Genome-wide identification and expression analysis of the cytokinin gene family in Brassica oleracea L. reveals its role in clubroot resistance

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Abstract

Background: cytokinins have important functions in regulating plant growth and response to abiotic stress. cytokinin family genes have been described in several plant species, but a comprehensive analysis of the cytokinin family genes in Brassica oleracea has not been reported to date, especially their roles in dealing with the invasion of P. brassicae. Results: Cytokinins are a class of phytohormones that promote cell division and differentiation and are thought to affect plant immunity to multiple pathogens. To reveal the mechanisms of the Brassica oleracea cytokinin family genes in response to clubroot disease, a total of 36 cytokinin genes were identified using a genome-wide search method. Phylogenetic analysis classified these genes into three groups. They were distributed unevenly across nine chromosomes in B. oleracea, and 15 of them did not contain introns. The results of colinear analysis showed that each cytokinin gene in the B. oleracea genome had at least one homologous gene in the Arabidopsis genome. A cis-element analysis indicated that these genes possessed several stress response cis-elements. The heatmap of the cytokinin gene family showed that these genes were expressed in various tissues and organs. Five and eight genes were up- and downregulated, respectively, in the susceptible material after inoculation. In addition, two and one genes were up- and downregulated, respectively, in resistant material. This may indicate that these cytokinin genes play important roles in the host plant response to clubroot disease. In addition, the results provide insights for better understanding the role of cytokinin in the B. oleracea–P. brassicae interaction. Conclusions: Our results are helpful to elucidate the role of cytokinin family genes in cabbage response to infection by P. brassicae, and lay a foundation for further study on the function of these genes. Keywords: Brassica oleracea, genome-wide, cytokinin family genes, clubroot

Background

Cytokinin is a plant hormone associated with cell division and differentiation, consisting of a glandular peptidep derivative with a substituted N^6-isoprenoid side chain (Miller et al. 1956; Shaw et al. 1994). Cytokinin plays important roles in the growth and development of plants. Cytokinin can promote cell proliferation in buds and increase the activity of meristematic tissues by regulating the PIN protein in shoots (Kieber et al. 2018; Waldie et al. 2018). In the roots, cytokinin not only acts as a signaling molecule regulating root growth (Zhao et al. 2015) but also determines the size of the roots by controlling cell differentiation (Ioio et al. 2007).

Cabbage (*Brassica oleracea* var. *capitata* L.) is a very important vegetable crop that is widely grown around the world, with great economic value. As a member of the U's triangle (Nagaharu 1935), cabbage is also of great theoretical significance for comparative and evolutionary analysis. Clubroot, caused by *Plasmodiophora brassicae* Woronin, which mainly invades cruciferous crops including cabbage, is a worldwide soil-borne disease (Yu et al. 2017; Rocherieux et al. 2004). The life cycle of *P. brassicae* includes two stages: the root-hair infection stage and the cortex infection stage. Resting spores germinate in a suitable soil environment to form primary zoospores, which then infect the root hairs and form primary plasmodia. The primary plasmodia can form secondary resting zoospores through a series of

complex processes. The secondary resting zoospores in turn infect cortical cells and form secondary plasmodia, which are the key cause of swollen roots (Rolfe et al. 2016). The clubroot hinders the absorption and transport of water and nutrients in plants (Pang et al. 2018), causing crop yield reduction.

Multiple signaling pathways are activated when plants are infected by *P. brassicae.* The formation of resting spores is restricted within cortex cells in resistant cabbage (Ning et al. 2018). The expression of cytokinins can significantly reduce clubroot symptoms and the content of the pathogen in susceptible lines (Chen et al. 2018). Chu et al. (2014) reported that the signaling and metabolic activity of ethylene (ET) and jasmonic acid (JA) were significantly upregulated in resistant lines compared to susceptible lines at 15 days after inoculation (dai). Schuller et al. (2014) found that brassinosteroid (BR) synthesis and signal perception were involved in clubroot development. Manoharan et al. (2016) reported that methyl salicylate (SA) plays an essential role in the response to a chronic pathogen during clubroot development in *B. oleracea*. In addition, the accumulation of sugar at the infected site can help plants reduce the risk of clubroot disease (Walerowski et al. 2018).

The formation of galls is the most typical symptom of plants infected by *P. brassicae* (Lisha et al. 2018). Microscopic observation of root infection showed that the galls are caused by cell elongation and division (Kobelt et al. 2000). The galls can be regarded as a powerful metabolic sink (Rausch et al. 1981) where auxins and cytokinins are synthesized in large amounts (Dekhuijzen and Overeem 1971Ando et al. 2005). Similar to clubroot disease, leguminous plants form tumors in the roots when they are infected by rhizobia (Miri et al. 2015). As a key endogenous plant signal, the role of cytokinin in this process is to induce the formation of a mass. Many phenomena indicate that cytokinin must play an essential role in the plant response to the invasion of *P. brassicae.* Cytokinin and auxin synergistically induce hypertrophy and hyperplasia by increasing the cell division rate and cell enlargement during the formation of a mass in the roots (Ludwig-Müller and Schuller 2008). The detection and analysis of five cytokinin synthase genes from *Brassica rapa* indicated that the expression of cytokinin synthase genes was increased during the primary developmental stage of clubroot disease (Ando et al. 2005; Schuller et al. 2014). Similarly, throughout the process of gall formation, cytokinin biosynthesis is upregulated, with downregulation of adenosine kinase (Ludwig-Müller et al. 2009).

RNA-seq is considered a powerful approach for detecting differentially expressed genes and is widely used to study the interactions between host plants and *P. brassicae*. Siemens et al. (2006) identified more than 1000 DEGs in infected individuals compared with the normal roots of *Arabidopsis*. A transcriptome study of *Brassica oleracea var. italica* and *Brassica macrocarpa Guss.* in different infection periods of *P. brassicae* showed that genes associated with glucosinolate biosynthesis, cell wall biosynthesis, and plant hormone signal transduction were activated much earlier in resistant lines (Zhang et al. 2016). In addition, comparative transcriptome analysis between susceptible and resistant Chinese cabbage varieties revealed that genes associated with pathogen-associated molecular patterns (PAMPs), pathogenesis-related (PR), calcium ion influx, hormone signaling, transcription factors, and cell-wall modification played important roles in the interactions between *B. rapa* and *P. brassicae* during the primary infection stages (Chen et al. 2016).

Although the function of cytokinin has been analyzed in various plant cultivars, such as *Arabidopsis* (Riou-Khamlichi et al. 1999), *Spirodela polyrhiza* (Kurepa et al. 2018), *Phaseolus lunatus* (Martin et al. 1999), rice (Ashikari et al. 2005), tomato (Chen et al. 2016), apple (Feng et al. 2017), potato (Lomin et al. 2018) and maize (Schmülling et al. 2003; Chettoor et al. 2017), the roles of the cytokinin gene family in cabbage with clubroot disease are currently unknown. In this study, we identified 36 cytokinin genes in *B. oleracea.* The phylogenetic relationships, chromosome locations, colinearity relationships, and gene structures of all detected genes as well as the *cis*-acting regulatory elements in promoters were then systematically analyzed. The transcription and expression patterns of cytokinin genes during different periods following inoculation with *P. brassicae* were also investigated. Our results will promote the understanding of the molecular mechanism of the cytokinin response to the development of clubroot disease in *B. oleracea.*

Results

Genome-wide identification and phylogenetic analysis of cytokinin genes in *B. oleracea*

A total of 36 cytokinin genes were identified, and the detailed information for each cytokinin gene is shown in Table 1. The proteins encoded by the cytokinin genes varied from 355 to 594 amino acids (aa) in length. Among these proteins, the *Bol024927* protein sequence was the longest, with 594 amino acids, and the *Bol006929* protein sequence was the shortest, with 355 amino acids. Among all of the proteins, 18 members shared similar localization to cytoplasm, three to the extracellular region, four to the vacuole, and eleven members were located in more than one compartment.

To study the evolutionary relationships of the cytokinin genes in *B. oleracea*, *Arabidopsis*, and *B. rapa*, 135 cytokinin genes were analyzed to construct an unrooted phylogenetic tree. The cytokinin genes were classified into three groups (Fig. 1), which contained 34 (class I), 32 (class II) and 56 (class III) members. The remaining 10 members could not be clustered into any group. The numbers of cytokinins from *B. oleracea, A. thaliana* and *B. rapa* in each class are represented in Supplemental Table S1 (Additional file 1).

Chromosomal distribution and differential retention of cytokinin genes in *B. oleracea*

The 36 cytokinin genes were assigned to nine chromosomes of *B. oleracea* (Fig. 2). The distribution of the cytokinin genes on each chromosome was uneven. Chromosome C07 contained the largest number (8) of cytokinin genes, followed by chromosomes C01, which contained six genes. Both chromosome C03 and C04 contained five genes. Three genes were located on C05. Chromosome C02, C06 and C08 each contained only two genes.

To obtain a better understanding of the evolutionary history of the cytokinin gene family in *B. oleracea* and *Arabidopsis*, duplications of putative cytokinin genes in the *B. oleracea* and *Arabidopsis* genomes were analyzed. Every cytokinin gene in the *B. oleracea* genome has at least one homologous gene in the *Arabidopsis* genome. In addition, segmentally duplicated gene pairs were observed among the 36

identified cytokinin genes in *B. oleracea* (Fig. 3; Additional file 2: Table S3). The *B. oleracea* genome contained 1–7 copies of each cytokinin gene found in *Arabidopsis*, while 13 of the *A. thaliana* cytokinin genes had no homologs in *B. oleracea* (Fig. 3; Additional file 3: Table S4). For example, *AT3G47930.1* contained only one homologous gene (*Bol024927*) in *B. oleracea*, while *AT2G41510.2* contained up to seven homologous genes (*Bol036074*, *Bol020547*, *Bol006929*, *Bol005172*, *Bol027725*, *Bol045724*, and *Bol035751*) in *B. oleracea*.

Structure and conserved motif analysis of cytokinin genes

The structures and phases of introns/exons were determined to further study the cytokinin genes. In general, genes with closer phylogenetic relationships exhibited similar genetic structures, such as *Bol006383* and *Bol028361* or *Bol035751* and *Bol036074*. Nearly half (16, 44.4%) of all the genes contained two or more introns, while 5 genes contained only 1 intron. The remaining 15 genes without introns were almost the same length. The length of each intron in different genes showed great divergence. For example, *Bol43933* contained 17 short introns, whereas *Bol006389* contained an extremely long intron (Fig. 4). The differences in the gene structure of *Bolcy* in *B. oleracea* might indicate the diversity of the potential biological functions of these genes.

The intron pattern is also related to the phylogenetic classification (Fig. 1; Fig. 4). Fifteen intron-free genes were classified into two subclasses. *Bol018140*, *Bol031036* and *Bol010168* all contained only one intron and showed a very close relationship. The same phenomenon occurred between *Bol035751* and *Bol036047*. Taken together, the distribution patterns of the introns in cytokinin genes with similar relationships were roughly the same.

To further reveal the functional diversification of cytokinin genes during their evolution, 10 conserved motifs were detected in the 36 cytokinin genes (Fig. 5). Among the 36 cytokinin proteins, motifs 1 and 3 existed in almost all of them, except for *Bol024927*, which exhibited no motifs. *Bol005172* contained motifs 4, 1, 3 and 6. *Bol022730* contained motifs 8, 1 and 3. *Bol043933* contained motifs 4, 1 and 3. *Bol006929*, *Bol020547* and *Bol045724* all contained motifs 1, 3, and 6. *Bol009917*, *Bol010168*, *Bol014258*, *Bol018140*, *Bol027725*, *Bol031036*, *Bol033608*, *Bol035751*, *Bol036074* and *Bol037999* only contained motifs 3 and 6. *Bol027388* contained the same motifs as *Bol024952* except for motif 9. Motifs 4, 8,1, 3, 9, 5, 10, 7, 6, and 2 were present in half of the family members, which may indicate similar functions of these proteins.

Cis-elements in the promoters of *B. oleracea* cytokinin genes

To further elucidate the regulatory mechanisms of *B. oleracea* cytokinin genes in response to clubroot disease, the promoter sequences were submitted to the PlantCARE database to identify *cis*-elements. Seventeen types of *cis*-acting regulatory elements were detected (Fig. 6). All 36 cytokinin genes contained 2-16 *cis*-elements related to light-responsiveness. Twelve cytokinin genes contained defense and stress-response-related *cis*-elements. MeJA-responsiveness *cis*-elements were detected in 28 chitinase genes, while *cis*-elements related to endosperm expression and anoxia-specific inducibility were only detected in

five and one cytokinin genes, respectively. Anaerobic induction *cis*-elements was detected in 28 cytokinin genes, as were drought induction *cis*-elements. The numbers of *cis*-elements related to circadian control, flavonoid biosynthesis and seed-specific regulation were relatively small, being detected in 2, 2 and 3 cytokinin genes, respectively.

Expression patterns of cytokinin genes in different organs and treatments

The RNA-Seq dataset (GSE42891) was examined to determine the expression levels of cytokinin genes in the leaves, stem, flowers, siliques, buds, calli and roots of *B. oleracea*. Most of the cytokinin genes exhibited different expression patterns (Fig. 7a). Fifteen were expressed in all organs, while the expression of two was not detected. Some genes were expressed in only one or two organs. For example, *Bol024949*, *Bol024952*, *Bol028359* and *Bol028360* were only expressed in siliques, whereas *Bol022730* and *Bol036628* were expressed in the flowers and buds. The various expression patterns suggested a broad range of biological functions of the cytokinin genes during the growth and development of *B. oleracea.*

To explore the relationship between cytokinin and clubroot disease, we tried to inoculate two cabbage materials, Xiangan 336 (resistant) and Jingfeng No. 1 (susceptible), and collected 8 different kinds of samples (Fig. 7b) for RNA-seq.

The expression patterns of most cytokinin genes under the eight different treatments were quite different (Fig. 7b). The expression levels of several genes (*Bol024952*, *Bol024949*, *Bol009917*, *Bol028359*, *Bol036628* and *Bol036948*) were almost the same in all treatments. Five (*Bol014258*, *Bol027725*, *Bol022730*, *Bol036074* and *Bol006388*) and six (*Bol020547*, *Bol027390*, *Bol018140*, *Bol006929*, *Bol024927* and *Bol028392*) genes were significantly up- and downregulated, respectively, in J7I compared with J7C. Similarly, three (*Bol020547*, *Bol006383* and *Bol005172*) and two (*Bol022730* and *Bol043933*) genes were significantly up- and downregulated, respectively, in X7I compared with X7C. Moreover, three (*Bol014258*, *Bol024330* and *Bol028363*) and four (*Bol018140*, *Bol010168*, *Bol037842* and *Bol045724*) genes were significantly up- and downregulated, respectively, in J28I compared to J28C, whereas the number of up- and downregulated genes in X28I compared to X28C were five (*Bol014258*, *Bol028392*, *Bol028361*, *Bol028363* and *Bol045724*) and five (*Bol027725*, *Bol018140*, *Bol027388*, *Bol028360* and *Bol024927*), respectively. In addition, some genes showed different expression levels in different inoculation periods in the same material. For example, *Bol027725*, *Bol022730* and *Bol036074* exhibited high expression levels in J7I but low levels in J28I. In contrast, *Bol028363*, *Bol011927* and *Bol006385* exhibited low expression levels in X7I but high levels in X28I.

Overall, after inoculation, the expression of *Bol027725*, *Bol036074* and *Bol006388* in the susceptible material were upregulated, and the expression of these genes in the resistant material was maintained at a low level. However, the expression patterns of *Bol006383* and *Bol005172* were exactly the opposite. The expression of *Bol027390*, *Bol006929*, *Bol024927* and *Bol028392* in the susceptible material was downregulated after inoculation, whereas the expression levels of these genes in the resistant material remained unchanged. In contrast, *Bol043933* showed an opposite expression pattern to the above two

genes. In addition, the expression patterns of *Bol020547* and *Bol045724* were completely opposite that of *Bol022730*, which was upregulated in susceptible material after inoculation but downregulated in resistant material. Generally, the expression patterns of cytokinin genes between Jingfeng No.1 and Xiangan 336 were quite different under the same treatment, possibly because of the difference in resistance between them.

To further examine these findings, quantitative reverse-transcription PCR (qRT-PCR) was performed to analyze the expression patterns of eight cytokinin genes under different treatments. The results showed that the expression levels of the eight genes were basically consistent with those in the heat map (Fig. 7b; Fig. 8). Generally, the expression levels of *Bol028363* and *Bol006383* were relatively low in each treatment. *Bol011927* and *Bol005172* showed similar expression patterns because of their higher expression levels in X28C and X28I and low expression levels in J28C and J28I. Overall, the expression levels of the 8 genes in X7I were relatively low.

Discussion

Cytokinins are closely related to various physiological activities in plants such as cell division, nutrient transfer and leaf senescence (Rioukhamlichi et al. 1999; Matsumotokitano et al. 2008; Amzallag et al. 1992; Merewitz et al. 2010). Additionally, they play a very important role in the plant responses to different types of adversity, including drought stress, heat stress, pathogen infection and pest infestation (Rivero et al. 2007; Liu et al. 2002; Babosha et al. 2009; Smigocki et al. 1995). However, the expression patterns cytokinin genes in response to *P. brassicae* have remained uncertain until now. In this study, we mainly analyzed the functions of the cytokinin gene family in the development of clubroot disease in *B. oleracea*. The members of cytokinin gene family detected in this study adopt an alpha+beta sandwich structure, with an antiparallel beta-sheet, in a ferredoxin-like fold. These structures are predominantly found in plant cytokinin dehydrogenase 1 proteins and are capable of binding both FAD and cytokinin substrates (Malito et al. 2004). To further understand this gene family, 36 genes were detected, and we then analyzed their colinearity, structures, chromosomal locations, *cis*-elements and expression patterns under different treatments. This research provides comprehensive information for a better understanding of the cytokinin gene family in *B. oleracea*.

Genes with few or no introns are thought to be expressed quickly in plants (Jeffares et al. 2008). Long introns can prolong the gene transcription time, so that genes with fewer introns will react more rapidly to biotic and abiotic stresses as well as the invasion of pathogens. Some stress-related gene families such as the *Hsp20* family (Peng et al. 2018), the leucine-rich repeat family (Zhou et al. 2016) and the GRF family (Sang et al. 2018) also contain few introns. Among the genes examined in this study, 15 genes were intronless, and 5 genes contained only 1 intron, potentially supporting the above hypothesis. In other words, these genes will be able to respond faster when plants are invaded by *P. brassicae*.

Many plants increase their resistance to pathogens by activating the signaling pathways of SA and JA (Cyclic et al. 2015; Xie et al. 2011). Furthermore, cytokinins can interact with SA and JA to strengthen

plant defense responses (Sano et al. 1994; Choi et al. 2010; Choi et al. 2011;). Exogenous application of JA or SA can significantly contribute to resistance to the biotrophic clubroot agent *Plasmodiophora brassicae* in *Arabidopsis*, broccoli and pak-choi (Séverine et al. 2015; Lovelock et al. 2013; Zhu et al. 2017). In this study, MeJA-responsive *cis*-elements were detected in 24 genes, while salicylic acid-responsive *cis*-elements were detected in 10 genes, which may indicate their different effects in the response to clubroot. Promoters control gene activity by binding to transcription factors. In this study, many types of *cis*-elements, such as stress-, hormone- and metabolism-related *cis*-acting regulatory elements were detected in the promoters of *B. oleracea* cytokinin genes (Fig. 6), possibly reflecting an interconnected induction mechanism involving transcription factors.

Gene duplication is a potential driver of biological evolution (Epstein et al. 1971). One of the characteristics of the eukaryotic genome is the existence of multiple gene families, which are groups of genes produced via the duplication and mutation of an ancestral gene. Interchromosomal replication is the main origin of gene duplication in eukaryotic genomes (Friedman et al. 2001). Three whole-genome duplications that have occurred in Arabidopsis over the past 350 million years have provided an opportunity for the large-scale expansion of certain classes of genes (Maere et al. 2005; Wang et al. 2015). In this study, we found that each homologous gene in Arabidopsis presented several copies in the *B. oleracea* genome; for example, AT1G26380.1 presented 3 copies, and AT5G44400.1 presented 5 copies (Fig. 3; Additional file 2: Table S3). This may suggest that the cytokinin genes were replicated during the evolution of *B. oleracea*, resulting in more diverse functions. Conserved genes are more likely to give rise to functional and persistent duplicates, thus contributing more to their long-term survival ability in eukaryotic genomes. Moreover, genes with many *cis*-regulatory regions, which are expressed in numerous tissues or encode multidomain proteins, will be preferentially preserved (Davis et al. 2004). The promoter region of each cytokinin gene examined in this study contained a large number of *cis*-elements, which may indicate that they have more diverse functions during gene evolution and thus have a greater chance to be retained.

According to the RNA-seq data of *B. oleracea*, a certain number of cytokinin genes, such as *Bol037842*, *Bol045724* and *Bol036074* exhibited incongruous expression patterns in various tissues, indicating that different cytokinin proteins may have diverse functions. Three genes, *Bol028363*, *Bol031036* and *Bol018140*, were relatively highly expressed in all of the investigated tissues under normal conditions, showing the expression characteristics of housekeeping genes (Lopes et al. 2013). Various expression patterns were observed under the different treatments in the two cultivars (Jinfeng No.1 and Xiangan 336). The invasion of *P. brassicae* can promote the division of root cells in susceptible plants and eventually lead to swelling of the roots (Siemens et al. 2006). Thus, the cytokinin-related genes may be activated by the invasion of *P. brassicae. Bol1027725*, *Bol022730* and *Bol036074* may be play an important role in the process of cell division after inoculation because they were upregulated in J7I compared to J7C. *Bol014258* and *Bol024330* showed the same expression characteristics between J28I and J28C; however, the opposite expression pattern was found for *Bol018140*, *Bol027388*, *Bol033608*, and *Bol006389*, which may indicate the different roles of these genes. Additionally, *Bol024952*,

Bol1009917 and *Bol1036948* were considered to have no effect on the response to pathogen invasion because they exhibited the same expression pattern under each treatment.

P. brassicae infects the root hairs of *oleracea* at 7 dai and the cortex cells at 28 dai (Ning et al. 2018). Therefore, genes with significant differential expression between Jinfeng No.1 and Xiangan 336 after inoculation may be crucial genes for the plant response to invasion by *P. brassicae. Bol022730* was upregulated in Jinfeng No.1 after inoculation but downregulated in Xiangan 336, possibly because its expression is inhibited by *P. brassicae* in resistant materials. In contrast, *Bol020547* and *Bol045724* were downregulated in Jinfeng No.1 after inoculation but upregulated in Xiangan 336. This may mean that these two genes play a key role in responding to infection by *P. brassicae*. Further analysis showed that the promoter regions of the two genes contained MeJA-responsiveness-related *cis*-elements, and *Bol020547* also contained a salicylic acid responsiveness-related *cis*-element. Therefore, *Bol020547* and *Bol045724* may be key genes in the *B. oleracea* response to clubroot disease, and their specific functions need to be further studied.

Conclusions

Here, a genome-wide analysis of *B. oleracea* cytokinin gene family was performed, and 36 cytokinin genes were confirmed. Subsequently, analyses of cytokinin genes on gene structures, phylogeny, chromosomal location, gene duplication and gene expression patterns were conducted based on bioinformatics and qRT-PCR methods. Five and eight genes were up- and downregulated, respectively, in the susceptible material after inoculation. In addition, two and one genes were up- and downregulated, respectively, in resistant material. This may indicating that cytokinin genes play important roles in the interaction between *B. oleracea* and *P. brassicae*. The study provides comprehensive information on the cytokinin gene family in *B. oleracea* and will aid in determining the cytokinin gene functions.

Methods

Identification of cytokinin family genes in *Brassica oleracea*

The cabbage whole-genome protein sequences were downloaded from the *Brassica oleracea* Genomics Database (www.ocri-genomics.org/bolbase/blast/blast.html). The HMM profile of the *Cytokin-bind* domain was first downloaded from the Pfam (http://www.sanger.ac.uk/ Software/Pfam/) database (Pfam: PF09265). The CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), SMART (http://smart.embl-heidelberg.de/) and Pfam (http://pfam.xfam.org/) databases were used to analyze the conserved domains.

Phylogenetic analyses of cytokinin genes

The amino acid sequences of cytokinin proteins derived from *B. oleracea*, *Arabidopsis*, and *B. rapa* were used for phylogenetic analysis. An unrooted neighbor-joining phylogenetic tree was constructed using MEGA 6.0 software (Tamura et al. 2013) with 1000 bootstrap test replicates.

Chromosomal location of cytokinin genes

All identified cytokinin genes were mapped to *B. oleracea* chromosomes using Mapdraw V2.1 (Liu and Meng 2003) based on the information available from the *B. oleracea* genome database (http://plants.ensembl.org/Brassica_oleracea/Info/Index).

Collinearity analysis of cytokinin genes

The microsyntenic relationships of the cytokinin genes in *B. oleracea* and *Arabidopsis thaliana* were detected through BLAST searches of these genes against the whole genomes of these crops. The physical locations of the cytokinin genes on each chromosome were obtained from the respective databases. Then, TBtools was used to visualize the relationships between the two crop cultivars.

Gene structure and conserved motif analyses of cytokinin genes

The exon-intron structures of *B. oleracea* cytokinin family genes were analyzed at the Gene Structure Display Server (GSDS, http:// gsds.cbi.pku.edu.cn/index.php) (Guo et al. 2007). The NCBI-CDD (https:// www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (Marchlerbauer et al. 2015) and the MEME program (http://meme-suite.org/index.html) (Machanick et al. 2011) were used to identify protein sequences and the conserved motifs, respectively.

Analysis of *cis*-acting elements in cytokinin genes

The upstream sequences (1.5 kb) of the initiation codons (ATG) of each cytokinin gene were submitted to PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) (Rombauts et al. 1999) to study *cis*-acting elements in the promoters of the cytokinin genes.

Plant materials and treatments

The *P. brassicae* used in this study was collected from Changyang, Hubei Province, China, and was identified as belonging to race 4 (Ning et al. 2018) based on the differential sets of Williams (1966). 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 bought from the market. Then they were sown in plastic cups (25 cm×10 cm) containing sterile nursey medium. *P. brassicae* inoculation was carried out when the seedlings had produced two real leaves by injecting 2 mL of the resting spore suspension at the roots using a transferpettor. Two kinds of treatment were performed for each cultivars. A treatment without inoculation was set as the control. Every treatment included 24 seedlings, with 8 seedlings consisting of one biological replicate. Then, the plants were transferred to a culture room with a 16 h photoperiod and 19-25℃ temperature.

For transcriptional analysis of the cytokinin genes after *P. brassicae* inoculation, the roots from plants subjected to 8 treatments (J7C, J7I, X7C, X7I, J28C, J28I, X28C, X28I) were collected (24 individuals per

treatment, 8 individuals per replicate). All of the samples were quickly frozen in liquid nitrogen and stored at −80℃ until RNA extraction.

Total RNA extraction, cDNA synthesis, and qRT-PCR analysis

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 cytokinin genes were designed with Premier 3.0, (Additional file 1: Table S2). qRT-PCR was carried out using ChamQ Universal SYBR qPCR Master Mix (2×) (Vazyme, Nanjing, China) 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, 0.4 μl of a 10 μM solution of each primer, 2 μl diluted cDNA and 7.2 μl ddH_2O. The PCR program was set as follows: 95℃ for 2 min, followed by 45 cycles of 95℃ for 15 s, 60℃ for 15 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.

Expression analysis of cytokinin genes using RNA-seq data

To assay cytokinin gene expression profiles, Illumina RNA-seq data from the 8 different treatments as well as various tissues, including the leaves, stems, flowers, siliques, bud, calluses and roots, were downloaded from the NCBI (GSM1052958–964). FPKM values were used to calculate gene expression levels, and the positively expressed genes were evaluated according to a default empirical abundance threshold of 1 FPKM. The fragments per kb of exon per million mapped reads (FPKM) algorithm was used in this study to normalize the gene expression values. Heat maps of hierarchical clustering were constructed using MeV4.9 and TBtools (https://github.com/CJ-Chen/TBtools) software.

Abbreviations

qRT-PCR: Quantitative real-time polymerase chain reaction; dai: Days after inoculation; ET: Ethylene; JA: Jasmonic acid; SA: Salicylate; BR: Brassinosteroid; aa: amino acids; DEGs: Differentially expressed genes; PAMPs: Pathogen-associated molecular patterns; PR: Pathogenesis-related; HMM: Hidden markov model; CDD: Conserved domain database; FPKM: Fragments perkilobase of transcript per million mapped reads; BLAST: Basic Local Alignment Search Tool; NCBI: National Center for Biotechnology Information

Declarations

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Availability of data and materials

Data of this study have been included in the article or as additional file.

Authors' contributions

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

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

Amzallag G N, Lerner H R, Poljakoffmayber A. Interaction Between Mineral Nutrients, Cytokinin and Gibberellic Acid During Growth of Sorghum at High NaCl Salinity[J]. Journal of Experimental Botany, 1992, 43(1):81-87.

Ando S , Asano T , Tsushima S , et al. Changes in gene expression of putative isopentenyltransferase during clubroot development in Chinese cabbage (*Brassica rapa* L.)[J]. Physiological & Molecular Plant Pathology, 2005, 67(2):59-67.

Ashikari M , Sakakibara H , Lin S , et al. Cytokinin Oxidase Regulates Rice Grain Production[J]. Science, 2005, 309(5735):741-745.

Babosha A V , Ryabchenko A S , Avetisyan T V . Effect of exogenous cytokinins on dynamics of development and differentiation of infectious structures of the pathogen of wheat powdery mildew[J]. Cell and Tissue Biology, 2009, 3(4):387-396.

Chen J , Piao Y , Liu Y , et al. Genome-wide identification and expression analysis of chitinase gene family in, *Brassica rapa*, reveals its role in clubroot resistance[J]. Plant Science, 2018:S0168945217309378.

Chen JJ, Pang WX, Chen B, Zhang CY, Piao ZY (2016) Transcriptome analysis of *Brassica rapa* near-isogenic lines carrying clubroot-resistant and-susceptible alleles in response to *Plasmodiophora brassicae* during early infection. Front Plant Sci 6:1183. https://doi.org/ 10.3389/fpls.2015.01183

Chen X J, Xia X J, Guo X, et al. Apoplastic H2 O2 plays a critical role in axillary bud outgrowth by altering auxin and cytokinin homeostasis in tomato plants[J]. New Phytologist, 2016, 211(4):1266-1278.

Chettoor A M, Evans M. Live-Cell Imaging of Auxin and Cytokinin Signaling in Maize Female Gametophytes.[J]. Methods in Molecular Biology, 2017, 1669:95.

Choi J , Choi D , Lee S , et al. Cytokinins and plant immunity: old foes or new friends?[J]. Trends in Plant Science, 2011, 16(7):0-394.

Choi J , Huh S U , Kojima M , et al. The Cytokinin-Activated Transcription Factor ARR2 Promotes Plant Immunity via TGA3/NPR1-Dependent Salicylic Acid Signaling in Arabidopsis[J]. Developmental Cell, 2010, 19(2):0-295.

Cyclic lipopeptide iturin A structure-dependently induces defense response in Arabidopsis plants by activating SA and JA signaling pathways[J]. Biochemical and Biophysical Research Communications, 2015, 460(4):1015-1020.

Chu M, Tao S, Falk K C, et al. Fine mapping of Rcr1 and analyses of its effect on transcriptome patterns during infection by *Plasmodiophora brassicae*[J]. Bmc Genomics, 2014, 15(1):1166.

Davis J C , Petrov D A . Preferential duplication of conserved proteins in eukaryotic genomes[J]. PLoS Biology, 2004, 2(3):E55.

Dekhuijzen, H. M., and Overeem, J. C. 1971. The role of cytokinins in clubroot formation. Physiol. Plant Pathol. 1:151-161.

Epstein C J. Evolution by gene duplication.[J]. American Journal of Human Genetics, 1971, 23(5):541.

Feng Y , Zhang X , Wu T , et al. Methylation effect on IPT5b gene expression determines cytokinin biosynthesis in apple rootstock[J]. Biochemical and Biophysical Research Communications, 2017, 482(4):604-609.

Friedman R, Hughes A L. Gene duplication and the structure of eukaryotic genomes.[J]. Genome Research, 2001, 11(3):373.

Guo A Y, Zhu Q H, Chen X, et al. [GSDS: a gene structure display server].[J]. Hereditas, 2007, 29(8):1023-1026.

Ioio R D , Linhares F S , Scacchi E , et al. Cytokinins Determine Arabidopsis Root-Meristem Size by Controlling Cell Differentiation[J]. Current Biology, 2007, 17(8):678-682.

Jeffares DC, Penkett CJ, Bähler J. (2008). Rapidly regulated genes are intron poor. Trends Genet. 2008;24(8):375–8.

Jutta Ludwig-Müller, Prinsen E , Rolfe S A , et al. Metabolism and Plant Hormone Action During Clubroot Disease[J]. Journal of Plant Growth Regulation, 2009, 28(3):229-244.

Jutta Ludwig-Müller, Schuller A . What can we learn from clubroots: alterations in host roots and hormone homeostasis caused by *Plasmodiophora brassicae*[J]. European Journal of Plant Pathology, 2008, 121(3):291-302.

Kieber J J , Schaller G E . Cytokinin signaling in plant development[J]. Development, 2018, 145(4):dev149344.

Kobelt, P., Siemens, J., and Sacristán, M. D. 2000 Histological characterisation of the incompatible interaction between Arabidopsis thaliana and the obligate biotrophic pathogen *Plasmodiophora brassicae*. Mycol. Res. 104:220-225.

Kosti M V, Lazaridou S, Bourazani N, et al. Discovering patterns of correlation and similarities in software project data with the Circos visualization tool[J]. Computer Science, 2011.

Kurepa J , Shull T E , Smalle J A . Cytokinin-induced growth in the duckweeds Lemna gibba and Spirodela polyrhiza[J]. Plant Growth Regulation, 2018.

Lisha P , Lili Z , Qinfei L , et al. Identification of Quantitative Trait Loci for Clubroot Resistance in *Brassica oleracea* With the Use of *Brassica* SNP Microarray[J]. Frontiers in Plant Science, 2018, 9:822-.

Liu X, Huang B, Banowetz G. Cytokinin effects on creeping bentgrass responses to heat stress: I. Shoot and root growth. (Turfgrass Science)[J]. Crop Science, 2002, 42(2):457-465.

Lomin S N, Myakushina Y A, Kolachevskaya O O, et al. Cytokinin perception in potato: new features of canonical players[J]. Journal of experimental botany, 2018, 69(16): 3839-3853.

Lopes-Caitar V S, Ccg D C M, Darben L M, et al. Genome-wide analysis of theHsp20 gene family in soybean: comprehensive sequence, genomic organization and expression profile analysis under abiotic and biotic stresses[J]. Bmc Genomics, 2013, 14(1):577-577.

Lovelock D A , Donald C E , Xavier A. Conlan…. Salicylic acid suppression of clubroot in broccoli (*Brassicae oleracea* var.italica) caused by the obligate biotroph *Plasmodiophora brassicae*[J]. Australasian Plant Pathology, 2013, 42(2):141-153.

Machanick P, Bailey T L. MEME-ChIP: motif analysis of large DNA datasets[J]. Bioinformatics, 2011, 27(12):1696-7.

Maere S , De Bodt S , Raes J , et al. Modeling gene and genome duplications in eukaryotes[J]. Proceedings of the National Academy of Sciences, 2005, 102(15):5454-5459.

Malito E, Coda A, Bilyeu K D, et al. Structures of Michaelis and product complexes of plant cytokinin dehydrogenase: implications for flavoenzyme catalysis.[J]. Journal of Molecular Biology, 2004, 341(5):1237-1249.

Manoharan R K , Shanmugam A , Hwang I , et al. Expression of salicylic acid-related genes in *Brassica oleracea* var. capitata during *Plasmodiophora brassicae* infection[J]. Genome, 2016, 59(6):379-391.

Marchlerbauer A, Derbyshire M K, Gonzales N R, et al. CDD: NCBI's conserved domain database.[J]. Nucleic Acids Research, 2015, 43(Database issue):D222.

Martin R C , Mok M C , Mok D W S . Isolation of a cytokinin gene, ZOG1, encoding zeatin O-glucosyltransferase from Phaseolus lunatus[J]. Proceedings of the National Academy of Sciences, 1999, 96(1):284-289.

Matsumotokitano M , Kusumoto T , Tarkowski P , et al. Cytokinins are central regulators of cambial activity[J]. Proc Natl Acad Sci U S A, 2008, 105(50):20027-20031.

Merewitz E B, Gianfagna T, Huang B R. Effects of SAG12-ipt and HSP18.2-ipt expression on cytokinin production, root growth, and leaf senescence in creeping bentgrass exposed to drought stress.[J]. Journal of the American Society for Horticultural Science, 2010, 135(3):230-239.

Miller C.O, Skoog F, Okomura FS, von Saltza MH, Strong FM (1956) Isolation, structure and synthesis of kinetin, a substance promoting cell division. J Am Chem Soc 78: 1345–1350.

Miri M , Janakirama P , Held M , et al. Into the Root: How Cytokinin Controls Rhizobial Infection[J]. Trends in Plant Science, 2015:S1360138515002320.

Nagaharu, U. (1935). Genome analysis in *Brassica* with special reference to the experimental formation of B. napus and peculiar mode of fertilization. Jpn J Bot, 7(7), 389-452.

Ning Y, Wang Y, Fang Z, et al. Identification and characterization of resistance for *Plasmodiophora brassicae* race 4 in cabbage (*Brassica oleracea* var. capitata)[J]. Australasian Plant Pathology, 2018, 47(5):531-541.

Pang W, Fu P, Li X, et al. Identification and Mapping of the Clubroot Resistance Gene CRd in Chinese Cabbage (*Brassica rapa* ssp. pekinensis).[J]. Frontiers in Plant Science, 2018, 9:653-.

Peng Z , Dongdong W , Ruoqiu W , et al. Genome-wide analysis of the potato Hsp20 gene family: identification, genomic organization and expression profiles in response to heat stress[J]. BMC Genomics, 2018, 19(1):61-.

Rausch, T., Butcher, D. N., and Hilgenberg, W. 1981. Nitrilase activity in clubroot diseased plants. Physiol. Plant. 52:467-470.

Riou-Khamlichi C , Huntley R , Jacqmard A , et al. Riou-Khamlichi, C. Huntley, R. Jacqmard, A. & Murray, J. A. Cytokinin activation of Arabidopsis cell division through a D-type cyclin. Science 283, 1541-1544[J]. Science, 1999, 283(5407):1541-1544.

Rioukhamlichi C, Huntley R, Jacqmard A, et al. Cytokinin Activation of Arabidopsis Cell Division Through a D-Type Cyclin[J]. Science, 1999, 283(5407):1541-1544.

Rivero R M , Kojima M , Gepstein A , et al. Delayed leaf senescence induces extreme drought tolerance in a flowering plant[J]. Proceedings of the National Academy of Sciences, 2007, 104(49):19631-19636.

Rocherieux J , Glory P , Giboulot A , et al. Isolate-specific and broad-spectrum QTLs are involved in the control of clubroot in *Brassica oleracea*[J]. Theoretical & Applied Genetics, 2004, 108(8):1555-1563.

Rolfe S A , Strelkov S E , Links M G , et al. The compact genome of the plant pathogen *Plasmodiophora brassicae* is adapted to intracellular interactions with host *Brassica* spp[J]. BMC Genomics, 2016, 17(1):272.

Rombauts S, Déhais P, Van Montagu M, et al. PlantCARE, a plant *cis*-acting regulatory element database[J]. Nucleic Acids Research, 1999, 27(1):295-6.

Sang S , Chunlai W , Chao H , et al. Genome-Wide Analysis of the GRF Family Reveals Their Involvement in Abiotic Stress Response in Cassava[J]. Genes, 2018, 9(2):110-.

Sano H , Seo S , Orudgev E , et al. Expression of the Gene for a Small GTP Binding Protein in Transgenic Tobacco Elevates Endogenous Cytokinin Levels, Abnormally Induces Salicylic Acid in Response to Wounding, and Increases Resistance to Tobacco Mosaic Virus Infection[J]. Proceedings of the National Academy of Sciences of the United States of America, 1994, 91(22):10556-10560.

Schmülling T, Werner T, Riefler M, et al. Structure and function of cytokinin oxidase/dehydrogenase genes of maize, rice, Arabidopsis and other cultivars[J]. Journal of Plant Research, 2003, 116(3):241-52.

Schuller A, Kehr J, Ludwigmüller J. Laser Microdissection Coupled to Transcriptional Profiling of Arabidopsis Roots Inoculated by *Plasmodiophora brassicae* Indicates a Role for Brassinosteroids in Clubroot Formation[J]. Plant & Cell Physiology, 2014, 55(2):392-411.

Séverine Lemarié, Robertseilaniantz A , Lariagon C , et al. Both the Jasmonic Acid and the Salicylic Acid Pathways Contribute to Resistance to the Biotrophic Clubroot Agent *Plasmodiophora brassicae* in Arabidopsis[J]. Plant & Cell Physiology, 2015, 56(11):2158.

Shaw, G. (1994) in Cytokinins: Chemistry, Activity and Function, eds. Mok, D. W. S. & Mok, M. C. (CRC Press, Boca Raton, FL), pp. 15–34.

Siemens J , Keller I , Sarx J , et al. Transcriptome Analysis of Arabidopsis Clubroots Indicate a Key Role for Cytokinins in Disease Development[J]. Molecular Plant-Microbe Interactions, 2006, 19(5):480-494.

Smigocki A C . Phenotype modification and enhanced tolerance to insect pests by regulated expression of a cytokinin biosynthesis gene[J]. Hortscience A Publication of the American Society for Horticultural Science, 1995.

Tamura K. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0[J]. Molecular Biology & Evolution, 2013, 30(12):2725-2729.

Voorrips R E. MapChart: Software for the Graphical Presentation of Linkage Maps and QTLs[J]. Journal of Heredity, 2002, 93(1):77-78.

Waldie T , Leyser O . Cytokinin Targets Auxin Transport to Promote Shoot Branching[J]. Plant physiology, 2018, 177(2).

Walerowski P, Gündel A, Yahaya N, et al. Clubroot disease stimulates early steps of phloem differentiation and recruits SWEET sucrose transporters within developing galls[J]. The Plant Cell, 2018, 30(12): 3058-3073.

Wang X, Kole C. The *Brassica rapa* Genome[M]. 2015.

Xie X Z , Xue Y J , Zhou J J , et al. Phytochromes Regulate SA and JA Signaling Pathways in Rice and Are Required for Developmentally Controlled Resistance to Magnaporthe grisea[J]. Molecular Plant, 2011, 4(4):688-696.

Yu F , Zhang X , Peng G , et al. Genotyping-by-sequencing reveals three QTL for clubroot resistance to six pathotypes of *Plasmodiophora brassicae* in *Brassica rapa*[J]. Scientific Reports, 2017, 7(1):4516.

Zhao Y, Cheng S, Song Y, et al. The interaction between rice ERF3 and WOX11 promotes crown root development by regulating gene expression involved in cytokinin signaling[J]. The Plant Cell, 2015, 27(9): 2469-2483.

Zhang X, Liu Y, Fang Z, et al. Comparative Transcriptome Analysis between Broccoli (*Brassica oleracea* var. italica) and Wild Cabbage (*Brassica* macrocarpa Guss.) in Response to *Plasmodiophora brassicae* during Different Infection Stages:[J]. Frontiers in Plant Science, 2016, 7(R106):1929.

Zhou F , Guo Y , Qiu L J . Genome-wide identification and evolutionary analysis of leucine-rich repeat receptor-like protein kinase genes in soybean[J]. BMC Plant Biology, 2016, 16(1):58.

Zhu H F, Gao Q Q, Xiao-Feng L I, et al. Effect of exogenous salicylic acid on seedling growth and clubroot resistance in pak-choi(Brassic campestris ssp. chinensis Makino)[J]. Acta Agriculturae Shanghai, 2017.

Liu RH, Meng JL (2003) MapDraw: a microsoft excel macro for drawing genetic linkage maps based on given genetic linkage data. Hereditas 25:317–321

Tables

Table 1 Information on *B. oleracea* cytokinin genes

Gene ID	Chr	Genomic Location	Gene Length (bp)	Protein Length (aa)	Predicted Localization
Bol028359	C01	7442998-7444581	1584	527	Cytoplasm
Bol028360	C01	7449780-7451360	1581	526	Cytoplasm
Bol028361	C01	7453035-7454639	1605	534	Cytoplasm
Bol028363	C01	7482109-7483644	1536	511	Cytoplasm
Bol028392	C01	7729897-7731465	1569	522	Cytoplasm
Bol031036	C01	30380413-30382523	2111	548	Chloroplast/Cytoplasm
Bol014258	C02	9886003-9888966	2964	518	Endoplasmic reticulum/Vacuole
Bol036074	C02	6024676-6027814	3139	517	Extracell
Bol011927	C03	50037656-50039251	1596	531	Cytoplasm
Bol020547	C03	12074968-12077007	2040	545	Vacuole
Bol024927	C03	39976312-39978905	2594	594	Chloroplast/ Vacuole
Bol024949	C03	40218413-40219993	1581	526	Cytoplasm
Bol024952	C03	40274541-40276136	1596	531	Cytoplasm
Bol005172	C04	39333424-39335907	2484	412	Vacuole
Bol006929	C04	529034-530361	1328	355	Vacuole
Bol027388	C04	21332565-21335127	2563	505	Cytoplasm
Bol027390	C04	21363848-21365476	1629	542	Cytoplasm
Bol037842	C04	35866576-35869681	3106	532	Cytoplasm
Bol018140	C05	22809900-22811690	1791	561	Chloroplast/Cytoplasm
Bol036628	C05	25011639-25013231	1593	530	Cytoplasm
Bol037999	C05	4599782-4602880	3099	465	Endoplasmic reticulum/Vacuole
Bol010168	C06	25330129-25332003	1875	561	Chloroplast/Cytoplasm
Bol027725	C06	2274860-2277663	2804	531	Extracell
Bol006383	C07	27111633-27113243	1611	536	Cytoplasm
Bol006385	C07	27055734-27058578	2845	537	Cell membrane/Cytoplasm
Bol006388	C07	27010262-27011857	1596	531	Cytoplasm
Bol006389	C07	27003157-27008056	4900	543	Cytoplasm
Bol022730	C07	7797363-7801639	4277	505	Endoplasmic reticulum
Bol024330	C07	41865890-41867509	1620	539	Cytoplasm
Bol033608	C07	45245680-45249406	3727	524	Endoplasmic reticulum
Bol036948	C07	40966319-40967965	1647	548	Cell membrane/Cytoplasm
Bol031324	C08	38898546-38900162	1617	538	Cytoplasm
Bol045724	C08	33615188-33616880	1693	361	Vacuole
Bol009917	C09	24618802-24620803	2002	587	Cytoplasm
Bol035751	C09	29462698-29465742	3045	520	Extracell
Bol043933	C09	38523554-38527278	3725	570	Chloroplast/Cytoplasm/Vacuole

Figures

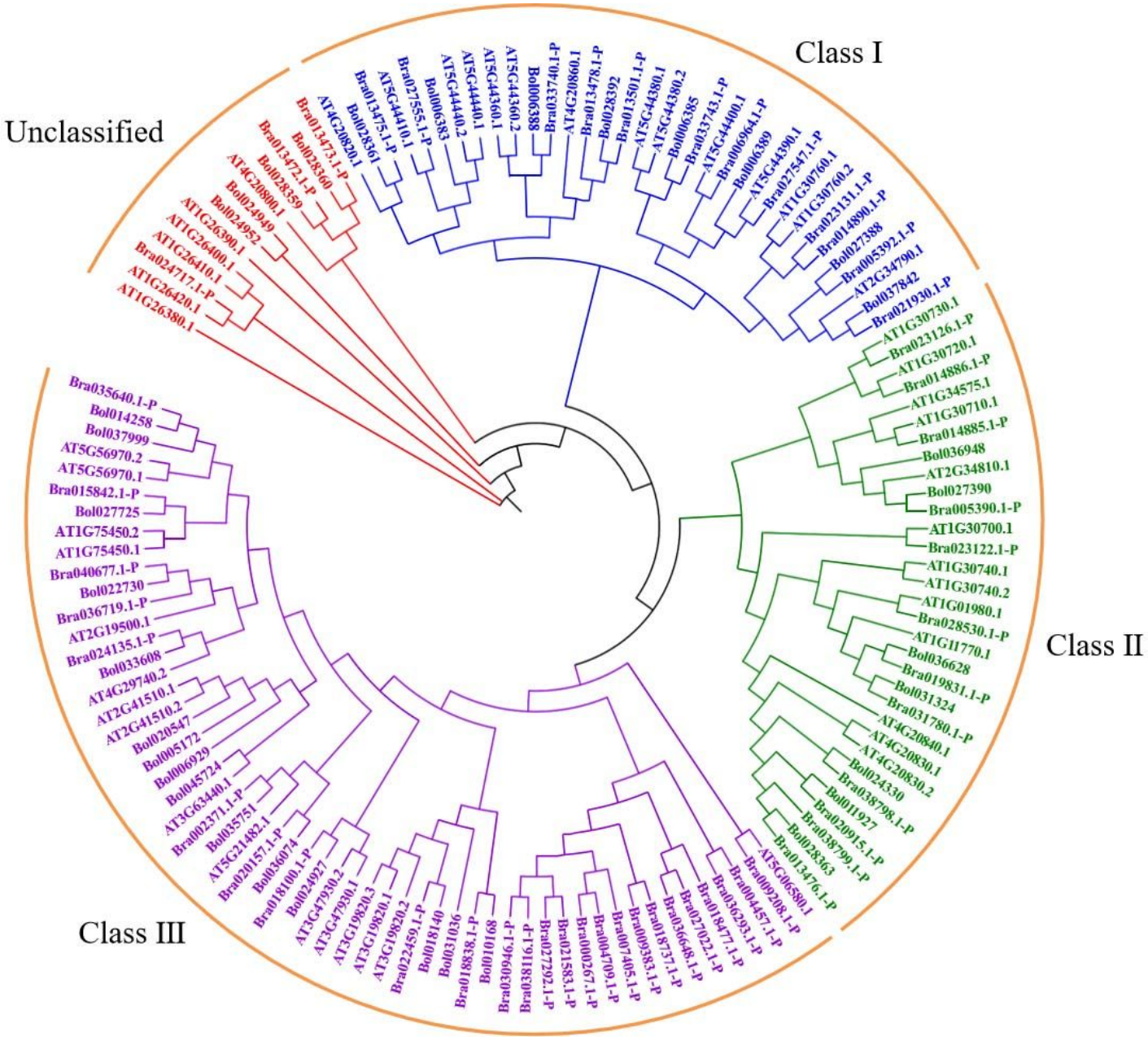

Figure 1

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

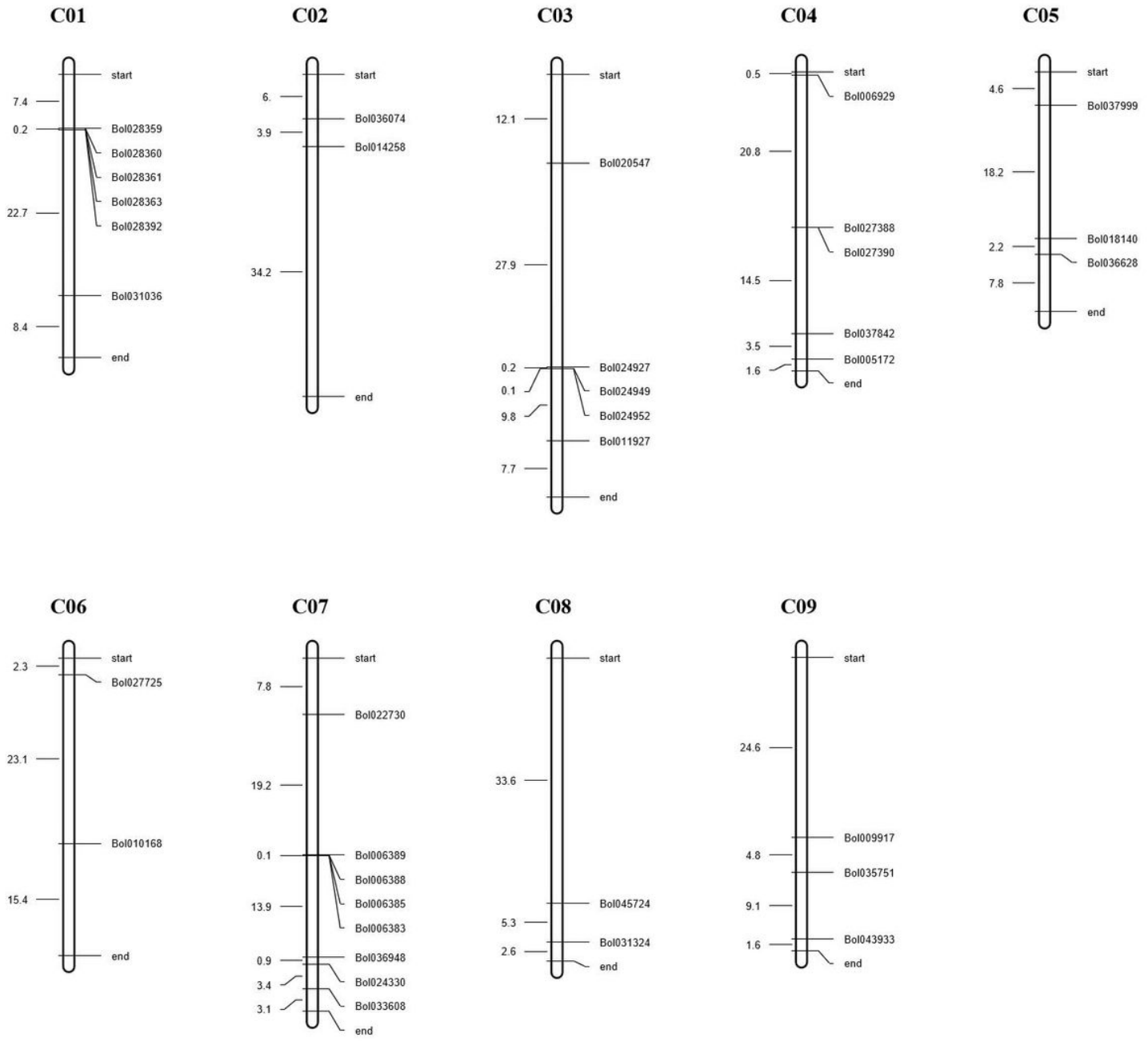

Figure 2

Distribution of cytokinin genes on B. oleracea. chromosomes. The chromosome number is indicated at the top of each chromosome. Gene names are indicated on the right side of each chromosome. The distance (Mb) between genes or from, the gene to the end of the chromosome is shown on the left side of each chromosome.

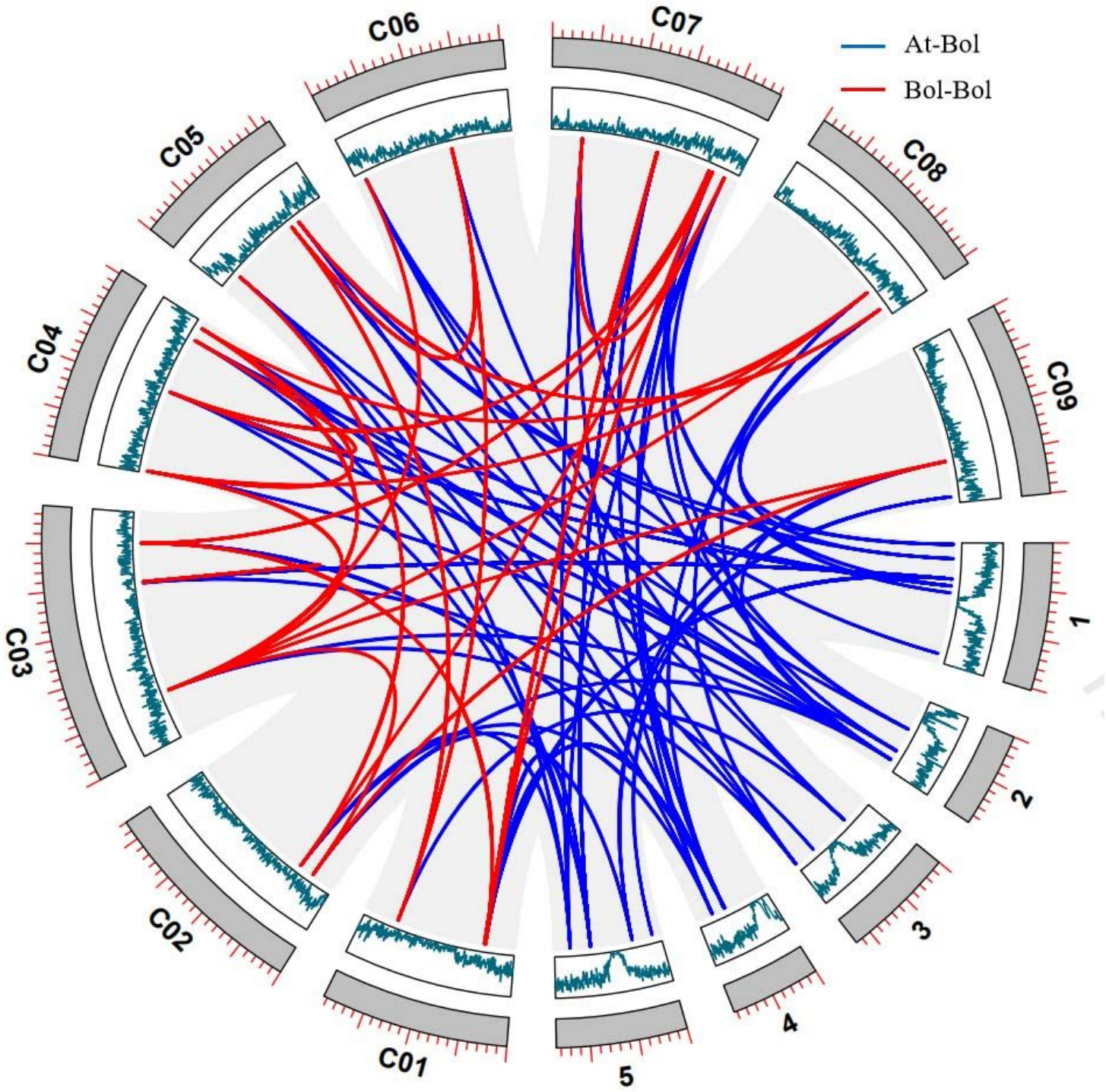

Figure 3

Syntenic relationships of B. oleracea and A. thaliana cytokinin genes in chromosome maps.C01-C09, nine chromosomes of B. oleracea. 1-5, five chromosomes of A. thaliana. Red lines, homologous genes between cabbage chromosomes. Blue lines, homologous genes between cabbage and A. thaliana chromosomes.

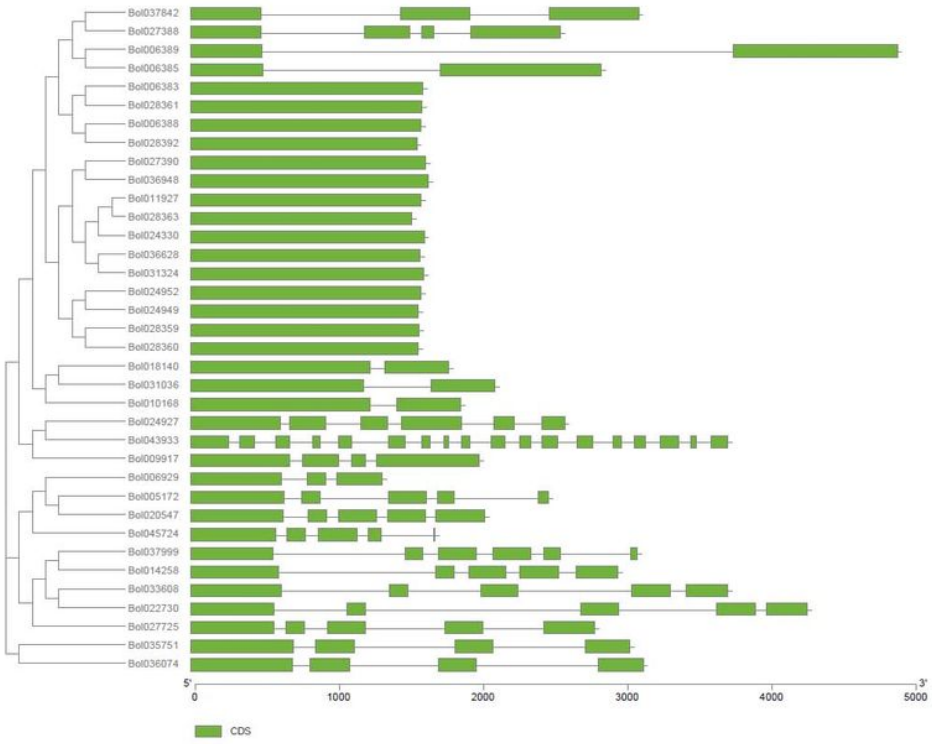

Figure 4

Gene structures of 36 cytokinin genes identified in B. oleracea. Exons are represented by green boxes, while introns are represented by gray lines.

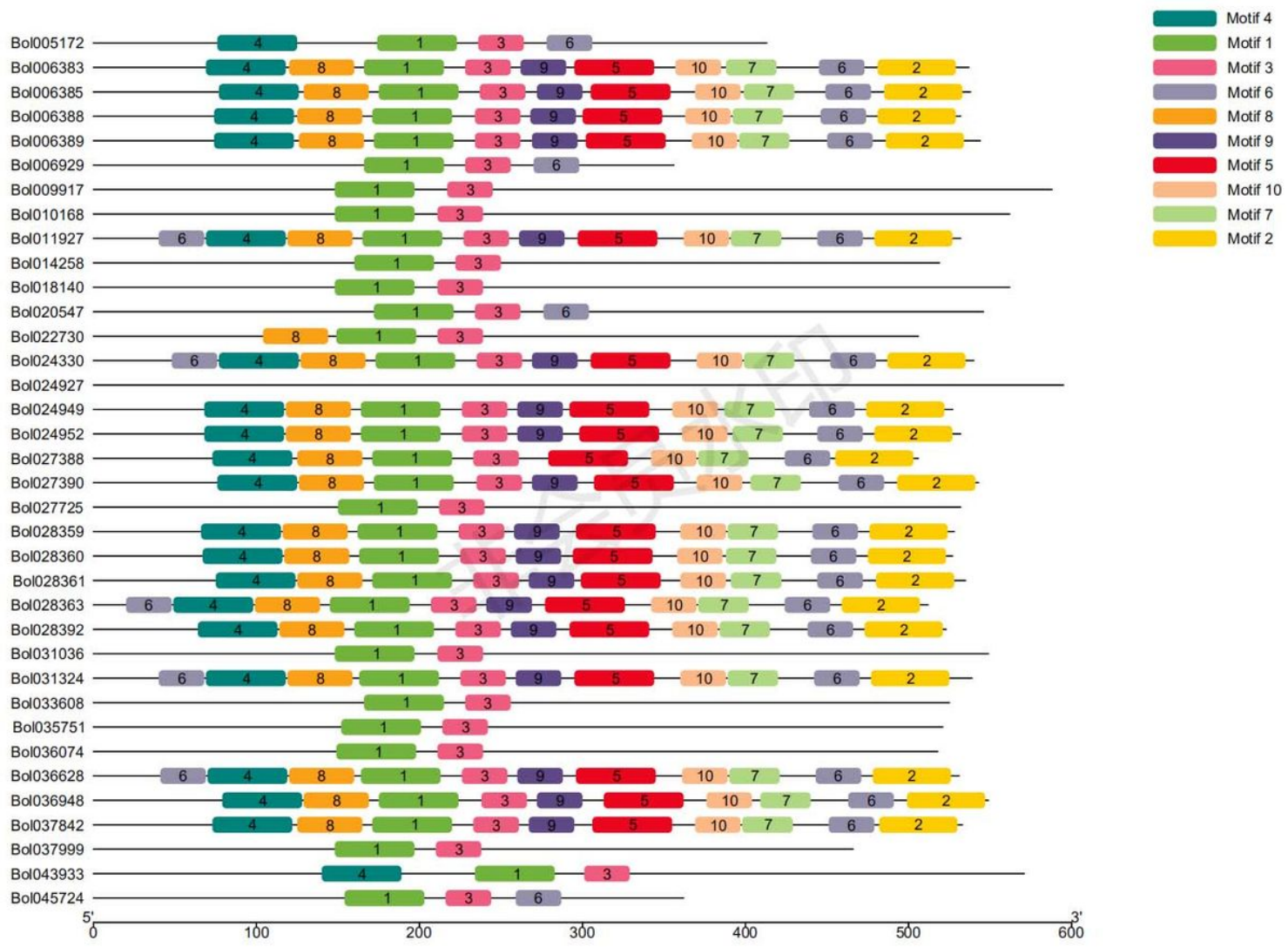

Figure 5

Distribution of conserved motifs in cytokinin genes. Ten putative motifs are indicated in different colored boxes. The lengths of the motifs in each protein are shown proportionally.

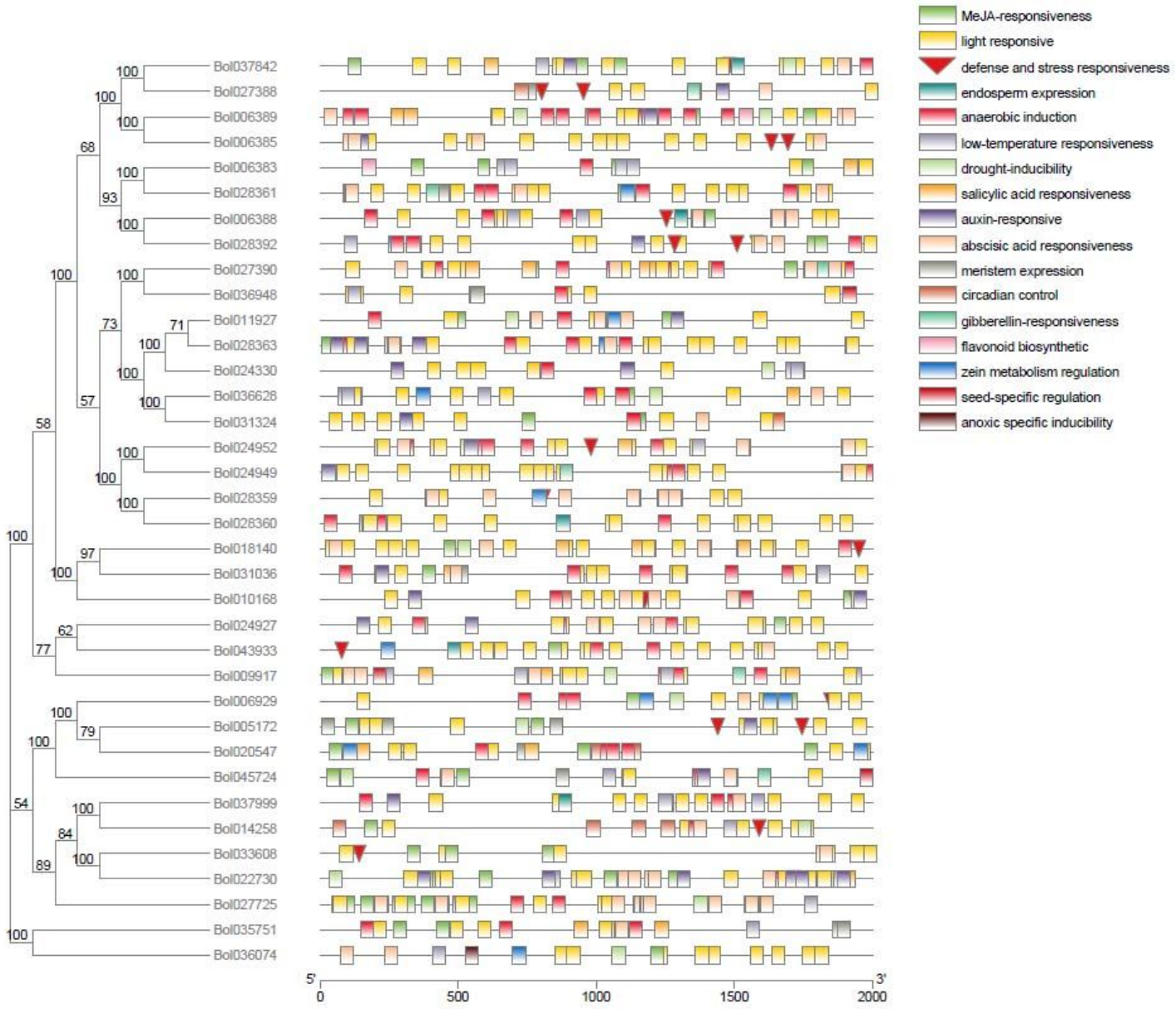

Figure 6

Predicted cis-acting elements in the promoter regions of cytokinin genes.

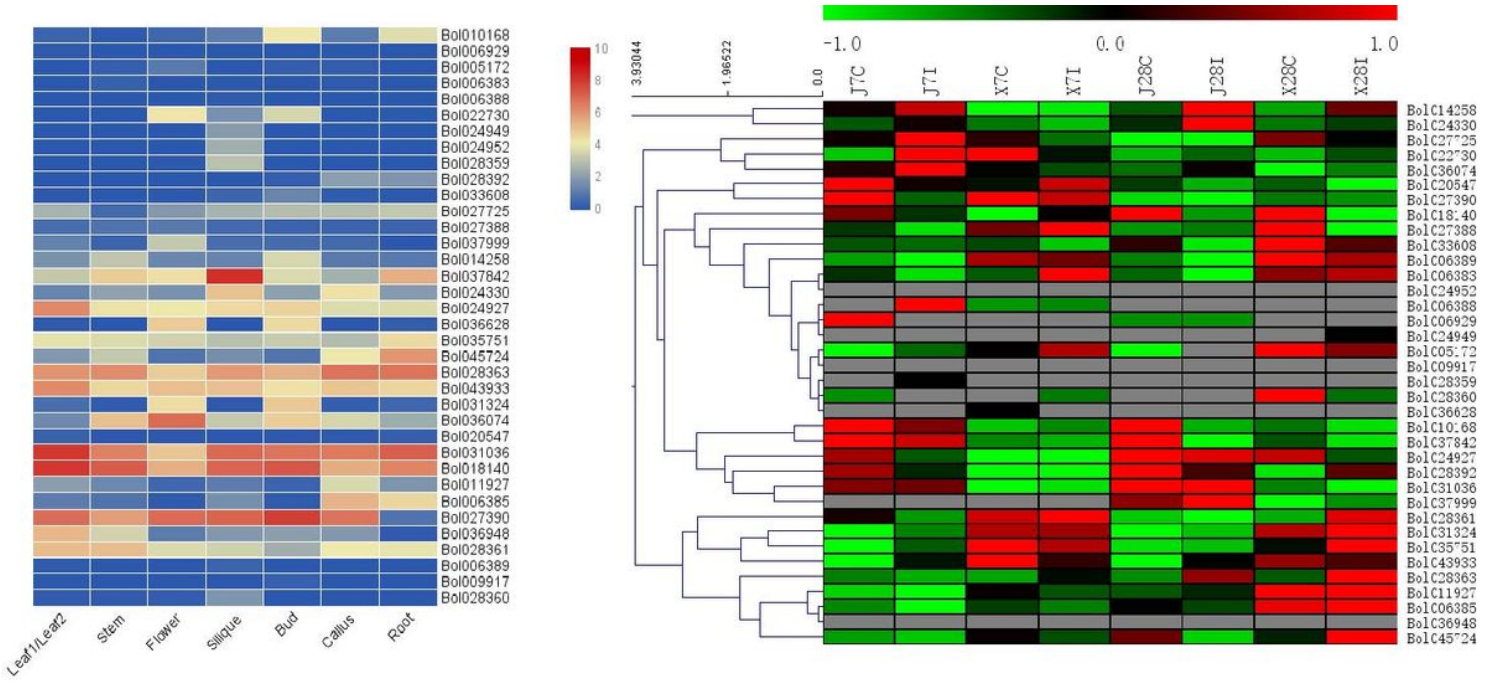

Figure 7

Expression patterns of cytokinin genes analyzed by RNA-Seq. a Heatmap showing cytokinin gene expression in different organs. b Expression profiles of cytokinin genes in different periods after the inoculation of two materials. J7C, Jingfeng No. 1 not inoculated at 7 days as a control. J7I, Jingfeng No. 1 inoculated at 7 days. X7C, Xiangan 336 not inoculated at 7 days as a control. X7I, Xiangan 336 inoculated at 7 days. J28C, Jingfeng No. 1 not inoculated at 28 days as a control. J28I, Jingfeng No. 1 inoculated at 28 days. X28C, Xiangan 336 not inoculated at 28 days as a control. X28I, Xiangan 336 inoculated at 28 days.

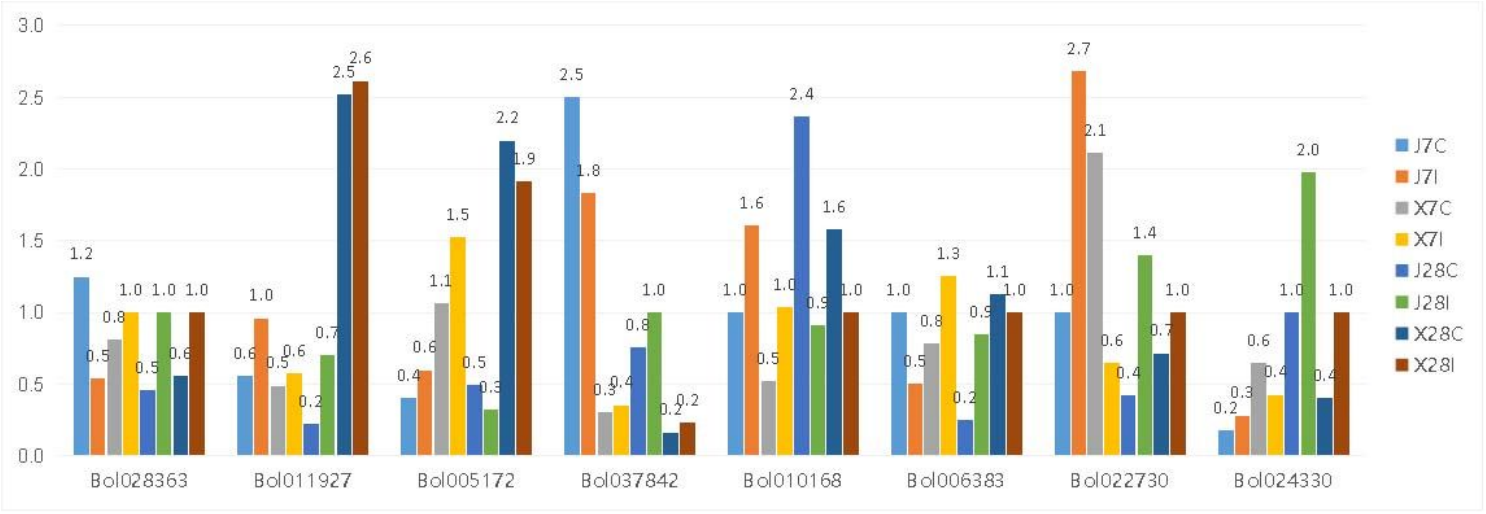

Figure 8

Expression levels of 8 cytokinin genes analyzed by qRT-PCR.

Supplementary Files

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