

Genome-Wide Identification of Two-Component Signal Transduction System Genes in Melon (*Cucumis melon* L.)

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Abstract

Two-component system (TCS) is responsible for cytokinin signaling, which plays critical roles in plant development and physiological process. This system is generally composed of two signaling factors, a histidine kinase (HK) and a response regulator (RR) that is associated with a histidine phosphotransfer (HP) protein. In this study, we performed systematic investigation on TCS genes in melon (*Cucumis melon* L.). We identified 44 TCS genes in melon, including 18 *HK(L)s* (9 *HKs* and 9 *HKLs*), 5 *HPs* (4 authentic and 1 pseudo), and 21 *RRs* (7 Type-A, 8 Type-B, and 6 pseudo). The classification and structure of these melon TCS members were introduced in detail as well. Our results provided new insights into the characteristics of the melon TCS genes and might benefit their functional study in future.

Keywords

Cytokinin, Melon, Two-Component System

1. Introduction

Cytokinins are essential for many of the physiological and developmental processes such as seed germination, functional root nodule establishment, lateral root development, shoot apical meristem maintenance, leaf expansion, flowering, circadian clock, nutrient mobilization, abiotic stress, and senescence [1] [2] [3] [4]. In eukaryotes such as yeast and plant, a two-component system (TCS)

has been reported for the transduction of cytokinin signal [5] [6]. This TCS consists of two signaling factors, a *histidine kinase* (*HK*) gene family and a *response regulator* (*RR*) gene family [7]. *HK* can sense the cytokinin signals by phosphorylating its conserved histidine residues. The phosphoryl group is then transferred to a conserved asparagine residue on the receiver (Rec) domain of an *RR*, which modulates the activity of concerned downstream genes directly or indirectly [1] [7]. In addition, *histidine phosphotransfer* (*HP*) genes are regarded as the mediators for the transfer of the phosphoryl group between the *HKs* and the *RRs* (Figure 1) [6] [8] [9].

Melon (*Cucumis melon* L.) is an economically important fruit crop that originates from Asian, with an average production during the past decade more than 29 million tons per year (FAOSTAT, 2017; <http://www.fao.org/faostat/en/#home>). This crop is mainly cultivated in tropical and temperate countries, especially in the Asian countries, with China leading the list [10] [11]. Although melon is a eudicot of interest for its specific biological properties [10] [12], there is still no genome-wide investigation on melon TCS genes. In this study, we examined the putative TCS genes and revealed that the melon genome contained a total of 44 members. Their classification and characteristics were also analyzed systematically. Our comprehensive analysis of the TCS genes might provide a framework for future functional dissection of TCSs in melon hormone signal transduction.

2. Materials and Methods

2.1. Data Collection

Protein sequence data of *Arabidopsis* AHKs, AHPs and ARR were downloaded from the NCBI databases (<https://www.ncbi.nlm.nih.gov/>). Genome sequence data of melon deposited in the website of *Cucurbit* Genomics Data (<http://cucurbitgenomics.org/organism/3>) were used for TCS gene identification and analysis.

2.2. Identification of the Putative Melon TCS Genes

Previously cucumber and watermelon TCS members have been successfully identified by using *Arabidopsis* TCS genes [13]. So we also used *Arabidopsis* TCS protein sequences as queries to search for the putative counterparts in melon by BLASTP with E-value of $1e-5$ [14]. The Pfam (<http://pfam.janelia.org>) and SMART (<http://smart.embl-heidelberg.de/>) tools were used to check whether these genes contained the structural characteristics and conserved domains of TCS elements, *i.e.*, HisKA (Histidine Kinase A phosphoacceptor) domain, HATPase (histidine kinase-like ATPase) domain, Hpt (histidine-containing phosphotransfer) domain, and Rec domain. Information of abbreviation notation used in this article was listed in Table A1. Thereafter the identity of melon TCS genes with *Arabidopsis* was analyzed by BLASTP against *Arabidopsis* databases in TAIR (<http://www.arabidopsis.org/>). Their CDS and protein sequences

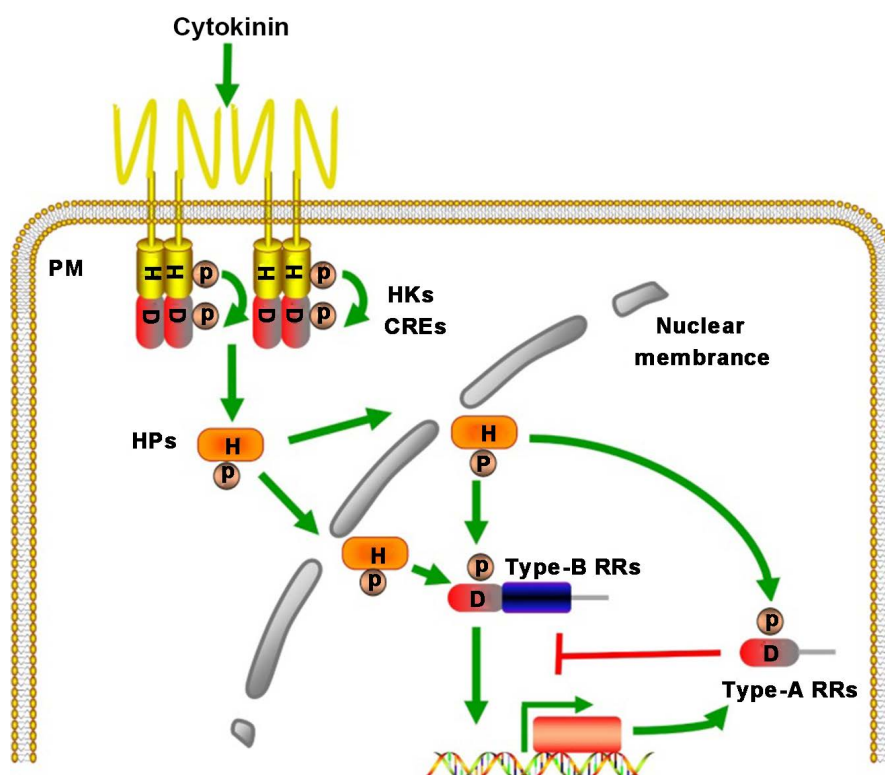


Figure 1. Model of the two-component signal transduction pathway. Cytokinin responses (CREs), histidine-kinases (HKs), histidine phosphotransfer proteins (HPs) and type-B response regulators (RRs) work as a positive regulatory loop and transfer the cytokinin from plasma membrane (PM) to nucleus. Type-B RR act as transcription factors to regulate some cytokinin targets, including type-A RRs. The type-A RRs can inhibit their own transcription, providing a negative feedback mechanism. H: phospho-accepting histidine residue. D: aspartate residue. P: phosphoric acid groups.

together with position information in melon genome were obtained from *Cucurbit* Genomics Database (<http://cucurbitgenomics.org/organism/3>). The transmembrane domains of melon TCS proteins were analyzed by TMHMM Server v.2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>).

2.3. Phylogenetic Analysis and Gene Structure Construction

Phylogenetic analysis of the full-length protein sequences was conducted using MEGA5 [15]. The evolutionary history was inferred using the Neighbor-Joining method with the following parameters: Poisson correction, pairwise deletion, and bootstrap (1000 replicates) [16] [17]. The DNA and cDNA sequences corresponding to each predicted genes were downloaded from the melon genome database (<http://cucurbitgenomics.org/organism/3>), and then the gene structures were analyzed using the Gene Structure Display Server online tool (<http://gsds.cbi.pku.edu.cn/>).

3. Results and Discussion

The TCS signaling is widely present in higher plant, including *Arabidopsis tha-*

liana [8] [9], rice (*Oryza sativa*) [18], lotus (*Lotus japonicus*) [19], soybean (*Glycine max*) [14] [20], maize (*Zea mays*) [21], *Physcomitrella patens* [22] [23], and wheat (*Triticum aestivum* L.) [24], as well as horticultural crops such as Chinese cabbage (*Brassica rapa*) [20], tomato (*Solanum lycopersicum*) [25], cucumber (*Cucumis sativus* L.) [13] and watermelon (*Citrullus lanatus*) [13] (Table 1). In *Arabidopsis*, there are 56 TCS genes and their functions have been extensively studied [8] [9]. To find the putative TCS members in melon, we performed a BLASTP search against the melon genome database by using 56 *Arabidopsis* TCS protein sequences. A total of 90 genes were selected as putative TCS genes including 26 *HK(L)s*, 6 *HPs*, 58 *RRs* in the melon genome. To confirm these putative melon TCS genes, the amino acid sequences of all 90 genes were further filtered by Pfam and SMART based on the presence of structural and conserved TCS elements. Finally, 44 typical TCS genes including 18 *HK(L)s*, 5 *HPs*, 21 *RRs* were identified in melon (Table 1). To better reflect the paralogous relationship, all melon TCS members were named according to their homology with *Arabidopsis* counterparts.

The identified 18 putative *CmHK(L)s* in melon were separated as 9 *CmHKs* and 9 *CmHK(L)s* according to the presence or absence of conserved residues required for histidine kinase activity (Figure 2(a)). Further they were classified to four distinct gene families: the typical *CmHK* family (four *cytokinin receptor*-like *CmHKs*, one *CKI1*-like *CmHK*, one *CKI2/AHK5*-like *CmHK*, and one *AHK1*-like *CmHK*), the *ethylene response (ETR)* homolog family (two *ETR1*-like *CmHKs*, one *ETR2*-like *CmHK(L)s*), the *phytochromes (PHY)* (six *PHY*-like *CmHK(L)s*) and the *pyruvate dehydrogenase kinase (PDK)* family (two *PDK*-like *CmHK(L)s*) (Table 2). The protein sequences of these *CmHK(L)s* ranged from 352 to 1261 amino acids, indicating great variations in their structures and possible functions (Table 2).

Table 1. Summary of the TCS gene number identified in plants.

Species	HK(L)	HP (pseudo-HP)	Type-A RR	Type-B RR	Type-C RR	Pseudo RR	Total	References
<i>Arabidopsis thaliana</i>	17 (9)	6 (1)	10	12	2	9	56	[8] [9]
<i>Oryza sativa</i>	14 (8)	5	20	7	0	5	51	[18]
<i>Lotus japonicus</i>	14	7	7	11	1	5	45	[19]
<i>Glycine max</i>	36 (15)	13(3)	18	15	3	13	98	[14] [20]
<i>Zea mays</i>	11 (3)	9 (2)	21	7	0	0	48	[21]
<i>Physcomitrella patens</i>	18	3	7	5	4	4	41	[22] [23]
<i>Triticum aestivum</i>	7	10	41	2	0	2	62	[24]
<i>Brassica rapa</i>	20(9)	8 (1)	21	17	4	15	85	[20]
<i>Citrullus lanatus</i>	19 (9)	6 (2)	8	10	1	5	49	[13]
<i>Solanum lycopersicum</i>	20 (11)	6 (2)	7	23	1	8	65	[25]
<i>Cucumis sativus</i> L.	18 (8)	7 (2)	8	8	0	5	46	[13]
<i>Cucumis melon</i> L.	18 (9)	5 (1)	7	8	0	6	44	This work

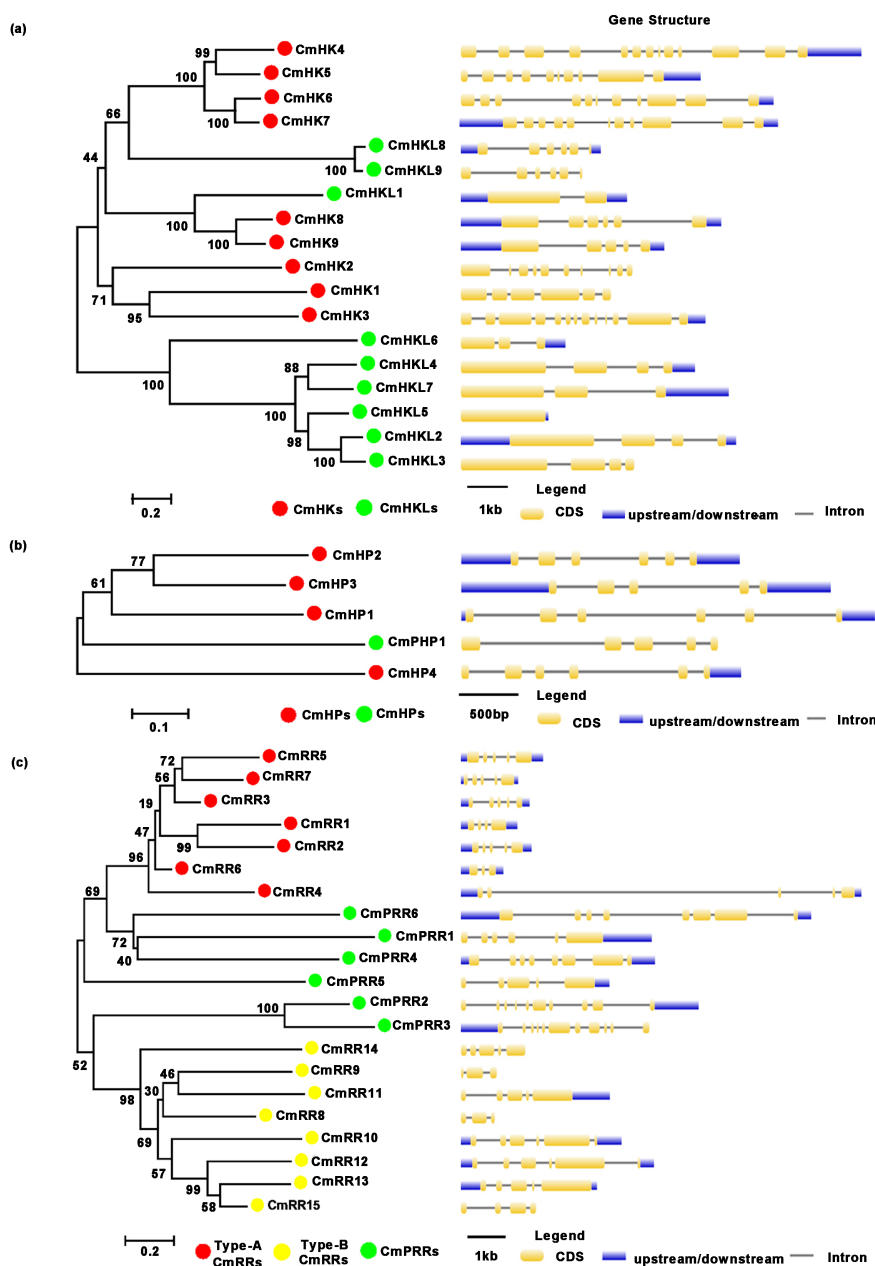


Figure 2. Phylogenetic relationship and gene structure of the Histidine kinases (a), Histidine phosphotransfer proteins (b) and Response regulators (c) in melon. The phylogenetic tree was constructed based on the Neighbor-Joining method by MEGA5. Bootstrap supports from 1000 replicates were indicated at each branch. The gene structure was analyzed using the Gene Structure Display Server online tool.

Four authentic *HPs* and one *pseudo-HP* (*PHP*) with a pseudo-*Hpt* domain were identified in melon genome (**Figure 2(b)**). The *CmHP1*, *CmHP2* and *CmHP3* had a close relationship with *AHP1* (**Figure 2(b)**, **Table 3**), a positive regulators in CK signaling [26], while the *CmHP4* was close to *AHP4* (**Table 3**), which was evolutionarily distinct from the other *AHPs* and functioned as a negative regulator in CK signaling [26]. *CmPHP1* exhibited the longest CDS and

Table 2. Features of *HK* genes in melon.

Gene name	Locus ^a	Features ^b	NO.of TM ^c	Family ^d	Chr.	Location	Length (CDS)	Length (AA)
CmHK1	MELO3C004183	HK, Rec	2	CKI1 like	5	24012076-24015727	3042	1013
CmHK2	MELO3C016937	HK	0	CKI2/AHK5 like	7	1011644-1015819	1827	608
CmHK3	MELO3C013758	HK, Rec	2	AHK1 like	6	35430786-35436735	3678	1225
CmHK4	MELO3C025982	CHASE, HK, Rec	3	AHK2 like	11	13051983-13061724	3786	1261
CmHK5	MELO3C022310	CHASE, HK, Rec	1	AHK3 like	11	29688783-29694615	2799	932
CmHK6	MELO3C020055	CHASE, HK, Rec	2	AHK4 like	10	11370534-11378139	3015	1004
CmHK7	MELO3C005250	CHASE, HK, Rec	2	AHK4 like	9	18109706-18117450	2970	989
CmHK8	MELO3C003906	GAF, HK, Rec	3	ETR1 like	5	19336090-19342422	2223	740
CmHK9	MELO3C015961	GAF, HK	3	ETR1 like	1	30737715-30742663	1914	637
CmHKL1	MELO3C006451	GAF, HKL, Rec	4	ETR2 like	6	3311645-3315687	2304	767
CmHKL2	MELO3C026502	GAF,PHY,PAS,HKL	0	PHYA like	3	22669205-22675899	3372	1123
CmHKL3	MELO3C026506	GAF,PHY,PAS,HKL	0	PHYA like	3	22702766-22706984	3462	1153
CmHKL4	MELO3C006717	GAF,PHY,PAS,HKL	0	PHYB like	6	5314892-5320586	3399	1132
CmHKL5	MELO3C024195	GAF, PHY, PAS	0	PHYC like	1	4097121-4099252	2061	686
CmHKL6	MELO3C024196	PAS, HKL	0	PHYC like	1	4094242-4096786	1326	441
CmHKL7	MELO3C002705	GAF,PHY,PAS,HKL	0	PHYE like	12	20683634-20690149	3081	1026
CmHKL8	MELO3C006749	HKL	0	PDK like	6	5676982-5680391	1110	369
CmHKL9	MELO3C003205	HKL	0	PDK like	8	30902754-30905707	1059	352

Note: a. Systematic names given to genes by *Cucurbit* Genomics Database. b. Features indicated conserved histidine-kinase (HK) domain, diverged histidine-kinase like (HKL) domain, receiver (Rec) domain, cyclases/histidine kinases associated sensory extracellular (CHASE) domain, cGMP phosphodiesterase/adenylyl cyclase/FhlA (GAF) domain, Per-ARNT-Sim (PAS) domain, and phytochrome (PHY) domain. c. Number of TM (transmembrane) from TMHMM Server v. 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>). d. The proteins belonged to which family in *Arabidopsis*, including cytokinin independent (CKI), *Arabidopsis* histidine-kinase (AHK), ethylene response (ETR), phytochrome (PHY), and pyruvate dehydrogenase kinase (PDK).

Table 3. Features of *HP* genes in melon.

Gene name	Locus ^a	Features ^b	NO.of TM ^c	Family ^d	Chr.	Location	Length (CDS)	Length (AA)
CmHP1	MELO3C015359	HPt	0	AHP1 like	2	1029369-1032563	456	151
CmHP2	MELO3C006593	HPt	0	AHP1 like	6	4414993-4417142	462	153
CmHP3	MELO3C021379	HPt	0	AHP1 like	11	27044881-27047733	393	130
CmHP4	MELO3C024439	HPt	0	AHP4 like	1	35201615-35203775	453	150
CmPHP1	MELO3C017877	Pseudo-HPt	0	AHP6 like	7	25647268-25649249	552	183

Note: a. Systematic names given to genes by *Cucurbit* Genomics Database. b. Features included conserved histidine-containing phosphotransfer (HPt) domain and a pseudo-HPt domain lacking the histidine phosphorylation site. c. Number of TM (transmembrane) from TMHMM Server v. 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>). d. The proteins belonged to *Arabidopsis* histidine phosphotransfer (AHP) family.

amino acid sequence (**Table 3**) and had close relationship with *Arabidopsis AHP6*, which functioned as a competitor of other *AHPs* and played a negative role in CK responses by interfering with phosphorelay [27].

There were 21 protein-coding genes in the melon genome that were predicted as *RRs* (**Table 4**). Also there were 6 genes encoding *RRs* without the essential residues that were required for biological activity, and were thus named as *pseudo-RRs* (*PRRs*) (**Table 4**). Among these *RRs/PRRs*, we identified seven type-A *RRs* (*CmRRI-7*), each of which contained a Rec domain along with short C-terminal extension (**Table 4, Figure 2(c)**). Furthermore, these type-A *CmRRs* exhibited close relationship to their homologs, namely, *ARR3*, *ARR5* and *ARR9* in *Arabidopsis* (**Table 4**). Genetic analysis suggests that *ARR3*, *ARR5* and *ARR9* could function as negative regulators in cytokinin signaling, thus possibly

Table 4. Features of *RR* genes in melon.

Gene name	Locus ^a	Features ^b	NO.of TM ^c	Family ^d	Chr.	Location	Length (CDS)	Length (AA)
Type-A response regulator in Melon								
CmRR1	MELO3C012031	Rec	0	ARR3 like	10	3154722-3156150	702	233
CmRR2	MELO3C017128	Rec	0	ARR5 like	2	24899213-24901003	687	228
CmRR3	MELO3C012470	Rec	0	ARR9 like	10	276889-278626	525	174
CmRR4	MELO3C010624	Rec	0	ARR9 like	3	8270800-8280939	732	243
CmRR5	MELO3C019056	Rec	0	ARR9 like	8	10595693-10597773	963	320
CmRR6	MELO3C005156	Rec	0	ARR9 like	9	16724305-16725377	468	155
CmRR7	MELO3C009770	Rec	0	ARR9 like	4	28260826-28262276	708	235
Type-B response regulator in Melon								
CmRR8	MELO3C006873	Rec	0	ARR2 like	6	6879744-6880601	603	200
CmRR9	MELO3C022469	Rec	0	ARR2 like	11	30889957-30890865	657	218
CmRR10	MELO3C006693	Rec, Myb	0	ARR2 like	6	5116428-5120493	1983	660
CmRR11	MELO3C010714	Rec, Myb	0	ARR11 like	3	29045246-29049016	1755	584
CmRR12	MELO3C016975	Rec, Myb	0	ARR12 like	7	687521-692408	2070	689
CmRR13	MELO3C010245	Rec, Myb	0	ARR12 like	2	15090353-15093797	2028	675
CmRR14	MELO3C017472	Rec, Myb	0	ARR12 like	2	21873514-21875144	1239	412
CmRR15	MELO3C004489	Rec, Myb	1	ARR12 like	5	26950179-26952081	861	286
Pseudo response regulator in Melon								
CmPRR1	MELO3C001999	Pseudo-Rec, CCT	0	APRR1 like	12	25825781-25830612	1671	556
CmPRR2	MELO3C003375	Pseudo-Rec, Myb	0	APRR2 like	4	685095-691112	1371	456
CmPRR3	MELO3C013874	Pseudo-Rec, Myb	0	APRR2 like	6	34410144-34415480	1680	559
CmPRR4	MELO3C005921	Pseudo-Rec	0	APRR5 like	9	24061601-24066514	2091	696
CmPRR5	MELO3C005336	Pseudo-Rec	0	APRR6 like	9	19505431-19509190	1686	561
CmPRR6	MELO3C003075	Pseudo-Rec, CCT	0	APRR7 like	8	29585304-29594173	2376	791

Note: a. Systematic names given to genes by Cucurbit Genomics Database. b. Features included receiver (Rec) domain, pseudo-receiver (Pseudo-Rec) domain, Myb-like DNA binding domain and CCT (CO, COL and TOC1) motif. c. Number of TM (transmembrane) from TMHMM Server v. 2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>). d. The proteins belonged to *Arabidopsis* response regulator (ARR) or *Arabidopsis* pseudo-response regulator (APRR) family.

participating in a negative feedback loop to reduce the plant sensitivity to cytokinins [28] [25].

There were eight type-B *RR* genes in melon, of which 6 members were transcription factors (TFs) and contained long C-terminal extensions with *MYB*-like DNA binding domains (Table 4). The other two type-B *RRs*, *CmRR8* and *CmRR9*, only had Rec domains and their *MYB*-like domains might be lost during the evolution of the melon *RR* family (Table 4). These type-B *CmRRs* shared high sequence similarities to their homologs, *ARR2*, *ARR11* and *ARR12*, in *Arabidopsis* (Table 4). It has been reported that *Arabidopsis* *ARR2* and *ARR12* play key roles in ethylene and CK signaling, respectively [29] [30] [31] [32] [33].

Six melon *PRRs* contained highly-diverged Rec domains (Table 4) and C-terminal extensions. Intriguingly, the CCT-domain and *MYB*-like domain in type-B *RRs* were also found in *CmPRR1/CmPRR6* and *CmPRR2/CmPRR3*, respectively. However, the exception occurred to the *CmPRR4* and *CmPRR5*, which lacked both the CCT and the *MYB*-like domains (Table 4). These great divergences should be paid more attentions on in the future study.

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Appendix

Table A1. Information of abbreviation notation.

Abbreviation	Annotation
CCT	CO, COL and TOC1
CHASE	cyclases/histidine kinases associated sensing extracellular
CK	cytokinin
CKI	cytokinin independent
CRE	cytokinin response
ETR	ethylene response
GAF	cGMP phosphodiesterase/adenylyl cyclase/FhlA
HATPase	histidine kinase-like ATPase
HisKA	His Kinase A (phosphoacceptor) domain
HK	histidine kinase
HKL	histidine-kinase like
HP	histidine phosphotransfer
HPt	histidine-containing phosphotransfer
PAS	Per-ARNT-Sim
PDK	pyruvate dehydrogenase kinase
PHY	phytochrome
PM	plasma membrane
Rec	receiver
RR	response regulator
TCS	two-component system
TM	transmembrane