horticulturae

MDPI

Article

Genome-Wide Identification and Expression Profiling of the *NCED* Gene Family in Cold Stress Response of *Prunus mume* Siebold & Zucc.

Ke Chen [1,2,3], Xue Li [1,2,3], Xiaoyu Guo [1,2,3], Lichen Yang [1,2,3], Like Qiu [1,2,3], Weichao Liu [1,2,3], Jia Wang [1,2,3] and Tangchun Zheng [1,2,3,*]

1 Beijing Key Laboratory of Ornamental Plants Germplasm Innovation and Molecular Breeding, National Engineering Research Center for Floriculture, School of Landscape Architecture, Beijing Forestry University, Beijing 100083, China; yanglichen2000@126.com (L.Y.)
2 Beijing Laboratory of Urban and Rural Ecological Environment, Engineering Research Center of Landscape Environment of Ministry of Education, School of Landscape Architecture, Beijing Forestry University, Beijing 100083, China
3 Key Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants of Ministry of Education, School of Landscape Architecture, Beijing Forestry University, Beijing 100083, China
* Correspondence: zhengtangchun@bjfu.edu.cn; Tel.: +86-106-233-6321

Abstract: The 9-cis-epoxy carotenoid dioxygenase (NCED) is an enzyme that is crucial in abscisic acid (ABA) biosynthesis, and its role is vital in plant development and abiotic stress. However, the function of the NCED family in Rosaceae plant species remains unclear. Through genome-wide screening, we identified 10, 10, 11, 12 and 13 *NCED* genes in *Prunus mume*, *Prunus apricot*, *Prunus salicina*, *Prunus persica*, and *Rosa chinensis*, respectively. Phylogenetic analysis showed that these *NCED* genes were divided into six groups. Gene structure analysis showed that the number and size of introns were relatively constant in each subfamily, while the motif composition differed significantly among them. Collinearity analysis revealed a high homology of *NCEDs* in the *Prunus* genus. Promoter cis-acting element analysis showed that eight *PmNCEDs* contained abscisic acid-responsive elements (ABRE). Furthermore, expression profile analysis based on qRT-PCR revealed that *PmNCED3*, *PmNCED8* and *PmNCED9* were up-regulated in response to low temperature stress, suggesting their significant role in the plant's response to cold stress. These findings provide insights into the structure and evolution of *PmNCEDs* and lay the foundation for further studies regarding their function during cold stress.

Keywords: *Prunus mume*; 9-cis-epoxy carotenoid dioxygenase; *NCED* gene; expression pattern; cold stress

Citation: Chen, K.; Li, X.; Guo, X.; Yang, L.; Qiu, L.; Liu, W.; Wang, J.; Zheng, T. Genome-Wide Identification and Expression Profiling of the *NCED* Gene Family in Cold Stress Response of *Prunus mume* Siebold & Zucc. *Horticulturae* **2023**, *9*, 839. https://doi.org/10.3390/horticulturae9070839

Academic Editor: Jiafu Jiang

Received: 25 June 2023
Revised: 20 July 2023
Accepted: 21 July 2023
Published: 23 July 2023

1. Introduction

Japanese apricot (*Prunus mume* Sieb. et Zucc.) is a perennial woody tree of the *Prunus* genus in the family Rosaceae, with attractive, colourful flowers in early spring that have important ornamental and economic value and are loved by people [1]. *P. mume* is widely planted in Asia and is mainly distributed in the south of China [2]. However, due to its limited tolerance to low temperatures, *P. mume* cannot be widely cultivated in all regions, severely limiting its economic and ornamental value [3]. Therefore, breeding for cold tolerant varieties is an important direction for *P. mume* breeding. It is very important to explore the cold-resistant genes and understand the expression patterns of candidate genes under low temperature stress for the breeding of new cold-resistant varieties of *P. mume*.

Under abiotic stress conditions such as low temperature, freezing damage, drought, salt and alkali, plants can adapt to or resist these stresses through self-regulation [4]. Abscisic acid (ABA) is a very important endogenous plant hormone that not only plays an important role in regulating plant growth and development, but also increases ABA content in response to various environmental stresses such as drought, high salinity and cold, which

can improve plant stress resistance under environmental stresses [5,6]. Studies have shown that the content of ABA in plants increases with the decrease in ambient temperature, while the content of ABA decreases with the increase in ambient temperature [7,8]. The concentration of ABA in the buds and roots of *Acer saggharum*, which was domesticated in winter, increased by about 10 times [9]. The ABA content of *Carpobrotus edulis* increased under cold stress [10]. Exogenous application of ABA could improve the frost resistance of *Vitis vinifera* [11]. Therefore, regulating the expression level of key ABA biosynthesis genes is crucial for enhancing plant stress resistance. ABA can be synthesized in plants through two pathways: the indirect pathway (C15 pathway) and the direct pathway (carotenoid pathway). The indirect pathway is the primary route for ABA synthesis in higher plant tissues [12].

9-cisepoxy-carotenoid dioxygenase (NCED) is a rate-limiting enzyme that is important in the synthesis and regulation of ABA through indirect pathways. It can induce the increase of endogenous ABA content and improve plant stress resistance under stress conditions [5,13–15]. The gene encoding NCED was initially isolated from ABA deficient mutants in maize [15,16] and subsequently identified in other plant species such as *A. thaliana* [15], *Solanum lycopersicum* [16], *Glycine max* [17], *V. vinifera* [18] and *Malus pumila* [19]. In *A. thaliana*, the *NCED* gene belongs to a multi-gene family with nine members. When induced by drought stress, *AtNCED3* can control endogenous ABA levels in plants, and overexpression of *AtNCED3* can reduce leaf transpiration rate and drought tolerance of *A. thaliana* [20]. Ectopic expression of *OsNCED5* and *OsNCED3* in *A. thaliana*, not only can enhance tolerance to drought stress and delay seed dormancy time, but also change the morphology of plants and leaves [21]. *CstNCED* gene can be induced by low temperature, drought, sorbitol, salt and exogenous ABA treatment [22]. Under salinity, low temperature and drought stress, *NCED* was closely related to endogenous ABA content in *Crocus sativus* [22]. Under the influence of drought, low temperature and high temperature, the expression of *MpNCED2* in *M. pumila* was significantly up-regulated, while the expression level of *MpNCED1* was affected by low temperature and high temperature, but not by drought [23]. In *Vigna unguiculata*, salt stress specifically induced *VuNCED1* expression, while cold (4 °C) or heat (40 °C) stress did not induce *VuNCED1* expression [24]. Cold stress and the application of exogenous ABA induced *CkNCED1* expression and ABA accumulation in *Caragana korshinskii* [25]. Furthermore, transgenic plants that overexpress the *NCED* genes can accumulate a lot of ABA and have stronger resistance to abiotic stress [20,26,27].

Cold injury is an important factor affecting the growth and distribution of *P. mume*, and the NCED gene family plays an important role in enhancing plant stress resistance, but the function of the NCED gene family in cold resistance of *P. mume* is still unclear. Here, 56 NCEDs family members were identified from 5 Rosaceae species and analyzed for their physicochemical properties, evolutionary relationships, structural characteristics, collinearity and expression pattern of *PmNCEDs* under different low temperature stress. These results contribute to a further understanding of the phylogenetic relationship of the NCED family in Roseaceae and the response mechanism of *P. mume* under low temperature stress.

2. Materials and Methods

2.1. Identification and Physicochemical Properties of NCEDs Gene Family

The genome data of *P. mume*, *P. apricot*, *P. salicina*, *P. persica*, *R. chinensis* and *A. thaliana* were downloaded from EnsemblPlants (https://plants.ensembl.org, accessed on 10 December 2022) [28]. The NCED protein sequences for *A. thaliana* were downloaded from UniProt (https://www.uniprot.org/, accessed on 10 December 2022). In TB tools [29], the NCED family members of five Rosaceae species were searched by BLAST GUI Wrapper Two Sequences Files (E = 10^{-5}). Subsequently, NCBI CD-Search (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi, accessed on 3 January 2023) and Pfam (https://pfam.xfam.org, accessed on 3 January 2023) were used to further analyze and identify the conserved domains of NCED proteins in five Rosaceae species.

Finally, after removing the redundant, incomplete domain and incorrect sequences, the remaining sequences can be regarded as NCED members of five Rosaceae species. The protein molecular weight (MW), and theoretical isoelectric point (*pI*) and subcellular location of five Rosaceae species NCEDs were predicted using ExPASY (http://web.expasy.org/protparam/, accessed on 20 January 2023) and WoLF PSORT (https://wolfpsort.hgc.jp/, accessed on 20 January 2023), respectively.

2.2. Phylogenetic Analysis

Phylogenetic analysis was conducted on the amino acid sequences of *A. thaliana*, *P. mume*, *P. apricot*, *P. salicina*, *P. persica* and *R. chinensis* NCED proteins. Protein sequences from six species were first compared using ClustalX [30], followed by the construction of a great likelihood tree using IQ-tree [31]. Finally, ChiPlot (https://www.chiplot.online/, accessed on 12 February 2023) was used to beautify the phylogenetic tree.

2.3. Chromosomal Localization and Collinear Analysis

The Multiple Collinearity Scan Toolkit (MCScanX) was utilized with default parameters in TB tools to analyze gene tandems [32]. Using MCScanX, collinearity of NCEDs in *P. mume*, *P. salicina*, *P. apricot*, and *A. thaliana* was analyzed and visualized in TB tools [29]. Additionally, TB tools were used to extract the location and length information of *NCED* genes on the chromosomes of five Rosaceae species and map their distribution on chromosomes.

2.4. Gene Structure and Protein Conserved Motif Analysis

To further understand the evolutionary relationships among the *NCED* genes of the five Rosaceae species, we used the online website MEME (https://meme-suite.org/meme/doc/meme.html, accessed on 24 February 2023) [33] to analyze the conserved motifs of five Rosaceae species NCED proteins and set the number of motifs to 10. Multiple sequence alignment of the full-length sequence of NCED proteins from the five Rosaceae species was performed using Muscle, and the results were trimmed using trimAI, followed by an automatic screening of amino acid substitution models using IQ-tree [31]. Finally, tree-structure-motif mapping was performed by TBtools [29].

2.5. Cis-Acting Regulating Element Analysis

The upstream 2-kb sequence of the initiation codon of *P. mume NCEDs* gene was extracted as the promoter region and submitted to the PlantCARE online website (https://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 25 February 2023) for analysis (Tables S1 and S2), and then screened and visualized by TBtools [30].

2.6. Expression Pattern Analysis

The tissues of flower buds, fruits, roots, stems and leaves from *P. mume* were collected and sequenced by Illumina HiSeq2000 [34]. Buds of the cultivar 'Zao Lve' of *P. mume* overwintered in the open ground in Beijing were used as test material at four different periods: EDI (Endodormant I, November), EDII (Endodormant II, December), EDIII (Endodormant III, January) and NF (Natural flush, February), respectively [35]. Stem samples of the cultivar 'Songchun' were collected from three places (Beijing [39°54′ N, 116°28′ E], Chifeng [42°17′ N, 118°58′ E], Gongzhuling [43°42′ N, 124°47′ E]) in autumn (October 2012), winter (January 2013) and spring (March 2013) [36]. Subsequently, the original data of RNA-seq obtained (Tables S3–S5) were analyzed and the gene expression heat map was drawn using TBtools [29].

To investigate the expression pattern of the cultivated variety 'Zao Lve' of *P. mume* in diverse cold stress situations, the grafted annual 'Zao Lve' was chosen as the test material. This material was collected from the greenhouse of Beijing Forestry University. It was treated at 4 °C for various durations (0, 1, 3, 5, 7, 9, 11 d) and at different temperatures (4, 0, −4, −8 °C) for 6 h. The control material was also maintained at 24 °C. The annual shoots of five plants under different treatment conditions were mixed for RNA-seq, and

there were biological repeats at the same time. They were frozen in liquid nitrogen and then stored at −80 °C for subsequent use. Total RNA was extracted using the RNA extraction kit (TIANGEN, Beijing, China) and reverse transcribed into cDNA as per the cDNA synthesis kit (Tiangen, Beijing, China). qRT-PCR primers were used and listed in Table S6. qRT-PCR was performed using the PikoReal real-time PCR system (Thermo Fisher Scientific, Waltham, MA, USA). We selected *PmPP2A* as the housekeeping gene [37]. To ensure accuracy, we conducted three biological replicates and three technical replicates, and calculated gene expression using $2^{-\Delta\Delta CT}$ [38].

3. Results

3.1. Genome-Wide Identification of NCEDs Gene Family in Five Rosaceae Species

In total, 56 *NCED* genes were characterized by Blastp comparison and conserved domain analysis of five Rosaceae species. There were 10, 10, 11, 12 and 13 *NCED* genes in *P. mume*, *P. apricot*, *P. salicina*, *P. persica* and *R. chinensis*, respectively. They were named successively according to their position distribution on chromosomes (Table 1). The *NCEDs* gene of five Rosaceae species encoded 75–1496 aa, among which the longest was *PmNCED1* (1496 aa) and the shortest was *PsNCED5* (75 aa). The molecular weight (MW) of the NCEDs was 8.36–168.43 kDa. The predicted *pI* ranged from 4.71 to 7.93, and most of the *pI* were less than 7, indicating they were mostly weakly acidic. Subcellular localization prediction of NCEDs in five Rosaceae species showed that 55.3% of 56 *NCED* genes were located in chloroplasts, which was congruous with the functional location of *NCED* genes in ABA biosynthesis pathway, while other genes were located in the nucleus, cytoplasm and mitochondria (Table 1).

Table 1. Basic information and physicochemical properties of *NCED* genes in five Rosaceae species.

Species	Gene Name	Gene ID	Chromosome Localization	Length (aa)	MW (kDa)	*pI*	Prediction of Subcellular Localization
Prunus mume	*PmNCED1*	*Pm005147*	Chr2	1496	168.43	5.83	Nucleus
	PmNCED2	*Pm005148*	Chr2	540	60.33	5.32	Chloroplast
	PmNCED3	*Pm005153*	Chr2	563	62.25	6.53	Mitochondrion
	PmNCED4	*Pm006647*	Chr2	586	64.73	6.21	Peroxisome
	PmNCED5	*Pm006977*	Chr2	89	10.13	5.54	Cell wall
	PmNCED6	*Pm008988*	Chr2	600	66.44	7.92	Chloroplast
	PmNCED7	*Pm010425*	Chr3	617	68.18	6.43	Chloroplast
	PmNCED8	*Pm011164*	Chr3	622	69.01	6.39	Chloroplast
	PmNCED9	*Pm016267*	Chr5	463	52.58	6.45	Peroxisome
	PmNCED10	*Pm017769*	Chr5	634	70.47	5.63	Chloroplast
Prunus apricot	*PaNCED1*	*PARG03953m01*	Chr2	600	66.43	7.92	Chloroplast
	PaNCED2	*PARG06146m02*	Chr2	586	64.69	6.21	Peroxisome
	PaNCED3	*PARG06146m01*	Chr2	604	66.52	6.48	Peroxisome
	PaNCED4	*PARG07910m01*	Chr2	565	62.47	6.20	Mitochondrion
	PaNCED5	*PARG07915m02*	Chr2	520	58.21	5.76	Cytoplasm
	PaNCED6	*PARG07915m01*	Chr2	267	30.14	5.44	Nucleus
	PaNCED7	*PARG07916m01*	Chr2	1073	120.20	5.61	Chloroplast
	PaNCED8	*PARG11107m01*	Chr3	627	69.57	6.63	Chloroplast
	PaNCED9	*PARG11892m01*	Chr3	617	68.12	6.43	Chloroplast
	PaNCED10	*PARG17947m01*	Chr5	615	68.35	5.69	Chloroplast

Table 1. *Cont.*

Species	Gene Name	Gene ID	Chromosome Localization	Length (aa)	MW (kDa)	*pI*	Prediction of Subcellular Localization
Prunus salicina	*PsNCED1*	*evm.model.LG01.539*	Chr1	597	65.77	6.21	Peroxisome
	PsNCED2	*evm.model.LG01.2322*	Chr1	565	62.55	6.36	Chloroplast
	PsNCED3	*evm.model.LG01.2327*	Chr1	176	19.63	5.63	Nucleus
	PsNCED4	*evm.model.LG01.2328*	Chr1	223	25.15	6.22	Nucleus
	PsNCED5	*evm.model.LG01.2329*	Chr1	75	8.36	4.71	Cytoplasm
	PsNCED6	*evm.model.LG01.2330*	Chr1	617	69.09	5.75	Chloroplast
	PsNCED7	*evm.model.LG01.2331*	Chr1	586	65.92	5.55	Cytoplasm
	PsNCED8	*evm.model.LG01.4261*	Chr1	605	66.82	7.93	Chloroplast
	PsNCED9	*evm.model.LG02.1859*	Chr2	615	68.39	5.76	Chloroplast
	PsNCED10	*evm.model.LG04.787*	Chr4	614	67.72	6.36	Chloroplast
	PsNCED11	*evm.model.LG04.1420*	Chr4	627	69.64	7.34	Chloroplast
Prunus persica	*PpNCED1*	*transcript:ONI26993*	Chr1	605	66.95	7.24	Chloroplast
	PpNCED2	*transcript:ONI30522*	Chr1	608	66.81	6.29	Peroxisome
	PpNCED3	*transcript:ONI30523*	Chr1	427	47.30	6.23	Peroxisome
	PpNCED4	*transcript:ONI33831*	Chr1	565	62.50	6.33	Chloroplast
	PpNCED5	*transcript:ONI33839*	Chr1	568	63.62	5.18	Nucleus
	PpNCED6	*transcript:ONI33840*	Chr1	617	68.96	5.64	Chloroplast
	PpNCED7	*transcript:ONI33841*	Chr1	555	62.49	6.01	Cytoplasm
	PpNCED8	*transcript:ONI20423*	Chr2	430	49.12	6.09	Cytoplasm
	PpNCED9	*transcript:ONI20422*	Chr2	547	61.83	5.97	Peroxisome
	PpNCED10	*transcript:ONI22509*	Chr2	615	68.70	5.77	Chloroplast
	PpNCED11	*transcript:ONI11006*	Chr4	617	68.16	6.43	Chloroplast
	PpNCED12	*transcript:ONI12196*	Chr4	632	70.35	6.63	Chloroplast
Rosa chinensis	*RcNCED1*	*transcript:PRQ54664*	Chr1	494	56.04	6.56	Peroxisome
	RcNCED2	*transcript:PRQ54676*	Chr1	631	71.20	7.70	Peroxisome
	RcNCED3	*transcript:PRQ56952*	Chr1	631	71.57	6.98	Chloroplast
	RcNCED4	*transcript:PRQ57420*	Chr1	624	69.89	6.27	Chloroplast
	RcNCED5	*transcript:PRQ57426*	Chr1	622	68.97	6.45	Chloroplast
	RcNCED6	*transcript:PRQ36937*	Chr4	613	67.29	6.81	Chloroplast
	RcNCED7	*transcript:PRQ41175*	Chr4	584	63.72	5.70	Chloroplast
	RcNCED8	*transcript:PRQ29478*	Chr5	688	75.95	6.74	Chloroplast
	RcNCED9	*transcript:PRQ30744*	Chr5	612	67.61	7.25	Chloroplast
	RcNCED10	*transcript:PRQ27514*	Chr6	330	37.34	4.71	Chloroplast
	RcNCED11	*transcript:PRQ27515*	Chr6	563	63.05	5.34	Cytoplasm
	RcNCED12	*transcript:PRQ27522*	Chr6	561	62.10	6.04	Chloroplast
	RcNCED13	*transcript:PRQ21333*	Chr7	303	34.62	5.56	Peroxisome

3.2. Phylogenetic Analysis of NCEDs Gene Family

The NCED phylogenetic tree of *A. thaliana*, *P. mume*, *P. apricot*, *P. salicina*, *P. persica* and *R. chinensis* was constructed using the full-length amino acid sequences (Figure 1). Based on the topological structure analysis of the evolutionary tree, six NCED gene species were divided into six subfamilies: Group I~Group VI. Among them, PmNCED6, PmNCED7 and PmNCED8 of *P. mume* formed Group I with AtNCED2, AtNCED3, AtNCED5, AtNCED6 and AtNCED9 directly related to ABA synthesis in *A. thaliana*. PmNCED4, PmNCED5 and AtCCD4 constitute Group II; PmNCED9 and AtCCD1 constitute Group III. PmNCED1 and PmNCED2 constitute Group IV. PmNCED3 and AtCCD8 constitute Group V. PmNCED10 and AtCCD7 constitute Group VI. Analysis of the affinities between the PmNCEDs and other species of NCEDs showed that most of the PmNCEDs belonged to the same branch as the PaNCEDs, indicating that the PmNCEDs were close in kinship to the PaNCEDs.

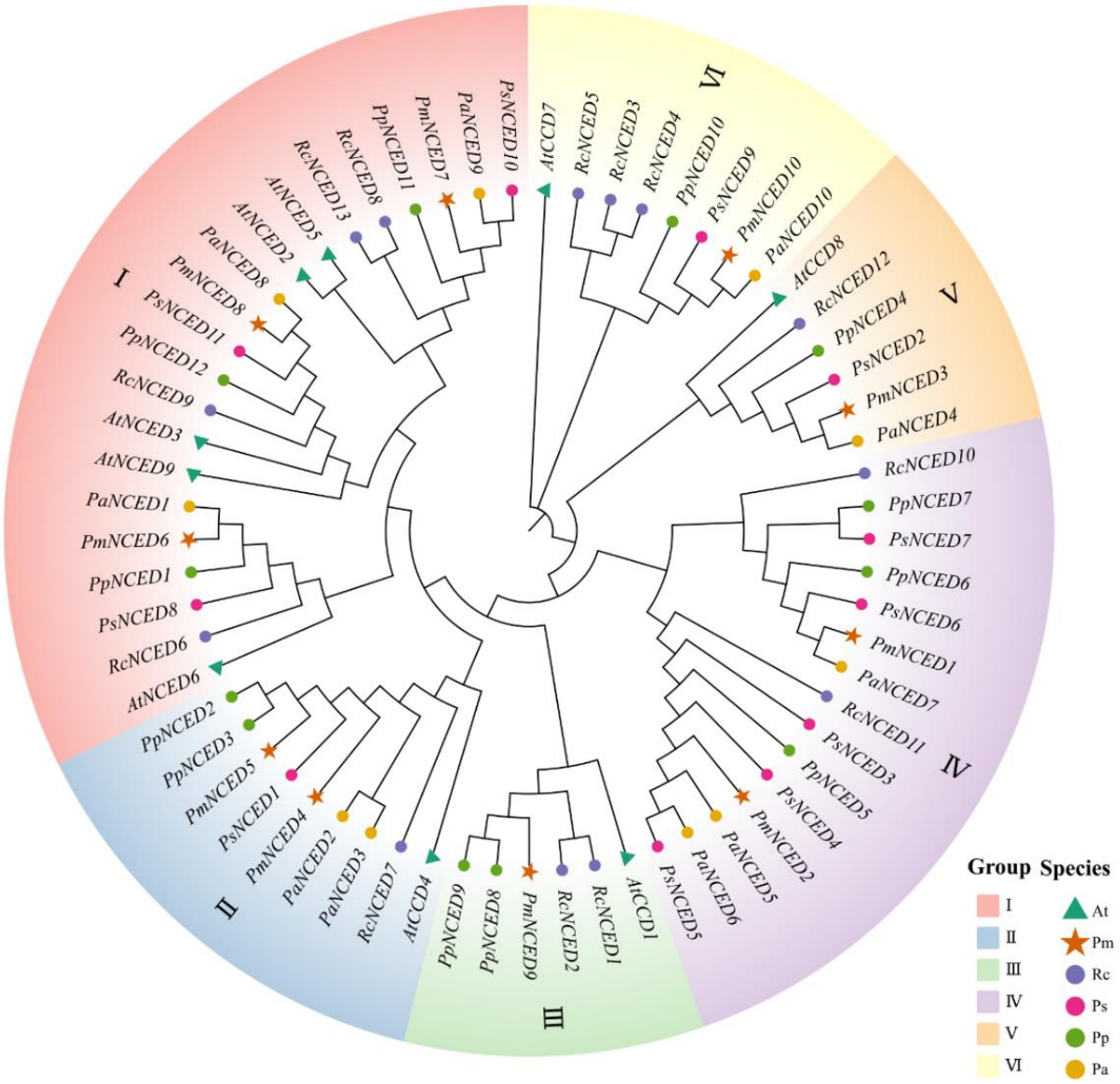

Figure 1. The ML Phylogenetic tree of NCEDs family in 5 Rosaceae species and *A. thaliana*. Roman numerals indicate subgroups. At: *A. thaliana*; Pm: *P. mume*; Rc: *R. chinensis*; Ps: *P. salicina*; Pp: *P. persica*; Pa: *P. apricot*.

3.3. Chromosomal Localization of NCEDs Gene Family in Five Rosaceae Species

The chromosome map showed that *NCED* genes were mostly unequally spread on the chromosomes of five Rosaceae plants (Figure 2). *PmNCEDs* and *PaNCEDs* were each distributed on chromosomes 2, 3 and 5 (Figure 2A,B), and chromosome 2 contained the highest number of *NCED* genes (6 *PmNCEDs* and 7 *PaNCEDs*), and we found that *PmNCED1* and *PmNCED2* of the *P. mume* NCED genes were adjacent to each other on chromosome 2 and were tandem repeats. *PsNCEDs* and *PpNCEDs* were each distributed on chromosomes 1, 2 and 4 (Figure 2C,D), with the largest number of *NCED* genes on chromosome 1 (8 *PsNCEDs* and 7 *PpNCEDs*) and *PsNCED9* alone on chromosome 2 (Figure 2C). *RcNCEDs* were randomly located on chromosomes 1, 4, 5, 6 and 7 (Figure 2E), with chromosome 1 containing a higher number of *NCED* genes (5).

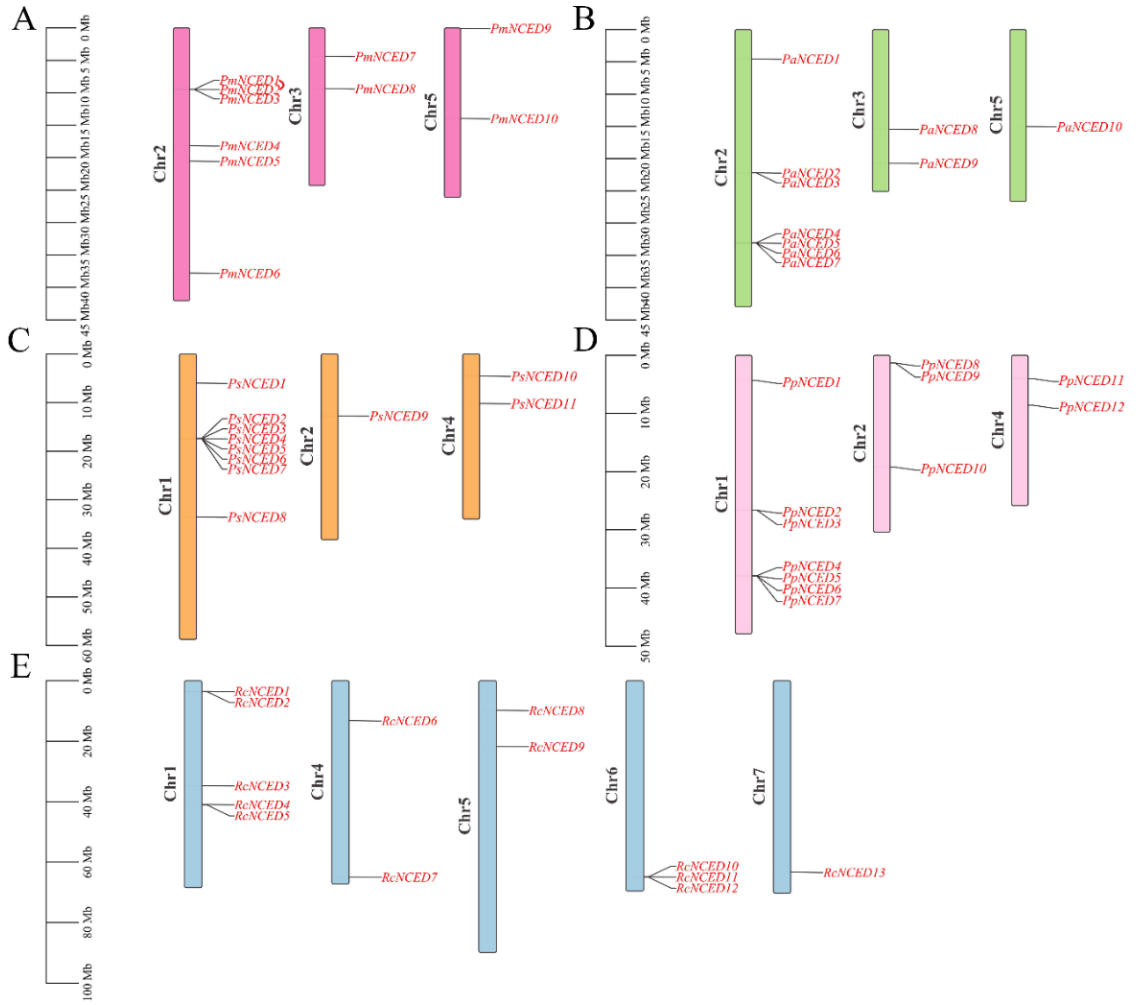

Figure 2. Chromosomal location of *NCED* genes in five Rosaceae species. (**A**–**E**). Chromosome distribution of *NCED* genes in *P. mum*, *P. apricot*, *P. salicina*, *P. persica*, and *R. chinensis*. Chromosome numbers are displayed on the left side of the bar, and the *NCED* genes are marked on the right side of the chromosomes. Scale on the left represents the chromosome length and is expressed in megabytes (Mb). Pm: *P. mume*; Pa: *P. apricot*; Ps: *P. salicina*; Pp: *P. persica*; Rc: *R. chinensis*.

3.4. Gene Structure and Conserved Motifs Analysis of NCED in Five Rosaceae Species

The structure and function of genes are closely related, which can reflect their evolutionary relationship. In order to further understand the phylogenic relationships between the *NCED* genes in five species of Rosaceae, the gene structures of intron/exon were compared according to their distribution patterns (Figure 3B). The results suggested that the *NCED* genes in Group I had no intron and only one exon. In Group II, except for *RcNCED7* and *PmNCED5*, only one exon had no intron, and other *NCED* genes contained 1–2 introns. The number of introns in Group IV was the most abundant, and the number of introns in *PmNCED1* was the highest, with a total of 22 introns. Therefore, with some exceptions, the number and size of introns in five Rosaceae species were relatively conservative in each subfamily, thus consolidating the taxonomy obtained in the phylogenetic tree (Figure 3A). However, intron-exon organization differs between subfamilies, suggesting that intra recombination such as insertion/deletion contributes to the amplification of the NCED family in five Rosaceae species besides their functional differentiation.

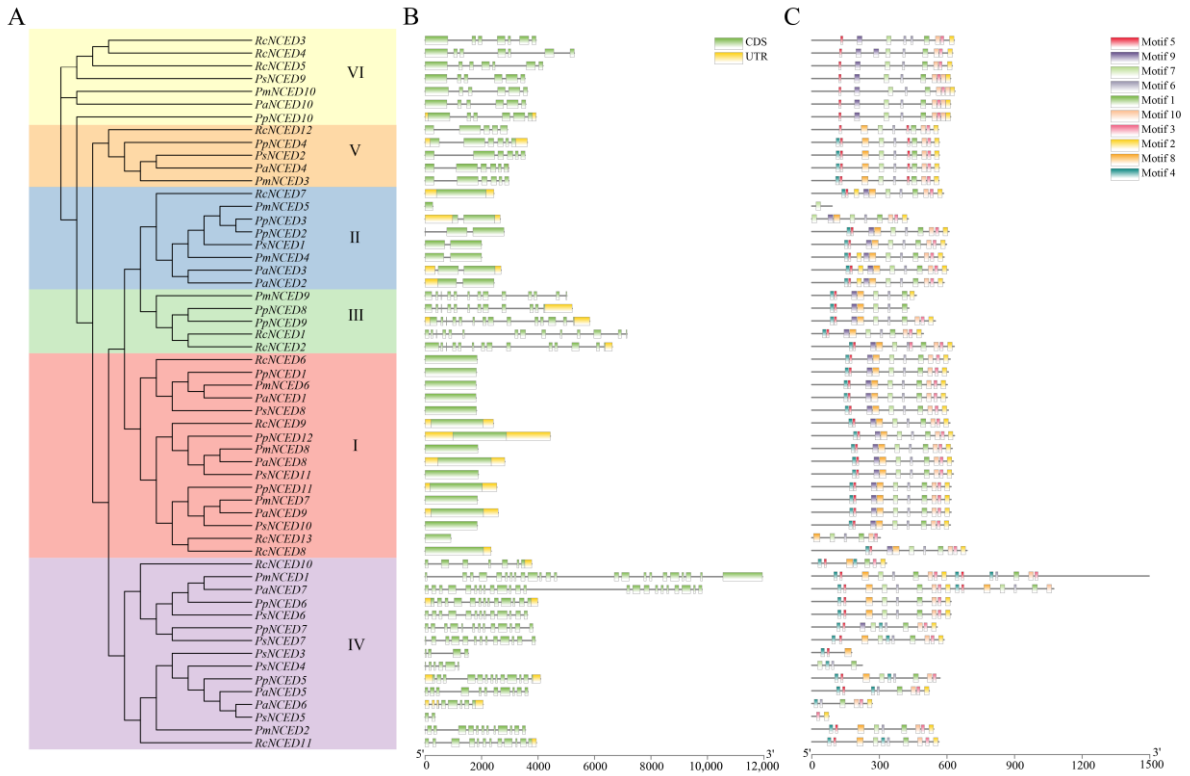

Figure 3. Gene structure and conserved motifs analysis of *NCED* gene family in five Rosaceae species. (**A**). Phylogenetic trees of NCED proteins from five Rosaceae species. I~VI represent 6 groups; (**B**). Exon-intron structure of *NCED* genes. The black lines represent introns; (**C**). The conserved motifs of *NCED* genes in five Rosaceae species. Conservative motifs are represented by different colored boxes. The black lines represent non-conserved sequences.

MEME was used to detect conserved motifs to further analyze the differences among *NCED* genes, where 10 conserved motifs were identified (Figure 3C). Of these, motifs 1, 2, 3, 5, 6, 7 and 10 are conserved in most NCEDs, which indicates that these motifs may be the reason for their common function. For example, Group I contains 10 conserved motifs except for RcNCED8 and RcNCED13; Group VI does not contain motif 4 and motif 8; Group V does not contain motif 9; and Group IV is more diverse. These data indicate that, in general, NCED isoforms with similar genetic structure also have similar motif composition and domain location, and belong to the same clade.

3.5. Collinear Analysis of NCEDs in Prunus Species and A. thaliana

Collinearity analysis was performed for four species: *P. mume*, *P. apricot*, *P. salicina* and *A. thaliana* (Figure 4). The number of collinear gene pairs of *P. mume*, *P. apricot*, *P. salicina* and *A. thaliana* were 6, 6 and 2, respectively, indicating that the homology of NCED family genes was high in *Prunus*. No corresponding homologous genes were found for *PmNCED2*, *PmNCED5*, *PmNCED6* and *PmNCED9* genes, suggesting that some genes were lost during the evolution of the *NCED* gene family of *P. mume*, or the *NCED* gene family of *P. mume* was expanded. *PmNCED10* was present in collinear gene pairs of *P. mume*, *P. apricot*, *P. salicina* and *A. thaliana*, suggesting that *PmNCED10* may be common in monocotyledonous and dicotyledonous plants, forming before species differentiation and having a longer evolutionary time.

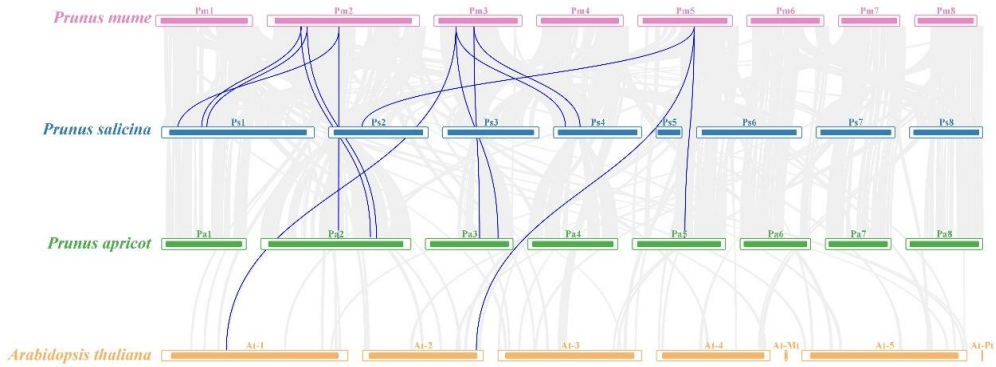

Figure 4. Collinear analysis of *NCED* genes in three *Prunus* species and *A. thaliana.* Gray lines in the background represent collinear relationships throughout the genome, and the blue lines mainly represent the collinear *NCED* gene pairs.

3.6. Analysis of Cis-Elements in the Promoters of PmNCEDs

The upstream 2-kb sequence of the initiation codon of the *PmNCEDs* was used for cis-element analysis (Figure 5). Except for *PmNCED9*, the promoter region of *PmNCED*s generally had light responsive elements (Figure 5A,B), such as G-box, GT1-motif, Box 4, MRE, CTt-motif, etc. Six *PmNCEDs* (*PmNCED1*, *PmNCED2*, *PmNCED3*, *PmNCED4*, *PmNCED5*, and *PmNCED10*) each contained a low-temperature response (LTR) element. *PmNCED2*, *PmNCED4* and *PmNCED7* also contain drought responsive component MBS. The promoter region of *PmNCED*s was also enriched with several cis-acting elements associated with phytohormone response (Figure 5A,B), such as the TCA-element associated with salicylic acid responsive; the P-box associated with gibberellin-responsive; the MeJA-responsive element G-box and CGTCA-motif. In addition, *PmNCEDs* contain an abscisic acid responsive element (ABRE), which accounts for 16.59% of all elements, except for *PmNCED7* and *PmNCED9*, of which the *PmNCED6* gene contains 12 elements (Figure 5A,C).

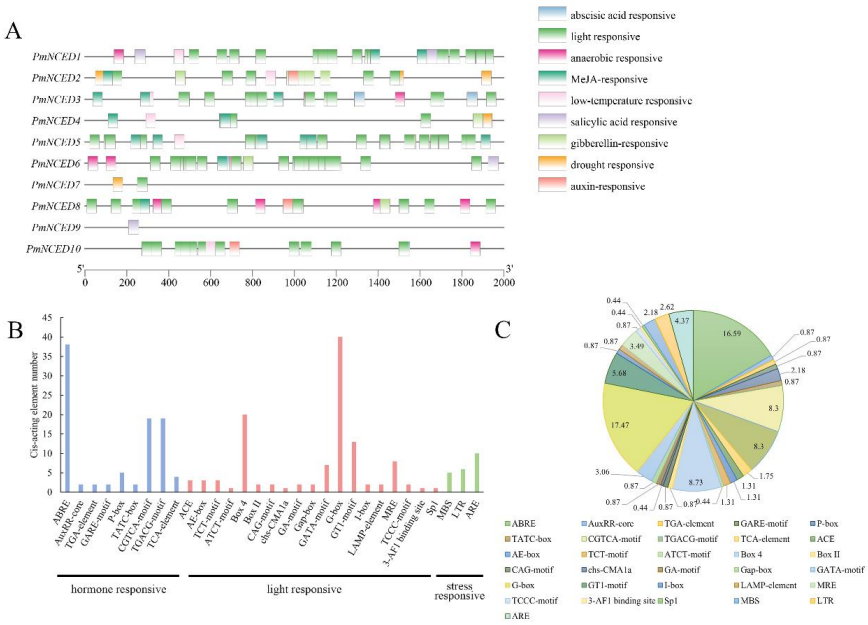

Figure 5. Cis-element analysis of *PmNCEDs* promoter. (**A**). Cis-elements in 2-kb sequence upstream of *PmNCEDs*. Different cis-elements are represented by round rectangles of different colors; (**B**). The number of *PmNCEDs* in each class of cis-elements. Cis-elements are divided into hormone, light and stress response classes; (**C**). Proportion of cis-elements involved in hormonal, light and stress responses in the *PmNCEDs* promoter (%).

3.7. Expression Pattern of PmNCEDs

Expression patterns of *PmNCEDs* were analyzed based on transcriptome data sets to further explore the function of *PmNCEDs* (Figure 6). *PmNCEDs* were expressed differently in the buds, leaves, roots and stems of *P. mume* (Figure 6A). The expression level of most *PmNCEDs* in different tissues was low, such as *PmNCED5* and *PmNCED10* were almost not expressed. *PmNCED7* was slightly expressed in buds, leaves, roots and stems, but hardly expressed in fruits. The expression level of *PmNCED8* in each tissue was higher than that of other genes. *PmNCED4* and *PmNCED9* were highly expressed in buds, while *PmNCED4* was almost not expressed in fruits and leaves, and the expression of *PmNCED9* in all tissues was high. Expression of *PmNCEDs* in buds of 'Zao lve' cultivated species at three endodermal developmental stages differs considerably under low temperature conditions (Figure 6B). In general, the expression levels of *PmNCED2*, *PmNCED5*, and *PmNCED6* were low, while there were no significant changes observed in the expression of *PmNCED9*. On the other hand, the expression levels of *PmNCED1* and *PmNCED3* were comparatively lower, but showed a mild up-regulation during the NF stage from EDIII to bud dormancy release. The expression level of *PmNCED7* showed a downward trend from EDI to NF. The expression level of *PmNCED8* was similar in the EDI stage and the EDIII stage, but down-regulated in the EDII stage and NF stage. However, the expression level of *PmNCED4* was a gradually cumulative one and reached the peak in the NF stage of bud dormancy release. The expression level in the stem of 'Songchun' was also different in Beijing, Chifeng and Gongzhuling in different seasons (Figure 6C). Among them, *PmNCED1*, *PmNCED2*, *PmNCED5* and *PmNCED10* were almost not expressed. *PmNCED3* was slightly expressed in autumn and winter in Beijing and Chifeng, but there was no obvious change trend from autumn to winter. The expression of *PmNCED4* was high in spring, but low in autumn and winter, and showed a downward trend from autumn to winter. *PmNCED8* was highly expressed in autumn and winter, but decreased from winter to spring. *PmNCED9* was highly expressed in the three regions in different seasons, but there was no obvious change trend. As the average winter temperature in three regions decreased, the expression of *PmNCED7* and *PmNCED8* showed an increase, and there was a noticeable change in the trend of *PmNCED7*, while the change of the two genes from autumn to winter was not obvious.

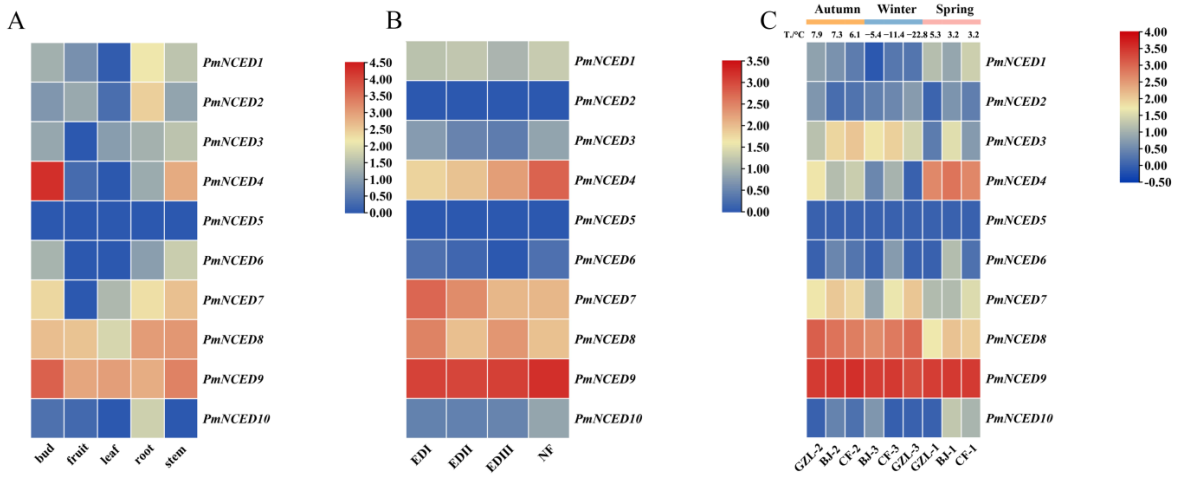

Figure 6. Expression pattern of the *PmNCEDs*. (**A**). The heatmap of the expression of *PmNCEDs* in different tissues; (**B**). The heat map of the expression of *PmNCEDs* in buds of *P. mume* 'Zao lve' at three stages of endo dormancy under low temperature conditions; (**C**). The heatmap of the expression of *PmNCEDs* in the stems of 'Songchun' from different regions and seasons.

Obtaining the expression levels of *PmNCEDs* through qRT-PCR was done to investigate their role in dealing with cold stress under various treatments (Figure 7). Under chilling treatment at 4 °C (Figure 7A), the expressions of *PmNCED4* and *PmNCED7* slightly changed

with the extension of chilling treatment time, but showed a downward trend overall. The expression levels of *PmNCED3* and *PmNCED6* were low on the whole but increased sharply on the 9th day. Although the expression levels of *PmNCED8* and *PmNCED9* fluctuated in a small range, the expression levels of the two genes remained high with the extension of cold treatment time. As the processing temperature gradually reduced (Figure 7B), the expression of *PmNCED6* and *PmNCED7* showed an overall downward trend. When the cool treating temperature decreased, there were some small fluctuations in *PmNCED4* expression, but there was no obvious regularity, with a slight upward trend overall. Compared to the expression of *PmNCED4*, the expression of *PmNCED8* fluctuated greatly, and peaked at −4 °C with the increase of chilling stress degree, and then decreased at −8 °C. At 0 °C, the expression level of *PmNCED3* reached its peak, then decreased at −4 °C, but slightly increased at −8 °C, overall higher than the control group. The expression level of *PmNCED9* increased gradually and peaked at −8 °C, although the range of change was small.

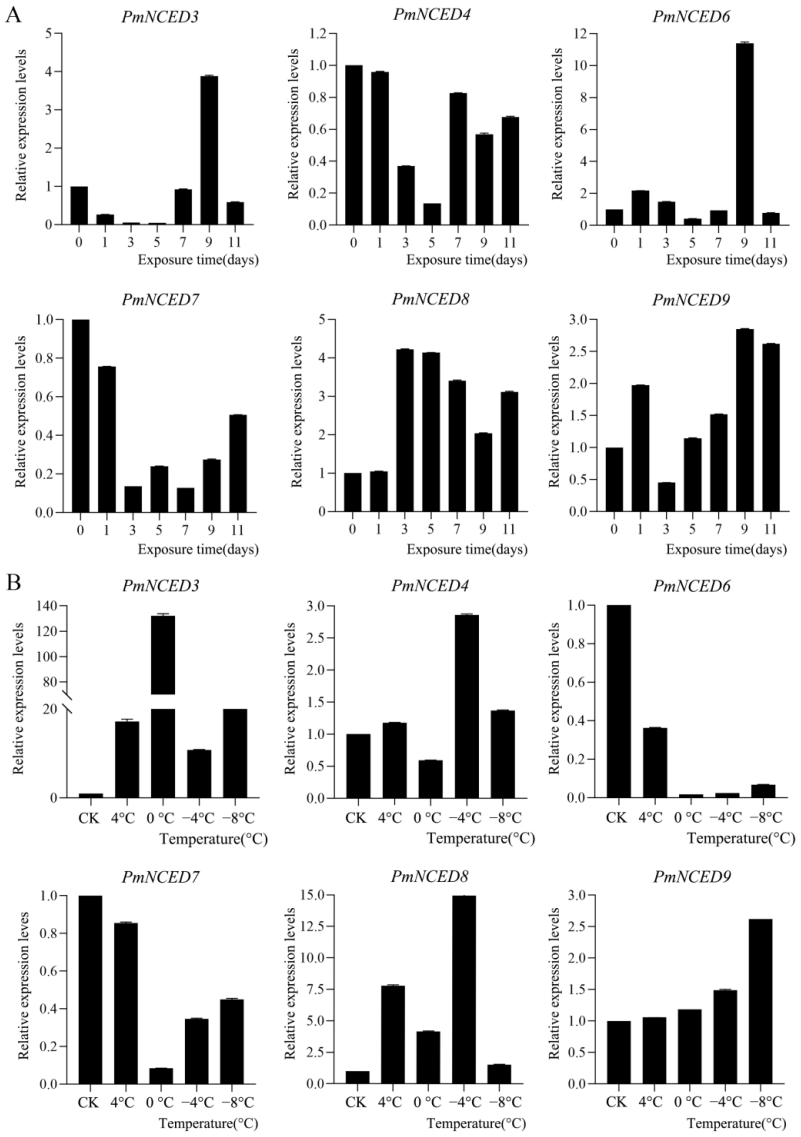

Figure 7. qRT-PCR analysis of *PmNCEDs* under low temperature treatment. (**A**). The expression level of *PmNCEDs* under 4 °C treatment for 0, 1, 3, 5, 7, 9,11 d; (**B**). The expression level of *PmNCEDs* under 4 °C, 0 °C, −4 °C, and −8 °C treatment for 6 h. The samples at 24 °C were used as the inner group control.

4. Discussion

As a typical abiotic stress factor, low temperature has a significant influence on plant development and crop yield [39]. Under the influence of climate change, severe weather such as cold and freezing often occurs in China (especially in the southern region). Cold and low temperature damage causes certain damage to the somatic structure of fruit trees and ornamental plants, causing disorder of material metabolism and even death, causing serious economic losses to the production of the fruit industry in China, thus restricting the healthy and sustainable development of the fruit industry [40]. Low temperature is also one of the most principal factors restricting the growth and distribution of *Prunus* genus. The main ornamental organ of *P. mume* is the flower, the low temperature in early spring may affect the normal growth and development of flowers and reduce the ornamental effect of flowers. Therefore, it is of great significance to explore the response and tolerance mechanism of *P. mume* to low temperature stress to improve its cold tolerance. ABA is a significant signaling molecule in plants under stress, and NCED is a key rate-limiting enzyme in ABA synthesis. In most species, the NCED family is a small gene family, which has been confirmed in plants such as *A. thaliana* [15], *S. lycopersicum* [16], *G. max* [17], *V. vinifera* [18] and *M. pumila* [19].

In this research, we identified 56 *NCED*s in total from five Rosaceae species, with *P. mume* and *P. apricot* identifying the same number of NCED family members. *R. chinensis* had the highest number of *NCED* genes (13), while *P. persica* and *P. salicina* had the next highest number of *NCED* family members, with 12 and 11 *NCED* genes, respectively. In contrast, only nine, five and five members of the *NCED* family were found in *A. thaliana*, *Oryza sativa* and *Zea mays* [41], respectively. This indicates a relatively consistent evolutionary relationship among the five Rosaceae species and shows the extension of the *NCED* gene family in the evolution of the *Prunus* genus. Gene replication events occur frequently in biological evolution, but only one pair of tandem replication events was found in the *PmNCED*s (Figure 2A). The collinearity results manifested that the number of collinearity gene pairs of *P. apricot*, *P. salicina* and *A. thaliana* was 6, 6, and 2, respectively, indicating that *P. mume* had a close homology relationship with the *NCED* of *P. apricot* and *P. salicina*, but a distant homology relationship with the *NCED* of *A. thaliana*. In addition, no corresponding homologous genes were found for *PmNCED2*, *PmNCED5*, *PmNCED6* and *PmNCED9*, suggesting that the *NCED* gene family of *P. mume* may have expanded.

Through the analysis of cis-elements, it was found that the promoter region of *PmNCEDs* contains a wide range of cis-elements involved in light response and phytohormone induction. Among them, ABRE related to abscisic acid responsive accounted for 16.59% of all components, TGACG-motif and CGTCA-motif related to MEJA- responsive accounted for 16.6%. At the same time, some genes also contain elements related to low temperature response, which is consistent with the existing studies on the involvement of *NCED* in the process of plant resistance. For example, transcriptional factor *NGATHA1* of *A. thaliana* activates *AtNCED3* to induce ABA biosynthesis through dehydration stress [42]. However, there is a positive feedback adjustment of ABA in the biosynthesis of ABA in plants under dehydration stress, and ABA induced *AtNCED3* expression requires the distal ABA response element ABRE [43].

Thus far, numerous studies have investigated how *NCED* genes react to environmental stresses such as drought, high temperatures, and excessive salt. In *Vigna unguiculata*, salt stress specifically induced *VuNCED1* expression, while cold (4 °C) or heat (40 °C) stress did not induce *VuNCED1* expression [24]. Cold stress and the application of exogenous ABA induced *CkNCED1* expression and ABA accumulation in *C. korshinskii* [25]. Under salinity, low temperature and drought stress, *NCED* was closely related to endogenous ABA content in *C. sativus* [22]. Under the influence of drought, low temperature and high temperature, the expression of *MpNCED2* in *M. pumila* was significantly up-regulated, while the expression of *MpNCED1* was affected by low temperature and high temperature, but not by drought [23]. Dehydration, salt stress, low temperature stress and drought stress can induce *SgNCED1* transcription and ABA accumulation [44]. In our study, under

low temperature stress, only *PmNCED8* reacted strongly, and the expression of *PmNCED9* showed an upward trend under cold stress, but its expression level changed little, while the expression of *PmNCED3* changed significantly, and its expression level changed unsteadily during long-term cold treatment (Figure 7). These results indicate that *PmNCED3*, *PmNCED8* and *PmNCED9* have different responses to low temperature stress.

By checking cis-elements of the *PmNCEDs*, it was found that only *PmNCED3* contained cold stress response elements, while *PmNCED8* and *PmNCED9* had no cold stress response elements (Figure 4). In addition, *PmNCED3* contains elements related to both abscisic acid response and MeJA-response, while *PmNCED8* contains elements related to MeJA-response. ABA and MeJA, as signaling molecules in response to abiotic stress, can participate in controlling the expression of resistance genes in plants, and exogenous hormones can enhance plant cold tolerance [45,46]. The promoter region of *MpNCED1* and *MpNCED2* contains regulatory elements such as ABRE, ARE and TCA-element, in addition, *MpNCED2* also contains CGTCA-motif and TGACG-motif relevant to MeJA-response [23]. Therefore, the response of *PmNCED3* and *PmNCED8* to low temperature stress may be due to the presence of elements related to abscisic acid response and MeJA-response.

5. Conclusions

A total of 56 *NCED* genes were identified in 5 species of Rosaceae through genome-wide screening and were divided into 6 subfamilies according to phylogenetic analysis. The analysis of motif and gene structure further proved the accuracy of classification. Evolutionary analysis showed that the homology of *NCEDs* genes in *P. mume*, *P. apricot* and *P. salicina* was high. qRT-PCR analysis showed that *PmNCED3*, *PmNCED8* and *PmNCED9* may be related to cold stress response. These results increased our understanding of the gene structure and the evolution of the NCED gene family in five Rosaceae specie, and lay a foundation for further study on the function of *PmNCEDs* under low temperature stress.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9070839/s1, Table S1: The promoter sequence of *PmNCEDs*; Table S2: Cis-acting element on the promoter of *PmNCEDs*; Table S3: Expression profiles of *PmNCED*s in different tissues; Table S4: Expression profiles of *PmNCEDs* during the process of flower bud dormancy release; Table S5: Expression profiles of *PmNCEDs* in different regions and seasons; Table S6: The primers of qRT-PCR used in this study.

Author Contributions: Conceptualization, T.Z.; Data curation, X.L., X.G., L.Y., L.Q. and W.L.; Funding acquisition, T.Z.; Investigation, K.C., X.L. and X.G.; Methodology, K.C. and W.L.; Project administration, T.Z.; Resources, J.W.; Software, K.C., X.L., L.Y., L.Q. and W.L.; Supervision, J.W.; Writing—original draft, K.C.; Writing—review & editing, T.Z. All authors have read and agreed to the published version of the manuscript.

Funding: The research was supported by the Fundamental Research Funds for the Central Universities (No. QNTD202306) and Beijing High-Precision Discipline Project, Discipline of Ecological Environment of Urban and Rural Human Settlements and the Special Fund for Beijing Common Construction Project.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Peng, T.; Guo, C.; Yang, J.; Wan, X.; Wang, W.; Zhang, J.; Bao, M.; Zhang, J. Transcriptome analysis revealed molecular basis of cold response in *Prunus mume*. *Mol. Breed.* **2023**, *43*, 34. [CrossRef] [PubMed]
2. Zhuo, X.; Zheng, T.; Zhang, Z.; Zhang, Y.; Jiang, L.; Ahmad, S.; Sun, L.; Wang, J.; Cheng, T.; Zhang, Q. Genome-wide analysis of the NAC transcription factor gene family reveals differential expression patterns and cold-stress responses in the woody plant *Prunus mume*. *Genes* **2018**, *9*, 494. [CrossRef] [PubMed]
3. Zhang, Q.; Zhang, H.; Sun, L.; Fan, G.; Ye, M.; Jiang, L.; Liu, X.; Ma, K.; Shi, C.; Bao, F.; et al. The genetic architecture of floral traits in the woody plant *Prunus mume*. *Nat. Commun.* **2018**, *9*, 1702. [CrossRef]
4. Hu, H.; Xiong, L. Genetic engineering and breeding of drought-resistant crops. *Annu. Rev. Plant Biol.* **2014**, *65*, 715–741. [CrossRef]

5. Shinozaki, K.; Yamaguchi-Shinozaki, K. Gene networks involved in drought stress response and tolerance. *J. Exp. Bot.* **2006**, *58*, 221–227. [CrossRef] [PubMed]
6. Lange, B.M.; Ghassemian, M. Genome organization in *Arabidopsis thaliana*: A survey for genes involved in isoprenoid and chlorophyll metabolism. *Plant Mol. Biol.* **2003**, *51*, 925–948. [CrossRef]
7. Kovács, Z.; Simon-Sarkadi, L.; Szucs, A.; Kocsy, G. Differential effects of cold, osmotic stress and abscisic acid on polyamine accumulation in wheat. *Amino Acids* **2010**, *38*, 623–631. [CrossRef]
8. Roychoudhury, A.; Paul, S.; Basu, S. Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress. *Plant Cell Rep.* **2013**, *32*, 985–1006. [CrossRef] [PubMed]
9. Bertrand, A.; Robitaille, G.; Castonguay, Y.; Nadeau, P.; Boutin, R. Changes in ABA and gene expression in cold-acclimated sugar maple. *Tree Physiol.* **1997**, *17*, 31–37. [CrossRef]
10. Fenollosa, E.; Gamez, A.; Munne-Bosch, S. Plasticity in the hormonal response to cold stress in the invasive plant *Carpobrotus edulis*. *J. Plant Physiol.* **2018**, *231*, 202–209. [CrossRef]
11. Wang, H.; Blakeslee, J.J.; Jones, M.L.; Chapin, L.J.; Dami, I.E. Exogenous abscisic acid enhances physiological, metabolic, and transcriptional cold acclimation responses in greenhouse-grown grapevines. *Plant Sci.* **2020**, *293*, 110437. [CrossRef]
12. Priya, R.; Siva, R. Analysis of phylogenetic and functional diverge in plant 9-cis-epoxycarotenoid dioxygenase gene family. *J. Plant Res.* **2015**, *128*, 519–534. [CrossRef] [PubMed]
13. Schwartz, S.H.; Qin, X.; Zeevaart, J.A. Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes, and enzymes. *Plant Physiol.* **2003**, *131*, 1591–1601. [CrossRef]
14. Ren, H.; Gao, Z.; Chen, L.; Wei, K.; Liu, J.; Fan, Y.; Davies, W.J.; Jia, W.; Zhang, J. Dynamic analysis of ABA accumulation in relation to the rate of ABA catabolism in maize tissues under water deficit. *J. Exp. Bot.* **2006**, *58*, 211–219. [CrossRef]
15. Tan, B.C.; Schwartz, S.H.; Zeevaart, J.A.; McCarty, D.R. Genetic control of abscisic acid biosynthesis in maize. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12235–12240. [CrossRef]
16. Burbidge, A.; Grieve, T.M.; Jackson, A.; Thompson, A.; McCarty, D.R.; Taylor, I.B. Characterization of the ABA-deficient tomato mutant notabilis and its relationship with maize Vp14. *Plant J.* **1999**, *17*, 427–431. [CrossRef]
17. Qin, X.; Zeevaart, J.A. The 9-cis-epoxycarotenoid cleavage reaction is the key regulatory step of abscisic acid biosynthesis in water-stressed bean. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 15354–15361. [CrossRef] [PubMed]
18. Zhang, R.; Yang, C.; Wang, C.; Wei, Z.; Xia, D.; Wang, Y.; Liu, G.; Wang, Y. Time-course analyses of abscisic acid level and the expression of genes involved in abscisic acid biosynthesis in the leaves of *Betula platyphylla*. *Mol. Biol. Rep.* **2012**, *39*, 2505–2513. [CrossRef]
19. Neves, D.M.; Filho, M.A.; Bellete, B.S.; Silva, M.F.; Souza, D.T.; Dos S Soares Filho, W.; Costa, M.G.; Gesteira, A.S. Comparative study of putative 9-cis-epoxycarotenoid dioxygenase and abscisic acid accumulation in the responses of sunki mandarin and rangpur lime to water deficit. *Mol. Biol. Rep.* **2013**, *40*, 5339–5349. [CrossRef] [PubMed]
20. Iuchi, S.; Kobayashi, M.; Taji, T.; Naramoto, M.; Seki, M.; Kato, T.; Tabata, S.; Kakubari, Y.; Yamaguchi-Shinozaki, K.; Shinozaki, K. Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in arabidopsis. *Plant J.* **2001**, *27*, 325–333. [CrossRef]
21. Huang, Y.; Jiao, Y.; Xie, N.; Guo, Y.; Zhang, F.; Xiang, Z.; Wang, R.; Wang, F.; Gao, Q.; Tian, L.; et al. *OsNCED5*, a 9-cis-epoxycarotenoid dioxygenase gene, regulates salt and water stress tolerance and leaf senescence in rice. *Plant Sci.* **2019**, *287*, 110188. [CrossRef] [PubMed]
22. Ahrazem, O.; Rubio-Moraga, A.; Trapero, A.; Gómez-Gómez, L. Developmental and stress regulation of gene expression for a 9-cis-epoxycarotenoid dioxygenase, *CstNCED*, ssolated from *Crocus sativus* stigmas. *J. Exp. Bot.* **2012**, *63*, 681–694. [CrossRef]
23. Xia, H.; Wu, S.; Ma, F. Cloning and expression of two 9-cis-epoxycarotenoid dioxygenase genes during fruit development and under stress conditions from malus. *Mol. Biol. Rep.* **2014**, *41*, 6795–6802. [CrossRef]
24. Iuchi, S.; Kobayashi, M.; Yamaguchi-Shinozaki, K.; Shinozaki, K. A stress-inducible gene for 9-cis-epoxycarotenoid dioxygenase involved in abscisic acid biosynthesis under water stress in drought-tolerant cowpea. *Plant Physiol.* **2000**, *123*, 553–562. [CrossRef] [PubMed]
25. Wang, X.; Wang, Z.; Dong, J.; Wang, M.; Gao, H. Cloning of a 9-cis-epoxycarotenoid dioxygenase gene and the responses of *Caragana korshinskii* to a variety of abiotic stresses. *Genes Genet. Syst.* **2009**, *84*, 397–405. [CrossRef]
26. Thompson, A.J.; Jackson, A.C.; Symonds, R.C.; Mulholland, B.J.; Dadswell, A.R.; Blake, P.S.; Burbidge, A.; Taylor, I.B. Ectopic expression of a tomato 9-cis-epoxycarotenoid dioxygenase gene causes over-production of abscisic acid. *Plant J.* **2000**, *23*, 363–374. [CrossRef] [PubMed]
27. Qin, X.; Zeevaart, J.A. Overexpression of a 9-cis-epoxycarotenoid dioxygenase gene in *Nicotiana plumbaginifolia* increases abscisic acid and phaseic acid levels and enhances drought tolerance. *Plant Physiol.* **2002**, *128*, 544–551. [CrossRef]
28. Bolser, D.M.; Staines, D.M.; Perry, E.; Kersey, P.J. Ensembl plants: Integrating tools for visualizing, mining, and analyzing plant genomic data. *Methods Mol. Biol.* **2017**, *1533*, 1–31. [PubMed]
29. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant* **2020**, *13*, 1194–1202. [CrossRef]
30. Thompson, J.D.; Gibson, T.J.; Higgins, D.G. Multiple sequence alignment using clustalW and clustalX. *Curr. Protoc. Bioinform.* **2002**. [CrossRef]

31. Nguyen, L.T.; Schmidt, H.A.; von Haeseler, A.; Minh, B.Q. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* **2015**, *32*, 268–274. [CrossRef]
32. Yang, L.; Li, P.; Qiu, L.; Ahmad, S.; Wang, J.; Zheng, T. Identification and comparative analysis of the rosaceae *RCI2* gene family and characterization of the cold stress response in *Prunus mume*. *Horticulturae* **2022**, *8*, 997. [CrossRef]
33. Bailey, T.L.; Williams, N.; Misleh, C.; Li, W.W. MEME: Discovering and analyzing DNA and protein sequence motifs. *Nucleic Acids Res.* **2006**, *34*, W369–W373. [CrossRef] [PubMed]
34. Zhang, Q.; Chen, W.; Sun, L.; Zhao, F.; Huang, B.; Yang, W.; Tao, Y.; Wang, J.; Yuan, Z.; Fan, G.; et al. The genome of *Prunus mume*. *Nat. Commun.* **2012**, *3*, 1318. [CrossRef]
35. Zhang, Z.; Zhuo, X.; Zhao, K.; Zheng, T.; Han, Y.; Yuan, C.; Zhang, Q. Transcriptome profiles reveal the crucial roles of hormone and sugar in the bud dormancy of *Prunus mume*. *Sci. Rep.* **2018**, *8*, 5090. [CrossRef]
36. Jiang, L. Physiological Changes and Gene Expression Pattern in Response to Low Temperature Stress in *Prunus mume*. Ph.D. Thesis, Beijing Forestry University, Beijing, China, 2020.
37. Wang, T.; Lu, J.; Xu, Z.; Yang, W.; Wang, J.; Cheng, T.; Zhang, Q. Selection of suitable reference genes for miRNA expression normalization by qRT-PCR during flower development and different genotypes of *Prunus mume*. *Sci. Hortic.* **2014**, *169*, 130–137. [CrossRef]
38. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data ysing real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [CrossRef]
39. Cai, H.; Tian, S.; Liu, C.; Dong, H. Identification of a *MYB3R* gene involved in drought, salt and cold stress in wheat (*Triticum aestivum* L.). *Gene* **2011**, *485*, 146–152. [CrossRef]
40. Ping, X.Y.; Zhou, L. Research prospects and progress on chilling injury of economic forest and fruit trees in south China. *Chin. J. Eco-Agric.* **2018**, *26*, 467–479.
41. She, K.; Xu, Z.; Li, X.; Luo, X.; Liu, Y. Sequence characteristics and bioinformatics analysis of *NCED* gene in maize. *Ningxia J. Agric. For. Sci. Technol.* **2010**, *5*, 14–17.
42. Sato, H.; Takasaki, H.; Takahashi, F.; Suzuki, T.; Iuchi, S.; Mitsuda, N.; Ohme-Takagi, M.; Ikeda, M.; Seo, M.; Yamaguchi-Shinozaki, K.; et al. *Arabidopsis thaliana NGATHA1* transcription factor induces ABA biosynthesis by activating *NCED3* gene during dehydration stress. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, E11178–E11187. [CrossRef]
43. Yang, Y.Z.; Tan, B.C. A distal ABA responsive element in *AtNCED3* promoter is required for positive feedback regulation of ABA biosynthesis in arabidopsis. *PLoS ONE* **2014**, *9*, e87283. [CrossRef]
44. Yang, J.; Guo, Z. Cloning of a 9-cis-epoxycarotenoid dioxygenase gene (*SgNCED1*) from *Stylosanthes guianensis* and its expression in response to abiotic stresses. *Plant Cell Rep.* **2007**, *26*, 1383–1390. [CrossRef]
45. Nakashima, K.; Yamaguchi-Shinozaki, K. ABA signaling in stress-response and seed development. *Plant Cell Rep.* **2013**, *32*, 959–970. [CrossRef]
46. Gao, Z.; Zhou, Y.; He, Y. Molecular epigenetic mechanisms for the memory of temperature stresses in plants. *J. Genet. Genom.* **2022**, *49*, 991–1001. [CrossRef]