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Genome-wide identification of the *PYL* gene family and expression of *PYL* genes under abiotic stresses in Chinese cabbage

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Abstract

The family of pyrabactin resistance 1 (PYR1)/PYR1-like (PYL) regulatory components of ABA receptors (RCAR) play a vital role in the initial step of ABA signaling. To understand the expression mode of *PYL* genes in response to various abiotic stresses in Chinese cabbage (*Brassica rapa ssp. pekinensis*), the members of *BrPYL* gene family were first identified with the relevant bioinformatics software. And then, the relative expressions of identified *BrPYLs* after heat, cold, drought, and salt treatments for 0, 24, 48, and 72 h were determined *via* real-time quantitative PCR. Here, we identified 24 *PYLs* in the *B. rapa* genome. Based on the phylogenetic analysis, these *BrPYL* genes were divided into three classes and distributed on ten chromosomes in Chinese cabbage. Most of *BrPYL* genes in the same group have similar gene structures and intron numbers. There were seven genes (*BrPYL5*, *BrPYL8*, *BrPYL22*, *BrPYL3*, *BrPYL18*, *BrPYL11*, and *BrPYL21*) from Group A with two introns and one gene (*BrPYL19*) from Group D with one intron. Analysis of conserved motifs suggested that every group contained motif 2 containing the Polyketide_cyc2 domain. Subsequently, the prediction of *cis*-acting elements indicated that *BrPYL* genes had 5 stress-related elements and 5 hormone-related elements, among which the number of *MYC* (dehydration reaction) was the highest, suggesting that *BrPYL* genes could respond to hormones and abiotic stresses. Expression patterns under four abiotic stresses showed that the expressions of *BrPYL4*, *BrPYL11*, *BrPYL21*, and *BrPYL23* responded to these stresses at different time points. To conclude, we identified the *BrPYL* genes and build the *BrPYLs* expression mode in response to various abiotic stresses. This study provides a theoretical basis for stress-resistance breeding of Chinese cabbage.

Keywords: abiotic stresses, *Brassica rapa* ssp. *pekinensis*, bioinformatics, gene expression pattern, *PYR/PYLs* gene family.

Introduction

Abscisic acid (ABA), as one of the five most important plant hormones, regulates the growth and development of plants, which is mainly reflected in the resistance to the different types of biotic and abiotic stresses, the seed maturation and germination, as well as cell metabolism of plants (Dalal *et al*. 2009, Lee and Luan 2011, Di *et al*. 2018). The synthesis of ABA improves the tolerance to different abiotic stresses in plants (Bai *et al*. 2019, Zhang *et al*. 2019). Lee and Luan (2011) have reported that plants would produce and accumulate more ABA to induce stomatal closure, reduce transpiration rate and water loss when subjected to drought conditions. In addition, related studies have shown that in transgenic *Arabidopsis* overexpressing *AtNCED3* (9-*cis*-epoxycarotenoid dioxygenase, a key enzyme involved in ABA biosynthesis pathway) results in higher ABA accumulation and decrease in transpiration rate under drought stress (Schwartz *et al*. 2003, Iuchi *et al*. 2010, Thompson *et al*. 2010). In rice,

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Abbreviations: NCED - 9-*cis*-epoxycarotenoid dioxygenase; PP2C - the type 2C protein phosphatases; PYR/PYL - the pyrabactin resistance 1 (PYR1)/PYR1-like (PYL)/regulatory components; SnRK2 - the sucrose nonfermenting 1-related protein kinases subfamily 2.
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OsbZIP23 positively regulates *OsNCED4* by binding to the promoter of *OsNCED4*, overexpression of *OsbZIP23* results in *OsNCED4* up-regulation and increase of ABA content (Wei *et al*. 2016). It has been reported that ABA signaling pathway is composed of three core components, which are the pyrabactin resistance (PYR)/PYR1-like (PYL) regulatory components of ABA receptor (RCAR, considered as PYLs for short), the sucrose nonfermenting 1-related protein kinases subfamily 2 (SnRK2), and the type 2C protein phosphatases (PP2C) (Zhang *et al*. 2017). ABA signaling pathways activate downstream kinases through receptors, and in the absence of ABA, kinases are inhibited by phosphatase. SnRK2 is in an autophosphorylation activity conditions while PP2C binds SnRK2 to maintain SnRK2 in an inactive state, making dephosphorylation of SnRK2. However, ABA can bind PYR1/PYLs to prevent the interaction between PP2C and SnRK2, making SnRK2 in autophosphorylation state. This activates ABA responsive gene expression by inducing downstream transcription factors (Soon *et al*. 2012). ABA plays a key role in regulating plant growth and development by inducing positive regulators. *SnRK2.2* and *SnRK2.3*, the homologous genes of *OST1* (open stomata), are activated by ABA to regulate ABA responses in seed germination, root growth, and gene expression (Nishimura *et al*. 2010).

The PYLs are important receptor proteins in ABA signal transduction pathway in plants. They can activate transcription factors downstream of signaling pathway to regulate the expression of related genes, and then enhance resistance to stresses. It has been reported that there were 14 *PYLs* in the *Arabidopsis thaliana* genomes (Bai *et al*. 2019). Simultaneously, the homologous genes were determined in other plant genomes, containing 14 *PYLs* in tomato (González-Guzmán *et al*. 2014), 12 *PYLs* in rice (Tian *et al*. 2015, Hou *et al*. 2020), 46 *PYLs* in *Brassica napus* (Di *et al*. 2018), 29 *PYLs* in tobacco (Bai *et al*. 2019), and 14 *PYLs* in rubber tree (Guo *et al*. 2017). It has been reported that when *OsPYL9* was overexpressed in transgenic *Arabidopsis* and rice plants, the tolerance to drought stress was enhanced significantly and leaf senescence was delayed (Zhao *et al*. 2016). The relative expressions of 14 *BnPYLs* in *B. napus* were determined by RT-qPCR under drought, heat, and salinity treatments, respectively. Among them, *BnPYL1-2*, *BnPYL1-3*, and *BnPYL7-2* responded significantly to abiotic stresses (Di *et al*. 2018). *PYLs* contributed to the transcription regulation of tomato fruit division and enlargement and resistance to drought stress (González-Guzmán *et al*. 2014). It was found that quinabactin (QB) is an ABA agonist, which has similar functions to ABA. In tomato, at least three *SlPYLs* (Sl8g076960, Sl6g061180, and Sl6g050500) were sensitive to QB, which inhibited tomato seed germination. In contrast, Sl3g007310 and Sl12g055990 had no sensitivity to 10 μM QB. These results demonstrate that QB can selectively combine with *SlPYLs*. Notably, tomato seed germination needs QB insensitive ABA receptors. Moreover, overexpression of *Sl3g007310* and *Sl6g050500* can enhance drought stress tolerance in *Arabidopsis* (González-Guzmán *et al*. 2014). It has been showed that in *Arabidopsis*, loss of the *pyr*/*pyl* in quintuple mutant 11458 (*PYL2* present, *PYR1* and *PYL1*, *PYL4*, *PYL5* and *PYL8* are knocked out) could enhance transpiration and lead to water loss, suggesting *PYL2* is involved in ABA-induced guard cell responses. However, *PYL4* and *PYL5* are essential for the responses to CO_2. The expression of *PYL4* or *PYL5* in guard cells contribute to increasing CO_2 sensitivity to render stomatal closure (Dittrich *et al*. 2019). Subjected to the biotic stress, plants also can accumulate more ABA to adjust plant physiological and developmental processes, which are conductive to decrease pathogen attacts (Lim *et al*. 2015). Some studies reported that ABA-induced stomatal closure prevents the pathogen from entering the ectoplasm (Melotto *et al*. 2006, Cho *et al*. 2008, Lee and Luan 2011). For instance, in the process of *Pseudomonas syringae* pv. *tomato (Pst)* DC3000 inoculation and PAMP (the pathogen-associated molecular pattern) treatment, overexpression of *RCAR3*, *RCAR4*, and *RCAR5* (ABA receptors) can maintain stomatal closure, thus improve tolerance to *Pst* DC3000 (Lim *et al*. 2015). Therefore, it is necessary to identify and analyze *PYLs* in plants to understand their functions. To date, some research on *PYLs* have been reported in many plant species. Chen *et al*. (2017) identified the members of *PYL* gene family in *A. thaliana* and analyzed the expression patterns of *AtPYL* genes under drought stress. Di *et al*. (2018) reported the *BnPYL* members in *Brassica napus* and the response mode of *BnPYLs* under drought, salinity, and heat stresses. Besides, it has been reported that the *AtPYL1* closely clustered with *BrPYL1* and overexpression of *BrPYL1* promoted the seed germination of *Arabidopsis* under ABA application (Li *et al*. 2017). Hou *et al*. (2020) identified 13 *MdPYLs* in *Malus domestica* and the results of yeast two-hybrid assays verified that MdPYLs had an interaction with two 2C protein phosphatases (MdPP2C65 and MdPP2C72). However, there is little information on *PYLs* in Chinese cabbage and the expression modes of *BrPYLs* in response to different abiotic stresses still have not been reported.

Chinese cabbage (*Brassica rapa* ssp. *pekinensis*, 2n = 20), belongs to *Brassicaceae* family (Sun *et al*. 2010). One of the most important leaf vegetables around the world (Duan *et al*. 2015), has undergone multitude cultivation and selection, mainly to provide vitamins and crude fiber for human beings (Huang *et al*. 2015). Its water content is up to 90 - 95 %. Better adaptability to growth environment and high yield make *B. rape* playing an important role in the domestic vegetable supply. Nevertheless, with the change and deterioration of growth environment, Chinese cabbage are subjected to different degrees of abiotic stresses, decreasing quality and yield (Ma *et al*. 2015). Hence, in this study, the identification and analysis of *BrPYLs* in Chinese cabbage has been carried out. And then the expression pattern of identified 24 *BrPYLs* response to four abiotic stresses (heat, cold, salinity, and drought) was performed with real-time quantitative PCR. Our study will lay foundation for the verification of candidate *PYL* genes and provide a theoretical basis for stress-resistance breeding in Chinese cabbage.

Materials and methods

Identification of the *BrPYLs* and chromosome distribution in *B. rapa* genome: We used the protein sequences of 14 *AtPYLs* downloaded from the *TAIR* database (https://www.arabidopsis.org/) (Lamesch *et al.* 2012) to search annotation sequences with the *BLASTP* tool with E-value < $1e^{-10}$ (the default value) in the *Brassica* database (http://brassicadb.org) (Wang *et al.* 2011). The results of the combined searches, and all non-redundant sequences were subjected to the *SMART* database (http://smart.embl-heidelberg.de/) for domain analysis. The sequences were only accepted if they simultaneously contained the conserved Polyketide_cyc2 domain as putative *BrPYL* gene models. The location of the *BrPYL* genes on 10 chromosomes was identified from the *Brassica* genome database and the distribution of *BrPYL* genes was drawn by *MapChart* (Voorrips 2002).

We used the online website (https://web.expasy.org/compute_pi/) to obtain the physical and chemical properties of *BrPYL* gene family. The subcellular localization of *BrPYLs* was forecasted with the *WoLF PSORT* (http://www.genscript.com/wolf-psort.html) (Xiong *et al.* 2016).

Phylogenetic analysis and gene structure of *BrPYLs*: To have a knowledge of the evolutionary relationship between *BrPYLs* and *PYLs* from other plants species, the protein sequences of 24 PYLs from *Brassica rapa* (Br), 14 from *Arabidopsis thaliana* (At), 12 from *Oryza sativa* (Os), and 46 from *Brassica napus* (Bn), were aligned by using *Clustalx* software (Chenna *et al.* 2003). We applied the software *MEGA7.0* to build phylogenetic tree, and the setting condition was the neighbor-joining (NJ) method with 1 000 bootstrap replicates (Saitou and Nei 1987).

The gene structures of the *BrPYL* genes were generated using *GSDS* (http://gsds.cbi.pku.edu.cn/) (Hu *et al.* 2015) by uploading the CDS sequences with the corresponding genomic DNA sequences from the genome database of 24 *BrPYLs* that were retrieved from the *Brassica* database (http://brassicadb.org).

Conserved motifs of *BrPYL* genes: The conserved motifs in the PYL proteins were identified with *MEME v. 5.1.1* (http://meme-suite.org/tools/meme) with the following parameter settings: the maximum number of motifs is 10, and the remaining parameters are the default values.

To predict the diverse *cis*-acting elements in promoters of the *BrPYL* genes, the 2 000 bp upstream of initiation codon (ATG) genomic sequences of *BrPYLs* were collected from the *Brassica rapa* genome in the *Brassica* database (http://brassicadb.org) and then 2 000 bp upstream of ATG was estimated using *PlantCARE* software (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot *et al.* 2002) to analyze for *cis*-acting elements.

Plants and treatments: The germinated seeds (*Brassica rapa* ssp. *pekinensis* (Lour) Hanelt, cv. Furui) were sowed in the plug filled with substrate (*Gansu Lvneng Ruiqi Biotechnology Co*., Wuwei) and the seedlings were grown under a 12-h photoperiod, an irradiance of 250 µmol m^{-2} s^{-1}, relative humidity of 80 %, and day/night temperatures of 25/18 °C. When two-leaf stage was reached, the neat seedlings were exposed to cold stress 10/5 °C (day/night), heat stress 35/20 °C (day/night), 2 % NaCl solution (salt stress), and drought stress (withholding irrigation) for 0, 24, 48, and 72 h, respectively. The leaf samples were collected at 0, 24, 48, and 72 h. All the collected samples were immediately frozen in liquid nitrogen and stored at -80 °C until use. Three biological repeats were performed.

Real-time quantitative PCR: Total RNA was isolated from collected samples using a *Plant RNA Extraction* kit (*Takara*, Tokyo, Japan) and RNA concentration was measured with micro spectrophotometer (*P200*, *Pultton Technology*, USA). The cDNA was synthesized by using a *PrimeScript*^{TM} RT reagent kit (*Takara*). The primers were synthesized using CDS sequences by *Shanghai Biology Company* (Shangai, China; Table 1 Suppl.). *BrACTIN* was used as an internal reference. The real-time qPCR was performed using *ABI 7500*), *Thermo Fisher*, Massachusetts, USA). The volume of reaction system was 20 mm^3, which contained 2 mm^3 of cDNA solution, 10 mm^3 of *2*SuperReal PreMix Plus* from *SYBR Green* kit (*Tiangen*, Shangai, China), 0.6 mm^3 of 10 µM forward and reverse primers, 0.4 mm^3 *50*ROX Reference Dye*^{A}, and 6.4 mm^3 of distilled deionized water. The amplification program conditions were as follows: 95 °C for 15 min, and 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Three replications were performed for each sample. We computed the relative expressions of the *BrPYL* genes with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). *SPSS 20.0* was used to analyze the significance of relative expressions and *Excel* was used to complete the histograms of relative expressions under each treatment.

Results

To identify the members of *BrPYL* gene family, the related sequences, such as genomic, CDS, and protein sequences, were obtained from the *Brassica* database (http://brassicadb.org). As shown in Fig. 1, twenty-four full-length genes coding ABA receptors (*PYLs*) were identified in the Chinese cabbage genome, which were located onto 10 chromosomes. All the *PYL* genes are assigned specific names (*BrPYR1*, *BrPYL1* to *BrPYL23*) according to the order of their positions on chromosomes (Fig. 1). Distribution of genes on chromosomes has shown that the numbers of genes located on chromosome 3 were the highest with six genes (*BrPYL7-11*) and *BrPYR1*, while *BrPYL18* and *BrPYL19* were mapped on chromosome 7 and chromosome 8, respectively.

As it is shown in Table 2 Suppl., according to the sequence analysis of these *BrPYL* genes, the length changed between 450 bp (*BrPYL*15) and 1 536 bp (*BrPYL*8) and the size of protein varied from 149 (BrPYL15) to 212 (BrPYL10 and BrPYL14) amino acids in length. In addition, the minimum molecular mass of the BrPYL proteins was 16 174.3 Da (BrPYL15), the maximum molecular mass of the BrPYL proteins was 23 885.7 Da

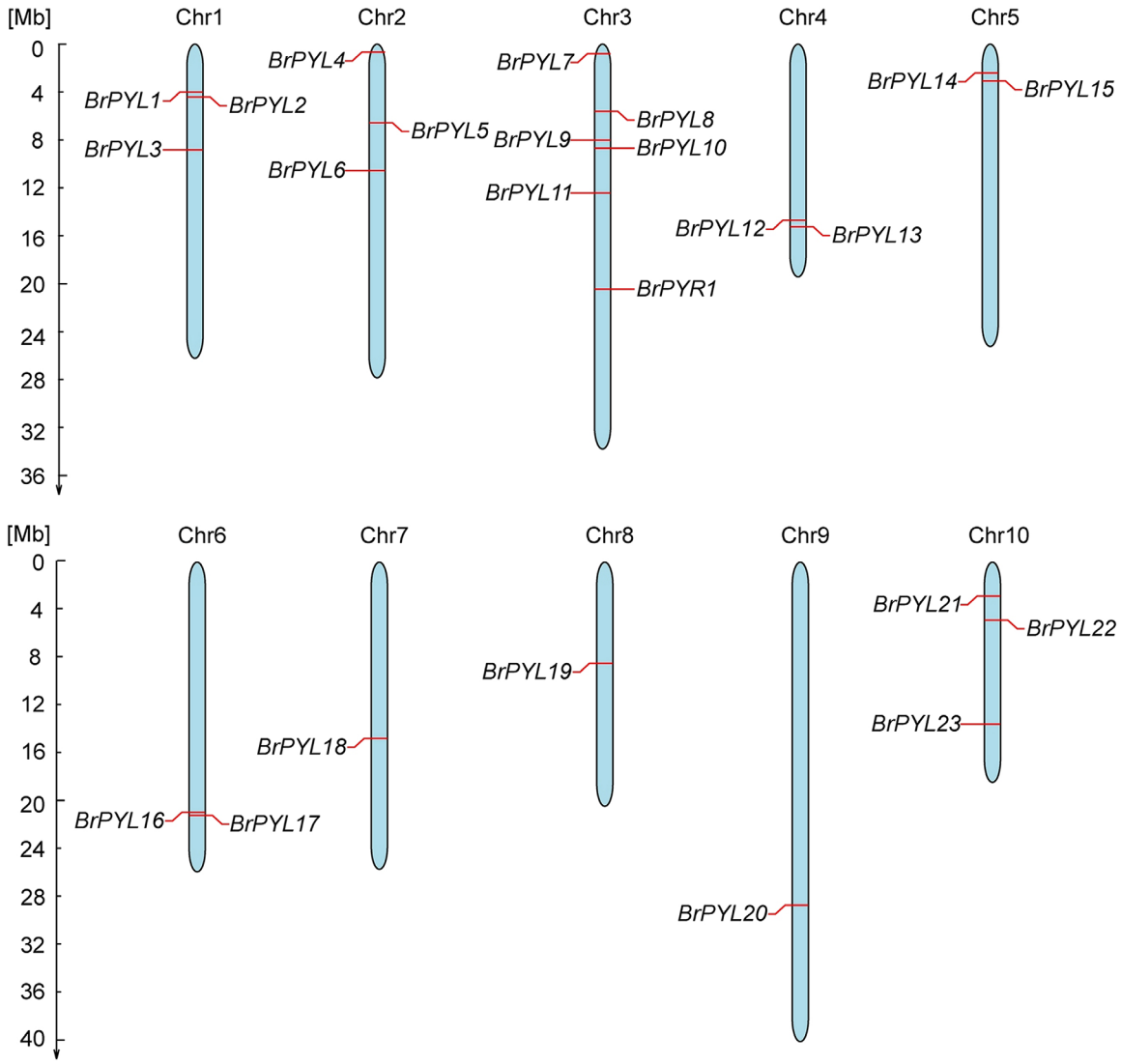

Fig. 1. Distribution of the *BrPYL* genes on 10 chromosomes in Chinese cabbage according to *Mapchart* software. The numbers of chromosomes are depicted at the top of each chromosome.

(BrPYL16). The pI ranged from 5.0 (BrPYL2, BrPYL16, BrPYL17, BrPYL19 and BrPYL20) to 9.0 (BrPYL6 and BrPYL15), which indicates most BrPYLs are acidic proteins (Table 2 Suppl.). Subcellular localization analysis indicated that *BrPYLs* were divided into five categories according to their location in the cell (Table 2 Suppl.), including 13 *BrPYLs* (*BrPYR1*, *BrPYL1*, *-3*, *-4*, *-5*, *-7*, *-8*, *-9*, *-11*, *-12*, *-21*, *-22,* and *-23*) located in the cytoplasm, 4 *BrPYLs* (*BrPYL2*, *-13*, *-16,* and *-20*) in nucleus, 4 *BrPYLs* (*BrPYL6*, *-10*, *-15,* and *-19*) in chloroplast, while *BrPYL14* and *BrPYL18* were located in mitochondria and cytoskeleton, respectively. Among the genes, the instability coefficient of 80 % of the proteins is greater than 40, indicating that their structures are unstable. The average hydrophobic index of all proteins was negative, which was predicted to be hydrophilic proteins.

To study the evolutionary relationship of *BrPYL*, a phylogenetic tree from 4 plant species containing *B. rapa* (Br), *A. thaliana* (At), *O. sativa* (Os), and *B. napus* (Bn) was built by using *MEGA7.0* software. Protein sequence alignment showed that *BrPYL*s were divided into three families named Group I, Group II, and Group III. As it is shown in the Fig. 2*A*, 14 *AtPYLs*, 12 *OsPYLs*, 24 *BrPYLs*, and 46 *BnPYL*s were clustered into three groups, among which Group II and Group I had the largest number (33) of members. Group III is the least with 30 members. Interestingly, according to the sequence alignment results, *BrPYL4* and *BnPYL5-5* from Group I had a highly homologous sequence, as well as *BrPYL16*, *BnPYL1-1*, and *BnPYL1-2*. In the Group II, the evolutionary relationship among *BrPYL10* and *BnPYL6-2*, *BrPYL17,* and *BnPYL11* were also close. Furthermore, *BrPYL22*, *BnPYL8-5*, and *BnPYL8-6* from Group III had a high homology, so do *BrPYL3*, *BnPYL10-1*, and *BnPYL10-2* (Fig. 2*A*). From these results we inferred that *BrPYLs* and *BnPYLs* may have the same ancestors. Related research reported that *Brassica napus* is a species formed by natural hybridization of *Brassica rapa* and *Brassica oleracea* for

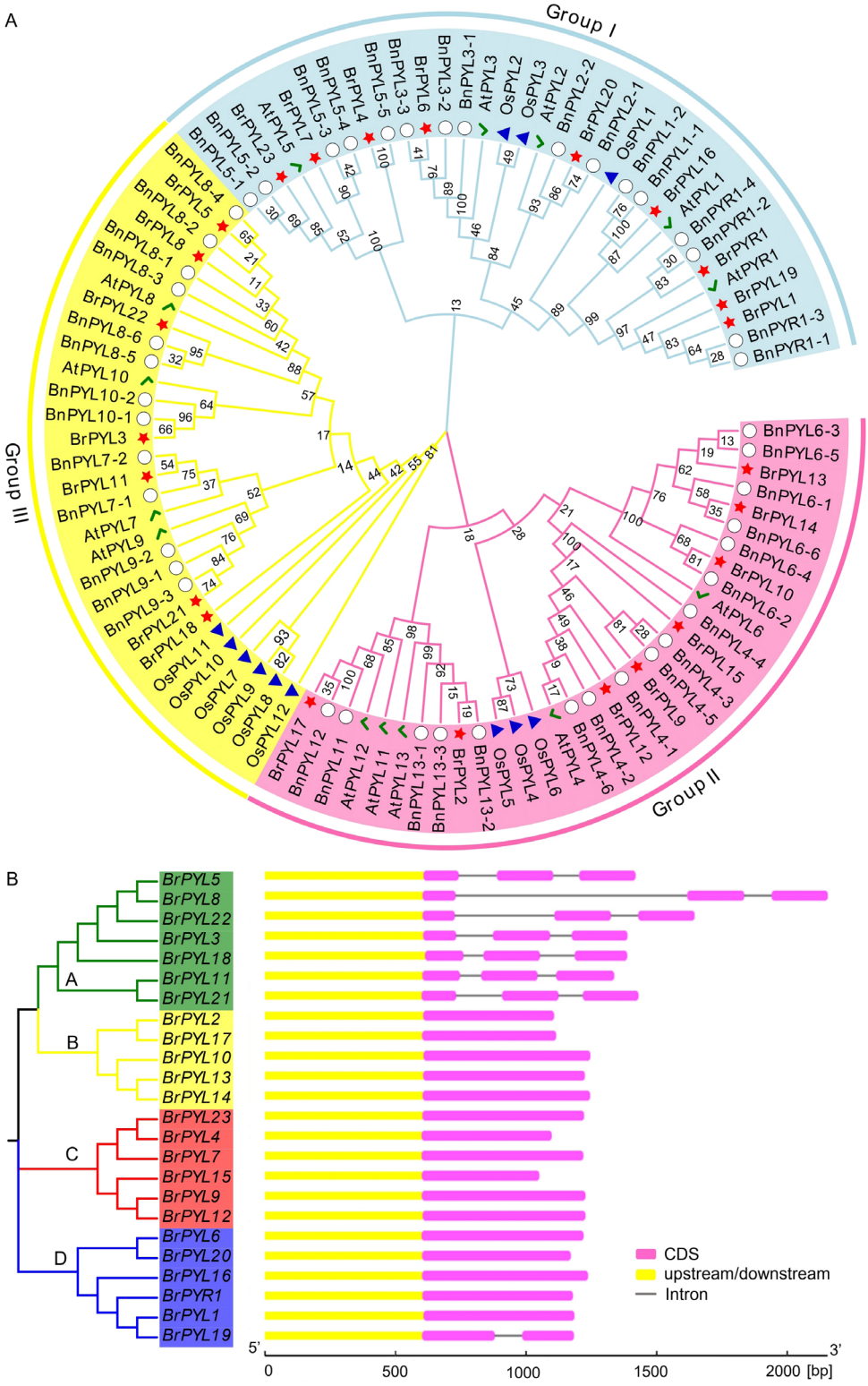

Fig. 2. *A* - Phylogenetic tree of the *PYL* genes from *B. rapa* (Br), *A. thaliana* (At), *O. sativa* (Os), and *B. napus* (Bn). In total, 14 *AtPYLs*, 12 *OsPYLs*, 24 *BrPYLs*, and 46 *BnPYLs* were included. According to the clustering of the PYL proteins, the tree was divided into three groups. The numbers of every branch represent genetic relationships among these species (the larger the number, the closer the relationship). *Red stars* represent *BrPYLs*, *green checks AtPYLs*, *blue triangles OsPYLs*, and *white circles BnPYLs*. *B* - Gene structure of the *PYL* genes in Chinese cabbage according to their phylogenetic relationships marked with different colors. The lengths and positions of introns are shown on the figure. The *pink boxes* and *gray lines* denote CDS and introns, respectively. CDS - coding sequences; bp - base pairs. A, B, C and D represent different groups of Chinese cabbage.

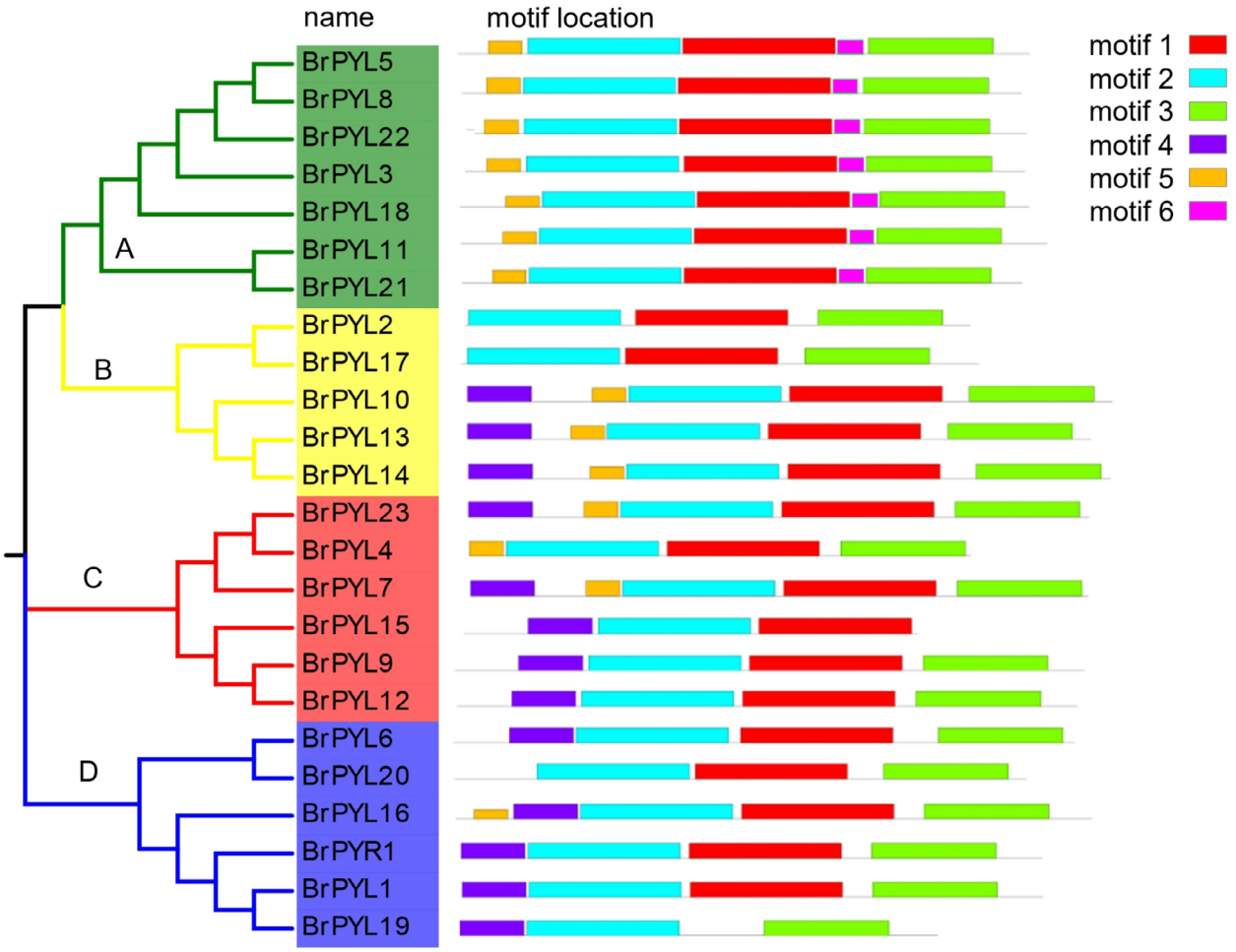

Fig. 3. The conserved motifs of the BrPYL proteins. These motifs were identified using *Multiple EM* for motif elicitation (*MEME*). Boxes of different colors correspond to different motif. A, B, C, and D represent different groups of Chinese cabbage.

thousands of years (Di *et al*. 2018).

To further comprehend the similarities and differences in gene structure of the *BrPYL* genes, we used server *GSDS* to analyze the exon/intron organizations of *BrPYLs* from the same group. Interestingly, in terms of gene structure, all *BrPYLs* had upstream, but no downstream, exhibiting highly conservative structure (Fig. 2*B*). Moreover, introns provide binding sites for binding proteins and may affect the structures and functions of genes. There was relevant evidence indicating that intron retention plays an important role in regulating gene expression under stresses (Fiume *et al*. 2004, Nogales *et al*. 2018). *BrPYL3*, *-5*, *-8*, *-11*, *-18*, *-21*, *-22* from Group A embraced 2 introns, and *BrPYL19* from Group C contained 1 intron, while other genes no introns (Fig. 2*B*). These results demonstrated that *BrPYLs* in the same group have similar gene structure.

Diverse motifs of PYL proteins were investigated by *MEME* program in which 6 motifs ranging from 6 to 50 amino acids in length are identified. As shown in Fig. 3, motif 6 only existed in BrPYL*5*, -8, -11, -18, -22, -23 from Group A. In addition, BrPYL proteins from Group A have the same motif, suggesting that they were conservative in evolution. BrPYL10, -13 and -14 from Group B own motif 4 and motif 5. Except for BrPYL19 without motif 1, BrPYL15 without motif 3, all other proteins with motif 1 and motif 3 were recognized. Interestingly, all BrPYLs contain motif 2 (Fig. 3), and the Polyketide_cyc2 domain, which is a unique functional domain of PYL protein motifs 1, 2, 3, 6. These results suggested that the structures of BrPYLs in one group were highly conservative, whereas the structures among groups were diverse.

As shown in Fig. 4*A*, *BrPYL* genes responded to different abiotic stresses. They had cold stress response element (LTR), drought-inducibility (MBS), dehydration reaction (MYC), defense and stress responsiveness (TC-rich), antioxidant response element (ARE), *etc*. In addition, there were five hormone-related elements involving abscisic acid-responsive element (ABRE), salicylic acid-responsive element (TCA-element), gibberellin-responsive element (GARE-motif), and MeJA-responsive element (CGTCA-motif and TGACG-motif). To conclude, the numbers of the LTR, MBS, MYC, and ARE were the highest in the Group A (Fig. 4*B*), including 10, 11, 23, and 17, respectively. The largest numbers of TC-rich (6) were

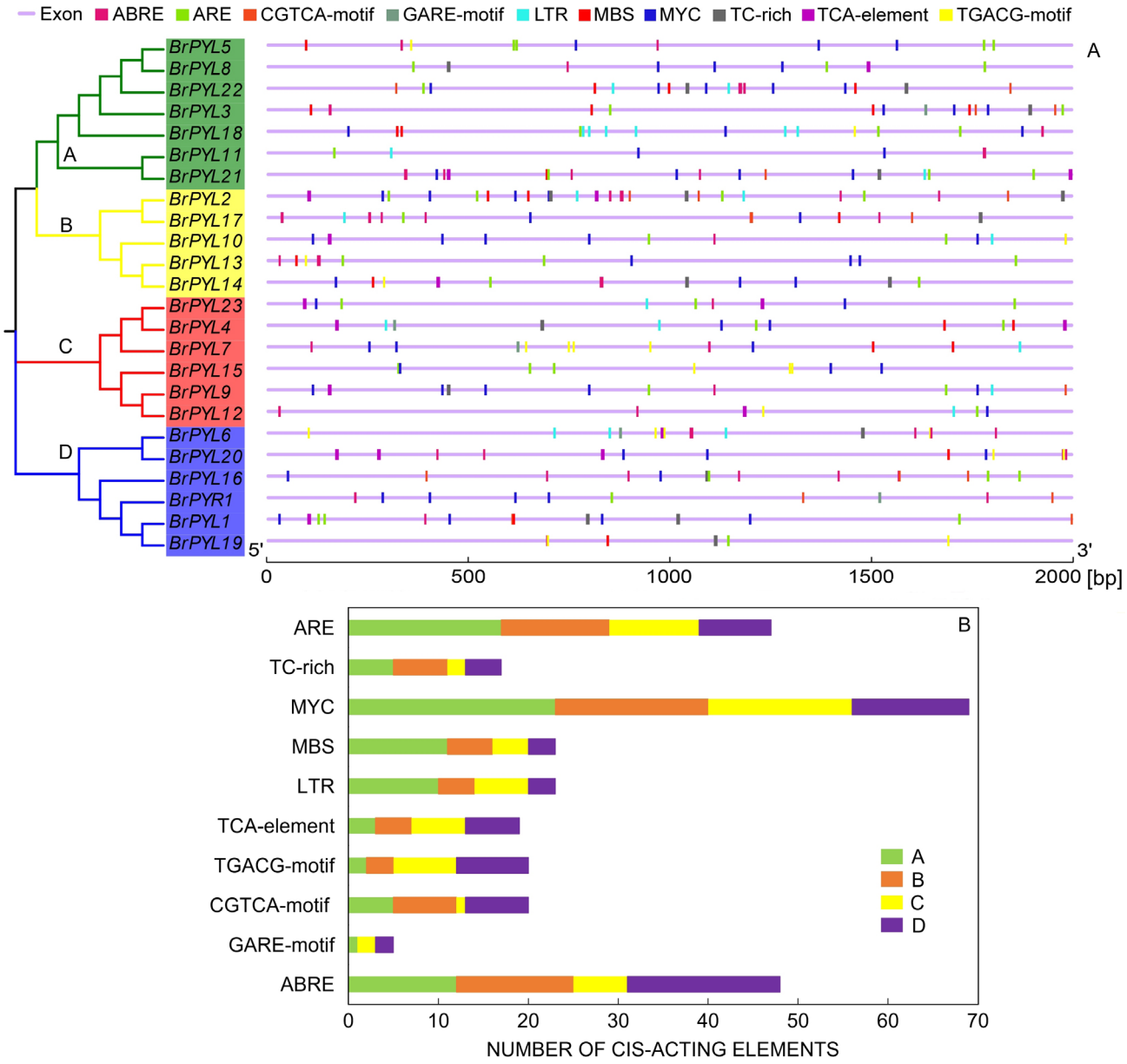

Fig. 4. Analysis on *cis*-acting elements of *BrPYL* gene family in Chinese cabbage. *A* - position of *cis*-acting elements of *BrPYLs*. *B* - numbers of *cis*-acting elements from four groups in Chinese cabbage. ABRE is abscisic acid response element; ARE is antioxidant response element; CGTCA-motif and TGACG-motif are methyl jasmonate response elements; GARE-motif is gibberellin response element; LTR is cold stress response element; MBS is involved in drought-inducibility; MYC is involved in dehydration reaction; TC-rich is involved in defense and stress responsiveness; TCA-motif is salicylic acid response element.

in Group B (Fig. 4*B*). However, there were no GARE-motif in the Group B. Additionally, the number of the GARE-motif and TCA-element from Group C was the same as that from Group D (Fig. 4*B*). 17 ABREs were recognized in the Group D. It is worth noting that in all *cis*-acting elements, the number of MYC was the highest. Hence, the above results indicated that transcriptional regulation in *BrPYL* genes might be mainly related to abiotic stresses.

As a core regulatory component of ABA signaling pathway, PYR/PYL can regulate relevant genes expression to protect plants under abiotic stresses (Hou *et al*. 2020). To further understand expression patterns of *BrPYL* genes under abiotic stresses, real-time qPCR was used to analyze the relative expressions of 24-identified *BrPYLs* after heat, cold, salt, and drought stresses for 0, 24, 48, and 72 h. As it is shown in the Fig. 1*A* Suppl., under heat stress, *BrPYL1* was up-regulated at 24, 48, and 72 h, while there was no significant difference between expressions of *BrPYL1* at each time point. The expressions of *BrPYL4*, *-10*, and *-14* were up-regulated dramatically at 24 h, among which *BrPYL10* and *BrPYL14* were over twofold higher than in CK (0 h). The expressions of *BrPYL8* and *BrPYL21* increased significantly at 48 h. In addition, *BrPYL8*, *-11*, *-14*, *-23* significantly increased at 72 h under heat treatment.

The expression of *BrPYL14* from Group B was over six times higher than that of CK (0 h) at 72 h. However, the expressions of *BrPYL2*, *-6*, and *-9* were down-regulated significantly at 48 h. *BrPYL3, BrPYL15, BrPYL17*, and *BrPYL21* were down-regulated significantly at 24 and 72 h. *BrPYL4, BrPYL12, BrPYL18*, and *BrPYL19* were down-regulated significantly at 48 and 72 h. Besides, *BrPYL7* and *BrPYL23* from Group C were down-regulated after heat treatment at 24 and 48 h. The expressions of *BrPYL5* and *BrPYL22* from Group A significantly decreased, as time went on.

As shown in Fig. 1*B* Suppl., *BrPYR1*, *-7*, *-9*, *-11*, and *-23* had similar expression patterns under cold stress, showing to be up-regulated at all the time points. The expressions of three genes (*BrPYL4*, *-11*, and *-18*) related to low temperature element (LTR) increased at 24 h, among which the expression of *BrPYL11* was five-fold higher than in CK (0 h) (Fig. 1*B* Suppl.). The transcriptions of *BrPYL3*, *-17*, and *-21* related to low temperature element (LTR) were up-regulated significantly at 48 h, which was significantly higher than in CK (0 h). *BrPYR1*, *BrPYL1*, *-7*, *-8*, *-9*, *-11*, and *-23* were significantly up-regulated at 72 h under cold treatment. However, the expressions of *BrPYL2*, *-3*, *-8*, *-10*, *-13*, *-14*, *-16*, *-17*, *-20*, and *-22* were dramatically down-regulated at 24 h under cold treatment (Fig. 1*B* Suppl.). However, with the increase of chilling stress time, the expression of *BrPYL22* decreased significantly.

Under drought stress (Fig. 1*C* Suppl.), five genes (*BrPYL2*, *-4*, *-6*, *-7,* and *-23*) had peak values at 24 h. *BrPYL7* was 15 times higher than in CK (0 h), indicating *BrPYL7* may significantly respond to drought stress. Six genes (*BrPYL9*, *-10*, *-13*, *-14*, *-15*, and *-21*) increased significantly at 48 h. *BrPYL9* and *BrPYL10*, *BrPYL14* and *BrPYL15* had similar expression trend: up-regulated significantly at 72 h. Besides, the expressions of two genes (*BrPYL7* and *BrPYL23*) were higher markedly than other genes at 72 h. Nevertheless, the expressions of *BrPYR1*, *BrPYL3*, *BrPYL20*, and *BrPYL21* decreased after drought treatment for 24 h, but increased significantly after 48 h, which indicates that these genes may response to drought stress in different periods of time.

As is depicted in the Fig. 1*D* Suppl. 2, among 24 genes, *BrPYL2*, *BrPYL4*, *BrPYL6*, *BrPYL17* and *BrPYL18*, having similar variation tendency, emerged peaks at 24 h, while they were all down-regulated after 24 h of salt stress. The expressions of seven genes (*BrPYL3*, *-13*, *-15*, *-16*, *-20*, and *-21*) were significantly up-regulated at 48 h. The expressions of *BrPYL11* and *BrPYL23* were higher significantly at 72 h than at other time points, among which the expression of *BrPYL11* was over five-fold higher than that of CK (0 h). The transcriptions of two genes (*BrPYL9* and *BrPYL12*) from Group B were down-regulated significantly. Five genes (*BrPYL3*, *BrPYL5*, *BrPYL8*, *BrPYL21*, and *BrPYL22*) from Group A decreased dramatically at 72 h. All *BrPYLs* within Group III were significantly down-regulated at 48 h.

Discussion

Abscisic acid can not only regulate plant growth and development, but also resistance to abiotic and biotic stressess (Lee and Luan 2011, Zhang *et al*. 2019). In our present study, twenty-four *BrPYLs* were identified in Chinese cabbage through bioinformatic analysis, and the expression patterns of *BrPYLs* were analyzed under different abiotic stresses.

Although *PYLs* have been identified in many plants, such as *Arabidopsis* (Zhang *et al.* 2019), tomato (Mou *et al*. 2015), wheat (Gordon *et al*. 2016), maize (Fan *et al*. 2016, Wang *et al*. 2018), and rice (Hou *et al*. 2020), the expression pattern of *PYL* genes to various stresses in *B. rapa* has not been reported. The results from the above research have shown that the number of *PYL* gene family members are different in different species, such as fourteen in tomato (González-Guzmán *et al*. 2014), nine in wheat (Gordon *et al*. 2016), and thirteen in maize (Fan *et al*. 2016, Wang *et al*. 2018). In this study, we have identified 24 *PYL* genes in *B. rapa*, which far outnumbers the 14 *AtPYLs* in *A. thaliana* (Zhang *et al*. 2019), indicating that the genome duplication and lose were likely to happen in the evolution of *B. rapa*. The chromosome distribution analysis of *BrPYLs* showed that Chr03 contained the most genes, in accordance with the *PYLs* distribution in *B. napus*. 46 *BnPYLs* origin from *B. rapa* and *B. oleracea*, of which 38 were located on chromosomes, and most of them were located on A3 and C3 chromosomes (Di *et al*. 2018). As predicted by subcellular localization in Table 2 Suppl., most of the genes (*BrPYR1*, *BrPYL1*, *-3*, *-4*, *-5*, *-7*, *-8*, *-9*, *-11*, *-12*, *-21*, *-22*, and *-23*) were located in the cytoplasm, and *BrPYL2*, *-13*, *-16,* and *-20* were localized in the nucleus. Many studies showed that *AtPYL4* exists in the plasma membrane and nucleus of plant cells, and *AtPYL4* interacted in an ABA independent manner with CAR1 (C2-domain abscisic acid-related proteins) and regulated ABA sensitivity (Rodriguez *et al*. 2014). In addition, *VvPYL4* which is highly homologous to *AtPYL4,* was localized in the cytoplasm and nucleus. *VvPYL4* positively regulated grapevine resistance to *Plasmopara viticola* (Liu *et al*. 2020). The ABA receptor-like gene *VvPYL9* from drought-resistance wild grapevine was also localized in cytoplasm and nucleus. The heterologous expression of *VvPYL9* in *Arabidopsis* enhances ABA sensitivity during seed germination and primary root growth and enhances drought tolerance of *Arabidopsis* plants (Liu *et al*. 2019). Therefore, subcellular localization of genes was the same, but the function is not completely the same. In our study, *BrPYL8* located in the cytoplasm was up-regulated under heat stress and cold stress, which may improve the tolerance of heat stress and cold stress, while *BrPYL13* located in the nucleus was up-regulated under drought and salt stresses, which suggested that *BrPYL13* may respond to salt and cold stresses. Thus, the subcellular localization of genes was different, and their functions may also be different. According to phylogenetic analysis, the 96 *PYL*s from *B. rapa*, *B. napus*, *A. thaliana*, and *O. sativa* were classified into three groups (Fig. 2*A*). Interestingly, we found that *AtPYL6* was homologous to *BnPYL6-2*, *BrPYL10,* and

BnPYL6-4, and *AtPYL8* was homologous to *BrPYL22*, *BnPYL8-6*, and *BnPYL8-5*. Di *et al*. (2018) have reported that *14AtPYLs* had one to six homologs in *B. napus*, among which *AtPYL6* and *AtPYL8* had six homologs in *B. napus*. These results indicated that members of the *BrPYLs* had higher homology to *A. thaliana* and *B. napus* than to *O. sativa*, which demonstrated most of *BrPYLs*, *BnPYLs*, and *AtPYLs* from the same ancestors are orthologous genes. In present study, seven genes (*BrPYL3*, *-5*, *-8*, *-11*, *-18*, *-21*, and *-22*) from Group A had 2 introns while *BrPYL19* from Group C embraced only one intron (Fig. 2*B*). Bai *et al*. (2019) have found that the *NtPYL* subfamily I (*NtPYL1* to *NtPYL18*) have one intron. Most members in the *NtPYL* subfamily II (*NtPYL19* - *27*) have two introns, only *NtPYL22* has one intron. All members in the *NtPYL* subfamily III have no intron, all of which suggested that the gene structures from the same group are highly conservative. Moreover, we speculated that these genes containing introns may have similar functions in defending abiotic stress *via* regulating gene expression.

Our study demonstrated that all proteins possess motif 2 containing Polyketide_cyc2 domain, which suggested *BrPYLs* have a high conservation. This result is consistent with BnPYLs conserved motifs. All BnPYL proteins contain motif 2 (Di *et al*. 2018). Combined with the evolutionary relationship, the results showed that most of BrPYL proteins in the same groups own the similar motifs, such as BrPYL proteins in Group A possess motif 6. Except for BrPYL4 from Group C and BrPYL20 from Group D, all BrPYLs from Group C and Group D contain motif 4 (Fig. 3). Zhang *et al*. (2017) have reported the similar results when identified *PYLs* in *Gossypium*, which showed that motif 8 and 10 emerged in subfamily I of GbPYLs, and motif 2 and 4 existed in subfamily III. *Cis*-acting elements are mainly involved in transcriptional regulation to hormone response, abiotic stress response, and development processes (Nishimura *et al*. 2010, Verma *et al*. 2019, Zhang *et al*. 2019). Some hormone-related elements, such as ABRE, CGTCA-motif, GARE-motif, TCA-motif, and TGACG-motif, exist in plants (Mishra *et al*. 2014, Ding *et al*. 2018, Li *et al*. 2019, Zhang and Li 2019). In this study, we found that the number of MYC is the largest. MYC is related to dehydration and mainly participates in drought stress. Six genes (*BrPYL3*, *-4*, *-11*, *-17*, *-18*, and *-21*) related to low temperature element (LTR) respond to cold stress. Hou *et al*. (2020) have also reported that the expressions of *MdPYL1*, *MdPYL6*, *MdPYL7*, and *MdPYL13* (related to LTR) in apple were gradually up-regulated with the increase of cold stress time. In addition, among the hormone related elements, ABRE was found the most abundant, similarly as in *B. napus* and *Medicago truncatula* (Nishimura *et al*. 2010, Di *et al*. 2018), indicating that ABRE plays an important role as a transcription factor in ABA signal pathway.

PYLs are key regulators of hormone ABA signaling pathway in plants. Under stress, PYLs can improve the tolerance of Chinese cabbage to stress. Thus, the expression patterns of 24 *BrPYLs* were performed after heat, cold, drought, and salt stress. The expression of *BrPYL1*, *-8*, *-10*, and *-14* in *B. rapa* were up-regulated at all time points under heat stress, which was consistent with *BnPYR1-3*, *BnPYL1-2*, *BnPYL3-1*, *BnPYL4-2*, *BnPYL5-4*, *BnPYL6-1*, *BnPYL7-2*, and *BnPYL9-2* (Di *et al*. 2018). When Chinese cabbage was subjected to cold stress, the relative expressions of six genes (Br*PYR1*, *BrPYL7*, *-8*, *-9*, *-11*, and *-22*) were up-regulated at all the time points. The expressions of three genes (*BrPYL4*, *-11*, and *-18*) related to low temperature element (LTR) were up-regulated at 24 h. The transcriptions of two genes (*BrPYL17* and *BrPYL21*) related to LTR were up-regulated significantly at 48 h. The similar results have been reported in apple, four genes (*MdPYL1*, *MdPYL6*, *MdPYL7*, and *MdPYL13*) containing LTR were up-regulated significantly under cold stress (Hou *et al*. 2020). Previous study has showed that overexpression of *OsPYL3* in *Arabidopsis* led to improved cold and drought stress tolerance (Lenka *et al*. 2018). *BrPYL22* with high homology to *OsPYL3* was significantly up-regulated under cold stress and drought stress at 48 h. In addition, the *BrPYL14* was significantly up-regulated under drought stress, in line with the expression of *BnPYL6-1* (Di *et al*. 2018). *BrPYL14* and *BnPYL6-1* have a high homology in phylogenetic tree (Fig. 2*A*), which implies that they might have the similar function in the evolutionary processes. *BrPYL22*, a homologous gene of *AtPYL8*, *BnPYL8-5*, and *BnPYL8-6*, was significantly up-regulated under drought stress, which was like the report in cotton (Chen *et al*. 2017). They have reported that overexpression of *GhPYL*26 homologous to *AtPYL8* can significantly defend drought stress in transgenic *A. thaliana*. In our study, some *BrPYLs* (*BrPYR*1, *BrPYL1*, *BrPYL8*, *BrPYL9*, *BrPYL19*, *BrPYL22*) were significantly down-regulated at different degrees under salt stress, which was consistent with the expression patterns of *BnPYL9-1* and *BnPYL9-2* (Di *et al*. 2018). The expressions of *BrPYL2*, *-3*, *-6*, *-11*, and *21* were up-regulated at diverse time points, suggesting that the response speeds of these genes to stress are different. These results indicated that *BrPYLs* could respond to abiotic stresses and enhance resistance.

To conclude, a total of 24 *PYLs* were identified from *B. rapa*. We analyzed the expressions of 24-identified *BrPYLs* and found that *BrPYL4*, *BrPYL11*, *BrPYL21*, and *BrPYL23* were markedly affected under cold, heat, drought, and salt stresses (Fig. 1 Suppl.), manifesting that these *BrPYLs* can effectively respond to various abiotic stresses. Our results will lay the foundation for further functional characterization of *BrPYLs* and for further research about improving resistance of abiotic stresses in Chinese cabbage.

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