figure 5

The expression of chitinase genes in different organs.

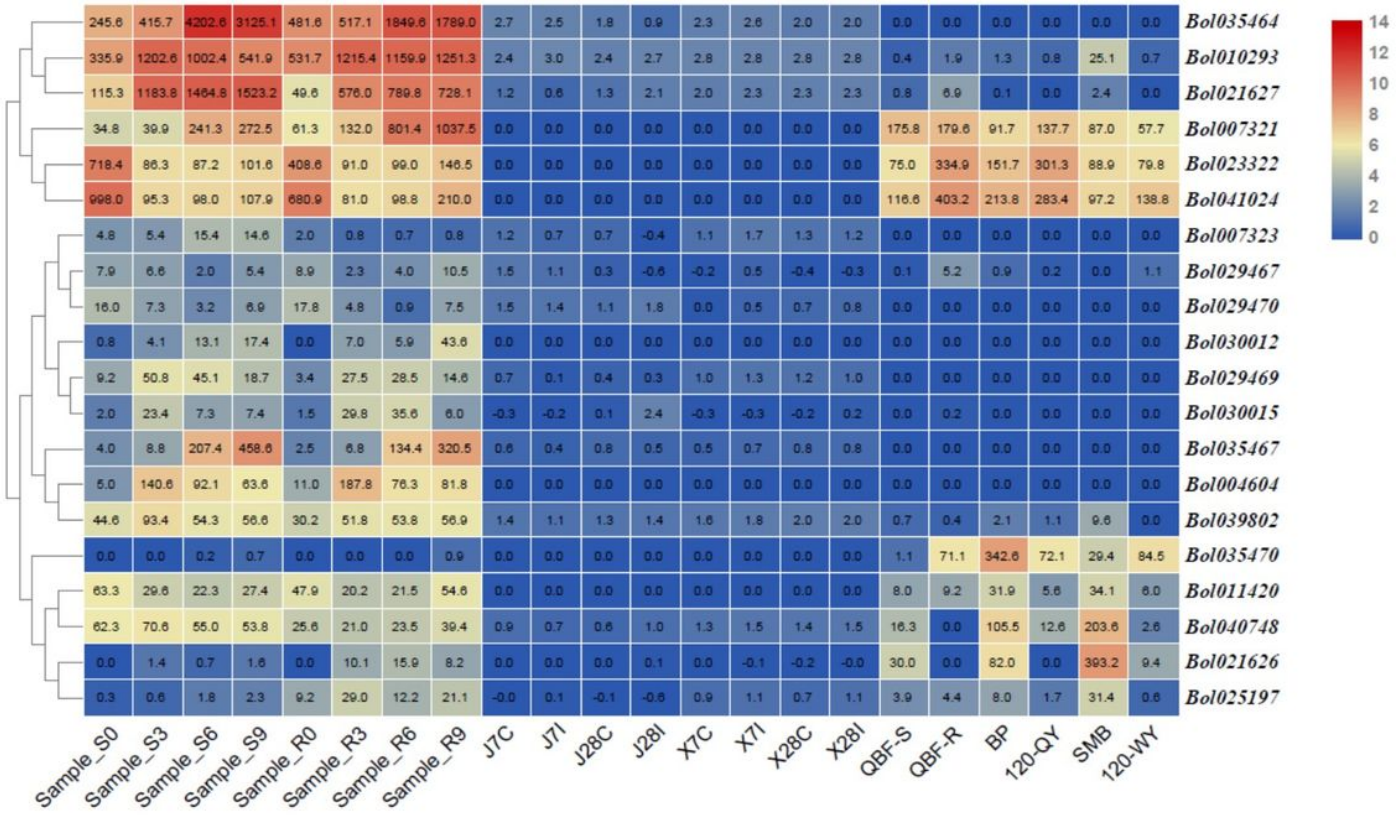

Figure 6

Expression patterns of chitinase genes analyzed by RNA-Seq. Sample S0, S3, S6 and S9 represented 01-20 inoculated by F. oxysporum at 0, 3, 6 and 9 days, respectively. Sample R0, R3, R6 and R9 represented 96-100 inoculated by F. oxysporum at 0, 3, 6 and 9 days, respectively. J7C, Jingfeng No. 1 not inoculated by P. brassicae at 7 days. J7I, Jingfeng No. 1 inoculated by P. brassicae at 7 days. X7C, Xiangan 336 not inoculated by P. brassicae at 7 days. X7I, Xiangan 336 inoculated by P. brassicae at 7 days. J28C, Jingfeng No. 1 not inoculated by P. brassicae at 28 days. J28I, Jingfeng No. 1 inoculated by P. brassicae at 28 days. X28C, Xiangan 336 not inoculated by P. brassicae at 28 days. X28I, Xiangan 336 inoculated by P. brassicae at 28 days. QBF-S, leaves with PM of D157 during pod setting stage. QBF-R, normal leaves of D157 during pod setting stage. BP, leaves with BS of W18 during heading stage. 120-QY, normal leaves of W18 during heading srage. SMB, leaves with DM of 01-20 during heading srage. 120-WY, normal leaves of 01-20 during heading srage.

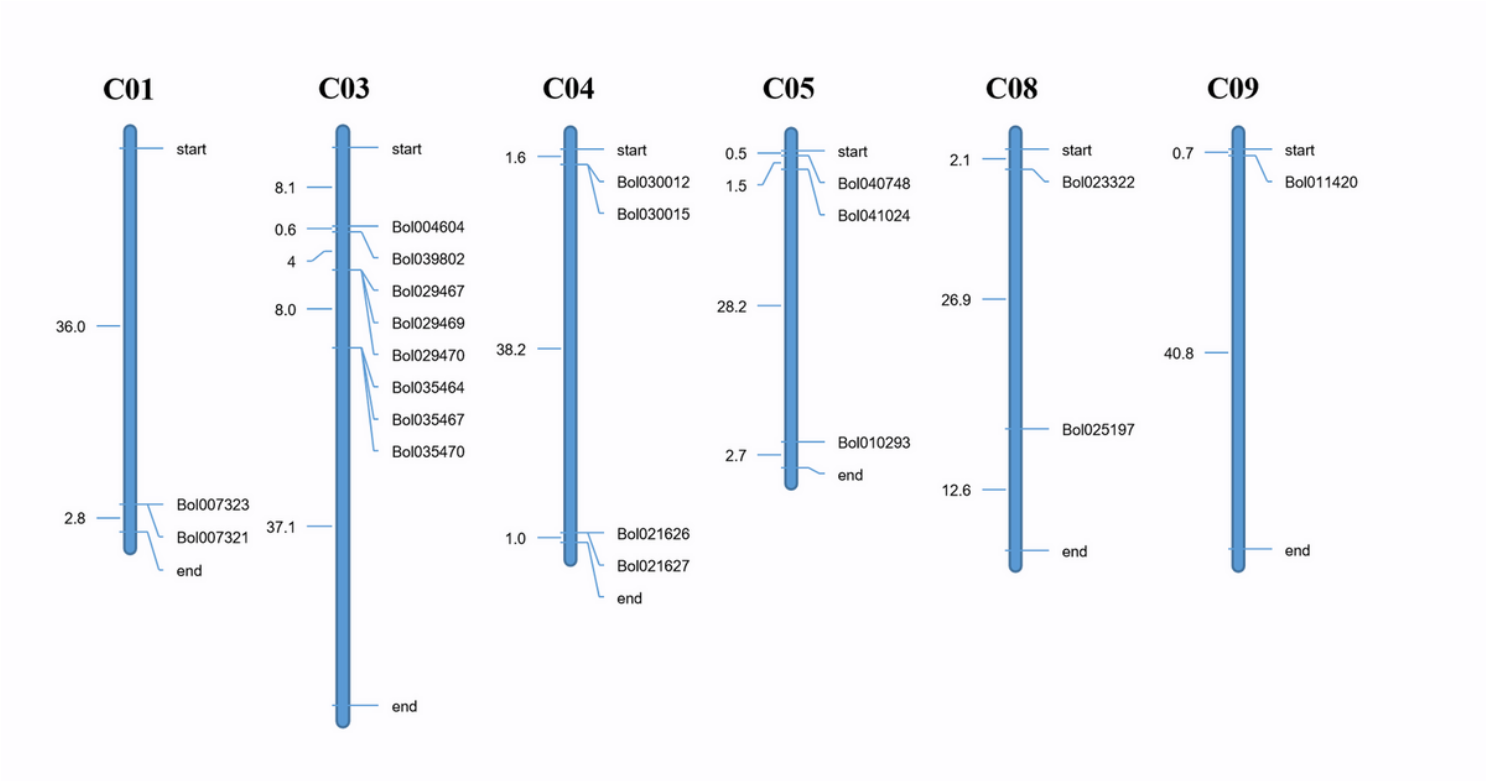

Figure 7

Distribution of chitinase genes on B.oleracea. chromosomes. The number on the top of each chromosome represented the cabbage chromosome number. Gene names are indicated on the right sides of each chromosome. The distance (Mb) between genes or genes to the ends of the chromosome are indicated on the left sides of each chromosome.

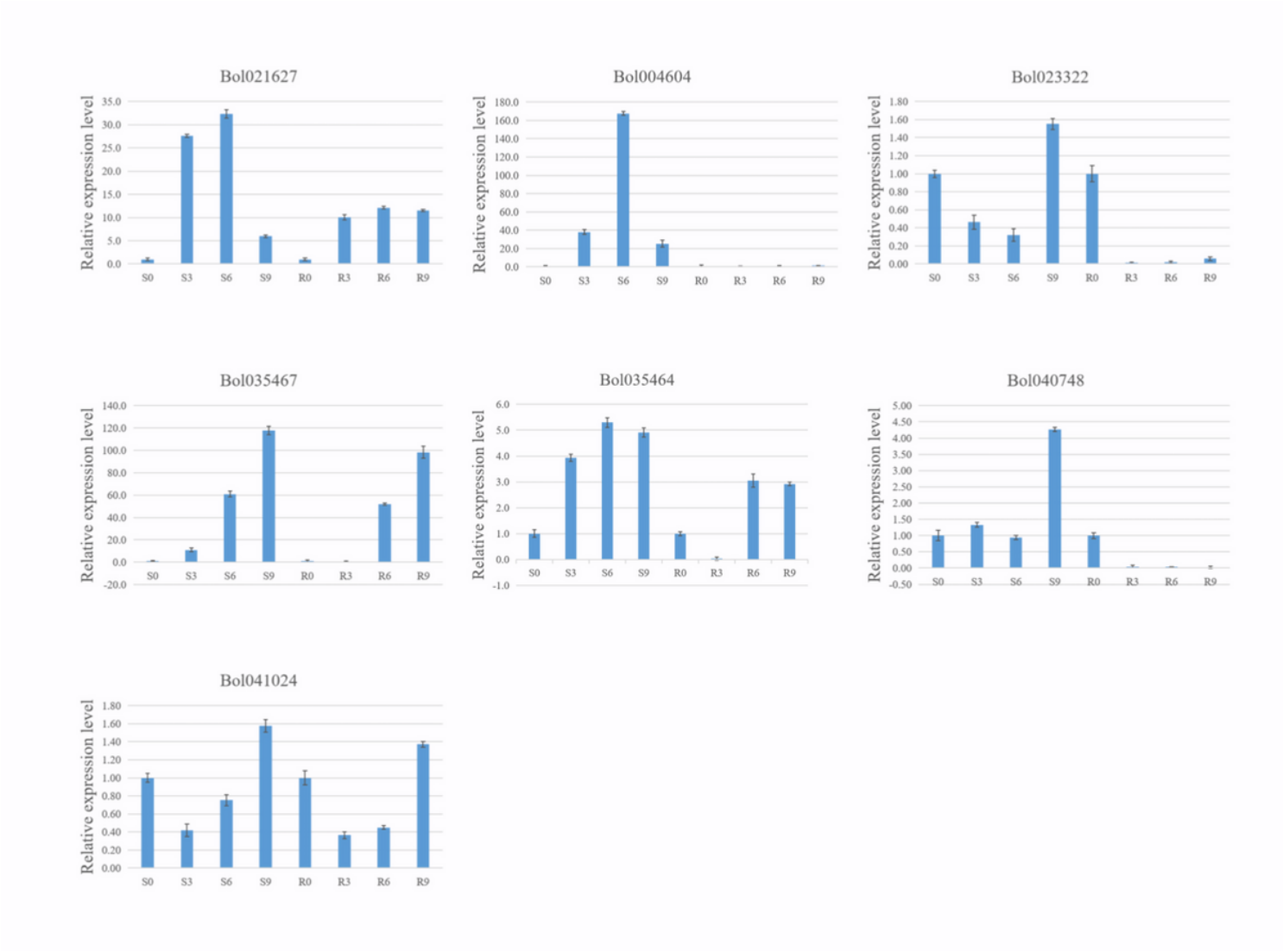

Figure 8

Expression level of chitinase genes under stress of F. oxysporum. Data are presented as the means ± SD.

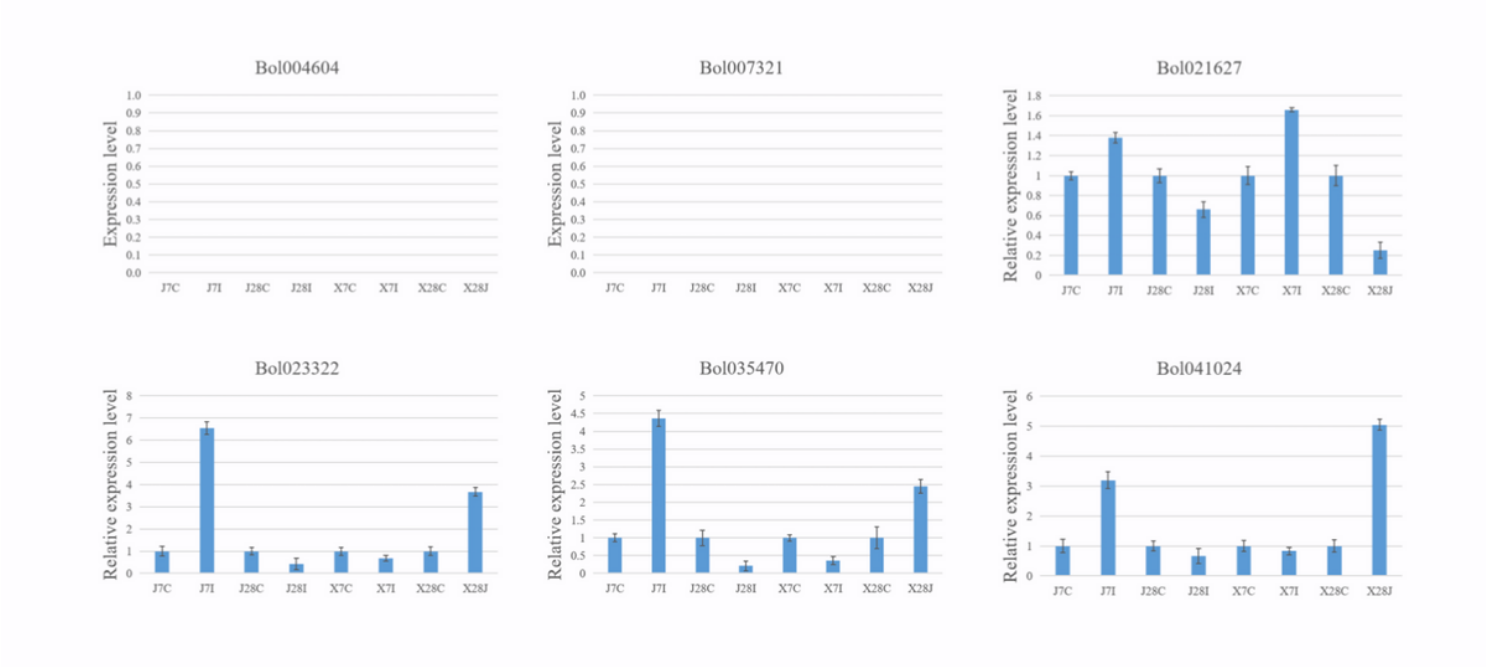

Figure 9

Expression level of chitinase genes under under stress of P. brassicae. Data are presented as the means ± SD.

Supplementary Files

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- Additionalfile3.xlsx
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