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The Genome-Wide Identification of the R2R3-MYB Gene Family in Chinese Flowering Cabbage and the Characterization of Its Response to *Pectobacterium carotovorum* Infection

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Abstract: Chinese flowering cabbage is an important bolting stem vegetable widely grown in southern China, but severe losses caused by soft rot disease are very common in this crop. The MYB transcription factor (TF) family is the largest TF family in plants and plays diverse roles in response to stresses. However, the responses of MYB TFs to biotic stress in Chinese flowering cabbage have not been systematically studied. Herein, 255 R2R3-MYB genes were identified in the genome of Chinese flowering cabbage and classified into 29 subgroups based on phylogenetic comparisons with *Arabidopsis thaliana*. Gene duplication events involved 182 gene duplication pairs, and we found that two tandem duplication events involving R2R3-MYB genes in Chinese flowering cabbage may also affect gene family expansion. Transcriptome data analysis indicated that MYB TF genes are highly enriched in differentially expressed gene (DEG) sets. Combined with phylogenetic and transcriptome analysis, we identified 12 R2R3-MYB genes that potentially play a role in the response to soft rot stress. Our research provides a foundation for further research on the response of R2R3-MYB genes to soft rot stress in Chinese flowering cabbage.

Keywords: R2R3-MYB; Chinese flowering cabbage; soft rot; gene expression



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1. Introduction

TFs are crucial regulators of gene transcription that form transcription initiation complexes with RNA polymerase II to participate in the transcription initiation process in eukaryotes [1]. Among the numerous TF families, the MYB TF family has the largest number of members and has been confirmed to be involved in various biological processes in plants [2]. The N-terminus of the MYB protein contains a highly conserved MYB DNA-binding domain which typically consists of 1~4 incomplete tandem repeats (R1, R2, R3, and R4) of approximately 50~53 amino acids, and each repeat forms three α helices. The second and third helices construct a helix–turn–helix (HTH) structure with three evenly spaced tryptophan (TRP) residues which participate in DNA binding [2]. Based on the number of incomplete repeats in the MYB domain, the MYB TF family can be classified into four subfamilies: 1R-MYBs, R2R3-MYBs, R1R2R3-MYBs, and 4R-MYBs [3].

In plants, the MYB gene was first identified in 1987 and has been confirmed to be involved in the biosynthesis of anthocyanins in maize aleurone layer tissue [4]. With the advancement of sequencing technology, an increasing number of plant genomes have been constructed, and members of the MYB TF family have been identified in various plants, such as *Arabidopsis*, rice, cotton, poplar, tobacco, and cassava [3,5–9]. Numerous studies have shown that MYB TFs can bind to MYB binding sites (MBSs) on target DNA, including the MYB core sequence (C/TNGTTG/A) and the AC element (ACCA/TAA/CT/C) [10–12].

Through the regulation of downstream target genes, MYB TFs play important roles in various aspects of the plant life cycle, including secondary metabolism, root hair development, flowering, hormone signal transduction, and abiotic stress responses [13–17]. Additionally, an increasing number of studies have revealed that MYB TFs are involved in plant pathogen resistance. For instance, the wheat MYB TF TaRIM1 has been confirmed to positively regulate wheat sharp eyespot resistance by activating disease resistance genes (*defensin*, *PR10*, *PR17c*, *nsLTP1*, and *chit1*) [18]. Gao et al. discovered that the overexpression of mustard *BjMYB1* enhances resistance to *Botrytis cinerea* [19]. Djami-Tchatchou et al. evaluated the expression of several tomato defense genes after being inoculated with *Pectobacterium carotovorum*; these genes were significantly upregulated and a maximum 14-fold increase in expression was detected for the MYB TF gene [20]. The induction of MYB TF gene expression was also found in pepper plants after being inoculated with *P. carotovorum* [21]. Research has shown that MYB TFs primarily regulate plant disease resistance in several ways. First, they mediate physical defense against plant pathogens by controlling the synthesis of secondary cell walls and cuticular wax in plant cells. Second, they influence the signaling of plant hormones, such as abscisic acid, jasmonic acid, ethylene, and salicylic acid, to mediate pathogen resistance. Third, they directly activate the expression of pathogen-defense-related genes. Fourth, they regulate the synthesis of flavonoids through the MYB-bHLH-WD40 complex to defend against pathogens [22]. Taken together, these findings indicate that the MYB TF can integrate multiple resistance genes and defense signaling pathways, playing a pivotal role in plant immune responses.

Chinese flowering cabbage (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee), a vegetable crop of the *Brassica* genus, is deeply beloved by the people of southern China [23]. With the expansion of its cultivation area in recent years, diseases of Chinese flowering cabbage have become increasingly severe, among which soft rot is one of the primary diseases threatening Chinese flowering cabbage. In *Brassica* crops, most soft rot is caused by *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*), a bacterial pathogen that primarily infects plants through wounds at the base of the petiole [24]. At the initial stage of disease development, the affected area appears to be water-soaked, subsequently expanding to a slimy and soft rot, accompanied by an unpleasant odor. Currently, there are limited varieties of Chinese flowering cabbage that are resistant to soft rot, and the pathogenesis of soft rot has not been determined. Therefore, research on genes related to soft rot responses is important for the development of Chinese flowering cabbage varieties with enhanced resistance to this disease.

In this study, we identified 255 R2R3-MYB family members from Chinese flowering cabbage and analyzed their systematic evolution, chromosomal distribution, and responses to soft rot stress. Combined with transcriptome and quantitative PCR (qPCR) data, the expression patterns of the BcMYBs in response to *Pcc* infection were explored. Our data provide a scientific basis for functional research on the R2R3-MYB TF family in Chinese flowering cabbage.

2. Materials and Methods

2.1. Plant Materials and Bacterial Plant Incubation

Seeds of the Chinese flowering cabbage cultivar ‘Youqing 49’ were sown in a soil mixture (peat moss, perlite, and vermiculite (8:1:1), Shengsheng Agriculture Co., Ltd, Guangzhou, China) and grown in a greenhouse at 20 °C under a 16 h light/8 h dark cycle at 50% relative humidity. The *Pcc* pathogen, which was isolated and identified in our laboratory, was cultured in LB medium overnight at 28 °C, after which the cells were harvested, resuspended in LB medium, and adjusted to an OD₆₀₀ of 0.5. Six leaf-stage plants were inoculated with *Pcc* pathogen liquid at the petiole, as previously described [24]. The inoculated leaves were collected at 0, 12, and 24 h following inoculation (hpi), and three biological replicates were performed. The samples were frozen immediately in liquid nitrogen and stored at −80 °C for RNA isolation.

2.2. Identification of R2R3-MYB TFs and Analysis of Their Physicochemical Properties

The known R2R3-MYB protein sequences of *Arabidopsis* were downloaded from the Arabidopsis Information Resource database (<http://www.arabidopsis.org/> (accessed on 30 January 2024)) and used as query sequences to preliminarily identify homologous R2R3-MYB protein sequences using TBtools (version 2.069) [25]. Subsequently, the hidden Markov model (HMM) profile of the MYB domain (PF00249) was downloaded from the Pfam database (<http://pfam.xfam.org/> (accessed on 30 January 2024)) to find the target protein sequences in the Chinese flowering cabbage protein database using the HMM search program of TBtools. The final Chinese flowering cabbage R2R3-MYB genes were renamed BcMYBs according to their sequence IDs. The physicochemical properties of the BcMYB proteins, including the number of amino acids, their molecular weight (MW), their theoretical isoelectric point (pI), and the grand average of hydropathicity (GRAVY), were calculated using the ExPASy website (<https://web.ExPASy.org/protparam/> (accessed on 30 January 2024)).

2.3. Chromosome Localization, Gene Duplication, and Phylogenetic Analysis

The genome annotation file (GFF3) and the associated genomic DNA sequences of Chinese flowering cabbage were used to map the physical location and length of chromosomes, which in turn determined the chromosomal distribution of BcMYB genes. The chromosomal distributions and synteny relationships of the BcMYB genes were graphically displayed using TBtools software (version 2.069). For phylogenetic analysis, the BcMYB and AtMYB protein sequences were subjected to multiple sequence alignment using the ClustalW tool in MEGA7.0 [26]. A phylogenetic tree was constructed using the maximum likelihood (ML) method with 1000 bootstrap replicates.

2.4. RNA Isolation and qPCR

Total RNA was isolated from Chinese flowering cabbage plants using a Plant RNA Extraction Kit (Omega Laboratories, Inc. Norcross, GA, USA), according to the manufacturer's protocol. cDNA was synthesized with a 1st-Strand cDNA Synthesis Kit (Takara, Dalian, China) in a 10 µL reaction mixture containing 1 µg of total RNA, according to the manufacturer's instructions. Quantitative PCR was performed with an ABI 7500 Real-Time PCR System (Applied Biosystems, Foster, CA, USA) using TB Green[®] qPCR Premix (Takara, Dalian, China). The BcTUB2 gene was used as an internal positive control. The relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method based on the mean value of three technical replicates [27]. The primers used for qPCR are listed in Supporting Information Table S1.

2.5. RNA Sequencing and Data Analysis

Total RNA was isolated from inoculated leaves (0 hpi, 12 hpi, and 24 hpi) and used for RNA library construction. Transcriptome sequencing was performed on the Illumina HiSeq platform at Sangon Biotech (Shanghai) Co., Ltd., Shanghai, China, with three biological replicates per treatment. The raw sequencing data were filtered to remove adapter sequences and obtain high-quality clean reads. The clean data were subsequently mapped to the *Brassica rapa* var. *parachinensis* reference genome [28]. HTSeq (v0.6.1) was used to count the read numbers mapped to each gene, and the fragments per kilobase of transcript sequences per million sequenced base pairs (FPKM) were calculated based on the length of the gene and read counts mapped to this gene [29]. A clustering heatmap of the expression patterns of the BcMYB genes was generated based on the FPKM values using TBtools software. TF enrichment analysis was performed via the R package "clusterProfile" [30].

3. Results

3.1. Identification and Analysis of the Physicochemical Properties of R2R3-MYB TFs in Chinese Flowering Cabbage

Using Pfam screening in conjunction with the reference *Arabidopsis* R2R3-MYB protein sequences, a total of 255 R2R3-MYB genes were found in the Chinese flowering cabbage genome, and these genes were named BcMYB1~BcMYB255 according to their sequence ID (Table S2). The physicochemical properties of the BcMYB proteins were analyzed using TBtools, as shown in Table S2. The number of amino acids in the BcMYB proteins ranged from 36 to 1156, with predicted protein molecular weights ranging from 4156.89 to 172,588.06 Da. The isoelectric points of these BcMYB proteins ranged from 4.66 to 10.31, with 104 acidic proteins ($pI < 6.5$), 24 neutral proteins ($6.5 < pI < 7.5$), and 127 alkaline proteins ($pI > 7.5$). The instability coefficients of these BcMYB proteins ranged from 23.95 to 81.66, and 20 members had instability coefficients less than 40, while the remaining 215 R2R3-MYB proteins had instability coefficients greater than 40, indicating that these proteins were unstable. The fat coefficients ranged from 43.81 to 99.35, indicating that the proteins encoded by the BcMYB genes were thermally stable. The hydrophilicity coefficients of all the 255 BcMYBs were less than 0, with a maximum value of -0.062 and a minimum value of -1.114 , indicating that all the BcMYBs were hydrophilic proteins.

3.2. Phylogenetic Analysis and Classification of the R2R3-MYB Gene Family

To investigate the evolutionary relationships of the BcMYB proteins, a total of 255 BcMYBs and 126 AtMYBs were used to construct a phylogenetic tree via the ML method. As shown in Figure 1, the sequence similarity and phylogenetic tree topology allowed us to divide the genes into 29 subgroups (S1~S29), which ranged in size from 6 to 23 R2R3-MYB members, and each subgroup contained AtMYBs and BcMYBs. This result suggested that BcMYBs and AtMYBs might have a close evolutionary relationship. Members in the same subgroup of the phylogenetic tree have high sequence similarity scores and are likely to have similar biological functions. Several members of AtMYBs in subgroups S5, S10, S17, and S29 have been reported to be involved in the biotic stress response [20], suggesting that the BcMYBs in these four subgroups are also likely to be involved in the biological stress response.

3.3. Chromosome Location and Collinearity Analysis of the BcMYBs

Based on the annotation information of the Chinese flowering cabbage genome, chromosomal localization analysis was performed using TBtools. As shown in Figure 2, 254 R2R3-MYB genes were distributed on all 10 chromosomes and 1 gene (BcMYB255) was located on the scaffold. Among these 10 chromosomes, chromosome 3 had the most R2R3-MYB genes, with a total of 44, while chromosome 4 had the least, with only 11. In addition, the R2R3-MYB gene was densely distributed on all 10 chromosomes, including the ends of chromosomes 1, 2, 5, 6, and 9; the top and middle of chromosome 3; and the bottom of chromosomes 7, 8, and 10. Subsequently, we used TBtools to investigate the duplication events of the genes to determine the expansion patterns of the BcMYBs. The collinearity relationships of the duplicated pairs in the R2R3-MYB gene family are shown in Figure 3. In total, we identified 182 pairs of highly similar paralogs that shared a high degree of identity through their protein sequences (Table S3). In addition, the substitution ratios of Ka to Ks mutations (Ka/Ks) in the above gene pairs were calculated. All 182 duplicated gene pairs had a Ka/Ks ratio < 1 , implying that R2R3-MYB genes in Chinese flowering cabbage are slowly evolving. The analysis also revealed two tandemly duplicated gene pairs located on chromosome 1 (BcMYB28 and BcMYB29) and chromosome 9 (BcMYB221 and BcMYB222) among the 255 BcMYB genes (Figure 2 and Table S3), indicating that these two tandemly duplicated genes might have maintained conserved functions.

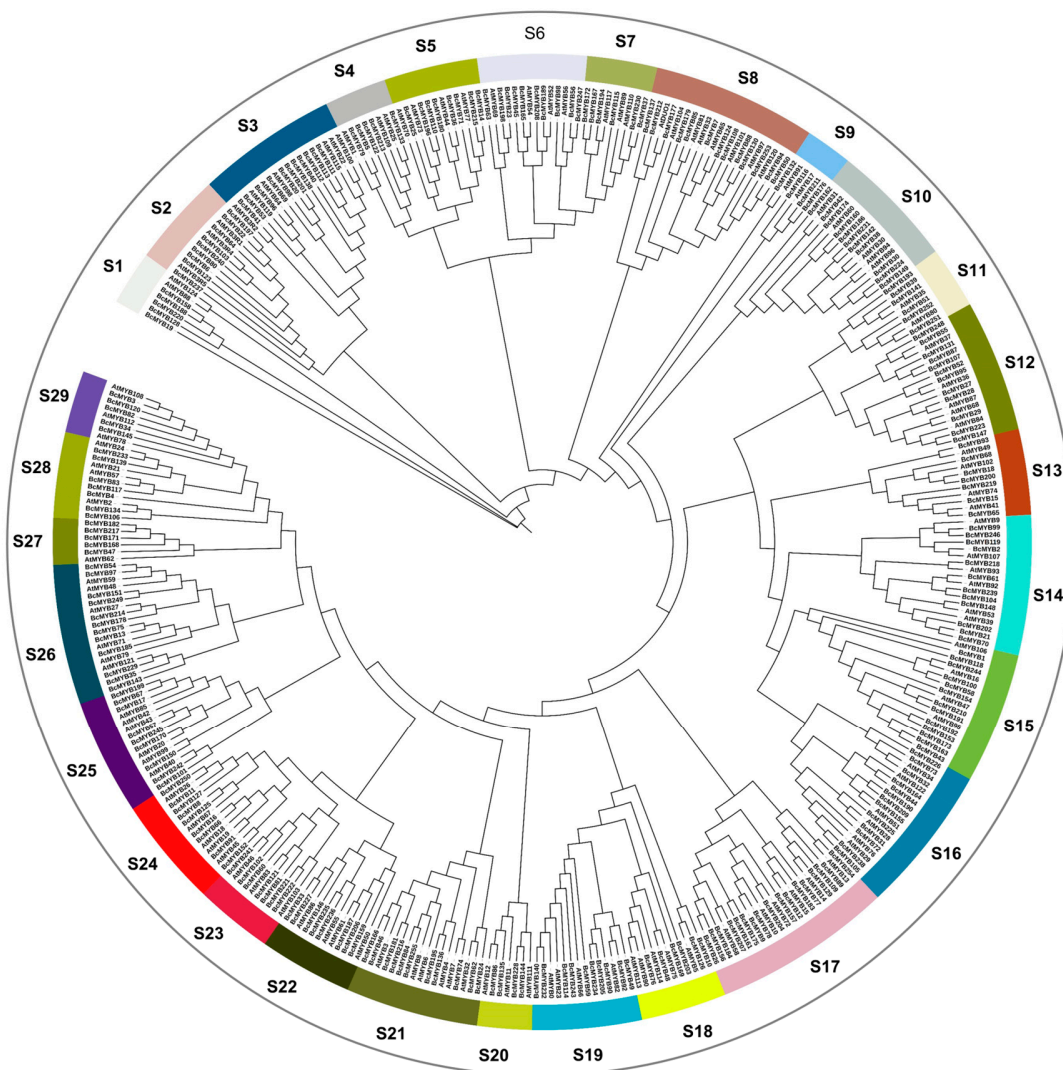


Figure 1. Phylogenetic analysis of R2R3-MYB proteins. The maximum likelihood phylogenetic tree included 255 R2R3-MYB proteins in Chinese flowering cabbage and 126 in *Arabidopsis*. At, *Arabidopsis thaliana*; Bc, *Brassica campestris*. The subgroups are distinguished by different colors.

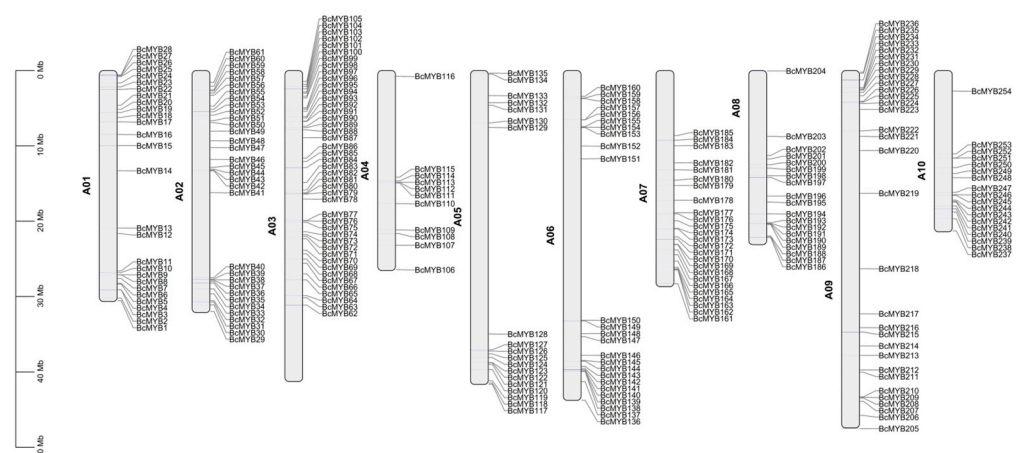


Figure 2. Chromosomal localization of the R2R3-MYB gene family in Chinese flowering cabbage. The *BcMYB* genes are shown on the right of each chromosome. The gene positions and the size of each chromosome can be estimated using the scale on the left of the figure. The length of each chromosome was estimated in megabases (Mb).

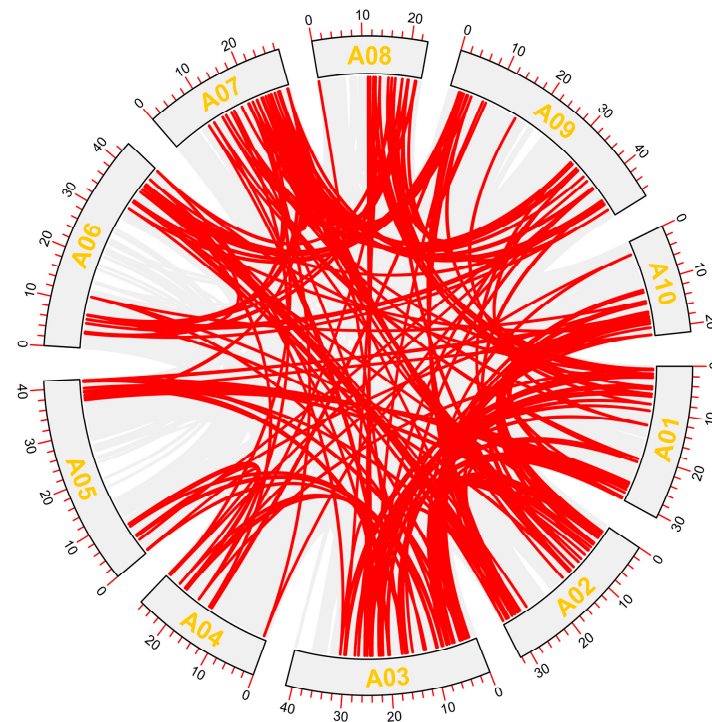


Figure 3. Depiction of duplicated *BcMYB* genes on the 10 Chinese flowering cabbage chromosomes. The gray lines indicate collinear blocks in the whole Chinese flowering cabbage genome and the red lines indicate duplicated R2R3-MYB gene pairs. The scale bars indicate the length of each chromosome and estimated in megabases (Mb).

3.4. Global Analysis of Differentially Expressed TF Genes in Response to *Pcc* Infection

Pcc had a strong pathogenic effect on Chinese flowering cabbage, and the infected area expanded with increasing inoculation time and changed from water stains to rotting (Figure S1). To further investigate the expression characteristics of the *BcMYBs* in response to *Pcc* infection, transcriptomic sequencing was performed on leaves infected with the *Pcc* pathogen using the Illumina platform. After quality control and filtering, 39.815–56.807 million valid sequences were obtained, with a Q30 value consistently above 94.65%, indicating high sequencing quality and meeting the requirements for subsequent analysis. Compared to those in the control group (Sr-0), 6890 and 7206 differentially expressed genes (DEGs) were identified in infected leaves at 12 hpi (Sr-12) and 24 hpi (Sr-24), respectively. Among the identified DEGs, 3221 genes were upregulated and 3669 genes were downregulated after 12 hpi. Moreover, there were 3487 upregulated genes and 3719 downregulated genes at 24 hpi (Figure 4). We then analyzed the DEG sets using the PlantTFDB (version 5.0) database (<https://planttfdb.gao-lab.org/> (accessed on 30 January 2024)) and identified 521 and 525 differentially expressed TF genes, respectively. These results indicated that the number of DEGs and differentially expressed TF genes were not affected by the duration of *Pcc* infection. To further understand the roles of these differentially expressed TF genes, enrichment analysis was performed. The top 10 TF families associated with the most significant enrichment in terms of *p* values are shown in Figure 5. Among these TF families, the MYB TF family was highly significantly enriched at both 12 hpi and 24 hpi. These comparative transcriptomic analyses suggest that MYB TFs may play a significant role in regulating the host immune response to *Pcc* infection.

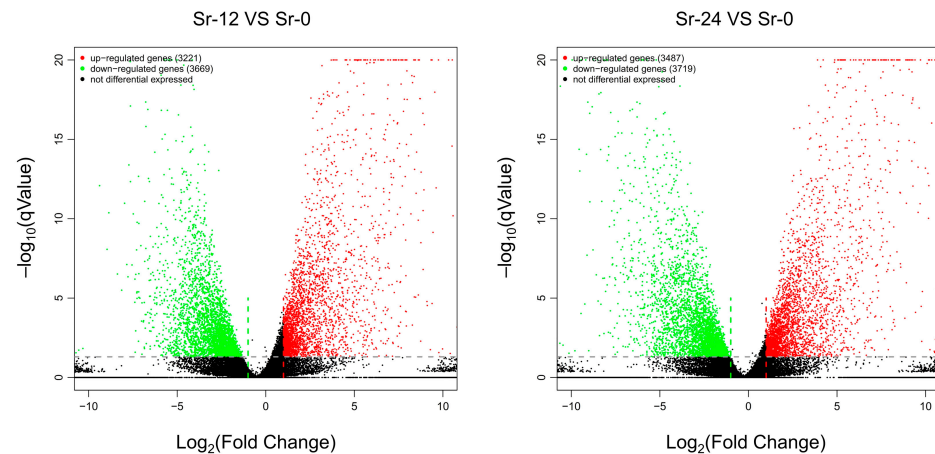


Figure 4. Volcano map of differentially expressed genes. The red dots indicate upregulated genes, the green dots indicate downregulated genes, and the genes not differentially expressed are indicated by black dots.

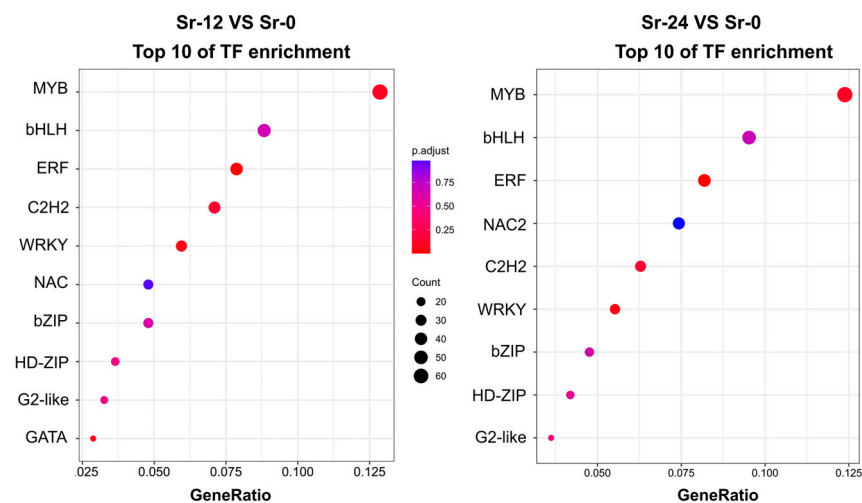


Figure 5. Differentially expressed TF gene enrichment analysis upon *Pcc* infection. Among these TF families, the MYB family was most significantly enriched at both 12 hpi and 24 hpi. Enrichment analysis was performed using the clusterProfile package. The size of black dots represents the number of genes. The smaller the *p*-adjust of a bubble, the greater its significance.

3.5. Response of *BcMYBs* to *Pcc* Infection

We found that the MYB TFs were highly enriched in response to *Pcc* infection; among these differentially expressed MYB TF genes, 38 were of the R2R3 type. To investigate the response of R2R3-MYB genes to *Pcc* infection in Chinese flowering cabbage, time course transcriptome data were analyzed. The expression of 38 differentially expressed MYB TF genes upon *Pcc* infection is shown in a heatmap. As shown in Figure 6, the 38 *BcMYBs* were clustered into four subgroups according to their expression patterns. The first subgroup included 10 genes whose expression was upregulated at 12 hpi and downregulated at 24 hpi. The second subgroup included eight genes whose expression continuously decreased. The third subgroup included three genes whose expression first increased and then decreased. The final subgroup included the most members, with 11 genes whose expression continuously increased. To validate the transcriptome data, a total of 12 *BcMYBs* from groups S5, S10, S17, and S29, which contain AtMYBs involved in biotic stress responses, were selected for qPCR detection. As shown in Figure 7, most of the 12 *BcMYBs* exhibited similar expression trends according to both qPCR and RNA sequencing. The results indicated that the 12 *BcMYB* genes might be involved in the response of Chinese flowering cabbage to *Pcc* infection.

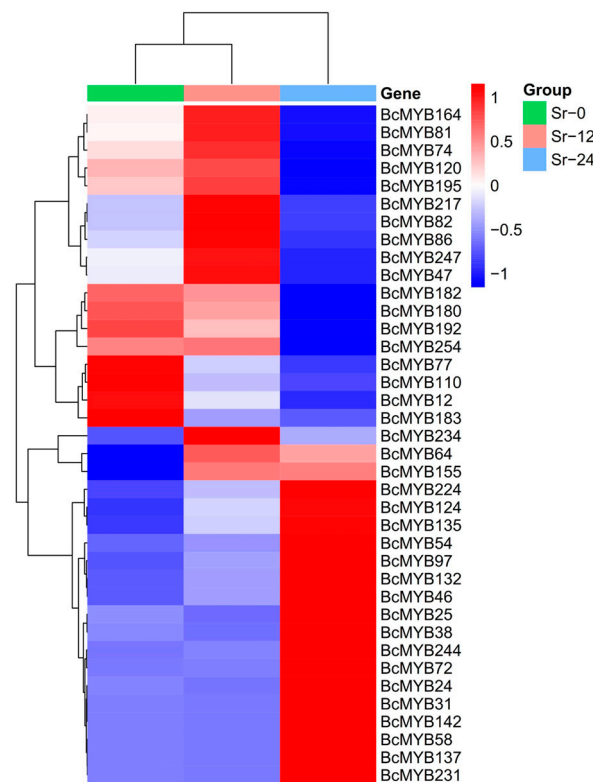


Figure 6. Expression patterns of *BcMYBs* in response to *Pcc* infection. Heatmap of the expression profiles of *BcMYBs* in Chinese flowering cabbage infected with *Pcc*. The color grade indicates the gene expression level: blue indicates low expression and red indicates high expression. Sr-0, Sr-12, and Sr-24 represent 0, 12, and 24 h of infection by *Pcc* pathogen, respectively.

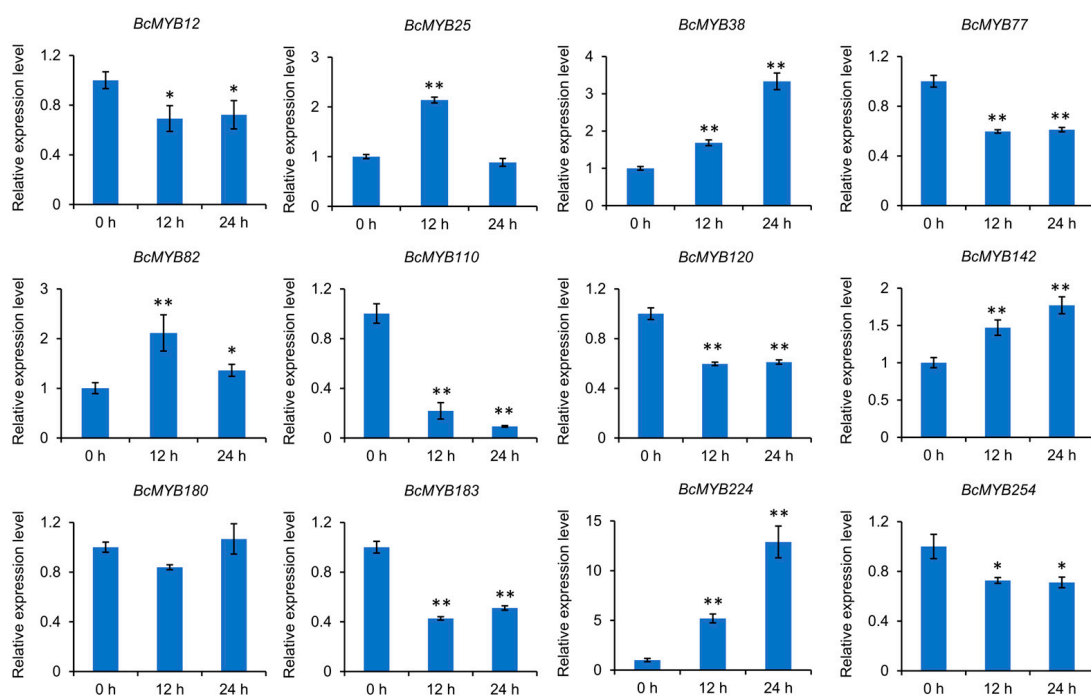


Figure 7. qPCR analysis of the *BcMYB* genes after *Pcc* infection. The expression levels of the *BcMYB* genes in response to *Pcc* infection. Six leaf-stage Chinese flowering cabbage plants were infected for 12 h or 24 h. *BcTUB2* served as an internal control. Error bars represent the standard deviation (SD; $n = 3$; t test: * $p < 0.05$, ** $p < 0.01$).

4. Discussion

MYB genes are widely distributed among higher plants and comprise one of the largest groups of transcription factors in the plant kingdom. As the most abundant TF family in plants, the MYB TF family participates in a variety of biological activities, including the response to pathogen infection. Although the roles of some plant MYB genes in response to pathogen infection have been revealed, there is still limited research on MYB genes, which are abundant in the plant kingdom. The systematic analysis of MYB TF genes in response to pathogen infection on a genome-wide scale is an effective approach for identifying potential genes related to disease resistance.

With the complete sequencing of genomes of more species, numerous R2R3-MYB genes have been identified. In this study, we conducted a comprehensive genome-wide analysis of the R2R3-MYB gene superfamily in Chinese flowering cabbage. A total of 255 R2R3-MYB genes were identified in Chinese flowering cabbage, whose number was markedly greater than that in *Arabidopsis* and equivalent to that in *Brassica rapa* ssp. *Pekinensis* [31]. Although the amount of available genome data for *Brassica* plants is much greater than that for *Arabidopsis*, an increasing number of studies have shown that variability in the number of gene family members in plants is not directly related to genome size and might be attributed to the number of gene duplication events during genome evolution [32]. The evolution of a gene family largely depends on the organization of the gene structure. Similar to other plant species [8], the R2R3-MYB gene family in Chinese flowering cabbage exhibits significant variations in its membership properties. The smallest BcMYB protein consists of only 36 amino acids, while the largest BcMYB protein comprises 1566 amino acids. There are also significant differences in the other physicochemical parameters. *Brassica* species have undergone genome triplication events, and the significant differences in the properties of the BcMYBs may indicate their functional divergence (Table S2). Based on phylogenetic research, the R2R3-MYB genes from Chinese flowering cabbage and *Arabidopsis* were divided into 29 subgroups (Figure 1), and each subgroup contained both AtMYBs and BcMYBs. These results indicated that these genes might be derived from a common ancestor and that the R2R3-MYB gene family could also undergo species-specific differentiation after separation. The biological functions of most BcMYB TFs have not been characterized, whereas most BcMYB proteins are clustered with known functions of *Arabidopsis* homologs. In *Arabidopsis*, AtMYB3, AtMYB4, AtMYB7, and AtMYB32, which are members of subgroup S21, have been reported to be involved in modulating phenylpropanoid biosynthesis [33,34], and AtMYB97, AtMYB101, and AtMYB120, which are from subgroup S8, have been confirmed to participate in pollen tube reception [35]. We found that 11 BcMYBs were clustered into subgroup S21 and 13 BcMYBs were clustered into subgroup S8, indicating that these genes might play a role similar to that of their *Arabidopsis* homologs. Like in most plants, each chromosome of Chinese flowering cabbage contains an unequal number of R2R3-MYBs. Although there was a highly similar distribution pattern of R2R3-MYB on the chromosome between *B. rapa* var. *pekinensis* and Chinese flowering cabbage [30], differences could still be found on chromosome A06 (Figure 2). BcMYBs were mainly distributed on both ends of the A06 chromosome; nevertheless, BrMYBs were also distributed on the middle of chromosome A06. Gene duplication has been confirmed to occur during the process of plant evolution, thereby contributing to the establishment of new gene functions [36]. Among these BcMYBs, there were 182 duplicated pairs but only 2 tandemly duplicated gene pairs, indicating the diversification of their functions (Figure 3; Table S3).

Several studies have functionally characterized the role of MYB TFs in biotic stress and the immune responses of model plants to agriculturally important insect pests [20]. In plants, the transcription of a large number of MYB TFs is induced in response to pathogen attack. For example, the *Arabidopsis* AtBOS1 (AtMYB108) gene is strongly induced by *Botrytis* infection [37], and MdMYB73 is strongly induced in apple fruits and transgenic calli after being inoculated with *Botryosphaeria dothidea* [38]. Currently, there is no information available about the role of BcMYBs in biotic stress, and RNA-Seq is a more robust method

used for revealing global gene expression patterns related to plant immunity in response to pathogen infection over time and for screening valuable genes related to resistance. To investigate the expression characteristics of the *BcMYB* genes in response to *Pcc* infection, we analyzed the transcriptome data. Compared with those of other TF families, the MYB TF family had the greatest proportion of these DEGs, which may be related to the number of their family members. Genes from the same subfamily are likely to share similar functions. In *Arabidopsis*, several MYB TFs, including AtMYB15, AtMYB30, AtMYB44, AtMYB96, and AtMYB108, have been confirmed to be involved in biotic stress [39–44]. Therefore, we focused on the members of the same subgroup (S5, S10, S17 and S29) as these AtMYBs. Among the 38 differentially expressed *BcMYB* genes, 12 *BcMYBs* (*BcMYB12*, *BcMYB25*, *BcMYB38*, *BcMYB77*, *BcMYB82*, *BcMYB110*, *BcMYB120*, *BcMYB142*, *BcMYB180*, *BcMYB183*, *BcMYB224*, and *BcMYB254*) were clustered into the above four subgroups. In response to *Pcc* infection, the expression of the three members of the S10 subgroup (*BcMYB38*, *BcMYB142*, and *BcMYB224*) continuously increased, and the expression of the S17 subgroup members (*BcMYB12*, *BcMYB77*, *BcMYB183*, and *BcMYB254*) decreased. Our results also revealed that the transcriptional levels of other differentially expressed *BcMYB* genes were significantly altered under soft rot stress (Figure 7). In *B. rapa* var. *pekinensis*, *BrMYB232* showed constitutive expression in response to *Pcc* infection, and *BrMYB19* and *BrMYB83*, who had close homologs to AtMYB108, were induced by *Fusarium* infection [45]. In this study, AtMYB108 clustered in subgroup 29, while the close homolog *BcMYB3* did not respond to *Pcc* infection, possibly because the response of MYB TF genes is pathogen-specific. In addition, we found that *BcMYB120* and *BcMYB82*, two other homologs of AtMYB108, exhibited opposite expression patterns. However, it is not clear whether they also differ in their biological functions. In brief, this study is the first to identify and preliminarily analyze R2R3-MYB genes in Chinese flowering cabbage and provides basic information for further investigations.

5. Conclusions

In summary, a total of 255 R2R3-MYB genes were identified from Chinese flowering cabbage in this study. Comprehensive bioinformatics analysis was performed to investigate phylogenetic relationships, chromosomal localization, and collinearity. RNA-seq was performed to investigate the dynamic expression patterns of *BcMYBs* in response to *Pcc* infection, revealing both similarities and differences in transcription among family members. Overall, this study provides preliminary evidence that *BcMYBs* may play important roles in soft rot stress responses in Chinese flowering cabbage. We selected these 12 *BcMYBs* as candidate genes for further functional studies.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae10040325/s1>. Table S1: Primers used in this study; Table S2: Properties of R2R3-MYB proteins in Chinese flowering cabbage; Table S3: Duplicated gene pairs of the R2R3-MYB gene family in Chinese flowering cabbage; Figure S1: Phenotype of Chinese flowering cabbage infected with soft rot disease.

Author Contributions: B.C. and H.Z. designed the research; S.L., X.Z., G.L. and D.J. performed the gene family analysis; S.L. and F.Y. performed the qPCR; Y.Z. analyzed the data; S.L. and B.C. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors have no conflicts of interest to declare.

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