# Genome-wide Identification of the CIPK Gene Family in Jasmine and Expression Analysis Under Salt Stress

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## Abstract

# Background

Various *CIPK* genes are involved in abiotic stress responses in plants. Despite the economic importance of jasmine (*Jasminum sambac*), and the availability of genome data, there are few reports analyzing the *CIPK* gene family. The aim of this study was to perform a phylogenetic analysis and characterization of the *CIPK* genes in jasmine.

# Results

In the present study, A total of 17 *CIPKs* were identified, which were unevenly distributed on chromosomes. The JsCIPK protein sequences contained 311–781 amino acids, with a molecular weight of 35.05–87.58 kDa. Phylogenetic analysis revealed that the 17 JsCIPKs could be divided into five classical branches. *JsCIPK* genes with higher homology showed greater similarity between conserved protein motifs. Collinearity analysis demonstrated that 13 gene pairs in Arabidopsis were collinear with the jasmine sequences. Various hormone-related response- and stress-induced elements were observed in the promoter region of *JsCIPK* genes. Furthermore, the expression of *JsCIPK* genes varied in different organs. Finally, the expression analyses of eight *JsCIPKs* under salt stress were performed.

# Conclusions

A systematic analysis of the CIPK gene family in Jasmine and expression analysis under salt stress was provided. These findings lay a foundation for future functional studies of JsCIPKs in jasmine related to growth and development and responses to abiotic stress.


## Background

Plants are frequently exposed to various abiotic stresses, such as salt, drought, cold, high temperature, and oxidative stress. In response, they have developed complex mechanisms to perceive external signals and adapt to various environmental stimuli through appropriate physiological and morphological changes [1]. For example, in unfavorable environments, plants can maintain a normal metabolic response by upregulating the expression of related genes and changing the structure of certain proteins to maintain the integrity of plant structure and function [2].

When plants respond to exogenous stimuli and biotic and abiotic stresses, intracellular calcium ion ($Ca^{2+}$) levels are regulated [3]. In turn, $Ca^{2+}$ plays a crucial role as a secondary messenger in plant stress signal transduction [4]. $Ca^{2+}$ sensors in plants can be classified into four types: calcin-dependent protein kinases (CDPKs), calmodulins (CaMs), calmodulin-like proteins (CAMLs), and calcineurin B-like proteins (CBLs) [5]. Among them, CBLs are specifically targeted by and interact with the CIPK family of

serine/threonine protein kinases, which are unique to plants [6], regulating downstream genes and leading to a range of physiological and biochemical changes [7]. More specifically, CBLs sense changes in $Ca^{2+}$ in plants and combine with CIPK to form a CBL-CIPK complex. This complex binds to the corresponding target proteins, phosphorylates them, and transmits downstream signals, improving the response of plants to stress [8].

CBL-CIPK protein complexes participate in various stress signal transmission networks in plants, such as drought, low temperature, high salt, and low potassium, in response to changes in the expression patterns of *CIPK* genes in plants [9]. CIPK proteins usually contain a conserved catalytic kinase domain at the N-terminus and a regulatory domain at the C-terminus. The former contains a serine/threonine protein kinase catalytic domain with an activation loop for phosphorylation. The C-terminal regulatory domain consists of the protein phosphatase interaction (PPI) motif and the autoregulatory NAF motif (also known as the FISL motif) [10]. The C-terminal regulatory domain is highly conserved and mediates the interactions between CIPK and CBL proteins [11].

With continuous improvements in genome sequencing technology, *CIPK* and *CBL* genes have been widely identified in many species, including Arabidopsis [12], rice [13], maize [6], apple [3], grape [14], pepper [15], rape [16], and tomato [17]. Significant differences in the number of *CIPK* family members exist among the different plants. Maize, rice, Arabidopsis, and tomato have 43, 31, 26, and 22 *CIPK* gene family members, respectively. In previous studies, various CBL-CIPK complexes were shown to play an important role in plant responses to abiotic stress and nutrient signaling cascades [14]. Because the sequences and structures of CBLs and CIPKs are highly conserved, scientists have hypothesized that the functions of CBLs and CIPKs are similar within and between species. For example, in Arabidopsis, the salt overly sensitive (SOS) pathway is a well-elucidated salt tolerance pathway [17] and similar SOS signaling networks exist in other species, such as begonia [18], poplar [19], and tomato [17]. Similarly, low temperature, drought, salinity, and other abiotic stresses have been shown to influence the transcription levels of 20 *OsCIPKs* in rice, among which overexpression of *OsCIPK3*, *OsCIPK12*, and *OsCIPK15* significantly improved the salt tolerance of transgenic plants, while overexpression of corn *ZmCIPK8* significantly improved drought resistance in tobacco (*Nicotiana tabacum*). Overexpression of wheat (*Triticum aestivum*) *TaCIPK14* enables tobacco to acquire stronger cold adaptation ability [20]. Overexpression of *AtCIPK21* in rice can reduce the accumulation of reactive oxygen species in transgenic plants under low-temperature stress [21]. In terms of cold stress, CIPK can induce the expression of $Ca^{2+}$-dependent protein kinases (CPK) and MAPK, and significantly increase the content of polyamines and the activity of antioxidant enzymes in plants. This may be the main way by which CIPK regulates cold resistance in plants.

Jasmine (*Jasminum sambac*) is a perennial evergreen shrub belonging to the Luteaceae family that can be primarily divided into three types: single-leaved, double-leaved, and multipetaled plants. Jasmine is native to tropical and subtropical India and the Persian Gulf. It has high ornamental, economic, edible, and medicinal value. Most varieties are resistant to cold, drought, frost, wet, and alkaline conditions, and deciduous vines are very cold- and drought-tolerant [22]. The morphology, cultivation management

technology, pest control, chemical composition, and extraction of essential oils have been studied in jasmine; however, there are few reports on the bioinformatics analysis of gene families in jasmine (*Jasminum sambac*). Extensive jasmine genomic data provide an opportunity for genome-wide identification of jasmine, as well as comparative genomic studies.

The aim of this study was to perform a bioinformatics analysis of the *CIPK* genes in jasmine using bioinformatics tools. Phylogenetic relationships, cis-acting regulatory elements prediction, and functional components were analyzed through comparisons of jasmine genome-wide-identified *CIPK* homologous sequences. These findings provide a basis for further studies on the biological functions of the *CIPK* genes in jasmine.

## Results

# Identification of members of the jasmine CIPK family

The NAF domain (PF03822) and pkinase domain (PF00069) were used as reference sequences. The HMM search method was used to identify potential members of the *JsCIPK* gene family, and BLASTP was used to search the jasmine protein database using 26 AtCIPKs as query sequences. The results of the two search methods were compiled, redundant sequences were removed from the dataset, and 17 JsCIPK protein sequences were identified. These putative *JsCIPK* genes were named *JsCIPK1– JsCIPK17* according to their locations on different chromosomes. These genes were unevenly distributed on the chromosomes, with only one *JsCIPK* gene each on chromosomes 8 and 13; two on chromosomes 1, 2, 6, and 11; three on chromosome 9; and four on chromosome 3 (Fig. 1).

Analysis of the physical and chemical properties revealed that the JsCIPK protein sequence ranged from 311 (JsCIPK5) to 781 (JsCIPK1) amino acids (Table 2), with an average amino acid number of 465. The molecular weight (Da) of the proteins ranged from 35049.82 (JsCIPK5) to 87584.34 (JsCIPK1). The theoretical pIs of the CIPKs ranged from 5.52 (JsCIPK10) to 9.24 (JsCIPK6). Analysis of the pI values showed that most of the CIPK proteins were basic; only JsCIPK1 was acidic. The instability indices of JsCIPK2, JsCIPK8, JsCIPK9, JsCIPK10, and JsCIPK17 were > 40, indicating that the proteins may be unstable, whereas the other JsCIPKs were stable. The grand average hydropathicity (GRAVY) was negative, indicating a hydrophilic protein. Subcellular localization showed that all of the proteins were localized to the plasma membrane.

**Table 1.** Gene-specific primers were designed for the expression analysis of eight *JsCIPK* genes by RT-qPCR

| | Sequences (5'–3') | |
|---|---|---|
| **Primers** | **Forward** | **Reverse** |
| *JsCIPK1* | GGCCTCAACCTTGCTTCTCT | GTGACCCTTGCGTCCAATCT |
| *JsCIPK2* | AGGTTTTGGCTCGAAAGGGT | TCCGGTGTGAACCATCTTGG |
| *JsCIPK6* | AGGCATGACGGAGCAAATCA | TCGACCCCTAGCGATTTTGG |
| *JsCIPK7* | GCACCGGAGGTATTAAGCCA | TACGCAGCAATGACACGGAA |
| *JsCIPK8* | GGGAGGCTTTTAGGGAAGGG | AACCAATCGCATGACGGAGA |
| *JsCIPK12* | ACGGCGCAAAAGTTGACATC | GTATCCGGGTTCGGGTCAAG |
| *JsCIPK13* | TCCAATCCATTTGGTGGCGA | GCACATCTTTCGGCATTCCC |
| *JsCIPK14* | TTGGCTATGATGGGGCAACC | GCTGAACGAAAGCCAAGGTG |
| *JsACTIN2* | TCTCTATGGTAACATTGTCCTG | ATCCAGACACTGTACTTCCTCT |

Table 2
Physiological characterization of *JsClPKs*

| Gene Name | Amino acids (no.) | Molecular wt (Da) | Isoelectric points (pH) | Instability Index | GRAVY | Subcellular location prediction |
|---|---|---|---|---|---|---|
| *JsClPK1* | 781 | 87584.34 | 9.2 | 34.08 | -0.3 | Plasma membrane |
| *JsClPK2* | 466 | 52539.46 | 8.83 | 44.22 | -0.293 | Plasma membrane |
| *JsClPK3* | 427 | 48192.72 | 9.12 | 31.36 | -0.292 | Plasma membrane |
| *JsClPK4* | 439 | 50258.81 | 7.14 | 34.35 | -0.438 | Plasma membrane |
| *JsClPK5* | 311 | 35049.82 | 8.14 | 37.59 | -0.251 | Plasma membrane |
| *JsClPK6* | 427 | 47803.13 | 9.24 | 32.77 | -0.336 | Plasma membrane |
| *JsClPK7* | 431 | 48451.73 | 7.25 | 36.73 | -0.162 | Plasma membrane |
| *JsClPK8* | 453 | 51333.05 | 8.7 | 40.09 | -0.333 | Plasma membrane |
| *JsClPK9* | 423 | 47904.25 | 9.13 | 43.32 | -0.261 | Plasma membrane |
| *JsClPK10* | 413 | 46984.35 | 5.52 | 49.4 | -0.387 | Plasma membrane |
| *JsClPK11* | 448 | 51180.02 | 8.95 | 32.16 | -0.372 | Plasma membrane |
| *JsClPK12* | 445 | 50465.36 | 9.09 | 37.73 | -0.324 | Plasma membrane |
| *JsClPK13* | 546 | 62336.68 | 8.68 | 30.32 | -0.462 | Plasma membrane |
| *JsClPK14* | 423 | 49621.04 | 7.63 | 35.65 | -0.462 | Plasma membrane |
| *JsClPK15* | 442 | 50283.96 | 8.91 | 36.71 | -0.415 | Plasma membrane |
| *JsClPK16* | 492 | 55492.27 | 9.18 | 37.35 | -0.28 | Plasma membrane |
| *JsClPK17* | 523 | 59268.7 | 9.04 | 40.28 | -0.274 | Plasma membrane |

# Phylogenetic analysis of the CIPK gene family in jasmine

To study the evolutionary relationship of CIPK proteins between jasmine and Arabidopsis, an ML phylogenetic tree was constructed using the CIPK amino acid sequences. The results showed that the 43 CIPKs could be divided into five subfamilies (named Groups I–V) as follows: Group I, JsCIPK2, JsCIPK3, JsCIPK5, JsCIPK6, JsCIPK13, and JsCIPK15; Group II, JsCIPK9; Goup III, JsCIPK11 and JsCIPK8; Group IV, JsCIPK12; and Group V, JsCIPK1, JsCIPK4, JsCIPK7, JsCIPK10, JsCIPK14, JsCIPK16, and JsCIPK17 (Fig. 2). These results also indicated that intron-rich *JsCIPKs* clustered into Group V, whereas intron-poor and intron-free *JsCIPKs* clustered into the other four groups, which was consistent with the results for Arabidopsis.

# Gene structure and conserved motifs of *JsCIPKs*

Structural analysis of *JsCIPKs* showed that the sequence length of the genes ranged from 1365 bp (*JsCIPK9*) to 6350 bp (*JsCIPK4*) and contained coding sequences (CDS) and untranslated region (UTR) with different numbers and lengths, respectively (Fig. 3). UTR statistics showed that *JsCIPK2*, *3*, *5*, *6*, *9*, *11*, *12*, *13*, *15*, and *17* had both exon start and end structures. Except for *JsCIPK1*, all the other members of the gene family contained a complete UTR region. CDS analysis showed that *JsCIPK1* and *JsCIPK16* contained 16 exons; *JsCIPK4*, *JsCIPK14*, and *JsCIPK17* contained 14 exons; and *JsCIPK7* and *JsCIPK10* contained 13 exons, respectively. There were four exons each in *JsCIPK5* and *JsCIPK13*, two in *JsCIPK8* and *JsCIPK12*, and only one in the other *JsCIPKs*. The *CIPK* gene family members were divided into intron-harboring and intron-less types, consistent with the results of other plant *CIPK* gene family members. These results indicated that homologues clustered in the same subfamily have similar genetic structures and conserved functions.

The conserved motifs of the jasmine CIPK protein family were analyzed using the MEME tool, and 10 motifs were predicted. The results showed that the motifs of most JsCIPK proteins were the same in type and sequence, and the higher the homology, the higher the similarity of the motifs. Among them, JsCIPK5 lacked motif 4–7, and JsCIPK10 lacked motif 5 (Fig. 3). A Batch CD-search was used to analyze the CIPK domains of jasmine. The predicted results showed the presence of the CIPK_C domain in the C-terminus of JsCIPKs protein sequences, whereas the PKc_like superfamily was present in the N-terminus of JsCIPKs protein sequence STKc_SnRK3, which had two different domains. The structure of the N-terminal kinase, the base of non-ferferent-1 (SNF-1) kinase, is similar to that of sucrose and matches the structural characteristics of the CIPK protein (Fig. 3). In this study, JsCIPK protein sequences were homologous, and all JsCIPKs clustered into five groups, I–V, according to the type and sequence of the motifs. We speculated that homologous proteins were more similar in the same group, and that there might be functional redundancy.

# Collinearity analysis of jasmine CIPK gene family

To further understand the phylogenetic relationship of *CIPKs*, a collinearity analysis diagram of Arabidopsis and *J. japonicum* genes, and a collinearity diagram of *CIPK* gene family members of each

species were constructed. The results showed that 13 gene pairs in Arabidopsis were collinear with jasmine genes (Fig. 4a). A collinear relationship was observed, including among *JsCIPK15*, *AtCIPK12*, *AtCIPK13*, *ATCIPK18*, *ATCIPK19*, *JsCIPK2*, *AtCIPK12*, *AtCIPK13* and *ATCIPK19*; *JsCIPK6* with *AtCIPK1* and *AtCIPK17*; *JsCIPK9* with *AtCIPK4* and *AtCIPK7*; and *JsCIPK12* with *AtCIPK14* and *AtCIPK22*. The collinearity pairs of Arabidopsis and jasmine included *JsCIPK3* and *AtCIPK22*; *JsCIPK4* and AtCIPK3; *JsCIPK13* and *AtCIPK15*; *JsCIPK16* and *AtCIPK23*; and *JsCIPK17* and *AtCIPK21*. In addition, the *CIPKs* of *J. japonicum* included four gene replication pairs: *JsCIPK1*:*JsCIPK16*, *JsCIPK2*:*JsCIPK15*, and *JsCIPK3*:*JsCIPK14* (Fig. 4b).

# Analysis of cis-acting elements of CIPK gene family members in jasmine

To explore the potential functions of *CIPK* genes, cis-acting element prediction was performed on the promoter region sequence 2000 bp upstream of the *JsCIPK* start site (Fig. 5). The results demonstrated that there were several cis-acting elements in the promoter region, and the types and quantities of cis-acting elements differed in different gene members. Eight components were identified in the promoter region of *CIPK*, including ABRE, TGA-element, TC-rich, GARE-motif, LRE, LTR, MeJA, and SA. Except for *JsCIPK4*, all of the other genes contained more than four types of functional elements. All of the *JsCIPKs* contained light-response elements, most of which contained methyl jasmine- and abscisic acid-response elements. Among these, 14 genes had abscisic acid cis-acting elements, 13 had methyl jasmine cis-acting elements, and most had low-temperature-, salicylic acid-, and gibberellin-response elements. Three gene family members had auxin-response elements and three had defense stress elements, suggesting that the three family members with defense stress elements might be involved in the abiotic stress response in jasmine.

# Expression analysis of *JsCIPK* genes

Heat map analysis revealed that *JsCIPK5* was not expressed in any organ. *JsCIPK1, JsCIPK2, JsCIPK6*, and *JsCIPK13* were strongly expressed in leaves; and *JsCIPK1*, *JsCIPK2*, and *JsCIPK13* were highly expressed in flowers. *JsCIPK2* and *JsCIPK12* were significantly expressed in roots, whereas *JsCIPK1, JsCIPK6*, and *JsCIPK13* were expressed in stems. The expression of *JsCIPK2* in roots was higher than that in the leaves and flowers, and the expression of *JsCIPK2* was relatively high in all tissues. Other *JsCIPK* genes were expressed at low levels in all tissues. Based on these results, the expression of most *JsCIPK* genes appeared to be tissue-specific (Fig. 6).

To determine the role of *JsCIPKs* on the response to salt stress, the expression of eight *JsCIPKs* were analyzed (Fig. 7). The results demonstrated that the expression of all *Js CIPKs* had varying degrees of response under salt stress. It is worth mentioning that the expression of *JsCIPK6* was down-regulated by salt stress at different time, and the expression of *JsCIPK8* was up-regulated significately.

# Discussion

The *CIPK* gene participates in plant development in Arabidopsis and rice (Kanwar et al., 2014); however, there are no corresponding reports in jasmine. In this study, 17 *CIPK* gene family members were screened through bioinformatics analysis, and all of these genes were comprehensively identified, including analysis of their evolutionary relationship, chromosome structure, and chromosome distribution.

The first plant *CIPK* was discovered in the model plant Arabidopsis in which 26 *CIPKs* have now been identified [23]. In addition, among Solanaceae plants, 15 *CIPK*s have been identified in eggplant [24] and 21 in pepper. Among the Gramineae, 34 *CIPK*s have been identified in rice [25], 51 in turnip [26], and 43 in maize [27]. Among woody plants, 16 *CIPK*s have been identified in plum and 20 in grapes [14]. *CIPKs* have also been detected in algae, mosses, ferns, Cucurbitaceae, and other plants. Analysis of the location of *CIPK*s on chromosomes showed that the number of *CIPK*s distributed on chromosomes 3, 6, and 9 was higher than that on other chromosomes, which is consistent with millet and maize in the Gramineae. The uneven distribution of *CIPKs* may be related to species evolution and genetic variation.

NAF- and PPI-conserved motifs mediate specific binding of CBL-CIPK and PP2C-CIPK signal transduction pathways, respectively. In this study, a motif5 deletion was observed in the gene structure of *JsCIPK5* and *JsCIPK10*. However, whether deletion of this motif affects the signaling pathway of CIPK remains to be verified. A Batch CD-search revealed that all JsCIPKs were composed of a highly conserved kinase domain and a relatively conserved regulatory domain, reflecting the highly conserved evolutionary characteristics of JsCIPKs in jasmine.

Analysis of gene expression profiling data showed that the majority of Group I *JsCIPKs* had significantly higher expression levels in different organs than that of other *JsCIPK* gene group members. However, a few members of Groups II and III also showed high expression. Subsequently, eight JsCIPKs with high expression levels in different organs were selected as the expression determination under salt stress. The results showed that most *JsCIPKs* were up-regulated under salt stress, and the expression level reached its highest after 1 h of salt stress treatment. It is worth mentioning that the expression of *JsCIPK6* was down-regulated by salt stress at different time, and the expression of JsCIPK8 was up-regulated significately.

In conclusion, 17 *JsCIPK* genes were identified in this study, and a comprehensive bioinformatics analysis was carried out to determine gene structure, physicochemical properties, chromosomal location, expression patterns in different tissues, and collinearity. Previous studies have shown that *CIPKs* are involved in various abiotic stress responses, including to drought, low temperature, high pH, and high salinity. The expression levels of *JsCIPK2* and *JsCIPK13* were very high in in jasmine roots, stems, leaves, and flowers, indicating that these two genes are important in the four major plant growth organs. In contrast, the expression of *JsCIPK6* was relatively high in leaves and stems, but low in flowers and roots, thus indicating that this gene is mainly involved in leaf and stem development. Besides, the expression of *JsCIPK6* and *JsCIPK8* was significately down-regulated and up-regulated by salt treatment, respectively. The functions of these two genes in response to salt stress can be further studied in the future. These

findings can lay the foundation for the genetic improvement of JsCIPKs in salt tolerant jasmine germplasm.

## Methods

# Identification and characterization of CIPK family members in jasmine

The 26 CIPK protein sequences of Arabidopsis were obtained from the TAIR website and downloaded locally. Three steps were performed to identify the members of the CIPK family in jasmine. First, the Hidden Markov Models (HMM) of Pkinase (PF00069) and NAF (PF03822) were downloaded from the Pfam database, and the protein database (E-value < 0.01) was searched using HMMER3 software [28]. The Arabidopsis CIPK protein sequences were used as seed sequences, and the jasmine protein sequences annotated using BLAST in the jasmine genomic database (https://github.com/maypoleflyn/Jasminum-sambac-genome) were downloaded to the local site for further analysis. If each CIPK member contained multiple genes with high homology, only the sequences with the highest homology were selected. To obtain all CIPKs encoded in the genome, the protein sequences of Jasmine CIPKs (JsCIPKs) were reverse translated into nucleotide sequences and further compared with the nucleotide sequences of the *J. sambac* genome to avoid missing any unannotated *CIPK* genes. Further comparative analysis was carried out using a conservative domain database (CCD) [29]. Eventually 17 jasmine *CIPK* genes were identified. The above results were analyzed using TBtools software. The number of amino acids, isoelectric point (pI), protein stability, and molecular weight of the JsCIPKs were analyzed using the ExPASy website [30]; and secondary structure prediction and subcellular localization was carried out using Subcellular Localization Predictor [31].

# Phylogenetic analysis and chromosomal mapping of JsCIPKs

The protein coding sequences of 26 *AtCIPK* and 17 *JsCIPK* genes were analyzed by multiple sequence alignment using Clustal X software. After sequence alignment, the matrix was used for phylogenetic tree analysis, and a phylogenetic tree was constructed using the Maximum Likelihood (ML) method in MEGA7 software (Arizona State University, Tempe, AZ, USA), with a bootstrap test value set at 1000 [32]. We extracted the chromosomal location data of the *JsCIPK* gene family members from genome annotation files and determined whole chromosome densities. TBtools was used for visual analysis, showing only those chromosomes containing *JsCIPK* members [33].

# Analysis of conserved motifs and domains of the jasmine CIPK family

Gene sequences were analyzed for gene structure, conserved motifs, conserved structural domains, and multiple sequence alignment. Clustal X was used for multiple sequence alignment analysis of the protein

sequences encoded by the 26 *AtCIPK* and 17 *JsCIPK* genes. The conserved protein motifs of JsCIPKs were analyzed using online prediction in MEME [34]. Promoter element prediction was performed using the PLANTCARE database [35]. Gene structure, conserved motifs, and cis-acting elements of all members of the *CIPK* gene family were visualized using the gene structure viewer in TB tools.

# Analysis of *CIPK* gene distribution and collinearity in jasmine

Based on the gene position number, the corresponding chromosomal location information of jasmine was extracted from the genome annotation files [36], visualized, and identified using MapChart software. Analysis of the colinear relationship between gene family members was performed by MCScanX [37].

# Prediction of cis-acting elements in the jasmine *CIPK* gene

The conserved *CIPK* motif was analyzed using the MEME suite [38]. The Amazing Optional Gene Viewer in TBtools was used to determine the structure of *JsCIPK*. The cis-elements of all promoter sequences (approximately 2000 bp) were identified using PlantCARE [36].

# Analysis of the expression patterns of *CIPK* genes in jasmine

RNA-seq data of jasmine root, stem, leaf, and flower organs were obtained from the NCBI SRA database under accession number SRR14317414-SRR14317417. Then the expression levels of 17 *JsCIPK* genes were collected and analyzed, and a heat map was generated using TBTools.

# Expression analysis of *JsCIPK* genes under salt stress

3-month-old jasmine were treated with 300 mM NaCl, and leaves were collected at 0, 1, 6, and 12 h for the expression analysis of *JsCIPK* genes by qRT-PCR analysis with specific primers (Table 1). Three biological replicates were performed for all treatments. Then, $2-\Delta\Delta$ CT method was taken as the analyzation of data.

## Abbreviations

calcin-dependent protein kinases: CDPKs; calmodulins: CaMs; calmodulin-like proteins: CAMLs; calcineurin B-like proteins: CBLs; the protein phosphatase interaction: PPI; salt overly sensitive: SOS; $Ca^{2+}$-dependent protein kinases: CPK; contained coding sequences: CDS; non-ferferent-1: SNF-1; qRT-PCR: Quantitative real-time PCR

## Declarations

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Not applicable.

### Authors' contributions

RW and LY conceived and designed the study. LY, RW, KC, TC, XH and XZ conducted the experiments and analyzed the data. LY and RW wrote the manuscript. All authors reviewed the manuscript.


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### Data availability statement

The jasmine genome, annotation file, and RNA-seq data were deposited in Github (https://github.com/maypoleflyn/Jasminum-sambac-genome).

### Ethics approval and consent to participate

Not applicable

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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# Figures

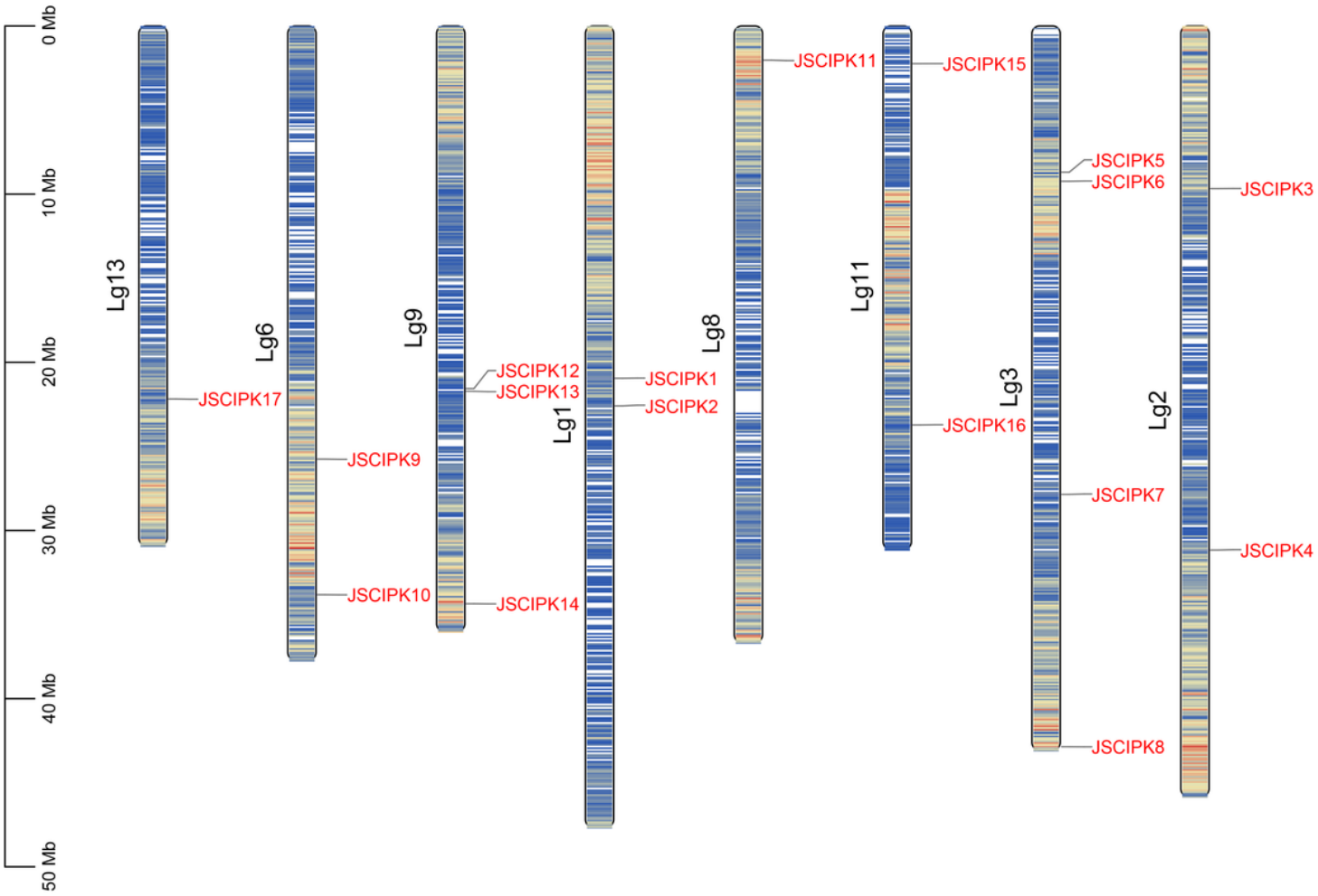
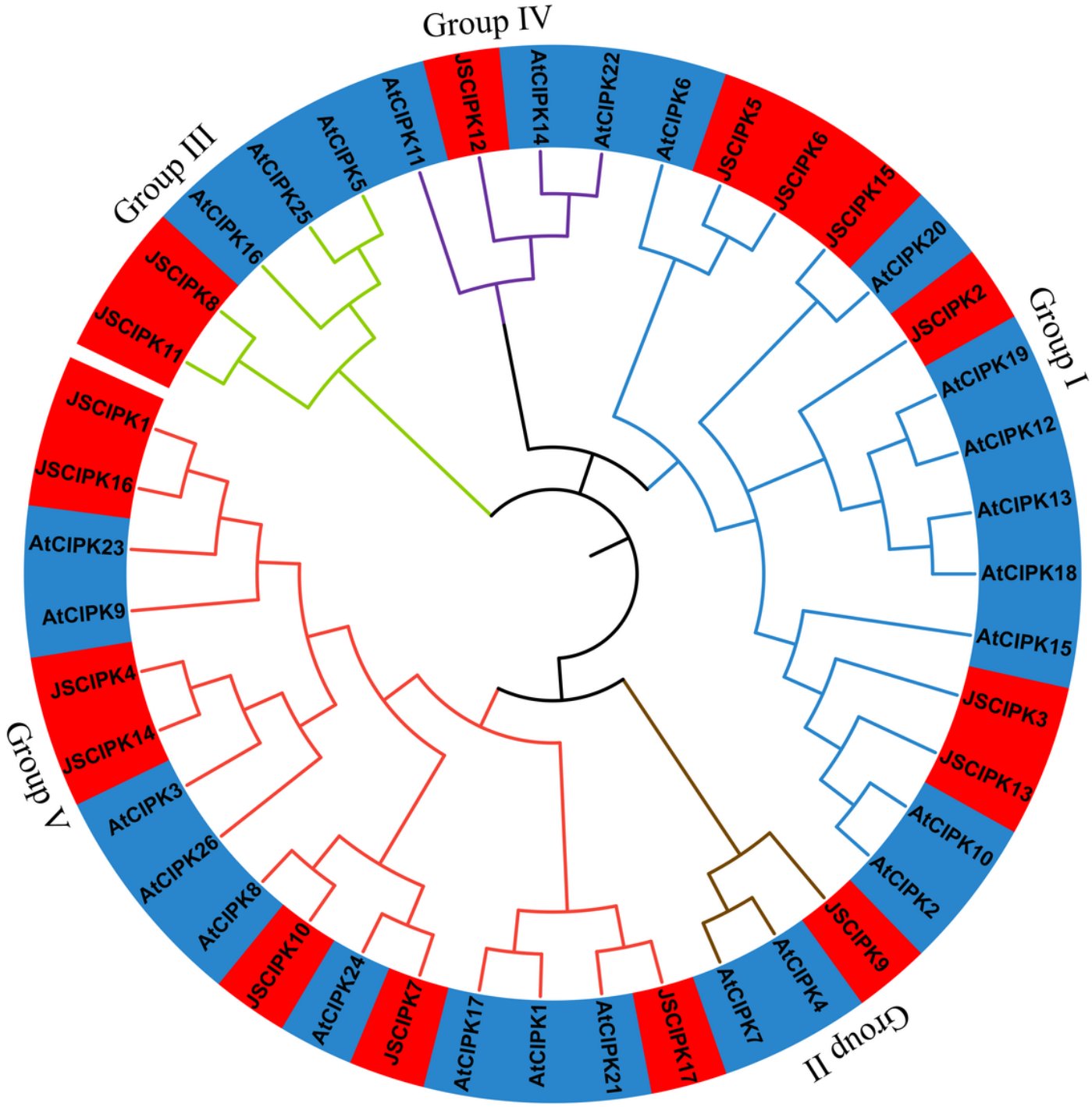



**Figure 1**

Chromosomal locations of *Jasminum sambac* (JS) *CIPK* genes.




## Figure 2

The phylogenetic analysis of CIPK proteins families across *Jasmine sambac* (JS) and *Arabidopsis thaliana* (At)


## Figure 3

Analyses on phylogenetic relationships, motifs, and gene structure of *CIPK* genes from *Jasminum sambac*. (**A**)Motif distribution of JsCIPK proteins. Protein motif architectures were indicated using Pfam and MEME online tool. (**B**) Exons and introns structures of *JsCIPK* genes

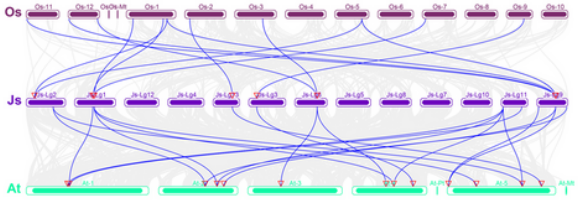



Figure4A.png

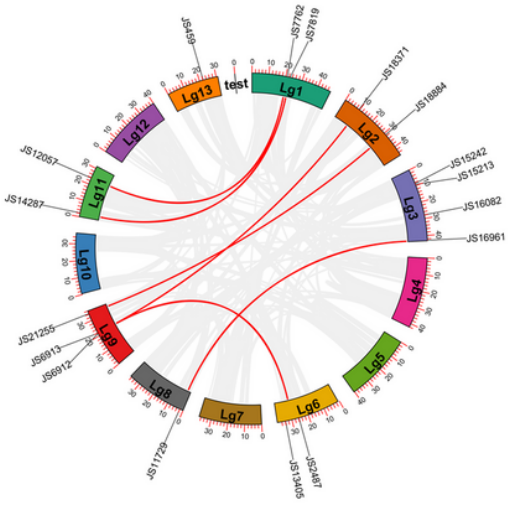
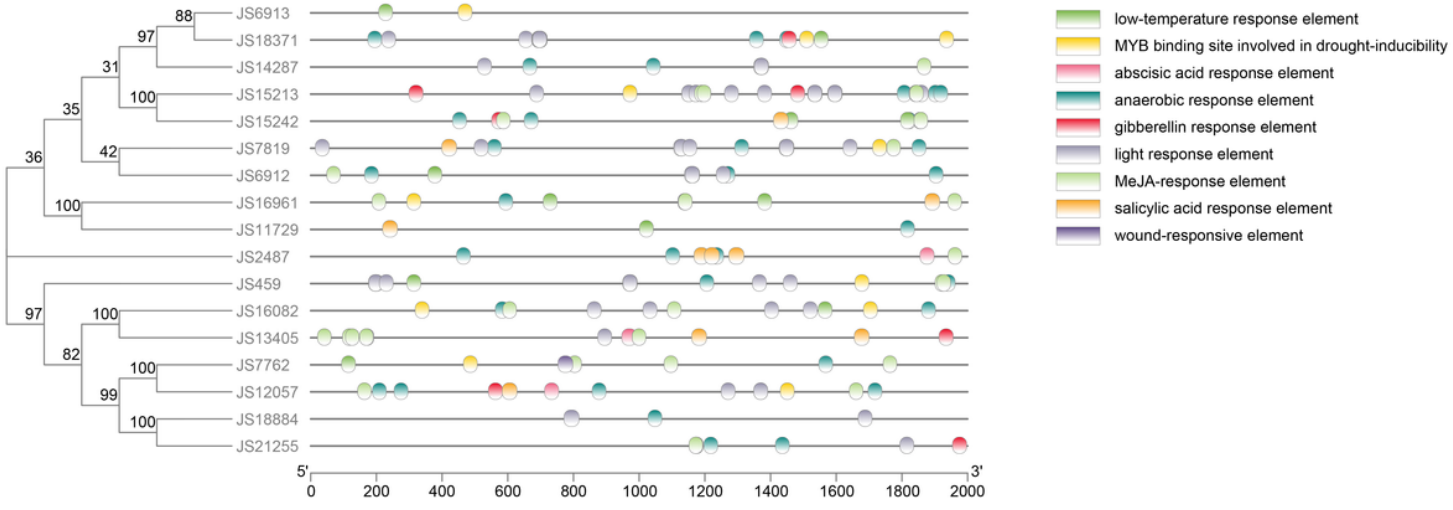



Figure4B.png

## Figure 4

Collinearity analysis of *CIPKs* in *Jasmine sambac* and *Arabidopsis thaliana*




## Figure 5

Types and number of cis-promoters involved in the stress response

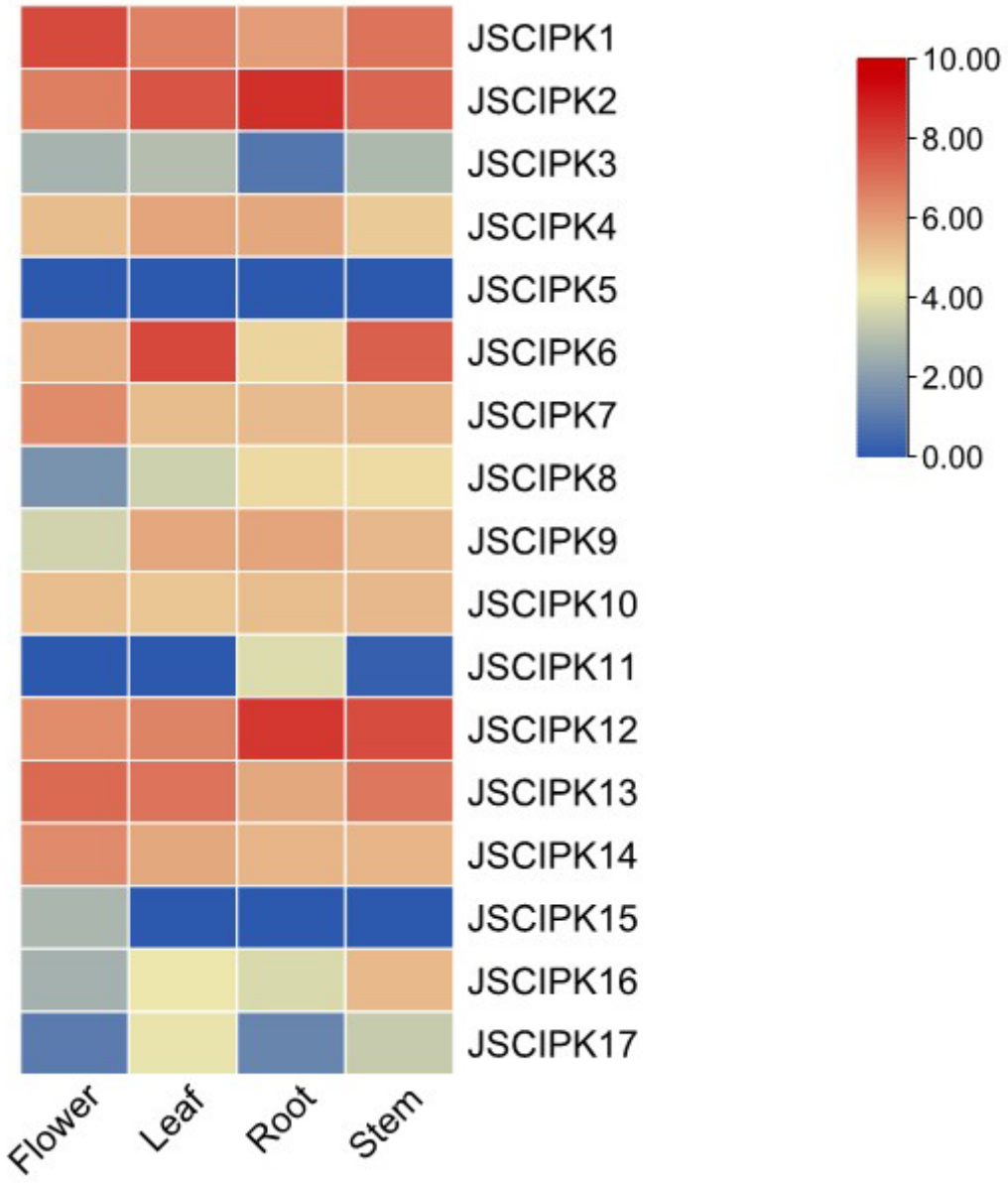
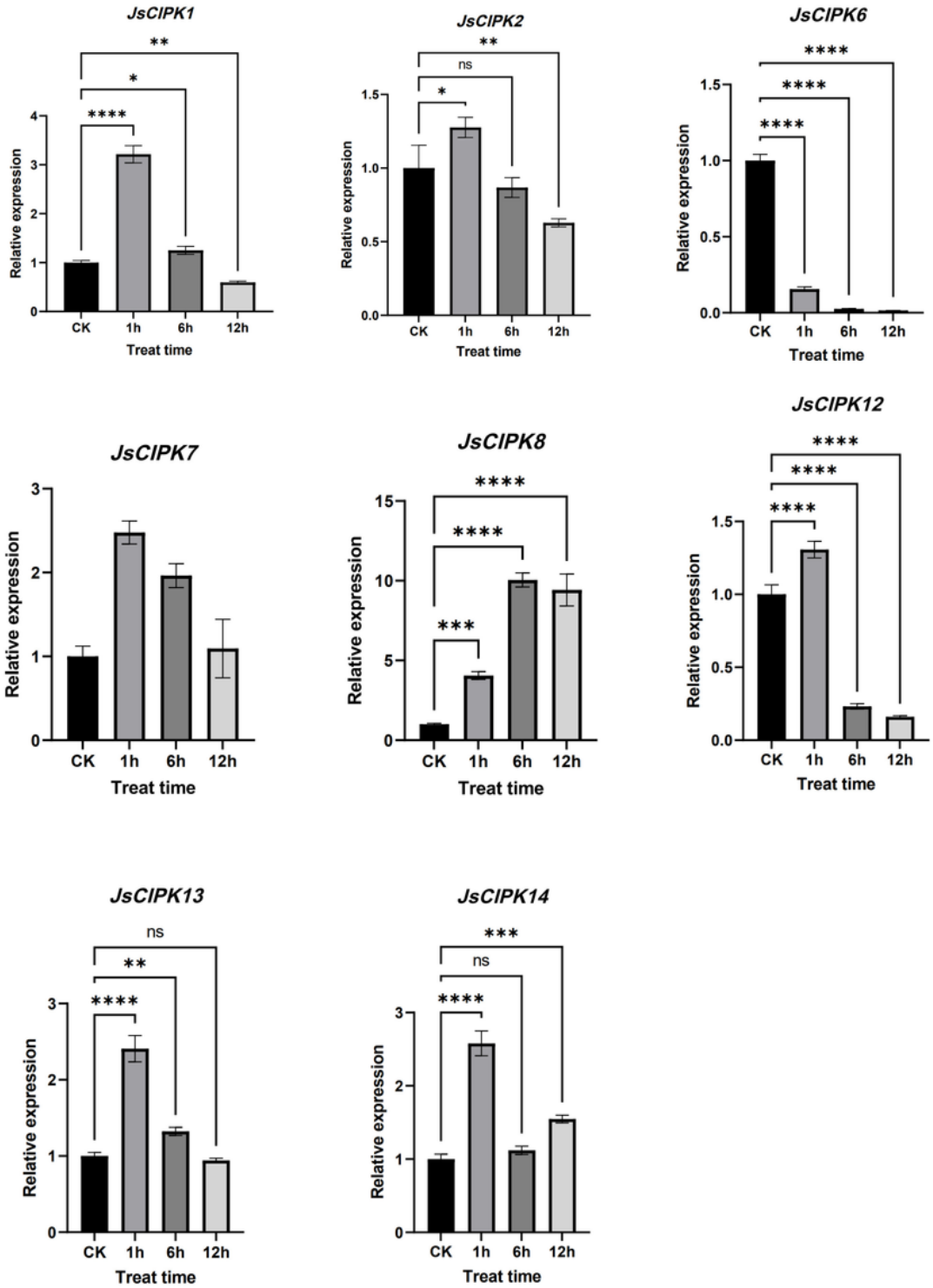



**Figure 6**

Relative mRNA expression levels of *CIPK* genes in the different jasmine tissue.




## Figure 7

Effect of salt stress (300 mM NaCl) on the expression of eight *JsCIPK* genes in leaves of jasmine. ns represents $P > 0.05$, * represents $P \leq 0.05$, **represents $P \leq 0.01$, *** represents $P \leq 0.001$ and **** represents $P \leq 0.0001$.